Excited-State Behavior of 7-Diethylaminocoumarin Dyes in AOT Reversed Micelles: Size Effects

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Photophysical properties of two 7-diethylaminocoumarin dyes belonging to the family of coumarinylbenzopyrano pyridines (BC I and BC II), and a third dye, 7-diethylamino-4-trifluoromethyl coumarin (C 35), are reported in Aerosol OT (AOT) and water-in-oil (*n*-heptane/AOT/water) microemulsions. In the case of BC I, emission arising from two different environments for the dye is observed. Results of the steady-state fluorescence anisotropy experiments also support the presence of two different environments. At low W_0 (=[water]/[AOT]) values ($0 < W_0 < 10$), the excited-state properties of the molecule in the interfacial region are found to be sensitive to the polarity of the surrounding microenvironment. The decrease in the fluorescence quantum yield at higher W_0 (≥ 10) values can be attributed to the intermolecular hydrogen bonding between the cyano (−CN) group of the dye and water molecules present in the micellar interface. These observations are explained on the basis of the comparable size of the probe dye molecule and the molecular dimensions of the interfacial region in the microemulsions. The large molecular size of BC II is also found to be the reason for the sensitivity of the dye to the microenvironment of *n*-heptane/AOT/water microemulsions. However, the effects are much smaller than those observed in BC I. A similar photophysical study involving C 35 is found to show no such effects. The smaller molecular size, charge neutrality, hydrophobic nature, and a preferential solvation in heptane are essentially the reasons for the results observed in C 35.

Introduction

The extreme sensitivity of the photophysical properties of the aminocoumarin dyes to the surrounding solvent medium has enabled them to be used as fluorescent probes to monitor changes in the microenvironments in organized media.[1−7] Aminocoumarin dyes have also been used to study solvation dynamics in zeolites, micelles, and reversed micelles.[8,9]

The reason for such a wide application range of the aminocoumarin dyes is due to the fact that on optical excitation they undergo an intramolecular charge transfer (ICT) from the electron-donating amino group to the electron-withdrawing carbonyl group of the coumarin ring. This results in a large excited-state dipole moment. With an increase in solvent polarity, the ICT states are successfully solvated. This decreases the energy gap between the lowest excited singlet state and the Franck−Condon ground state, resulting in a large Stokes shifted fluorescence emission. Varying the position and nature of substitutions on the coumarin nucleus can alter spectral and other photophysical properties of these dyes.

The 7-diethyl aminocoumarin dyes have a flexible diethylamino substitution at the 7-position. In homogeneous solvents the photophysical properties of such aminocoumarin dyes having strong electron-accepting substitution at the 3- or 4-position have mainly been explained using the twisted intramolecular charge transfer (TICT) hypothesis,[10−13] the umbrella-like motion (ULM) of the amino nitrogen that assumes a double bond character in the excited singlet state,[14] and the formation of solute−solvent complexes.[15]

However, photophysical studies, other than solvation dynamics,[8,9] of flexible 7-dialkylaminocoumarin dyes in restricted environments containing water, such as alkane/AOT/water reversed micelles, have been found to be less interesting. In addition to being neutral in charge, small in size, hydrophobic in nature, and short-lived in the excited state, these molecules exhibit preferential solvation in the apolar hydrocarbon phase of the reversed micelles. The effects of the interfacial properties of the reversed micelles and the addition of water thereafter are thus concealed beneath the strong fluorescence observed from the dye solubilized in the organic phase.

Recently, several groups have synthesized coumarin heterodimers and explored the possibility of applying them as laser dyes,[16] as organic scintillators,[17] and as triplet sensitizers.[18,19] The role of solvent in the intramolecular charge-transfer processes in such dyes has also been studied.[19−21] These dyes, in addition to being sensitive to the surrounding solvent medium, have an added advantage of being able to sense a multitude of medium effects at the same time. This is due to the large molecular size of the dye. The long molecular axis of these dyes can be up to 15 Å.[20] Since the size of these dyes is comparable to the dimensions of the interfacial region of the reversed micelles, the investigation of their photophysical properties in such organized media (reversed micelles and water-in-oil microemulsions) is likely to be interesting.

In this paper we report mainly the photophysical properties of a coumarinylbenzopyrano pyridine derivative, BC I (Chart 1), in AOT reversed micelles. The spectroscopic characteristics of BC I have recently been investigated in polar aprotic solvents[13] and in dioxane−water mixtures.[21] In the latter, there was some evidence of a weak intermolecular hydrogen bond between the cyano nitrogen and water in the ground state.

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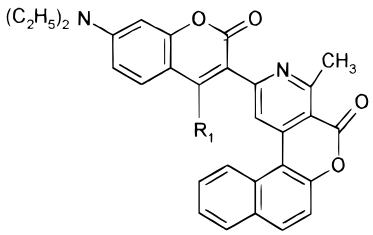
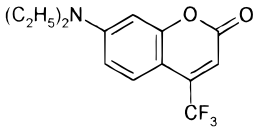
CHART 1: Dye Structures



BC I: R_1 = CN
BCII: R_1 = H



C 35

However, a considerable decrease in fluorescence quantum yield of the dye with an increase in the concentration of water observed in the dioxane−water mixtures was related to polarity, as suggested earlier in polar aprotic solvents,[13] rather than to hydrogen-bonding. The nonradiative deactivation rates can be correlated with the rate of intramolecular charge-transfer processes in the dye. Theoretical calculations (AM1) on another dye belonging to the same family show that, for such dye molecules, the *second* coumarin moiety, which is twisted out-of-plane with respect to the *parent* coumarin in the ground state, is likely to assume a planar conformation, hence extending the π-electron conjugation in the excited singlet state.[20] The dependence of the photophysics of BC I on the concentration of water in dioxane[21] and its structural properties (possible coplanarity of the two coumarin rings in the excited singlet state) thus make an interesting study of the intramolecular charge-transfer processes of the dye in systems encapsulating water, such as reversed micelles.

