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An Understanding of the Modulation of Photophysical Properties of Curcumin inside a Micelle Formed by an Ionic Liquid: A New Possibility of Tunable Drug Delivery System

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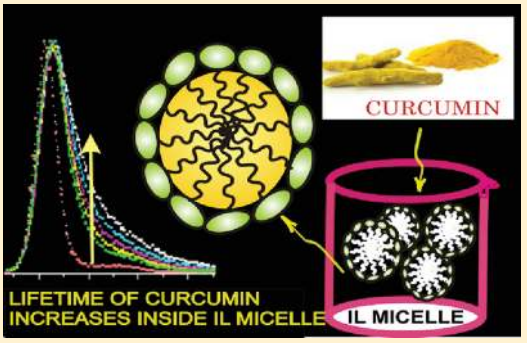
Supporting Information

ABSTRACT: The present study reveals the modulation of photophysical properties of curcumin, an important drug for numerous reasons, inside a micellar environment formed by a surfactant-like ionic liquid (IL-micelle) in aqueous solution. Higher stability of the drug inside IL-micelle in the absence and presence of a simple salt (sodium chloride) as well as considerably large partition coefficient (K_p = 8.59 × 10^3) to the micellar phase from water make this system a well behaved drug loading vehicle. Remarkable change in fluorescence intensity with a strong blue-shift implies the gradual perturbation of intramolecular hydrogen bond (H-bond) present within the keto−enol group of curcumin along with considerable formation of intermolecular H-bond between curcumin and the headgroup of surfactant-like IL. Very fast nonradiative decay channels in curcumin mainly caused by the excited state intramolecular proton transfer (ESIPT) are thus depleted remarkably in the presence of IL-micelle of reduced polarity and as a result of restricted rotational and vibrational degrees of freedom when bound to the micelle. Moreover, time-resolved results confirm that not only the keto−enol group of curcumin is playing here but also the phenolic hydroxyl groups are also responsible for such modulation in photophysical properties. From a thermodynamic point of view, our system shows good correlation with its stability parameters (higher binding constant with very less hydrolytic degradation rate ∼1%) and higher negative value of binding enthalpy of interaction ($-\Delta H$) than total free energy change ($-\Delta G$) implies that the nature of binding interaction is enthalpy driven not entropy alone. Summarizing all the above observations, we have concluded that the modulation of the intramolecular proton transfer is due to the presence of both intermolecular proton transfer as well as strong hydrophobic interaction between curcumin and the IL-micelle.



1. INTRODUCTION

Curcumin (diferuloylmethane), the Indian solid gold, the major active component of turmeric, *Curcuma longa* L. (Zingiberaceae), is used as a spice in curry and as a coloring agent in yellow mustards, cosmetics, pharmaceuticals, and hair dyes.[1] It has attracted great interest in recent years because of its antioxidant, anti-inflammatory, and potential cancer chemopreventive activities.[2−9] Recently, curcumin has also been found to bind to α-amyloid proteins in models of Alzheimer's disease.[10] Moreover, curcumin can inhibit the metabolic action of aflatoxin B_1,[11] aminopeptidase N,[12] lipoxygenase,[13] cyclooxygenase,[14] and ornithine decarboxylase.[15] Finally, it seems to have a potential in the treatment of cystic fibrosis[16] and can be considered as a model substance for the treatment of HIV infection.[17−19] In considering potential mechanisms for its range of biological activities, it is important to acknowledge the inherent chemical features of the curcumin molecule. Although the systematic name for curcumin 1, 7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepatadiene-3,5-dione, implies that curcumin is a α-diketone tautomer. X-ray crystal structure analysis has established that curcumin and its bisacetoxy derivative exist as a keto−enol form.[20,21] Recently, the ^{1}H and ^{13}C NMR studies have confirmed the presence of the enol form in nonpolar, polar, and protic solvents with NMR spectroscopy.[22] Theoretical results also support the existence of the enolic form having perfect resonance between the two phenolic rings, and the electron density is distributed on the entire molecule.[23−27] The enolic form can exist in different cis and trans isomeric forms depending on the temperature, polarity, or hydrogen bonding nature of the solvents.[28,29] Formation of the cis keto−enol structure becomes preferred because of its large dipole moment (7.7 and 10.8 D in the gas and solution phases, respectively), which leads to formation of a strong intramolecular H-bond, as well as the extended conjugation of the molecular backbone compared with that of the diketo form.[23,30] In fact, the keto−enol form can be considered as coexisting in two equivalent keto−enol tautomers which may interconvert between each other via an intramolecular hydrogen atom transfer (IHT) process.[26] Very recently, Adhikari et al. proposed that cyclocurcumin also undergoes photoinduced

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cis−trans isomerization and the trans form is more stable than the cis form.[31] Several recent studies in various solvents,[32−38] micelles,[36,38,39] vesicles,[35,40,41] etc., confirm the fastest non-radiative S_1-decay kinetics of curcumin proceeds through an excited state intramolecular proton transfer (ESIPT) between the hydroxyl group and the keto group of curcumin followed by radiationless excited-tautomer decay in closed cis-enol tautomer. Support to the occurrence of radiationless decays of curcumin through ESIPT paths has been provided in a recent femtosecond fluorescence upconversion study.[42]

