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Photophysics of the phenoxazine dyes resazurin and resorufin in direct and reverse micelles

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ABSTRACT

The photophysics of the phenoxazin-3-one dyes resazurin and resorufin was studied in a micellar solution of cetyltrimethylammonium chloride and in reverse micelles of 1,4-bis(2-ethylhexyl)sulfosuccinate and benzylhexadecyldimethylammonium chloride. Absorption and fluorescence emission spectra, as well as fluorescence lifetimes and T–T transient absorption spectra were determined as a function of surfactant concentration. In the presence of direct micelles of cetyltrimethylammonium chloride, both dyes displayed red shifts in the absorption and fluorescence spectra together with a simultaneous fluorescence lifetime increase. The electrostatic attraction between the anionic dyes and the positive micellar interface favors the location of the dyes closer to the head groups of the surfactant molecules. In reverse micellar systems the spectral properties depended upon the charge of the surfactant and water content. In the case of 1,4-bis(2-ethylhexyl)sulfosuccinate, at low water content both dyes were incorporated into the interface; as the water content increased their spectral properties tended to those in pure water. In contrast, in the case of cationic surfactant, the dyes were located in the interfacial pseudophase as a result of electrostatic interactions.

1. Introduction

The study of the behavior of synthetic dyes in organized media is of interest because of the resemblance of these systems with many biological and chemical structures in nature. In an organized media e.g., micelles, reverse micelles, calixarenes, cyclodextrins, the reactants are confined within a small region, a few nanometer in size. The local properties like polarity, viscosity and pH are vastly different from those in homogeneous medium. Consequently, the physical and chemical properties of dyes may undergo dramatic changes in these systems. In particular, the study of dye–surfactant interactions is of great interest. Although a lot of research work has been done in this area it is still important for the theory and technology of dyeing.

The dye–surfactant interactions have also been the subject of many studies in view of the fact that they mimic many biological processes taking place between large organic molecules and biomembrane and can act as a model redox system. In fact, the study of organic dyes in a micellar medium is important for understanding the thermal and light induced reactions in biomembranes [1]. Such reactions go through the involvement of excited and free radical species whose behavior in a micellar medium can be significantly different from that in a homogeneous aqueous medium. Furthermore, the understanding of the interactions between ionic dyes and charged surfaces are of interest in numerous applications ranging from the design of electronic devices to the characterization of drug-delivery systems. In many applications of electronic energy transfer, which may include the photosensitized reactions and the micelle enhanced emission detection in analytical techniques, donor, acceptor, and micelle concentrations may be high enough to allow considerable energy transport between neighboring micelles [1].

Resazurin (RZ) is a heterocyclic *N*-oxide dye that is often used to study biological materials [2,3]. Most of these applications are based on the oxygen atom transfer reaction with the dye as donor. In this way RZ is reduced to the strongly fluorescent product resorufin (RF) (Scheme 1) which can be used as a target fluorescent probe, by a thermal reaction using organic compounds or enzymes as catalysts [4–8] or by a photochemical reaction [9,10]. Resorufin has also been used as a probe molecule to study the reorientation of solvent molecules, and has shown an interesting chemistry and photochemistry in protic solvents which strongly depends on temperature, viscosity and structure of the solvent [11,12].

In previous work we reported on the photophysics and photochemistry of resazurin and resorufin in aqueous solutions [13–15].

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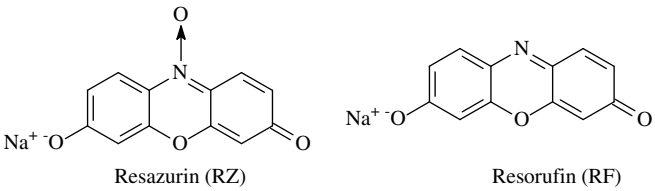

Scheme 1.

The RZ photoreaction in the presence of amines leads to deoxygenation of the N-oxide group giving RF. This photoreaction is highly dependent on the amine structure and is efficient only in the case of tertiary aliphatic amines. The absorption and fluorescence properties of these dyes are dependent on pH. Here we present results concerning the influence of cationic direct and reverse micelles on the spectroscopic properties of the singlet and triplet excited states of these dyes.

