

Exciplex-type Behavior and Partition of 3-Substituted Indole Derivatives in Reverse Micelles Made with Benzylhexadecyldimethylammonium Chloride, Water and Benzene[¶]

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ABSTRACT

The fluorescence properties of 3-methylindole (MI), 3-indoleacetic acid (IAA), 3-indoleethyltrimethylammonium bromide (IETA), L-tryptophan (Trp) and tryptamine hydrochloride (TA) were studied in reverse micelles solutions made with the cationic surfactant benzylhexadecyldimethylammonium chloride (BHDC) in benzene as a function of the molar ratio water/surfactant R ($= [\text{H}_2\text{O}]/[\text{BHDC}]$). The fluorescence quenching of the model compound MI by benzene in cyclohexane solutions and by BHDC in benzene solutions were also studied in detail. The fluorescence of MI in benzene is characteristic of a charge-transfer exciplex. The exciplex is quenched by the presence of BHDC, due to the interactions of the surfactant ion pairs with the polar exciplex. In reverse micelle solutions at low R values, all the indoles show exciplex-type fluorescence. As R increases, the fluorescence behavior strongly depends on the nature of the indole derivative. The anionic IAA remains anchored to the cationic interface and its fluorescence is quenched upon water addition due to the increases of interface's micropolarity. For IETA, TA and Trp an initial fluorescence quenching is observed at increasing R , but a fluorescence recovery is observed at $R > 5$, indicating a probe partition between the micellar interface and the water pool. For the neutral MI, the fluorescence changes with R indicate the partition of the probe between the micellar interface and the bulk benzene pseudophase. A simple two-site model is proposed for the calculation of the partition constants K as a function of R . In all cases, the calculation showed that even at the highest R value, about 90% of the indole molecules remain associated at the micellar interface.

INTRODUCTION

The excited state properties of indolic compounds have been widely investigated, mainly due to the biological relevance of these molecules. The interest on the fluorescence of these molecules also arises from the utility of the indole chromophore as an intrinsic probe of the structure and function of proteins and enzymes (1,2).

Reverse micelles can be considered as a very simple model of biological systems. They present three different sites for location of small probe molecules: *i.e.* the internal water pool, the micellar interface formed by the monolayer of surfactant molecules and the external organic phase (3–5). It is therefore of interest to investigate the excited state behavior of indolic compounds in these systems. Fluorescence techniques have been extensively employed to determine the partition of fluorescent probes and/or quenchers in reverse micelles and in several other organized systems (6,7). In particular the fluorescence properties of indole derivatives were previously used to gain information on its distribution among the three regions of the reverse micellar system formed by the negatively charged surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT)[†] dispersed in *n*-heptane (8,9).

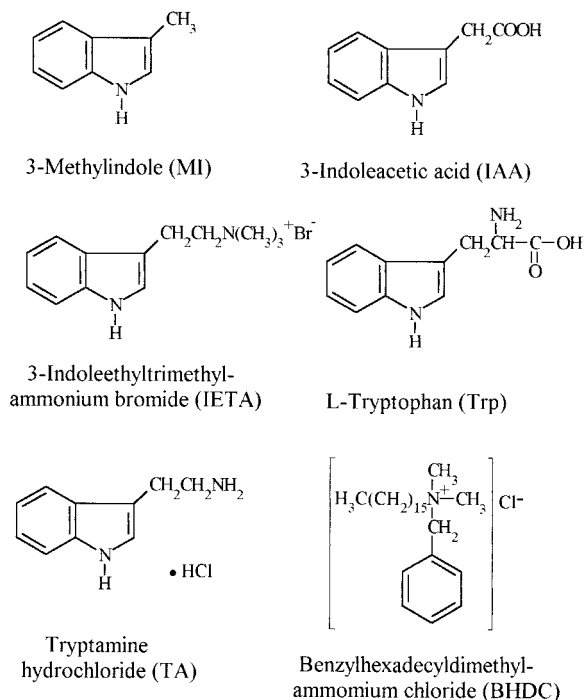
In this work we present a study of the fluorescence properties of indole derivatives in reverse micelles formed by the cationic surfactant benzylhexadecyldimethylammonium chloride (BHDC) dispersed in benzene. Not only does the electrical charge of the interface differ from the previous investigations in AOT, but the organic solvent also presents a particular medium for the excited state behavior of indolic compounds. In fact, it was previously demonstrated that in benzene and other aromatic solvents, the fluorescence of indole and its derivatives is greatly quenched, and the emission changes to an exciplex-type behavior (10). Therefore, it is expected that in the BHDC–benzene system the indoles may present an exciplex-like fluorescence emission. We have previously presented the use of exciplexes to sense the prop-

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[†]Abbreviations: AOT, sodium bis(2-ethylhexyl) sulfosuccinate; BHDC, benzylhexadecyl-dimethylammonium chloride; IAA, 3-indoleacetic acid; IETA, 3-indoleethyltrimethylammonium bromide; MI, 3-methylindole; SV, Stern–Volmer; TA, tryptamine hydrochloride; Trp, L-tryptophan.