Surfactant molecules solubilize in hydrocarbon solvents with the charged groups pointing inward and the hydrocarbon tails extended into the bulk solvent, forming reversed micelles.[22] In view of its ease of preparation, stability, and ability to solubilize large amounts of water, Aerosol OT (AOT, sodium 1,4-bis(2-ethylhexyl) sulfosuccinate) is the most widely used surfactant in the study of reversed micelles.[23] Water added to the AOT reversed micelle is solubilized in the polar core, forming a water pool surrounded by a layer of surfactant molecules.[23] In *n*-heptane/AOT reversed micelles, the radius of such a water pool is found to be $2W_0$ Å (W_0 = [water]/[AOT]).[24] The bulk properties (polarity, viscosity, and hydrogen-bonding ability, etc.) of the water inside the pool (free water) and that confined at the interface (bound water) vary with an increase in W_0.[25] The interfacial region is also known to have high ionic strength and a heterogeneous micropolarity.[22−25]

As a comparative study, the photophysical properties of BC II and 7-diethylamino-4-trifluoromethylcoumarin (C 35) (Chart 1) in AOT reversed micelles are also presented. The molecular size of BC II is comparable to that of BC I, but unlike that in BC I the fluorescence is unquenched even in highly polar solvents.[13] The estimated radius of C 35 dye is 5 Å.[12] The spectral and photophysical properties of C 35 in homogeneous solutions are found to be extremely sensitive to the polarity of the surrounding solvent medium.[11,12,14,26,27] To our knowledge, this is the first study of the photophysical properties (other than solvation dynamics) of flexible aminocoumarin dyes in reversed micelles.

Experimental Section

(a) Materials. BC I and BC II are kind gifts from Dr. S. Pal (India). The details of purification of BC I are given elsewhere.[21] The purity of BC II has been checked by thin-layer chromatography and also by comparing the absorption and fluorescence excitation spectra in pure solvents. C 35 and *n*-heptane (spectroscopy grade), both from Aldrich, and AOT (Sigma ultra grade) from Sigma are used as received. The molar ratio of residual water/AOT, as determined by Karl−Fischer titration, was found to be 0.2. Doubly distilled water was used in the preparation of water-in-oil microemulsions.

(b) Sample Preparation. The solutions used in the first experiments were all prepared from a stock solution containing 5 mM BC I in dichloromethane. This stock solution was in turn diluted to another stock solution containing 5 μM dye in *n*-heptane. Binary mixtures of *n*-heptane/AOT were prepared by adding the required amount of AOT into 5 mL each of the second stock solution. For preparation of ternary *n*-heptane/AOT/water mixtures a stock solution containing 5 μM BC I in a binary solution of 0.1 M AOT in *n*-heptane was used. Solutions with different W_0 were prepared by adding appropriate amounts of doubly distilled water, using calibrated micropippets, to the binary stock solution. Solutions were sonicated for 2−3 min in order to achieve a homogeneous ternary mixture. The samples were incubated for 12−18 h at room temperature before carrying out any measurements. An identical method was adopted to prepare solutions of BC II and C 35.

(c) Methods. Absorption spectra were recorded on a JASCO V-560 UV−vis spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS50B luminescence spectrophotometer using a perpendicular geometry arrangement for excitation and emission. Corrected fluorescence spectra were obtained using the correction file provided by the manufacturer. Fluorescence quantum efficiencies (Φ_f) were determined using rhodamine 101 in ethanol and quinine sulfate (BDH, U.K.) in 0.1 N H_2SO_4 as standards.[28] The error in the estimation of Φ_f is ±5%. Fluorescence lifetimes (τ_f) were determined with a PTI LS-1 time-correlated single-photon-counting instrument using a hydrogen flash lamp. Fluctuations in the pulse jitter and intensity were corrected by making an alternate collection of scattering and sample emissions. In this way 10% of the total number of counts at the maximum were collected each time. Data analysis was carried out using the curve-fitting program supplied by the manufacturer. Quality of the fits was determined by the reduced χ^2 and a high Durbin−Watson parameter (>1.7).[29]

Results and Discussion

1. BC I in *n*-Heptane/AOT Binary Mixtures. In *n*-heptane, BC I shows a partly structured absorption spectrum in the visible region. Addition of AOT to an *n*-heptane solution of BC I results in a very small increase in absorbance and a small bathochromic shift of the wavelength of peak absorbance. A significant broadening of the absorption spectrum at the red-edge region (λ > 520 nm), where the dye shows practically no absorbance in *n*-heptane solutions, is observed, as shown in Figure 1a, at and above an AOT concentration of 0.05 M. A broadening of the absorption spectrum of BC I with an increase in solvent polarity has also been found in the earlier studies in pure solvents[13] and in dioxane−water mixtures.[21]

Corrected fluorescence spectrum in *n*-heptane shows characteristic features of 7-aminocoumarin dyes in that it is more structured than the absorption spectrum. The peak at 510 nm is more intense than the one at the red-edge at 550 nm (Figure

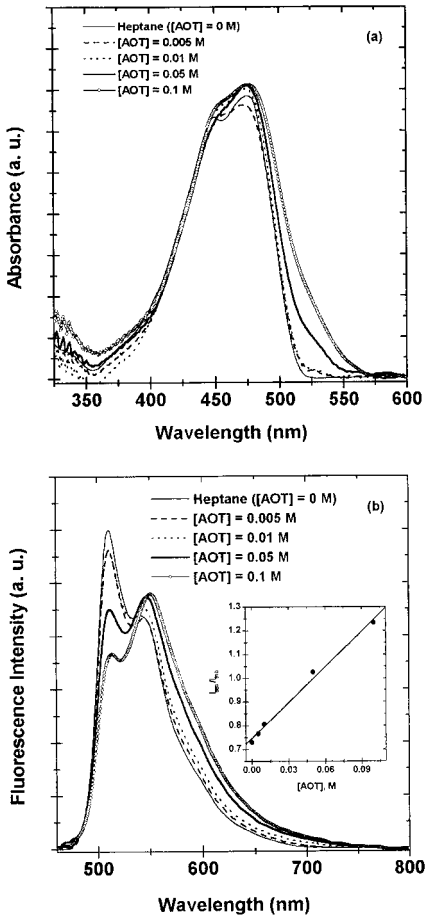


Figure 1. (a) Absorption spectrum of BC I at different concentrations of AOT in heptane/AOT solutions. (b) Fluorescence spectrum of BC I at different concentrations of AOT in heptane/AOT solutions. Inset shows a plot of the ratio of the fluorescence intensity of the peak at 550 and 510 nm of BC I against the concentration of AOT in heptane.

