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# Exploring the locations of different groups of a cationic surface-active 3*H*-indole probe molecule in AOT-based water-in-oil microemulsions

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#### **Abstract**

We report herein the spectral and photophysical studies of the locations of different groups of a probe molecule, i.e., [2-(p-hexylamino)phenyl-3,3-dimethyl-5-ethoxycarbonyl-3H-indole]methyldioctadecylammonium iodide (1) in the microemulsion of sodium bis(2-ethylhexyl)sulfosuccinate (AOT). Two models were suggested: (1) the aliphatic chains, the hexyl group together with the part from the amino nitrogen to indolic nitrogen penetrate into the interface of the microemulsion, while the part from the indolic nitrogen to the right side resides outside the interface; (2) the aromatic main chain of 1 lies flat at the inner interface. The measured spectral characteristics, micropolarity, and fluorescence lifetime of 1 support the two models. © 2001 Elsevier Science B.V. All rights reserved.

#### 1. Introduction

It is well known that fluorescent probe molecules are often used to study the physicochemical properties of microheterogeneous media, e.g., micropolarity and microviscosity [1–3]. However, the reported values of these properties for similar systems are not identical, partly resulting from the fact that the locations of different probes in such systems might be different [4–8]. Thus, it should be better to investigate the location of a fluorescent probe when it is used in sensing a certain microheterogeneous medium.

In the past few years, the fluorescent substituted 3*H*-indoles have been used to probe successfully

the mean structural properties of aqueous micelles [4,9–11,15], reversed micelles [4,12], surfactant vesicles [13,14], and cyclodextrins (CD's) [16–21]. As a member of the family of 3H-indoles, the cationic surface-active molecule 1 (see Fig. 1) has attracted significant attention in the recent past [15,16]. In a previous paper, the study on the interaction between 1 and aqueous micelles was performed. The locations of the different parts of 1 in the SDS micelle were suggested [15]. However, the results are preliminary and more experimental evidences are necessary to elucidate this kind of problem. In addition, a certain medium better than the SDS micelle should be chosen to avoid the formation of the coacervate phase between 1 and the SDS micelle [15].

As an extension of the previous paper, the interactions of 1 with AOT-based reversed micelles or water-in-oil (w/o) microemulsions have been

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$$[H_{3}C \xrightarrow{N^{+}} (CH_{2})_{17}CH_{3} \\ \downarrow \qquad \qquad \qquad I$$

$$X \qquad \qquad Y$$

$$2 \qquad NH_{2} \qquad CN$$

$$3 \qquad N(CH_{3})_{2} \qquad CN$$

$$4 \qquad NH_{2} \qquad CO_{2}Et$$

$$5 \qquad N(CH_{3})_{2} \qquad CO_{2}Et$$

Fig. 1. Molecular structures of 1-5.

studied. We first want to obtain a relatively clear picture about the locations of different parts of 1 in the w/o microemulsion composed of AOT, n-heptane, and water. The key is to ascertain the location of the indolic nitrogen of 1 in the microemulsion. We also attempt to get some information on the structural property of the AOT-based microemulsion reflected by the spectral and photophysical characteristics of 1. It is believed that the information on the location of 1 will make the structural property of the AOT-based microemulsion sensed by 1 more convincible.

#### 2. Experimental

#### 2.1. Materials

1 was a generous gift from Professor Durocher in Department of Chemistry at University of Montreal, Canada. The synthesis and purification of 1 has been described elsewhere [15]. Analytical grade reagents sodium hydroxide, sodium carbonate, sodium bicarbonate, potassium acid phthalate, hydrochloric acid, sulfuric acid, n-hexanol, n-pentanol, n-butanol, n-propanol, methanol, and Triton X-100 (Fluka) were used without further purification. AOT (Sigma, 99% purity) was purified by the methods of El Seoud and Da Silva

[22]. *n*-Heptane was redistilled over metallic sodium. Cetyltrimethylammonium bromide (CTAB, Shanghai, China) was recrystallized from an ethanolacetone mixture (80:20, v/v) and dried under vacuum.