2. Experimental

2.1. Materials

Resazurin (RZ) and resorufin (RF) were from Sigma and were used as supplied. Sodium 1,4-bis(2-ethylhexyl)sulfosuccinate (AOT) from Sigma was dried under vacuum over P_2O_5. The surfactant benzylhexadecyldimethylammonium chloride (BHDC) from Fluka was twice recrystallized from ethyl acetate and dried under vacuum. Cetyltrimethylammonium chloride (CTAC) (Kodak) was purified by recrystallization. Benzene and heptane (Sintorgan HPLC grade) were used as received. Water was purified through a Millipore Milli-Q system. The pH was adjusted to pH = 10 by the incorporation of a concentrated NaOH solution. Reverse micelles solutions were prepared with AOT 0.2 M in *n*-heptane and BHDC 0.1 M in benzene. The water content, $w = [H_2O]/[\text{surfactant}]$ was varied between 0 and 30 adding water at pH 10.

2.2. Measurements

Absorption spectra were determined on a Hewlett Packard 6453E diode array spectrophotometer. Fluorescence spectra were measured out at room temperature in air equilibrated solutions with a Spex Fluoromax spectrofluorometer. Fluorescence quantum yields were determined relative to cresyl violet in methanol [16]. Fluorescence lifetime measurements were performed with an Edinburgh Instruments OB 900 time correlated single-photon counting fluorometer. Transient absorption measurements were made using a laser flash photolysis equipment previously described

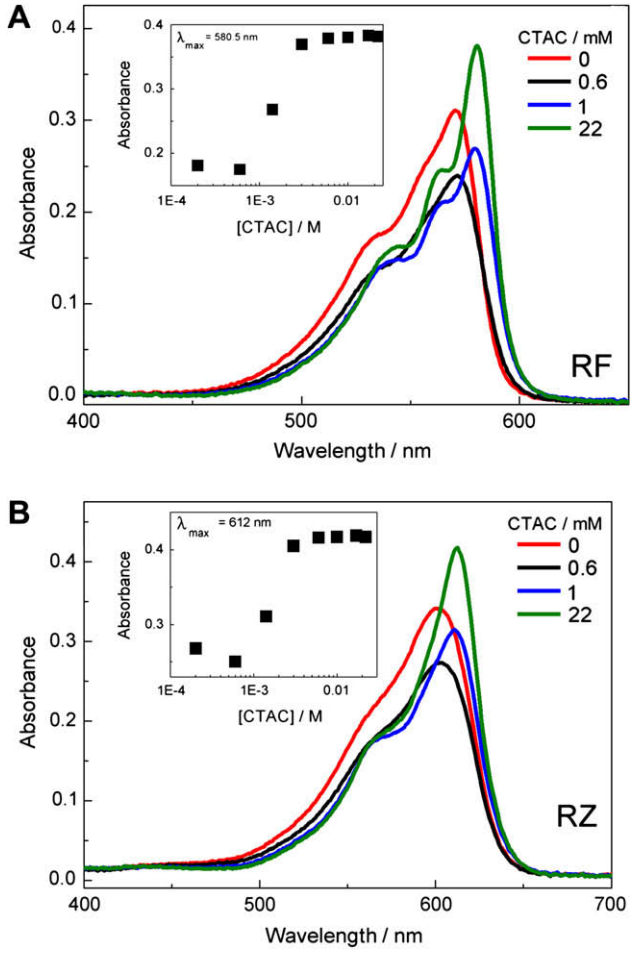

Fig. 1. Absorption spectra of the dyes (5×10^{-6} M) in water at pH 10 as a function of CTAC concentration. Inset: absorbance at the maximum as a function of CTAC concentration. (A) RZ; (B) RF.

