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EXPLORING THE CHARGED NATURE OF SUPRAMOLECULAR MICELLES BASED ON P-SULFONATOCALIX[6]ARENE AND DODECYLTRIMETHYLAMMONIUM BROMIDE

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ABSTRACT

The aggregation of supramolecular amphiphiles formed from hexamethylated p-sulfonatocalix[6]arene (SC6HM) and dodecyltrimethylammonium bromide (C_{12}TAB) was studied by capillary electrophoresis experiments and by kinetic probes. The hydrolysis of 4-methoxybenzenesulfonyl chloride (MBSC) was used to investigate the micropolarity of the micellar aggregates and their ability to solubilize and stabilize labile organic compounds against hydrolysis. Further insights were obtained using a more sophisticated kinetic probe: the basic hydrolysis of *p*-nitrophenylvalerate (NPV). This probe provides information on the ionic composition of the micellar interface and on the potential of the aggregates to be used as nanoreactors. The results obtained revealed that the charge of the micellar aggregates can tuned from anionic to cationic through the adjustment of the C_{12}TAB:SC6HM molar ratio and confirmed that these micelles have good solubilization properties. On the other hand, the kinetics of the of *p*-nitrophenylvalerate basic hydrolysis suggest that, in the concentration range comprised between the first and second CMC's, Br^- anions do not take part of the micellar structure.

INTRODUCTION

Surfactants are amphiphiles, molecules that contain hydrophobic and hydrophilic segments.[1,2] Compounds of this sort are ubiquitous in biological systems, as main components of membranes bilayers, and also find use in numerous technological and industrial applications: from common cleaning detergents to oil recovery, pharmaceutical and cosmetic formulations, drug delivery systems, catalysis and synthesis of nanostructured advanced materials, to name a few. All of these applications arise from the ability of these molecules to adsorb at surfaces and interfaces and to self-assemble into more or less defined aggregates of nanometric dimensions.

Conventional surfactants consist of a polar or ionic head group connected to a hydrophobic alkyl chain. Despite of their relative structural simplicity when dissolved in water these compounds can become involved in complex equilibria between free monomers, interfacial adsorption and aggregation. These phenomena have been the subject of numerous studies, but are not yet fully understood.[3–5] In addition to conventional surfactants, in recent years a new class of amphiphilic compounds emerged, the so-called supramolecular amphiphiles. The main difference between conventional and supramolecular amphiphiles is that in the former case the hydrophilic and hydrophobic segments are connected via covalent bonds, while in the latter case these segments are hold together by non-covalent interactions or dynamic covalent bonds.[6–8] While, as discussed above, the adsorption and aggregation properties of conventional surfactants are not fully understood, the field of supra-amphiphiles is in its infancy and the physico-chemical basis for the aggregation behavior of this special class of amphiphilic compounds is yet to be established.

The aggregation of supramolecular amphiphiles based on water soluble *p*-sulfonatocalix[n]arenes and cationic organic guests has attracted the attention of some research groups during the last years.[9–26] The stability and final architecture of the self-assembled aggregate depend strongly on the concentration ratio and structural features of both guest and host. For example, highly flexible *p*-sulfonatocalix[n]arenes methylated at the lower rim form micelle-like aggregates in the presence of cationic surfactants while native *p*-sulfonatocalix[n]arenes, which are conformationally less mobile due to intramolecular hydrogen

bonding between the hydroxyl groups, self-assemble into unilamellar vesicles and multilamellar nanoparticles in the presence of positively charged amphiphiles.[9–26]

The micellar aggregation of hexamethylated *p*-sulfonatocalix[6]arene (SC6HM) in the presence of cationic surfactants displays very interesting behavior and shares some features with oppositely charged polyelectrolyte/surfactant systems that deserve to be investigated in detail.[9,12,13] The critical micelle concentration (CMC_0) of pure dodecyltrimethylammonium bromide ($C_{12}TAB$) is CMC_0=14 mM and in the presence of SC6HM is shifted to CMC_1 = 0.2 mM. The magnitude of the shift seems to be rather insensitive to the calixarene concentration (at least from 0.1 to 5 mM). Similarly, below the charge neutralization point, the aggregation number (N) also seems to be fairly independent of the SC6HM and $C_{12}TAB$ concentrations (N≈20-25). For a constant concentration of SC6HM and above the charge neutralization point, N increases linearly with the concentration of $C_{12}TAB$. Despite of this increase, it was also observed that only a small fraction of added surfactant is absorbed by the micelles and the main fraction remains free in bulk solution. As a consequence, when the concentration of free surfactant reaches a value equal to the CMC_0 a second aggregation process is observed (CMC_2).

