

Intramolecular Proton Transfer Effects on 2,6-diaminopyridine

A. Antony Muthu Prabhu · S. Siva ·
R. K. Sankaranarayanan · N. Rajendiran

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Abstract The photophysical behaviour of 2,6-diaminopyridine (DAP) has been studied in solvents of different polarity, pH, β -cyclodextrin (β -CD) and compared with 2-amino pyridine (2AP). The inclusion complex of both molecules with β -CD are analysed by UV-visible, fluorimetry, FT-IR, ^1H NMR, SEM and AM1 methods. The solvent studies shows i) DAP gives more red shifted absorption and emission maxima than 2AP molecule and ii) addition of amino group in 2AP effectively increase the resonance interaction in the pyridine ring. A regular red shift observed in acidic pH solutions suggests intramolecular proton transfer (IPT) present in both molecules. β -CD studies indicates i) in pH \sim 7, a regular red shifted absorption and emission maxima observed in AP molecules suggests pyridine ring encapsulated in to the β -CD cavity (1:1 inclusion complex formed) and ii) in pH \sim 1, a blue shifted absorption maxima noticed in 2AP, is due to protonated amino group deeply encapsulated in to the hydrophobic part of the β -CD cavity.

Keywords 2,6-diaminopyridine · β -cyclodextrin · Intramolecular proton transfer · Inclusion complex

Introduction

Intramolecular proton transfer (IPT) is currently one of the most attractive topics of interest as a primary function for photoelectronic devices as well as a basic mechanism of

biological and chemical energy conversion [1, 2]. The process of excited state intramolecular proton transfer which typically shows a largely Stokes shifted fluorescence band has become a field of active research due to its widespread implications as UV stabilization, stimulated radiation production, information storage, fluorescent solar concentrator as well as environmental probes in biomolecules [3, 4]. Solvent effects on proton transfer to solvent raise interesting problems concerning static and dynamic processes, particularly intriguing is the question, when the solvent which is also the proton acceptor behaves as a continuous medium or whether its molecular properties indispensable in understanding proton transfer and proton salvation dynamics. The proton transfer of aromatic molecules which are hydrogen bonded in the ground state undergoes changes markedly depending on the kind of substitution on the aromatic ring or functional group.

Further, protomeric tautomerism of heteroaromatic compounds involving the transfer of hydrogen between cyclic nitrogen and an immediately adjacent substituent atom in the ring has been a field of much interest in experimental as well as theoretical chemistry [5–7]. The simplest molecules having stable tautomeric structure are 2-hydroxy pyridine and 2-aminopyridine (2AP). The tautomerisation in 2AP is accomplished by IPT between the amino and nitrogen atoms. A number of studies on AP molecules have indicated that comparable amounts of both conformers are present in liquid media as supported by FTIR, NMR and dipole moment studies [8, 9], in spite of the fact that imino structure is highly dimmerized in nonpolar solvents [10].

The nature of the emission spectra depends largely on proton accepting ability of the solvent medium. Several investigations have been directed to the identification of the solvent dependent proton transfer reaction of *ortho* amino or *ortho* hydroxyl compounds. Solvent properties are

A. A. M. Prabhu · S. Siva · R. K. Sankaranarayanan ·
N. Rajendiran (✉)
Department of Chemistry, Annamalai University,
Annamalai nagar,
608 002 Tamilnadu, India
e-mail: drrajendiran@rediffmail.com

thought to play an important role in the tautomerization of *ortho* amino or *ortho* hydroxyl aromatic compounds [11–14]. One can easily select solvents which should inhibit or promote intra or intermolecular proton transfer. S. Mukherjee *et al.*, [11–14] discussed the excited state IPT can take place in 4-methyl-2,6-diformyl phenol molecule only in non-polar solvents. Because, non-polar solvents offer as environment where intramolecular H-bond remains undisturbed IPT cannot take place in polar solvents, since the intramolecular H-bond is ruptured by strong interaction with solvents. It is also shown that the spectral changes occur in protic solvents are due to the complete transfer of proton to the solvent and formation of anion. In view of the wide occurrence of such processes, it seems too desirable to obtain further information about the number of conformers and their dependence on the solvent, pH and β -CD characteristics. In this regard, we have examined the spectral properties of 2,6-diaminopyridine in different solvents, pH, β -CD and compared with 2-aminopyridine.

Experimental

Instruments

Absorption spectral measurements were carried out with a Hitachi Model U-2001 UV-visible spectrophotometer and fluorescence measurements were made using a Shimadzu RF 5301 spectrofluorometer. The pH values in the range 2.0–12.0 were measured on Elico pH meter model LI-120. FT-IR spectra were obtained with Avatar-330 FT-IR spectroscopy using KBr pelleting. The range of spectra was from 500 to 4000 cm^{-1} . Microscopic morphological structure measurements were performed with JEOL JSM 5610 LV- scanning electron microscope (SEM).

Reagents and materials

DAP, 2AP and β -CD were obtained from Sigma-Aldrich chemical company and used as such. The purity of the compound was checked by similar fluorescence spectra when excited with different wavelengths. Triply distilled water was used for the preparation of aqueous solutions. Solutions in the pH range 2.0–12.0 were prepared by adding the appropriate amount of NaOH and H_3PO_4 . A modified Hammett's acidity scale (H_0) [15] for the solutions below pH \sim 2 (using a H_2SO_4 - H_2O mixture) and Yagil's basicity scale (H_-) [16] for the solutions above pH \sim 12 (using a NaOH- H_2O mixture) were employed. The solutions were prepared just before taking measurements. The concentration of the AP solutions was the order of 2×10^{-4} to 2×10^{-5} mol dm^{-3} . The concentration of β -CD solution was

varied from 1×10^{-3} to 1×10^{-2} mol dm^{-3} . The solid inclusion complex was prepared by co-precipitation method.

