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Paramagnetic Relaxation Enhancement Experiments: A Valuable Tool for the Characterization of Micellar Nanodevices

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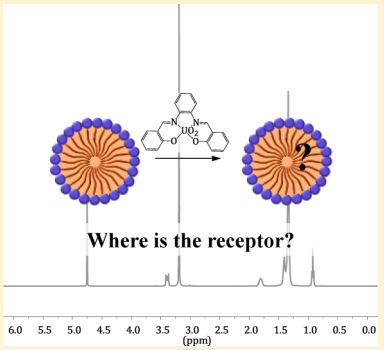
Supporting Information

ABSTRACT: Micellar incorporation of hydrophobic molecular receptors is a promising strategy to obtain efficient nanodevices that work in water. In order to fully evaluate the potential of this approach, information on the localization and orientation of the receptor inside the micelle are necessary. Systematic studies undertaken on a uranyl−salophen receptor incorporated into CTABr and CTACl micelles show that nuclear magnetic resonance paramagnetic relaxation enhancement (NMR-PRE) experiments are particularly suitable to provide this type of information. The effect on the measurements of surfactant concentration, nature of the surfactant polar head, and ionic strength is also reported. Notably the normalization procedure applied to the obtained data can be considered of general application, thus enabling the comparison of information collected for different types of supramolecular micelle/receptor systems.



INTRODUCTION

The development of molecular receptors able to recognize specific guests with high affinity and selectivity in organic media is a major topic in supramolecular chemistry. The possibility of using these receptors in an aqueous environment in order to detect physiologically important anions and pollutants species, although highly appealing, remains challenging. The first problem encountered in the majority of cases is the poor solubility of the receptors in water. A solution to this issue can be obtained through their incorporation into micelles to form water-soluble nanoscale devices.[1−5]

Micelles are extensively used to incorporate hydrophobic compounds in a wide variety of industrial and recovery processes as well as in an assortment of consumer products among which are pharmaceuticals.[6−8] Micelles have also attracted interest in a more fundamental context where they are used as model membranes for the study of the interaction of various peptides, proteins, and organic molecules with lipid membranes.[9−11]

The potentialities and the straightforwardness of this strategy are manifest as no difficult and time-consuming synthetic modifications need to be brought to the receptor skeleton to make it hydrophilic. Moreover, the receptor/micelle system shows in many cases enhanced binding properties. For example, micellar incorporation of hydrophobic platinum porphyrins exhibit good oxygen sensitivity and response time.[4] Another example is the one reported by Rebek and co-workers where the induced conformational reorganization of a resorcinarene-cavitand incorporated into dodecylphosphocholine micelles significantly increases its binding capacity toward a series of different guests.[2,12] We, too, have recently shown that the salophen-UO_2 complex **1**, shown in Figure 1, completely

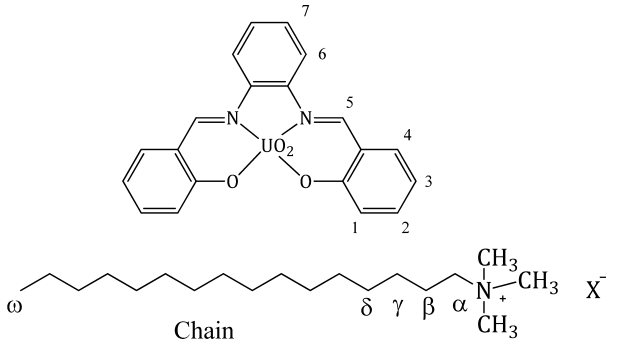


Figure 1. Chemical structure and proton labeling of the uranyl−salophen receptor **1** and of surfactants CTAX (X = Br^-, Cl^-).

insoluble in water, exhibits very good binding affinity and selectivity for aqueous fluoride (K of the order of 10^4 M^{-1}) if incorporated into cationic cetyltrimethylammonium bromide (CTABr) micelles.[13] The observed affinity is 2 orders of magnitude larger than the one observed when the same receptor is made water-soluble by appending two glucose units on the ligand skeleton, confirming in this way the true supramolecular character of the micelle−receptor system.[14]

