NMR Evidence of Slow Monomer−Micelle Exchange in a Calixarene-Based Surfactant

Nuno Basilio,[†] Luis García-Río,*[,†] and Manuel Martín-Pastor[‡]

Departamento de Química Física, Facultad de Química, Laboratorio Integral de Dinámica e Estructura de Biomoléculas José R. Carracido, Unidade de Resonancia Magnética, RIAIDT, Edif. CACTUS, Universidad de Santiago, 15782 Santiago, Spain

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The self-aggregation of amphiphilic *p*-sulfonatocalix[4]arene was studied by fluorescence and NMR techniques. Pyrene fluorescence spectra were used to determine the critical micelle concentration and the value obtained was confirmed using Diffusion-Ordered NMR Spectroscopy (DOSY). It was found from the ^{1}H NMR spectra that exchange between the monomers in the bulk and in the micelles is slow on the NMR time scale. This finding was confirmed by 2D EXSY experiments and the rate constants of the process at different temperatures were extracted using the latter technique.

Introduction

When dissolved in water, surfactant molecules tend to form aggregates above their critical micelle concentration (cmc). Micelles are the most common and the most thoroughly studied aggregates observed in surfactant solutions.[1] Micellar aggregates are short-lived dynamic species. Rapid exchange occurs between surfactant molecules in the micelles and those in the surrounding bulk solution. For single chain surfactants, the exchange is relatively fast with characteristic times of the order of 1 ns to 1 ms, and rapid techniques such as ultrasonic absorption,[2,3] pressure-jump,[4] temperature-jump,[5] and stopped-flow dilution techniques are required for their measurement.[6] Since the exchange rate is too fast on the NMR time scale, the NMR spectra of surfactant molecules show averaged signals for free and self-assembled monomers.[5] However, it has been found that for some cationic gemini[7,8] and fluorocarbon-hydrocarbon hybrid[9] surfactants the exchange is slow on the NMR time scale and it is possible to observe separate signals for monomers in the bulk solution and in the micelles. This situation allows the rate constants for the process to be determined by NMR techniques.

Because of their facile modification,[10] calixarenes are particularly attractive for the construction of "surfactants with a host-guest recognition site".[11] Shinkai et al. showed that *p*-sulfonatocalixarenes bearing appropriate alkyl groups at the lower rim form micelles in aqueous solution.[11] The same researchers also demonstrated that the conformation of the calixarene is a crucial parameter in modulating the aggregation behavior[12] with the cone shaped conformation being ideal for the formation of globular micelles. After Shinkai's pioneering work, several examples of amphiphilic calixarenes appeared in the bibliography. For instance, Strobel et al.[13] showed that the nature of the ionic headgroup is a key parameter in the aggregation behavior of calixarene amphiphiles; carboxylated calixarenes form vesicles whereas calixarenes with trimethylammonium head groups form micelles. Hirsch's group[14] used calix[4]arene as a building block to construct an amphiphile that assembled into completely uniform and structurally precise micelles, which are spontaneously formed by exactly seven monomers in aqueous solution and are nondeformable upon drying. Of special interest is the case of stimuli-responsive assemblies. It was shown that vesicles formed by calixarene derivatives transform into micelles on dropping the pH from 7 to 5 with subsequent release of encapsulated guests.[15] Houmadi et al. presented amphiphilic calix[6]arene-based systems with an aggregation architecture that responded both to pH and metal ions.[16]

Recently, it has been reported that water-soluble nucleotide-calix[4]arene self-assembles into micelles and that the exchange between dimers and micelles is slow on the NMR time scale.[17] To the best of our knowledge, this example and the water-soluble guanidinium calixarene derivatives[18] are the only examples of this phenomenon in amphiphilic calixarene systems to have been reported.

In this work, we present the results of combined ^{1}H NMR, diffusion NMR, and fluorescence studies on the micellization of *p*-sulfonatocalix[4]arene bearing hexyl chains at the lower rim (SC4TH).