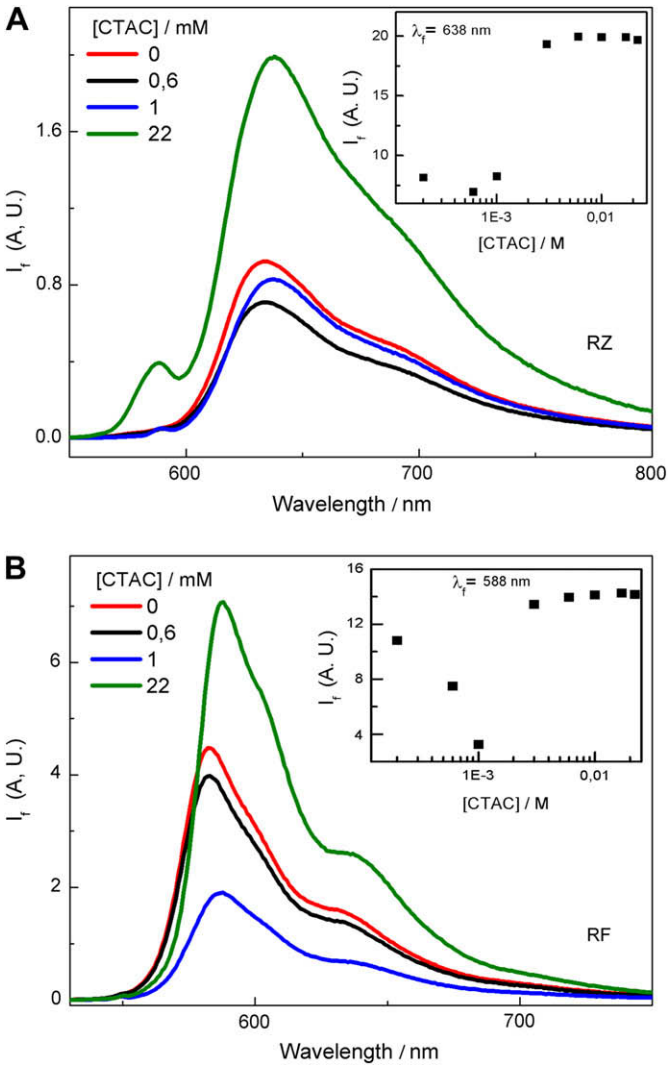

Fig. 2. Corrected Fluorescence spectra of the dyes (5×10^{-6} M) in water at pH 10 as a function of CTAC concentration. Inset: fluorescence intensity at the maximum as a function of CTAC concentration. (A) RZ; (B) RF.

[17] in samples subjected to a continuous bubbling with high purity argon. All measurements were carried out at 25 °C with the exception of those in BHDC/benzene reverse micelles for which 32 °C was employed in order to favor the stability of the microemulsion.

Quantum yields of triplet species (Φ_T) were determined using zinc tetraphenylporphyrin (ZnTPP) triplet state as a reference actinometer. Values of 7.3×10^4 M^{-1} cm^{-1} and 0.83 were used for the absorption coefficient and quantum yield of ZnTPP triplet state, respectively [18]. The molar absorption coefficients of triplet dyes were determined by the ground-state depletion technique [19]. For both dyes, the negative absorption of the difference transient spectra matches the ground-state band. This is consistent with the lack of photoproduct formation under our conditions of laser experiments, and permits the application of the ground-state depletion method to determine the molar absorption coefficients of the triplet state.

3. Results and discussion

3.1. CTAC micelles

The UV–visible absorption spectrum of RZ in basic aqueous solutions consists of an intense absorption band at 602 nm ($\varepsilon = 42{,}000$ M^{-1} cm^{-1}) and a weak band at 380 nm. The bands are assigned to the $\pi \rightarrow \pi^*$ transition of the phenoxazin-3-one, and to the weak $n \rightarrow \pi^*$ transition of the *N*-oxide, respectively [13]. The visible spectrum of RF is characterized by an intense band centered at 572 nm ($\varepsilon = 51{,}000$ M^{-1} cm^{-1}) with a shoulder at 535 nm. The absorption of the dyes increases linearly with the concentration in the range 0.5–20 μM. This indicates that the aggregation of the dye is not appreciable in this concentration range. No changes were observed in the absorption spectra as a function of increasing pH in the range 7.5–11.5 (p*K* RF = 5.8; p*K* RZ = 5.5) [13].