To better understand the physicochemical basis of interaction of curcumin with other biological/chemical molecules, it is necessary to study its fundamental spectroscopic and physicochemical properties. However, the major problem with curcumin is its extremely low solubility in aqueous solution (2.99×10^{-8} M) and its poor bioavailability, which limits its clinical efficacy.[42−44] Curcumin is stable at low pH in aqueous alcohol solutions but undergoes hydrolysis and chemical degradation at basic pH.[45,46] Even under physiological pH conditions, degradation was significant. To resolve this serious issue, attempts have been made through encapsulation in polymeric micelles, liposomes, polymeric nanoparticles, lipid-based nanoparticles, and hydrogels to increase its aqueous solubility and bioavailability.[47−54] Encapsulation of hydrophobic drug molecules inside an aqueous nanoparticulate system has been attempted so as to deliver such drugs with their full potential. In our present work, we have used a micellar system formed by an ionic liquid (IL), 1-butyl-3-methylimidazolium octyl sulfate ($[C_4mim][C_8SO_4]$), in aqueous solution. A special feature of this IL is that it can form micelles (CMC = 31−37 mM) in water.[55] In recent studies, there is a tremendous interest to investigate photophysical, chemical properties of many compounds in room temperature ionic liquids (RTILs). Moreover, the effect of addition of polar solvent to RTILs and RTILs containing microemulsions has received much attention. Several groups prepared and characterized RTILs containing micelles and microemulsions.[56−60] The self-aggregation behavior of RTILs in aqueous solution has recently attracted much attention due to structural similarities of RTILs with ionic surfactants, and the thermodynamics and phase equilibria of the ionic liquid water mixtures have been studied in detail[61] because this knowledge is of great importance to the development of extraction methods. Another reason for choosing $[C_4mim][C_8SO_4]$ is due to its greenness with respect to other commonly used RTILs such as $bmimPF_6$, $bmimBF_4$, etc. $[C_4mim][C_8SO_4]$ can undergo modest biodegradation, while other commonly used RTILs show negligible biodegradation.[62−64] Generally, the C_4mim ionic liquids are treated as poor candidates in close bottle tests (OECD 301D) for their biodegradability.[65] 1-Butyl-3-methylimidazolium octylsulfate was the only ionic liquid that experienced a significant level of degradation (25%), but the level of achievement was 60%.[62] If the side chain of the imidazolium cation contains an ester group, then it was found to have higher biodegradability with all of the anions that were tested. The combination of such ester containing cation and the octyl sulfate anion shows the highest biodegradability in this series (in closed bottle test).[62] However, all these ILs have been proved that they are toxic enough for using in our body system and it is still now a debatable issue.[62,66−71] However, there is now a growing interest in the materials applications of ILs which utilize novel tunable physical and chemical properties and more recently the chemical properties, the toxicity, a biological property has been one of the most highly debated topics in this field.[72] Indeed, toxicity of the ILs is also a tunable property of ILs, and given the similarities between many common IL building blocks and active pharmaceutical ingredients (APIs) or API precursors.[73] Research on modulation of molecular architecture of ionic liquids makes them a wide range of newly formed compounds having tunable active pharmaceutical ingredients with the advantage of higher solubility, bioavailability, modest stability, etc. For example, lidocaine docusate, ranitidine docusate, and didecyldimethylammonium ibuprofen are the newly synthesized drugs which have controlled and modified efficacy compared to their precursors.[74] An ionic liquid-in-oil microemulsion (Tween80/span20/dimethylimidazolium dimethylphosphate) has been used as a potential carrier of the sparingly soluble drug acyclovir (ACV) for excellent solubility and skin permeation enhancing effect.[75] Recently, it was observed that ionic liquid can act as dual functional antimicrobial agents and plasticizers in medical devices.[76]

Herein, we have carried out a spectroscopic investigation of curcumin in a micellar environment formed by an ionic liquid. We have also determined the partition coefficient of curcumin inside IL-micelle and their binding constant in aqueous solution. A temperature dependent study as well as a salt addition study has also been carried out to get an idea about the location of the drug molecule and the nature of interaction with the micelle. We also want to monitor the hydrolytic stability of this compound inside IL containing micelle which can be useful for its use as a drug carrier in the near future. Our main objective is to unravel the photophysical properties of curcumin and its nature of hydrogen bonding and hydrophobic interaction with RTILs containing confined environment. Another important aspect to understand the sensitivity of the fluorescence properties of curcumin with change in polarity around its microenvironment in micelle is also discussed here. More importantly, by using simple photophysical spectroscopy, here we want to get detailed knowledge about a stable drug−micelle system, which has a wide range of tunable properties to make it very special in pharmaceutical research.

2. EXPERIMENTAL SECTION

2.1. Materials. $[C_4mim][C_8SO_4]$ was obtained from Fluka and purified according to the literature procedure.[77] Curcumin obtained from Sigma Aldrich (purity ∼81%) was purified by the column chromatographic technique, and the purity of the sample used (∼99%) was checked by the HPLC technique.[38] The structures of the IL and curcumin are given in Scheme 1. Milli-Q water was used to prepare all solutions. The concentration of curcumin in the experiment was kept at 4×10^{-6} M.

Instruments and Methods. The absorption and emission spectrum was measured with a Shimadzu (model UV 2450) UV−vis spectrophotometer and Jobin Yvon-fluoromax-3. All the fluorescence emission spectra and steady state anisotropy at a particular wavelength were corrected for the wavelength sensitivity of the detection system. Steady state anisotropy is defined as[78]

$$r_0 = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}} \quad (1)$$

where G is the correction factor. I_{VV} and I_{VH} are the fluorescence decays polarized parallel and perpendicular to the polarization of the excitation light, respectively.

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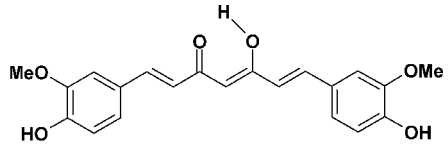
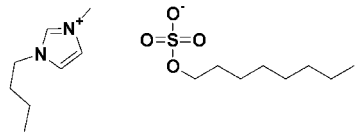
Scheme 1. Structure of Curcumin and the Ionic Liquid



Curcumin



1-Butyl-3-methylimidazolium Octyl Sulfate (bmimOs)

Fluorescence lifetimes were obtained from a time-correlated single photon counting (TCSPC) spectrometer using nano-LED (IBH, U.K.) as the light source at 408 nm. The experimental setup for picosecond time correlated single photon counting (TCSPC) has been described elsewhere.[79] Briefly, the samples were excited at 408 nm using a picosecond laser diode (IBH, Nanoled), and the signals were collected at the magic angle (54.7°) using a Hamamatsu microchannel plate photomultiplier tube (3809U). The instrument response function of our setup is 90 ps.

The average fluorescence lifetimes for the decay curves were calculated from the decay times and the relative contribution of the components using the following equation[78]

$$\tau_{AV} = \tau_1 a_1 + \tau_2 a_2 \quad (2)$$

where τ_1 and τ_2 are the first and second components of decay time of curcumin and a_1 and a_2 are the corresponding relative weightage of these components.

