Fluorescence Anisotropy of Ionic Probes in AOT Reverse Micelles: Influence of Water Droplet Size and Electrostatic Interactions on Probe Dynamics

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Fluorescence anisotropies of two structurally similar ionic probes, rhodamine 110 and fluorescein, were measured in di(2-ethylhexyl) sodium sulfosuccinate (AOT) reverse micelles as a function of the mole ratio of water to surfactant W. This study was undertaken to explore the influence of water droplet size and electrostatic interactions on the rotational diffusion of the probe molecules. It was noticed that at $W = 1$ and 2, the anisotropy decays of both the probes display single-exponential behavior and for a particular value of W, the time constants sensed by rhodamine 110 and fluorescein are identical. Moreover, an increase in the reorientation time was observed from $W = 1$ to 2. These observations indicate that, at $W = 1$ and 2, it is the overall rotation of micelle which is responsible for the decay of the anisotropy and also rule out the possibility of internal rotation of the probes within the reverse micelles. However, from $W = 4$ to 20, the anisotropy decays of the probes could only be described by a biexponential function with two time constants. The rotational diffusion of rhodamine 110 and fluorescein in the above-mentioned range of W was rationalized using the two-step model. The average reorientation time decreases with an increase in W for both the probes, and this decrease is pronounced in the case of fluorescein compared to that in rhodamine 110. The decrease in the average reorientation time with W is due to the change in the micellar packing within the core. The significant reduction in the average reorientation time of fluorescein is a consequence of repulsive electrostatic interactions between the negatively charged probe and the anionic head groups of the surfactant AOT.

1. Introduction

In an attempt to explore the nature of the nanoscopic water, especially the effect of confinement, and also understand the dynamics of the solutes located in the interfacial region, numerous studies have been performed in reverse micelles.[1–20] Reverse micelles formed with the anionic surfactant, di(2-ethylhexyl) sodium sulfosuccinate (AOT) have received considerable attention due to their ability to solubilize large quantities of water and form well-defined and well-characterized water droplets over a wide composition range. The AOT reverse micelles can be pictured as water droplets that are coated by a layer of surfactant molecules and are dispersed in a nonpolar solvent. However, in reality the picture is more complex as the confinement of water molecules in nanocavities will have a profound bearing on the strength, structure, and mobility of the hydrogen bonded network. In a recent study, Fayer and coworkers[7] have demonstrated that the dynamics of nanoscopic water is primarily governed by the presence of confinement rather than the nature of the confining interface. Using ultrafast infrared pump−probe spectroscopy, they observed that the orientational relaxation of water molecules is very similar in the nanopools of both anionic AOT and nonionic Igepal CO 520 reverse micelles, wherein the respective mole ratios of water to surfactant were chosen such that the sizes of the water droplets are identical.

Understanding the mobility of medium-sized solute molecules solubilized in the cores of the reverse micelles is another important aspect, because these aggregates are used for performing chemical reactions in organic phase but by hosting the reactants in the water pool.[16] Furthermore, the sizes of the water droplets present in these confined geometries have a significant bearing on the solute rotation. As a result a number of investigations have been undertaken to examine the rotational diffusion of organic solutes in reverse micelles[9–20] and a majority of these studies have been carried out in AOT reverse micelles.[9–17,20] Despite these studies, a comprehensive understanding of the solute rotation in these systems is yet to emerge. Besides there is a considerable disparity among the results available in literature, which is probably due the characteristics such as size, charge, and nature of the functional groups present on the probe molecules employed in these investigations. Moreover, early studies have used steady-state fluorescence anisotropy to examine the solute rotation in AOT reverse micelles.[9,12,13,15] Even though some of these investigations utilized time-resolved methods,[9–11,13] the time resolution was limited by the pulse width of the excitation sources. Time-resolved anisotropy measurements with picosecond resolution are also available in literature,[14,16,17,20] and the results of some these studies can be summarized in the following manner.

