THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

Characterization of Multifunctional Reverse Micelles' Interfaces Using Hemicyanines as Molecular Probes. I. Effect of the Hemicyanines' Structure

Fernando Moyano, Silvina S. Quintana, R. Dario Falcone, Juana J. Silber, and N. Mariano Correa

J. Phys. Chem. B, **2009**, 113 (13), 4284-4292• DOI: 10.1021/jp8105502 • Publication Date (Web): 04 March 2009

Characterization of Multifunctional Reverse Micelles' Interfaces Using Hemicyanines as Molecular Probes. I. Effect of the Hemicyanines' Structure

Fernando Moyano, Silvina S. Quintana, R. Dario Falcone, Juana J. Silber, and N. Mariano Correa*

Departamento de Química, Universidad Nacional de Río Cuarto, Agencia Postal # 3, (X5804ZAB) Río Cuarto, Argentina

Received: December 1, 2008; Revised Manuscript Received: January 27, 2009

In this work, we report the behavior of two different hemicyanines, *trans*-4-[4-(dimethylamino)styryl]-*N*-methylpyridinium iodide (HC) and 4-[4-(dihexadecylamino)styryl]-*N*-methylpyridinium iodide (DIA), in water/sodium 1,4-bis-2-ethylhexylsulfosuccinate (AOT)/benzene reverse micelles media using absorption and emission spectroscopy in addition to the steady-state and time-resolved fluorescence emission techniques. Our results show that the AOT reverse micelles interface has the nontrivial deaggregation property, a result that may have potential application for the preparation of dye lasers, which require a noninteracting monomeric form of the dye. Also, we show that the water interacts with a different region of the AOT moiety depending on the external organic solvent used and, in addition, we also present a nice, simple, and noteworthy method that helps to examine the presence or the absence of organized media. In conclusion, our results show that HC and DIA are powerful dyes to characterize simultaneously different interfaces' properties as they can be used to sense, at the same time, fluidity and specific interactions at the interface. These results are important because those properties are the key for molecular recognition.

Introduction

Reverse micelles and water-in-oil microemulsions have attracted considerable attention due to their ability to host hydrophilic components in organic solvents. These systems are suitable media for processes that involve hydrophobic and hydrophilic reactants providing "microreactors" for a variety of chemical and biological reactions.[1−4]

Several surfactants are able to aggregate in nonaqueous solvents to yield reverse micellar systems. Small solute particles can be located in three different compartments: (a) the external organic solvent, (b) the micellar interface formed by a surfactant monolayer, and (c) the internal polar core. A common anionic surfactant used to form reverse micelles is AOT (sodium 1,4-bis-2-ethylhexylsulfosuccinate). AOT has a well-known V-shaped molecular geometry, giving rise to stable reverse micelles without cosurfactant.[2,5] In addition, it has the remarkable ability to solubilize a large amount of water with values of W ($W = [H_2O]/[AOT]$), as large as 40−60 depending on the surrounding nonpolar medium, the solute, and the temperature. However, the droplet size depends only on W.[2,5]

In general, for a fluorophore in a bulk nonviscous solvent, the dipolar relaxation of the solvent molecules around it in the excited state is much faster than its fluorescence lifetime. Hence, the wavelength of maximum emission usually is independent of the excitation wavelength. However, it does show excitation wavelength dependence if the dipolar relaxation of the solvent molecules is slow in the excited state, such that the relaxation time is comparable to or longer than the fluorescence lifetime. Such a shift in the wavelength of maximum emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of the absorption band, is known as the red edge excitation shift (REES).[7,8] Wavelength-selective fluorescence comprises a set of approaches based on the red edge effect in fluorescence spectroscopy. This approach can be used to directly monitor the microenvironment and dynamics around a fluorophore in a motion-restricted media such as organized media like reverse micelles or vesicles.[9,10]

Among small molecules, dyes based on the hemicyanine (aminostyrylpyridinium) chromophore have been investigated to establish the relation between molecular structure and their solvatochromism.[11−17] Also they have been used in various applications such as fluorescence markers or sensors,[18−20] frequency converters,[21] and as materials for use in nonlinear optics.[22]

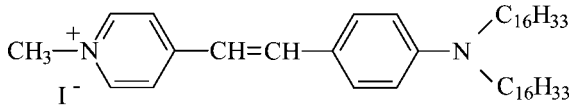
It has been shown that upon enhancing the solvent's polarity the absorption bands shift to higher energy while the emission bands shift to lower energy. The effects were assigned to the intramolecular charge transfer in the processes of the dyes excitation and to the interaction of these charge shifts with the environment. The symmetrical solvatochromism was explained considering that the excitation starts from a ground state where the positive charge lies mainly on the pyridinium (isoquinolinium) ring of the dyes and the emission starts from an excited state, where the positive charge lies mainly on the aniline ring where the positive charge is relocated after excitation.[12−14]

Very recently,[23] we have performed a solvatochromic study on two different hemicyanines (Scheme 1): *trans*-4-[4-(dimethylamino)styryl]-*N*-methylpyridinium iodide (HC) and 4-[4-(dihexadecylamino)styryl]-*N*-methylpyridinium iodide (DIA) in homogeneous media in order to gain more insight about its spectroscopic behavior. The results demonstrate, for the first time, that the cationic hemicyanines undergo a specific interaction with the medium through the electron donor capacity of the solvents as measure by the β solvent parameter. The energy of the absorption band shifts bathocromically with β while the energy of the emission band shifts hypsochromically. It seems that the positive charge located at the pyridinium ring of the

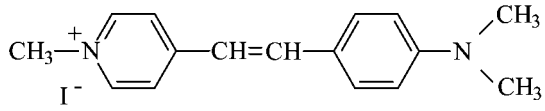
* To whom correspondence should be addressed. E-mail: mcorrea@exa.unrc.edu.ar.

10.1021/jp8105502 CCC: $40.75
Published on Web 03/04/2009

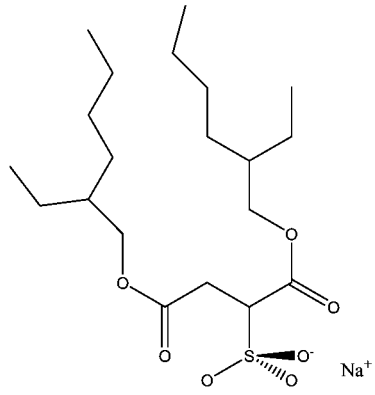
SCHEME 1: Molecular Structure of the Molecular Probes Used: DIA, HC, and the AOT Surfactant



DIA



HC



AOT

HC and DIA in the ground state interacts with the electrons in a different way than the positive charge located in the aromatic dialkylamino group in the excited state.

In this work, we investigated the behavior of the cationic hemicyanines HC and DIA in water/AOT/benzene reverse micelles media using absorption and emission spectroscopy in addition to the steady-state and time-resolved fluorescence emission techniques. Other authors have investigated the photophysics of HC and another similar hemicyanine *N*-*n*-heptyl-4-(2-(4-(dimethylamino)phenyl)ethenyl)pyridinium bromide in water/AOT/*n*-heptane or cyclohexane reverse micelles at [AOT] = constant and varying the water content.[24,25] They have found that both hemicyanines interact electrostatically through the sulfonate group of the AOT molecule and this interaction dominates the medium polarity effect on their absorption spectra. Both studies have focused on the water structure inside reverse micelles using the hemicyanines as molecular probe. As they only have used a single AOT concentration, a lot of outstanding information about the reverse micelles formation and interface properties is missing.

Our results show that the AOT reverse micelles interface has the nontrivial deaggregation property, a result that may have potential application for the preparation of dye lasers, which require a noninteracting monomeric form of the dye. Also, we show that the water interacts with a different region of the AOT moiety depending on the external organic solvent used. Thus, HC and DIA are significant dyes to characterize different interfaces' properties simultaneously as they can be used to sense, at the same time, fluidity and specific interactions at the interface.