We have calculated the quantum yield of curcumin with the following equation[78]

$$\Phi_X = \Phi_{ST}\left(\frac{A_X}{A_{ST}}\right)\left(\frac{Abs_X}{Abs_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right) \quad (3)$$

where Φ_X and Φ_{ST} are the quantum yield of the experimental solution and standard solution taken. A_X and A_{ST} are the integrated area under the fluorescence curve, and Abs_X and Abs_{ST} are the absorbances. Quinine sulfate in 0.1 N H_2SO_4 solutions was taken as a standard solution.

For size measurement, we have done dynamic light scattering measurement using a Malvern NanoZS employing a 4 mW He−Ne laser (λ = 632.8 nm). Temperature was maintained by using a circulating temperature bath.

3. RESULTS AND DISCUSSION

3.1. Steady State Results.

3.1.1. Effect of IL Concentration.

3.1.1.a. UV-Fluorescence Results. Although curcumin is barely soluble in water, the solubility is considerably increased in the presence of a surfactant-like long chain ionic liquid containing aqueous solution. The presence of the micellar phase is the reason for this increment, and it indicates that the partition of curcumin to the micellar phase from the bulk water is large enough. Absorption and fluorescence spectra of curcumin have already been well characterized by different groups in different kinds of solvents and media.[8,38,80−82] Curcumin shows strong absorption bands at 426, 423, and 432 nm in TX-100, DTAB, and SDS micelles, respectively.[83] In our study, we have obtained a structured and intense peak at 431 nm with another peak at 350 nm in water, but it gradually blue-shifted to 423 nm upon addition of IL (Figures 1 and 2 in the Supporting Information). Curcumin absorption is $\pi-\pi^*$ type in nature, and gradual increment in absorbance is observed with addition of IL in aqueous solution. The presence of an additional peak at 350 nm and absence of any shoulder at 442 nm in pure water and the absence of the peak at 350 nm and presence of the shoulder peak at 442 nm after addition of the IL suggests that curcumin is present in a vastly different environment in the presence of IL in water. Close inspection of the UV spectra shows that it is very similar to the UV spectra that were observed inside TX-100 and DTAB micelle and in methanol and DMSO.[84] However, such a shoulder in the absorption peak is absent for SDS micelle and such observation was explained by the presence of strong interaction of water with curcumin located in the Stern layer. This information helps us to consider that in our case curcumin is located far inside from the hydrodynamic shear surface of the micelle.

Similar to absorption spectra, a large blue-shift of 30 nm is obtained for fluorescence spectra upon addition of the ionic liquid up to 60 mM (Figure 1).

Measurement of the fluorescence Stokes shift of a probe molecule is a very useful technique to measure reorganization of the probe's local environment. Because of changes in the chromophore's properties (charge distribution, polarizability, size, etc.), the excited-state energy minimizes at a different value

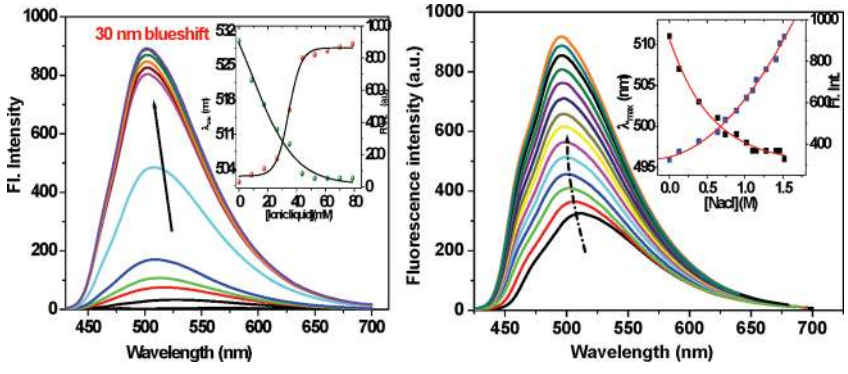


Figure 1. Fluorescence spectra of curcumin (left) with change in [bmimOs] and (right) with change in [NaCl]. Plots of variation of emission intensity and emission maxima of curcumin are given in the inset.

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of the reorganization coordinate compared to the ground state. Absorption occurs from molecules equilibrated to a distribution about the ground-state minimum. If there is no environmental relaxation, the fluorescence occurs at the same frequency as the absorption, and there is no Stokes shift. If the environment relaxes completely, the fluorescence is red-shifted, and the full equilibrium Stokes shift is observed. Moreover, Stokes shifts are highly sensitive to local motions, weakly sensitive to collective internal motions, and completely insensitive to overall tumbling. Herein, the absorption and emission spectra of curcumin show a Stokes shift of 4716 cm^{-1} and it is shifted to 3423 cm^{-1} in 60.8 mM IL-micellar solution (Table 1).

Table 1. Stokes Shift and $E_T(30)$ Values of the Curcumin−bmimOs System

curcumin in	$E_T(30)$	Stokes shift (cm^{-1})
7.6 mM bmimOs	49.34	4427
15.2 mM	48.44	4242
22.8 mM	47.65	4053
30.4 mM	47.02	3938
38.0 mM	46.52	3815
45.6 mM	45.95	3703
53.2 mM	45.39	3584
60.8 mM	44.87	3452

