Dibenzo[*a,c*]phenazine: A Polarity-Insensitive Hydrogen-Bonding Probe

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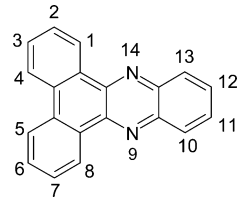
A derivative of phenazine, dibenzo[*a,c*]phenazine (DBPZ), can be used as a very good hydrogen-bonding probe unlike its parent phenazine molecule. Steady-state absorption and fluorescence studies reveal that DBPZ is completely insensitive to polarity of the medium. However, DBPZ can form a hydrogen bond very efficiently in its first excited singlet state. The extent of this excited-state hydrogen-bond formation depends both on size and on hydrogen-bond donor ability of the solvents. Time-resolved fluorescence studies and theoretical calculations also suggest that this hydrogen-bond formation is much more favorable in the excited state as compared to the ground state. In the excited state, the electron density is pushed toward the nitrogen atoms from the benzene rings, thereby increasing the dipole moment of the DBPZ molecule. Although the dipole moment of DBPZ increases upon photoexcitation, like other polarity probes, the molecule remains fully insensitive to the polarity of the interacting solvent. This unusual behavior of DBPZ as compared to simple phenazine and other polarity probes is due to the structure of the molecule. Hydrogen atoms at the 1 and 8 positions of DBPZ are sterically interacting with a lone pair of electrons on the proximate nitrogen atoms and make both of the nitrogen atoms inaccessible to solvent molecules. For this reason, DBPZ cannot sense the polarity of the medium. However, DBPZ can only sense solvents, those that have hydrogen with some electropositive nature, that is, the hydrogen-bond donating solvents. Hydrogen being the smallest among all elements can only interact with the lone pair of electrons of nitrogen atoms. Thus, DBPZ can act as a sensor for the hydrogen-bond donating solvents irrespective of their dielectrics.

Introduction

Hydrogen bonding is one of the fundamental elements of chemical structure and reactivity of water, proteins, and DNA building blocks of life.[1−3] Intermolecular hydrogen bonding is a site-specific local interaction between hydrogen donor and acceptor molecules that plays a dominant role in various forms of molecular recognition processes. The nature of the hydrogen bond in solution is of particular interest, and it has been probed by diverse experimental and theoretical methods. It is evident from the literature that there are quite a large number of phenazine derivatives,[4,5] which show significant differences in their dipole moment in the ground and excited states on photoexcitation. In such cases, the solvent molecules undergo reorganization around the solute molecules according to their own dipole moment and hydrogen-bonding capacity to minimize the total energy of the solute−solvent system. For this dipole−dipole interaction, the emission spectra of those molecules depend very much on the polarity of the solvent employed. Such polarity probes show considerable red shift of the fluorescence maxima in the polar solvents as compared to nonpolar medium. This phenomenon is popularly known as solvation.[6−8] However, it is a very rare observation in the literature that a molecule with a dipole moment higher in the excited state as compared to the ground state remains insensitive to polarity (or dielectric) but sensitive to the hydrogen-bonding capacity of the solvent. In this Article, we would like to report on such a molecule, that is, dibenzo[*a,c*]phenazine (DBPZ), and its activity as an efficient hydrogen-bonding probe unlike its parent phenazine molecule. Because of the planar structure and rigid framework of the fused ring system, the conformational and configurational relaxation processes are not feasible enough in DBPZ. Only the solvent relaxation process becomes important in its first excited singlet state. Photoexcitation of DBPZ results in significant changes in the spatial charge distribution around its nitrogen atoms like other polarity probes. Yet surprisingly DBPZ remains insensitive to the polarity of the solvents. The structure of the molecule might be responsible for its peculiar behavior.

CHART 1



Steady-state absorption and fluorescence studies reveal that DBPZ forms hydrogen bonding with hydroxylic solvents in the excited state. The extent of hydrogen-bond formation depends on the hydrogen-bond donor ability (α) and steric bulk of the solvent. Time-resolved fluorescence studies suggest that the hydrogen-bond formation is much more facilitated in the excited