Scheme 1. Chemical structure of 3-substituted indole derivatives and the cationic surfactant BHDC.

erties of reverse micellar systems, in particular the interfacial region (11,12). In the previous work, the exciplex was formed with pyrene derivatives and a neutral quencher. In the present case, the probe and the organic solvent form the exciplex. To our knowledge, the possibility of the use of this kind of exciplex for probing microenvironments was not explored. Our results show that the emission of the indole–benzene exciplex is quenched in BHDC media. From the effect of the water content in the reverse micelle system on the emission spectra of the indoles, several conclusions could be obtained about the location and partition effect of the indolic compounds in this medium.

EXPERIMENTAL

Materials. All the chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) in their highest purity degree. Structural formulae are shown in Scheme 1. 3-Indoleacetic acid (IAA) was recrystallized several times from ethanol adding small amounts of a concentrated HCl aqueous solution. The surfactant BHDC was twice recrystallized from ethylacetate and dried under vacuum. All the organic solvents were from Sintorgan (Buenos Aires, ARG) (high-performance liquid chromatography grade) and used as received. Water was triply distilled.

Preparation of the solutions. Reverse micellar solutions with IAA, L-tryptophan (Trp), tryptamine hydrochloride (TA) and 3-indoleethyltrimethylammonium bromide (IETA) were prepared by the addition of a small amount of the indole derivatives dissolved in water to a 0.1 M BHDC benzene solution. Incorporation of MI into the BHDC solutions was performed by addition of few microliters of a stock benzene solution. The water to surfactant molar ratio R ($= [\text{H}_2\text{O}]/[\text{BHDC}]$) was adjusted with neutral water for all the indoles except for IAA, where the water pool was formed with NaOH aqueous solutions at pH 9 in order to assure total deprotonation of the carboxylic group (pK_a ca 5). The mixture was shaken or mildly sonicated for a few minutes until a visually clear solution was obtained. In all cases, the final concentration of the indole derivatives was ca 5×10^{-5} M. Under these conditions, and taking into account

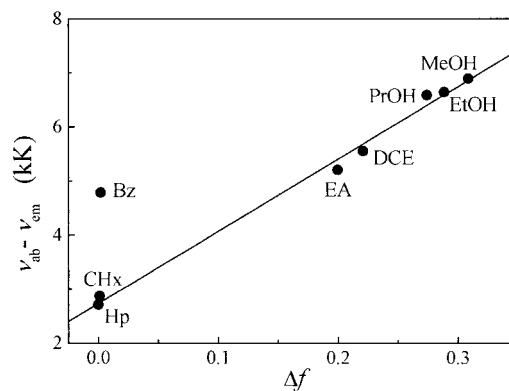


Figure 1. Stokes shifts in kK ($=1000 \text{ cm}^{-1}$) for 3-methylindole in several solvents: *n*-heptane (Hp), cyclohexane (CHx), benzene (Bz), ethylacetate (EA), 1,2-dichloroethane (DCE), 1-propanol (PrOH), ethanol (EtOH) and methanol (MeOH). $\Delta f = ([\epsilon - 1]/[2\epsilon + 1]) - ([n^2 - 1]/[2n^2 + 1])$. Regression data (benzene data is not considered): intercept = 2.74 ± 0.10 kK, slope = 13.36 ± 0.46 kK $^{-1}$, $R = 0.997$.

the aggregation number of BHDC in benzene at the different R values (13), the indole to micelles molar ratio was always <0.3 . In all cases R was varied up to 23.6, because above this value the solutions showed phase separation.

Methods. The absorption spectra were recorded with a Shimadzu UV-2102PC spectrophotometer. Steady-state fluorescence spectra were measured with a Spex Fluoromax spectrofluorometer in air-equilibrated solutions. The excitation wavelength was 290 nm. In all cases the fluorescence spectra of the indole derivatives were corrected by subtracting the small residual fluorescence of the medium (BHDC–benzene–water solutions). Fluorescence quantum yields were determined by using as standard the fluorescence of MI in water ($\Phi_f = 0.34 \pm 0.02$) (14). In all cases the quantum yields were corrected for the difference in refractive index of the solvents.

Lifetime measurements were performed by using the single photon counting technique with an Edinburg OB900 apparatus. Excitation was at 290 nm, whereas emission was observed at the fluorescence maximum of the indoles (ca 340 ± 10 nm). All experiments were performed by duplicate and under controlled temperature of 25°C, in order to avoid problems related with the precipitation of BHDC at lower temperatures.