Results and discussion

Effect of solvents

The absorption spectral data, $\log \epsilon_{\text{max}}$ and fluorescence spectral data of DAP and 2AP in the solvents of different polarity and hydrogen bond forming tendency are compiled in Table 1. The absorption spectra of both molecules are red shifted from cyclohexane to methanol but compared with methanol it is blue shifted in water. In all solvents, absorption maxima of DAP is red shifted than 2AP indicates the addition of amino group in 2AP effectively increase the absorption and emission behavior. Absorption and fluorescence solvatochromic shifts of DAP are found to be more than that of 2AP suggests intramolecular hydrogen bonding or intramolecular proton transfer (IPT) present in between the amino and =N- atom.

It is already reported, that 2AP molecule undergoes a tautomerism of the imine-enamine type (Scheme 1). The possibility of the imino pyridine for the APs are first occurred by Marckwald many years ago [17–19]. The FTIR spectrum of DAP shows the absorption frequency at 3445 cm^{-1} is due to NH_2 monomer, the broad absorption frequency at 3207 cm^{-1} is responsible for dimmer structure and the absorption frequency at 3392 cm^{-1} is due to free NH_2 group present in this molecule. In 2AP, NH_2 monomer frequency appears at 3446 cm^{-1} , dimmer frequency appears at 3205 cm^{-1} and free NH_2 group frequency appears at 3353 cm^{-1} . The structure of both molecules analysed by semi empirical quantum mechanical calculations method (CAche and AM1, Scheme 1) and FTIR studies suggest imine-enamine type tautomerism and dimmers are present in both molecules. Three kinds of transition are possible in both AP molecules: (i) n - π^* , (ii) π - π^* and (iii) charge transfer. The nature of the transition depends upon the type of solvent used and the nature of the substituents. There are well known procedures for establishing the nature of the transition. The data in Table 1 shows high values of the molecular extinction coefficient and a small change in the absorption spectra in polar and hydrogen bonding solvents suggests n - π^* transition is not present in both molecules [20]. Distinction between π - π^* and charge transfer bands can be made from a correlation of the magnitude of the Stokes shift with the nature of the substituent and solvent. In general, large Stokes shift and greater solvent dependence are observed for the charge transfer band. Our data in Table 1 clearly shows that the lowest energy transition in the neutral AP molecule is π - π^* character [21, 22].

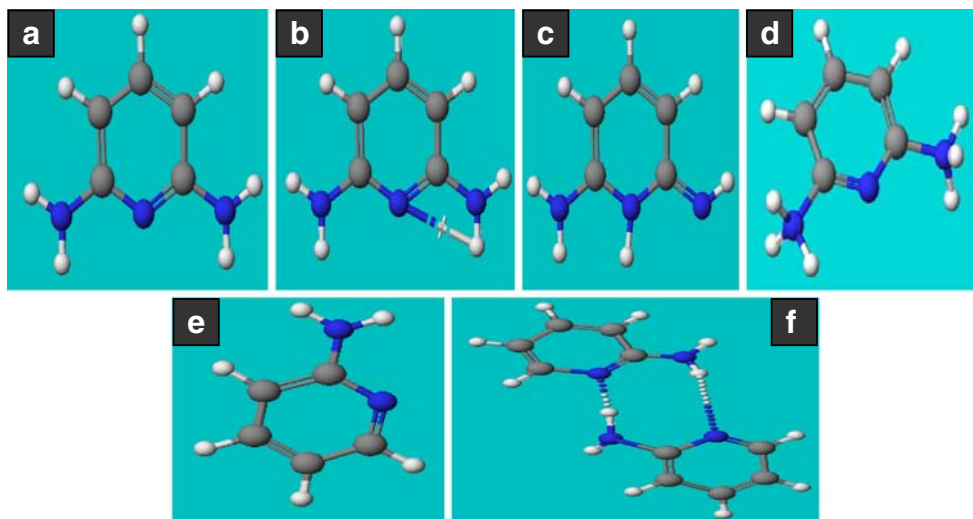
Table 1 Absorption and fluorescence spectral data of amino pyridines in selected solvents

Solvents	2,6-Diaminopyridine				2-Aminopyridine				Pyridine			
	λ_{abs}	$\log \epsilon$	λ_{flu}	ν_{ss}	λ_{abs}	$\log \epsilon$	λ_{flu}	ν_{ss}	λ_{abs}	$\log \epsilon$	λ_{flu}	ν_{ss}
Cyclohexane	295	3.55	337	4413	291	4.56	327	3724	251	4.55	360	11984
	230	3.94			238	4.60						
Dioxane	305	3.67	342	3547	292	4.58	340	4770				
	243	3.97			240	4.66						
Acetonitrile	305	3.68	352	4377	291	4.63	340	4953	251	4.64	320	8511
	246	3.95			234	4.72						
<i>t</i> -Butyl alcohol	307	3.51	355	4405	295	4.65	345	5205				
	242	3.96			234	4.73						
2-Propanol	307	3.50	356	4483	296	4.67	350	5212				
	242	3.95			235	4.75						
Methanol	307	3.97	358	4640	294	4.66	350	5380	257	4.65	350	10662
	242	4.02				4.73						
Water	303	3.71	365	5663	291	4.64	360	6528	256	4.60	356	10897
	240	3.78			228	4.72			253	4.66		
									251	4.74		
Correlation coefficient												
(i) $E_{\text{T}}(30)$				0.875				0.923				
(ii) BK				0.752				0.790				

Solvents can interact either with =N- or amino group. The interaction of solvents with the lone pair of the amino group will lead to a blue shift and the solvent interaction with hydrogen atom of the amino group or lone pair of

the =N- will lead to a red shift both in absorption and fluorescence. Thus, in water the blue shifted absorption maxima suggests the formation of hydrogen bond with the lone pair of the amino group (solute) inhibiting its

Scheme 1 CCache structure and Bond distance (\AA) of DAP and 2AP: (a) amino, (b) IPT, (c) imino, (d) dication, (e) amino 2AP and (f) dimmer structure 2AP



Bond distances (\AA)

2AP: $\Delta H = 33.5466$ kcal/mol, $\text{NH} - {}^4\text{H} = 5.80$, $\text{NH} - {}^6\text{H} = 4.57$

DAP: $\Delta H = 35.8017$ kcal/mol, $\text{NH}^1 - \text{NH}^3 = 4.73$,
 $\text{NH}^2 - \text{NH}^4 = 6.42$, $\text{NH}^1 - \text{CH}^3 = 5.79$, $\text{NH}^2 - \text{CH}^3 = 5.82$

interaction with the π -cloud. The red shift in aprotic solvents is due to the usual dipole-dipole effect on the π - π^* transition or hydrogen donating character of the amino group [21, 22]. So the above shifts are mainly due to interactions of the solvents with the amino group of DAP. The regular red shift in pyridine is due to lone pair of the pyridine nitrogen to the solvents.