Visser et al.[14] have investigated the rotational diffusion of rhodamine B and octadecylrhodamine B in AOT reverse micelles and observed that the anisotropy decays of these probes follow biexponential behavior with two time constants. They have ascribed the two time constants to the internal rotation of the probe and the overall rotation of the micelle. However, as the mole ratio of water to surfactant W increased, it has been noticed that the experimentally obtained time constant for the overall rotation of the micelle was found to be much smaller than the calculated one using the Stokes−Einstein−Debye (SED) hydrodynamic model.[21] The explanation provided by them was that the time constant for the internal rotation of the probe is also appearing in the longer component. Wittouck et

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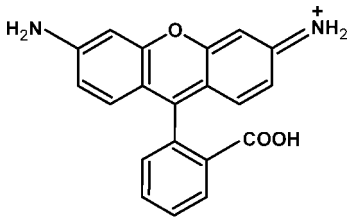
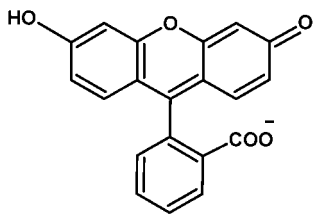


Rhodamine 110



Fluorescein

Figure 1. Molecular structures of the ionic probes used in the study.

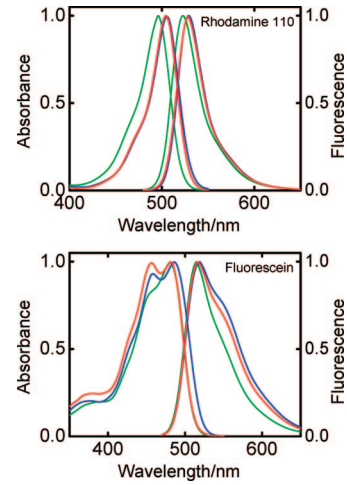


Figure 2. Absorption and emission spectra of rhodamine 110 and fluorescein in AOT reverse micelles at $W = 1$ (blue) and $W = 20$ (red). The spectra of the probes in water (green) are also shown for comparison.

al.[16] have measured the fluorescence anisotropy of a cationic probe cresyl violet in AOT reverse micelles as function of W. For $W < 10$, their elucidation of the data is consistent with that of Visser et al.[14] However, for $W > 10$, it has been suggested that the motion of micellar segments also needs to be considered. In a recent study, Spry et al.[20] have investigated the rotational diffusion of anionic probe, 8-methoxy-pyrene-1,3,6-trisulfonate (MPTS), in AOT reverse micelles with varying amounts of water. It has been noticed that at $W = 1$, the anisotropy decay of MPTS can be fitted with a single exponential function and the time constant describes the overall rotation of the micelle. In the region, $W = 2-5$, they observed restricted rotation of the probe with a large residual anisotropy at long times. From $W > 5$, the residual anisotropy vanishes and the rotational diffusion becomes similar to that observed in bulk water. Although the results published in the literature and the explanations offered are consistent, the overall trends appear to be contrasting and seem to depend on the nature of the probes used in these studies.

In an attempt to provide a better appreciation of solute rotational diffusion in AOT reverse micelles, the present study was undertaken and the following strategy has been adopted. To find out whether the charge and size of the probe molecules will have a bearing on their mobility, two structurally similar probes, rhodamine 110 and fluorescein, were chosen (see Figure 1). It is evident from the figure that rhodamine 110 and fluorescein have a close structural resemblance but possess distinct electrical properties. Since AOT is a negatively charged surfactant, the interaction of these probes with the surfactant head-groups is expected to be dissimilar. This work, essentially deals with fluorescence anisotropy measurements of positively charged rhodamine 110 and negatively charged fluorescein in AOT reverse micelles in the W range of $1-20$, and isooctane is used as the organic phase. It will be interesting to find out how the water droplet size and electrostatic interactions are going to influence the solute rotation in the AOT reverse micelles.

2. Experimental Section

The probes rhodamine 110 (chloride salt) and disodium fluorescein were obtained from Exciton. The surfactant AOT and the solvent isooctane were purchased from Aldrich and Merck, respectively. All these chemicals are of the highest available purity and were used without further purification. Deionized water from Millipore A-10 was used in the preparation of the samples. In all the measurements, the concentration of AOT was maintained at 0.1 M whereas that of the probes in the range of $10^{-5}-10^{-6}$ M.

Absorption and Fluorescence spectra were recorded using a Chemito Spectrascan UV 2600 spectrophotometer and a Hitachi F-4010 spectrofluorometer, respectively. Time-resolved fluorescence measurements were carried out using time-correlated single-photon counting (TCSPC)[22] facility at the Tata Institute of Fundamental Research, Mumbai and details of the system have been described elsewhere.[23] The excitation source was a frequency-doubled output of a picosecond Ti:sapphire laser (Tsunami, Spectra Physics). The samples containing the probes rhodamine 110 and fluorescein were excited at 440 nm and the emission was monitored at 550 nm. The decays measured in this manner are convoluted with the instrument response function (IRF), which was measured by replacing the sample with a solution that scatters light. The full width at half-maximum (FWHM) of the IRF is about 50 ps. All the measurements were carried out at 298 K. Each measurement was repeated at least $2-3$ times and the average values are reported. Fluorescence and anisotropy decay parameters were obtained from the magic angle and anisotropy decays, respectively, with the help of iterative reconvolution methods as described in literature.[24,25] Details concerning the analysis of the fluorescence and anisotropy decays have been mentioned in our earlier publication.[26] Viscosities of isooctane and 0.1 M solution of AOT in isooctane at 298 K were measured with the help of Cannon calibrated viscometer (size 25).