Emission maxima of curcumin inside TX-100 and DTAB micelle[83] are at ∼500 nm which is very close to that in our system. Similar values of Stokes shift of curcumin were found for TX-100, DTAB micelle,[83] and also in the presence of HSA[85] compared to our system. It was reported that curcumin is strongly bound in the hydrophobic pocket in the proteins and situated inside the palisade layer of TX-100, DTAB[83] micelle. Accordingly, it can be guessed that the microenvironment around curcumin inside IL-micelle is hydrophobic in nature. Upon gradual addition of IL, the emission maximum of curcumin is blue-shifted with steep increment as the polarity around curcumin molecule is continuously changed. Moreover, spectral widths of fluorescence spectra also decrease considerably with increase in IL-concentration. This absorption and emission behavior of curcumin suggests that the S_1 state has large intramolecular charge transfer character inside the IL-micellar aqueous solution. We got an apparent idea about the polarity of the micellar environment by using a plot of Stokes shift versus $E_T(30)$ of a series of alcohols (C_nOH, where n = 1, 2, 3, 4, 5, 6, 8) as a reference scale (Figure 2a). Increment in fluorescence intensity with decrease in $E_T(30)$ upon addition of IL clearly proves that curcumin undergoes a favorable transfer from water-rich region to more hydrophobic region of the micelle, and a linear plot of Stokes shift with the solvent polarity parameter $E_T(30)$ values of the surroundings of curcumin proves the presence of strong hydrogen bonding interaction present in this IL micelle−curcumin system (Table 1, Figure 2b). Strong blue shift in emission spectra with considerable increase in quantum yield (0.051) in the IL micellar phase clearly indicates that the polarity around the drug is less than that in the bulk water phase. This can be explained by the fact that weaker H-bonding interaction in an environment having lower $E_T(30)$ reduces the efficiency of the nonradiative deactivation process. Increment in lifetime is also observed with a decrease in $E_T(30)$ value which is discussed in a forthcoming section.

3.1.1.b. Determination of Partition Coefficient. To establish curcumin and the IL-micelle as a potential drug loaded system, one should have the quantitative idea of penetration of the drug into the micellar system when normally dissolved in a solvent. Strong support of the existence of curcumin inside micelle was confirmed by determination of the partition coefficient. By using fluorescence data in eq 4, one can get the quantitative estimation of the extent of penetration of curcumin and also a qualitative idea about the interaction of curcumin with the surfactant-like IL.[86−88]

$$\frac{(I_\infty - I_0)}{(I_t - I_0)} = 1 + \frac{[\text{water}]}{K_p} \times \frac{1}{[\text{surfactant}]} \quad (4)$$

where I_∞, I_t, and I_0 are the fluorescence intensities of curcumin at saturated IL concentration, an intermediate IL concentration, and in the absence of IL, respectively. K_p is the partition coefficient of curcumin to the micellar phase from the bulk water. From the slope of the $(I_\infty - I_0)/(I_t - I_0)$ vs $[\text{surfactant}]^{-1}$ plot, we got the value of $K_p = 8.59 \times 10^3$ which is quite large for such a newly studied system (Figure 3). Such considerable partitioning of the drug inside IL micelle indicates the favorable interaction between the drug and the micelles leading to a stable system.

3.1.1.c. Determination of Steady State Anisotropy (r_0). Moreover, we found another way to monitor the transfer of curcumin by measuring its degree of rotation which is the reflection of restriction imposed by the surfactant assembly. From a systematic observation of the change in steady state anisotropy with addition of surfactant, one can get fruitful information about the microenvironment around the probe

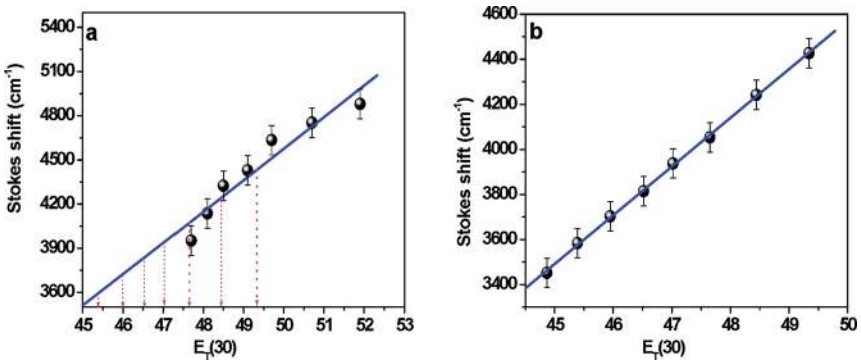

Figure 2. (a) Plot of Stokes shift vs $E_T(30)$ of a series of alcohols (C_nOH, where n = 1, 2, 3, 4, 5, 6, 8). (b) Plot of Stokes shift vs $E_T(30)$ of the surrounding environment of curcumin at different concentrations of IL.

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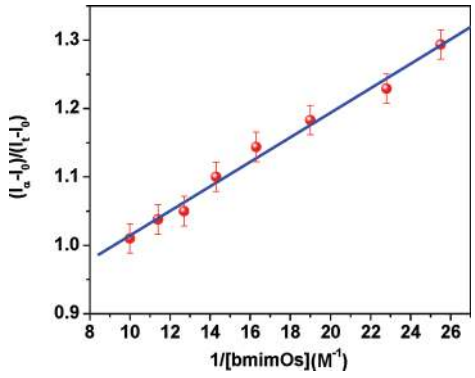

Figure 3. Plot of $(I_\infty - I_0)/(I_t - I_0)$ vs $[\text{bmimOs}]^{-1}$ for partition coefficient determination.

molecule.[78,89−92] Steady state anisotropy is thus a direct proof of the firmness of the drug moiety, and we got gradual increment in anisotropy (r_0) upon addition of IL followed by a plateau at higher concentration by using eq 1. Around the CMC, there is an inflection indicating the change in microenvironment sensed by the drug molecule inside the micelle. A higher value of anisotropy (Figure 4) above the

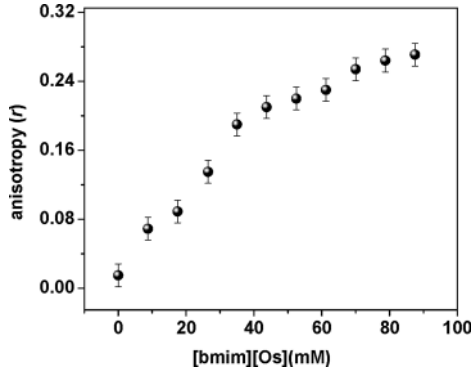

Figure 4. Steady state anisotropy vs concentration of bmimOs.

critical micellar concentration (CMC) implies that the drug molecule is rigidly held inside the micellar region. Gradual increase in anisotropy reflects gradual increase in rigidity of the drug inside micelle, which in turn means that the drug is going to a more and more restricted region inside the micelle. This fact was corroborated by a huge blue shift in the steady state emission spectra.