The fluorescence spectrum is regularly red shifted as the polarity and proton donor capacity of the solvent increase (Fig. 1). Generally, the fluorescence spectra results can be explained by charge migration from the amino group to the heterocyclic ring upon excitation. That is, the charge density of the nitrogen atom decreases resulting in an increase in the proton donor ability of the amino group. In APs, the absorption and emission spectra are red shifted in polar and non-polar solvents. This is probably a similar type of tautomers present in both molecules. In the case of pyridine, the H-bonding interactions of solvent molecule with the pyridine nitrogen destabilized the closely spaced higher energy (n, π^*) state, thereby decreasing the singlet-triplet interaction and they increasing fluorescence quantum yield. In general, such interactions facilitate the charge transfer from the amino group to the partially positively charged H-bonded ring nitrogen which stabilizes the charge transfer (CT) state as indicated by the red shift of the spectrum in the presence of H-bonding solvents. In the case of DAP, a second type of H-bonding interaction, where the amino lone pair acts as donor is also possible. Usually, the H-bonding interactions of the solvent molecules with the amino group of aromatic amines enhances molecular fluorescence as a result of destabilization of the CT state, thus reducing the CT character of the emitting state (π - π^*). The fluorescence enhancement of DAP in H-bonding solvents can also be explained using the same principles [21, 22]. The fluorescence spectrum in each solvent is broader, that is having broader full width at half maximum

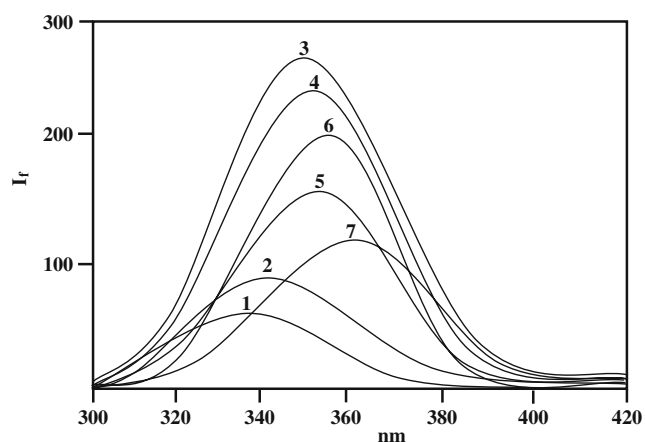


Fig. 1 Fluorescence spectra of DAP in selected solvents: (1) cyclohexane, (2) dioxane, (3) ethyl acetate, (4) acetonitrile, (5) 2-propanol, (6) methanol, (7) water

height (FWHM). The FWHM of the fluorescence spectrum is not increased in polar solvents. This suggests that different species are present in both AP molecules.

In cyclohexane and aprotic solvents, the fluorescence intensities are very weak (Fig. 1). This can be explained as follows: it has been reported that in nitrogen heterocycles, (e.g., quinoline, isoquinoline and acridine) there is a $^1n\pi^*$ state very close to the (π - π^*) state [21, 22]. In fact, in hydrocarbon solvents, the lowest excited singlet state in these molecules is of $^1n, \pi^*$ type. Most of these nitrogen heterocycles are weakly or non-fluorescent in non-polar and aprotic solvents. However, in protic solvents, they become strongly fluorescent due to a reversal of n, π^* and π, π^* states [21, 22]. Thus, π - π^* is the lowest energy transition in these molecules in protic solvents. Lim [23] and Hochstrasser [24] have also explained by this vibronic interaction between the $^1n, \pi^*$ and $^1\pi, \pi^*$ states. Since both APs are heterocyclic, their fluorescence properties can be similarly explained. Although $^1\pi, \pi^*$ is the lowest excited singlet state in the solvents studied in this work, a small contribution from a close-lying $^1n, \pi^*$ state is evident from the absorption and fluorescence data. In protic solvents, the energy gap between these two states is probably very small. As a result, vibronic interactions between the states are high. This reduces the symmetry of the $^1\pi, \pi^*$ state and thus increases the Franck-Condon factor. Consequently, the rate of radiation less decay increases. This explains the low fluorescence intensity of DAP in cyclohexane and aprotic solvents. However, in protic solvents, the H-bonding interaction of the solvent molecules with the lone pair of pyridine nitrogen destabilizes the $^1n, \pi^*$ state but stabilizes the $^1\pi, \pi^*$ state, thus reducing the energy gap between the states and in turn, decreases the vibronic interaction.

It is also noted that, the fluorescence excitation spectrum of DAP in methanol shows same spectral shape depending on the emission wavelength (330, 345, 360 nm) (Fig. 2). The emission intensities of DAP in cyclohexane is very weaker than other solvents. Further, the emission spectrum of DAP in water is also shows same feature depending on the excitation wavelength (290, 320 and 340 nm). These results suggest that the different species may be present in both molecules (Scheme 1). In non polar solvents, the emission intensities are very low indicates the presence of dipolar structure in both molecules. The emission in water is strongly pH dependent (discussed in latter) with the Stokes shift reaching its maximum value in acidic aqueous solutions (303 to 330 nm). The broad Stokes shifted emission at acidic pH is common in molecules having an electron withdrawing group such as =N- and COOH attached to an aromatic nucleus. However, the nature of such emission is not always easy to ascertain, since it can be the result of a variety of causes, including dimmer formation in the ground states (or other kinds of aggregates)