In previous work, we were able to estimate the concentration of free $C_{12}TAB$ and the micellized $C_{12}TAB$:SC6HM ratio above the CMC_1 from self-diffusion coefficients obtained by DOSY NMR experiments.[12] The results illustrate that the concentration of free $C_{12}TAB$ is approximately constant and equal to the CMC_1 for total concentrations of surfactant slightly below the charge neutralization point. This means that above the CMC_1, all surfactant molecules added to the solution are transformed into micelles, as a consequence of the highly cooperative micellization process. Above the charge neutralization point the concentration of free $C_{12}TAB$ increase linearly with the total surfactant concentration until a second aggregation process is observed and a new plateau is reached. This behavior suggests that the aggregation mechanism switches from cooperative to non-cooperative around the charge neutralization point, switching again to cooperative above the second aggregation process. The dependence of the *micellized* $C_{12}TAB$:SC6HM ratio on the total surfactant concentration also provides important information for the complete understanding of the system. Within the non-cooperative range there is an

increase in this parameter from 4 to 8 suggesting a transition from negatively charged to positively charged micelles (SC6HM is a hexanion). Above the second aggregation process the *micellized* C_{12}TAB:SC6HM ratio shows a significant increase due to the dilution of the SC6HM-rich micelles within the new aggregates. While it is not clear if the Br^- anions participate in the stabilization of the cationic SC6HM-rich micelles it is obvious that they must be present in the aggregates formed above the second aggregation process.

With the objective of getting further insights into this intriguing and complex behavior we decided to investigate this system using the hydrolysis of 4-methoxybenzenesulfonyl chloride (MBSC) and the basic hydrolysis of *p*-nitrophenylvalerate (NPV) as kinetic probes, together with capillary electrophoresis experiments. Despite being far less popular than fluorescent or colorimetric probes, reactivity probes provide a powerful methodology to investigate the aggregation, counterion binding and interfacial properties of self-organized systems based on amphiphilic compounds.[27–31]

EXPERIMENTAL SECTION

All chemicals used were of the highest commercially available purity and none required further purification. Hexamethylated *p*-sulfonatocalix[6]arene was available from previous studies.[9,12,13] 4-Methoxybenzenesulfonyl chloride (MBSC) and *p*-nitrophenylvalerate (NPV) stock solutions were prepared in acetonitrile, due to their instability in water. The final acetonitrile concentration in the reaction medium was 1% (v/v).

Kinetic runs were initiated by injecting 30 μL of MBSC or NPV stock solution into a 1 cm path length cuvette containing all other compounds dissolved in 2970 μL of water. Reaction kinetics were recorded by measuring the absorbance changes at 295 nm in the case of MBSC or at 400 nm for the basic hydrolysis of NPV in a Cary 50 UV–Vis spectrophotometer equipped with a cell holder thermostated at (25.0±0.1)^{o}C. The MBSC and NPV concentrations were always 1.0 x 10^{-4} M and 2.6 x 10^{-5} M, respectively. The absorbance-time data of all kinetic

experiments were fitted by first-order integrated equations, and the values of the pseudo-first-order rate constants (k_{obs}) were reproducible to within 3%.

Electrophoretic analysis were made on an Agilent CE^{3D} capillary electrophoresis system, with on-column diode-array detection at 25±0.5 °C and electropherograms were monitored at 200 nm for SC6HM and 235 nm for thiourea using data treatment software (HP Chemstation). Fused-silica capillaries (Microtube, Araraquara, Brazil) with total length 48.5 cm, effective length 40.0 cm, and 50 μm i.d. were used in all experiments. The capillaries were conditioned by flushing with 1 M NaOH (5 min), deionized water (5 min) and electrolyte solution (10 min). Between experiments, the capillary was reconditioned by flushing 2 min with the background electrolyte (BGE) containing 5 mM sodium tetraborate (TBS), pH = 9.2. In order to evaluate the interaction of C_{12}TAB with SC6HM, the BGE containing 5 mM TBS was enriched with C_{12}TAB in concentrations ranging from 0.04 mM to 50 mM. Samples containing SC6HM 0.5 mM, 140 mg/L of thiourea (fixed; electroosmotic flow marker) and were diluted with BGE with different contents of C_{12}TAB. The pressure, analysis voltage and time of sample injections were performed in accordance with the concentration of C_{12}TAB: +50 mBar, + 25 kV, 5s for the 0.04 mM to 0.12 mM; -50 mBar, +25 kV, 5s to see the SC6HM and -50 mBar, -25 kV, 5s to see the EOF for the 0.16 mM to 2 mM concentrations; 2.2mM to 50 mM was used -50 mBar, +25kV, 5s. Electroosmotic mobility was calculated from the migration time of thiourea (neutral marker). Electropherograms were plotted in effective mobility scale using the following equation:

$$\mu_{ep} = \frac{L_{eff}}{E}\left(\frac{1}{t_{cal}} - \frac{1}{t_{eof}}\right) \quad (1)$$

where μ_{ep} is the effective mobility, L_{eff} is the total capillary length, E the applied electric field, t_{cal} is the apparent detection time, and t_{eof} is the detection time of the neutral marker. The effective mobility was calculated according procedures described previously.[32]