3.1.1.d. Determination of Binding Constant (K_b). The organized micellar media can serve to protect the compound from external perturbations in solution; also, entrapment within the micellar cavity may result in appreciable modulation in rates of various physical and/or chemical processes. To what extent it can serve as a quite good carrier of any compound, one should know the stability or the binding constant of that compound with the surfactant in solution. Quantitative estimation of binding ability between curcumin and the IL micelles in water was determined by the method as described by Almgren et al.[93] This method can be described by the following equation

$$\frac{(I_\infty - I_0)}{(I_t - I_0)} = 1 + \frac{1}{K_b[\text{M}]} \tag{5}$$

where I_0, I_t, and I_∞ are the emission intensities of curcumin in the absence of IL, at an intermediate concentration of IL, and at a condition of saturation, respectively, and K_b is the binding constant with the micellar system when [M] is described by the following way

$$[\text{M}] = \frac{[\text{S}] - \text{CMC}}{N_{agg}} \tag{6}$$

in which [S] is the IL-surfactant concentration and N_{agg} is the aggregation number of the micellar system. Variation in $(I_\infty - I_0)/(I_t - I_0)$ vs $[\text{M}]^{-1}$ gives a linear plot from which we have easily determined the binding constant. From Figure 5, we have

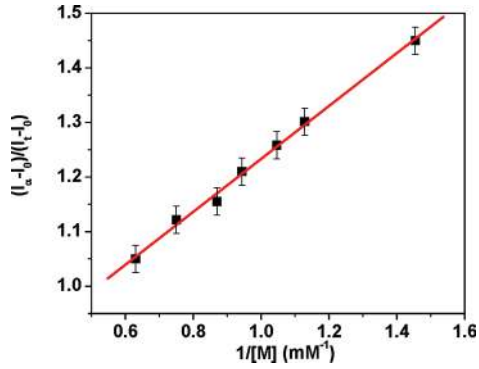

Figure 5. Plot of $(I_\infty - I_0)/(I_t - I_0)$ vs $[\text{bmimOs}]^{-1}$ for binding constant determination.

obtained a value of 2.08×10^4 M^{-1} for the binding constant which is large enough and support for a favorable interactive system. Such high value of binding constant can be attributed to some specific type of interactions such as strong van der Waals interaction between the hydrophobic octyl chain of the IL and two phenyl moieties of curcumin or maybe π-stacking between the aromatic headgroups of the micelle and the two phenyl moieties of curcumin. The pH of our micellar solution is 6.88 in which curcumin is in neutral form, which means the participation of the hydroxyl group of the two phenyl moiety as a H-bonding site cannot be ruled out.

3.1.2. Effect of Salt. A monotonic increase in emission spectra is observed for NaCl addition to the micellar solution (Figure 1). During salt addition, curcumin undergoes considerable blue-shift from 512 to 496 nm but very negligible shift is observed in absorption spectra. This clearly indicates that the presence of salt has no effect on ground state but a profound effect on excited state phenomenon; i.e., ESIPT of curcumin is largely influenced by the addition of a high molar concentration of salt. It is quite expected because salts are known as a good candidate for H-bond breaking and H-bond making substances. The presence of NaCl facilitates the formation of intermolecular H-bonding and slows down the ESIPT process. It was reported that $bmim^+$ and Os^- show strong H-bonding interaction and addition of salt affect their network.[94] As a consequence, a new network of H-bonding can be formed between salt and surfactant. Moreover, in the absence of strong H-bonding between surfactant cation and anion, the chance of formation of intermolecular H-bonding between curcumin and surfactant anion also cannot be ruled out.

We have successfully determined the CMC of the IL-surfactant by using curcumin fluorescence in the presence of

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different salt concentrations, and it was found that the CMC is drastically decreased in the presence of a high concentration of NaCl (Figure 6). The CMC is lowered to 21 mM from 34 mM

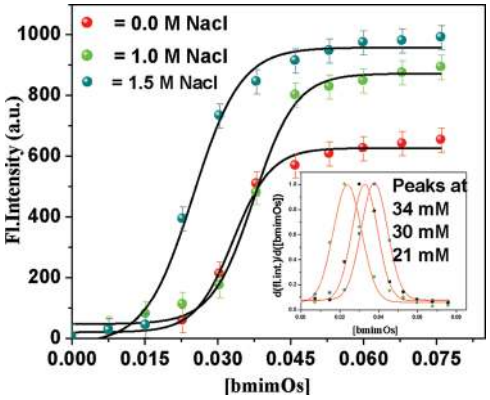

Figure 6. Plot of fluorescence intensity vs [bmimOs] at different salt concentrations (differential plots are given in the inset).

in the presence of 1.5 M NaCl, which means that the micellar transition of curcumin is more favorable and more stable in the presence of salt. Now the reason behind the monotonic increase in fluorescence spectra of curcumin with the help of the aggregation behavior of bmimOs molecules in the presence of various amounts of salt can be clearly understandable. We have used pyrene quenching study by cetylpyridinium chloride (CPC) in the absence as well as in the presence of NaCl and determined the aggregation number of bmimOs micelle by using the following equation[95]

$$\ln \frac{I_0}{I_Q} = \langle n \rangle = [\text{quencher}]\left\{\frac{N_{agg}}{[\text{bmimOs}] - [\text{cmc}]}\right\} \quad (7)$$