3. Results and Discussion

Absorption and fluorescence spectra of rhodamine 110 and fluorescein in AOT reverse micelles at $W = 1$ and 20 and also in water are given in Figure 2. The is no change in the absorption as well as emission spectra of rhodamine 110 in AOT reverse micelles at the two extreme values of W used in the study, which indicates that there is no significant variation in the environment experienced by the probe molecule with an increase in the water content. In bulk water, however, these spectra are blue-shifted compared to that in AOT reverse micelles. The anionic probe,

fluorescein, can exist as either monoanion or dianion in water depending on the pH, and in the pH range of 6−10, the monoanion−dianion equilibrium prevails.[27] In AOT reverse micelles, the absorption band of fluorescein is structured with the maxima centered around 455 and 480 nm, which is the characteristic of the monoanion. In contrast, only a shoulder is present at 455 nm in the absorption spectrum of fluorescein in water with a peak around 480 nm, which is an indication that monoanion and dianion forms are present in solution. Both the monoanion and dianion of fluorescein emit in the range of 480−650 nm, but the emission spectrum of the monoanion has a shoulder around 550 nm.[27] Based on this logic, it is evident from Figure 2 that fluorescein exists as monoanion in AOT reverse micelles from $W = 1-20$, and in water, both the forms are in equilibrium. Even though both these forms are present in water, one can preferentially excite the monoanion form of fluorescein using 440 nm light.

Fluorescence lifetimes, τ_f, of rhodamine 110 and fluorescein were measured in AOT reverse micelles and also in water to ascertain their location. The fluorescence decays of both the probes at all values of W and in water could be fitted with a single-exponential function. The lifetime of rhodamine in water is 4.1 ns, and in AOT reverse micelles, it is around 3.7 ns, which does not vary with W. The lifetime of fluorescein in water is 3.5 ns, and this value is comparable to the one reported in literature[27] for the monoanion, which is 3.7 ns. Since 440 nm excitation wavelength was employed, emission from the fluorescein monoanion was observed. Unlike the lifetimes of rhodamine 110, τ_f values of fluorescein decrease from 4.5 to 4.0 ns with an increase in W from 1 to 20 in AOT reverse micelles, and these numbers are higher than the ones obtained in water. Thus, from these results it can be inferred that both rhodamine 110 and fluorescein are not solubilized in water but are located at the micelle−water interface within the cores of the AOT reverse micelles.

Since both the probes used in this study contain a carboxylate group, excited-state proton transfer is possible in these systems. In fact, for fluorescein, this process has been thoroughly investigated in aqueous buffer systems.[27,28] Alvarez-Pez et al.[27] have observed two lifetimes and two pre-exponential factors (one with a negative amplitude) for fluorescein in aqueous 1 M phosphate buffer solutions in the pH range of 5.9−7.7. Moreover, the lifetimes were found to be pH dependent and these results are indicative of excited-state proton transfer reactions. However, in 1 mM phosphate buffer solutions, they did not observe any excited-state proton reactions. In the case of AOT reverse micelles, determination of parameters such as pK_a and excited-state pK_a is not straightforward because the influence of additives that are need for the variation of pH, such as alkali and buffer, on the structure of the reverse micelles is not known. Thus, in the absence of these data, one must be careful to discount the influence of protonation and deprotonation on the lifetimes and reorientation times of the probes in the AOT reverse micelles. The single exponential fluorescence decays observed for rhodamine 110 and fluorescein in AOT reverse micelles are perhaps an indication that the excited-state proton transfer is not operative in the present case.