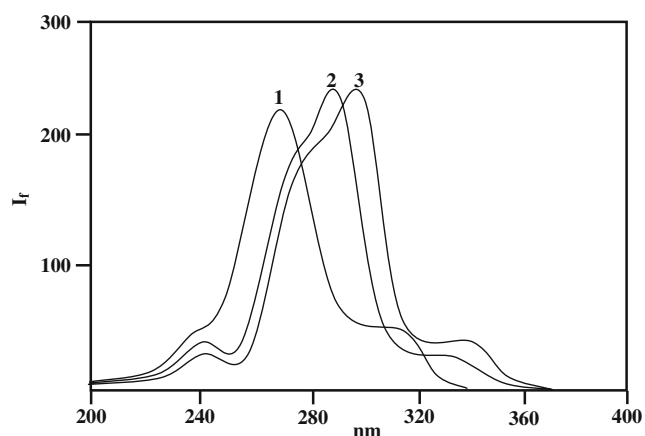


Fig. 2 Excitation spectra of DAP in methanol: (1) 330 nm, (2) 345 nm, (3) 360 nm

excimer formation or charge transfer processes. In both APs, the donor is amino group and the acceptor is the tertiary nitrogen atom. In an acidic medium, the tertiary nitrogen atom becomes more conjugated with the aromatic π -system, in a situation in which there is a marked charge separation within the molecule. The small Stokes shift in polar and nonpolar solvents would indicate a primarily dipolar interaction between the solute and the solvent molecules.

The Stokes shifts [$\bar{\nu}_{\text{abs}}(\text{max}) - \bar{\nu}_{\text{flu}}(\text{max})$] increases with an increase in the polarity of H-bonding of the solvents indicating that the dipole moment of the molecules increases upon excitation. The increase in the dipole moment in the S_1 state suggests both molecules are more polar in the S_1 state. Empirically or theoretically derived solvents parameters like $f(D,n)$ [25], BK [26] and $E_T(30)$ [27] values are accurate parameters of solvent polarity have been used by several authors to correlate molecular spectroscopic properties. Among these parameters, $f(D,n)$ and BK parameters are taken into account of solvent polarity alone, whereas $E_T(30)$ incorporates both solvent polarity and hydrogen bonding effects. From the correlation of Stokes shifts with any one of these parameters on idea of about the type of interaction between the solute and solvent can be obtained. The solvatochromic shifts reveal that the hydrogen bonding interactions are present along with dipole interactions. In order to confirm this, we have used the above solvent parameters and the values are compared with Stokes shifts of AP molecules (Table 1). Figure 3 shows the plots of Stokes shifts vs $f(D,n)$, BK and $E_T(30)$ parameters. The increase in Stokes shifts from cyclohexane to water in AP molecules are found to be more in accordance with $E_T(30)$ than with other parameters. As mentioned earlier, $E_T(30)$ parameter incorporates both H-bonding and solvent polarity effects, whereas the BK and $f(D,n)$ parameters represent only solvent polarity

effects. Since H-bonding interactions are predominant in the solvatochromic shifts of both molecules, $E_T(30)$ gives good correlation than the other two parameters.

Effect of proton concentration

Absorption and emission spectra of both APs have been studied in the acidity/basicity range from $H_0 -10$ to $H_+ 16$. In the S_1 state, four prototropic species- dication, monocation, neutral and monoanion have been observed. The absorption maxima of both APs are shown in Table 2. With the decrease on pH from 8, the absorption spectra of both APs are regularly red shifted and the absorption spectra so obtained in DAP/2AP does not resemble with 2AP/pyridine respectively. In DAP, the difference in the red shifted maxima (303 to 330 nm) is greater than 2AP (287 to 296 nm). Further increase on proton concentration, the absorption spectra is again red shifted in 2AP, whereas, the absorption maxima largely blue shifted in DAP (330 to 299 nm). The former red shifted spectrum is due to formation of monocation and the latter red shifted spectrum in 2AP and blue shifted maximum in DAP is due to the formation of dication. The absorption spectra of both compounds does not change when the basicity is increased until $H_+ 14$, however, a red shift is noticed at the highest basic concentration ($H_+ 17$). The red shifted spectrum is due to the formation of monoanion of both molecules.

The unusual regular red shift in the absorption spectra of the monocation (MC) is due to presence of IPT between the two groups (NH_3^+ and $=N^-$) which has caused a large red shift in the absorption spectra of the MCs. Further, the literature results [21, 22] shows, even after protonating the amino group, the protonation at $=N^-$ and $-NH_2$ lead to red shift and blue shift respectively, if $\pi-\pi^*$ is

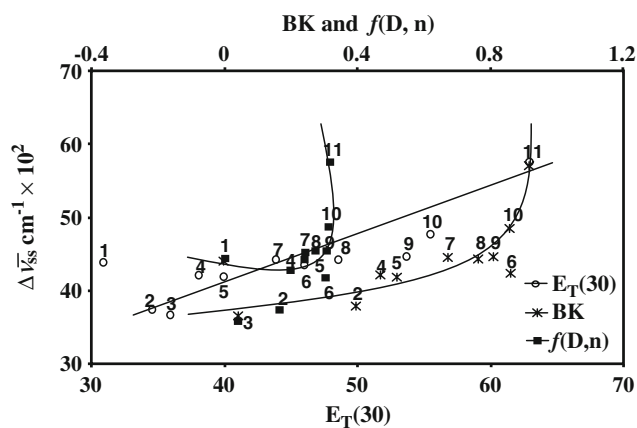


Fig. 3 Plot of Stokes shifts (cm^{-1}) of DAP vs $E_T(30)$, BK and $f(D,n)$ solvent parameters: (1) cyclohexane, (2) diethyl ether, (3) dioxane, (4) ethylacetate, (5) methylacetate, (6) acetonitrile, (7) *t*-butyl alcohol, (8) 2-propanol, (9) ethanol, (10) methanol, (11) water

Table 2 Absorption and fluorescence prototropic maxima (nm) of amino pyridines in aqueous and β -CD medium

Species	DAP				2AP			
	Aqueous medium		β -CD medium		Aqueous medium		β -CD medium	
	λ_{abs}	λ_{flu}	λ_{abs}	λ_{flu}	λ_{abs}	λ_{flu}	λ_{abs}	λ_{flu}
Dication	299	390	298	390	300	365	298	365
	211		210		229		231	
Monocation	303–330	370	328	370	293–296	370	291	370
	241		241		229		230	
Neutral	305	362	298	363	289	360	286	355
	238		235		227		228	
Monoanion		422				400		
		331						

nf=non-fluorescent

the lowest energy transition and the system is not involve charge transfer or IPT.