#### 2.2. Instruments

Absorption spectra were recorded on an UV-3000 (Shimadzu) spectrophotometer using 1 cm-path quartz cells. Fluorescence spectra were measured on a FL-4500 (Hitachi) spectrofluorimeter. Both the excitation and emission bandpasses were 5 nm. Each solution was excited near the absorption wavelength maximum using 1 cmpath quartz cells. Fluorescence lifetime measurements were made on a multiplexed time-correlated single-photon counting fluorimeter (Horiba, Mode Naes-1100). The fluorescence lifetime was determined from data on the fluorescence transient waveform of the material to be tested and the lamp waveform data using the least-squares iterative deconvolution method. The error in the lifetime measurement is less than 10%. The excitation and emission wavelengths were 395 and 485 nm, respectively. 5000 counts were collected for each sample. More details on the apparatus for the fluorescence lifetime measurements were described elsewhere [23].

#### 2.3. Methods

Tridistilled water was used throughout the experiments. AOT-based microemulsions with different pH values were prepared according to the method used by Chang et al. [24]. The pH value 9.5 was adjusted by adding a buffer solution of sodium carbonate and sodium bicarbonate. The pH values 7.0, 5.2 and 4.0 were adjusted by adding a series of buffer solutions with different ratios of potassium acid phthalate and sodium hydroxide, respectively. The pH value 2.2 was adjusted using a solution of potassium acid phthalate and hydrochloric acid. The apparent pH of the microemulsions was determined by directly immersing a complexed electrode in the transparent microemulsions. This apparent pH value was the same as that of the buffer solutions indicating that the

pH of the water pool is close to that of the added initial buffer system. The concentration of 1 for absorption spectra was  $4 \times 10^{-6}$  to  $5 \times 10^{-6}$  M, while those for steady-state fluorescence and timeresolved fluorescence measurements were  $1 \times 10^{-6}$ and  $3 \times 10^{-6}$  M, respectively. Stock solutions of 1 in methanol and of AOT (0.5 M) in n-heptane were prepared for all analyses. For the sample solutions of 1 in pure solvents and in the 'dry' AOT reversed micelle (the molar ratio of water to surfactant R = 0), an aliquot of the stock solution of 1 was injected into the solvents or the reversed micelle directly. In order to prepare sample solutions of 1 in AOT-based microemulsions, the following procedures were performed: (1) an aliquot of the stock solution of 1 was added to volumetric flasks; (2) the methanol was evaporated completely under nitrogen bubble; (3) required amounts of the stock solutions of AOT were added to maintain the concentration of AOT at 0.1 M; (4) appropriate amounts of buffer solutions and n-heptane were finally added. The above mixtures were sonicated for 20-30 min to achieve transparent solutions, which were then incubated for 4-6 h at room temperature before carrying out any measurements. For the Triton X-100 (0.015 M) and CTAB (0.015 M) reverse micelles, sample solutions were prepared in a solvent mixture of 80% (v/v) n-heptane and 20% (v/v) n-hexanol following the above method for AOT samples. The pH values of all samples except for those for the pH effect study were maintained at 9.5. All measurements were done at room temperature.

#### 3. Results and discussion

#### 3.1. Absorption and fluorescence spectra

Fig. 2 displays the absorption and fluorescence spectra of 1 in AOT-based microemulsions at various R-values, and in Triton X-100-based and CTAB-based microemulsions at R = 20.

One can see from Fig. 2 that on going from water to AOT-based microemulsions, besides the remarkable increases in the molar absorption coefficient and in the relative fluorescence intensity, blue shifts in absorption and fluorescence spectra

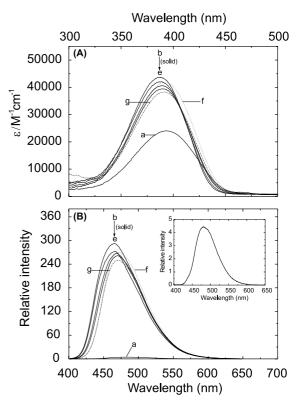


Fig. 2. Absorption (A) and fluorescence (B) spectra of 1 in water (a), in the AOT-based microemulsion at R=0 (b); 5 (c); 10 (d); 20 (e), and in the CTAB-based (f, dot) and Triton X-100-based (g, dash dot dot) microemulsions. The insert shows the fluorescence spectrum of 1 in water.

