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Photophysics of norharmane in micellar environments: a fluorometric study

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Abstract

Steady state photophysics of norharmane (NHM) has been studied in different aqueous micellar environments. In aqueous solution at pH 7, excitation of the neutral species promotes a rapid transfer of proton giving rise to the corresponding cationic emission. Aqueous micelles differing in their surface charge characteristics interact with the fluorophore differently. The dependence of the fluorescence of the probe molecule on different micelles has been exploited to determine the critical micellar concentrations (CMCs) of the surfactants. The binding constant (K) and free energy change (ΔG) for the interaction of norharmane with the micelles have been evaluated from the fluorescence data. The probable location of the probe in the micelles has also been suggested. Polarity of the microenvironment around the probe has been determined for CTAB and TX-100 micellar systems from a comparison of the variation of fluorescence properties of the two prototropic species in water–dioxane mixture with varying composition.

Keywords: Photophysics; Norharmane; Critical micellar concentration; Microenvironment; Polarity

1. Introduction

Owing to the diverse application of the micellar systems, principally because of the likeliness of the micellar environments with those of proteins, enzymes, etc., and thus mimicking the biological systems, attention has been drawn to the micellar effects on the nature and facts of various photophysical processes [1–3]. Aqueous micellar environments modify a number of photophysical/photochemical processes because of a change in the micropolarity and steric rigidity inside the micellar microenvironments compared to the situation in the bulk aqueous phase. Ionic micelles, due to the presence of the surface charge, can modify a reaction to a great extent when there is a charge separation during the process [4,5].

Norharmane (9H-pyrido[3,4-b]-indole) (NHM) (Scheme 1) belongs to the group of naturally occurring alkaloids. It is a compound linked by a common tricyclic system, adjacent positions being occupied by indole and pyridine rings. The pyridine ring in NHM is π-deficient and the indole ring is π-excess resulting in the existence of two

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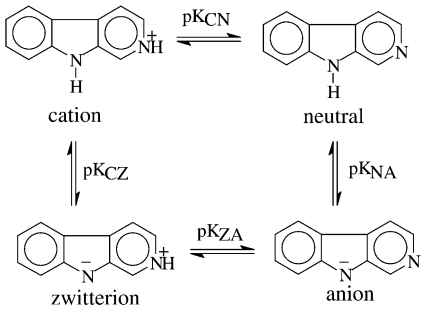

Scheme 1. Different acid–base equilibria for Norharmane; CN: cation–neutral, NA: neutral–anion, ZA: zwitterion–anion, and CZ: cation–zwitterion.

functional sites for acid–base chemistry; in the ground state, the electron density is highest in the mid-plane of NHM, i.e. the pyrrole ring and on excitation to the S_1 state, the electron density migrates to both ends of the molecule, especially to the pyridine nitrogen [6,7]. The wide use of NHM as photosensitizer towards a variety of systems, including bacteria, fungi, viruses, insects, etc. [8,9] and fluorescence standard [10] for biological systems, has drawn the attention of photoscientists. It is also suggested that these compounds are formed as photoproducts from tryptophan in human lenses [11]. A large number of photophysical studies have been made on NHM in different environments and under different conditions [10–17]. The photophysical and/or photochemical properties of NHM have been shown to be very much influenced by the solvents [12,18–20].

Based on the prototropic studies, a number of acid–base equilibria have been proposed for the molecular system (Scheme 1), depending on the pH of the solution. Literature reports indicate that in aqueous medium between pH 1 and 10, only the cationic species emits; at pH ~ 12.3 emissions of all the three species, viz., neutral, cation and zwitterion, have been recorded, and the anionic species starts absorbing only at a pH of approximately 14 [18,20,21].