The fluorescence spectra of various prototropic species of the both DAP is shown in Table 2. The fluorescence characteristics of both molecules are different from its absorption characteristics. When the pH is decreased from 8.0, the fluorescence maximum is red shifted and given maximum of 370 nm. Further increase on hydrogen ion concentration, 2AP gives a blue shifted fluorescence spectrum whereas DAP gives red shifted maximum suggest that in both molecules, the former species is monocation formed by protonation of =N- atom and the latter species is dication obtained by the protonation of the amino group.

DAP possesses three protonation sites (one lone pair of the tertiary nitrogen atom and two amino groups) and two deprotonation sites (two amino group). The monocation absorption is strongly red shifted in comparison with the neutral species. If the protonation had occurred at the amino group, the absorption spectrum of DAP/2AP should have been blue shifted and resembles the 2AP/pyridine absorption spectrum as noticed in other aromatic amines [28–32], where the absorption and fluorescence spectra of the protonated species resembles those of the parent molecules.

However, it is interesting to note that, the spectrum of the monocation species is regularly red shifted relative to that of neutral form of the 2AP/ pyridine molecules. The red shifted spectrum is most likely due to stronger solute-solvent interaction of the positively charged monocation species with the water molecules. Further, if the dication occurred at the pyridinium nitrogen atom, then one should have expected a red shift in the absorption spectrum. However, this is in contrast to 5-aminoquinoline in which the monocation occurs at the ring nitrogen [21, 22]. The pK_a value of the corresponding ammonium ion is much higher than that of aniline (4.5) suggests that the IPT interaction of the amino group of both APs with its pyridine ring is much stronger than in aniline (Table 3). Thus, in the case of 2AP, the second protonation occurs at the ring nitrogen to produce dication species as indicated by the red shift of the long wavelength absorption band. Therefore, the red shifted absorption spectrum around pH 6.5 can be ascribed to the monocation species. The pK_a values of the dication species of APs are lower than that of pyridine (4.5). The low basicity of the ring nitrogen of the protonated APs are obviously due to the extended conjugation and the presence of a positively charged NH_3^+ group

Table 3 pK_a (pK_a^*) values of different prototropic equilibrium of all APs in the S_0 and S_1 states

Equilibrium	DAP				2AP			
	Aqueous medium		β -CD medium		Aqueous medium		β -CD medium	
	pK_a abs	pK_a^* FT	pK_a abs	pK_a^* FT	pK_a abs	pK_a^* FT	pK_a abs	pK_a^* FT
Dication Monocation	4.2	4.1	4.2	4.2	4.2	4.2	4.2	4.1
Monocation Neutral	6.7	6.7	6.6	6.5	6.7	6.6	6.6	6.6
Neutral \rightleftharpoons Monoanion	14.6	14.8			14.5	14.0		

Table 4 Absorption and fluorescence maxima (nm) of DAP and 2AP in β -CD medium

β -CD concentration (M)	DAP						2AP					
	pH~8			pH~1			pH~8			pH~1		
	λ_{abs}	$\log \epsilon$	λ_{flu}	λ_{abs}	$\log \epsilon$	λ_{flu}	λ_{abs}	$\log \epsilon$	λ_{flu}	λ_{abs}	$\log \epsilon$	λ_{flu}
Without β -CD	304	4.32	361	331	4.61	394	291	3.50	360	300	3.55	370
	238	4.41		241	4.63		229	4.13		229	4.17	
0.002	306	4.39	363	331	4.65	394	297	3.54	365	299	3.63	370
	241	4.46		242	4.68		229	4.13		229	4.19	
0.006	306	4.46	364	331	4.66	394	298	3.70	370	298	3.76	370
	241	4.49		241	4.73		228	4.15		228	4.19	
0.010	306	4.59	365	331	4.68	394	298	3.79	370	297	3.84	370
	241	4.53		240	4.78		229	4.16		229	4.21	
K (M^{-1})	152		132	435		468	251		163	637		345
ΔG (-ve)	8.1		12.1	10.1		12.2	8.2		12.4	10.4		12.5

which reduces the charge density of the pyridine nitrogen atom. A similar results has also been observed by Dey *et al.* [21, 22].

To check the above point, the absorption and emission spectra of pyridine have been taken in different solvents and acid concentration. This shows that the monocation maxima of DAP/ 2AP are not similar to the spectra of 2AP/ pyridine respectively. The unusual red shift observed in the absorption and emission spectra of DAP/2AP monocation does not resemble with 2AP/ pyridine suggest that IPT interactions takes place from amino to pyridine ring. Thus, in DAP due to presence of IPT a larger red shifted monocation maximum is observed.

To test the IPT process in both molecules, the absorption and fluorescence spectra of DAP in cyclohexane are recorded with addition of trifluoroacetic acid (TFA). An addition of TFA in cyclohexane gives the similar red shifted monocation and a dication maximum indicates IPT present in both AP molecules. Further, an addition of 1% water does not change the spectral maximum of cyclohexane rule out the formation of an exciplex and solvent relaxation. These results suggest that the red shifted monocation maximum is due to hydrogen of the NH_3^+ group interact with =N- atom of the pyridine ring.