where I_0 and I_Q are the fluorescence intensities of pyrene in the absence of cytlpyridinium chloride (CPC), $\langle n \rangle$ is the mean occupancy number of the quencher molecule and in the presence of CPC, respectively, N_{agg} is the aggregation number, and CMC is the critical micellar concentration of bmimOs micelles. By using the slope of the straight line obtained from the plot of $\ln(I_0/I_Q)$ vs [CPC] (Figure 3, Supporting Information), we have calculated the aggregation number or bmimOs micelle at different salt concentrations. The aggregation numbers of bmimOs micelle are 39, 54, and 84 in the presence of 0.0, 1.0, and 1.5 M NaCl, whereas CMC values are 34, 30, and 21 mM, respectively. The overall result of change in CMC and aggregation is an increase in the number of bmimOs micelle in the solution (using eq 6) from 0.15 to 0.22 mM. Moreover, the size of the micelle is also getting increased in the presence of salt and it was obtained by using dynamic light scattering study (DLS). The size of the micelle changes from 2.0 to 5.00 nm almost 3-fold after addition of salt up to 1.5 M NaCl. As a result, the number of curcumin molecules partitioned to the micelles is also increased. We have also checked the nature of binding in the presence of salt, and a high binding constant (6.98×10^4 M^{-1}) was found at a concentration above the CMC in the presence of 1 M NaCl. We have also monitored the absorption spectra of curcumin inside IL-micelle in the presence of 1 M NaCl with a certain time interval, and we found that the drug is stable enough inside the micellar environment, as depicted in Figure 7. In the presence of salt, the rate of degradation of the curcumin–

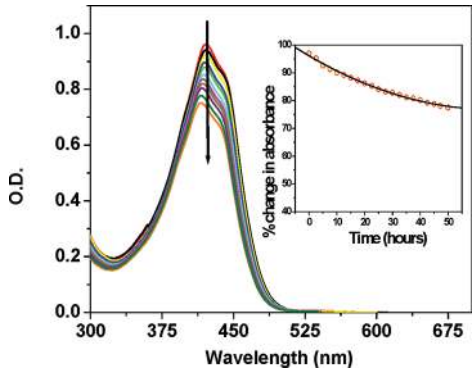

Figure 7. Absorption spectra of curcumin in the presence of 1 M salt with a gradual time interval. Variation of absorbance with time is given in the inset.

micelle system is 0.399%/h, which is quite low compared to that in absence of salt, 1.22%. The higher stability of curcumin inside bmimOs micelle in the presence of salt concentration suggests the usefulness of this drug–micellar system.

3.1.3. Effect of Temperature. Upon an increase in temperature, the fluorescence intensity of curcumin decreases sharply, as shown in Figure 9 (inset). The nature of interaction between curcumin and IL surfactant was characterized by measuring the change in thermodynamic parameters with temperature. We have increased the temperature form 10 to 60 °C at a constant IL concentration (35 mM). Binding constant (K_b) values are decreasing with an increase in temperature, and we have determined the change in enthalpy (ΔH) and change in entropy (ΔS) by plotting ln K_b vs $1/T$ (Figure 8) using the

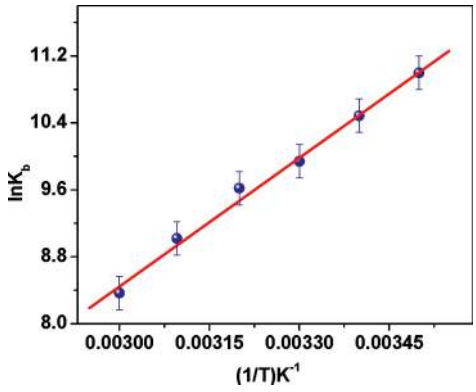

Figure 8. van't Hoff plot of curcumin in bmimOs micelle at different temperatures.

van't Hoff equation [$\ln K_b = (-\Delta H/R)/T + \Delta S/R$]. Change in Gibbs free energy was calculated by using the following equation: $\Delta G = -RT \ln K_b$, where R is the universal gas constant and T is the absolute temperature (Table 2). The calculated binding enthalpy and entropy are ΔH (kcal/mol) = −10.17 and ΔS (cal/mol) = −13.72 over this temperature range. From Table 2, we can see that the value of change in enthalpy is greater than the total free energy change during this interaction. Similar negative values of thermodynamic parameters were noticed for curcumin–lipid[35,40] and curcumin–HSA[85] interactions in which it was concluded that the binding is enthalpy driven and binding is guided by both H-bonding and hydrophobic interactions. In our study, the binding enthalpy ($-\Delta H$) value for transfer or binding of curcumin to

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Table 2. Change in Binding Constant and Free Energy (ΔG) at Different Temperatures[a]

temperature (35.0 mM bmimOs)	$K_b/10^4$ (M^{-1})	ln K_b	$-\Delta G$ (kcal/mol)
283 K	5.97	10.99	6.29
293 K	3.58	10.49	6.15
303 K	2.08	9.94	6.01
313 K	1.35	9.51	5.88
323 K	0.83	9.02	5.76
333 K	0.42	8.37	5.60

[a] ΔH (kcal/mol) = −10.17 and ΔS (cal/mol) = −13.72 in this temperature range.

micelle from water is distinctly negative and larger than the total free energy change ($-\Delta G$), indicating the binding interaction is an enthalpy driven process which is governed by H-bonding interaction between the keto−enol group of curcumin and the negative group of the surfactant as well as hydrophobic interaction between aromatic rings of curcumin and the long alkyl chain of IL.

4. TIME-RESOLVED RESULTS

The change in fluorescence lifetime helps to get a clear idea about the modulation of photophysical properties such as radiative and nonradiative deactivation channels brought about by encapsulation of the probe molecule. Unlike the steady state fluorescence measurements, there are not many reports on the fluorescence lifetimes of curcumin. The competing nonradiative processes shortened the fluorescence lifetimes considerably. The fluorescence decay profiles, obtained from time correlated single photon counting (TCSPC) studies, in most of the organic solvents showed a multiexponential fit, and the fluorescence lifetime values as well as their relative amplitudes varied significantly with the nature of the solvent.[38,80,96−98] Due to the complex nature, in most of the studies, average fluorescence lifetimes (τ_{AV}) were only compared where τ_{AV} can be expressed by eq 2.