RESULTS AND DISCUSSION

The MI–benzene exciplex

The fluorescence band of indole derivatives is solvent dependent, being less structured and redshifted in polar media (15,16). However, the fluorescence maximum of the indoles in benzene and in others aromatic solvents cannot be explained in terms of solvation effects. Figure 1 shows the Stokes shifts observed for MI (that can be considered as model compound for the rest of the 3-substituted indole derivatives) plotted following the Lippert–Mataga relationship (Eq. 1) (17).

$$v_{ab} - v_{em} \cong \frac{2(\mu^* - \mu)^2}{a^3 hc} \left(\frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) + \text{const} \quad (1)$$

It can be seen that benzene diverges from the linear correlation. Typically, deviation of the Lippert–Mataga correlation results from specific solvent interactions, such as hydrogen bonding or charge-transfer interactions.

Figure 2 shows the emission changes produced by adding benzene to a cyclohexane solution of MI. At benzene concentrations lower than 0.3 M, the shape of the fluorescence

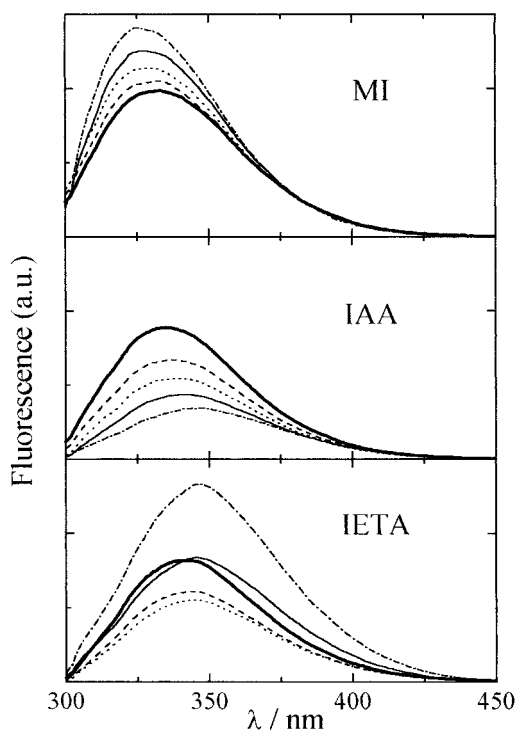


Figure 4. Fluorescence spectra of MI, IAA and IETA in 0.1 M BHDC–benzene reverse micelles at several values of R : (—) 1.4; (---) 2.8; (···) 5.6; (-·-) 12.5; and (- - -) 23.6.

thylammonium cation). In fact, from the measured lifetime of 6.2 ns for MI in benzene a bimolecular rate quenching constant by BHDC $k_q^{\text{BHDC}} \approx 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ is obtained, that is close to the diffusional limit in benzene.

At higher BHDC concentrations, the surfactant molecules start to form larger aggregates with unknown stoichiometry. This process reduces the effective ion pair concentration and the downward curvature in the SV plot is produced. This effect is observed for exciplex quenching by BHDC, whichever fluorescence quantum yields or decay times are used, indicating the decrease of the effective quencher concentration (18). The redshift and broadening of the emission band of the exciplex can be associated with an increment of the local polarity of the micellar aggregates as the BHDC concentration increases (25).

Fluorescence properties of MI in BHDC reverse micelles

The fluorescence spectra and parameters of MI as a function of the water content of the reverse micelle solution are shown in Figs. 4 and 5, respectively. Table 1 collects the fluorescence parameters for the indoles in homogeneous solution and in BHDC media.

The effect of added water can be analyzed in terms of the behavior of the MI–benzene exciplex. When R increases at a given BHDC concentration, the emission yield slightly increases. This may be rationalized in terms of the reduced quenching efficiency of BHDC on forming the reverse micelles. In the presence of water nearly all the surfactant molecules are forming reverse micelles and surfactant ion pairs

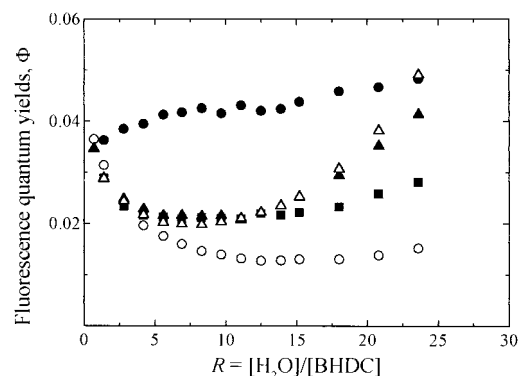


Figure 5. Fluorescence quantum yield Φ , in 0.1 M BHDC–benzene–water solutions as a function of the water content, $R = [\text{H}_2\text{O}]/[\text{BHDC}]$ for indole derivatives: (●) MI; (○) IAA; (△) IETA; (▲) TA; and (■) Trp.

are no longer available for the quenching process (see above).