Studies on the proton transfer in NHM have been made in different solvents like water, dichloromethane, chloroform, ethanol, and acetonitrile–acetic acid mixture [18–21]. Keeping in mind the wide application of the fluorescent molecules susceptible to proton transfer reactions as probes in the studies of biophysical interest, in the present work, we have studied the fluorescence behavior of norharmane in different aqueous micellar environments that are the simple mimics of the real bio-systems. In particular, to study the effects of different micellar surface charge on the photophysics of NHM, common surfactants sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB) and triton X-100 (TX-100) have been used for the generation of anionic, cationic and non-ionic micelles, respectively. Different environmental characteristics have been determined through the study like critical micellar concentrations (CMCs) polarity around the probe. The probe–micelle interaction has also been studied through the determination of the binding constants for the three micellar systems.

2. Experimental section

Norharmane procured from Aldrich was further purified by recrystallizing from ethanol. SDS, CTAB and TX-100 (all from Aldrich) were used as received. Analytical grade hydrated copper sulfate (Loba Chemie, India) was used without further purification for the quenching studies. 1,4 dioxane (Aldrich, spectroscopy grade) was used for the polarity measurement experiments. Triply distilled water was used throughout the experiment. The micellar solutions were prepared freshly to avoid aging [22]. Concentration of NHM was kept at ca. 2×10^{-5} M in all the solutions.

Shimadzu MPS 2000 absorption spectrophotometer and Spex fluorolog-2 spectrofluorimeter were used for the absorption and emission spectral studies, respectively.

3. Results and discussion

3.1. Absorption study

The absorption spectrum of norharmane in aqueous solution shows two bands with maxima at 348 nm and 372 nm corresponding to the neutral and the cationic species, respectively [20]. Gradual addition of SDS (Fig. 1) to the aqueous solution of NHM changes the absorption spectrum signifi-

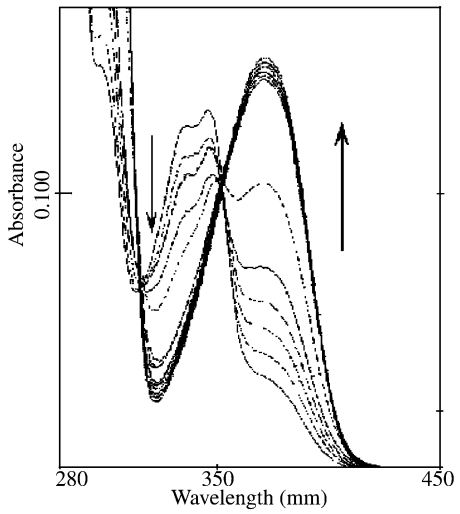

Fig. 1. Absorption spectra of NHM in aqueous SDS micellar environment. SDS concentrations in different curves are: 0.0 mM, 0.6 mM, 0.9 mM, 1.2 mM, 1.5 mM, 2.4 mM, 3.6 mM, 5.1 mM, 6.0 mM, 10.0 mM, 15.0 mM and 18.0 mM, respectively.

cantly. The band at 372 nm corresponding to the cationic species is enhanced with a concomitant decrease in the band at 348 nm corresponding to neutral species resulting in an isosbestic point at 352 nm. In a sharp contrast to this observation, there was hardly any change in the absorption spectrum with the addition of CTAB or TX-100 in the air equilibrated aqueous solution of NHM. The absorption study, thus, reflects that the presence of CTAB and TX-100 in the solution does not modify the ground state prototropic equilibrium of NHM noticeably, while the ground state prototropic equilibrium is favored remarkably towards the cationic species in SDS micellar environment. This is justified considering the additional electrostatic stabilization of the cationic species by the anionic surface charge of the SDS micellar units.