All the studies were focused on excited-state intramolecular hydrogen atom transfer (ESIPT) of curcumin because this phenomenon has been associated with the medicinal properties of other pigment molecules. It was reported that the fluorescence lifetime of curcumin in methanol has a dominant component with a time constant of roughly 130 ps.[38,80] This decay component, however, was assigned to different molecular processes, i.e., solvation[80] and ESIPT[38] in the two studies. Recently, it was observed that ESIPT occurs with a time scale of approximately 100 ps. Furthermore, the similarity between the time scale of ESIPT and the measured fluorescence lifetime indicates that ESIPT is a major photophysical process in the deactivation of the excited state. In our work, we have also given attention to the average lifetime of curcumin, giving thrust only to the proton transfer mechanism. The lifetime of curcumin was detected at different concentrations of IL and was found gradual increment from 103 to 158 ps (Table 3). There was also an increment upon gradual addition of NaCl to the micellar solution (Table 4). Similar to steady-state, time-resolved decay also shows a decrement in lifetime with increase in temperature (Table 5). The decays in all cases were biexponential in nature, and we got considerable change in lifetime, which confirms the modulations of photophysical character of curcumin when encapsulated inside IL-micelle. Previously, many studies have shown that the ESIPT rate of curcumin can be modulated in the presence of a surfactant assembly[83,38,99] and it was found that the curcumin lifetime is shortened mainly due to its fast ESIPT process. Moreover, it was also concluded in those works that intermolecular H-bonding occurs between curcumin and water inside micellar layers, and curcumin and surfactant. In our study, we have obtained quite similar values of time constants that were obtained for TX-100 micelle. For TX-100 micelle, it was concluded that the presence of strong intermolecular H-bonding between oxygen of the C−O bonds in TX-100 and curcumin slows down the intramolecular proton transfer rate within the keto−enol group. Methanol, ethanol like protic polar solvents also provide a similar environment by forming an intermolecular H-bond with the keto−enol group of curcumin, and retardation of intramolecular proton transfer makes the lifetime longer compared to nonpolar solvent like cyclohexane. Longer time constants of curcumin inside IL-micelles also suggest that there is a strong perturbation in the intramolecular H-bond in between the keto−enol group by forming strong intermolecular H-bonding with the anion of the IL. Moreover, it was reported that DMSO, DMF like H-bond accepting solvents show a much lower lifetime (155−175 ps) than that of ethyl acetate, benzonitrile, and glycerol triacetate like aprotic polar solvents (230−250 ps). In latter solvents, dipole−dipole interaction perturbed the intramolecular H-bond, but the in case of the former solvents, existence of multiple intermolecular H-bonds between the keto−enol group and two phenolic OH groups of curcumin and the surfactant headgroups is possible. The present study shows the lifetime of curcumin is very close (110−180 ps) to that of DMSO, DMF, and it implies that formation of multiple intermolecular H-bonds induces significant nonradiative deactivation through hydrogen stretching vibrations.

Table 3. Fluorescence Lifetime, Quantum Yield, Non-Radiative Rate and Radiative Rate Constants of Curcumin in bmimOs Micelle at Different Concentrations

curcumin in	τ_1/ps (a_1)	τ_2/ps (a_2)	$\tau_{AVERAGE}$/ps	quantum yield (Φ_X)	K_{nr} (s^{-1})/10^9	K_r (s^{-1})/10^8
0.0 mM bmimOs						
7.6 mM	61 (0.73)	215 (0.27)	103	0.0118	9.59	1.10
15.2 mM	79 (0.79)	224 (0.21)	110	0.0178	8.92	1.62
22.8 mM	89 (0.82)	238 (0.18)	115	0.0255	8.47	2.21
30.4 mM	94 (0.84)	260 (0.16)	124	0.0345	7.79	2.77
38.0 mM	104 (0.86)	290 (0.14)	130	0.0398	7.39	2.98
45.6 mM	118 (0.87)	298 (0.13)	141	0.0435	6.78	3.08
53.2 mM	126 (0.88)	305 (0.12)	148	0.0461	6.44	3.11
60.8 mM	138 (0.89)	318 (0.11)	158	0.0507	5.93	3.20

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Table 4. Fluorescence Lifetime, Quantum Yield, Non-Radiative Rate and Radiative Rate Constants of Curcumin in bmimOs Micelle at Different Salt Concentrations

curcumin in 35.0 mM bmimOs	τ_1/ps (a_1)	τ_2/ps (a_2)	$\tau_{AVERAGE}$/ps	quantum yield (Φ_X)	K_{nr} (s^{-1})/10^9	K_r (s^{-1})/10^8
0.0 M	76 (0.60)	193 (0.40)	123	0.034	7.85	2.76
0.13 M	96 (0.65)	195 (0.35)	130	0.038	7.40	2.92
0.39 M	111 (0.68)	196 (0.32)	137	0.041	7.00	2.99
0.64 M	124 (0.73)	199 (0.27)	145	0.048	6.57	3.31
0.89 M	136 (0.76)	200 (0.24)	152	0.053	6.23	3.49
1.02 M	147 (0.80)	199 (0.20)	158	0.058	5.96	3.67
1.15 M	161 (0.82)	205 (0.18)	169	0.063	5.55	3.72
1.28 M	171 (0.85)	212 (0.15)	178	0.067	5.24	3.76
1.408 M	178 (0.86)	218 (0.14)	184	0.073	5.03	3.96

Table 5. Fluorescence Lifetime, Quantum Yield, Non-Radiative Rate and Radiative Rate Constants of Curcumin in bmimOs Micelle at Different Temperatures

temperature (35.0 mM bmimOs)	τ_1/ps (a_1)	τ_2/ps (a_2)	$\tau_{AVERAGE}$/ps	quantum yield (Φ_X)	K_{nr} (s^{-1})/10^9	K_r (s^{-1})/10^8
283 K	120 (0.67)	260 (0.33)	166	0.051	5.71	3.07
293 K	111 (0.72)	230 (0.28)	140	0.041	6.85	2.92
303 K	83 (0.87)	209 (0.23)	120	0.035	8.04	2.91
313 K	78 (0.85)	254 (0.15)	105	0.028	9.26	2.67
323 K	70 (0.88)	272 (0.12)	94	0.020	10.40	2.12
333 K	65 (0.92)	268 (0.08)	82	0.010	12.04	1.22