However, even at $R = 23.6$, the fluorescence quantum yield is lower than that observed in benzene (Table 1). This means that even when the reverse micelles are formed a quenching effect is taking place. In this case the operating

Table 1. Fluorescence quantum yields Φ , half bandwidth $\Delta\nu$, and maximum λ , for the indole derivatives in homogeneous solutions and in 0.1 M BHDC/benzene solutions at several R values at 25°C

Compound/solvent	Φ	λ (nm)	$\Delta\nu$ (kK)
MI			
Benzene	0.062 ± 0.005	324 ± 1	4.74 ± 0.05
Cyclohexane	0.44 ± 0.01	305 ± 1	3.51 ± 0.05
Water	0.34 ± 0.01	370 ± 1	5.02 ± 0.05
0.1 M BHDC			
$R = 1.4$	0.036 ± 0.004	333 ± 1	5.17 ± 0.05
$R = 12.5$	0.042 ± 0.004	328 ± 1	4.96 ± 0.05
$R = 23.6$	0.048 ± 0.004	326 ± 1	4.85 ± 0.05
IAA			
Benzene	0.051 ± 0.005	318 ± 1	4.70 ± 0.05
Methanol	0.21 ± 0.02	353 ± 1	5.60 ± 0.05
Water	0.32 ± 0.03	362 ± 1	6.91 ± 0.05
0.1 M BHDC			
$R = 1.4$	0.032 ± 0.004	335 ± 1	4.91 ± 0.05
$R = 12.5$	0.013 ± 0.004	346 ± 1	5.10 ± 0.05
$R = 23.6$	0.015 ± 0.004	347 ± 1	5.10 ± 0.05
IETA			
Water	0.48 ± 0.03	350 ± 1	5.16 ± 0.05
0.1 M BHDC			
$R = 1.4$	0.029 ± 0.004	342 ± 1	4.80 ± 0.05
$R = 12.5$	0.022 ± 0.004	345 ± 1	4.97 ± 0.05
$R = 23.6$	0.049 ± 0.004	346 ± 1	5.06 ± 0.05
TA			
Water	0.31 ± 0.01	351 ± 1	5.08 ± 0.05
0.1 M BHDC			
$R = 1.4$	0.029 ± 0.004	341 ± 1	4.88 ± 0.05
$R = 12.5$	0.022 ± 0.004	344 ± 1	5.00 ± 0.05
$R = 23.6$	0.041 ± 0.004	346 ± 1	5.01 ± 0.05
TRP			
Water	0.16 ± 0.01	351 ± 1	5.14 ± 0.05
0.1 M BHDC			
$R = 1.4$	0.029 ± 0.004	343 ± 1	4.92 ± 0.05
$R = 12.5$	0.022 ± 0.004	346 ± 1	5.03 ± 0.05
$R = 23.6$	0.028 ± 0.004	347 ± 1	5.07 ± 0.05

mechanism is the collisional deactivation of the exciplex by the reverse micelles as a whole (26,27). This mechanism considers the penetration of the polar exciplex into the micellar interface, where it is deactivated by the increment of polarity of the microphase and/or by a distortion of the molecular arrangement of the exciplex that enhances back electron transfer processes to the ground state. Therefore, the quenching process should be dependent on the concentration of the water pools [WP]. The water pool concentration is calculated by the ratio $[BHDC]/N_{ag}$, where N_{ag} is the micellar aggregation number at different R (13). In fact, the SV plots at $R = 5, 10$ and 20 (N_{ag} 300, 400 and 750, respectively [13]) by using the ratio Φ_o/Φ_R (with Φ_o as the fluorescence quantum yield of MI in benzene, and Φ_R the quantum yield as function of the water pool concentration keeping the same R value) vs [WP] were linear (data not shown). The linearity of these plots is an indication that reverse micelles with well-defined size and aggregation numbers are produced in the whole BHDC concentration range. The SV quenching constants by the water pools K_{SV}^{WP} ($= k_q^{WP}\tau_o^{exc}$) were 3100, 3560 and 5550 M^{-1} , respectively. However, taking into account the fluorescence lifetime of the MI–benzene exciplex (τ_o^{exc} ca 6.2 ns), the bimolecular rate quenching constants k_q^{WP} are almost one order of magnitude larger than those expected for a quenching process exclusively controlled by diffusion (27). Therefore, in spite of the lack of an upward curvature in the SV plots, an extra static quenching contribution is most likely present in the quenching process. The static component can be present if the MI–benzene exciplex is also formed in the micellar interface. The values of the fluorescence maximum (λ) and half width ($\Delta\nu$) are larger in micellar solutions than in pure benzene, indicating the presence of more than one population of excited states. In other words, this means that even at $R = 23.6$, MI is not quantitatively located in the organic phase and a partition of MI between benzene and the micellar interface is occurring.