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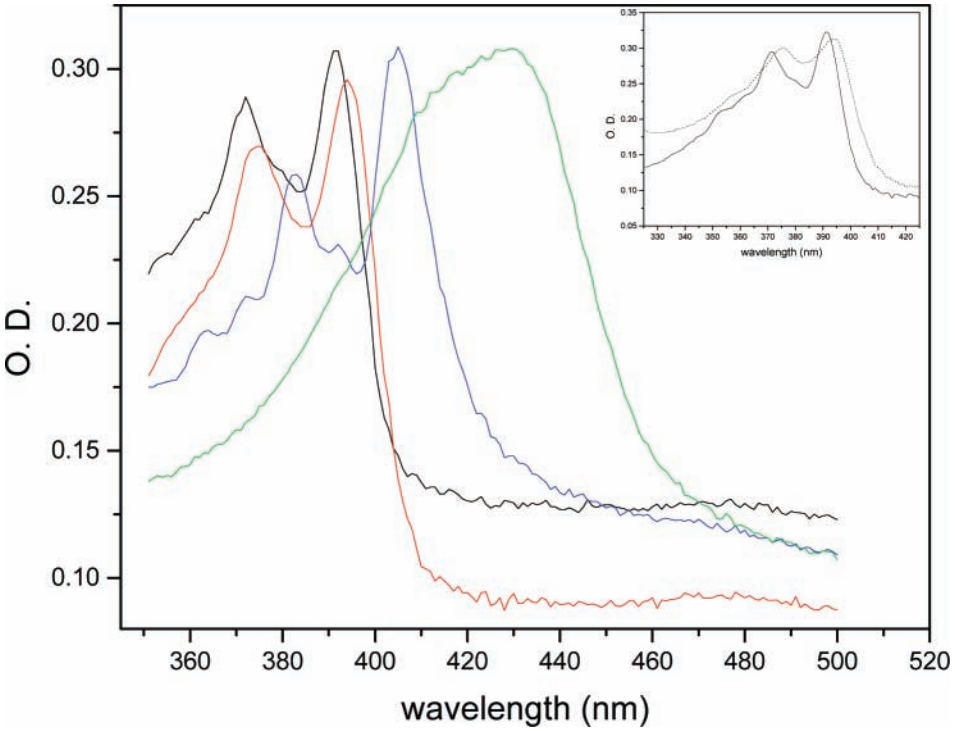

Figure 1. Absorption spectra of DBPZ (1×10^{-5} M) in MeCN (black), 63% (v/v) water−MeCN (red), 2% MeCN−water mixture (blue), and MeCN−$HClO_4$ mixture (green). Inset shows the absorption spectra of DBPZ (1×10^{-5} M) in MeCN (−) and in TFE (- - -).

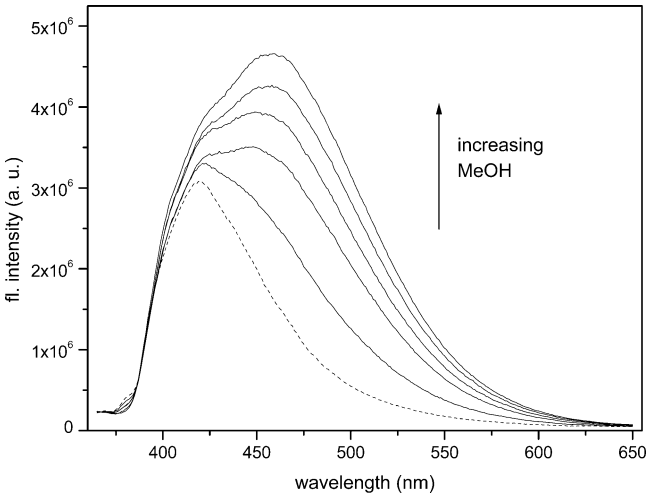

Figure 2. Steady-state fluorescence spectra of DBPZ (1×10^{-5} M) ($\lambda_{ex} = 350$ nm) in MeCN with increasing amount of MeOH (v/v): 0% (- - -), 23%, 48%, 58%, 73%, and 100%.

state than in the ground state. This experimental observation is further supported by the theoretical calculations.

Experimental Section

DBPZ (Chart 1) was synthesized in the laboratory using the method mentioned in ref 9. It was purified by repeated crystallization with the purity checked by thin-layer chromatography (TLC), melting point, and mass spectroscopy. UV spectroscopy grade methanol (MeOH), ethanol (EtOH), 2-propanol (ISP), *tert*-butanol (*t*-But), trifluoroethanol (TFE), acetonitrile (MeCN), cyclohexane (CH), tetrahydrofuran (THF), and toluene were obtained from Spectrochem and used without purification. Water was triply distilled. Urea was purchased from Aldrich. DBPZ is soluble in all organic solvents mentioned above. To study the effect of water, we diluted the stock solution of DBPZ in MeCN with water. The maximum water concentration was 54.44 M instead of 55.56 M (for pure water). Therefore, the actual solvent composition was 2% MeCN−water mixture for the highest water concentration in the medium.

UV−vis absorption spectra were recorded on a Shimadzu UV-2101-PC absorption spectrophotometer at 298 K. The steady-state fluorescence measurements were made in a Spex Fluoromax-3 spectrofluorimeter using a 1 cm path length quartz cuvette. During all of the steady-state fluorescence studies, samples were excited at 350 nm (λ_{ex}) using 2 nm band-passes for excitation and emission. The fluorescence quantum yield was measured with reference to quinine sulfate in 1 N H_2SO_4 (quantum yield = 0.7)[10] by comparing the area of fluorescence and absorbance at the excitation wavelength, using the formula

$$\Phi_f = \Phi_r(I_f/I_r)(OD_r/OD_f)(n_f/n_r)^2$$

where Φ is the quantum yield, I is the integrated fluorescence intensity, OD is the optical density, and n is the refractive index. The subscripts r and f refer to the reference fluorophore quinine sulfate and DBPZ, respectively.