Experimental Section

Sodium 1,4-bis(2-ethylhexyl)sulfosuccinate (AOT) (Sigma >99% purity) was used as received. AOT was kept under vacuum over P_2O_5 to minimize H_2O absorption. The absence of acidic impurities was confirmed through the 1-methyl-8-oxyquinolinium betaine (QB) absorption bands.[26]

trans-4-[4-(Dimethylamino)styryl]-*N*-methylpyridinium iodide (HC) was synthesized through the modification of a known method.[24,27] 4-Picoline (Sigma) was dissolved in acetone (0.5 M) with an excess of methyl iodide (Fluka) and the mix was refluxed for 14 h. The crystals were purified with absolute ethanol (Sintorgan) and then were added to a solution of *N*,*N*-dimethylaminobenzaldehyde (0.5 g), piperidine (2 mL), and ethanol (30 mL). The mixture was refluxed under N_2 atmosphere for 4 h. The solution was cooled until the HC crystals precipitated from the solution. The crystals were recrystallized in a 1:1 ethanol:heptane solution, and the purity was checked by silica gel 250 μm thin-layer chromatography plates, TLC (Analtech) in a chloroform−methanol 2:1 v/v mix where the $R_f = 0.76$.[27]

The fluorescent probe 4-[4-(dihexadecylamino)styryl]-*N*-methylpyridinium iodide (DIA), from Molecular Probes (Eugene, OR), was used as received.

Ultrapure water was obtained from Labonco equipment model 90901-01.The stock solutions of AOT in the hydrocarbon solvent were prepared by mass and volumetric dilution. To obtain optically clear solutions, they were shaken in a sonicating bath. To introduce the probe, a concentrated solution of HC and DIA was prepared in acetonitrile (Sintorgan HPLC quality). The appropriate amount of these solutions to obtain the desired final concentration of hemicyanines in the micellar system was transferred into a volumetric flask, and the acetonitrile was removed by bubbling dry N_2. Benzene was added to the residue, and the resulting solution was used to prepare the surfactant containing samples. The appropriate amount of stock surfactant solution to obtain a given concentration of surfactant in the micellar media was transferred into the cuvette, and the water was added using a calibrated microsyringe. The amount of water present in the system is expressed as the molar ratio between water and the AOT ($W = [H_2O]/[AOT]$).

The absorption spectra were measured by using Shimadzu 2401 equipment at 25 ± 0.1 °C unless otherwise indicated. A Spex Fluoromax apparatus was employed for the fluorescent measurements. Corrected fluorescence spectra were obtained using the correction file provided by the manufacturer. The path length used in the absorption and emission experiments was 1 cm. λ_{max} was measured by taking the midpoint between the two positions of the spectrum where the absorbance is equal to 0.9 × A_{max}. The uncertainties in λ_{max} are about 0.1 nm.

Fluorescence decay data were measured with the time-correlated single photon counting technique (Edinburgh Instrument FL-900) with a PicoQuant subnanosecond pulsed LED PLS 450 (emitting at 450 nm) < 600 ps fwhm, collecting a total number of 10 000 counts. Fluctuations in the pulse and intensity were corrected by making an alternate collection of scattering and sample emission. The choice between a single- or a double-exponential fit was made on the basis of the presence or absence of any observed deviation from random fluctuation in residual plots and the values of the chi-squared parameter (χ^2).[28] For the best fit, χ^2 must be around 1.0−1.2.[29] It must be noted that in the case of single-exponential fit we have tried to perform multiexponential fitting but the statistics of the decays were not improved at all or sometimes they became worse. In all cases, we did not observe negative amplitude in the fitting at any

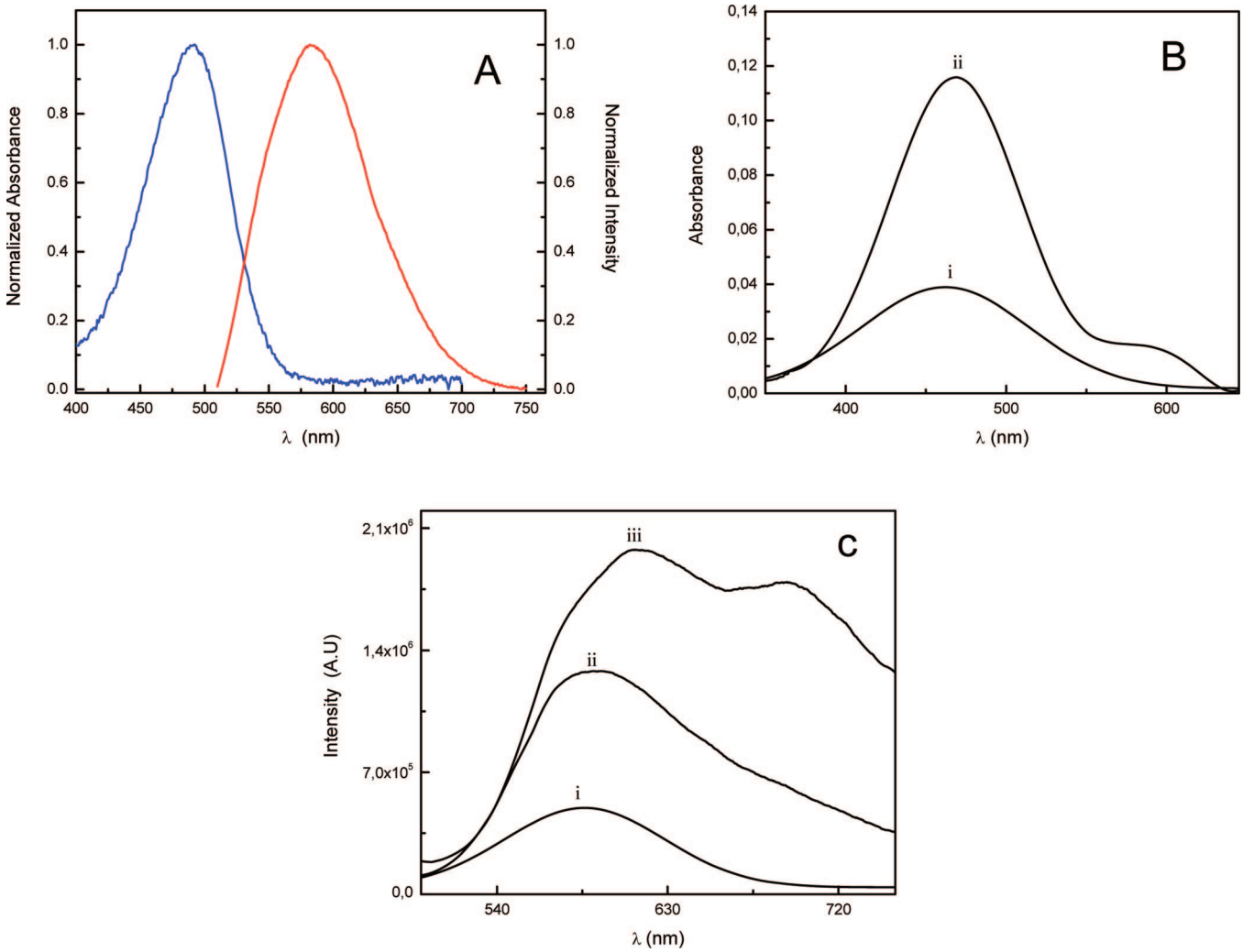


Figure 1. (A) Normalized absorption (blue line) and emission spectra (red line) of DIA in benzene. [DIA] = 1×10^{-6} M. (B) Absorption spectra of HC in benzene at different concentrations. [HC]/M (i) 3×10^{-6} and (ii) 2×10^{-5}. (C) Emission spectra of HC in benzene at different concentrations. [HC]/M (i) 1×10^{-6}, (ii) 1×10^{-5}, and (iii) 4×10^{-5}.

detection wavelength, which means that there were no rise components for HC inside AOT reverse micelles. In other words, HC does not experience an excited-state process leading to a new emitting state different from the initially excited state.[30]

Results and Discussion

I. DIA and HC in Benzene. We have shown[23] that HC and DIA in solvents where the β parameter[31] is low or nil, such as the hydrocarbons, experience an aggregation process due to its low solvent solubilities. It seems that the tendency of these cationic dyes to aggregate is enhanced when their positive charge is poorly solvated.