Experimental Section

All commercial reagents were of the highest purity available and were used as received. *p*-Sulfonatocalix[4]arene (SC4) was prepared by *ipso*-sulfonation of *p*-tert-butylcalixarene in H_2SO_4 at 80 °C. SC4TH was synthesized from SC4 following literature procedures.[19] The product was characterized by ^{1}HNMR and ^{13}CNMR spectroscopy and MALDI-TOF mass spectrometry. SC4TH, ^{1}H NMR (400 MHz, D_2O, ppm) δ = 0.96 (t, 12H), 1.42 (br, 24H), 2.02 (q, 8H), 3.43 (d, 4H), 3.93 (t, 8H), 4.44 (d, 4H), 7.35 (s, 8H); ^{13}C NMR (400 MHz, D_2O, ppm) 158.0, 137.3, 134.5, 126.0, 75.6, 32.0, 30.30, 30.1, 25.9, 22.7, 13.8; MALDI-TOF-MS *m*/*z* ($M + 4H - 3Na^+$) 1103.4; calcd 1103.4.

The fluorescence spectra of pyrene were measured on a Cary Eclipse instrument with an excitation wavelength of 334 nm. The pyrene concentration was kept constant at 0.4 μM. ^{1}H NMR and Diffusion-Ordered NMR Spectroscopy (DOSY) spectra were recorded at 25 °C on a Varian Inova 400 spectrometer. The DOSY spectra were acquired with the standard stimulated echo pulse sequence using LED and bipolar gradient pulses.[20] Square-shaped pulsed gradients of 2 ms duration were applied

* To whom correspondence should be addressed. E-mail: luis.garcia@usc.es.

[†] Departamento de Química Física, Facultad de Química.

[‡] Laboratorio Integral de Dinámica e Estructura de Biomoléculas José R. Carracido, Unidade de Resonancia Magnética, RIAIDT, Edif. CACTUS.

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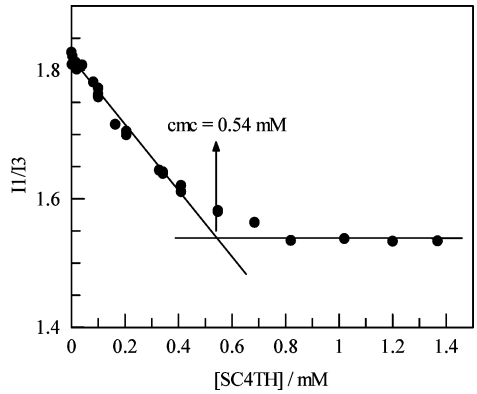


Figure 1. Plot of the *I*1/*I*3 ratio in pyrene fluorescence vs concentration of SC4TH; [pyrene] = 0.4 × 10^{-3} mM, excitation at 334 nm.

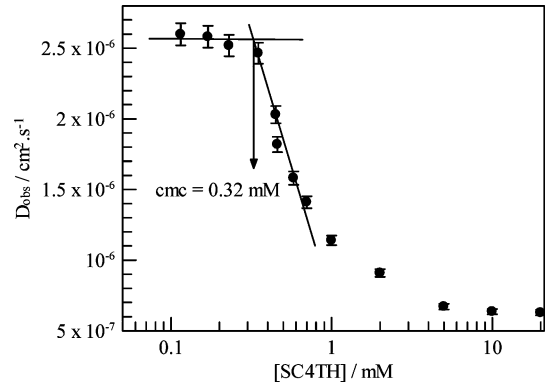
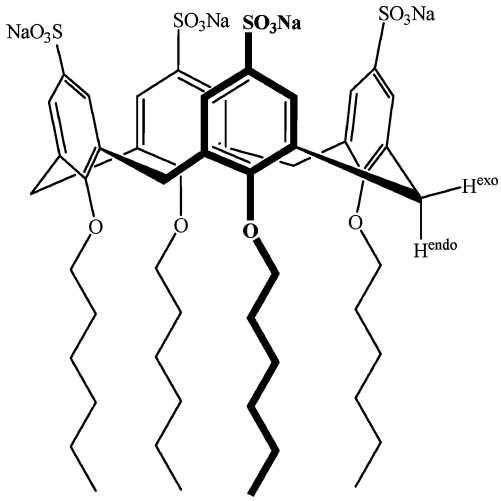