RESULTS AND DISCUSSION

Electrophoresis. The quantification of the concentration of *free* C_{12}TAB and of the *micellized* C_{12}TAB:SC6HM ratio from self-diffusion coefficients required approximations regarding the diffusion coefficients of the aggregates.[12] In order to confirm the previous analysis, the charged nature of the aggregates was examined using capillary electrophoresis (CE).[32] CE separations are based on measurements of the difference in mobility of charged compounds (or aggregates) as a function of applied voltage. Thus, the interaction between SC6HM and C_{12}TAB was examined by CE, using the conditions described in the experimental section and the experimental results are shown in Figures 1A to 1E.

As can be seen in Figure 1A, when the surfactant concentrations are in the range of 0.04 mM to 0.12 mM, the calixarene peak (labeled as 2) shows up after the EOF marker (peak 1). The results is consistent with expectations, since the CE analysis were performed in the counter electrosmotic flow mode, and the results are indicating that the SC6HM:C_{12}TAB aggregate is negatively charged. Subsequently, as the concentration of C_{12}TAB increases (values in the range of 0,16 – 2,0mM C_{12}TAB, Figures 1B and 1C), shows that additional surfactant monomers are incorporated into the SC6HM:C_{12}TAB aggregate and, as a consequence, there is a considerable decrease in electrophoretic mobility, which reaches null electrophoretic mobility values with 3mM of C_{12}TAB (Figure 1 D). Experiments with surfactant concentration in the range of 3 to 18 mM, when the surfactant is in large excess in relation to the calixarene, the aggregate formed between surfactant and calixarene is positive charged. Finally, when [C_{12}TAB] > CMC, micelles of C_{12}TAB are formed and the SC6HM molecules will be distributed between the micelles, in a typical cationic aggregate (Figure 1E). The change in electrophoretic mobility as a function of surfactant concentration can be observed in Figure 2.