1b). With an increase in the concentration of AOT from 0 to 0.1 M, a gradual change in the fluorescence spectrum is also observed. The ratio of the fluorescence intensity of the two peaks, I_{550}/I_{510}, follows, as shown in the inset of Figure 1b indicating a linear relationship with the concentration of AOT in the binary mixture. Although there is no bathochromic shift of the fluorescence, an overall broadening of the fluorescence spectrum at the red-edge with an increase in the concentration of AOT is clearly observed. No change in the fluorescence spectra upon changing the excitation wavelength was observed for BC I in the binary mixture.

The above findings show that the dye is sensing an increasingly polar microenvironment in both the ground and excited states. The sensitivity is, however, overshadowed by the preferential solvation of the dye in *n*-heptane. Thus, the structured emission profile is retained at all AOT concentrations. This is also reflected in the small decrease in the fluorescence quantum efficiency (Φ_f) of the dye with an increase in the concentration of AOT. The small decrease of Φ_f from 0.84 in pure *n*-heptane to 0.80 in *n*-heptane/0.1 M AOT solutions is, however, close to the experimental error.

In *n*-heptane, the fluorescence lifetime of the dye is ~3 ns and is independent of the emission wavelength. Fluorescence decay measured at 510 nm (and at 550 nm for [AOT] < 100 mM) is exponential and almost independent of the concentration of AOT in the binary solution. The lifetime obtained at this wavelength is similar to the lifetime of the dye in *n*-heptane. On the other hand, biexponential decays, with lifetimes of 3.2 and 4.5 ns, are obtained at 550 nm for an AOT concentration of 100 mM. Fluorescence decays measured at 610 nm are all monoexponential. Fluorescence lifetimes measured at 610 nm change from ~3 to 4.3 ns as the concentration of AOT is increased. The lifetime obtained at higher AOT concentrations is similar to that found in moderately polar homogeneous solutions (chloroform and dichloromethane)[13] and for low water concentrations in dioxane−water mixtures.[21]

These observations could mean that at higher AOT concentrations, the dye is already sensing both the apolar solvent phase and the micellar phase. The absence of an isosbestic point in the absorption spectra, however, rules out any specific interaction in the ground state. This is because BC I and AOT have essentially hydrogen-bond-accepting sites. In the absence of ionic interaction, the association of the dye in the micellar phase due to hydrophobic interactions between the dye and the hydrophobic tail of AOT is one possibility. In addition to the hydrophobic interactions, the electron donor part of BC I (diethylamino group) can be expected to be aligned close to the polar headgroup of the surfactant, giving rise to dipole−dipole interactions.[30−32] In turn, this can lead to the presence of dye molecules in the interface of the reversed micelles (see below). A third reason for the spectral changes could be the formation of a weak hydrogen bond between the cyano substitution in BC I and the water molecules intrinsically present in AOT. The possibility of the formation of such a weak hydrogen bond between the dye and water molecules has been established from a recent study of the photophysical properties of the dye in dioxane−water mixtures.[21] All these could be contributing to the reversal in the ratio of the two emission peaks at 510 and 550 nm with an increase in AOT concentration. However, since the extent of decrease in fluorescence yield is negligible, the hydrogen-bonding effects, even if present, could be very weak. An equilibrium for partition of BC I between the two pseudophases is, however, not achieved even in the excited state, and this is evident from the conspicuous absence of an isoemissive point (see Figure 1b).

2. BC I in *n*-Heptane/AOT/Water Microemulsions. On addition of water to the reversed micelles, further broadening, albeit small, of the absorption spectra at the red-edge and a global decrease in the absorbance is observed (Figure 2a). No isosbestic point, characteristic of an equilibrium between the dye molecules and water in the occupancy of the micellar interface, is found. Interestingly, the wavelength of maximum absorption is almost unchanged for all W_0 values (W_0 = [water]/[AOT]). Since the dye is insoluble in bulk water, it could be occupying the interfacial region of the micelle. A similar broadening of the absorption spectrum at the red edge was observed in a recent study on a xanthene dye: Nile red in *n*-heptane/AOT/water microemulsions.[33] This was also explained as due to solubilization of the dye molecules in the interfacial region. In view of the preferential solvation of the dye in *n*-heptane, the spectral shifts are perhaps not apparent in AOT reversed micelles.

Steady-state fluorescence spectra were recorded for two different wavelengths of excitation: 470 and 540 nm.

(a) Excitation at 470 nm. The fluorescence spectrum, unlike the absorption spectrum, shows greater sensitivity to the presence of water molecules in the reversed micelle. Representative spectra are shown in Figure 2b. For $W_0 = 0$ the ratio of the fluorescence intensity of the two peaks at 510 and 550 nm (I_{550}/I_{510}) is <1. On addition of 1 mol of water ($W_0 = 1$) this undergoes a reversal and becomes greater than unity. This