Fluorescence properties of ionic indoles in BHDC reverse micelles

The fluorescence spectra of the all indoles in reverse micelles at $R = 1.4$ (the lowest R value) are similar, and present the characteristic features of the indole–benzene exciplex-type emission, as Fig. 4 shows for MI, IAA and IETA. However, the fluorescence behavior with the increment of R depends on the indole derivative (Fig. 4). The fluorescence parameters in homogeneous solutions and in 0.1 M BHDC reverse micelles at some R values are shown in Table 1. On the other hand, the absorption spectra of all indole derivatives in BHDC reverse micelles solutions were similar to those observed in homogeneous media.

For all indoles, the fluorescence quantum yields in BHDC reverse micelle solutions are about of one order of magnitude lower than those observed in homogeneous solutions of nonaromatic solvents. However, the dependence on the water content of the emission of the ionic indole derivatives differs from that observed for the neutral MI (Fig. 5). The emission maximum of IAA shifts to the red, together with a band broadening and a reduction of the fluorescence quantum yield as R increases up to 10, where a plateau is reached (Table 1). Since IAA is in its anionic form, it may be con-

sidered as completely associated to the positive micellar interface. The values of λ and $\Delta\nu$ increase with R , however they are much lower than those observed in pure water but larger than in benzene (Table 1).

For IETA, TA and Trp, the fluorescence maximum and bandwidth also increase with R , approximating the values in water, Table 1. In addition, the fluorescence quantum yields for these ionic derivatives are similar to that observed for IAA up to $R \approx 5$ (Fig. 5). However, for IETA, TA and Trp a fluorescence recovery is observed at higher R values, showing IETA a larger effect (Fig. 5). This particular behavior can be understood by considering the detergent-like nature of the ionic indole derivatives, and the balance between electrostatic and hydrophobic interactions between the probes and the micellar interface. At $R < 5$ the water pool is not completely formed and the water molecules can be considered to be “bound” to the micellar interface, solvating the polar head and the chloride counterion of the surfactant (28,29). Under this condition, the size of the water pool is almost negligible and a simple two-pseudophase model can describe the microemulsion: the micellar aggregate and the bulk solvent. Thus, the ionic indole derivatives are able to reside at the interface as cosurfactants, with their polar heads toward the water pool and the indole moiety directed to the benzene phase. Since the aggregation number of the aggregates decreases with R while the curvature radius increases (13), it is expected that more solvent molecules are soaking the micellar interface. In that case, the exciplex formation between the indole group and the benzene is favored at the interface. Thus, the fluorescence quenching and the red bandshift up to $R < 5$ are due to the increment on the local polarity by the incorporation of water at the interface. This behavior resembles very closely that observed for exciplexes formed between pyrene (11) or naphthalene (30) detergent-like probes and amines in reverse micelles solutions. In those cases, the exciplex fluorescence quantum yields and lifetimes decrease, while the emission maxima shift to the red. Since these exciplexes were formed at the interface (11,30) this behavior denotes an increment on the polarity of the micellar interface as R increases.

On the other hand, at $R > 5$, the surfactant’s polar heads and their counterions become fully solvated (28,29). Thus, the proportion of “free” water molecules present in the water pool increases until the water environment resembles that of bulk water (31,32). Thus, the progressive increment on the fluorescence quantum yield for IETA, TA and Trp is explained on the basis of a gradual displacement of the indoles toward the water pool, where the indole fluorescence cannot be quenched by benzene and/or BHDC. The effect is larger for the cationic probes IETA and TA than for the zwitterion Trp, since the probe displacement is favored by a larger electrostatic repulsion between the cationic probes and the cationic micellar interface. Instead, the anionic IAA can be considered anchored to the cationic interface, and therefore behave as an exciplex bounded to the micellar interface (11,30).

In spite of the formation of a water pool with properties resembling those of water, the fluorescence quantum yield of the ionic indole derivatives in reverse micelles is much lower than in water. This result can be explained assuming that the displacement of the cationic indole toward the water

pool is not complete. Thus, most of the indole molecules remain at the micellar interface, probably due to a balance of hydrophobic and electrostatic interactions at the micellar interface that favors the comicellization of the probes.

Partition effects of indoles in BHDC reverse micelles

According to the above results, two partition cases may be distinguished for these indole derivatives in BHDC reverse micelles: (1) the partition of the neutral MI between the micellar interface and the benzene pseudophase; and (2) the partition of the ionic derivatives IETA, TA or Trp between the micellar interface and the water pool. On the other hand, the anionic IAA remains anchored to the cationic micellar interface, and therefore is not partitioned either with the water pool and/or with the benzene pseudophases.