When trying to appreciate the full potential of this approach, information on the localization and orientation of molecular receptors within the micelle is of paramount importance. Nuclear magnetic resonance spectroscopy (NMR), through the monitoring of changes in the chemical shifts and relaxation

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times of surfactant protons or via intermolecular surfactant−receptor NOEs, has proved to be efficient in this context yielding information on the loci of incorporated molecules in micelles.[15−23]

Paramagnetic relaxation enhancement (PRE) experiments can also be considered as an important NMR tool, which can be used to gain further information on these systems. To date, it has mainly been used to determine the position, orientation, and insertion depth of peptides in membrane-mimicking micelles,[24−28] but as we show here, it can be applied also to much smaller systems. PRE experiments consist in the measurement of the increase in longitudinal or transverse relaxation rates induced by the presence of a paramagnetic species.[29] The enhancement is distance dependent and can consequently provide information on the distance between the paramagnetic species and the nuclei under investigation. In the case of micelles, the paramagnetic species can either be specifically covalently bonded to the surfactant[30−32] or can simply be a water-soluble probe, which remains in the solution surrounding the micelles.[24,25,33]

In our previous study devoted to the fluoride binding of receptor **1** incorporated into CTABr micelles, we mentioned some preliminary PRE experiments, which were confirmed by NOE measurements.[13] Here, we report an extensive and systematic analysis of the effect of different parameters on PRE measurements carried out on cetyltrimetylammonium (CTAX) systems. We have considered the effect of the counterion (X = Cl, Br), of the ionic strength, and also of surfactant concentration. The study clearly points out that through a normalization procedure it is possible to compare data obtained for different systems in a reliable and meaningful way.

EXPERIMENTAL SECTION

Materials. Receptor **1** was synthetized as previously reported.[34] $K_3[Cr(CN)_6]$ (99.99% purity) was purchased from Aldrich and used without further purification. CTABr and CTACl surfactants were purchased from Aldrich and crystallized using standard procedures before use.[35] D_2O (99.9 atom %D) was purchased from Aldrich.

Solutions of CTABr and CTACl were prepared by dissolving the surfactant in D_2O. Then, 50 mM surfactant solutions containing receptor [**1**] (∼1 mM) were prepared by adding a known quantity of receptor, weighed with precision, to the surfactant solution and stirring for a minimum of 30 min.

NMR Spectroscopy. All measurements were carried out at 30 °C in order to be above the Krafft temperature of both surfactants.[36−38]

^{1}H NMR spectra were recorded on a Varian spectrometer operating at 9.4 T (399.9 MHz for ^{1}H).

Amounts of 500 μL of the different solutions were placed in the NMR tube. For the PRE experiments, aliquots (∼5 μL) of a concentrated D_2O solution of $K_3[Cr(CN)_6]$ (2.7 mM) were added using micrometric Hamilton syringes.

T_1 measurements were undertaken using the classical inversion recovery (180−τ−90−acquisition) sequence, with 16 points and the delay varying between 0 and 6s. The delays were adapted according to the amount of added chromium salt. Data treatment was performed using the Varian VNMRJ software.

To obtain the T_1 values, eq 1 was adjusted to the values of the signal integrals

$$I_\tau = I_\infty[1 - (1 - \cos\alpha)\, e^{-\tau/T_1}] \quad (1)$$

where I_τ is the signal integral after a delay τ, I_∞ is the signal integral after full relaxation, and α is the angle of inversion. The angle is left as a parameter in case of incomplete inversion.

For each signal monitored, the increase in the longitudinal relaxation rate induced by the paramagnetic species ($\Delta(1/T_1)$; the difference between the longitudinal relaxation rate measured in the presence and in the absence of the paramagnetic species) was plotted as a function of the concentration of paramagnetic species. A linear regression was undertaken with the experimental data points from which the relaxivity value was derived (slope of the line).