3.2. Fluorescence study

Considering that 352 nm is the isosbestic point in the probe—SDS series and that the absorption spectrum remains practically unaltered in the probe—CTAB and probe—TX-100 series, for the fluorometric studies the probe was excited at 352 nm so as to maintain the same absorbance in a series of micellar solutions. Room temperature fluorescence spectrum of the aqueous solution of NHM (at pH 7) shows a single and unstructured band peaking at 450 nm ascribed to the cationic species [20]. With an increase in the concentration of SDS in aqueous solution of the fluorophore, the fluorescence band shows an initial decrease followed by an increase along with a small bathochromic shift of approximately 10 nm (Fig. 2a). In contrast to the CTAB and TX-100 systems to be discussed later, addition of SDS does not lead to the development of a second emission band corresponding to the neutral species of NHM. In conformity with the absorption study, observation of only the cationic emission band indicates that the cationic species is stabilized in the SDS environment and the effect can be assigned to an electrostatic interaction between the anionic surface charge of the SDS micellar units and the cationic species of NHM. A similar enhancement in the cationic fluorescence during the excited state intermolecular proton transfer reaction of carbazole in SDS micelle was shown by Chattopadhyay et al. [5]. A slight lowering in the environmental polarity, due to the addition of SDS till the critical micellar concentration (CMC) is attained (and micelles are formed), may be assigned to be responsible for the initial decrease in the fluorescence of the cationic species of NHM. It is important to mention here that an increase in the cationic emission of NHM in the micellar environment suggests that the species does not penetrate into the micellar core; rather it sits closer to the micellar surface where it is stabilized.

Gradual addition of CTAB and TX-100 to the aqueous solution of NHM, changes the emission band dramatically. A new blue shifted structured emission band with peak at 380 nm develops at the cost of cationic band at 450 nm (Fig. 2b,c) resulting in isoemissive points at 409 nm and 414 nm in CTAB and TX-100 micellar environments, respectively. Consistent with the existing literature, the structured 380 nm emission has been assigned to the neutral species of NHM [20]. Observation of the isoemissive point confirms that the prototropic interconversion is limited within the cationic and the neutral species only.