Therefore, in a first approximation it is possible to assume a simple two-site model for the partition of the indoles in BHDC reverse micelles. This is supported by the progressive changes with R of the fluorescence parameters toward the values observed in homogeneous solutions (Table 1).

Thus, within this framework the partition of the indoles in BHDC reverse micelles can be described by the partition constant defined in (Eq. 2) (6,7).

$$K = \frac{[\text{In}]_b}{[\text{In}]_f} \quad (2)$$

with $[\text{In}]_b$ the indole concentration at the micellar interface and $[\text{In}]_f$ the probe concentration in the water pool or benzene pseudophases, depending on the nature of the indole. Equation 2 can be rewritten in terms of the indole fraction bound to the BHDC micellar interface f_b , of that dissolved in the pseudophases f_f and the volume of the interface V_b and of the pseudophases, V_f .

$$K = \frac{f_b V_f}{f_f V_b} \quad (3)$$

Since the absorption spectra of the indoles were independent of the media composition, the total fluorescence quantum yield, Φ_T , may be expressed by (Eq. 4).

$$\Phi_T = f_b \Phi_b + f_f \Phi_f = f_b \Phi_b + (1 - f_b) \Phi_f \quad (4)$$

where Φ_b refers to the fluorescence quantum yield of the indole bound to the interface and Φ_f corresponds to the quantum yield in the pseudophase, which in the present case can be taken as the fluorescence quantum yield of the ionic indoles in water or in benzene for MI. Equation 4 yields f_b if a reasonable assumption is made, which is that the Φ_b value corresponds to that observed for an indole derivative totally located at the micellar interface. This is the case of IAA, which is anchored at the BHDC micellar interface at all R values. Then the fluorescence behavior of the fraction of the indoles incorporated at the interface can be considered very close to that showed by IAA. This assumption is supported by the very close fluorescence quantum yields for all the indoles in BHDC solutions at $R = 1.4$ (Table 1), and the similar fluorescence quantum yield behavior of the all the ionic derivatives at $R < 5$ (Fig. 5).

Since the volumes of the aqueous and organic pseudophases depend on the amount of water dissolved in the microemulsion, (Eq. 3) must be rearranged as follows for the

Table 2. Partition constants ($K = [\text{In}]_b/[\text{In}]_f$) calculated for 3-substituted indoles derivatives in 0.1 M BHDC reverse micelles at 25°C as a function of R

R	MI*	IETA†	TA†	Trp†
4.2	24 ± 2	37 ± 7	16 ± 3	12 ± 3
9.7	16 ± 1	28 ± 3	15 ± 2	8 ± 2
15.2	12 ± 1	23 ± 2	15 ± 2	9 ± 2
20.8	10 ± 2	15 ± 2	11 ± 1	9 ± 1
23.6	9 ± 1	12 ± 2	10 ± 1	10 ± 2

* $K_{\text{interface/benzene}}$.

† $K_{\text{interface/water pool}}$.

case of the neutral MI (Eq. 5), while for IETA, TA and Trp (Eq. 6) must be used.

$$K = \left(\frac{f_b}{1 - f_b} \right) \frac{V_{Bz}}{V_b} = \left(\frac{f_b}{1 - f_b} \right) \frac{(V_T - V_b - V_w)}{V_b} \\ = \left(\frac{f_b}{1 - f_b} \right) \left(\frac{V_T}{[\text{BHDC}]V_b^\circ} - \frac{V_w^\circ}{V_b^\circ} R - 1 \right) \quad (5)$$

$$K = \left(\frac{f_b}{1 - f_b} \right) \frac{V_w}{V_b} = \left(\frac{f_b}{1 - f_b} \right) \frac{[\text{H}_2\text{O}]V_w^\circ}{[\text{BHDC}]V_b^\circ} \\ = \left(\frac{f_b}{1 - f_b} \right) \frac{V_w^\circ}{V_b^\circ} R \quad (6)$$

where V_{Bz} and V_w are the volume of the benzene and aqueous pseudophases, respectively, and V_T is the total volume of the solution. $V_w^\circ = 18 \text{ cm}^3 \text{ mol}^{-1}$ and $V_b^\circ = 443 \text{ cm}^3 \text{ mol}^{-1}$ are the molar volume of water and BHDC (13), respectively. Table 2 presents the partition constant values K as a function of R for MI, IETA, TA and Trp, as determined with (Eqs. 5 and 6). The error bars were determined from the standard deviation of two sets of experiments. From Table 2 it is observed that K decreases with R for MI, IETA and TA, but it is almost independent of R for Trp.