Figure 2. Variation of observed diffusion coefficient, D_{obs}, for SC4TH with concentration in D_2O at 25 °C.

with a power level linearly incremented from 2.1 to 64.3 G cm^{-1} in 20 steps. To obtain reliable results for the diffusion coefficient, the diffusion time Δ of the experiment was optimized for each sample to a value between 60 and 200 ms. The raw data were processed using the MestreC program (Mestrelab Research Inc.). Two-dimensional EXSY experiments were carried out on a Varian Inova 750 spectrometer at 25 °C with mixing times 0 (reference) and 200 ms. Rate constants were calculated from the peak intensities using the EXSYCALC program (Mestrelab Research Inc.).



SC4TH

Results and Discussion

First, steady state fluorescence spectra of pyrene were used to determine the cmc of SC4TH. The relative changes in the intensities of the first and the third vibronic bands in the pyrene fluorescence spectrum as a function of SC4TH concentration were used, since this ratio is very sensitive to the polarity of the microenvironment.[21] A plot of the results obtained from the fluorescence study of pyrene in the presence of SC4TH is shown in Figure 1. The breakpoint at 0.54 mM was identified as the cmc.

In an effort to corroborate the cmc and to elucidate the various types of aggregates that SC4TH can form, a DOSY study was carried out at different calixarene concentrations. The results are represented in Figure 2. The cmc was taken as the point at which the diffusion coefficient abruptly drops to lower values, thus indicating the presence of aggregates in solution. The obtained value of 0.32 mM is lower than that obtained by fluorescence and this discrepancy can be attributed to the differences expected between D_2O and H_2O. It is worth noting that the diffusion coefficients level off at about 10 mM and therefore micellar growth can be ruled out. The diffusion coefficients can be used to determine the hydrodynamic radius of the species in solution using the Stokes−Einstein equation. When micelles are present in solution the diffusion coefficient of monomers and aggregates is given by the following expression

$$D_{obs} = \frac{cmc}{[SC4TH]}D_m + \frac{([SC4TH] - cmc)}{[SC4TH]}D_M \quad (1)$$

where D_m and D_M refer to the diffusion coefficients of the monomer and the micelle, respectively. The hydrodynamic radius of the monomer and/or micelle was calculated with the Stokes−Einstein equation from the diffusion coefficient at concentrations below and above the cmc. The hydrodynamic radii obtained below and above the cmc were 9.6 (monomer) and 42.7 Å (aggregates), respectively, and the latter radius is consistent with the formation of micellar assemblies. The radius of 42.7 Å seems too much for a spherical micelle composed of C6 chains and suggest that the micelles have another geometry, rather than spherical. For an oblate ellipsoidal aggregate we obtain a length for the major semiaxis, a = 46 Å, being the length minor semiaxis, b, equal to the length of the surfactant molecule.

In previous studies, the ^{1}H NMR mutiplicities of the methylene protons, H_{endo} and H_{exo}, of calix[4]arenes have been used as an indication of the calixarene conformation.[22] In the present study, the signals for H_{endo} and H_{exo} of SC4TH appear as doublets at concentrations above and below the cmc, indicating that SC4TH is fixed in the cone conformation both in the monomeric and micellized states.

Shinkai et al.[23] found that the ^{1}H NMR chemical shifts of an amphiphilic *p*-sulfonatocalix[4] arene bearing butyl groups at the lower rim at concentrations below the cmc remain constant, but above the cmc the H_{endo} and OCH_2 proton signals are shifted to higher field on increasing the calixarene concentration. The authors suggested that the butyl groups form the micellar core and the calixarene rings form stacks at the micellar surface.