In this work, we will show first the results in benzene because this is the organic solvent used to prepare the reverse micelle systems. It must be noted that the β parameter for benzene is very low[31,32] and thus hemicyanines are prone to aggregate as was discussed above.

Figure 1A shows the absorption and emission spectra of DIA in benzene. Both spectra consist of a single band with the absorption maxima wavelength around 490 nm and an emission maxima around 580 nm, which is independent of excitation wavelength. This indicates that only one species is present in this nonpolar solvent. DIA in benzene was also explored using time-resolved emission spectroscopy. The experiments were carried out at $\lambda_{exc} = 450$ nm, at two emission wavelengths, $\lambda_{em} = 590$ and 650 nm, and at two DIA concentrations, 5×10^{-7} and 1×10^{-6} M. The fluorescence decay of the molecule in benzene fits well to a single-exponential function with a fluorescence lifetime of $\tau = 0.40$ ns $\pm$ 0.10, $\chi^2 = 1.16$, results that confirm that only one species is present in this solvent at both concentrations tested. Due to its very low solubility in this solvent, it was not possible to explore at higher concentrations, but 1×10^{-6} M is the concentration that we use in this work.

On the other hand, the situation is quite different for HC. Figure 1B shows the absorption spectra of HC in benzene at two different concentrations, i.e., 3×10^{-6} and 2×10^{-5} M. Figure 1C shows the emission spectra of HC in benzene at [HC] = 1×10^{-6}, 1×10^{-5}, and 4×10^{-5} M. As can be observed at concentration $\sim 10^{-6}$ M, the absorption and emission spectra consist of a single band centered at 470 and 583 nm, respectively. However, at higher concentration, around 10^{-5} M, the absorption and emission spectra show the appearance of a new band at lower energy: $\lambda_{abs} = 590$ nm and $\lambda_{em} = 695$ nm, respectively. Moreover, the HC absorbance at 470 nm does not obey the Lambert−Beer law over the concentration range used (results not shown). It is well-known that cationic hemicyanines are molecules that tend to aggregate to give J- and H-aggregates.[23] Thus, HC monomer absorbs at 470 nm and emits at 583 nm while the aggregate absorption band peaks at 590 nm and emits at 695 nm. The absence of a clear isosbestic point

TABLE 1: Fluorescence Lifetime (τ, ns) of HC in Benzene at Different Dye Concentrations

[HC]/M	$\lambda_{exc} = 450$ nm $\lambda_{emi} = 590$ nm	$\lambda_{exc} = 450$ nm $\lambda_{emi} = 670$ nm
3×10^{-6}	$\tau = 0.19 \pm 0.04$ $\chi^2 = 1.17$	$\tau = 0.20 \pm 0.04$ $\chi^2 = 1.20$
2×10^{-5}	$\tau_1 = 0.22 \pm 0.06$ $\tau_2 = 1.17 \pm 0.08$ $\chi^2 = 1.22$	$\tau_1 = 0.20 \pm 0.05$ $\tau_2 = 1.20 \pm 0.04$ $\chi^2 = 1.11$

in the absorption spectrum of HC suggests that the aggregation process may include higher order aggregates beyond simple monomer−dimer equilibrium. Because its absorption spectrum appears at lower energy relative to the monomer, we conclude HC forms J-aggregates,[33,34] that is, aggregates with dye molecules arranged head-to-tail in a slanted stack.[33,34] Emission at lower energy relative to the monomer has also been reported for other J-aggregates.[35−38]

We also explored HC aggregation using time-resolved emission spectroscopy. The measurements were performed for HC in benzene at two different concentrations, 3×10^{-6} and 2×10^{-5} M, and two different emission wavelengths as given in Table 1. At low concentration, where the monomer dominates the absorption spectrum, the fluorescence decay fits well to a single-exponential decay with a time constant corresponding to the fluorescence lifetime of the monomer, $\tau = 0.20 \pm 0.02$ ns, which is independent of the emission wavelength used. Probing the higher concentration with excitation at 450 nm and detection at 590 and 680 nm, the region where the contribution from the aggregate should be higher, the fluorescence decay exhibits a biexponential decay with the monomer lifetime and a longer one, $\tau_2 = 1.2\ 0 \pm 0.04$ ns, that corresponds to the HC aggregate species, both lifetimes being emission wavelength independent. It is known[10,39] that change of the fluorescence lifetime with the emission wavelength is observed for fluorophores that exist in environments of restricted mobility while independent fluorescence lifetimes with emission wavelength are expected for relaxed ones.

II. DIA and HC in AOT Reverse Micelles. Having characterized the spectroscopy of the hemicyanines in benzene solution, we utilize it to probe different characteristics of the AOT reverse micelle's interfaces. We also want to explore the ability of the AOT reverse micelles to deaggregate dyes as was previously demonstrated working with other molecular probes.[40,41] Thus, we prefer to work at [HC] $= 2 \times 10^{-5}$ M where the presence of HC aggregates in benzene was demonstrated.

Reverse Micelles at W = 0. Figure 2A−D shows the absorption and emission spectra of DIA and HC in benzene/AOT reverse micelles as a function of AOT concentration respectively; [DIA] $= 1 \times 10^{-6}$ M; [HC] $= 2 \times 10^{-5}$ M. For both hemicyanines the data show that as the [AOT] increases both the absorption and the emission bands shift hypsochromically even though it is well-known that the AOT reverse micelle micropolarity increases as the AOT concentration increases.[2] As was previously discussed, the absorption bands shift hypsochromically while the emission bands shift bathochromically as the polarity of the medium increases.[23] The unexpected result inside the reverse micelles seems to indicate that other effect rather the medium polarity dominates the hemicyanines photophysics in the microheterogeneous media. Since HC and DIA bear a positive charge in their moieties and AOT has a negative charge in its polar headgroup, it is very likely that the Coulombic interaction between the dyes and the surfactant headgroup have an influence on the hemicyanines solvatochromic behavior. This interaction was invoked before to explain anomalous behavior of similar hemicyanines inside *n*-heptane/AOT/water reverse micelle systems.[24,25]

Recently, it was shown that not only the polarity is the responsible for the HC and DIA spectroscopic results in homogeneous media and we demonstrate that the cationic hemicyanines undergo a specific interaction with the medium through to the electron donor ability of the solvents as measured by the β solvent parameter. The absorption band shifts bathochromically as β increases while the emission band shifts hypsochromically.[23] Thus, since the AOT polar head is a good electron donor environment,[2] this possibility should also be taken into account in conjunction with the Coulombic attraction interaction to fully explain the hemicyanines' absorption and emission blue shift bands inside reverse micelles. In the ground state, the positive charge is located in the hemicyanine's N pyridinium atom (Scheme 1) and it interacts electrostatically with the AOT sulfonate negative charge. Thus, as the surfactant concentration increases, HC and DIA inside AOT reverse micelles experience this interaction, being closer to the AOT sulfonate group rather than the more electron donor (higher β)[32] succinate ester group. Upon excitation, the hemicyanines' excited states shift the positive charge toward the N of the aniline group. Thus, we expect that the charge in the excited state is now near the succinate ester group and the tails region of the AOT reverse micelles interface. This interface domain offers a lower polarity and a higher electron donor medium in comparison with the environment that surrounds the hemicyanines' ground state.