Absorption spectra in the presence of CTAC are presented for both dyes in Fig. 1. The detergent produces a red shift in the spectral maximum, from 603 to 612 nm for RZ and from 570 to 581 nm for RF. The absorbance at the maximum initially decreases with increasing concentration of CTAC and a sharp increase can be observed in both cases when the concentration of CTAC is ca. 1 mM and it remains constant for [CTAC] > 3 mM (inset Fig. 1). This change is coincident with the critical micellar concentration of CTAC (cmc = 1.5 mM [20]).

The effect of CTAC on the fluorescence spectra is shown in Fig. 2. A red shift is again observed in the presence of the detergent, but in this case to a minor extent than in the absorption. The fluorescence intensity, measured at the red shifted maximum, initially decreases with increasing concentration of CTAC and thereafter a sharp increase is observed at [CTAC] ca. 1 mM and it remained constant for [CTAC] > 3 mM. The absorption and emission spectra of both dyes at [CTAC] beyond the cmc are very similar to those in ethanol (not shown). The effect of CTAC on the fluorescence quantum yield and lifetime was also investigated. Both quantities show a noticeable increment in the presence of the cationic micelles. The photophysical properties of the dyes are collected in Table 1.

Fluorescence lifetimes as a function of CTAC concentration are shown in Fig. 3. At all concentrations the decay profiles were characterized by a single exponential. In both cases the lifetimes begin to increase when [CTAC] becomes of the order of the cmc until reaching a constant value. This value is similar to that in methanolic solutions, 1.08 ns for RZ and 5.19 ns for RF [17].

Table 1
Photophysical properties of rezasurin and resorufin in different media

Dye	Medium	Absorption	Fluorescence	Triplet state
		λ_{max} (nm)	λ_{max} (nm)	λ_{max} (nm)
		ε (M^{-1} cm^{-1})	τ_F (ns)	ε (M^{-1} cm^{-1})
			Φ_F	Φ_T
RZ	H_2O	602	634	400
	pH 10	42,000 ± 3000	0.65	6500 ± 600
			0.11 ± 0.01	0.08 ± 0.01
RZ	CTAC	612	638	390
		50,000 ± 2800	1.08	6600 ± 700
			0.25 ± 0.01	0.12 ± 0.01
RZ	AOT	604	635	400
	$w = 20$	43,000 ± 2800	0.74	6500 ± 600
			0.10 ± 0.01	0.08 ± 0.01
RZ	BHDC	623	639	400
	$w = 20$	53,000 ± 2700	1.40	6700 ± 700
			0.14 ± 0.01	0.11 ± 0.02
RF	H_2O	572	583	700
	pH 10	51,000 ± 2800	2.90	17,000 ± 2000
			0.41 ± 0.01	0.04 ± 0.005
RF	CTAC	580	588	700
		64,000 ± 2800	5.19	26,000 ± 3000
			0.83 ± 0.01	0.004 ± 0.002
RF	AOT	572	584	700
	$w = 20$	58,500 ± 2800	2.94	19,500 ± 2000
			0.39 ± 0.01	0.04 ± 0.005
RF	BHDC	588	592	700
	$w = 20$	54,000 ± 2800	4.33	11,500 ± 800
			0.77 ± 0.01	0.02 ± 0.004