A region of the ^{1}H NMR spectra of SC4TH at a series of concentrations is shown in Figure 3. Below the cmc (0.23 mM), the spectrum essentially shows a single set of sharp

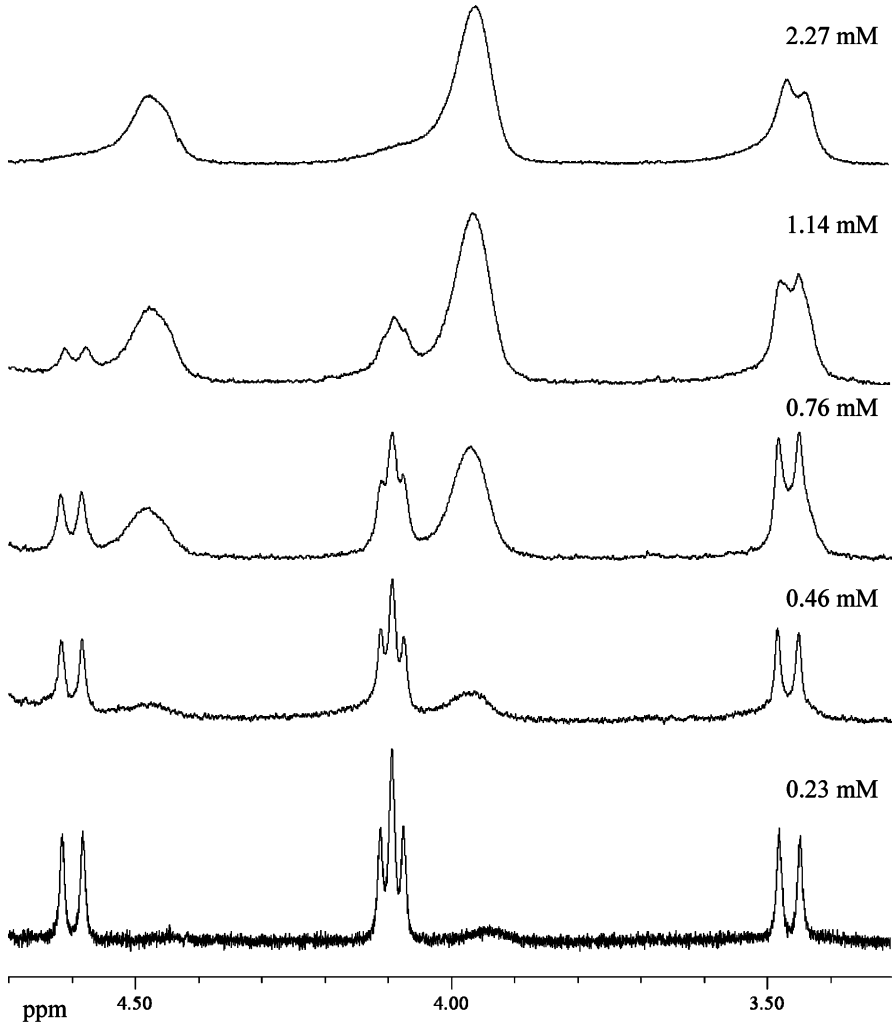


Figure 3. ^{1}H NMR spectra of SC4TH at different concentrations in D_2O at 25 °C. The doublet at 4.60 ppm, triplet at 4.1 ppm, and doublet at 3.45 ppm correspond to the protons H_{endo}, OCH_2, and H_{exo}, respectively.

signals and, as mentioned above, these show the multiplicity expected for this compound in the cone conformation. When the concentration is above the cmc, a new set of broad signals is also observed in the spectrum and the intensity of these signals increases linearly with the concentration. Eventually, at 2.5 mM only the set of broad signals is visible.