It is worth highlighting that the hemicyanines' counterions are iodide which is a fairly efficient fluorescent quencher. It can be thought that because the effective I^- concentration near the chromophore is high at $W = 0$, there could be an effective hemicyanines quenching by I^- inside the reverse micelles. Surprisingly, Figure 2D shows a dramatic intensity increase for HC as the AOT concentration increases, which discards the possibility of such quenching process. Probably the strong AOT−HC interaction avoids this phenomenon. Moreover, there are other scenarios that can be considered to explain the intensity increases that Figure 2D shows. The hemicyanine dyes' spectroscopy being sensitive to the electric field,[12,28] it is possible that once located at the AOT reverse micelles interfaces, where it is known that the ionic strength is quite high,[1,2] their electronic spectrum should change. We have tested the sensitivity of the HC's absorption and emission spectrum to the ionic strength by comparing them in water and in NaCl−water saturated solution (results not shown). The results show that both absorption and emission bands increase their absorbance and intensity, respectively, in the salts' saturated water solution. This seems to indicate that hemicyanines experience the ionic strength and the electric field of the AOT reverse micelles interface (Figure 2). Also, it is known[17] that there is a significant increase in the fluorescence quantum yield of the hemicyanines with the increase in the viscosity of the medium. The AOT reverse micelle interface has a much higher microviscosity value at $W = 0$ than benzene.[2]

As was previously mentioned, we also want to explore the effect that the AOT reverse micelle has on the HC aggregation process previously discussed in subsection I. Figure 2C shows the HC absorption spectra at different [AOT] and [HC] $= 2 \times 10^{-5}$ M. As can be observed, the band that peaks at $\lambda = 590$ nm, which was assigned to the HC aggregate species in benzene, disappears as the [AOT] increases. It seems that, unlike its behavior in benzene, HC does not aggregate inside AOT reverse micelles and its distribution can be explained through Poisson's

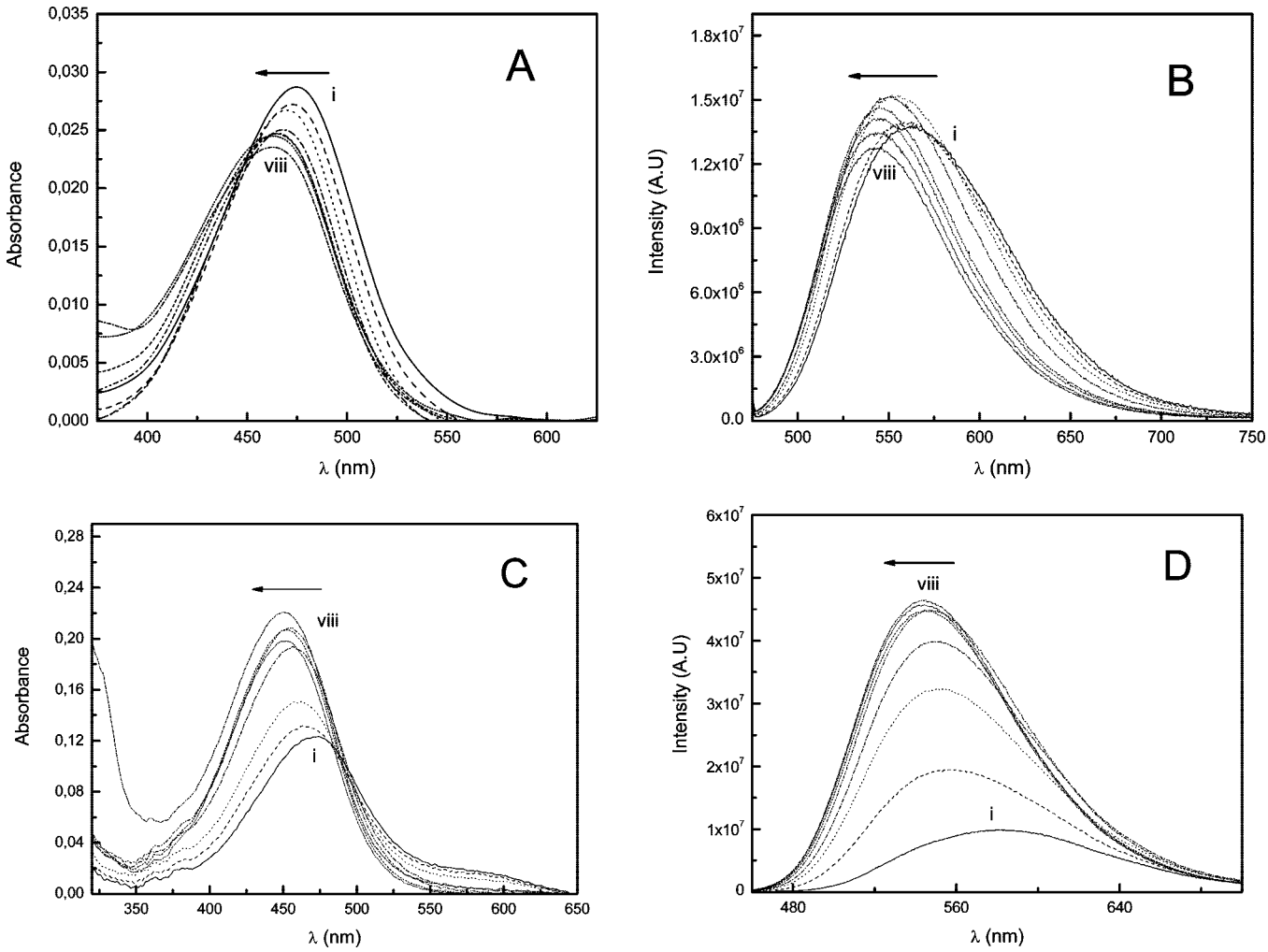


Figure 2. Absorption (A) and emission (B) spectra of DIA in AOT/benzene reverse micelles at different AOT concentrations, $W = 0$. [AOT]/M: (i) 1×10^{-3}, (ii) 2×10^{-3}, (iii) 7×10^{-3}, (iv) 1.9×10^{-2}, (v) 7.4×10^{-2}, (vi) 0.1, (vii) 0.2, and (viii) 0.3. [DIA] $= 1 \times 10^{-6}$ M. Absorption (C) and emission (D) spectra of HC in AOT/benzene reverse micelles at different AOT concentrations, $W = 0$. [AOT]/M: (i) 0, (ii) 1×10^{-3}, (iii) 5×10^{-3}, (iv) 1×10^{-2}, (v) 5×10^{-2}, (vi) 0.1, (vii) 0.2, and (viii) 0.3. [HC] $= 2 \times 10^{-5}$ M.

statistics.[42] At surfactant concentrations above the critical micelle concentration (cmc), the occupation number is

$$n = \frac{[\text{dye}]}{[\text{RM}]} \tag{1}$$

where [RM] represents the concentration of reverse micelles

$$[\text{RM}] = \frac{[\text{surfactant}] - \text{cmc}}{N_{\text{agg}}} \tag{2}$$

with N_{agg}, the aggregation number, the number of surfactant molecules in the RMs.[43] If on average, $n < 1$, then fewer than one molecule occupies any given reverse micelle and the environment leads to the complete deaggregation of dye.[37,38,41] Thus, at [AOT] = 0.1 M, and for [HC] $= 2 \times 10^{-5}$ M, considering an operational cmc for AOT $= 2 \times 10^{-3}$ M[44] and N_{agg} at $W = 0$ around 20,[45] leads to $n = 0.004$. Consequently, finding more than one dye molecule in any reverse micelle would be very unlikely and the aggregation process that HC undergoes in benzene does not occur in the reverse micelles. It is worth highlighting that Zouni et al.[46] have reported kinetics evidence for disaggregation of a related dye, RH421, which strongly aggregates in aqueous solution in the presence of lipid vesicles made with dimyristoylphosphatidylcholine. The observed kinetic behavior of the dye is consistent with a disaggregation of the dye aggregates in water and its incorporation of dye monomers into the vesicle membrane.