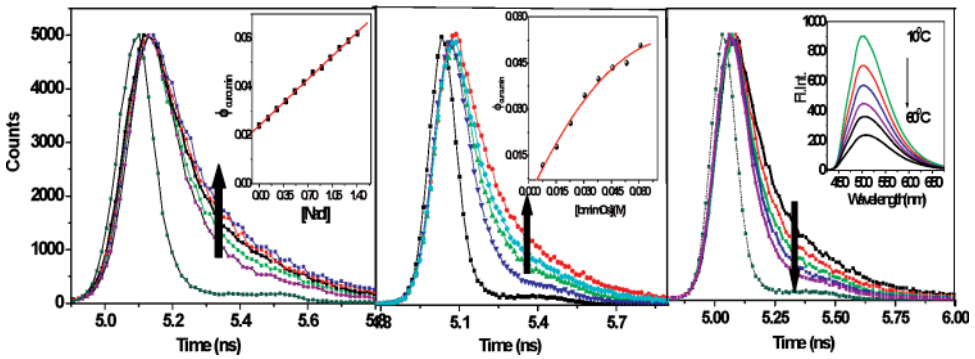

Figure 9. Time-resolved decay plot of curcumin (left) with change in NaCl concentration, (middle) with change in concentration, and (right) with change in temperature.

In analogy to literature reports of modulations of excited state photophysics of the curcumin in constrained environments of proteins, micelles, and cyclodextrin, the increase of lifetime associated with increasing IL concentration (Figure 9) seems attributable to diminution of nonradiative decay channels through reduction of rotational/vibrational degrees of freedom resulting from its encapsulated state inside micellar environments. To further construe the modulations in excited state behavior of curcumin, we have calculated the radiative (k_r) and nonradiative (k_{nr}) decay rate constants using the following two equations:

$$k_r = \frac{\phi_F}{\tau_{AV}} \qquad \frac{1}{\tau_{AV}} = k_r + k_{nr}$$

where k_r and k_{nr} are the radiative and nonradiative rate constants, respectively, and ϕ_F is the fluorescence quantum yield of curcumin. From Table 1, we can see reduction in the nonradiative decay path accompanied by increment in the radiative decay path for encapsulated curcumin upon gradual addition of IL (Figure 10). Similar results were also observed in the case of salt addition to micellar solution. The nonradiative rate constant of curcumin is highest in cyclohexane (1.5×10^{10} s^{-1}) which is larger than the value observed inside our system. However, in strong hydrogen−bond donating/accepting solvent like methanol, DMF, and DMSO,[36,38] the nonradiative rate constants are quite close to each other and also similar to k_{nr} inside IL−micelle, indicating our previous supposition that the excited state of curcumin is perturbed by the presence of intermolecular H-bonding interaction between IL-micelle and

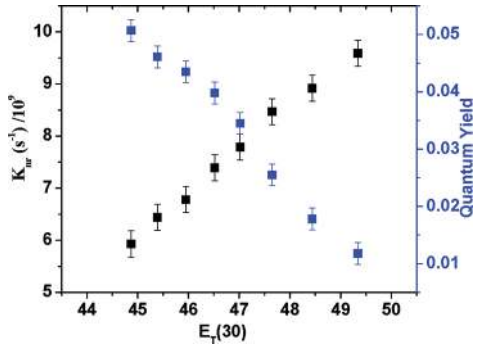

Figure 10. Variation of nonradiative rate constant (black points) and variation of quantum yield (blue points) with polarity of the medium in E_T(30) scale in IL-micelle.

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curcumin. In other words, this fact established that the excited state proton transfer is the main photophysical process for the nonradiative deactivation of the excited curcumin molecule.

In summary, we have successfully observed the modulation of the photophysical properties of curcumin inside an IL-micelle, a promising drug delivery system under various conditions, and also obtained useful information about the stability of this drug−micelle system. A high value of partition coefficient as well as binding constant of curcumin from water to micellar phase confirms the preferential location of the drug inside the micelle. Considerable increase in anisotropy value with increase in IL concentration supports the encapsulation of the drug. In our study, we have used curcumin for a special job to determine the CMC of the IL in water by using its very sensitive fluorescence properties with change in polarity around its microenvironment and we have successfully determined the value of the CMC of the IL in the absence of salt and in the presence of salt. Considerable blue-shift in absorption spectra as well as in emission spectra having a structural change points toward the existence of a strong H-bonding environment. Remarkable enhancement in steady state fluorescence intensity inside IL micelle seems to be the manifestation of the effect of perturbation of intramolecular H-bond in a microenvironment having reduced polarity compared to bulk water. A longer lifetime inside IL-micelle in the absence or presence of salt also supports the formation of an intermolecular H-bond between the keto−enol group of curcumin and the surfactant head-group. The salt effect and temperature effect also give the idea about the mechanism and nature of interaction which is attributed mainly to hydrogen bond as well as hydrophobic interaction. A higher negative value of binding enthalpy proves the process is mostly enthalpy driven. Significant depletion of nonradiative decay channels of curcumin inside IL-micelle matches very well with other examined confined systems like surfactant micelle,[83,100] membrane,[35,40] proteins,[85] cyclodextrin,[101,102] supramolecular assembly,[103] etc., and provides a huge area of possibility of curcumin-IL system to be studied as a successful drug carrier vehicle.

CONCLUSION

We have presented detailed information about the modulation of the photophysical properties of a potent ESIPT molecule, namely, curcumin, upon interaction with surfactant-like ionic liquid, bmimOs, a new class of surfactant in aqueous solution. In particular, the remarkable enhancement in fluorescence spectra with a distinct blue-shift along with considerable change in time-resolved spectra dictates the sensitivity of the interaction of curcumin with the IL-micelle. By using this very property, we have successfully calculated the CMC of the IL in water. Higher encapsulation efficiency was also determined along with an enthalpy guided favorable binding interaction in the presence of salt and in the absence of salt. A very low value of the hydrolytic degradation rate of curcumin encapsulated inside micelle makes it a smart nanocompartment to handle it with ease. Our study opens a promising area of research that can provide a wide range of designed nano-compartments having tunable properties only by changing the cation or the anion which helps to monitor some specific interaction present within that system.

ASSOCIATED CONTENT

Supporting Information

Absorption spectra of curcumin inside IL−micelle upon addition of IL and salt. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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