Time-resolved anisotropy measurements were performed to understand the rotational diffusion of rhodamine 110 and fluorescein in AOT reverse micelles. For the sake of comparison, anisotropy decays of the two probes were also measured in water. Anisotropy decays of rhodamine 110 and fluorescein in water and AOT reverse micelles at $W = 1$ and 2 could be adequately fitted with single exponential function. However, from $W = 4-20$, a biexponential function of the form given below was needed to fit the anisotropy decays.

$$r(t) = r_0[\beta\exp(-t/\tau_{r1}) + (1-\beta)\exp(-t/\tau_{r2})] \quad (1)$$

In the above equation, τ_{r1} and τ_{r2} are the two time constants associated with the decay of the anisotropy, β is the percentage contribution of τ_{r1}, and r_0 is the initial anisotropy whose value depends on the angle between absorption and emission transition dipoles. To compare the anisotropy decays measured at different values of W, the average reorientation time, $\langle\tau_r\rangle$, was calculated using eq 2.

$$\langle\tau_r\rangle = \beta\tau_{r1} + (1-\beta)\tau_{r2} \quad (2)$$

The anisotropy decay parameters along with the fluorescence lifetimes of rhodamine 110 and fluorescein are given in Tables 1 and 2, respectively. Inspection of the Tables reveals the following features. The reorientation times of both the probes are identical in water, which is 110 ps. Since the IRF of the TCSPC setup used in this study is 50 ps, reorientation times of the order of 100 ps can be measured with an uncertainty of about 10%. In case of biexponential fits, the accuracy of the recovered time constants also depends on the ratio of the longer to the shorter component. For rhodamine 110 and fluorescein in AOT reverse micelles, the ratio of τ_{r1} to τ_{r2} is 4−5 and 7−8, respectively. Thus, these parameters can be recovered fairly accurately, and the uncertainties mentioned in the tables are the average deviations obtained from three independent measurements. Another point that needs some attention is the magnitude of r_0 obtained during the analysis. Even though not mentioned in Tables 1 and 2, the r_0 values recovered for the two probes in AOT reverse micelles and in water are in the range of 0.32−0.34, which are lower than the theoretical limit of 0.4. From steady-state anisotropy measurements, the r_0 value of

TABLE 1: Fluorescence Lifetimes and Anisotropy Decay Parameters of Rhodamine 110 in AOT Reverse Micelles as a Function of W at 298 K[a]

W	τ_f/ns	β	τ_{r1}/ns	τ_{r2}/ns	$\langle\tau_r\rangle^b$/ns
1.0	3.68	1	3.23 ± 0.09		3.2
2.0	3.69	1	3.5 ± 0.1		3.5
4.0	3.69	0.88 ± 0.01	3.8 ± 0.1	0.78 ± 0.05	3.4
7.0	3.67	0.86 ± 0.01	3.5 ± 0.1	0.64 ± 0.05	3.1
10.0	3.67	0.83 ± 0.03	3.5 ± 0.1	0.61 ± 0.02	3.0
13.0	3.66	0.75 ± 0.01	3.3 ± 0.1	0.62 ± 0.04	2.6
17.0	3.67	0.76 ± 0.03	2.9 ± 0.1	0.69 ± 0.05	2.4
20.0	3.69	0.71 ± 0.04	3.0 ± 0.1	0.78 ± 0.02	2.4
water	4.06	1	0.11 ± 0.01		0.11

[a] The uncertainties on lifetimes are less than 2%. [b] Calculated using eq 2.

TABLE 2: Fluorescence Lifetimes and Anisotropy Decay Parameters of Fluorescein in AOT Reverse Micelles as a Function of W at 298 K[a]

W	τ_f/ns	β	τ_{r1}/ns	τ_{r2}/ns	$\langle\tau_r\rangle^b$/ns
1.0	4.52	1	3.28 ± 0.07		3.36
2.0	4.32	1	3.5 ± 0.1		3.5
4.0	4.18	0.84 ± 0.02	3.6 ± 0.2	0.43 ± 0.09	3.1
7.0	4.09	0.76 ± 0.02	3.1 ± 0.1	0.45 ± 0.04	2.5
10.0	4.08	0.69 ± 0.02	2.9 ± 0.2	0.44 ± 0.03	2.1
13.0	4.07	0.65 ± 0.01	2.8 ± 0.2	0.44 ± 0.07	2.0
17.0	4.01	0.63 ± 0.02	2.6 ± 0.1	0.36 ± 0.01	1.8
20.0	4.03	0.60 ± 0.01	2.5 ± 0.1	0.35 ± 0.06	1.6
water	3.46	1	0.11 ± 0.01		0.11

[a] The uncertainties on lifetimes are less than 2%. [b] Calculated using eq 2.