RESULTS AND DISCUSSION

Micelles are characterized by size, shape, and aggregation number (number of surfactant molecules that compose the micelles, N) but also by the surfactant's critical micellar concentration (the surfactant concentration above which the micelles can form; CMC). Surfactant molecules such as cetyltrimethylammonium bromide and chloride (CTAX; X = Br or Cl) ionize in aqueous solution, and the corresponding micelles are aggregates of CTA^+ ions. The counterions that stay near the micellar surface do not fully neutralize the ammonium head groups, which bear a residual fractional charge equal to α. The values of the above-mentioned parameters for CTACl and CTABr micelles are given in Table 1. The differences in the size, shape, and charge of these two types of micelles can be explained by the fact that hydrated Cl^- is larger than hydrated Br^- and penetrates less into the Stern layer and is consequently less effective in neutralizing the head-groups.[39]

Table 1. Micellar Parameters of 50 mM CTAX Solutions[39]

	CMC (mM)	N	α	Semiminor axis $b = c$ (Å)	Semimajor axis a (Å)
CTABr	∼1	145	0.26	24.0	33.7
CTACl	∼1	107	0.28	23.0	27.1

The assigned ^{1}H NMR spectra of a 50 mM CTACl solution is shown in Figure 2. The spectrum of an analogous CTABr solution (not shown) is very similar, exhibiting only very small differences in chemical shifts (<0.09 ppm).

PRE experiments were undertaken on CTABr and CTACl micelles under different experimental conditions. The longitudinal relaxation rate of the different surfactant protons were measured in the presence of increasing amounts of the

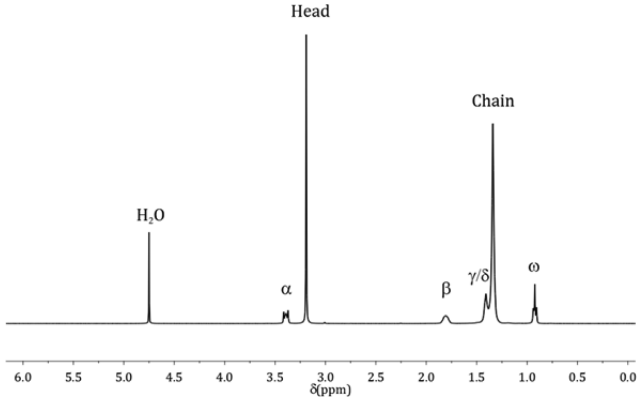

Figure 2. ^{1}H NMR 400 MHz spectra with assignment of a 50 mM CTACl solution in D_2O at 30 °C.

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paramagnetic species ($K_3[Cr(CN)_6]$).[29,40] The addition of the chromium salt did not induce any chemical shift variations. During the experiments, the signals of the trimethylammonium polar head, α-CH_2, β-CH_2, and ω-CH_3, were monitored. The signal around 1.4 ppm, which corresponds to both the γ and δ CH_2 protons, was also monitored (after signal deconvolution). The PRE data obtained for this signal corresponds to the weighted average of the data pertaining to the 2 methylene groups and is biased toward the δ CH_2.

Relaxation enhancements induced by the presence of the paramagnetic species can be described by different models detailed in the literature.[24,26,29,33,41−44] The model required to interpret the data depends on the motional correlation between the probed molecule and the paramagnetic species. As in our case, the paramagnetic probe stays in the solution surrounding the micelles, PRE is best described by the second-sphere interaction model, which considers that the two molecules are rotationally correlated.[24,33] Once the interaction is averaged over the volume in which the paramagnetic species can be located, the enhancement is dependent on the inverse of the third power of the distance.[26,41,42] When plotting the relaxation rate enhancement, $\Delta(1/T_1)$, as a function of paramagnetic species concentration, a linear relationship will be obtained, and the slope, known as the relaxivity (Φ), is directly proportional to $1/r^3$; the greater the slope, the closer the probed nuclei is to the micellar interface.

Figure 3 is an example of the data obtained (50 mM CTABr solution) and shows that a linear relationship is indeed

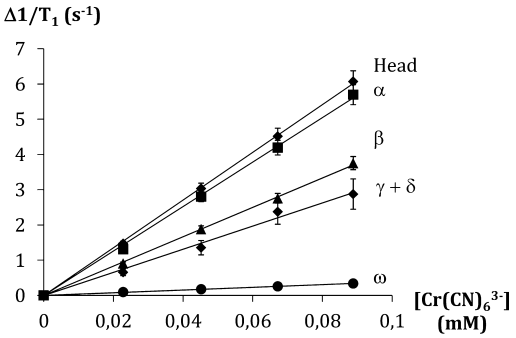

Figure 3. Relaxivity plot for the different CTABr protons (50 mM solution, 30 °C).

obtained. Relaxivities were determined for CTABr and CTACl micelles at different surfactant concentrations (see Table 2).