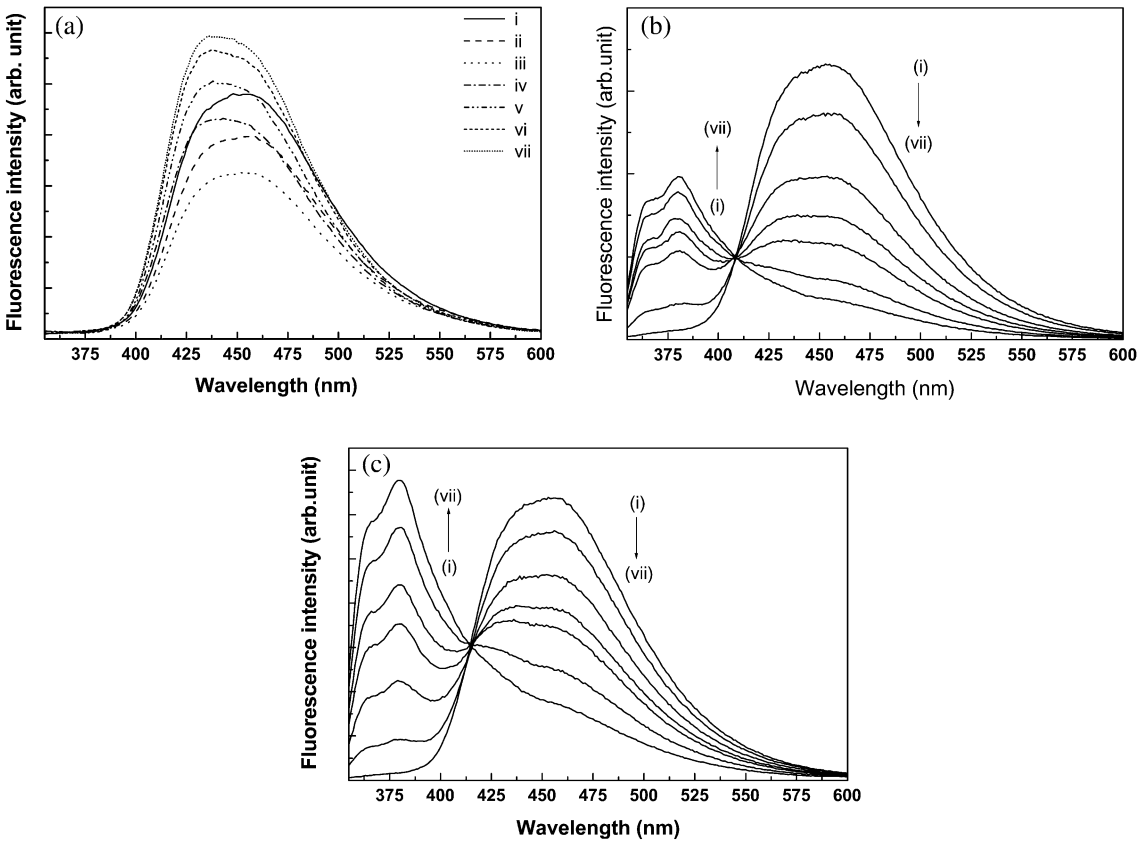

Fig. 2. Emission spectra of NHM solutions as a function of (a) SDS, (b) CTAB and (c) TX-100 concentrations ($\lambda_{exc} = 352$). Curves (i)–(vii) correspond to 0.0, 0.60, 0.9, 1.2, 6.0, 9.0, 27.0 mM in (a); 0.0, 0.9, 1.8, 3.0, 4.8, 7.2, 11.4 mM in (b) and 0.0, 0.6, 1.2, 2.4, 4.8, 8.8, 15.2 mM in (c).

Since the absorption spectrum remains undisturbed with the addition of CTAB and TX-100, the change in the fluorescence properties of NHM is ascribed to the excited state prototropic process. An enhancement in the neutral emission at the cost of the cationic emission in these environments indicates that the excited state proton transfer (ESPT) reaction is favored towards the neutral species, i.e. the deprotonation process is disfavored in the photoexcited state.

The fluorometric behavior of NHM in aqueous CTAB and TX-100 are somehow alike in nature. These observations can be explained considering a lowering in the protopropic character as well as micropolarity around the fluorophore within the micellar environments. It is known that protic solvents (capable of proton donation) have specific influence on the photophysics of NHM. Thus, in water NHM gives only cationic emission as mentioned earlier, while in pure dioxane only the neutral emission exists. A fluorometric study in a varying composition of water–dioxane mixture, to be discussed in Section 3.4, shows a similar enhancement of the neutral emission of NHM and a concomitant decrease in the corresponding cationic emission when the dioxane proportion is increased in the solvent mixture. Since an increase in the dioxane proportion in water–dioxane mixture lowers the polarity of the environment, the variation in the fluorescence behavior of NHM in CTAB and TX-100 indicates that the polarity around the fluorophore bound to the micelles is

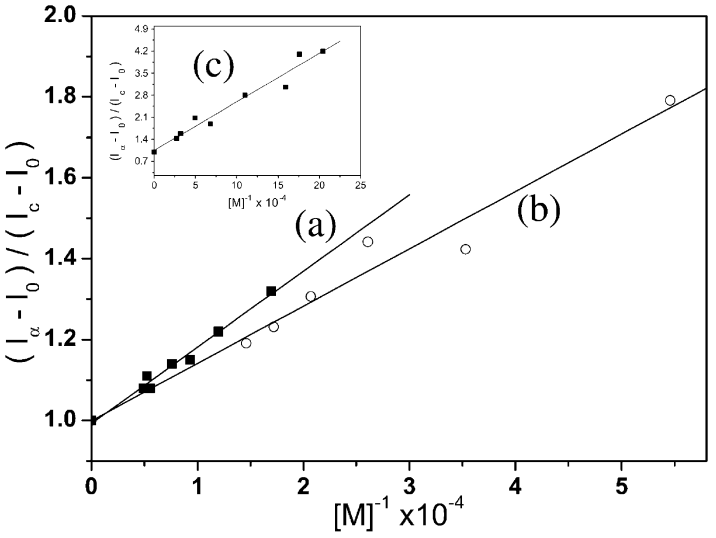

Fig. 3. Plot of $(I_\alpha - I_0)/(I_c - I_0)$ against $[M]^{-1}$ in (a) SDS, (b) CTAB and (c) TX-100. Plot (c) is given separately because of the difference in the scaling of the $[M]^{-1}$ axis.

appreciably less than that in the bulk aqueous phase, assuming the variation in the protic character to change parallel in dioxane/water and micelle/water systems.