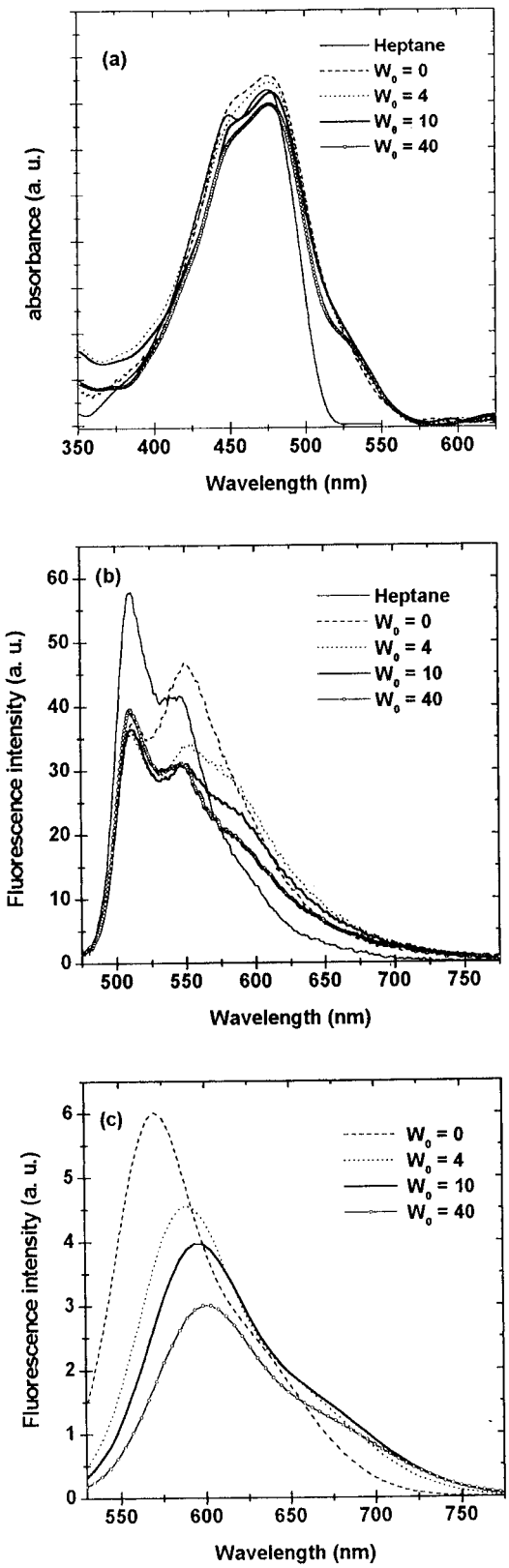


Figure 2. (a) Absorption spectrum of BC I at different values of W_0 in AOT reversed micelles. (b) Fluorescence emission spectrum of BC I at different values of W_0 in AOT reversed micelles with $\lambda_{exc} = 470$ nm. (c) Fluorescence emission spectrum of BC I obtained at different values of W_0 with $\lambda_{exc} = 540$ nm.

structured emission profile is retained at all values of $W_0 \geq 1$, and it resembles the fluorescence spectrum of the dye in pure *n*-heptane. This is a reflection of the hydrophobic character of the dye. However, even for the highest amount of water added to the reversed micelle, the ratio does not reach the value observed in pure *n*-heptane solution.

The broadening of the fluorescence around 600 nm that was observed on addition of 0.1 M AOT to *n*-heptane gradually changes to a shoulder with an increase in W_0. The intensity in this region decreases with an increase in the amounts of water added (Figure 2b). An overall decrease in the fluorescence intensity and the appearance of a shoulder around 590 nm are observed on addition of water.

The sensitivity of the fluorescence emission of the dye to the concentration of water in the reversed micelles (Figure 2b) is masked by its preferential solvation in *n*-heptane. To extract the fluorescence spectrum associated with the dye in a reversed micelle, a synthetic spectrum is constructed by subtracting the fluorescence spectrum in bulk *n*-heptane from that in the ternary mixture, both normalized at 510 nm. Henceforth, these synthetic spectra will be referred to as "heptane-subtracted spectra". The spectral maxima and bandwidths of the heptane-subtracted spectra hence obtained are found to be similar to that obtained on direct excitation of micelle-associated BC I molecules at 540 nm (see below).

Fluorescence lifetime (τ_f) in *n*-heptane is independent of detection wavelength and is 3.05 ns. A complex picture emerges from the measurements of fluorescence lifetimes in reversed micelles and microemulsions. Fluorescence decays measured at 510 nm and for $W_0 = 0$ are all monoexponential. The lifetime obtained at this wavelength is ~3 ns and is similar to that of the dye in *n*-heptane. This is also found to be invariant to W_0. On the other hand, biexponential decays are obtained, for all values of W_0, at the detection wavelength of 550 nm. For $W_0 = 0$, the first lifetime (τ_{F1}) is similar to that observed in *n*-heptane solutions and the second lifetime (τ_{F2}) is comparable to that observed in moderately polar aprotic solvents[13] and for smaller concentrations of water in dioxane−water mixtures (~4 ns).[21] With an increase in W_0, both τ_{F1} and τ_{F2} decrease. Fluorescence decays collected at $\lambda = 600$ nm are monoexponential. The fluorescence lifetime changes from ~4.3 to 3.1 ns as W_0 is increased from 0 to 40. The lifetime obtained at this wavelength and at lower W_0 is similar to that found as τ_{F2} above. All the results are summarized in Table 1.

The biexponential decay observed at 550 nm can be argued to be due to a bimodal distribution of the chromophores, or in other words, to the partitioning of the dye molecules between the oil and the micellar environment with no equilibrium between them. However, for this argument to be true, one of the two lifetimes obtained at 550 nm should correspond to that obtained in pure heptane and be invariant to the changes in W_0. Evidently, this is not the case observed (Table 1). At higher W_0 values the fluorescence characteristics observed at 550 nm could be due to those dye molecules present exclusively in the interfacial region and simultaneously experiencing the microenvironments of the oil−micelle interface as well as micelle−water interface. In other words some dye molecules could also be present in the vicinity of the alkyl tails of the surfactant. The penetration of water toward the oil−micelle interface of the AOT microemulsions is not unlikely.[22] The presence of water in this region can be of great significance to the photophysical properties of the probe dye, more so when the probe dye is insoluble in water but sensitive to the presence of water in its vicinity. Such an observation has also been made by Laia and Costa[34] in the recent study of the fluorescence quenching of a squaraine dye (water insoluble) by water in AOT reversed micelles. We have found that the fluorescence quantum

TABLE 1: Fluorescence Lifetimes and Pre-exponential Factors Obtained for BC I in AOT Reversed Micelles at Different Detection Wavelengths and W_0 Values, $\lambda_{exc} = 470$ nm

Detection Wavelength = 510 nm

	W_0										
	0	1	2	3	4	6	8	10	20	30	40
τ_f (ns)	3.1	3.0	2.9	2.9	3.2	3.0	3.2	3.0	3.2	3.0	2.9