Table 2. Relaxivity Values (mM^{-1} s^{-1}) of the CTABr and CTACl Protons for Different Surfactant Concentrations[a]

	[CTAX] (mM)	$^+N(CH_3)_3$	α-CH_2	β-CH_2	γ+δ-CH_2	ω-CH_3
CTABr	50	68	64	42	33	4
	25	119	115	76	64	7
	10	303	294	189	155	18
CTACl	50	146	160	105	73	9
	25	191	201	132	84	13
	10	406	411	272	170	33
	5	692	726	487	213	53

[a]Estimated error: 5% except for γ+δ-CH_2 (15% due to deconvolution).

A decrease of the relaxivity values is observed for all concentrations and for both surfactants when going from the micelle surface to the core confirming that the paramagnetic species does not indeed penetrate into the micelles. As the number of free surfactant molecules, which would experience high relaxivities, is negligible compared to the number of molecules involved in the micellar aggregates, these relaxivity values are indeed characteristic of the micelles

It is noticeable that the relaxivities values in the CTACl systems are systematically higher than those of the CTABr systems. As hydrated chloride is less effective in neutralizing the positive charge of the polar head,[39] the negatively charged paramagnetic species interacts probably more efficiently with it than in the case of CTABr micelles.

It is interesting to point out that, even if not significant in all cases, the relaxivity of the α-CH_2 seems to be systematically higher than that of the headgroup in the case of the CTACl micelles. This highlights differences in the way the paramagnetic species enters into the Stern layer of these micelles, which is known to be less densely packed in the case of the CTACl micelles.[45]

A decrease in the relaxivities values is observed for both types of micellar systems with surfactant concentration. In the range monitored, the increase in surfactant concentration simply leads to more micelles in solution.[46−48] The decrease in the ratio of paramagnetic species to micelle is at the origin of the observed effect. Indeed, when plotting the relaxivities as a function of the inverse of surfactant concentration (Figure 4), a linear correlation is observed.

The effect of counterion concentration on the relaxivity values was also monitored. In the case of the CTACl micelles, which experience no significant effect in the presence of added salt,[46] relaxivity values were reproducible in the presence of 100 mM of KCl (see the Supporting Information). In the case of the CTABr micelles, it was not possible to obtain reproducible data when working in the presence of 50 mM added KBr as the micelles precipitate.[49]

Enhancing chloride concentration leads to a significant decrease in the relaxivity of the surfactant protons. As the effective micellar fractional ionization (α) does not change in the presence of the added salt, the increase of the dielectric constant of the medium[50] and the resulting decrease of the electrostatic interaction between the polar head and the chromium salt must be at the origin of this effect. In this context, it is interesting to point out that dynamic light scattering (DLS) experiments have shown that increasing the salt concentration leads to a screening of the repulsion between positively charged micelles.[48]

From the above, it is obvious that relaxivity values are sensitive to experimental conditions, which will be problematic when wanting to compare different systems, such as micelles containing different types of receptors or composed of different surfactants. As a solution to this problem, we undertook to normalize the relaxivity values, dividing them by the value obtained for the protons of the polar head. Table 3 reports the normalized values. They are clearly not dependent on experimental conditions and can thus be reliably used to compare different micellar systems such as receptor **1** incorporated in CTABr and CTACl micelles.