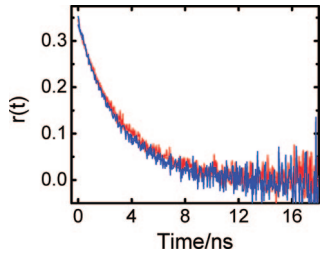


Figure 3. Anisotropy decay curves of rhodamine 110 (blue) and fluorescein (red) in AOT reverse micelles at $W = 1$. The smooth curves passing through the data points were obtained by single-exponential fits with $\tau_r = 3.23 \pm 0.09$ ns and 3.28 ± 0.07 ns for rhodamine 110 and fluorescein, respectively.

rhodamine 110 was found to be 0.380 ± 0.005.[29] The r_0 value of fluorescein should be comparable since it is structurally similar to rhodamine 110. A value of 0.38 for r_0 implies that the angle between absorption and emission transition dipoles is 10.5°. However, the recovered r_0 values from the time-resolved anisotropy measurements are lower by 10−15% compared to the one obtained from the steady-state measurement. This observation points to the fact that a fast depolarization process, which is beyond the time resolution of the experimental setup used in this study, is operative in these systems. The probable fast process could be the free rotation of the phenyl group around the single bond. Since this process is expected to be much faster compared to τ_{r1} and τ_{r2}, it is unlikely to have a bearing on the recovery and subsequent interpretation of the two anisotropy decay constants.

In AOT reverse micelles at $W = 1$, τ_r values of rhodamine 110 and fluorescein are the same within the limits of experimental error but this number is significantly longer compared to the one measured in water. As W increases from 1 to 2, the reorientation time increases by about 10%, and even at $W = 2$, τ_r values of both the probes are identical. From $W = 4$ to $W = 20$, τ_{r1} and τ_{r2} values of rhodamine 110 are considerably longer compared to that of fluorescein and these parameters decrease with an increase in W for both the probes. To illustrate these observations in a pictorial manner, anisotropy decays of the probes in AOT reverse micelles at $W = 1$ are represented in Figure 3 whereas those at $W = 4$, 10, and 20 are given in Figure 4. The average reorientation times of the probes as function of W are plotted in Figure 5. These observations were rationalized based on the sizes of the AOT reverse micelles at different values of W. As mentioned earlier, the water droplets present in AOT reverse micelles have well-defined spherical structures, and the relationship between water pool diameter d_{wp} and W is given by[5]

$$d_{\mathrm{wp}} = 0.29W + 1.1 \text{ nm} \tag{3}$$

Eq 3 holds up to $W = 20$, and from this relationship, the calculated radii of the water pools at $W = 1$ and 2 are 0.7 and 0.84 nm, respectively. The long axial radii of the probes rhodamine 110 and fluorescein measured with the aid of Corey−Pauling−Koltun (CPK) space filling models are 0.67 and 0.64 nm, respectively, which indicates that the probes can be accommodated inside the water pools of the AOT reverse micelles at $W = 1$ and 2. In case of AOT, it has been established that the thickness of the surfactant monolayer, l_c, is dependent on the organic solvent used and in most cases a value of 1.0 nm is a good approximation.[16] Thus, the hydrodynamic radius of AOT reverse micelles is given by $r_h = 0.5d_{\mathrm{wp}} + 1.0$, and using the values of r_h at different values of W, the time constants

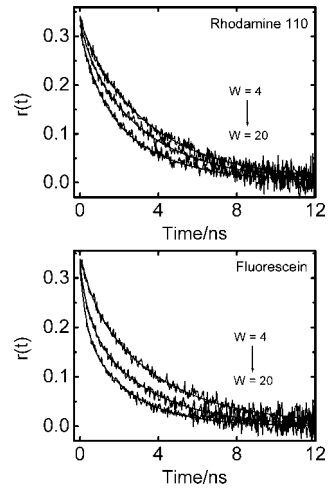


Figure 4. Anisotropy decay curves of rhodamine 110 and fluorescein in AOT reverse micelles at $W = 4$, 10, and 20. It is evident from the figure that as the water content increases the anisotropy decays become faster for both the probes. The smooth curves passing through the data points were obtained by biexponential fits.