of 1 occur. Similar phenomena were observed for 1 in the SDS micelle [15]. According to the previous studies [9,13,25,26], water can act as a hydrogen bond donor to the electron lone pair of the nitrogen atom of the amino or substituted amino groups in 3H-indoles, causing reduced conjugation of the phenyl ring with the indolic moiety leading to a blue shift in the absorption band. Therefore, when the anilino moiety of 3H-indoles penetrates the interfaces of micelles or microemulsions where the nitrogen atom is protected from water interactions, a red shift could be observed in the absorption band. On the contrary, when water acts as hydrogen bond donor to the electron lone pair of the indolic nitrogen, the conjugation of the phenyl ring with the indolic moiety is increased and thus a red shift should occur in the absorption band. Therefore, when 3H-indoles transfer from

water to micelles or microemulsions where the indolic nitrogen also approaches the interface and is protected to some extent from water interactions, a blue shift effect in the absorption band exists. The total effect accompanying the transferring of 3*H*-indoles from water to micelles or microemulsions might be red shift or blue shift, depending on which effect in the above two is predominated. Indeed, the effect of blue shift has been found only for 1 interacting with the SDS micelle [15], while the effect of red shift has been observed for some other 3*H*-indoles interacting with the SDS or CTAB micelles [9,10].

Here, it should be helpful to mention the interactions between β-CD and the following 3Hindole probe molecules with similar structures (see Fig. 1): 2-(p-aminophenyl)-3, 3-dimethyl-5-cyano-3*H*-indole 2-(p-dimethylaminophenyl)-3, **(2)**, 3-dimethyl-5-cyano-3*H*-indole (3), 2-(*p*-aminophenyl)-3, 3-dimethyl-5-carboethoxy-3*H*-indole (4), and 2-(p-dimethylaminophenyl)-3, 3-dimethyl-5carboethoxy-3*H*-indole (5). When 2–4 transfer from water to 0.4 mM aqueous solution of  $\beta$ -CD, in which only the 1:1 inclusion complex is formed, the situations of the shifts in the absorption band are much different [19,20]. Both 2 and 4 have amino group, however, red shift for the former and no shift for the latter were observed [19,20]. Similarly, both 3 and 5 are with dimethylamino group, however, no shift for the former and blue shift for the latter were found [19,20]. The fact that 4 and 5 are more deeply entrapped into the  $\beta$ -CD cavity than 2 and 3, respectively, can account for the different behaviors between 2 and 4, and between 3 and 5 [19,20]. On the other hand, the different behaviors between 2 and 3, and between 4 and 5, should be ascribed to the fact that the electron lone pair in the amino group is more available than that in the dimethylamino group [20,21]. The above results strongly suggest that a balance between the aforesaid two effects, which are responsible for the red shift and the blue shift, respectively, does exist.

Now, going back to the situation of 1 interacting with the AOT-based microemulsion, we believe that the nitrogen atom in the substituted amino group of 1 is deeply entrapped into the interface such that the indolic nitrogen penetrates

the interface to some extent. Only in this way, the blue shift in the absorption band of 1 in the AOTbased microemulsion is understandable. It is quite clear that this phenomenon results from the strong electrostatic attraction between the cationic nitrogen of 1 and the anion of AOT. For comparison, the absorption and fluorescence spectra of 1 in the Triton X-100-based and CTAB-based microemulsions at R = 20 are also shown in Fig. 2. In the former case, no electrostatic interaction exists, while in the latter case, strong electrostatic repulsion takes place. One can infer that in these two cases, especially in the later case, the indolic nitrogen approaches or penetrates the interface to a smaller extent than in the case of AOT-based microemulsion. This is the reason why blue shifts going from water to the Triton X-100-based and CTAB-based microemulsions (R = 20) are smaller than that to the AOT-based microemulsion (R = 20) as shown in Fig. 2.

#### 3.2. pH effects

Our recent pH effect studies of 1 and some other substituted 3*H*-indoles in water, micelles, or  $\beta$ -CD have shown that the first preferred site of protonation is the indolic nitrogen atom [9,10,25,26]. Thus, the pH effect study provides us a way to judge the extent of the approaching or the penetration of the indolic nitrogen of 1 into the interface of the AOT-based microemulsion. For this purpose, the pH effect study has been carried out. Fig. 3 illustrates the absorption spectra of 1 in AOT-based microemulsions (R = 20) at various pH values.

Fig. 3 shows that a red-shifted, highly intense, and structured absorption band is formed when the pH is reduced from 9.5 to 2.2. An isosbestic point is also observed suggesting the presence of two species only [9,10,15]. These results can be interpreted in terms of the protonation of the indolic nitrogen atom to form the monocation [25,26].