In the excited state, DAP gives an unusual red shifted dication (DC) may be due to the strong IPT interactions present between the two NH_3^+ groups and =N- atom which has causes a large red shift emission maximum. This is because, protonated amino groups interact with tertiary nitrogen atom through IPT process, hence a red shifted maximum is observed in 2AP molecule. The larger red shift observed in DAP monocation indicates strong IPT interaction is present in this molecule. Interestingly, trication is not formed in DAP, because (i) in DAP, all the three nitrogen's

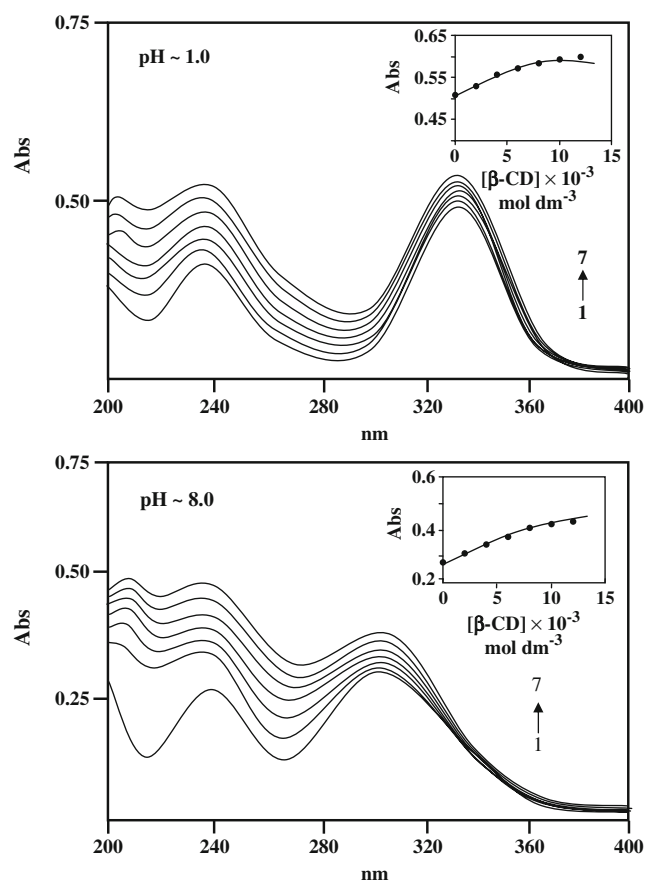


Fig. 4 Absorption spectra of DAP at different β -CD concentrations (mol dm^{-3}): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008, (7) 0.01. Insert Fig: absorbance vs. β -CD concentration

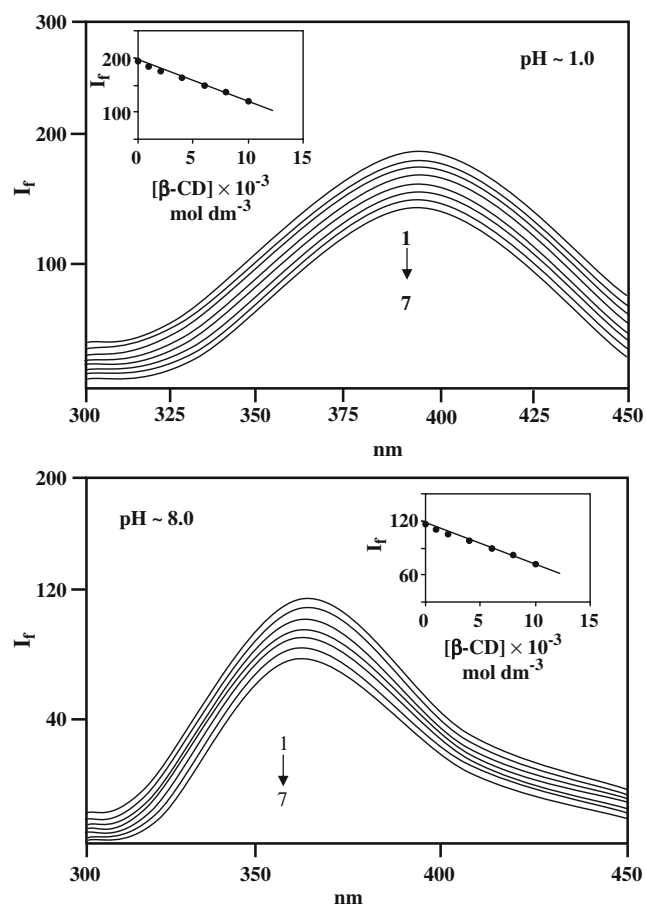


Fig. 5 Fluorescence spectra of DAP in different β -CD concentrations (mol dm^{-3}): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008, (7) 0.01. Inset Fig: fluorescence intensity vs. β -CD concentration

are present in the adjacent positions and (ii) IPT interactions prevent the formation of trication. From the above discussions we conclude IPT will be the predominant in both AP molecules.

Effect of β -CD

Table 4 depicts the absorption and fluorescence spectral maxima of DAP and 2AP in different β -CD concentrations ($\text{pH} \sim 8$ and $\text{pH} \sim 1$) are shown in and Fig. 4. In $\text{pH} \sim 8$, the absorption maxima of DAP is slightly red shifted, but in $\text{pH} \sim 1$, the absorbance increased at the same wavelength. This is because, neutral species present in former pH ($\text{pH} \sim 8$), whereas in latter ($\text{pH} \sim 1$) cationic species are present. When the β -CD concentration is increased in $\text{pH} \sim 8$ solution, the absorption maxima of 2AP is regularly red shifted from water to 0.01 M β -CD, whereas it is slightly blue shifted in $\text{pH} \sim 1$. Compared to 2AP, in both pH β -CD solutions, no significant absorption spectral shift is noticed in DAP, however, DAP absorbance slightly increases with an increasing β -CD concentration (i.e. close to each other), whereas in 2AP,

absorbance significantly increased. The above results suggest both molecules are transferred from more protic environments (bulk aqueous phases) to less protic environments (β -CD cavity) [33–38]. The red shifted absorption spectrum and increase of absorptivity generally observed for the inclusion complexes [39–44]. Isosbestic point could be noticed in both molecules indicates the presence of a single equilibrium involving 1:1 complexation between AP molecules with β -CD.