This variation can be associated with the important structural changes produced at the interface and into the water pool upon water addition. As R increases, the aggregation number N_{ag} also increases together with a reduction of the curvature radius of the micellar aggregate, reducing the void volume at the interface (13). This effect allows the displacement of MI toward the benzene phase. Furthermore, the rigidity of the micellar core (the water pool + the interface) is strongly reduced with R , with the largest changes produced at $R < 10$ (31,32). This effect favors the displacement of the ionic indoles toward the water pool, reducing their concentration at the interface. It is interesting to note that when the cationic compounds IETA and TA are compared, a larger change on K with R is observed for the trimethylammonium salt. Probably, the methyl groups at the ammonium polar head of IETA are favoring hydrophobic interactions with the interface at low R values. On the other hand, for Trp the partition constant is almost independent of R , probably due to its zwitterionic nature that allows a better electrostatic interaction with the double electrical layer of the interface, which is formed by the BHD^+ cation and the Cl^- anion of the surfactant.

In all cases, at high R values the partition constants tend

to a similar value ($ca\ 10 \pm 2$), indicating that about 90% of the indole molecules are located in the interface. This result can be related with the detergent-like nature of the indole derivatives which favors the associations of the probes with the surfactant layer in the micellar interface. However, it should be noted that the K values for MI are upper limit values, due to the quenching effect of the reverse micelles upon the exciplex-like fluorescence of MI in benzene (see above). This should be not the case for the ionic indoles, since they are into the micellar core (interface and/or water pool).

In summary, our results point that the fluorescence of the 3-substituted indole derivatives in BHDC–benzene reverse micelles correspond mainly to indole–benzene exciplex-type fluorescence, occurring at the micellar interface and/or in the bulk organic pseudophase. The exciplex is formed between the indole moiety and the benzene solvent. At $R < 5$, truly reverse micelles are not formed, and therefore is expected that all the probes are strongly associated with the micellar aggregates. On increasing R up to the formation of truly reverse micelles ($R > 5$), the neutral MI is partitioned between the interface and the organic pseudophase, where the exciplex is dynamically quenched by the reverse micelles as a whole. On the other hand, the anionic IAA remains anchored to the cationic interface with its indole moiety oriented toward the organic pseudo phase. In this case as R increases the exciplex is quenched by the increment of the interface's micropolarity. For the cationic IETA and TA, a partition of the probes from the micellar interface toward the water pool is observed as R increases. For Trp, the partition is almost independent on R , mainly due to the zwitterionic nature of the aminoacid that allows a better association with the micellar interface, keeping a balance between electrostatic and hydrophobic interactions.