Table 2 shows the fluorescence lifetime of HC in AOT/benzene reverse micelles at two AOT concentrations and different emission wavelengths. Interesting conclusions can be obtained from the data. At low AOT concentration, where the aggregation process would be favored, HC presents two lifetimes, $\tau_1 = 0.41$ ns and $\tau_2 = 1.21$ ns, which are independent of the detection emission wavelength. The value of τ_1 is close to the lifetime value obtained in benzene for the HC monomer species. However, the τ_1 value is a little higher than the one obtained in benzene. The differences could be explained considering the strong electrostatic interaction between HC monomer and AOT molecules invoked before. We have shown previously[23] an increase in the fluorescence lifetime of DIA and HC inside DOPC bilayer with respect to the value in pure water, a result that was explained considering the interaction between the dyes with the electron-rich region of the polar head of the phospholipid. Moreover, other related compounds like the zwitterionic hemicyanine RH421 also shows a substantial increase in its fluorescence lifetime when it is dissolved in solvents where it engages in specific interactions with the

TABLE 2: Fluorescence Lifetimes (τ, ns) of HC and DIA in AOT/Benzene Reverse Micelles at Different [AOT] and W (λ_{exc}= 450 nm)

	AOT/benzene $W = 0$			
[HC]/M	[AOT] = 5×10^{-3} M		[AOT] = 0.2 M	
2×10^{-5}	$\lambda_{emi} = 580$ nm $\tau_1 = 0.41 \pm 0.04$ $\tau_2 = 1.21 \pm 0.06$ $\chi^2 = 1.21$	$\lambda_{emi} = 670$ nm $\tau_1 = 0.45 \pm 0.03$ $\tau_2 = 1.22 \pm 0.02$ $\chi^2 = 1.22$	$\lambda_{emi} = 543$ nm $\tau = 0.47 \pm 0.10$ $\chi^2 = 1.17$	$\lambda_{emi} = 605$ nm $\tau = 0.78 \pm 0.08$ $\chi^2 = 1.19$
	AOT/benzene/water $W = 10$			
[HC]/M	[AOT] = 5×10^{-3} M		[AOT] = 0.2 M	
2×10^{-5}	$\lambda_{emi} = 578$ nm $\tau = 0.28 \pm 0.04$ $\chi^2 = 1.14$	$\lambda_{emi} = 660$ nm $\tau = 0.31 \pm 0.06$ $\chi^2 = 1.17$	$\lambda_{emi} = 576$ nm $\tau = 0.27 \pm 0.08$ $\chi^2 = 1.20$	$\lambda_{emi} = 660$ nm $\tau = 0.29 \pm 0.05$ $\chi^2 = 1.21$
	AOT/benzene $W = 0$			
[DIA]/M	[AOT] = 5×10^{-3} M		[AOT] = 0.2 M	
3×10^{-6}	$\lambda_{emi} = 556$ nm $\tau_1 = 0.60 \pm 0.03$ $\tau_2 = 1.20 \pm 0.05$ $\chi^2 = 1.16$	$\lambda_{emi} = 630$ nm $\tau_1 = 0.75 \pm 0.04$ $\tau_2 = 1.81 \pm 0.08$ $\chi^2 = 1.17$	$\lambda_{emi} = 543$ nm $\tau_1 = 0.63 \pm 0.03$ $\tau_2 = 1.50 \pm 0.05$ $\chi^2 = 1.09$	$\lambda_{emi} = 605$ nm $\tau_1 = 0.82 \pm 0.05$ $\tau_2 = 1.85 \pm 0.02$ $\chi^2 = 1.19$
	AOT/benzene/water $W = 10$			
[DIA]/M	[AOT] = 5×10^{-3} M		[AOT] = 0.2 M	
3×10^{-6}	$\lambda_{emi} = 588$ nm $\tau = 0.79 \pm 0.10$ $\chi^2 = 1.13$	$\lambda_{emi} = 670$ nm $\tau = 0.95 \pm 0.06$ $\chi^2 = 1.17$	$\lambda_{emi} = 590$ nm $\tau = 0.74 \pm 0.02$ $\chi^2 = 1.22$	$\lambda_{emi} = 670$ nm $\tau = 1.08 \pm 0.10$ $\chi^2 = 1.24$

microenvironment.[47] The value of τ_2 is comparable to the HC aggregate species obtained in benzene and thus it is assigned to this species. Here, it is worth discussing the independence of the lifetime values with the emission wavelength. Fluorescence lifetime serves as sensitive indicator for the local microenvironment where a solute exists because differential extent of solvent relaxation around a given fluorophore could be expected to give rise to differences in its fluorescence lifetime.[7,10,23,25] In a motion-restricted media such as organized systems like reverse micelles or vesicles, lifetime is dependent on the excitation and emission wavelengths. Observation at shorter wavelengths of the emission spectra selects the unrelaxed fluorophores with shorter lifetimes because this population decays both at the rate of fluorescence emission at the given excitation wavelength and at the rate of the emission at longer (nonobserved) wavelengths. On the other hand, the observation at longer wavelength (red edge) of the emission selects for the more relaxed fluorophores, which have spent enough time in the excited state to allow larger extent of solvent relaxation.[10] Thus, fluorescence lifetimes can be used to directly monitor the microenvironment and dynamics around a fluorophore in a motion-restricted environment as was previously demonstrated for HC and DIA in DOPC LUV.[23] Thus, the fact that the lifetimes are independent of the emission wavelength at [AOT] $= 5 \times 10^{-3}$ M can be interpreted considering the absence of AOT reverse micelles in benzene with the presence of HC. Even though in the literature values have been reported for the "operational" cmc for AOT/benzene around 10^{-4} M[48] to 10^{-3} M,[44] it is known that this value depends on the experimental method and/or probes used for its determination.[2] It seems to us that the strong electrostatic interaction between HC and AOT monomer molecules decreases the surfactant free molecules in solution, increasing the operational cmc value. Following the decrease of the absorption value at $\lambda = 590$ nm with the increase in the surfactant concentration shown in Figure 2C, it is possible to estimate the value of the operational cmc in this system (results not shown). The cmc value obtained is 1×10^{-2} M, which confirms the discussion shown above.

On the other hand, Table 2 shows that the situation is quite different for DIA in AOT/benzene reverse micelles media at [AOT] $= 5 \times 10^{-3}$ M. DIA presents two lifetimes which now are wavelength emission detection dependent. At $\lambda_{em} = 556$ nm, $\tau_1 = 0.60$ ns and $\tau_2 = 1.20$ ns and at $\lambda_{em} = 630$ nm, $\tau_1 = 0.70$ ns and $\tau_2 = 1.81$ ns. It must be noted that there is no evidence of DIA aggregation process at concentration around 10^{-6} M as was previously discussed. Thus, DIA's lifetimes assignation in reverse micelles have to be discussed properly. The fact that the fluorescence lifetimes values are wavelength emission dependent probably indicates that now, at [AOT] $= 5 \times 10^{-3}$ M, there are reverse micelles in contrast to what was observed with HC at the same [AOT]. Thus, DIA is located in a motion-restricted media and emits from two different microenvironments. The τ_1 value is close to the one obtained in benzene but, here this value is higher and wavelength dependent. We assign this value to DIA at the oil side of the AOT reverse micelles interfaces far from the sulfonate group. The τ_2 value is close to the one obtained for HC interacting electrostatically with the AOT sulfonate group. DIA being a hemicyanine related to HC, both have the same chromophore group which holds a positive charge and thus we assign τ_2 for DIA species that emits located at the polar side of the reverse micelles interface close to the AOT sulfonate group interacting through electrostatic interaction. Recently,[23] we have demonstrated that DIA interacts less than HC with an electron donor environment (β parameter) because of the long aliphatic chain of DIA which may delocalize and hinder the positive charge. Thus, we expect a weaker electrostatic interaction between DIA with AOT than HC with AOT.