Emission studies at 77 K were made using a Dewar system having a 5 mm o.d. quartz tube. The freezing of the samples at 77 K was done at the same rate for all of the samples. Triplet state emissions were measured in a Hitachi F-4010 spectrofluorimeter equipped with phosphorescence accessories at 77 K. All of the samples were made in pure ethanol and in a 1:1 MeCN−toluene mixture for low-temperature measurements. The samples were excited at 350 nm using a 10 nm band-pass, and the emission band-pass was 1.5 nm. The cryosolvent used in the experiment was always found to form a clear glass. Singlet state fluorescence lifetime was measured using a time-correlated-single-photon counting (TCSPC) spectrophotometer (Edinburg). The sample was excited at 358 nm. The lifetime is obtained on performing deconvolution technique, which is based on a convolution integral. The software was supplied by Edinburg.[11]

In the theoretical study, the structures were model built using the software MOLDEN,[12] and ab initio energy minimizations

TABLE 1: Fluorescence Maxima and Quantum Yield of DBPZ in Different Hydrogen-Bonding Solvents with Their Hydrogen-Bond Donating Ability

solvent	λ_{max} (nm)	Φ_f	α^a
63% H_2O (v/v)	486	0.0759	1.17
58% TFE (v/v)	470	0.0486	1.51
MeOH	462	0.0204	0.93
EtOH	426	0.0133	0.83
ISP	422	0.0126	0.76
t-But	420	0.0115	0.68
MeCN	420	0.0089	0.19
THF	420	0.0088	0.00

[a] Hydrogen-bond donor ability from ref 18.

and structure optimizations were carried out with 6-31G**[13] basis sets using GAMESS-US.[14] For ground-state calculations, we have used the restricted Hartree−Fock (RHF) wave functions, while for excited state we have used Multi Configuration Self-Consistent Field (MCSCF)[15] wave functions. We have used GUGA option of GAMESS in the MCSCF calculation to probe for single excitation where one of the doubly filled orbitals is converted to two singly occupied active orbitals. Subsequent to geometry optimization, we have also carried out normal-mode frequency calculation by numerically diagnolizing the Hessian matrices. We have used the ESP fit charges (q_i) to calculate the absolute dipole moment ($|\mu|$) using the formula

$$|\mu| = (\mu_x^2 + \mu_y^2 + \mu_z^2)^{1/2}$$

where μ_x, etc., are calculated as

$$\mu_x = \sum_{i=1}^{34} 4.8 x_i q_i$$

The values of x_i, y_i, and z_i are obtained by subtracting the center of mass from the atomic coordinates, and 4.8 is a conversion factor.

Results and Discussion

Steady-State Absorption Study. DBPZ shows two absorption peaks at 371 and 391 nm (Figure 1) in organic solvents of different polarity and hydrogen-bonding capacity (e.g., CH, THF, MeOH, EtOH, ISP, *t*-But, MeCN, etc.). The absorption spectrum of DBPZ is red-shifted by 3 nm in TFE (Figure 1, inset) as compared to the above-mentioned protic and aprotic solvents. When water is gradually added to the solution in MeCN, it has been observed that the spectrum of DBPZ remains unchanged even on addition of water up to 63% (v/v) (i.e., 35 M of water). Further addition of water results in a red shift of ~13 nm of the above absorption peaks from 371 and 391 nm to 384 and 404 nm, respectively. This bathochromic shift is first presumed due to the protonation at two nitrogen atoms of DBPZ. However, Markgraf et al.[16] reported that due to steric inhibition of solvation of conjugate acid the pK_a of DBPZ is very low (~0.3). When perchloric acid was added to the MeCN medium containing DBPZ, a broad structureless band appears at ~428 nm, which is due to the protonated form of DBPZ.[17] Thus, we can infer that the red-shifted peaks at 384 and 404 nm in water (Figure 1) are not due to the protonated form of the compound as obtained on addition of perchloric acid. This red shift of the absorption peak by 13 nm may be due to the change of solvent dielectric from MeCN to water, which is characteristic of a $\pi\pi^*$ transition of DBPZ.

TABLE 2: Fluorescence Lifetime of DBPZ in Different Solvents

solvent	λ_{em} (nm)	lifetime τ (ns)	χ^2
63% H2O (v/v) or 35 M H_2O	420	0.73 (88.1%)	1.08
		4.60 (11.9%)	
	486	0.08 (7.4%)	0.94
		6.60 (92.6%)	
MeOH	420	0.70 (98%)	1.09
		3.69 (2%)	
	462	0.87 (98%)	0.98
		3.85 (2%)	
EtOH	426	0.58 (92.7%)	0.88
		3.80 (7.3%)	
ISP	422	0.55 (88.4%)	1.13
		1.96 (11.6%)	
58% TFE (v/v)	420	0.71 (98%)	1.10
		3.89 (2%)	
	470	1.87 (52.8%)	0.92
		4.19 (47.2%)	
urea in 63% (v/v) water	486	0.09 (6.8%)	1.03
		6.04 (93.2%)	
45 M H_2O	420	0.26 (90.27%)	0.74
		5.92 (9.73%)	
54.44 M H_2O	420	0.14 (90.67%)	0.77
		5.75 (9.33%)	