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REFERENCES

- Eftink, M. R. and C. A. Ghiron (1984) Indole fluorescence quenching studies on proteins and model systems: use of the inefficient quencher succinimide. *Biochemistry* **23**, 3891–3899.
- Beechem, J. M. and L. Brand (1985) Time-resolved fluorescence of proteins. *Annu. Rev. Biochem.* **54**, 43–71.
- Fendler, H. J. (1982) *Membrane Mimetic Chemistry*. Wiley–Interscience: New York.
- Luisi, P. L. and B. E. Straub (Editors) (1984) *Reverse Micelles*. Plenum: New York.
- Encinas, M. V. and E. A. Lissi (1986) Solubilization of neutral molecules in AOT inverse micelles in *n*-heptane. *Chem. Phys. Lett.* **132**, 545–548.
- Lissi, E. A. and E. A. Abuin (1995) *Solubilization in Surfactant Aggregates*, Chap. 9 (Edited by S. D. Christian and J. F. Scamehorn), pp. 297–332. Marcel Dekker, New York.
- Silber, J. J., A. Biasutti, E. Abuin and E. Lissi (1999) Interactions of small molecules with reverse micelles. *Adv. Colloid. Interface Sci.* **82**, 189–252.
- Lissi, E. A., M. V. Encinas, S. G. Bertolotti, J. J. Cosa and C. M. Previtali (1990) Fluorescence quenching of indolic compounds in reverse micelles of AOT. *Photochem. Photobiol.* **51**, 53–58.
- Lissi, E. A., M. V. Encinas, S. G. Bertolotti, J. J. Cosa and C. M. Previtali (1990) Photophysical studies of indole alkanolic acids and tryptamine in reverse micelles of sodium dioctyl sulfosuccinate. *Photochem. Photobiol.* **52**, 981–986.
- Rivarola, C. A., S. G. Bertolotti, C. D. Borsarelli, J. J. Cosa, C. M. Previtali and M. G. Neumann (1996) Hydrogen bonding and charge transfer interactions in exciplexes formed by excited indole and monosubstituted benzenes in cyclohexane. *Chem. Phys. Lett.* **262**, 131–136.
- Borsarelli, C. D., J. J. Cosa and C. M. Previtali (1992) Exciplex formation between pyrene derivatives and *N,N'*-dimethylaniline in aerosol OT reverse micelles. *Langmuir* **8**, 1070–1075.
- Borsarelli, C. D., J. J. Cosa and C. M. Previtali (1993) Interface effect on the properties of exciplexes formed between pyrene derivatives and *N,N'*-dimethylaniline in reversed micelles. *Langmuir* **9**, 2895–2901.
- Jada, A., J. Lang, R. Zana, R. Makhoulfi, E. Hirsch and S. Candau (1990) Ternary water-in-oil microemulsions made of cationic surfactants, water, and aromatic solvents. 2. Droplets sizes and interactions and exchange of material between droplets. *J. Phys. Chem.* **94**, 388–395.
- Ricci, R. W. and J. M. Nesta (1976) Inter- and intramolecular quenching of indole fluorescence by carbonyl compounds. *J. Phys. Chem.* **80**, 974–979.
- De Lauder, W. B. and P. H. Wahl (1971) Effect of solvent upon the fluorescence decay of indole. *Biochem. Biophys. Acta* **243**, 153–163.
- Tatischeff, I., R. Klein, T. Zemb and M. Duquesne (1978) Solvent interaction with the indole chromophore. *Chem. Phys. Lett.* **54**, 394–398.
- von Lippert, E. (1957) Spectroskopische bestimmung des dipolmomentes aromatischer verbindungen im ersten angeregten singulettzustand. *Z. Electrochem.* **61**, 962–975.
- Borsarelli, C. D., J. J. Cosa and C. M. Previtali (1995) On the formation of BHDC reversed micelles in benzene probed by the 1-methylpyrene-*N,N'*-dimethylaniline exciplex. *J. Colloid. Interface Sci.* **179**, 34–36.
- Kitamura, N., S. Imabayashi and S. Tasuke (1983) Exciplex quenching by quaternary ammonium salts in chloroform. *Chem. Lett.* 455–458.
- Hartley, R. and L. R. Faulkner (1985) Quenching and possible coulombic complexation of the pyrene-*N,N'*-dimethylaniline exciplex by tetraalkylammonium salts. *J. Am. Chem. Soc.* **104**, 7254–7255.
- Chesta, C. A., J. J. Cosa and C. M. Previtali (1987) The effect of added salts on electron transfer reactions involving exciplexes: the *N,N'*-dimethylaniline-photosensitized dechlorination of 4-chlorobiphenyl. *J. Photochem.* **39**, 251–261.
- Grosso, V. N., C. M. Previtali and C. A. Chesta (1998) Salt induced charge separation in photoinduced electron transfer reactions. The effect of ion's size. *Photochem. Photobiol.* **68**, 481–486.
- Sigvartsen, T., B. Gestblom, E. Noreland and J. Songstad (1989) Conductometric and dielectric behaviour of solutions of tetrabutylammonium perchlorate in solvents of low and medium permittivity. *Acta Chem. Scand.* **43**, 103–115.
- Goodson, B. E. and G. B. Schuster (1984) Exciplex ionic dissociation in nonpolar solvent induced by multipolar salt complexes. *J. Am. Chem. Soc.* **104**, 7254–7255.
- Correa, N. M., M. A. Biasutti and J. J. Silber (1996) Micropolarity of reversed micelles: comparison between anionic, cationic, and nonionic reverse micelles. *J. Colloid. Interface Sci.* **184**, 570–578.
- Sato, C. and K. Kikuchi (1992) Quenching mechanism of exciplex fluorescence by inverted micelles. *J. Phys. Chem.* **96**, 5601–5604.
- Kikuchi, K. and J. K. Thomas (1988) Reactions of exciplexes with water pools in hydrophobic media. *Chem. Phys. Lett.* **148**, 245–248.
- McNeil, R. and J. K. Thomas (1981) Benzylhexadecyldimethylammonium chloride in microemulsions and micelles. *J. Colloid. Interface Sci.* **83**, 57–65.
- Costa, S. M. B., J. M. F. M. Lopes and M. J. T. Martins (1986) Steady-state fluorescence quenching kinetics of water-soluble

- zinc porphyrins in reversed micelles. *J. Chem. Soc., Faraday Trans. 2* **82**, 2371–2378.
30. Geladé, E., N. Boens and F. C. De Schryver (1982) Exciplex formation in dodecylammonium propionate reversed micellar system. *J. Am. Chem. Soc.* **104**, 6288–6292.
31. Zinsli, P. E. (1979) Inhomogeneous interior of aerosol-OT microemulsions probed by fluorescence and polarization decay. *J. Phys. Chem.* **83**, 3223–3231.
32. Borsarelli, C. D. and S. E. Braslavsky (1997) Nature of the water structure inside the pools of reverse micelles sensed by laser-induced optoacoustic spectroscopy. *J. Phys. Chem. B* **101**, 6036–6042.