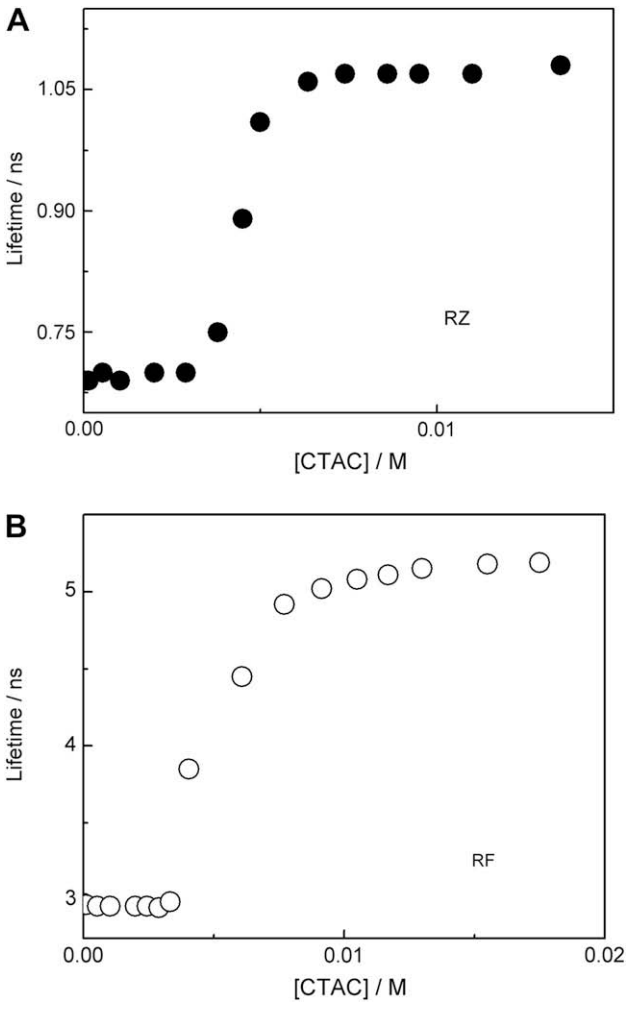

Fig. 3. Fluorescence lifetime of the dyes in water at pH 10 as a function of CTAC concentration. (A) RZ; (B) RF.

All these results can be understood in terms of the incorporation of the negative dye molecules to the positive CTAC micelles, where the dyes sense a medium of polarity similar to ethanol.

The triplet states of the dyes were also investigated in the presence of CTAC. In Fig. 4 the transient absorption spectra of RZ and RF in water and CTAC micelles are shown. They can be assigned to the triplet state of the dyes. It can be seen that the presence of the surfactant scarcely affects the shape and position of the bands. This is in agreement with the very small changes in the T–T absorption spectra on going from water to an alcoholic solvent [17]. However, a diverse effect of CTAC on the absorption can be observed. While the T–T absorption of RZ presents a large increment in the presence of the surfactant that of RF decreases noticeably. Triplet quantum yields were determined as described in the experimental section and are collected in Table 1. For both dyes triplet yields are low being lower for the most fluorescent dye, RF. The decrease in Φ_T for this dye can be correlated with the high increase in fluorescent yield, Φ_F in the presence of CTAC micelles. A lesser effect of CTAC on the Φ_F and Φ_T of RZ is observed and both quantities present a slight increase in the micellar medium.

3.2. AOT reverse micelles

In Fig. 5 the absorption spectra of the both dyes in reverse micelles of AOT/heptane are shown. At low w both dyes present a broad and slightly structured absorption in the ranges 400–600 nm (RF) and 400–550 nm (RZ), respectively. These spectra resemble those in water at pH 4. With increasing the water content an increase of absorption intensity is observed, and at $w \geq 20$ the spectra are similar to those in water at pH 10. Similar behavior was found for the fluorescence emission. These results may be understood in terms of incorporation of the dyes to the interfacial region of the reverse micelles co-micelling with AOT. At low w values all the water molecules are tightly bound to the surfactant head groups at the polar cores of reversed micelles and due to the negative head groups of AOT an apparent low pH is sensed in the interfacial region [21]. Therefore, the spectra under these conditions may be assigned to the neutral form of the dyes. At higher w the dye molecules move towards the center of the water pool due to electrostatic repulsion and present spectral properties similar to those in pure water. However, it can be concluded from a comparison of the spectra that RF is more tightly bound to the interface, since at $w = 12.8$ it still presents the absorption blue shifted, while at the same w RZ is practically all in a medium similar to pure water. It is commonly accepted that at this value of w the water pool in AOT systems is closely similar to an aqueous phase. Similar behavior was reported in literature for other systems. Politi and coworkers [22] studied the deprotonation of the excited states of the dye pyranine in microemulsions of AOT. They observed that at high w the properties of the dye were similar to pure water. They suggest