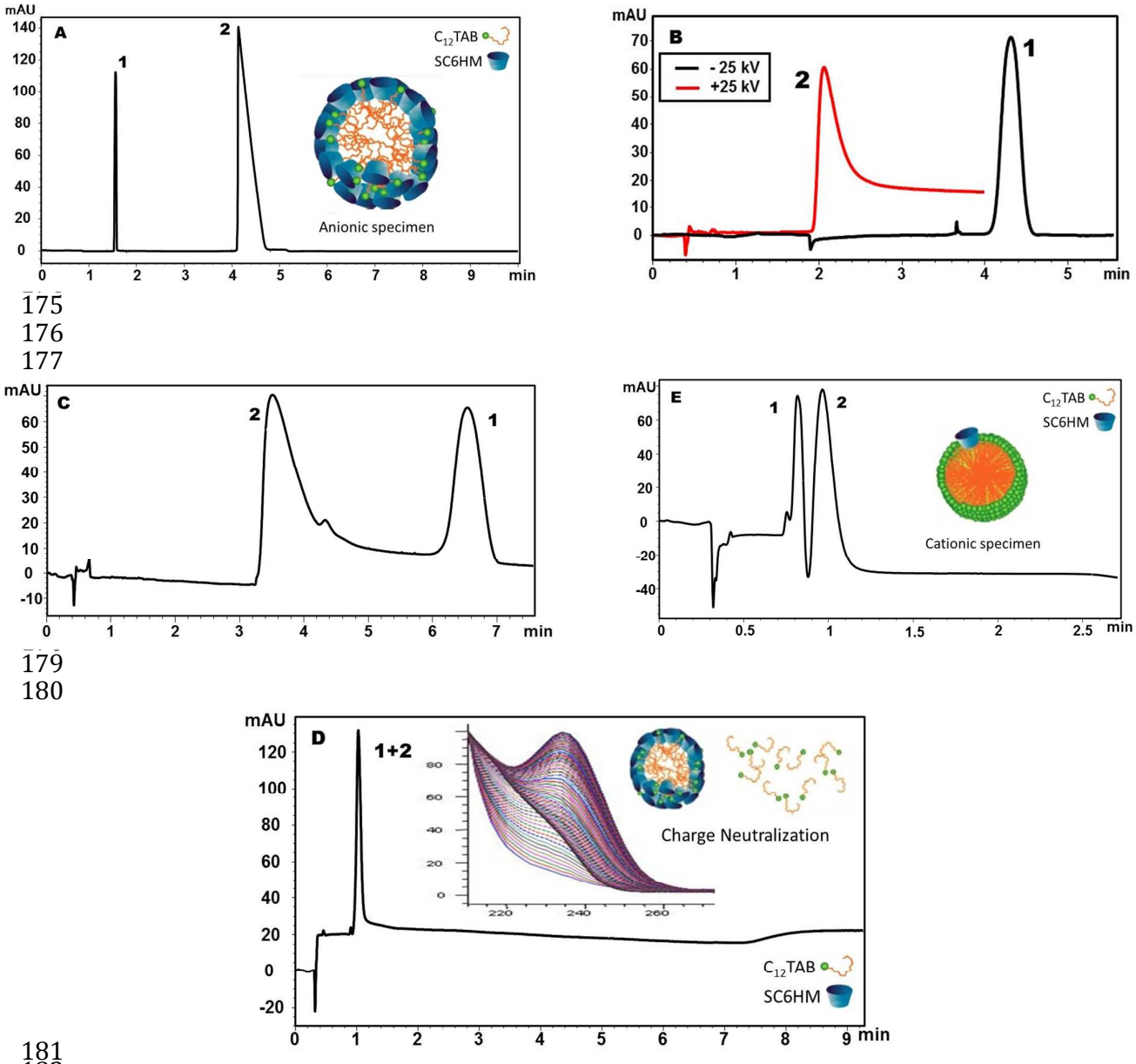


Figure 1.(A)Electropherogram profile of concentration of C_{12}TAB varying from 0.04 mM to 0.12 mM (+50mBar, +25kv, 5s); (B) 0,16 mM to 2,0 mM (-50 mBar, +25 kv, 5s to SC6HM and -50 mBar, -25kv, 5s to EOF; (C) 2,2 mM – 2,8 mM (-50 mBar, +25kv, 5s); (D) EOF + SC6HM (neutral) at ~3mM (-50 mBar, +25kv, 5s); (E) electropherogram> 3,4mM to 50 mM of C_{12}TAB (-50 mBar, +25kv, 5s). 1-EOF; 2-SC6HM;

The analysis of the mobility of the calixarene as a function of the concentration of $C_{12}TAB$ shown in Figure 2, allows observing several critical points. The first one is around 0.2 mM, and indicates the onset of the incorporation of surfactant molecules into the calixarenes and is denominated as (CMC_1), where the anionic species is predominant. After this point the electrophoretic mobility increases, crossing the null electrophoretic mobility value around 3mM, which corresponds to a 6:1 ratio of $C_{12}TAB$ molecules for each SC6HM, in good agreement with previous conclusions based on NMR data. Increasing the concentration of surfactant above 3.0 mM, the SC6HM:$C_{12}TAB$ aggregates become positively charged. These values are consistent with those reported in a previous study performed using conductivity analysis.[12] The third inflexion point, at 18mM, indicates CMC_2 of free surfactant in good agreement with that previously obtained by conductivity.[12] Overall, the results described here provide direct evidence for the transformation of anionic into cationic micelles in this system.

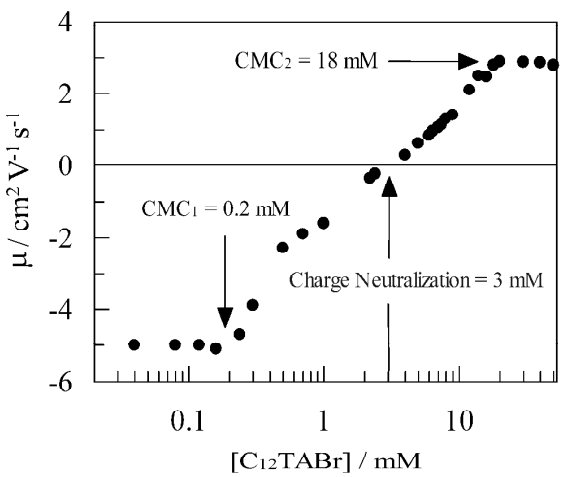


Figure 2. Dependence of the electrophoretic mobility data with the concentration of $C_{12}TAB$ in the presence of 0.5 mM of SC6HM.