It is pertinent to point out here that we have not used buffer media for the present study. To check whether or not a change in the solution pH due to the addition of the surfactants is responsible for the observed change in the fluorometric behavior of the probe, we measured the pHs of the different solutions that we used during our studies. The change in the solution pH was insignificantly small to be responsible for the marked change in the observed fluorescence spectra as confirmed by the blank runs. Thus, solution pH was ruled out to be a responsible factor for the observed changes.

In order to see the interaction between the probe and the micellar units, the binding constants between the probe and micelles have been determined from the fluorescence intensity data for the individual species of NHM following the method described by Almgren et al. [23]. We have,

$$(I_\alpha - I_0)/(I_c - I_0) = 1 + (K[M])^{-1}, \quad (1)$$

where I_0, I_c and I_α are the fluorescence intensities of the particular species of NHM considered in the absence of surfactant, at an intermediate surfactant concentration, and at a condition of complete micellization, respectively; K being the binding constant and $[M]$, the micellar concentration. The micellar concentration $[M]$ is determined by

$$[M] = (S - \mathrm{CMC})/N, \quad (2)$$

where S represents the surfactant concentration and N is the aggregation number of a micellar system. The values of N for SDS, CTAB and TX-100 have been taken as 62, 60 and 143, respectively, following the works of Saroja et al. [24].

The binding constant (K) values have been determined from the slope of the plots of $(I_\alpha - I_0)/(I_c - I_0)$ against $[M]^{-1}$ (Fig. 3). In CTAB and TX-100 micellar systems where two emissions exist, the data were plotted considering both cationic and neutral species of NHM individually. Representative plots monitoring the neutral species are presented in Fig. 3. The estimated binding constant values ($\pm 15\%$) are in the range of values for some other systems reported earlier [23,24]. In CTAB and TX-100 micelles, the binding constants determined considering the neutral and the cationic species are nearly the same and thus confirm each

Table 1
Binding constants and free energy changes for NHM–micelle interaction

Surfactant	Binding constant $(K)\times10^{-4}$/l mol^{-1}	ΔG^0/kJ mol^{-1}
SDS	5.70	27.31
CTAB	7.05	27.84
TX-100	6.50	27.64

other. From the K values, the free energy changes for the probe–micelle binding process for different micellar systems have been calculated at ambient temperature. The values are presented in Table 1.

3.3. Determination of CMCs

Variation of the fluorescence intensities of different prototropic species of NHM as a function of surfactant concentration serves as a sensitive parameter to determine the critical micellar concentrations (CMCs). For SDS, the fluorescence intensity of the cationic band is plotted against the concentration of the surfactant. In CTAB and TX-100, where two emissions exist, the fluorescence intensity of both the species of NHM was plotted as a function of the respective surfactant concentration. Representative plots monitoring the neutral species are presented in Fig. 2 b,c, respectively. All the plots give rise to a number of break points,