Figure 5 shows the emission spectra of DAP in different β -CD concentrations ($\text{pH} \sim 8$ and $\text{pH} \sim 1$). In $\text{pH} \sim 1$, no significant spectral changes observed, but it is red shifted in $\text{pH} \sim 8$. In both pH solutions, upon increasing the concentration of β -CD, the fluorescence intensities of both molecules are decreased from zero to higher β -CD concentrations. The fluorescence intensities are decreased along with increasing the β -CD concentration confirming that both molecules completely encapsulated in the β -CD cavity. The above results indicate the formation of different type of inclusion complex between both molecules with β -CD. As mentioned earlier, the spectral red shift in $\text{pH} \sim 8$ suggests the amino group interact with β -CD secondary -OH groups. Further, the decreasing fluorescence intensities in the same wavelength in $\text{pH} \sim 1$ solution shows the pyridinic ring and amino group deeply encapsulated in the β -CD cavity [39–44]. In $\text{pH} \sim 8$, the absorption spectra regularly red shifted in β -CD solutions are due to protonation takes place in pyridine nitrogen because it is well known fact that CDs are good proton donors and protonation on pyridine nitrogen give red shifted maxima [39, 40].

The binding constant for the inclusion complex formation has been determined by analysing the changes in the intensity of absorption and fluorescence maxima with the β -CD concentration. In order to determine the stoichiometry of the inclusion complex, the dependence on β -CD of the DAP and 2AP absorbance and fluorescence have been analysed by using the Benesi-Hildebrand equation 1:1 complex [45] (Eq. 2) between DAP and β -CD as shown below:

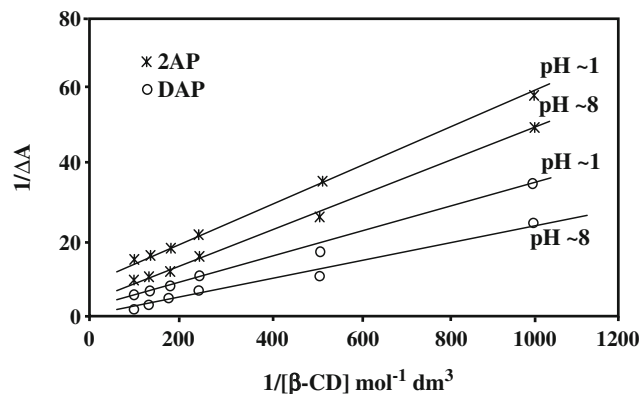


Fig. 6 Absorption spectra of Benesi-Hildebrand plot for the complexation of DAP and 2AP with β -CD: Plot of $1/(A-A_0)$ vs $1/[\beta\text{-CD}]$

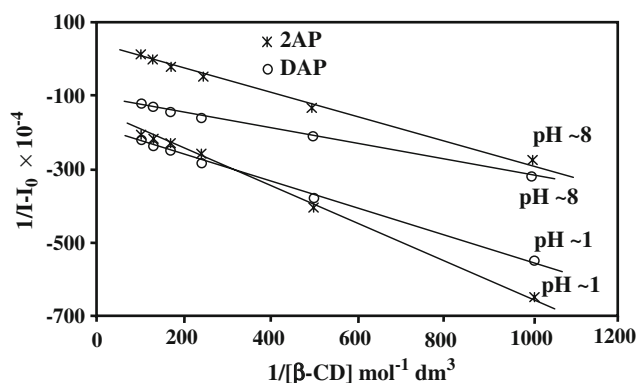
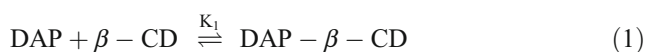


Fig. 7 Fluorescence spectra of Benesi-Hildebrand plot for the complexation of DAP and 2AP with β -CD: Plot of $1/I-I_0$ Vs $1/[\beta\text{-CD}]$



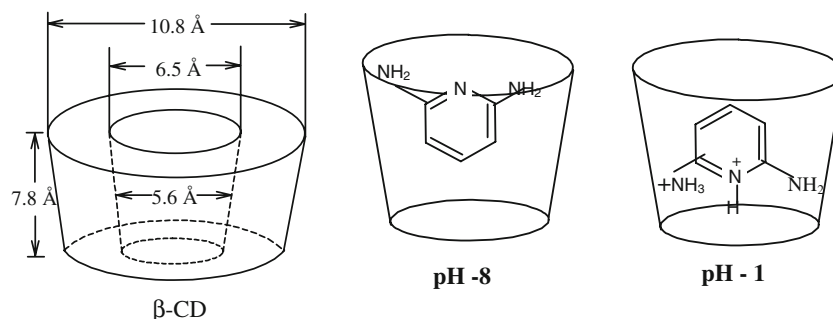
$$\frac{1}{I-I_0} = \frac{1}{I' - I_0} + \frac{1}{K(I-I_0)[\beta\text{-CD}]} \quad (2)$$

where K is the formation constant, I_0 is the initial absorption/fluorescence intensity of free DAP, I' the absorption/fluorescence intensity of β -CD inclusion complex and I is the observed absorption, fluorescence intensity. According to Eq. (2), a plot of $1/I-I_0$ versus $1/[\beta\text{-CD}]$ (both absorption and fluorescence) gives a linear relationship as shown in Figs. 6 and 7. This analysis reflects the formation of 1:1 inclusion complex between APs and β -CD complex. The ' K ' values obtained for 1:1 complexes are given in Table 4. The values of binding constant were calculated from the slope and the intercept of the plot. The binding constants of both pH solutions are significantly changed reveal that different inclusions are associated with both AP molecules. The quantitative comparison of binding constants of the two complexes of both pH solutions suggests β -CD provides a better site to accommodate of deep inclusion of 2AP in the β -CD cavity.