Steady-State and Time-Resolved Fluorescence Study. DBPZ has a fluorescence peak (λ_{max}) at 420 nm in aprotic solvents of different polarity, for example, CH, THF, and MeCN. On addition of protic solvents, the fluorescence peak is red-shifted with a concomitant increase in fluorescence quantum yield (Φ_f). Figure 2 shows that on addition of protic solvent like MeOH the fluorescence peak of DBPZ is shifted from 420 to 462 nm with simultaneous increase in Φ_f value. We can identify this highly fluorescent species at 462 nm as excited-state hydrogen-bonded species. A similar type of observation is found with EtOH, ISP, and *t*-But. However, with these alcohols the extent of bathochromic shift of λ_{max} and the enhancement in Φ_f of DBPZ are low as compared to those with MeOH (Table 1). As the solvent is changed from MeOH to *t*-But, steric crowding around the −OH group increases with the number of methyl groups introduced on the carbon bearing hydroxyl group. This hinders the approach of the solvents toward DBPZ molecules. Moreover, the electron-donating inductive effect (+I effect) of the methyl groups decreases the hydrogen-bond donor ability (α) of the −OH group of bulky alcohols, which is also supported by the values as reported by Kamlet et al. Therefore, the excited-state hydrogen bonding with *t*-But becomes least feasible, which is reflected in the extent of red shift of λ_{max} and change in Φ_f values of DBPZ as compared to other alcohols. A careful examination of Figure 2 reveals that in solution of MeOH at room temperature, DBPZ shows dual-fluorescence behavior. The fluorescence spectra consist of two emission bands with maximum at 420 and 462 nm, arising from the first excited singlet state of the non-hydrogen-bonded (or free) DBPZ molecule and the excited-state hydrogen-bonded species respectively. This suggests that the DBPZ molecule in its first excited singlet state remains in equilibrium between the free DBPZ molecule and the excited-state hydrogen-bonded form. For higher alcohols EtOH, ISP, and *t*-But, this equilibrium is mostly favored toward the free DBPZ molecule. Instead of simple alcohols, the same experiment was repeated with TFE, which has greater hydrogen-bond donor ability than alcohols and water also.[18] We have found that the fluorescence intensity of DBPZ increases up to the addition of 58% of TFE (v/v) in MeCN. At this condition, the λ_{max} of DBPZ is red-shifted to 470 nm. On further addition of TFE, the fluorescence intensity

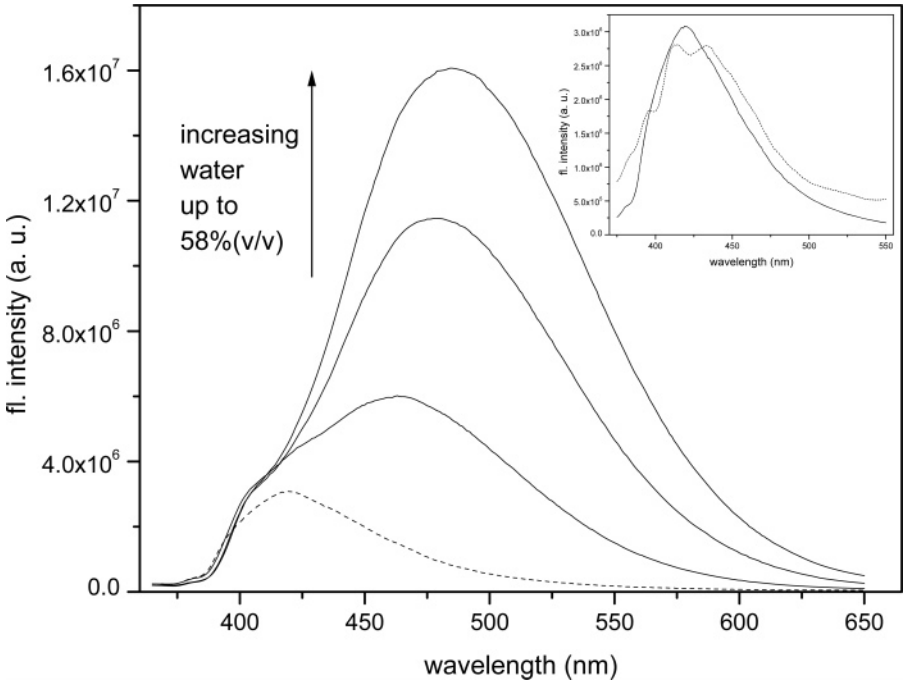

Figure 3. Steady-state fluorescence spectra of DBPZ (1×10^{-5} M) ($\lambda_{ex} = 350$ nm) in MeCN with increasing amount of water (v/v): 0% (- - -), 23%, 48%, and 58%. Inset shows the fluorescence spectra of DBPZ ($\lambda_{ex} = 350$ nm) in pure MeCN (−) and in 2% MeCN−water mixture (- - -).

of DBPZ decreases gradually and the λ_{max} is reverted to 426 nm. Although TFE is much bulkier than MeOH, it shows greater red shift of λ_{max} and Φ_f value than the latter because of its greater hydrogen-bond donating capacity than MeOH. Next, we tried the experiment with water, which is smaller in size as compared to all of the alcohols, and its hydrogen-bond donating capacity is greater than all of the alcohols except TFE. The fluorescence intensity of DBPZ increases up to addition of 63% (v/v) water in MeCN and then decreases with further addition of water. In the presence of 63% (v/v) water in MeCN, the λ_{max} is shifted from 420 to 486 nm (Figure 3) with the highest Φ_f values (Table 1), and the equilibrium remains strongly in favor of the excited-state hydrogen-bonded species as compared to the free DBPZ molecule. Thus, it is evident that the excited-state hydrogen bonding of DBPZ with protic solvents depends both on the size and on the hydrogen-bond donor ability of the solvents. Above 63% (v/v) of water concentration, the fluorescence intensity decreases and λ_{max} is blue-shifted and split into two parts, one at 410 nm and the other at 434 nm (inset of Figure 3). Figure 4 shows the variation of Φ_f values with the concentration of water. It is evident from Figure 4 that the quantum yield of DBPZ increases up to 63% (v/v) of water−MeCN mixture (i.e., 35 M with respect to water concentration) and then suddenly decreases. The reason for this unusual behavior of water as well as TFE will be discussed later.