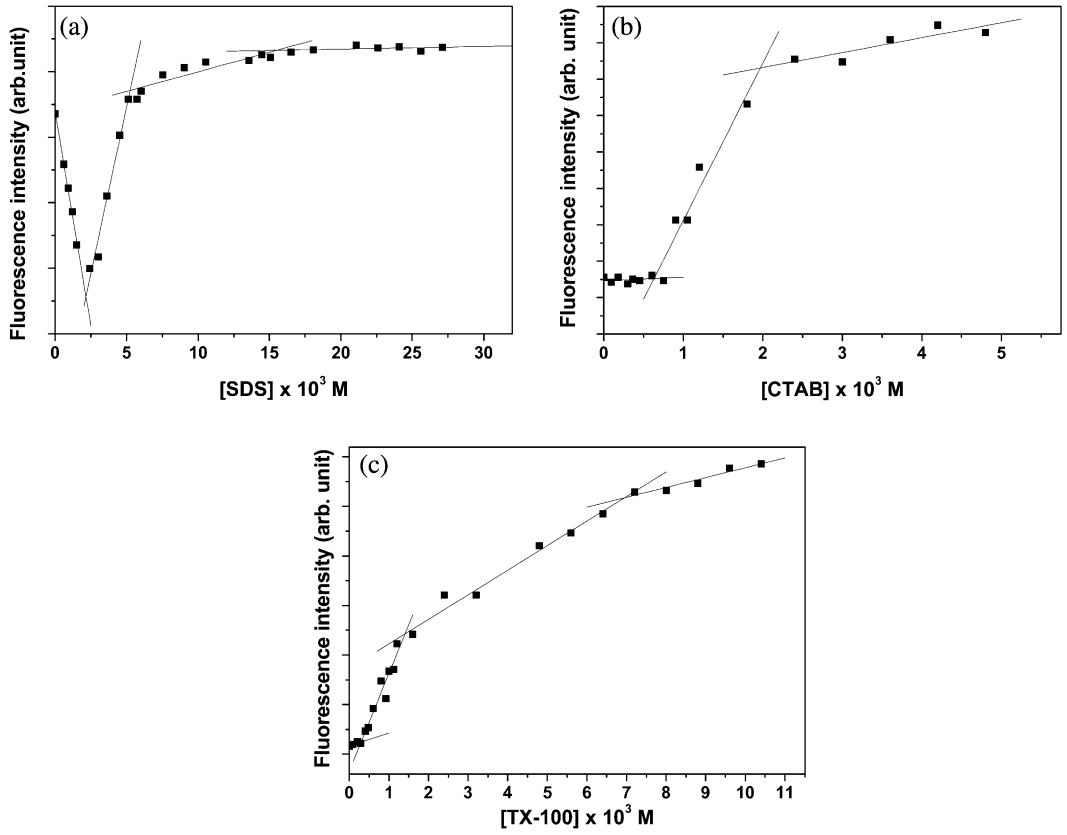

Fig. 4. Variation of fluorescence intensity of a prototropic species of NHM as a function of concentration of (a) SDS, (b) CTAB and (c) TX-100. (a) Refers to cationic species, and (b) and (c) refer to the neutral species of the probe (for detail see text). λ_{exc}= 352 nm.

Table 2
CMCs for aqueous SDS, CTAB and TX-100 systems

Surfactant	Estimated CMCs (mM)			Literature CMCs (mM)			Ref.
	CMC_1	CMC_2	CMC_3	CMC_1	CMC_2	CMC_3	
SDS	2.2	5.2	15.4	1.5	5.0	14.7	[28]
				2.0	6.0	15.0	[26]
				3.0	–	12–13	[32]
CTAB	–	0.65	1.95	0.30	0.75	–	[25]
				0.25	1.00	–	[26]
				–	0.70	1.6	[35]
TX-100	0.26	1.35	6.9	0.20	0.90	–	[28]
				0.29	–	5.4	[30]
				0.25	–	7.3	[33]

which, in tune with the existing literature, are corresponded to the critical micellar concentrations (CMCs). The CMC values obtained considering the neutral and cationic species are same within experimental limit. Multiple CMCs are obtained for all the systems studied (Fig. 4).

By now, multiple break points (or CMCs) are established from a number of current scientific reports [26–30]. Certain physical properties such as conductivity, surface tension, and osmotic pressure, when plotted against surfactant concentration, show multiple break points assigned to different CMCs [31]. Fishman and Eirich have shown two break points for SDS at 3 and 12–13 mM when they plotted reduced viscosity against the surfactant concentration [32]. Basu has shown two break points for TX-100 at 0.25 and 7.3 mM when he plotted non-radiative rate constants against surfactant concentration [33]. Multiple break points for all the surfactants have been shown by Sarkar et al. while monitoring fluorescence quantum yield of 2-(2′-hydroxyphenyl) benzimidazole against surfactant concentrations [34] although they did not report them as CMCs. Similarly, while plotting the fluorescence polarization anisotropy of different fluorescent probes against the surfactant concentrations, Dennison et al. and Chaudhury et al. have observed multiple break points which they have assigned as CMCs for the three surfactants same as those used in the present experiment [30,35]. Table 2 presents the determined CMC values along with the literature values for comparison.