The fact that the hydrogen ion in the water pool of the microemulsion can interact with the indolic nitrogen indicates that it might reside on the boundary between the interface and the water pool of the microemulsion, or it penetrates the interface

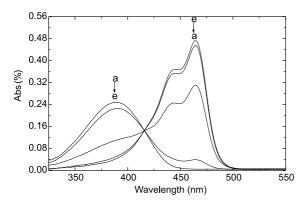


Fig. 3. Absorption spectra of 1 in AOT-based microemulsions (R=20) at various pH values: 9.5 (a); 7.0 (b); 5.2 (c); 4.0 (d); 2.2 (e).

only to a small extent. In previous studies on the 1:2 inclusion complexes of **2** or **3** with  $\beta$ -CD, it was found that **2** or **3** are totally entrapped in a hydrophobic environment except for the junction of the two cyclodextrins, where the indolic nitrogen is located [20]. Obviously, the microenvironments of the indolic nitrogen in the microemulsion and in the 1:2 inclusion complex should be similar to a certain extent.

### 3.3. Polarity of the microenvironment of 1 in the AOT-based microemulsion

To estimate quantitatively the micropolarity of the interface of the microemulsion, which in turn reflects the nature of the locating site of 1, the correlation of spectral shifts with different polarity parameters in the homogenous environment is attempted. No correlation can be made when all solvents of different nature, i.e., non-polar, polar and polar protic, are plotted on the same scale. But separately, non-polar or polar protic solvents can be fitted to the refractive index and dielectric function, respectively. A similar phenomenon has been observed before [9,13,20]. An excellent linear relationship with a correlation coefficient r = 0.99(see Fig. 4) can be obtained when plotting the Stokes shift  $(v_A - v_F)$  against the dielectric constant (D) in alcohols of increasing chain length and methanol-water mixtures for 1. The linear equation is given by

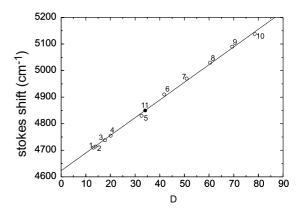


Fig. 4. Stokes shift of **1** as a function of dielectric constant (*D*). 1, *n*-hexanol; 2, *n*-pentanol; 3, *n*-butanol; 4, *n*-propanol; 5, methanol; methanol/water mixture: 6, 80:20; 7, 60:40; 8, 40:60; 9, 20:80; 10, water; 11, the AOT-based microemulsion at R = 20

$$(v_A - v_F) \text{ cm}^{-1} = 6.71D + 4620.$$
 (1)

Substituting the values of Stokes shifts of 1, we obtain the polarity values for AOT-based microemulsions, which are listed in Table 1.

It can be seen from Table 1 that the D value reported by 1 is 3.0 for the dry reversed micelle of AOT. With the addition of water, the D value increases rapidly at  $R \le 10$  and then reaches a plateau. One can note that the D value at R = 20 is 34.3, which is equivalent to that in methanol. In other words, the mean local environment of 1 in the AOT-based microemulsion at R = 20 is similar to that in methanol. This should be in agreement with the qualitative analysis on the whole picture of the location of 1 in the microemulsion and also with our previous results about the state of water in the AOT microemulsion [27].

Table 1 Stokes shifts of 1 and dielectric constants (D) estimated for AOT-based microemulsions at various R values

R	Stokes shift <sup>a</sup> (cm <sup>-1</sup> )	D
0	4640	3.0
5	4750	19.4
10	4820	29.8
15	4830	31.3
20	4850	34.3

<sup>&</sup>lt;sup>a</sup> Stokes shift is defined as  $(\nu_A - \nu_F)$ , where  $\nu_A$  and  $\nu_F$  are taken at the center of mass of the absorption and fluorescence bands, respectively.