A combination of NMR experiments permitted the assignment of the set of broad signals of SC4TH. The signals for protons H_{endo} and OCH_2 (see Figure 3) were shifted most toward higher field with respect to the analogous signal in the other set. For the other protons, the broadening was also evident but the signals were essentially unshifted with respect to those of the other set (e.g., H_{exo} in Figure 3).

The results of 2D EXchange Spectroscopy (EXSY), in addition to DOSY experiments, confirmed that the set of broad signals in the spectra corresponded to monomer units of SC4TH that are attached to/incorporated into the micelle and undergo a slow chemical exchange equilibrium (on the chemical shift time scale) with the set of sharp signals of the free monomer in bulk solution. The EXSY spectra shown in Figure 4 were acquired with a short mixing time (40 ms) to avoid NOE effects. These spectra clearly show the presence of cross peaks between the two sets of signals for H_{endo} (or OCH_2), thus confirming the existence of slow chemical exchange between them. For two site chemical exchange, the peak amplitude in 2D-EXSY spectra is related to the exchange rate constant, k', the relaxation rate, and the mixing time, t_m, by expression 2,[24] where A and R are (3) and (4), respectively.

$$A = e^{(-Rt_m)} \tag{2}$$

$$A = \begin{vmatrix} I_{11}/M_1 & I_{12}/M_2 \\ I_{21}/M_1 & I_{22}/M_2 \end{vmatrix} \tag{3}$$

$$\mathbf{R} = \begin{vmatrix} -R_1 - k'_1 & k'_{-1} \\ k'_1 & -R_2 - k'_{-1} \end{vmatrix} \tag{4}$$

In the amplitude matrix A, I_{11}, I_{12}, I_{22}, and I_{21} are the two-dimensional peak amplitudes measured for sites 1 and 2 in an experiment with a certain mixing time, and M_1 and M_2 are the equilibrium magnetization values obtained from the analogous 2D-EXSY experiment acquired with a mixing time of zero.

The exchange matrix $\mathbf{R}$ contains the kinetic parameters to be determined, namely the exchange rate of the direct and reverse exchange reaction equilibrium (k'_1 and k'_{-1}) and the proton longitudinal relaxation rates of the two sites (R_1 and R_2). These parameters can be obtained directly by first diagonalizing A and then calculating the eigenvector matrix $\mathbf{X}$ and its inverse $\mathbf{X}^{-1}$ so that $\mathbf{XD}\,\mathbf{X}^{-1} = A$ where $\mathbf{D}$ is the diagonal eigenvalue