Our estimated CMC values for these surfactant systems agree well to the CMC values reported earlier. Interpretation of the multiple CMCs is not, however, obvious at the moment. Very often one break is considered as CMC. The higher one is generally assigned to some phase transformation corresponding to a change in micelle size and/or shape [5], and the break point at a lower surfactant concentration is assigned to pre-micellar aggregation. Proper interpretation of multiple CMCs is still awaited.

3.4. Polarity of the micellar microenvironment and probable location of the fluorophore

Micelles are characterized by three distinct regions: a non-polar core formed by the hydrocarbon tails of the surfactant, a compact stern layer having the head groups, and a relatively wider and diffuse Gouy–Chapman layer that encompasses majority of the counter ions [1]. Depending on the nature of the probe and the micelle, a probe molecule may bind either to the non-polar core of micelles or to the micelle–water interface.

We have studied the fluorescence behavior of NHM in water–dioxane mixture of varying composition (Fig. 5). From Fig. 5 it is evident that with a decrease in water proportion in the solvent mixture the neutral band intensity increases at the cost of the cationic band. This means that as the polarity of the microenvironment around the probe is reduced, the cationic band is destabilized resulting in an increase in the neutral band. Since the

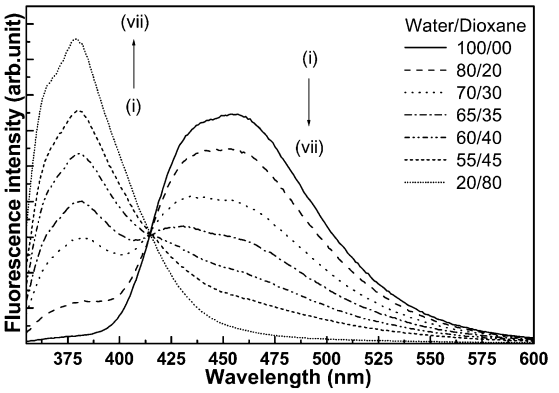

Fig. 5. Emission spectra of NHM in water–dioxane mixture under the same experimental condition. Composition of the solvent mixture is given in the inset. $\lambda_{exc} = 352$ nm.

water/dioxane mixture resembles with the micellar environment [36], the variation of the logarithm of the ratio of neutral to cation band intensities of NHM in the water/dioxane mixture has been compared to that of the micellar environments for studying the microenvironment around the probe in the latter media. The increase in the neutral to cation relative fluorescence yield in the CTAB and TX-100 micellar environment indicates that the microenvironment around the probe in both the cases is less polar than the bulk water. In order to get a quantitative measure of the polarity at the binding site of the micelle with the fluorophore empirical solvent polarity parameter, $E_T(30)$, based on the transition energy for the solvatochromic intramolecular charge transfer absorption of the betaine dye 2,6-diphenyl-4-(2,4,6 triphenyl-1-pyridino) phenolate as developed by Reichardt has been used [37,38]. Representative plot monitoring the log of the neutral to cation intensity of the fluorophore in water–dioxane mixture vs. $E_T(30)$, as presented in Fig. 6, establishes a linear correlation between the two parameters.

Comparing the value of the log of the neutral to cation intensity ratio of NHM in CTAB and TX-100 environments, with the above correlation, we have determined the micropolarity around the probe to be 54.1 and 54.3, respectively (Fig. 6). The values are in agreement with the literature reports for the interfacial polarities of these micellar systems estimated earlier using betaine dye [39], 1-anilino-8-napthalene sulfonate (ANS) [40] and 4-*N*,*N*-dimethylamino-3-hydroxyflavone [30]. The present experiment, thus, suggests that the probe does not penetrate into the micellar core; rather it binds with the micellar units at the interfacial region.