Time-resolved fluorescence studies also reveal the formation of an excited-state hydrogen-bonded complex of DBPZ with protic solvents. The prominent red shift in fluorescence maxima in the presence of MeOH, TFE, and water led us to measure the lifetime of the species at two emission wavelengths, one at 420 nm, where DBPZ remains free, and the other at the fluorescence maxima of the hydrogen-bonded DBPZ with the respective protic solvents. Table 2 depicts the fluorescence lifetime of DBPZ in different protic solvents. The lifetime of the hydrogen-bonded species decreases with bulkier alcohols. The maximum lifetime of the hydrogen-bonded species is obtained in water. The values of lifetime of DBPZ in MeCN, THF, and *t*-But are too small to be measured precisely by our existing setup.

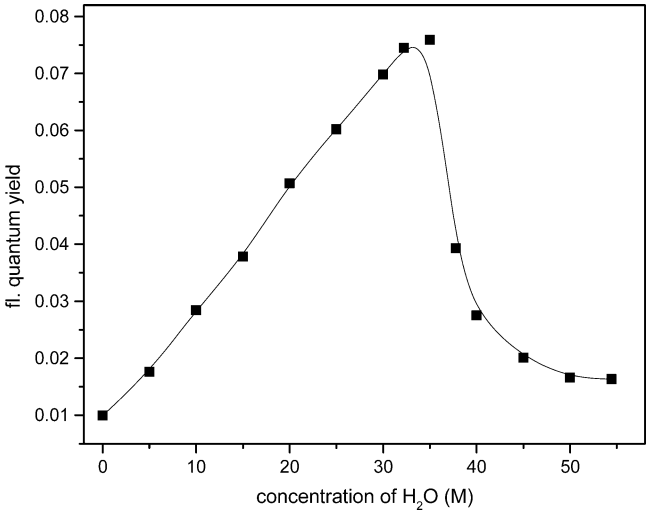

Figure 4. Variation of fluorescence quantum yield of DBPZ with concentration of water in MeCN.

To verify the fact that the peak at 486 nm in water with greater fluorescence intensity is due to the formation of excited-state hydrogen bonding, we added urea keeping the water concentration unaltered. We observed that with increasing urea concentration the fluorescence intensity decreases at 486 nm (Figure 5) while the absorption spectrum remains unaltered (inset of Figure 5). The time-resolved study also reveals that the lifetime of DBPZ decreases in the presence of urea (Table 2). In other words, urea itself forms hydrogen bonds extensively with water and thereby hinders the formation of excited-state hydrogen-bonded species between DBPZ and water. Recent investigations[19] of the structure of urea−water solutions using neutron diffraction analysis and isotope labeling on the water and urea hydrogen atoms and on the nitrogen atom of urea reveal that urea incorporates readily into water, forming pronounced hydrogen bonds with water at both the amine and the carbonyl head groups. So, less water molecules are available for DBPZ in the presence of urea that decreases the extent of hydrogen