The changes in electrophoretic mobility, shown in Figure 2, can be conveniently used to estimate zeta potentials (ζ_m) by means of equation 2, which relates the charge of the micelle to its mobility:

$$\zeta_m = \frac{\mu \eta}{\varepsilon_0 \varepsilon f(\kappa R_m)} \quad (2)$$

where η is the viscosity of the medium, $f(\kappa R_m)$ corresponds to Henry's function, κ is the Debye-Hückel shielding parameter (m^{-1}), R_m is the radius of $C_{12}TAB$ micelle, and ε_o and ε correspond to the vacuum permittivity and the relative permittivity of the solvent.[33,34]

Initially, the zeta potential of the $C_{12}TAB$ micelle was calculated above the CMC_2 of free surfactant, where the behavior should be closely related to a solution of $C_{12}TAB$ without additives. In order to proceed with the calculation of Zeta potential, we adopted the reported radii values (R_m) of the $C_{12}TAB$ micelle of 20 Å.[35] Considering the ionic strength of the supporting electrolyte (TBS = 0.005 M), it is possible to estimate a value of $\kappa = 2.3 \times 10^8\ m^{-1}$, and accordingly, under our experimental conditions κR_m for $C_{12}TAB$ micelles is 0.46, which allowed us to calculate the value of Henry's function $f(\kappa R_m)$ = 0.69, following Ohshima's approximation.[34]

Using the value of Henry´s function was possible to estimate the zeta potentials of the aggregates, and as shown in Table 1, the Zeta potential (ζ_m, in mV) above CMC_2 is 50.4 millivolts, a value which is consistent with those reported in the literature for $C_{12}TAB$ micelles. The mobility of the SC6HM anionic calixarene, for $[C_{12}TAB] \leq CMC_1$ is constant and probably reflects the mobility of the individual SC6HM molecules or of small aggregates. A crucial point is the behavior at CMC_1 (~0.2 mM), which corresponds to the formation of anionic aggregates of the calixarenes supramolecularly complexed with $C_{12}TAB$ and we could calculate a zeta potential of -47.2 millivolts, using a micellar radius of 20 Å and the same approximations described above to calculate Henry´s function. Between CMC_1 and CMC_2 the change in Zeta potential follows linearly the changes in mobility and, it is interesting to observe that the charge neutrality of the aggregate is attained when the $[C_{12}TAB]$ = 3.0 mM, which corresponds exactly to a ratio of ([surfactant]/[calixarene]) = 6, which is the theoretically expected neutralization point for an optimum effect of the complexation. Above a ratio of 6 surfactant molecules for each calixarene, the aggregate becomes increasingly positively charged, finally reaching zeta potential values which are closely similar to that of a pure $C_{12}TAB$ cationic micelle.[35]

Table 1. Zeta potential values for each different aggregate formed.[a]

Point	Charge of Aggregate	Concentration (mM)	ζ_m (mV)
CMC_2	Cationic Micelle	18.0	50.40
Neutral point	Neutral point	3.0	0
CMC_1	Anionic Micelle	0.2	-47.15

[a][SC6HM] = 0.5 mM, T = 25ºC.

Hydrolysis of MBSC. Another important feature to be investigated in the present system is the capacity of these supramolecular micelles to solubilize guest molecules or ions and test their potential to be used as nanoreactors. Using simple reactions as kinetic probes will allow these parameters to be investigated as well the micellar polarity and effect of the micellar charge on the compartmentalization of the reactants.

The rate of the solvolysis of MBSC is highly sensitive to the polarity of the (micro)environment in which the probe is located. Therefore this reaction has been recurrently used as a probe to investigate micellar and host-guest systems.[30,36] In general, the reaction rate decreases as the probe is transferred from bulk water to more apolar medium such as the micellar interior/interface or the cavity of a macrocyclic container. The results depicted in Figure 3-left suggest that MBSC does not interact with SC6HM in this concentration range, or that the observed rate constant for the solvolysis does not vary significantly from the bulk to the SC6HM pseudo-cavity: or both. SC6HM is highly flexible and is believed to exist in solution as a mixture of several interconverting conformers, thus lacking a defined structure/cavity.[37,38] As a result of this poorly defined geometry, SC6HM usually displays weaker binding abilities comparatively to more pre organized derivatives. The instability of MSBC in aqueous solution

precludes the utilization of more conventional techniques to confirm or rule out the complexation of MSBC with SC6HM.