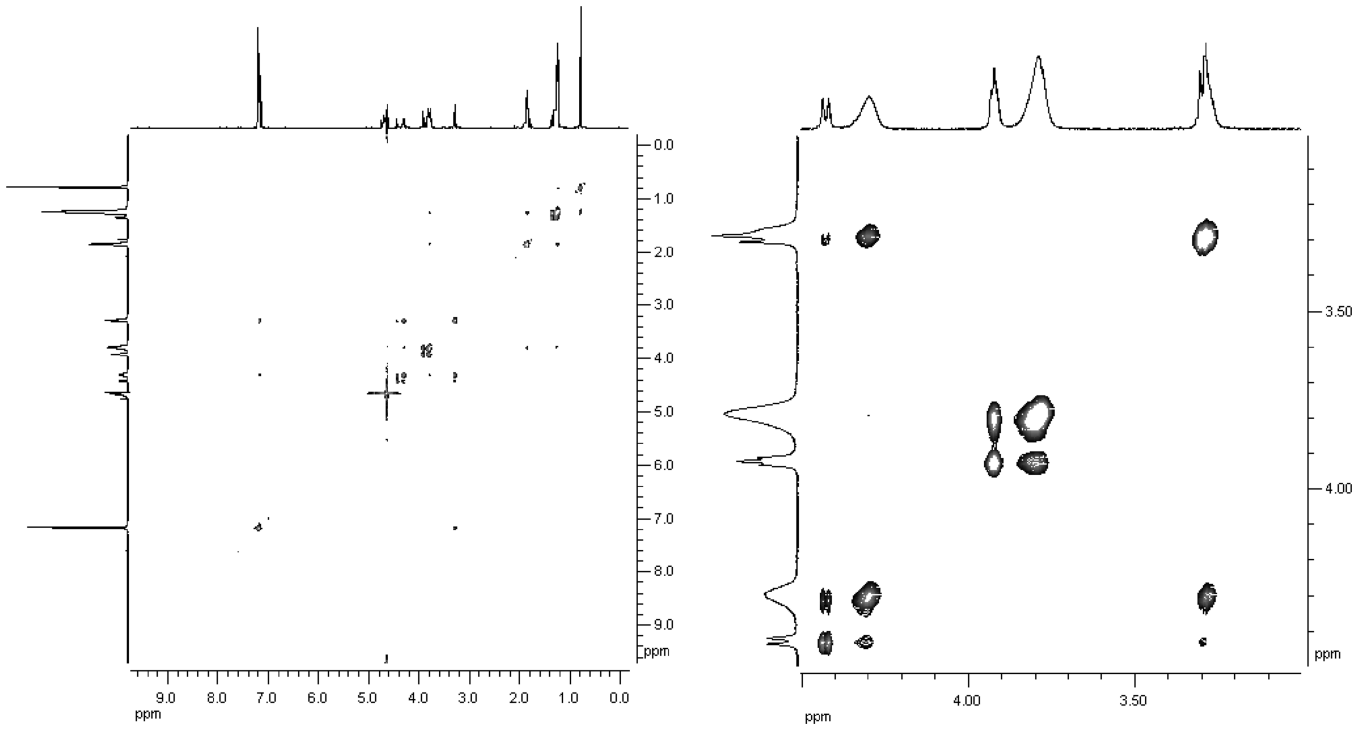


Figure 4. Two-dimensional EXSY spectrum of SC4TH (1 mM in D_2O) acquired with a mixing time of 200 ms at 25 °C. The full view spectrum is on the left. The spectrum on the right is an expansion showing the region for protons H_{endo}, OCH_2, and H_{exo}.

matrix. The solution to this equation is given 5 where ln **D** = diag (ln λ_i), with λ_i the ith eigenvalue of *A*. Thus **R** can be directly calculated from *A*

$$\mathbf{R} = -\frac{\ln A}{t_m} = -\frac{\mathbf{X}(\ln \boldsymbol{D})\mathbf{X}^{-1}}{t_m} \quad (5)$$

The two magnetization exchange rate constants, k'_1 and k'_{-1}, were obtained from 2D-EXSY experiments with a mixing time of 200 ms and these values correspond to the exchange from monomer to micelle and from micelle to monomer, respectively. The global exchange rate constant (k') can be calculated from the sum of k'_1 and k'_{-1} (an approximation due to the system being in equilibrium[24]). In this paper, the monomer exchange between aggregate and solution can be represented by reaction 6. Since the magnetization exchange between monomers in the bulk and the micelle is a first order reaction, the relationship between the magnetization rate constants (k') and the reaction rate constants (k) can be found in eqs 7 and 8

$$S + S_{n-1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_n \quad (6)$$

$$k'_1 = k_1[S] \quad (7)$$

$$k'_{-1} = k_{-1} \quad (8)$$

Two-dimensional-EXSY experiments were repeated at a series of temperatures and the exchange rates determined are given in Table 1. The calculated values for the global exchange rate constants (k') are of the same order of magnitude as those obtained for gemini surfactants[8] and amphiphilic nucleotide-calix[4]arenes[17] and are up to 9 orders of magnitude slower than those for conventional surfactants.