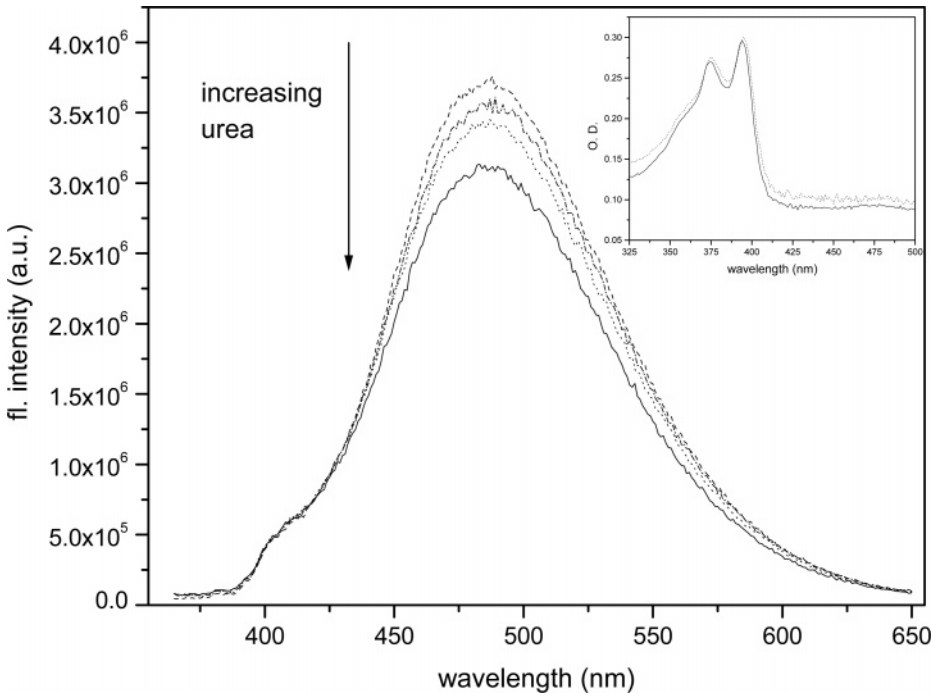

Figure 5. Steady-state fluorescence spectra of DBPZ (1×10^{-5} M) ($\lambda_{ex} = 350$ nm) in 58% water−MeCN mixture with increasing concentration of urea: 0 M, 4 M, 5 M, 6.3 M. Inset shows absorption spectra of DBPZ (1×10^{-5} M) in 58% water−MeCN mixture in the absence (−) and in the presence (- - -) of urea.

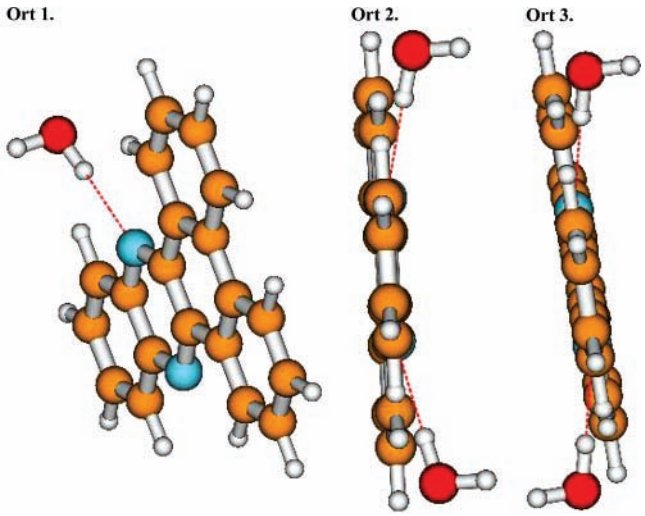

Figure 6. Geometrically optimized structures of DBPZ−$1H_2O$ (Ort 1) and two possible structures of DBPZ−$2H_2O$ (Orts 2 and 3) systems. The color code is red, oxygen; blue, nitrogen; yellow, carbon; and white, hydrogen.

bonding of DBPZ with water and thus diminishes the fluorescence intensity.

Reason for Differential Behavior of TFE and Water. Like many nitrogen-containing heterocyclic molecules, DBPZ has two kinds of excited states, nπ^* and $\pi\pi^*$, which have different emission probabilities. A high emission probability is predicted for the dipole allowed ($\pi \rightarrow \pi^*$) transition, while a lesser emission probability is expected for the (n$\rightarrow\pi^*$) transition because of the space forbidden character of the latter. For DBPZ, like other *N*-heterocyclics, the $^1\pi\pi^*$ and ^{1}nπ^* states are energetically close to each other. The absorption spectra of DBPZ show bathochromic shifts of 3 and 13 nm in TFE and water, respectively. Simultaneously, the room-temperature fluorescence spectra were also red-shifted in hydroxylic solvents. These observations suggest that the lowest excited singlet state of DBPZ has some $\pi\pi^*$ character because the energy of the $\pi\pi^*$ state decreases in the presence of protic solvents due to the formation of a hydrogen bond.[20] In contrast, a simple phenazine molecule shows a blue shift in low temperature absorption[21] and room-temperature fluorescence spectra[22] in the presence of hydroxylic solvents, which suggest that the lowest excited singlet state has some nπ^* character and thus has low emission probability. DBPZ also shows intense well-structured phosphorescence at 77 K in organic glasses. The red shift of phosphorescence spectra in EtOH as compared to that recorded in 1:1 MeCN−toluene mixture suggests that the lowest triplet excited state of DBPZ has also some $\pi\pi^*$ character. One thing still remains to be explained: why the fluorescence intensity decreases and a blue shift in λ_{max} is observed after addition of a certain volume of TFE or water. As discussed earlier on addition of the solvents, TFE and water, the fluorescence intensity of DBPZ initially increases and λ_{max} is red-shifted. This suggests that with TFE or water the first excited singlet state of DBPZ becomes more and more stabilized. As a result, the singlet state lifetime of the hydrogen-bonded DBPZ is greater as compared to the lifetime in non-hydroxylic solvents. After addition of a certain proportion of TFE or water, the energy of the first excited singlet state decreases to such an extent that efficient internal conversion occurs between the first excited singlet state and ground state of DBPZ, and, consequently, the fluorescence intensity decreases. In case of water, maximum steady-state fluorescence intensity is observed at 35 M of water concentration. On further increment of content of water, the fluorescence intensity as well as the fluorescence lifetime of DBPZ decrease as shown in Table 2 with concentration of water as 45 and 54.44 M. As the concentration of water increases, the energy gap between the first excited singlet state and the ground state decreases, efficient internal conversion occurs, and fluorescence intensity decreases. The decrease is much more prominent with water than with TFE. Although TFE has much greater hydrogen-bond donor ability as compared to water, its bulkier size hinders its approach

TABLE 3: Dipole Moment Values of DBPZ in Different Environments

	dipole moment (D)			
	GAMESS values[a]		from ESP-fit charge[b]	
systems	g.s.	e.s.	g.s.	e.s.
free DBPZ	0.43	9.28	0.44	9.45
Ort 1	2.47	9.28	2.54	10.30
without H_2O			0.77	9.31
Ort 2	3.22	10.67	3.29	10.69
without H_2O			0.58	9.20
Ort 3	2.18	11.94	2.21	11.95
without H_2O			0.38	9.10

[a] Values are always for the whole systems, even considering relevant water molecules. [b] Values calculated for the whole systems as well as for the DBPZ component only.

toward DBPZ as compared to that of water. Therefore, greater bathochromic shift of DBPZ is observed in water both in the absorption and in the emission spectra as compared to TFE.