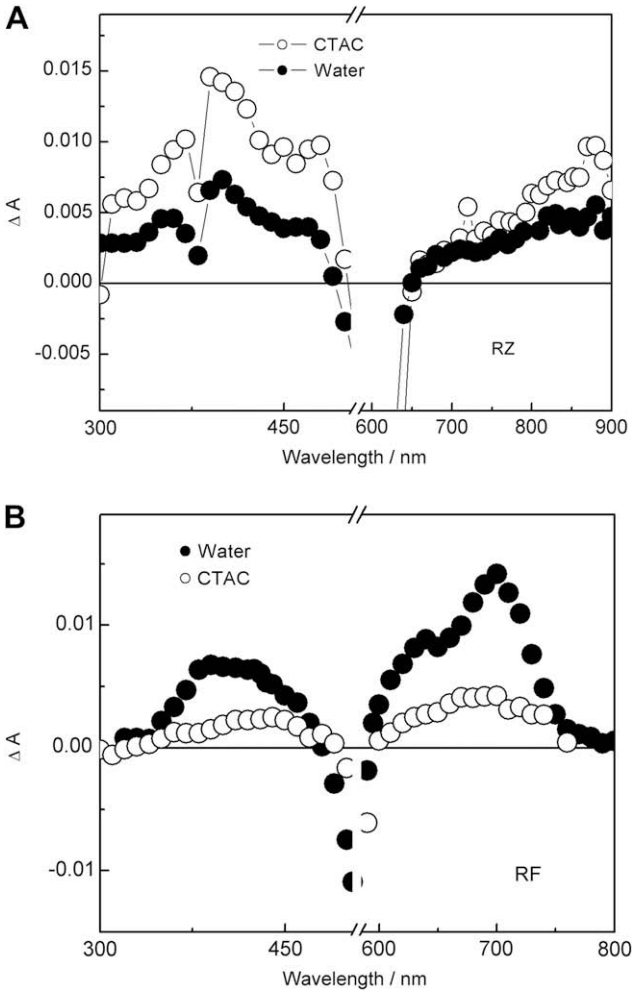

Fig. 4. Transient absorption of RZ and RF 5×10^{-6} M in water at pH 8 and CTAC 0.05 M at 5 μs after flash. (A) RZ; (B) RF.

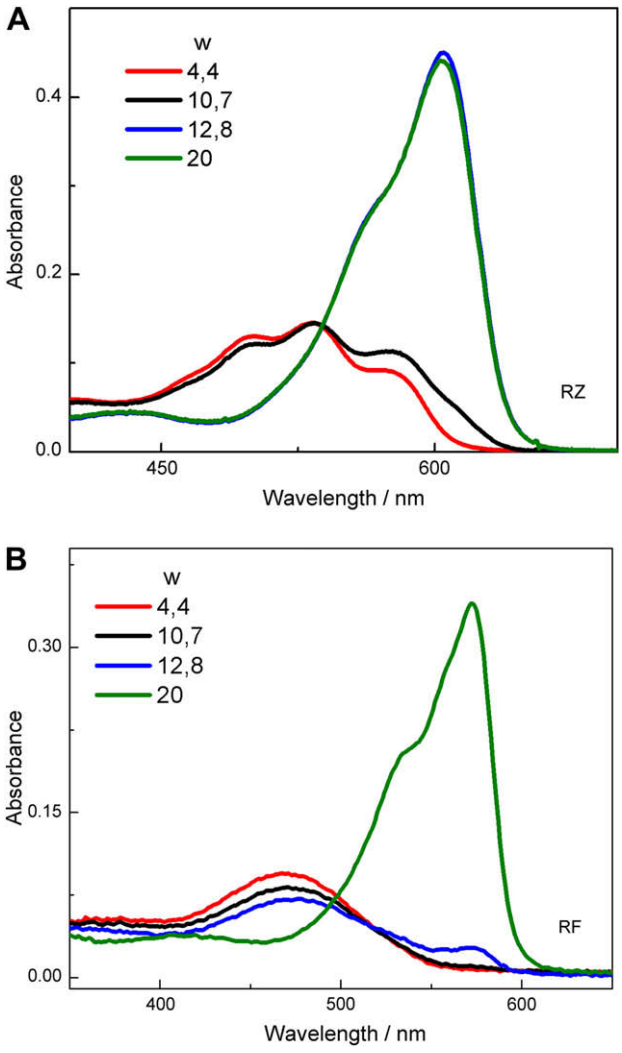

Fig. 5. Absorption spectra of RZ and RF in AOT 0.2 M as a function of the water content of the reverse micellar system. (A) RZ; (B) RF.