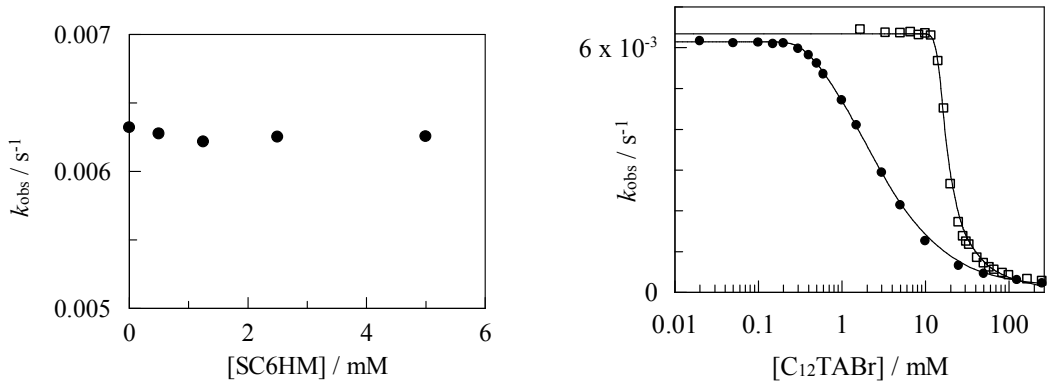


Figure 3.(Left) Influence of the SC6HM on the observed rate constant (k_{obs}) for the hydrolysis of MBSC. **(Right)** Variation of k_{obs} with [C_{12}TABr] in the absence (open squares) and in the presence of 5 mM of SC6HM (filled circles). All kinetic experiments were conducted at 25 ºC in water with 1% acetonitrile (v/v).

Figure 3-right shows the changes observed for k_{obs} as a function of the C_{12}TABr concentration in the absence and presence of 5 mM of SC6HM. The plot can be divided into two regions: a first one that corresponds to low surfactant concentration where the rate of the hydrolysis of MBSC is essentially constant and a second one above a certain critical concentration, that is indentified by the point at which the k_{obs} starts decreasing. This point is identified as the onset of micelle formation (the critical micelle concentration, CMC) and the decrease observed for k_{obs} is attributed to the incorporation of MBSC within the micellar aggregates, a lower polarity microenvironment that inhibits the hydrolysis of the probe. The CMC_0 and CMC_1 values obtained from the kinetic data, 13mM and 0.25 mM in the absence and presence of 5 mM of SC6HM, respectively, are in good agreement with those previously obtained by other techniques.[12,13]

The kinetic data can be quantitatively analyzed through the application of the pseudophase formalism. This simple theory considers that molecules and ions present in solutions are distributed between two well-differentiated environments: bulk water and a

micellar pseudophase. For reactions taking place in the sub-second and slower timescales the partition of the reactants between the bulk and the micellar pseudophase can be considered as a fast equilibrium so that the observed rate constant is given by the molar fraction weighted average of the rate constants corresponding to the reaction taking place in the two environments.

As shown elsewhere, k_{obs} is given by equation 3:[39]

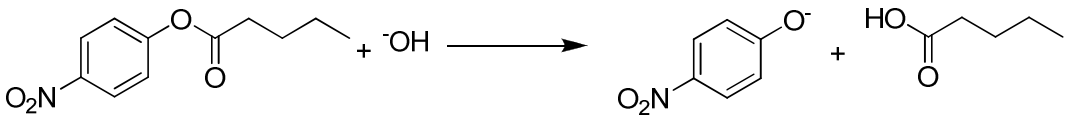
$$k_{obs} = \frac{k_w + k_m K_S^m [D_n]}{1 + K_S^m [D_n]} \quad (3)$$

Where k_w is the rate constant for the hydrolysis reaction taking place in bulk water, k_m is the rate constant for the reaction taking place in the micellar pseudophase, K_S^m is the binding constant of MBSC between the bulk and the micelles (defined as $K_S^m = [MBSC]_m/[MBSC]_w\,[D_n]$) and $[D_n]$ is the concentration of micellized surfactant that can be approximated as $[D_n]$ = $[\text{surfactant}]_0$-CMC; where $[\text{surfactant}]_0$ is the total concentration of surfactant. At this point it must be stressed that the approximation used to obtain $[D_n]$ does not apply for low concentrations of SC6HM because after neutralization of the calixarene charge, the concentration of free surfactant increases significantly before a second aggregation process take place.[12] Nevertheless, for concentrations of SC6HM with charge neutralization point well above the CMC_0 of pure surfactant (14 mM), this behavior is not observed, or insignificant.[13] In the present work the concentration of SC6HM (5 mM) corresponds to a charge neutralization point of 30 mM, well above the CMC_0.

As can be observed in Figure 3-right, the k_{obs} data is satisfactorily fitted by equation 3. Fitting was achieved with k_w = (6.3±0.1)x10^{-3} s^{-1}, k_m = (1.6±0.4)x10^{-4} s^{-1} and K_S^m = (2.7±0.2)x10^2 M^{-1} in the absence of SC6HM and k_w = (6.1±0.1)x10^{-3} s^{-1}, k_m = (1.7±0.2)x10^{-4} s^{-1} and K_S^m = (4.3±0.1)x10^2 M^{-1} in the presence of 5 mM of SC6HM. The results obtained in the absence of SC6HM are in good agreement with those reported in the literature.[30] When both sets of results are compared it can be seen that k_m has the same value in the absence and in the presence of calixarene. However it was shown previously that, in the case of MBSC, this

parameter does not indicate a significant dependence on the hydrophobic character or the interfacial charge and polarity of the micellar aggregates.[30,40] On the other hand, the K_S^m value shows a significant increase in the presence of SC6HM suggesting that the mixed micelles are more hydrophobic than pure aggregates, with the absolute value being close to that observed for C_{16}TAC (K_S^m = [4.7±0.3]x10^2 M^{-1}).[30]