At the highest water content (54.44 M), the red-shifted excited-state hydrogen-bonded species at 486 nm totally disappears, and the fluorescence peak appears with blue shift (inset of Figure 3). Actually, DBPZ exists in equilibrium between the free and hydrogen-bonded form in the presence of hydroxylic solvents, which has also been evident from Figure 2. In case of water, the excited-state hydrogen-bonded species is very much fluorescent in nature. As a result, it masks the fluorescence of the free DBPZ molecule. At higher concentration of water when the fluorescence of the excited-state hydrogen-bonded species is quenched due to internal conversion, then only the fluorescence of the free DBPZ appears prominently (inset of Figure 3). For this reason, at much higher concentration of water the fluorescence peak of DBPZ is blue-shifted and fluorescence quantum yield decreases. In water, the fluorescence peak of DBPZ appears at 410 and 434 nm.

The evidence of this excited-state hydrogen bonding was also found during the studies of the photoinduced electron-transfer reaction between DBPZ and organic amines in triplet states.[23] A prominent magnetic field effect (MFE) was observed for radical ion pairs generated in homogeneous MeCN−water mixture formed through photoinduced electron-transfer reactions. The observation of MFE requires diffusion, spin flipping, and geminate recombination of the radical ion pairs. When the participating radical ions are very close to each other, the exchange interaction will hinder spin conversion (singlet−triplet energy gap of solvent separated ion pair is large). On the other hand, at large distance of separation between the radical ion pairs, the geminate characteristics of the radical ion pairs and their spin correlation are lost and MFE decreases. For this reason, MFE is maximized at an optimum inter-radical distance. Therefore, this observation of MFE was explained by considering inter-radical hydrogen bonding via the intervening water molecules, which helps to sustain the geminate characteristics and hence the spin correlation in the radical ion pairs. Thus, this excited-state hydrogen bonding is also reflected in the triplet state studies of DBPZ.

Theoretical Study. To delineate the structure of the excited-state hydrogen-bonded complex between DBPZ and water, where maximum red shift in emission spectra is found, we carried out extensive model building followed by ab initio quantum chemical calculations. We have done structure optimization for a simple DBPZ molecule, DBPZ−1H_2O (ort 1), DBPZ−2H_2O (Figure 6), and DBPZ−MeCN systems in the ground and excited states to understand the effect of hydroxylic solvents on DBPZ. For the DBPZ−2H_2O system, there are two possible conformations: (i) where two water molecules reside in the same side of the molecular plane (ort 2) and (ii) where two water molecules reside in the opposite side of the molecular plane (ort 3). For the DBPZ−MeCN system, the structure of the complex cannot be optimized because in this case on geometry optimization the MeCN molecule goes far from the DBPZ molecule and no specific interaction can take place between them.

The dipole moment of the free DBPZ molecule is 0.43 and 9.28 D in the ground and excited states, respectively. This suggests that the charge separation is much greater within the molecule in the excited state as compared to the ground state. The electrostatic surface potential (ESP)-fit charges (calculated using MOLDEN software) on the nitrogen atoms of DBPZ are −0.5009 and −0.6479 in the ground and excited states, respectively. This indicates that the probability of hydrogen-bond formation is greater in the excited state. We can calculate the dipole moment of the whole system as well as the DBPZ component of the complex systems using the method mentioned in the Experimental Section. Table 3 shows the dipole moment values of all of the systems, considering the relevant water molecules also, as obtained from the orbitals (GAMESS values) and by calculating from ESP-fit charges, and they are in very good agreement. The intrinsic dipole moments of the DBPZ component are calculated from the ESP-fit charges as shown in Table 3 for both the ground and the excited states. The dipole moment values of the only DBPZ molecule in different orientations (e.g., orts 1, 2, and 3) without the water molecule(s) are close to the free DBPZ molecule (calculated either by GAMESS software or by ESP-fit charge). Thus, we can say that in presence of water the dipole moment of DBPZ is comparable to the free one in the ground and excited states. Table 4 depicts the hydrogen-bond length (HB length), hydrogen-bond energy (E_{HB}), −O−H bond length, and −O−H bond stretching frequency (ν_{-O-H}) for free water and DBPZ−H_2O systems in the ground and excited states. Hydrogen-bond energy in the ground state $(E_{HB})_{g.s.}$ is defined as $(E_{HB})_{g.s.} = E_{Ort} - (E_{DBPZ} + E_{H_2O})$, where E_{DBPZ}, E_{H_2O}, and E_{Ort} stand for the optimized energies of free DBPZ, free water molecule, and DBPZ−H_2O complex in the ground state. Similarly, the $(E_{HB})_{e.s.}$ is defined as $(E_{HB})_{e.s.} = E_{Ort}^* - (E_{DBPZ}^* + E_{H_2O})$, where E_{DBPZ}^* and E_{Ort}^* stand for the optimized energies of free DBPZ and DBPZ−H_2O complex in the excited state. Because Basis Set Superposition Error (BSSE)[24] is meaningless in the excited-state complex, we have not calculated that in either system. In case of −O−H bond length and ν_{-O-H}, we consider the hydrogen of the water molecule(s) that is participating in the