The results obtained at [AOT] = 0.2 M are also gathered in Table 2. Now, HC shows a fluorescence decay that exhibits a monoexponential decay with τ that corresponds to HC monomer species interacting with AOT polar head. This value is

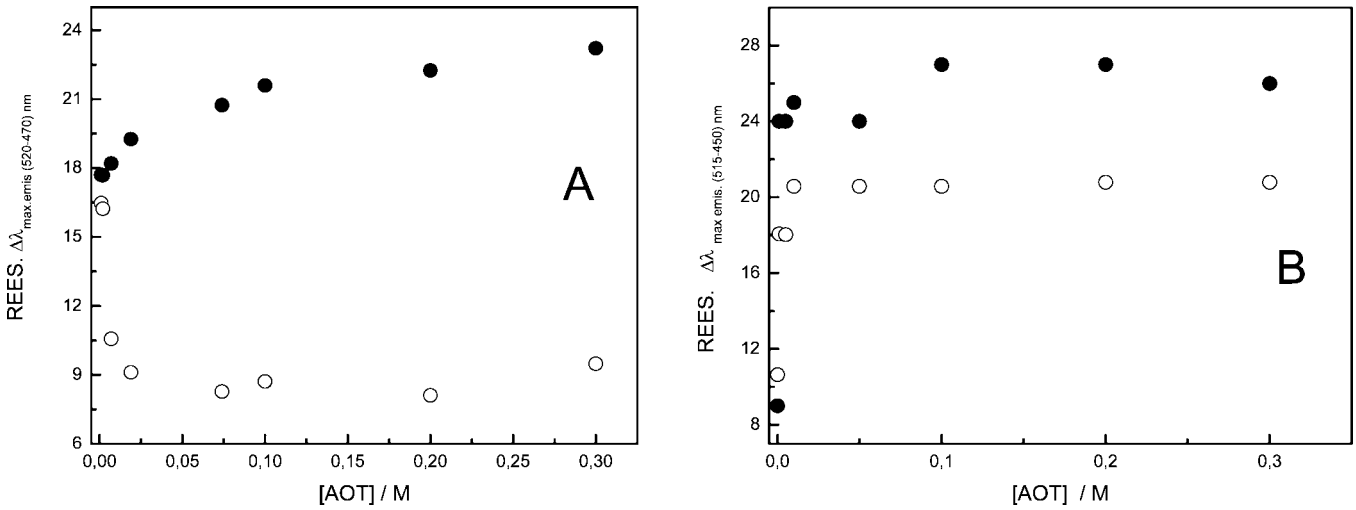


Figure 3. (A) REES ($\lambda_{max\ emi}$ at λ_{exc} 520 nm $-$ $\lambda_{max\ emi}$ at λ_{exc} 470 nm) values for DIA in AOT/benzene reverse micelles at different AOT concentrations. [AOT]/M: (i) 1×10^{-3}, (ii) 2×10^{-3}, (iii) 7×10^{-3}, (iv) 1.9×10^{-2}, (v) 7.4×10^{-2}, (vi) 0.1, (vii) 0.2, and (viii) 0.3. (●) $W = 0$. (○) $W = 10$. [DIA] $= 1 \times 10^{-6}$ M. (B) REES ($\lambda_{max\ emi}$ at λ_{exc} 515 nm $-$ $\lambda_{max\ emi}$ at λ_{exc} 450 nm) values for HC at different AOT concentrations. [AOT]/M: (i) 0, (ii) 1×10^{-3}, (iii) 5×10^{-3}, (iv) 1×10^{-2}, (v) 5×10^{-2}, (vi) 0.1, (vii) 0.2, and (viii) 0.3. (●) $W = 0$. (○) $W = 10$. [HC] $= 2 \times 10^{-5}$ M.

wavelength emission dependent which means that HC is located in a motion-restricted media, the AOT reverse micelles interface, in contrast to what was found at [AOT] $= 5 \times 10^{-3}$ M. Also, the presence of the reverse micelles has favored the HC deaggregation process as eqs 1 and 2 predict.

On the other hand, DIA shows a fluorescence decay that exhibits a biexponential decay, as was previously found and discussed at [AOT] $= 5 \times 10^{-3}$ M.

Red-Edge Excitation Shift (REES) Study. It is known[7,9] that a shift in the maximum fluorescence emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of absorption band, is called the red edge excitation shift (REES) and this tool can be used to directly monitor the microenvironment and dynamics around a fluorophore in a motion-restricted media such as organized media like reversed micelles[41] or vesicles.[23,49] We want to explore the dynamics of the AOT reverse micelles using HC and DIA as molecular probes and this powerful technique.

Figure 3, A and B, shows the shift in the maxima of the fluorescence emission of DIA and HC in AOT reverse micelles media at $W = 0$ as a function of the excitation wavelength at different AOT concentrations being $\Delta\lambda_{em} = (\lambda_{em(exc\ 520nm)} - \lambda_{em(exc\ 470nm)})$ and $\Delta\lambda_{em} = (\lambda_{em(exc\ 515nm)} - \lambda_{em(exc\ 450nm)})$ for DIA and HC, respectively. The results for both dyes show that, as the AOT concentration increases, the magnitude of the REES also increases concomitant up to [AOT] $\sim$ 0.05 M. Also, a huge REES value, around 20 nm for DIA and 26 nm for HC, was found for both hemicyanines. The appearance of such REES cannot be attributed only to the solvent relaxation process.[7,9,24] A value around 26 nm is too large for a regular solvent relaxation phenomenon (usually <10 nm), so we think that also the electrostatic interactions between the dyes and the AOT polar head invoked before are responsible for the REES magnitude.

Reverse Micelles at W = 10. Contrary to what was observed at $W = 0$, when water is added to the reverse micellar system, the absorption and emission bands shifting are expected for this kind of hemicyanines.[23] Figure 4, A, B, C and D, shows that the absorption band shifts hypsochromically and the emission band shifts bathochromically as the AOT concentration increases. As shown by our previous results in homogeneous media,[23] those shifts indicate that DIA and HC experience a more polar and a less electron donor environment as the water content increases, probably sensing the water presence. Thus, we show that the encapsulated water interacts with different regions of the AOT moiety depending on the external organic solvent used to create the reverse micelles media. When benzene is the organic solvent, water molecules penetrate the oil side of the interface to hydrate the AOT succinate ester group. On the contrary, when saturated hydrocarbons solvents are the organic pseudophase, water molecules interact through hydrogen bond with the AOT sulfonate group without penetration to the interface.[2,50] Therefore, in benzene HC is being solvated by the water molecules which are more polar (higher π^*) and less electron donor (lower β) molecules than the AOT succinate ester group.[31,32]

It must be taken into account that there is a clear decrease in the HC emission intensity at [AOT] $> 10^{-3}$ M (Figure 4D) that is different at the feature observed at $W = 0$ (Figure 2D). Futhermore, the fluorescence quantum yield and the emission lifetime of HC in water[25] are quite low in comparison with all the other solvents which probably indicates that HC senses a water environment at the reverse micelles interface. Figure 4B suggests that this is not the case for DIA where the intensities always increase with the surfactant concentration.

Figure 4C shows the HC absorption spectra at different [AOT] and [HC] $= 2 \times 10^{-5}$ M at $W = 10$. As can be observed, the band that peaks at $\lambda = 590$ nm, which was previously assigned to the HC aggregate species in benzene, disappears as the [AOT] increases. Moreover, it is clear that the HC deaggregation process is favored when water is present in the reverse micelles media. As was shown in the $W = 0$ section (Figure 2C), from Figure 4C we have estimated the value of the operational cmc in this system (results not shown) which gives a value of cmc around 2×10^{-3} M. Thus, the formation of AOT reverse micelles is favored at $W = 10$ rather than in the absence of water and now the operational cmc value is close to the one reported in the literature for AOT/benzene reverse micelle.[47]

Table 2 shows DIA and HC fluorescence lifetimes in water/AOT/benzene reverse micelles at two AOT concentrations and at different emission wavelengths at $W = 10$. HC shows a fluorescence decay that exhibits a monoexponential decay at both AOT concentrations and at both emission wavelengths. Interestingly, the long emission lifetime observed at $W = 0$ at [AOT] $= 5 \times 10^{-3}$ M which was assigned to HC aggregate