Basic hydrolysis of NPV. Because the hydrolysis of MBSC does not provide significant information on the interfacial properties of the micellar aggregates, another probe reaction was used to investigate it. Bimolecular reactions of hydrophobic organic compounds and ionic reactants are very convenient probes to get insights into the ionic composition of micellar interfaces.[27]The basic hydrolysis of nitrophenyl esters has become an iconic reaction in studies concerning self-assembled amphiphilic systems based on cationic surfactants. In this work the basic hydrolysis of *p*-nitrophenylvalerate was selected as a probe reaction (SCHEME 1) to investigate the ionic composition of micellar aggregates formed from SC6HM:C_{12}TAB supramphiphile.



SCHEME 1. Basic hydrolysis of p-nitrophenylvalerate.

As a basis for the study of the basic hydrolysis of *p*-nitrophenylvalerate (NPV) in the complex system formed by SC6HM and C_{12}TAB, the influence of each component was investigated separately. Figure 4-left shows the changes observed on the rate of the reaction in the presence of increasing concentration of SC6HM. The results suggest that NPV forms a complex with SC6HM (with K = 13±6 M^{-1} obtained from the non-linear data fit assuming a 1:1

complex) which stabilizes the guest in basic conditions (k_{obs} decreases) probably due to repulsion of $^{-}$OH ions by the sulfonate groups of the host. Nevertheless, the association constant is very low, probably due to the lack of preorganization of the receptor, and for 5 mM of SC6HM the fraction of complexed NPV is very low (ca. 6%).

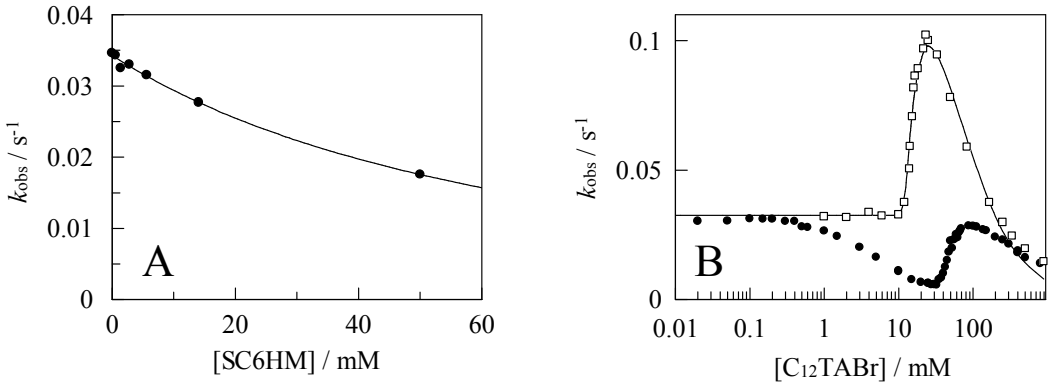


Figure 4.(Left) Influence of the SC6HM on the observed rate constant (k_{obs}) for the basic hydrolysis of NPV. **(Right)** Variation of k_{obs} with [C_{12}TABr] in the absence (open squares) and in the presence of 5 mM of SC6HM (filled circles). All kinetic experiments were conducted at 25 ºC in water with [NaOH] = 5 mM and 1% acetonitrile (v/v).

Figure 4-right shows the dependence of k_{obs} with the concentration of C_{12}TABr in the absence of SC6HM (open squares). The data follow the expected trend for bimolecular reactions of organic compounds with anionic reactants in the presence of cationic micelles.[27] The k_{obs} is constant for concentrations of surfactant below the CMC_0 (12 mM). It worth noting that this value is slightly below that determined above due to the higher salt concentration (NaOH). Above this point the apparent reaction rate increases due to the incorporation of NPV and $^{-}$OH in the micellar surface, increasing the local concentrations of both reactants. The reaction rate reaches a maximum value and then decreases for higher concentration of surfactant. This behavior is attributed to the competitive binding of inert Br^{-} anions that displace reactive $^{-}$OH anions to the bulk aqueous solution.