#### 3.4. Lifetime measurements

Lifetime measurements have been performed for 1 in AOT-based microemulsions at various Rvalues ranging from 0 to 20. In order to resolve the multiple fluorescence lifetimes, different exponential fittings were tried. However, the value of  $\chi^2$  for double exponential decay fitting was bad, and higher exponential fittings did not improve the statistics any further. The fluorescence decay profiles are best described by a single exponential function. The lifetime obtained is  $1.80 \pm 0.10$  ns, which is not affected significantly by the R-value within experimental error. The values of  $\chi^2$  for AOT-based microemulsions at R = 0, 5, 10, 15, 20, are 1.19, 1.49, 1.29, 1.29 and 1.10, respectively. This result indicates that 1 can only recognize one location in the AOT-based microemulsion and that the local environment sensed by 1 cannot be changed remarkably by the increasing of the R-value.

The fluorescence quenching of 1 in water has been reported recently [15]. The phenomenon is also exhibited in Fig. 2B. The measured lifetime for 1 in water is only 0.28 ns [15]. A plausible mechanism is the formation of a non-emissive twisted intramolecular charge transfer (TICT) state [15], which results from the strong hydrogen bonding interactions at the indolic nitrogen [15]. In methanol, however, the hydrogen bonding interactions at the indolic nitrogen are not strong enough such that the TICT state is not formed [9,13,20,25,26]. Similarly, even in the AOT-based microemulsion at R = 20, the microemulsion with a highest water content in this work, it seems that the hydrogen bonding interaction between water and the indolic nitrogen of 1 is relatively weak. In other words, water cannot approach easily the indolic nitrogen, and thus the water solution layer around the indolic nitrogen is small. This should be the main reason why the lifetime for 1 in AOTbased microemulsions is long and the TICT state does not exist. It seems to indicate that the interaction of the bound water and the trapped water in the interface of the AOT microemulsion with 1 is weak.

On the basis of the measured physicochemical properties of 1, and also considering the hydro-

phobic interactions of the two aliphatic chains and the hexyl group with the interface of the microemulsion, the strong electrostatic interaction between the cationic nitrogen and the AOT anion as well as the location of the indolic nitrogen, one can now give a relatively clear picture of the locations of different groups of 1 in the AOT-based microemulsion. One possible picture is shown in Fig. 5A, in which the two aliphatic chains, the hexyl group together with the part from the amino nitrogen to the indolic nitrogen penetrate the interface, while the part from the indolic nitrogen to the right side resides in the water pool. According to [19,20], and the analyses in Section 3.1, one can see that in the 1:1 inclusion complexes between 2-5 and  $\beta$ -CD, the left part of these smaller 3H-indoles fits the  $\beta$ -CD cavity, while the right part is located in water. Only in this way, the different situations of the shifts in the absorption bands of the 1:1 inclusion complexes can be explained (see Section 3.1). This strongly suggests that the part from the indolic nitrogen to the right end of the 3H-indoles probably resides in a water environment in micelle or microemulsion systems. The second possible

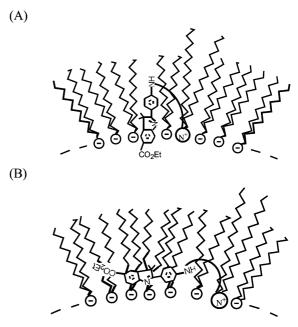


Fig. 5. The approximate picture of the location of 1 in the AOT-based microemulsion.

picture is shown in Fig. 5B, in which the aromatic main chain of 1 may lie flat in the inner interface of the microemulsion. In the above two pictures, both the amino nitrogen and the indolic nitrogen protect from the water interactions to a certain extent. However, the extent should not be the same. In addition, it seems that the interface of the microemulsion will disturb to a greater extent in the second picture than in the first one, owing to the relatively large size of 1. Nevertheless, it is difficult to know which picture is more plausible. Indeed, it is very possible that a real picture is between the above two.

#### 4. Concluding remarks

The spectral characteristics of absorption and steady-state fluorescence indicate that the amino group of 1 is deeply entrapped into the interface of the AOT-based microemulsion such that the indolic nitrogen of 1 can also penetrate the interface to some extent. The micropolarity of the interface of the microemulsion sensed by 1 shows that its mean microenvironment is similar to that in methanol. It was also found from the fluorescence lifetime measurements that the water solution layer around the indolic nitrogen is relatively small. On the other hand, the pH effect study reveals that a proton can get close enough to the indolic nitrogen for protonating it. Combining the above results, one can infer that the indolic nitrogen should reside at the inner interface, but be close to the boundary between the interface and the water pool. Therefore, the picture of the locations of different groups of 1 in the AOT-based microemulsion can be obtained as shown in Fig. 5.

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