The normalized relaxivity values obtained for the CTABr and CTACl micelles, with and without the receptor, are reported in Table 4. From these data, it is clear that, in both types of micelles, the receptor is located at the micelle surface with its

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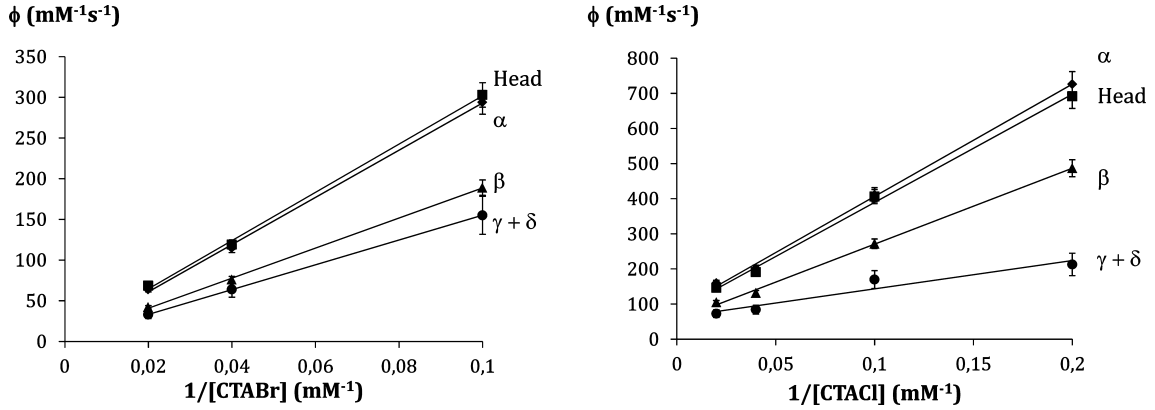

Figure 4. Relaxivity of the CTABr and CTACl protons as a function of the inverse of surfactant concentration.

Table 3. Normalized Relaxivities of the CTAX Nuclei at Different Surfactant Concentrations[a]

	[CTAX] (mM)	$^{+}N(CH_3)_3$	α-CH_2	β-CH_2	γ/δ-CH_2	ω-CH_3
CTABr	50	1.00	0.94	0.62	0.48	0.06
	25	1.00	0.97	0.64	0.54	0.06
	10	1.00	0.97	0.62	0.51	0.06
CTACl	50	1.00	1.09	0.72	0.50	0.06
	25	1.00	1.05	0.69	0.44	0.07
	10	1.00	1.01	0.67	0.42	0.08
	5	1.00	1.05	0.70	0.31	0.08

[a]Estimated error: 10% except for γ/δ-CH_2 (30% due to deconvolution).

binding site well exposed. Indeed protons 1 and 2 of the receptor exhibit the highest relaxivities. In the case of CTABr micelles, the values are, however, higher with proton 1 clearly more exposed. Its relaxivity greater than that of the polar head protons even suggests some preferential interaction with the negatively charged paramagnetic species, which is not observed in the case of the CTACl micelles. This could be due to the fact that the incorporation of molecules in CTABr micelles increases their size and changes the packing of the polar heads in the Stern layer.[51]

CONCLUSIONS

PRE experiments have been undertaken on CTABr and CTACl micelles under different experimental conditions with the aim of testing the possibility to obtain information about the localization and orientation of molecular receptors incorporated into micelles. In our case, the receptor was a uranyl−salophen complex, which, by incorporation into micelles, is made water-soluble. The results highlight that relaxivities determined via PRE measurements are strongly dependent on different parameters such as surfactant concentration, nature, and concentration of the counterion. By applying a normalization procedure to the obtained data, we have shown that it is possible to compare different micelle systems in a reliable way. The protocol can be considered of general use allowing the comparison of systems composed by different surfactants and/or different receptors.

ASSOCIATED CONTENT

Supporting Information

Table showing the effect of counterion concentration on relaxivity values. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Table 4. Normalized Relaxivities for the Surfactant (50 mM) and the Receptor Nuclei (1 mM)

	$^{+}N(CH_3)_3$	α-CH_2	β-CH_2	ω-CH_3	H1	H2	H3	H4	H5	H6	H7
CTABr[a]	1.00	0.95	0.63	0.05							
1-CTABr[a]	1.00	0.89	0.58	0.05	1.7	0.69	0.33	0.17	0.12	0.13	0.14
CTACl	1.00	1.03	0.67	0.06							
1-CTACl	1.00	0.94	0.62	0.06	0.88	0.57	0.27	0.15	0.11	0.14	0.13

[a]Experiments repeated in this work are in total accordance with data reported previously. Estimated error = 10%.

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