TABLE 1: Monomer−Micelle Exchange Rate Constants for SC4TH (1 mM) in D_2O at Different Temperatures

T/K	k'_1/s^{-1}	k_{-1}/s^{-1}	k'/s^{-1}
298.15	2.51	7.42	9.93
308.15	3.55	9.90	13.45
313.15	6.85	10.42	17.27
318.15	7.85	11.85	19.69

On considering the equilibrium for the monomer exchange (eq 6), one can write $k_1[S][S_{n-1}] = k_{-1}[S_n]$. As micelles with aggregation numbers $n - 1$ and n have approximately the same probability and the concentration of surfactant monomers in equilibrium with the micelles equals the cmc, then $k_1$cmc = k_{-1}. On the basis of this assumption, we can calculate a value for the forward bimolecular rate constant, k_1, $k_1 = 7.8 \times 10^3$ dm^3 mol^{-1} s^{-1} at 25.0 °C. For conventional surfactants, it is considered that the micelle behaves as a liquid-like drop[25] and the monomer association reaction does not involve slow structural reorganization or bond formation, meaning that k_1 is close to the diffusion-limited rate. Our results show that for SC4TH the forward rate constant is several orders of magnitude slower than the diffusion rate. The explanation for this unconventional result presumably lies in the fact that the sole barrier felt by the monomers entering the micelle arises from long-range electrostatic repulsions due to the micellar charge.[25] As SC4TH is a preorganized surfactant with four negative charges in the upper-rim, these effects could be much more important in this system and this could increase the activation barrier and consequently slow down the rate constant for the monomer association.

As expected, the exchange rate constants increase with temperature. A graphical representation of ln k against the inverse of temperature ($1/T$) is represented in Figure 5. As can

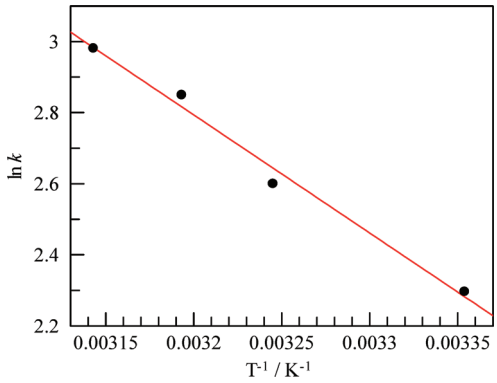


Figure 5. Arrhenius plot for the SC4TH monomer−micelle exchange rate. [SC4TH] = 1 mM in D_2O.

be observed, the exchange rate follows Arrhenius behavior and from the slope of the linear fit to the experimental data points we obtained an activation energy of 27.6 kJ mol^{-1}. For the sake of comparison, we applied the same treatment to the amphiphilic nucleotide-calix[4]arene dimer-micelle exchange rates given in ref 17. The representation of ln k against $1/T$ shows linear behavior with an activation energy of 46.2 kJ mol^{-1} obtained. The activation energy for this system is higher than that obtained in this work for SC4TH, possibly reflecting the fact that surfactant molecules are in a more stable dimerized form (involving a higher energy difference between the dimer and the transition state of the process). A more likely explanation could be the fact that the hydrophobic moiety of these calixarenes is based on the highly bulky *tert*-butyl calixarene cavity rather than on more flexible alkyl chains, thus slowing the interchange between free and micellized surfactant molecules due to steric effects.

Conclusions

In this study, we examined the aggregation properties of *p*-sulfonatocalix[4]arene tetrahexyl ether by means of NMR techniques and using pyrene as a fluorescence probe. The cmc was determined by fluorescence and DOSY techniques. The slight discrepancy between the two values obtained was attributed to known differences between H_2O and D_2O. In addition, diffusion experiments allowed us to confirm that this surfactant forms micelles in solution with a hydrodynamic radius of 4.27 nm. We also found that in contrast to conventional surfactants the exchange rate between free monomers in solution and those in the micelle is slow on the NMR time scale. The rate constants were determined using 2D EXSY experiments and these constants were found to be several orders of magnitude lower than those of conventional surfactants and comparable to those of other amphiphilic calixarenes and gemini surfactants.

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