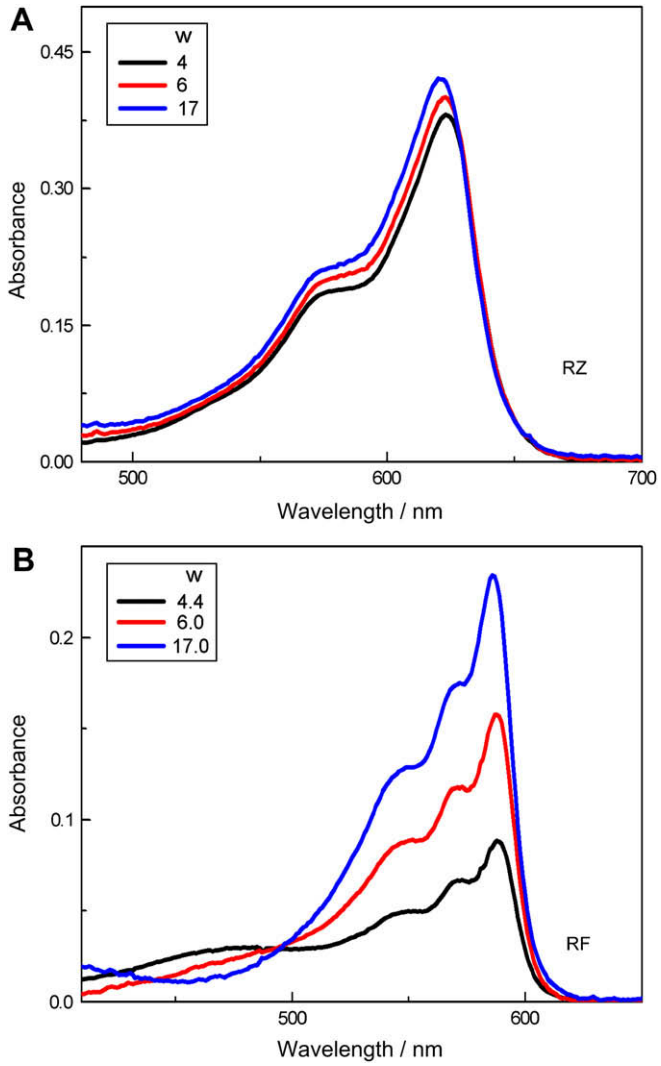

Fig. 6. Absorption spectra of RZ and RF in BHDC/benzene 0.1 M as a function of w at 32 °C. (A) RZ; (B) RF.