In SDS, the probe molecule shows only one band (cationic), so the above technique could not be applied for the determination of micropolarity around the probe in this environment. However, to assign the probable location of the fluorophore in the SDS micelle, heavy atom induced fluorescence quenching study has been performed using copper ion as quencher. The idea behind the experiment is the following. The ionic quencher is not supposed to be available in the micellar core due to the very low micropolarity in the region. It is expected to be available in aqueous phase as well as in the micelle–water interfacial region. Moreover, being positive in nature, the availability of the quencher in the interfacial zone is supposed to depend on the surface charge of the micellar units and it is expected to be remarkably more in SDS micellar system that has a negative surface charge. Hence, had the fluorophore been located into the micellar core, there should not be appreciable fluorescence quenching due to the lack of the

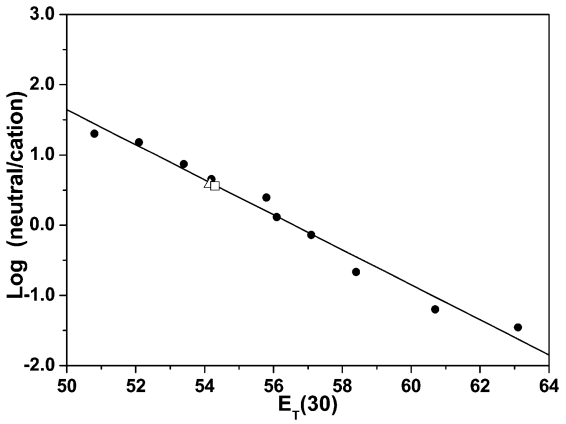

Fig. 6. Variation of log of neutral to cation fluorescence yield of NHM in water–dioxane mixture against $E_T(30)$. The experimental values of the former parameter in CTAB and TX-100 environments have been inserted in the figure.

quencher (Cu^{2+}). Had the probe been situated in the micelle–water interface, the Cu^{2+}-induced quenching of its fluorescence is supposed to be remarkably different from that in the aqueous medium.

Thus, for SDS system, where the polarity dependent fluorescence technique could not be exploited, the Cu^{2+}-induced fluorescence quenching of the probe in this environment relative to that in pure aqueous phase can be useful in determining whether the fluorescing moiety is located in the interfacial region or in the non-polar micellar core. The very efficient quenching of the fluorescence of NHM in SDS micelle compared to that in aqueous medium ($K_{SV}^{SDS} = 1200\ mol^{-1}$ and $K_{SV}^{water} = 20\ mol^{-1}$) indicates that the fluorophore moiety is very much available to the quencher for interaction. This leads to the proposition that the fluorophore is not embedded into the non-polar core of the micelle; rather it binds to the micelle in the micelle–water interfacial region where there is a rich concentration of Cu^{2+} due to the electrostatic interaction between the cationic quencher and the anionic surface charge of the SDS micellar units. Different authors have suggested that different probes can locate themselves in the micelle–water interfacial regions [41,42]. Schematic pictures have been given by the authors and the reader is referred to the concerned figures.

It is known that large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV) are systems mimicking more to the real bio environments than the micellar systems do. However, micellar systems are simpler than the lipid environments. Photophysical studies of the natural alkaloid norharmane will be extended to LUVs and SUVs with a better understanding of the phenomena through the present study.

4. Conclusion

The work reports the study of the photophysics of norharmane, a biological photosensitizer, in different aqueous micellar environments. The photophysical behavior of NHM is modified remarkably in these media compared to that in pure aqueous phase. This has been exploited to determine the CMCs of the micelles. In the anionic surfactant SDS, norharmane is incorporated in micelles as its cation, whilst in CTAB and Triton X-100 it goes in as the neutral form. The present study reveals that the probe does not penetrate into the micellar core and binds to the micelles at the micelle–water interfacial region. Micropolarity around the probe in CTAB and TX-100 micelles has also been determined in terms of $E_T(30)$.

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