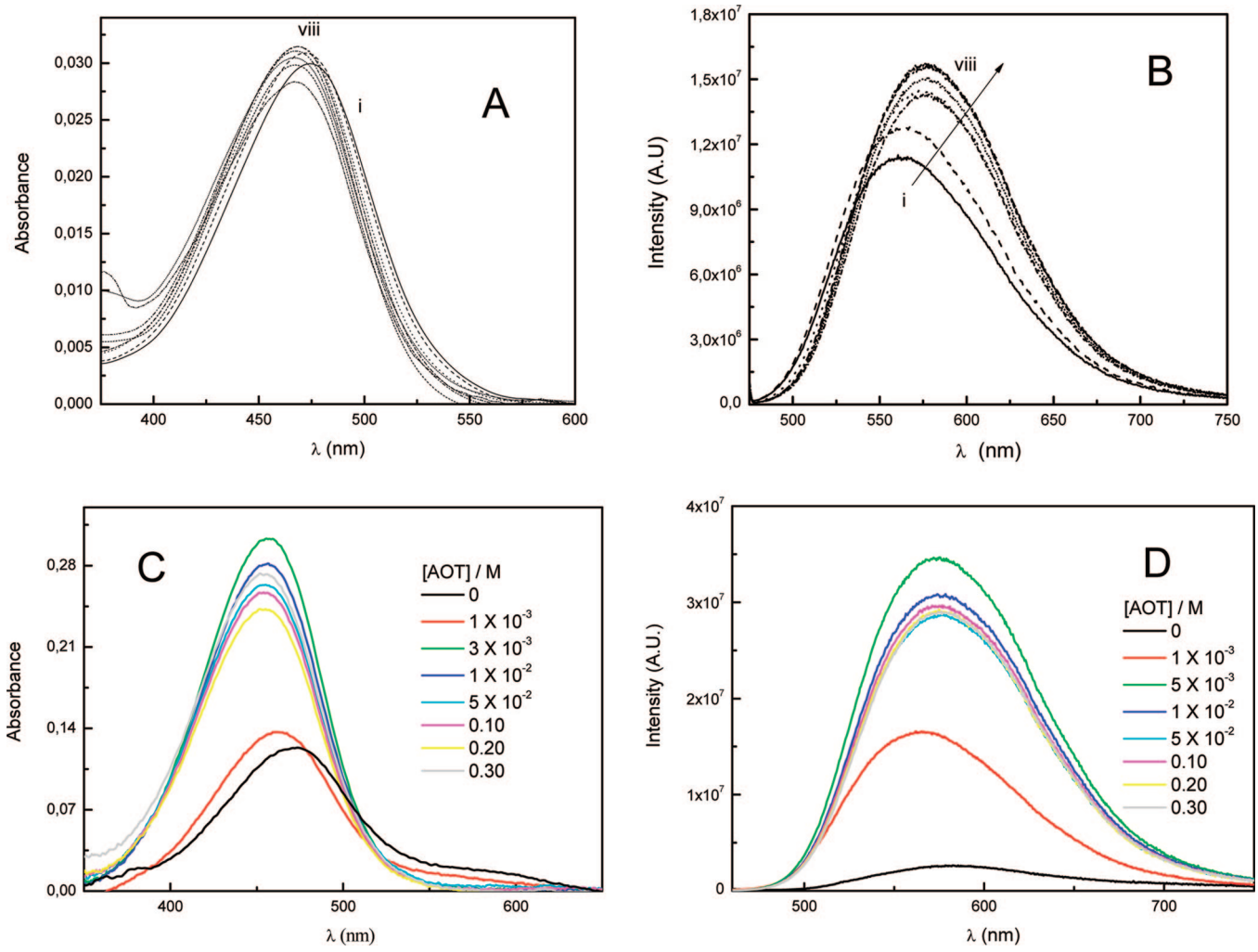


Figure 4. Absorption (A) and emission (B) spectra of DIA in AOT/benzene reverse micelles at different AOT concentrations, $W = 10$. [AOT]/M (i) 1×10^{-3}, (ii) 2×10^{-3}, (iii) 7×10^{-3}, (iv) 1.9×10^{-2}, (v) 7.4×10^{-2}, (vi) 0.1, (vii) 0.2, and (viii) 0.3. [DIA] $= 1 \times 10^{-6}$ M. Absorption (C) and emission (D) spectra of HC in AOT/benzene reverse micelles at different AOT concentrations $W = 10$. [HC] $= 2 \times 10^{-5}$ M.

species in benzene is now not present. The τ value obtained corresponds to HC monomer species inside the reverse micelle media. The fact that the aggregate species does not exist at $W = 10$ confirms the presence of reverse micelles at this low [AOT] at $W = 10$ as eqs 1 and 2 predict. Moreover, the τ value obtained is lower than the one obtained at $W = 0$, probably indicating the presence of water in the HC's microenvironment. The independence of the fluorescence lifetimes with the emission wavelength can be interpreted considering that HC exists in a less restricted environment in comparison with its location in the absence of water.[2] Here, an interesting question arises with regard to the HC−AOT interaction in the presence of water. Is this interaction still present? Figure 3B shows that the REES value for HC in water/AOT/benzene system at $W = 10$ is around 20 nm, which is quite large for a nonrestricted motion environment as was previously discussed. Thus, the REES value can indicate that the HC−AOT interaction is present even with water encapsulated in the system. It seems that the emission lifetimes value is not as sensitive as REES for sensing specific interactions.

DIA shows a fluorescence decay feature similar to HC, i.e., exhibits monoexponential decays, at both AOT concentrations and at both emission wavelengths, which are emission wavelength dependent. The emission lifetime value that corresponds to DIA−AOT species at $W = 0$ (τ around 1 ns) is no longer present at $W = 10$. It seems that the electrostatic interaction between DIA and the AOT sulfonate group weakens because the formation of hydrogen bonds between water and the oxygen atoms of the sulfonate group,[51] which result in the removal of DIA from the sulfonate group. Similar competition between molecular probes and water for the AOT polar head has been reported in the literature.[52,53] The dramatic decrease in the REES value for DIA at $W = 10$ shown in Figure 3A confirms the absence of the electrostatic interaction between DIA and AOT. Also, the REES values suggest that DIA exists in a motion-restricted environment inside the reverse micelles (REES around 9 nm). Thus, the τ value obtained at $W = 10$ is assigned to the DIA species located at the oil side of the AOT reverse micelles interfaces, far from the sulfonate group and also far from the water pool.

Conclusion

The behaviors of the cationic hemicyanines HC and DIA were studied in benzene as well as in water/AOT/benzene reverse micelles media, using absorption, emission, and time-resolved spectroscopies. The results in benzene show that HC experiences an aggregation process at concentration around 10^{-5} M giving J-aggregates. On the other hand, it was not possible to investigate DIA at concentration similar to HC because of its low solubility in benzene. Thus, there is no evidence of DIA aggregate species at concentration around 10^{-6} M.

Dissolved in the AOT reverse micelle systems, both dyes behave differently. HC experiences strong electrostatic interactions with the AOT anionic polar headgroup that cannot be disrupted with the presence of water in the reverse micelles. Moreover, at $W = 0$ this electrostatic interaction perturbs the reverse micelle formation, increasing the operational cmc in comparison with the value in the absence of HC. Once the reverse micelle is formed, HC incorporates as monomer at the AOT reverse micelles interface. At $W = 10$ and in contrast to $W = 0$, the fluorescence lifetimes values are independent of the emission wavelength which suggests that HC is in a fluid microenvironment but the REES values is too large for a molecule that exists in a nonrestricted motion environment. Thus, the REES value reflects that the HC−AOT interaction is still present with water encapsulated. It seems that the emission lifetime's value is not as sensitive as REES for sensing specific interactions.

For DIA at $W = 0$ the results also show the electrostatic interaction with the AOT polar headgroup but weaker than the one found for HC−AOT. At $W = 10$ there is a competition with water for the AOT interaction and DIA is expelled from the AOT sulfonate group to the oil side of the interface where there is no water. The emission lifetimes and the REES values at $W = 10$ show DIA in a restricted motion environment and the absence of the electrostatic DIA−AOT interaction.