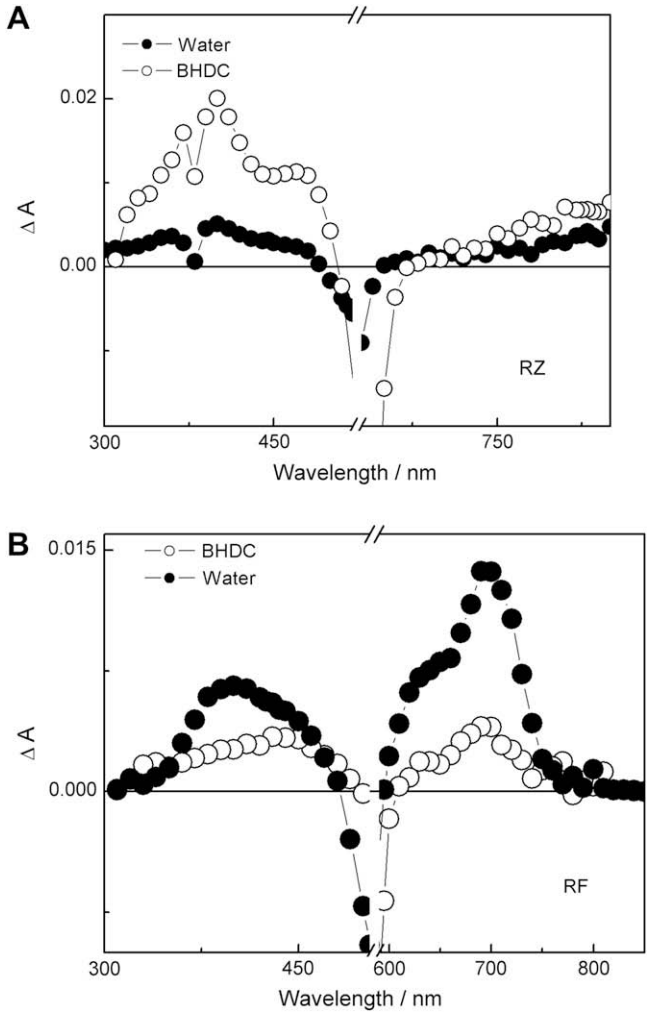

Fig. 7. Transient absorption spectra of RZ after 2 μs and RF in BHDC 0.1 M at 1 μs after flash at $w = 20$. (A) RZ; (B) RF.

that it remains located in the water pool at high water content. Okasaki and Tiroyama [23] studied the localization of organic acids as a function of pH in reverse micelles of AOT. The neutral forms of the acids were located in interface, but at high pH they move to the water pool as an ionized form.

The transient absorption spectra of both dyes in reverse micelles of AOT after laser flash excitation at 532 nm were recorded. They were very similar to those in water at pH 10 and were assigned to the triplet state of the dyes. No changes were observed as a function of the water content for $w > 20$.

3.3. BHDC reverse micelles

The changes in the absorption spectra of both dyes in BHDC/benzene as a function of w are shown in Fig. 6. For RF a weak absorption band located at 470 nm is present at low w together with a more intense band at 588 nm. The first band disappears and the second one increases in intensity with the increase in w. By comparison with the spectrum in benzene (not shown) the absorption at 470 nm can be assigned to the absorption of RF comicellizing in the positive interface and close to the bulk organic phase. The absorption band centered in 588 nm is red shifted with respect to pure water (572 nm) and can be attributed to the dye in the interfacial region close to the polar heads of BHDC, but oriented towards the water pool. This change was probably brought out by changes in the properties of the interfacial pseudophase produced by increasing the size of the water pool, such as a lower curvature and microviscosity. On the other hand, for RZ the absorption spectrum is also red shifted by ca. 20 nm with respect to pure water and it is scarcely affected by the water content of the microemulsion. In this case the negative dye molecules remain tightly bound to the positive interface and the presence of the N-oxide groups orients the molecules towards the more polar region of the interface. The maximum and shape of the fluorescence emission spectra are not influenced by the presence of BHDC when excited at 588 and 622 nm for RF and RZ, respectively.

Fluorescence lifetimes in BHDC as a function of w were determined. At all w values the decay profiles were characterized by a single exponential. Lifetimes were similar to the values observed in isopropanol (1.40 and 4.33 ns for RZ and RF, respectively). This behavior reveals that the dyes sense microenvironments of polarity similar to alcohol. It can be observed in Table 1 that fluorescence lifetimes and fluorescence quantum yields are higher than those in pure water in both cases. These facts, together with the absorption and emission spectra at high w demonstrate that the dyes do not migrate towards the water pool at high w.

The triplet state of RZ and RF were also investigated by laser flash photolysis. In Fig. 7 the transient absorption spectra in water and BHDC micelles are shown. They can be assigned to the triplet

state of the dyes. It can be seen that the spectrum in BHDC is very similar to that in water; nevertheless, the triplet quantum yields are quite different. For RF it is noticeable less than in water while the opposite occurs for RZ. Triplet quantum yields were determined as discussed in the experimental section and they are also collected in Table 1. These results are in line with the increment in the fluorescence quantum yield observed in the reverse micellar media for RF while the effect for RZ is less important. This is similar to what happens in the CTAC direct micelles.

In summary, it can be concluded that the photophysics of both dyes in aqueous solution is highly influenced by the presence of cationic micelles, presenting properties similar to an organic solvent of intermediate polarity. In AOT/heptane reverse micelles at low water content the dyes are incorporated into the negative interfacial region, moving to the water pool as the water content increases. In BHDC/benzene system the dyes remain in the interface at all *w* values, but a distinct behavior can be notice for both dyes. While RF penetrates close to the bulk organic phase at low *w* and afterwards changes to a more polar medium, RZ is, at all *w* values, in the aqueous region of the interface.

Acknowledgments

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