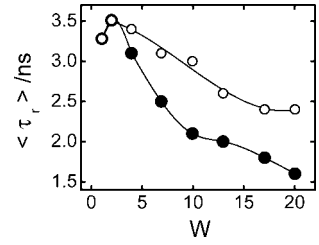


Figure 5. Variation of average reorientation times of rhodamine 110 (○) and fluorescein (●) in AOT reverse micelles with W. The lines passing through the data points are drawn as visual aid.

for the overall rotation of the AOT micelles (τ_M) were obtained from the SED relation:

$$\tau_{\mathrm{M}} = \frac{4\pi r_h^3 \eta}{3kT} \tag{4}$$

In the above equation, η is the viscosity of isooctane, which is 0.474 mPa s at 298 K. The parameters k and T are the Boltzmann constant and absolute temperature, respectively. The τ_M values obtained from eq 4 are 2.4 and 3.0 ns at $W = 1$ and 2, respectively. The experimentally measured reorientation times of the probes in AOT reverse micelles at these values of W are 3.2 and 3.5 ns, respectively. By varying the r_h values in the range of 5−10% in eq 4, an exact replication of the experimentally measured numbers can be achieved. However, it is also possible that the presence of charged probes in the small cavities of the AOT reverse micelles in a way alters the hydrodynamic radii of these reverse micelles. Since both the probes are sensing an identical reorientation time at a given value of W and also due to the fact that there is an enhancement in the τ_r value with an increase in the water content, it is only logical to rationalize that the measured reorientation times at $W = 1$ and 2 represent the overall rotation of the AOT reverse micelles. It must be noted that at low values of W, the recovery of single time constant from the anisotropy decays is an indication of the absence of internal rotation of the probes within the reverse micelles. This result is similar to the one obtained by Spry et al.[20] for the anionic probe MPTS in AOT reverse micelles at $W = 1$.

At this juncture, it is relevant to include some discussion concerning the choice of the viscosity that needs to be

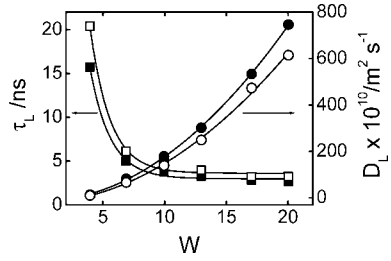


Figure 6. Plots of time constants for lateral diffusion for rhodamine 110 (□) and fluorescein (■) in AOT reverse micelles as a function *W*. Also shown in the figure is the variation of lateral diffusion coefficients with *W* for rhodamine 110 (○) and fluorescein (●). The lines passing through the points are drawn as visual aid.

employed in eq 4 for calculating the τ_M values of AOT reverse micelles. It must be borne in mind that the presence of 0.1 M AOT in isooctane increases the viscosity of the solution. However, in the absence of intermicellar interactions, a micellar aggregate senses the viscosity of the surrounding solvent rather than the bulk viscosity of the solution. In a recent study, Spry et al.[20] have considered the viscosity of heptane while calculating the reorientation time of AOT reverse micelles whereas Wittouck et al.[16] have used the solution viscosity for the same purpose. To find out how the τ_M values of AOT reverse micelles are altered if solution viscosity is used instead of solvent viscosity, the following exercise was performed. The viscosity of 0.1 M solution of AOT in isooctane was estimated by eq 5.[30]

$$\eta_{solution} = \eta_{solvent}(1 + [\eta]C) \quad (5)$$

In the above equation, *C* is the concentration of AOT in g cm^{-3} and $[\eta]$ is the intrinsic viscosity, which is 3.01 $cm^3\ g^{-1}$ for AOT in heptane at 298 K. Assuming that the value of $[\eta]$ is the same in isooctane, the viscosity of 0.1 M solution of AOT in isooctane was calculated, which is 0.537 mPa s. This number is almost identical to the experimentally measured value of 0.524 mPa s. Incorporation of 0.537 instead of 0.474 mPa s in eq 4 results in τ_M values of 2.7 and 3.4 ns at $W = 1$ and 2, respectively. These values are about 13% longer than ones mentioned earlier and improve the agreement with the experimentally measured numbers.

From $W = 4$ to $W = 20$, however, the rotational diffusion of both the probes follows a more complex pattern and inspection of Tables 1 and 2 reveals the following features. In case of rhodamine 110, τ_{r1} decreases by 27% as *W* increases from 4 to 20, and for fluorescein, this decrease is 44%. In contrast, there is no systematic variation in the values of τ_{r2} for both the probes. The decrease in β with an increase in *W* is 24% and 40% for rhodamine 110 and fluorescein, respectively. The average reorientation time decreases by 42% for the cationic probe, and this number is 94% for the anionic probe. Another point, which needs to be noted, is that the τ_{r1} values of both the probes are significantly lower than the time constants for the overall rotation of the micelles. Moreover, if τ_{r1} represents the overall rotation of the micelle, its values should have increased with an increase in *W*, but the recovered numbers follow an opposite trend. It may also be possible that the parameter τ_{r1} is not recovered accurately during analysis of anisotropy decay curves. To rule out this possibility, τ_{r1} was fixed to the calculated overall rotation time of the reverse micelle at each value of *W* and the analysis was repeated. However, such a procedure did not yield statistically satisfactory fits. Based on these facts, one can conclude that τ_{r1} does not represent the time constant for the overall rotation of the AOT reverse micelles.