Detection Wavelength = 550 nm

W_0	τ_{F1} (ns)	A_1	τ_{F2} (ns)	A_2
0	3.0	0.68	4.5	0.32
1	3.0	0.69	4.6	0.31
2	3.2	0.75	4.7	0.25
3	3.2	0.81	5.1	0.19
4	2.5	0.4	4.1	0.6
6	2.6	0.82	4.2	0.18
8	2.0	0.61	3.6	0.39
10	2.5	0.67	4.0	0.33
20	1.9	0.27	3.3	0.73
30	1.6	0.27	3.2	0.73
40	1.7	0.36	3.1	0.64

Detection Wavelength = 610 nm

	W_0										
	0	1	2	3	4	6	8	10	20	30	40
τ_f (ns)	4.2	4.3	4.3	4.3	4.3	3.6	3.6	3.3	3.3	3.1	3.0

efficiency and the lifetime of BC I are very sensitive to the concentration of water in dioxane.[21]

The observation of biexponential fluorescence decays is a characteristic feature in reversed micelles and microemulsions. These are generally explained using a model based on adsorption equilibrium between the oil or water-bound and micelle-bound dye molecules.[35] Under such conditions, if the rate of the equilibrium is slower than the lifetime of the dye, biexponential fluorescence decays are obtained. In such situations one of the preexponential factors is usually negative. The converse results in monoexponential decays. Such a general picture, however, is not applicable in the present study because of the fact that monoexponential decays are obtained for emission wavelengths of 510 and 600 nm. The two lifetimes τ_{F1} and τ_{F2} could then correspond to the dye molecules sensing the oil−micelle interface and micelle−water interface, respectively. The quenching of τ_{F1} is likely due to the fact that although the dye is preferentially solvated in *n*-heptane, it is also sensing the polarity associated with the bound water in the oil−micellar interface.

(b) Excitation at 540 nm. Since a broadening of the absorption spectra at the red-edge has been observed on addition of AOT to *n*-heptane, selective excitation of the dye molecules absorbing in this region was attempted. Exciting the dye at 540 nm in reversed micelles results in a single broad fluorescence emission with a peak at about 572 nm for $W_0 = 0$. As observed earlier on excitation at 470 nm, the fluorescence quenches with increasing W_0. A bathochromic shift of about 25 nm is also observed on increasing W_0 (Figure 2c). No fluorescence was observed on excitation of BC I at this wavelength in normal *n*-heptane solutions (figure not shown). This proves that the fluorescence spectrum obtained in Figure 2c is contributed by BC I molecules that are solubilized in the reversed micelle and that they absorb at wavelengths greater than 520 nm. The extent of bathochromic shift and the quenching of fluorescence are, however, found to be smaller than that observed earlier in homogeneous polar aprotic solvents[13] and in dioxane−water mixtures.[21]

TABLE 2: Photophysical Properties of BC I in AOT Reversed Micelles, $\lambda_{exc} = 540$ nm

W_0	Φ_f	$k_r \times 10^{-8}$ (s^{-1})	$k_{nr} \times 10^{-8}$ (s^{-1})	$\Delta\nu^a$ ($\lambda_{exc} = 540$ nm) (cm^{-1})	$\Delta\nu^a$ heptane-subtracted (cm^{-1})
0	0.58	1.38	1.0	2183	2382
2	0.47	1.10	1.23	2197	2442
4	0.47	1.10	1.23	2309	2479
6	0.38	1.05	1.72	2274	2474
8	0.37	1.04	1.77	2274	2473
10	0.35	1.05	1.97	2384	2506
20	0.31	0.94	2.09	2348	2564
30	0.30	0.97	2.26	2347	2560
40	0.28	0.92	2.36	2454	2540

[a] Estimated error is ±10%.

At a particular W_0, the fluorescence spectrum obtained on excitation at 540 nm is similar to the corresponding "heptane-subtracted spectrum" mentioned earlier. A comparison of the bandwidths ($\Delta\nu$) of these two spectra is given in Table 2. It is clear that within the limits of experimental error of ±10%, the two bandwidths are equal.

Fluorescence decays obtained for excitation at 540 nm are all monoexponential. The lifetimes obtained are very similar to the τ_{F2} values obtained above. The photophysical quantities obtained on excitation at 540 nm are also listed in Table 2. The corresponding radiative and nonradiative rate constants, k_r and k_{nr}, respectively, calculated using standard equations are also given in Table 2. The polarity of the microenvironment at $W_0 = 40$ is close to that experienced by the dye in a solvent whose polarity is intermediate to that of tetrahydrofuran (THF) and acetonitrile (ACN).[13] This observation is based on the values of Φ_f, τ_f, and λ_f obtained at $W_0 = 40$ and in THF and ACN. Thus, in the interfacial region, BC I can be assumed to undergo a polarity-induced quenching. In addition, the cyano substitution in the dye can also form a weak hydrogen bond with the water present at the interface. An intermolecular hydrogen bond between the −CN group and water has been found to contribute to the fluorescence quenching of BC I in dioxane−water mixtures.[21] In the reversed micelles of AOT, the microviscosity decreases with an increase in water (W_0)[36] because of the fact that the water bound at the interface is structured. This is also a contributing factor for fluorescence quenching because of the decrease in free volume and enhanced freedom of rotation of the dye, etc.

(c) Fluorescence Excitation Spectra. Fluorescence excitation spectra (FES) of the dye in *n*-heptane at emission wavelengths of 510, 550, and 600 nm resemble the absorption spectrum. On addition of 0.1 M AOT ($W_0 = 0$), FES show considerable changes. FES measured at emission wavelengths of 550 and 600 nm is red-shifted compared to that measured at 510 nm (Figure 3a). For $W_0 = 40$ and as shown in Figure 3b, FES measured at emission wavelengths of 510 and 550 nm overlap. This can be attributed to the hydrophobic character of the dye. BC I hence moves away from the water pool and gets preferentially solvated in the organic phase. On the other hand FES monitored at 600 nm overlaps very well with the absorption spectrum in the red-edge. This proves that the fluorescence observed on excitation at 540 nm is predominantly contributed by the dye molecules absorbing at $\lambda > 520$ nm. These are the dye molecules solubilized in the interfacial region of the reversed micelle.