TABLE 4: Hydrogen-Bonding Parameters

	HB length (Å)		E_{HB} (kcal/mol)		−O−H bond length (Å)[a]		ν_{-O-H} (cm^{-1})[b]	
systems	g.s.	e.s.	g.s.	e.s.	g.s.	e.s.	g.s.	e.s.
Ort 1	2.19	2.05	−4.8481	−6.4420	0.9477	0.9535	4096.07	4005.75
Ort 2	2.21	2.05	−9.3662	−12.6638	0.9474	0.9530	4065.56	3959.27
Ort 3	2.20	2.06	−9.4955	−12.6675	0.9474	0.9529	4063.49	3958.37

[a] −O−H bond length for free water is 0.95 Å. [b] ν_{-O-H} for free water is 4027.09 cm^{-1}.

hydrogen-bond formation with DBPZ. For all of the systems, the hydrogen-bond length is shorter in the excited state as compared to the ground state, indicating a stronger interaction in the excited state. This fact is corroborated with the E_{HB} values, which are significantly larger in the excited state than in the ground state. This suggests that hydrogen-bond formation is favorable in the excited state. Furthermore, the −O−H bond length in free water molecule is 0.95 Å, which is somehow elongated in the complex excited state, and simultaneously ν_{-O-H} also decreases as compared to free water (4027.09 cm^{-1}). This reduction in −O−H bond strength of water is a signature of hydrogen-bond formation. The greater value of ν_{-O-H} in the ground state as compared to free water molecule probably suggests the reluctance of the water molecule toward the hydrophobic DBPZ molecule, which may be attributed to the sparing solubility of DBPZ in aqueous medium.

Analysis of energetics of the free DBPZ molecule and DBPZ−H_2O complexes in the ground and excited states shows that with addition of water molecules the energy difference between the ground and excited states decreases. For the free DBPZ molecule, the energy difference between the ground and the excited states is 93.93 kcal/mol, while for ort 1, ort 2, and ort 3 the values of energy difference between ground and excited states are 92.33, 90.63, and 90.76 kcal/mol, respectively. This is also corroborated with the experimental results, where the fluorescence intensity is red-shifted with increasing water concentration.

One relevant question still arises that, although a considerable increase in the dipole moment occurs on photoexcitation of DBPZ, why does the molecule remain insensitive to the polarity of the medium, unlike other phenazine derivatives? The structure of the molecule is responsible for this peculiar behavior. Dias et al.[25] have reported that the 1,8 protons of DBPZ experience moderate deshielding by the nitrogen atoms as compared to its hydrocarbon homologue dibenzo[*a*,*c*]anthracene (DBA). As a result, greater chemical shift for 1,8 hydrogens was found for DBPZ (9.3 ppm) as compared to DBA (8.7 ppm) in the ^1H NMR spectroscopy. The protons in the 1 and 8 positions sterically interact with the lone pair of electrons of the proximate nitrogens. For this reason, bulky solvent molecules cannot interact with the nitrogens, and DBPZ remains insensitive to polarity of its environment. However, hydrogen being the smallest element can come closer to the nitrogen lone pair. For this reason, only those solvents containing hydroxylic groups can interact with DBPZ due to their hydrogen-bond donor ability. Thus, DBPZ acts as a polarity-insensitive hydrogen-bonding probe.

Conclusions

Steady-state absorption and fluorescence behavior of DBPZ have been investigated in various kinds of media, such as protic and aprotic organic solvents of different polarities and also in aqueous environment. DBPZ is not a polarity probe as its absorption and emission spectra remain unchanged in organic solvents of different polarity. However, DBPZ can recognize the solvents with different hydrogen-bond donor ability. Actually, DBPZ exists in equilibrium between the free molecule and hydrogen-bonded complex formed in its first excited singlet state in protic medium. The higher is the hydrogen-bond donor capacity of the solvent, the more the equilibrium is shifted toward the hydrogen-bonded complex, and consequently a red shift in the fluorescence maxima is observed. The formation of a hydrogen bond in the excited state is also supported by the theoretical studies. The structure of the molecule and its change in dipole moment ($\Delta\mu$ = 8.85 D) on photoexcitation are responsible for this excited-state hydrogen bonding with protic solvents. Normal alcohols show only the bathochromic shift and increase in Φ_f with increase in hydrogen-bond donor ability. In the presence of TFE/water, a maximum red shift in λ_{max} is found around some particular concentration, and on further increase in TFE/water concentration there is efficient internal conversion between the excited and ground singlet states, and thus Φ_f decreases. Therefore, it is evident that DBPZ is a good sensor for the protic solvents, and it can sense the presence of protic solvents in an aprotic environment.

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