TABLE 3: Order Parameters, Cone Angles, and Lateral and Wobbling Diffusion Coefficients for Rhodamine 110 in AOT Reverse Micelles Obtained from Anisotropy Decay Parameters Using the Two-Step Model

W	*S*	θ^0	$D_L \times 10^{10}/m^2\ s^{-1}$	$D_W \times 10^{-8}/s^{-1}$
4.0	0.94	16.4	10.4	0.24
7.0	0.93	17.7	66.6	0.35
10.0	0.91	20.2	139.5	0.47
13.0	0.87	24.4	248.9	0.65
17.0	0.87	24.4	473.5	0.55
20.0	0.84	27.2	614.2	0.58

TABLE 4: Order Parameters, Cone Angles, and Lateral and Wobbling Diffusion Coefficients for Fluorescein in AOT Reverse Micelles Obtained from Anisotropy Decay Parameters Using the Two-Step Model

W	*S*	θ^0	$D_L \times 10^{10}/m^2\ s^{-1}$	$D_W \times 10^{-8}/s^{-1}$
4.0	0.92	19.0	13.6	0.63
7.0	0.87	24.4	81.6	0.95
10.0	0.83	28.1	178.7	1.25
13.0	0.81	29.8	302.2	1.39
17.0	0.79	31.5	533.5	1.91
20.0	0.77	33.1	745.8	2.14

From these observations it appears that both τ_{r1} and τ_{r2} each represents a combination, rather than individual diffusion processes. It is a well-known fact that when probe molecules experience restricted rotation in normal micelles the anisotropy decay parameters are often interpreted using the two-step model.[31–38] However, this model has seldom been applied in case of reverse micelles.[8,18,19] According to the two-step model,[39–42] the probe molecule located at the interface of a spherical micelle undergoes a slow lateral diffusion on or inside the curved surface and a fast wobbling motion in an imaginary cone. Under the assumption that the slow and fast motions are separable, the experimentally measured anisotropy decay parameters are related to the model parameters by the following equations.[31]

$$\frac{1}{\tau_{r1}} = \frac{1}{\tau_L} + \frac{1}{\tau_M} \quad (6)$$

$$\frac{1}{\tau_{r2}} = \frac{1}{\tau_W} + \frac{1}{\tau_L} + \frac{1}{\tau_M} \quad (7)$$

$$\beta = S^2 \quad (8)$$

In the above equations, τ_L and τ_W are the time constants for lateral diffusion and wobbling motion, respectively, and β is the square of the order parameter *S*, which follows the inequality $0 \le S^2 \le 1$.[31] For each value of *W*, the time constants for the lateral diffusion, wobbling motion, and the order parameters were obtained with the aid of eqs 6–8. If lateral diffusion of the probes is one of the processes responsible for the decay of anisotropy, then it should become faster with an increase in the size of the reverse micelle. In other words, the value of τ_L should decrease with an increase in *W*, because an increase in the water content of the AOT reverse micelle reduces the compactness of the micelle−water interface within the core. In fact, this has been observed and Figure 6 gives plots of τ_L as a function of *W* for both rhodamine 110 and fluorescein. It is evident from the figure that τ_L decreases by a factor of 6 for both the probes as *W* increases from 4 to 20. However, the decrease is not uniform, as τ_L abates by a factor of 3 from $W = 4$ to $W = 7$ and only by a factor of 2 for *W* values from 7 to 20. This is because the contribution of the overall rotation of the micelle

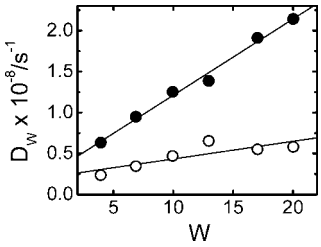


Figure 7. Plots of wobbling diffusion coefficients for rhodamine 110 (○) and fluorescein (●) in AOT reverse micelles as a function *W*. The lines passing through the points are drawn as visual aid.

to τ_{r1} is significant at low values of *W*. From the τ_L values, lateral diffusion coefficients were obtained using eq 9:[32]

$$D_L = \frac{r_{wp}^2}{6\tau_L} \tag{9}$$

where r_{wp} is the radius of the water pool. Since the probes are located at the micelle−water interface within the core, r_{wp} defines the radius of the curved surface on which lateral diffusion takes place. Plots of D_L vs *W* are also displayed in Figure 6, and as expected, the trends in the variation of the diffusion coefficient with *W* contrasts that of τ_L.