In summary, HC and DIA are powerful dyes to characterize different interfaces' properties as they can be used to sense, at the same time, fluidity and specific interactions at the interface. These results could be important because those properties are the key for molecular recognition.

Acknowledgment. Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Río Cuarto, Fundación Antorchas, and Agencia Nacional de Promoción Científica y Técnica is gratefully acknowledged. J.J.S., N.M.C., and R.D.F. hold research positions at CONICET. F.M. and S.S.Q. thank CONICET for research fellowships.

References and Notes

(1) De, T. K.; Maitra, A. *Adv. Colloid Interface Sci.* **1995**, *59*, 95.
(2) Silber, J. J.; Biasutti, M. A.; Abuin, E.; Lissi, E. *Adv. Colloid Interface Sci.* **1999**, *82*, 189.
(3) Biasutti, M. A.; Abuin, E. A.; Silber, J. J.; Correa, N. M.; Lissi, E. A. *Adv. Colloid Interface Sci.* **2008**, *136*, 1.
(4) Baruah, B.; Roden, J.; Sedgwick, M.; Correa, N. M.; Crans, D. C.; Levinger, N. E. *J. Am. Chem. Soc.* **2006**, *128*, 12758.
(5) De, T. K.; Maitra, A. *Adv. Colloid Interface Sci.* **1995**, *59*, 95.
(6) Moulik, S. P.; Paul, B. K. *Adv. Colloid Interface Sci.* **1998**, *78*, 99.
(7) Lakowicz, J. R. In *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic: New York, 1999.
(8) Milhaud, J. *Biochim. Biophys. Acta* **2004**, *1663*, 19.
(9) Chattopadhyay, A. *Chem. Phys. Lipids* **2003**, *122*, 3.
(10) Chattopadhyay, A.; Murkherjee, S.; Raghuraman, H. *J. Phys. Chem. B* **2002**, *106*, 13002.
(11) Binnemans, K.; Bex, C.; Venard, H.; De Leebeeck, H.; Görller-Walrand, C. *J. Mol. Liq.* **1999**, *83*, 283.
(12) Ephardt, H.; Fromherz, P. *J. Phys. Chem.* **1991**, *95*, 6792.
(13) Ephardt, H.; Fromherz, P. *J. Phys. Chem.* **1993**, *97*, 4540.
(14) Fromherz, P. *J. Phys. Chem.* **1995**, *99*, 7188.
(15) Mishra, A.; Behera, R. K.; Behera, P. K.; Mishra, B. K.; Behera, G. B. *Chem. Rev.* **2000**, *100*, 1973.
(16) Shim, T.; Lee, M.; Kim, S.; Sung, J.; Rhee, B. K.; Kim, D.; Kim, H.; Yoon, K. B. *Mater. Sci. Eng., C* **2004**, *24*, 83.
(17) Shim, T.; Lee, M. H.; Kim, D.; Ouchi, Y. *J. Phys. Chem. B* **2008**, *112*, 1906.
(18) Kostenko, O. M.; Kovalska, V. B.; Volkova, K. D.; Shaytanov, P.; Kocheshev, I. O.; Slominskiy, Y. L.; Pisareva, I. V.; Yarmoluk, S. M. *Fluoresc. J.* **2006**, *16*, 589.
(19) Korbakov, N.; Timmerman, P.; Lidich, N.; Benayahu, U.; Saar, A.; Yitzchaik, S. *Langmuir* **2008**, *24*, 2580.
(20) Ner, Y.; Grote, J. G.; Stuart, J. A.; Sotzing, G. A. *Soft Matter* **2008**, *4*, 1448.
(21) Lagadic, I.; Lacroix, P. G.; Clement, R. *Chem. Mater.* **1997**, *9*, 2004.
(22) Gao, F.; Wang, C.; Zeng, H.; Ma, S. *Colloids Surf. A* **2008**, *321*, 7.
(23) Moyano, F. M.; Silber, J. J.; Correa, N. M. *J. Colloid Interface Sci.* **2008**, *317*, 332.
(24) Hof, M.; Lianos, P.; Laschewsky, A. *Langmuir* **1997**, *13*, 2181.
(25) Kim, J.; Lee, M. *J. Phys. Chem. A* **1999**, *103*, 3378.
(26) Correa, N, M.; Biasutti, M. A.; Silber, J. J. *J. Colloid Interface Sci.* **1995**, *172*, 71.
(27) Lunkenheimer, K.; Laschewsky, A. *Prog. Colloid Polym. Sci.* **1992**, *89*, 239.
(28) Pham, T. H. N.; Clarke, R. J. *J. Phys. Chem. B* **2008**, *112*, 6513.
(29) O'Connor, D. V.; Phillips, D. *Time-Correlated Single Photon Counting*; Academic Press: New York, 1983; Chapter 6.
(30) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic: New York, 1999.
(31) Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. J. *J. Org. Chem.* **1983**, *48*, 2877.
(32) Abboud, J-L. M.; Notario, R. *Pure Appl. Chem.* **1999**, *71*, 645.
(33) Kasha, M.; Rawls, H. R.; El-Bayoumi, M. *Pure Appl. Chem.* **1965**, *11*, 371.
(34) McRae, E. G.; Kasha, M. *Physical Processes in Radiation Biology*; Academic Press: New York, 1964; p 23.
(35) Wilkinson, F.; Worrall, D. R.; Vieira Ferreira, L. F. *Spectrochim. Acta* **1992**, *48 A* (2), 135.
(36) Ban, T.; Kasatani, K.; Kawasaki, M.; Sato, H. *Photochem. Photobiol.* **1983**, *37* (2), 131.
(37) Falcone, R. D.; Correa, N. M.; Biasutti, M. A.; Silber, J. J. *Langmuir* **2002**, *18*, 2039.
(38) Togashi, D. M.; Costa, S. M. B.; Sobral, A. J. F. N.; Gonsalves, A. M. d'A. R. *J. Phys. Chem. B* **2004**, *108*, 11344.
(39) Chattopadhyay, A.; Murkherjee, S. *Biochemistry* **1994**, *33*, 5089.
(40) Falcone, R. D.; Correa, N. M.; Biasutti, M. A.; Silber, J. J. *Langmuir* **2002**, *18*, 2039.
(41) Correa, N. M.; Levinger, N. E. *J. Phys. Chem. B* **2006**, *110*, 13050.
(42) Atik, S. S.; Thomas, J. K. *J. Am. Chem. Soc.* **1981**, *103*, 3543.
(43) Lang, J.; Jada, A.; Mallaris, A. *J. Phys. Chem.* **1988**, *92*, 1946.
(44) Peyrelasse, J.; Boned, C. *J. Phys. Chem.* **1985**, *89*, 370.
(45) Riter, R. E.; Undiks, E. P.; Levinger, N. E. *J. Am. Chem. Soc.* **1998**, *120*, 6062.
(46) Zouni, A.; Clarke, R. J.; Holzwarth, J. F. *J. Phys. Chem.* **1994**, *98*, 1732.
(47) Koti, A. S. R.; Periasamy, N. *J. Fluoresc.* **2000**, *10*, 177.
(48) Ueda, M.; Schelly, Z. A. *Langmuir* **1989**, *5*, 1005.
(49) Moyano, F.; Biasutti, M. A.; Silber, J. J.; Correa, N. M. *J. Phys. Chem. B* **2006**, *110*, 11838.
(50) El Seoud, O. A.; Correa, N. M.; Novaki, L. P. *Langmuir* **2001**, *17*, 1847.
(51) Moran, P. D.; Graham, A.; Bowmaker, A.; Cooney, R. P.; Barlett, J. R.; Woolfrey, J. L. *Langmuir* **1995**, *11*, 738.
(52) Correa, N. M.; Silber, J. J. *J. Mol. Liq.* **1997**, *72*, 163.
(53) Correa, N. M.; Durantini, E. N.; Silber, J. J. *J. Colloid Interface Sci.* **1998**, *208*, 96.

JP8105502