On the basis of the FES, it can be assigned to two different environments for BC I in the ternary mixture of *n*-heptane/AOT/water. The FES monitored at 510 nm should exclusively correspond to the dye molecules in the bulk organic phase. The

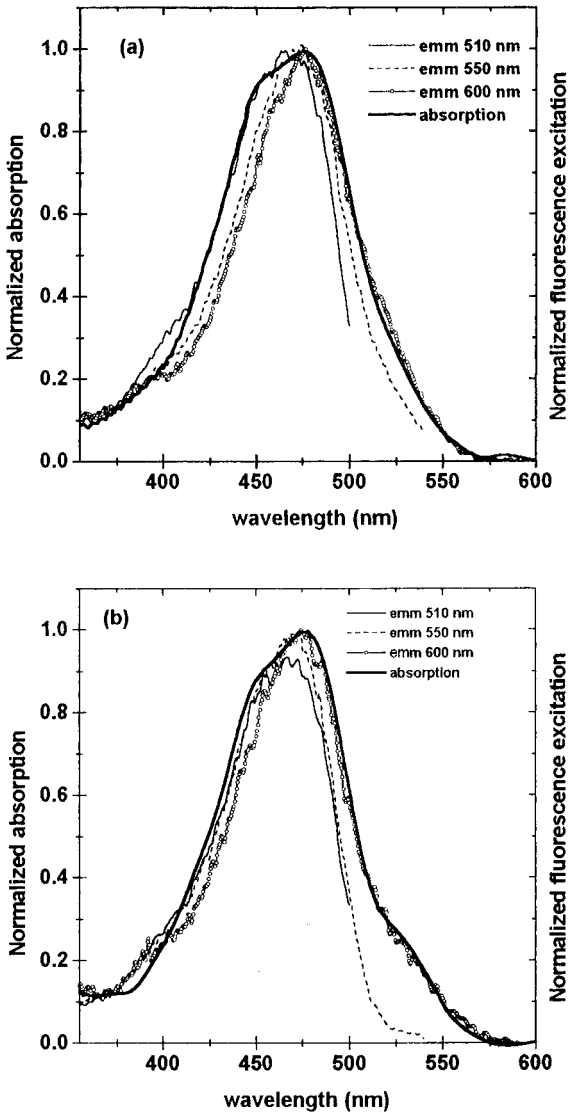


Figure 3. Fluorescence excitation spectra of BC I in reversed micelles collected at different λ_{em}, viz., 510, 550, and 600 nm: (a) $W_0 = 0$; (b) $W_0 = 40$. The corresponding absorption spectrum is also shown.

FES monitored at 550 nm is contributed partly by those dye molecules solubilized in the organic phase and partly by those solubilized in the micellar phase and sensing the oil−micelle interface. The FES measured at an emission wavelength of 600 nm especially gives evidence of the dye molecules sensing the micelle−water interfacial region (absorbing at $\lambda > 520$ nm).

BC I and AOT are neutral and anionic species, respectively. Hence hydrophobic, rather than electrostatic, interactions should favor association of dye and AOT. The results obtained from the present study show that in reversed micelles the dye molecules exist as two noninteracting spectroscopic species and that they can be selectively excited. The spectral and temporal characteristics hence obtained reflect the microenvironment sensed by the dye molecules and the nature and extent of interaction between the solute, AOT, and water at the interface.

(d) Steady-State Fluorescence Anisotropy. Fluorescence anisotropy is the most reliable and widely used technique to ascertain the location of the probe dye in a heterogeneous environment. We have used steady-state fluorescence emission anisotropy to obtain better insight into the location of the probe in the microemulsions. In the fluorescence depolarization measurements in microheterogeneous systems, rotational diffusion of the dye as well as that of the microheterogeneous medium surrounding it is included. Steady-state fluorescence anisotropy (r) was calculated using the relation

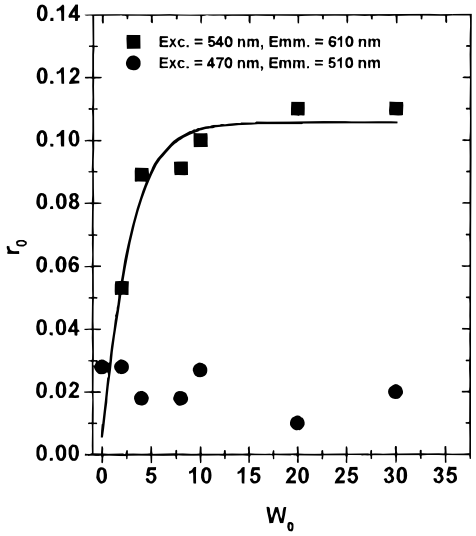


Figure 4. Steady-state fluorescence anisotropy of BC I measured for different excitation and emission wavelengths plotted against W_0 in AOT reversed micelles, at room temperature.

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}) \quad (1)$$

where G is the correction factor for detector sensitivity to the polarization direction of emission and I_{VV} and I_{VH} represent the vertically and horizontally polarized emission intensity obtained on excitation with vertically polarized light. Excitation of BC I was carried out at two different wavelengths (λ_{exc}): 470 and 540 nm. Fluorescence anisotropy was calculated for different W_0 values. Results of the fluorescence anisotropy calculated at 510 nm ($\lambda_{exc} = 470$ nm) and 610 nm ($\lambda_{exc} = 540$ nm) are shown in Figure 4. In either case fluorescence anisotropy, calculated at 550 nm, was equal to that calculated at other wavelengths. Fluorescence anisotropy measurements (summarized in Figure 4) reflect the different spectroscopic character of BC I dye molecules in the microemulsions. The rotational diffusion of the dye in an organic solvent causes fluorescence depolarization, and this is the reason for the very small value obtained for excitation at 470 nm. Thus, the photophysics obtained for excitation at this wavelength is predominantly determined by the behavior of dye molecules present in the organic solvent. Excitation at 540 nm on the contrary gives a nonzero value of anisotropy that shows a nonlinear increase with an increase in W_0.