Wobbling motion is another process responsible for the decay of the anisotropy in micelles and reverse micelles. The wobbling-in-a-cone model describes the relaxation of the solute when its rotational diffusion is restricted and the extent of restriction is defined by the cone angle.[42] Thus, if wobbling motion is the sole process that is operative then the anisotropy decay displays residual anisotropy at long times. The magnitude of the cone angle θ depends on the order parameter and was obtained from the following relation.[31]

$$\theta = \cos^{-1}\{0.5[(1 + 8|S|)^{0.5} - 1]\} \tag{10}$$

The wobbling diffusion coefficients, D_W, for the probes were calculated from the parameters τ_W, *S,* and θ with the help of eq 11.[40,41]

$$D_W = \frac{1}{[(1 - S^2)\tau_W]}\left[\frac{\cos^2\theta(1 + \cos\theta)^2}{2(\cos\theta - 1)}\left\{\ln\left(\frac{(1 + \cos\theta)}{2}\right) + \frac{(1 - \cos\theta)}{2}\right\} + \frac{(1 - \cos\theta)}{24}(6 + 8\cos\theta - \cos^2\theta - 12\cos^3\theta - 7\cos^4\theta)\right] \tag{11}$$

The calculated order parameters, cone angles, and lateral and wobbling diffusion coefficients of rhodamine 110 and fluorescein in AOT reverse micelles are given in Tables 3 and 4, respectively. The variation of D_W with *W* for both the probes is presented in Figure 7. It is evident from the tables and also from Figures 6 and 7 that these diffusional processes become faster with an increase in the water content, which is due to a change in the micellar packing within the core of these reverse micelles. In other words, the diffusional processes tend to be less restrained with an increase in the droplet size. In fact, this has also been reflected by a decrease in the order parameter, which is a measure of the spatial restriction of the motion, with an increase in *W*. Another issue, which needs to be mentioned here is that variation of these parameters with *W* is more pronounced in case of fluorescein compared to rhodamine 110. This is because of the repulsive electrostatic interactions between the negatively charged fluorescein and the anionic head groups of the surfactant AOT. Thus, it would be interesting to carry out similar type of measurements in reverse micelles formed with a positively charged surfactant such as cetyltrimethylammonium bromide.

From this study, it has become evident that the rotational diffusion of structurally analogous cationic and anionic probes is similar in AOT reverse micelles. However, this statement cannot be generalized in view of the reports available in literature.[16,20] As mentioned earlier, the results of Wittouck et al.[16] and Spry et al.[20] indicate that the rotational diffusion of the positively charged cresyl violet and negatively charged MPTS in AOT reverse micelles are quite contrasting and are also very different from what has been observed for rhodamine 110 and fluorescein in the present work, especially at high values of *W*. Thus, apart from the charge on the probe molecule, it is probable that the shape and the manner in which it is anchored at the micelle−water interface within the core, do play a significant role in dictating its rotational diffusion.

Conclusions

In this work, an attempt was made to assess the role of water droplet size and charge of the probe molecules on their rotational diffusion in AOT reverse micelles. For this purpose, fluorescence anisotropies of two structurally similar cationic (rhodamine 110) and anionic (fluorescein) probes were measured in AOT reverse micelles as a function of *W*, and the important conclusions are as follows. At $W = 1$ and 2, the anisotropy decays of both the probes report the overall rotation of the AOT reverse micelles, and the internal rotation of the probes in the micelles is absent at these values of *W*. In contrast, from $W = 4$ to $W = 20$, lateral and wobbling diffusion are mainly responsible for the decay of the anisotropy and both these motions are coupled to the overall rotation of the micelles. Although, the trends observed in the rotational diffusion of both rhodamine 110 and fluorescein in AOT reverse micelles appear to be the same, it has to be noted that the influence of electrostatic interactions cannot be overlooked. This conclusion is based on the observed faster diffusion of fluorescein compared to rhodamine 110 in the reverse micelles of AOT, which has negatively charged head groups.

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