The results depicted in Figure 4-right can be quantitatively analyzed through the application of the pseudophase ion exchange model (equations 4 and 5).[27] In equations 4 and 5 k_m, K_s^m and $[D_n]$ are the same as in equation 3 while m_{OH}, β and K_{OH}^{Br} are the concentration of ^-OH ions in the micellar pseudophase (defined as the mole ratio $[^-OH]/D_n$), the degree of counterion binding (which is assumed to be constant) and the ionic exchange equilibrium constant ($K_{OH}^{Br} = [OH]_w[Br]_M/[OH]_M[Br]_w$), respectively. By computing the values of m_{OH} from equation 5, using previously determined values for $\beta = 0.8$ and $K_{OH}^{Br} = 17$, equation 4 can be used to fit the k_{obs} data represented in figure 4-right. As can be observed the theoretical curve fits reasonably well the experimental data for concentrations of surfactant below ca. 200 mM but for higher concentration it starts to deviate from the data points. This behavior is well documented and is related to the known limitations of the pseudophase ion exchange model for high concentrations of surfactant. [27,39] From the fitting procedure the following values were obtained: k_w = 6.5 ± 0.1 M^{-1} s^{-1}, K_s^m = (1.4 ± 0.2) x 10^2 M^{-1} and k_m = 8.5 ± 0.9 s^{-1}. k_m is an apparent rate constant that is related to the bimolecular rate constant (k'_m) for basic hydrolysis of the NPV located within the micellar aggregates through the relation $k_m = k'_m / V_m$, where V_m is the molar volume of the Stern layer (0.14 M^{-1}).[41-43] From this relation, k'_m = 1.2 $M^{-1}s^{-1}$ can be obtained. When compared with k_w it is clear that the observed rate enhancement is due to an increase in the local concentrations of the reactants rather than to a true catalytic effect.

$$k_{obs} = \left(\frac{k_w[HO^-]_0 + (k_m K_s^m - k_w)m_{OH}[D_n]}{(1 + K_s^m[D_n])}\right) \quad (4)$$

$$m_{OH}^2 + \left\{\frac{(K_{OH}^{Br}[Br^-]_0 + [HO^-]_0)}{(K_{OH}^{Br} - 1)[D_n]} - \beta\right\}m_{OH} - \frac{\beta[HO^-]_0}{(K_{OH}^{Br} - 1)[D_n]} = 0 \quad (5)$$

In the presence of 5 mM of SC6HM (figure 4-right, closed circles), the k_{obs} values follow a different trend from that observed in the absence of calixarene. At 0.2 mM (CMC_1) the apparent rate constant starts dropping due to the incorporation of NPV into the mixed

SC6HM:C12TAB micelles that form above this concentration. Drawing a parallel with the behavior observed in the absence of SC6HM, the observed decrease in k_{obs} suggests that the micellar aggregates formed in the concentration range between 0.2 and 30 mM do not contain exchangeable $Br^-/^-OH$ counterions because in this case a change in k_{obs} should be observed. This means that within this concentration range the micelles are exclusively formed by SC6HM and $C_{12}TAB$. Above 30 mM the k_{obs} values display a sharp increase, reach a maximum value and drop again. Further, for high concentrations of $C_{12}TAB$ the kinetic data attain the values obtained in the absence of SC6HM. This behavior is typical of cationic micelles with exchangeable counterions and indicates that the mixed micelles are gradually transformed into "quasi like" micelles as the concentration of SC6HM becomes more diluted within the aggregates.

CONCLUSIONS

This work demonstrates that supra-amphiphiles formed from SC6HM and $C_{12}TAB$ aggregate into micellar aggregates with tunable degrees of ionization. For low $C_{12}TAB$:SC6HM molar ratios the micelles are negatively charged. As the concentration of $C_{12}TAB$ increases the negative micellar charge decreases and reaches neutrality at $C_{12}TAB$:SC6HM ≈ 6. Above this point the micelles become positively charged and their ability to solubilize more $C_{12}TAB$ decreases substantially. As a result the fraction of free $C_{12}TAB$ also increases until it reaches a new critical concentration leading to a second aggregation process.

The kinetics of the MBSC hydrolysis and NPV basic hydrolysis show that $C_{12}TAB$:SC6HM micelles have the ability to solubilize hydrophobic compounds within their structure. This result together with their reduced CMC demonstrate the higher potential of $C_{12}TAB$:SC6HM based supramphiphiles compared with conventional surfactants. The basic hydrolysis of NPV was shown to be a very effective probe to investigate the ionic composition of the micellar interface in this complex system. The results suggest that Br^- do not participate in the micelles formed between the first and second CMC's. Above this last point, the micellized $C_{12}TAB$:SC6HM

ratio increases significantly and the counteranions are transferred into the micellar interface screening the electrostatic repulsion between positively charged $C_{12}TAB$ molecules.

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GRAPHICAL ABSTRACT