The two different values of r obtained on excitation at different wavelengths gives support to the arguments made above, on the basis of the fluorescence spectral characteristics and lifetime, that the dye molecules are solubilized in different environments (oil and micelle). Qualitatively, the increase in r with an increase in W_0 can be explained as due to the increase in hydrodynamic micellar volume with an increase in W_0.[37,38] These findings can be understood by considering the molecular dimensions of the probe dye BC I and reversed micelle.

The hydrodynamic radius of the *n*-heptane/AOT/water reversed micelle is given by[39] $r_h = 15 + 1.75W_0$ Å. Thus, with an increase in W_0, the radius of the water pool increases, thereby increasing the hydrodynamic radius of the reversed micelle. However, the size of the interfacial region is unaffected by the increase in r_h. In micelles or reversed micelles biexponential decays can also be obtained when there are two noninteracting

spectroscopic species of the probe molecules with no equilibrium between them.[40] In such cases no rise term will be observed in the fluorescence decay. An elongated probe molecule with size comparable to the dimensions of the micellar interface can be expected to show such an interesting behavior.

As mentioned earlier in the Introduction, BC I is a large molecule with size comparable to that of the micellar region of the reversed micelle. In view of its large size, although the probe molecules solubilize in the microheterogeneous medium spanning from the organic phase into the interface, the spectroscopic nature of the dye is specific and depends on the orientation of the probe molecule and rotational diffusion of the dye. Such a situation is evident from the steady-state fluorescence anisotropy measurements (Figure 4), wherein the value of r is shown to be dependent on the wavelength of excitation and from the fluorescence decays collected at different emission wavelengths.

It can be observed from Table 1 that the fluorescence lifetime measured at 610 nm and the two lifetimes measured at 550 nm (τ_{F1} and τ_{F2}) are nearly invariant to W_0 (within the instrumental resolution of the nanosecond spectrometer) in the region $0 < W_0 < 6$. Quenching of the fluorescence lifetime becomes significant for $W_0 \geq 6$. A similar threshold value of W_0 above which the quenching of the fluorescence of BC I becomes more important is also evident from parts b and c of Figure 2.

A recent NMR spectroscopic study of toluene/AOT/water solution has shown that[41] for $W_0 < 2$ un-ionized hydrates are formed with water molecules apparently trapped in small compartments. Molecular simulation of the reversed micelles in apolar solvents also showed that neither spherical micelles nor a water pool is formed below $W_0 = 2$,[42] implying that it is unlikely that any water pool is formed. At this value of W_0 the AOT aggregates remain compact and rigid.[43] Reversed micelles containing water continue to form up to $W_0 = 4$ during which immobilized water gradually disappears, and this continues up to $W_0 = 10$. Above this value of W_0 free water appears in the center of the water pool and microemulsions are formed.[41] Durocher and co-workers[32] have shown that the effective dielectric constant of the reversed micelle increases monotonically up to $W_0 = 5$ and that it becomes nearly invariant to a further increase in W_0. Hasegawa et al.[37] have found that the microviscosity in the vicinity of the AOT micellar interface remains constant for $W_0 \geq 10$.

On the basis of the above information on the physical and dielectric properties of AOT reversed micelles, the observed photophysical properties of BC I can be explained as follows. In the region $0 < W_0 < 6$ where the concentration of bound water is very small, BC I experiences primarily an increasingly polar environment. The fluorescence spectral shifts and the quenching of fluorescence efficiency and lifetime hence are all analogous to that observed in moderately polar aprotic solvents[13] and for lower concentration of water in dioxane−water mixtures.[21] The effect of intermolecular hydrogen-bonding in this region is likely to be very small. For $W_0 \geq 10$ there is no increase in polarity[32] or viscosity[37] of the micellar interface and only a small bathochromic shift of the emission maxima is observed. Fluorescence efficiency and lifetime on the other hand show considerable quenching as W_0 is increased to 40. The decrease in the fluorescence efficiency and lifetime of BC I for $W_0 \geq 10$ can be attributed to the hydrogen-bonding between the cyano group and the water molecules in the micellar interface. This explains the role of cyano substitution in determining the photophysical properties of BC I in water-containing environments. This is consistent with the results obtained by us in SDS micelles wherein the fluorescence quantum efficiency and lifetime are found to be invariant to the increase in the concentration of the surfactant (below and above the critical micelle concentration). This suggests that in SDS micelles, BC I (a) is solubilized at the micellar interface, (b) always experiences the same polar environment, and (c) is presumably hydrogen-bonded to the water molecules.

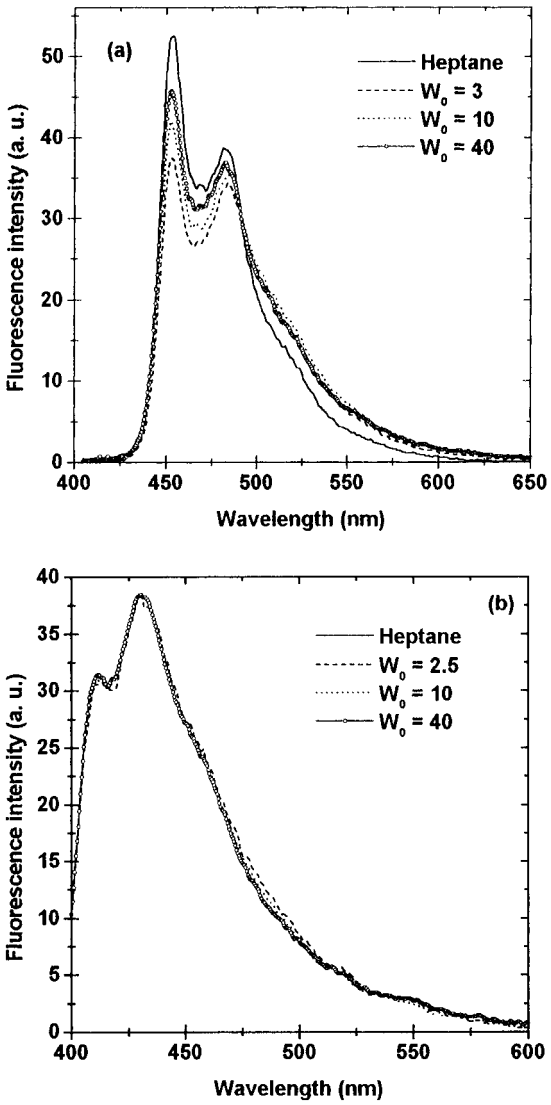


Figure 5. Fluorescence emission spectra of (a) BC II and (b) C 35 in heptane and for different W_0 values in AOT reversed micelles.

3. Study of Other Aminocoumarin Dyes in Reversed Micelles. *(a) BC II in n-Heptane/AOT/Water Microemulsions.* A further support of the role of the molecular size of the flexible aminocoumarin dye in determining its photophysical properties in reversed micelles is provided from the results obtained for BC II in AOT reversed micelles. BC II differs from BC I in the absence of the cyano substitution (Chart 1). In homogeneous solvents the solvatochromic behavior of BC II is similar to that of BC I.[13] However BC I undergoes rapid fluorescence quenching with an increase in solvent polarity, while the extent of quenching of BC II is comparatively small.[13,21] The fluorescence spectra of BC II in reversed micelles are shown in Figure 5a. The broadening of the spectra with an increase in W_0 is evident. The extent of broadening of the spectra and of the spectral shift is smaller when compared to that observed for BC I. This difference can be attributed to the absence of additional hydrogen-bonding acceptor sites in BC II. Further preferential solvation in heptane and the hydrophobic character

of the dye are both evident from the structured emission spectrum of BC II for all W_0 values.

(b) C 35 in n-Heptane/AOT/Water Microemulsions. Spectral and photophysical properties of C 35 have also been investigated in AOT reversed micelles. The most important features of this dye, applicable in the present study, are small molecular size, neutrality of charge, and dramatic decrease of fluorescence efficiency and lifetime in polar solvents. The fluorescence efficiency (lifetime) decreases from nearly unity (4.5 ns) in heptane to 0.01 (0.34 ns) in water.[12,14]

In bulk heptane C 35 shows a structured absorption spectrum with peaks at 375 and 395 nm. The structure in the absorption spectrum is retained for all values of W_0 in AOT reversed micelles. A broadening of the absorption spectrum at the red-edge ($\lambda > 420$ nm), where the dye shows no absorbance in bulk heptane solution, is also observed in reversed micelles. Such a broadening of the absorption spectrum has also been observed earlier in C 153[8b] and also in the present study of BC I (Figure 2a) and can now be regarded as a characteristic feature of aminocoumarin dyes in reversed micelles. The absorbance in this region can be attributed to the dye molecules solubilized in the interfacial region of the reversed micelles.

The fluorescence spectrum of C 35 in bulk heptane is also structured with peaks at 410 and 430 nm. As is the case with the absorption spectrum, the structured profile is retained at all values of W_0 (Figure 5b). A marked difference from the fluorescence spectra of BC I and BC II is evident from this figure. No broadening of the fluorescence spectrum at the red-edge, as is the case with BC I and BC II, is observed with an increase in W_0. The fluorescence efficiency of C 35 remains invariant to the increase in W_0 in the reversed micelle and is also found to be nearly equal to that of the dye in bulk heptane.

The fluorescence lifetime of the C 35 in bulk heptane is measured to be 4.9 ns and is independent of the emission wavelength. In reversed micelles, the fluorescence decay of the dye collected at 410 and 430 nm can be fitted with biexponential fits. Global analysis of the fluorescence decays could be successfully performed, and lifetimes of 2.9 and 4.2 ns could be extracted. The lifetime of the long-lived component is similar to that of the dye in bulk heptane, while a value of 2.9 ns is comparable to that observed for C 35 in moderately polar solvents.[26] No definite correlation between the pre-exponentials factors and W_0 could be established.

The invariance of the absorption and emission spectra and of the fluorescence efficiency and lifetime of C 35 to the amount of water added to the reversed micelles implies that the dye molecules always experience the same surrounding environment. This is unlike the significant quenching of fluorescence efficiency and lifetime of BC I observed for the same increase in W_0 values.

The photophysical properties of C 35 in reversed micelles, as described in the Introduction, are reminiscent of that of other bichromophoric dyes that are small in geometry (size), neutral in charge, and hydrophobic in nature and that show a preferential solvation in the hydrocarbon solvent. Such dye molecules, although they show an interaction with the surfactant molecules and reside at the interface, are preferentially solvated in the bulk organic solvent. This is the reason for the biexponential decays observed at 410 and 430 nm in reversed micelles. The interesting photophysical properties of C 35 observed in polar homogeneous solvents are then not reflected in reversed micellar environments. The effect of the heterogeneous environment of the interfacial region of the reversed micelles does not affect the spectral and photophysical properties of this dye.

Conclusions

The present work shows that the evaluation of the photophysical properties of large aminocoumarin dyes with molecular dimensions comparable to that of the interfacial region of the reversed micelles makes an interesting study. BC I, a hydrophobic aminocoumarin derivative, is preferentially solvated in the organic phase. However, in view of its size it experiences a multitude of microheterogeneous environments and hence polarity. Thus, BC I exhibits steady-state fluorescence anisotropy, fluorescence quantum efficiency, and a lifetime that is very sensitive to the excitation wavelength. At lower W_0 (<10) values, the dye experiences a polar microenvironment and hence undergoes a polarity-driven quenching, in a manner analogous to that found earlier in pure aprotic solvents and dioxane−water mixtures. The −CN substitution present on the dye, in addition, also forms a weak hydrogen bond with the water present in the micellar interface, and this becomes important for $W_0 \geq 10$. The fluorescence characteristics of BC II are additional evidence of the importance of the molecular size of flexible aminocoumarin dyes to enable their study in reversed micelles. The photophysical study of the other aminocoumarin dye C 35 in reversed micelles is found to be uninteresting. This is due to the fact that dyes such as C 35 remain predominantly in the bulk organic phase and do not respond to the changes in the polarity or viscosity of the interfacial region of the reversed micelles.

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