Of the two species (neutral, cation) we should note that, the β -CD can readily include the protonated species than

neutral species. By assuming this orientation for the AP molecules in the β -CD cavity is easy because cyclodextrin cavity favours the protonated form of the guests. This is because the lone pair of amino group may interact with -OH group of the β -CD. Further, in β -CD, the spectral red shifts at pH ~ 8 suggest that, amino group is located in the polar medium. This implication is based on the following reasons: The large rim of β -CD contains 12 secondary hydroxyl groups, and thus provides an environment qualitatively similar to polyhydroxy alcohols [46]. It is well known that substituents of aromatic/ heterocyclic rings capable of hydrogen bonding can bind the -OH groups of the β -CD edges. The energy involved in such hydrogen bond interaction is responsible for the higher/lower binding constants found, when compared to those of the substituted/ unsubstituted molecules. In pH ~ 1 , the higher formation constant in APs implies that the protonated APs are more easily embedded in the β -CD cavity than neutral AP. Further blue shift at pH ~ 1 suggest, protonated amino group present in the interior part of the β -CD cavity. Since the dipole-dipole interaction between the APs and β -CD is lowered in the less polar environment (hydrophobic part) a blue shift is observed in pH ~ 1 solution. The formation of the 1:1 guest: host complex is clearly demonstrated in Scheme 2. A large blue shift is observed in the absorption spectra indicate the interaction between both heterocyclic moieties may be hindered by the complexation.

This is further supported by the following observations: the ground state geometry of DAP and 2AP are optimized by using AM1 method (Scheme 1 and 2). In DAP, the vertical distance between $\text{NH}^2\text{-NH}^4 = 6.42 \text{ \AA}$, $\text{NH-H}^6 = 4.90 \text{ \AA}$, $\text{NH}^1\text{-CH}^3 = 5.79 \text{ \AA}$ and $\text{NH}^2\text{-CH}^3 = 5.82 \text{ \AA}$. The internal diameter of β -CD is approximately 6.5 \AA and its height is 7.8 \AA . Considering the shape and dimensions of β -CD and AP molecules, both APs can completely encapsulated in the β -CD cavity, because the length of both AP molecules are lower than that of upper/lower rim of β -CD cavity. Taking into account the dimensions of the AP and β -CD, the complex can be located as shown in Scheme 2. The neutral and monocation maxima of both APs in β -CD



Scheme 2 Proposed inclusion complex structure of DAP

medium is not significantly red or blue shifted than aqueous medium (Tables 3 and 4), again confirms the amino group may interact with -OH group of β -CD molecule.

The free energy change can be calculated from the formation constant (K) by eqn.

$$\Delta G = -RT \ln K$$

The ΔG values for the association of the both molecules with β -CD are given in Table 4. The ΔG values are negative which suggests that the inclusion process proceeded simultaneously at 303 K.

Prototropic reactions in β -CD medium

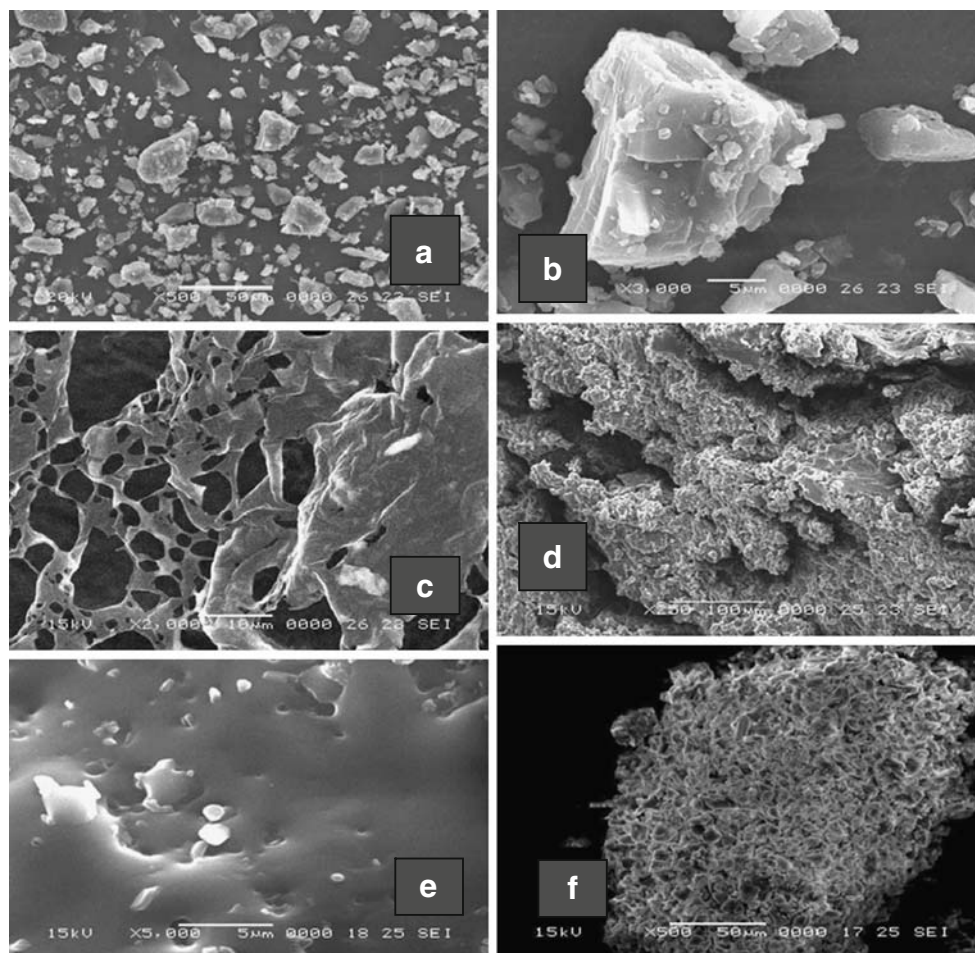
To know the effect of β -CD on the prototropic equilibrium between neutral, monocation and dication on the pH dependent changes in the absorption and emission spectra of both molecules in aqueous solution containing β -CD have been recorded and are shown in Table 2. The absorption and emission maxima of both APs have been studied in 6×10^{-3} M β -CD solutions in the pH range from 1 to 10. On comparison with aqueous medium, the absorption

maxima of both AP molecules are not significantly shifted in β -CD medium. The ground and excited state pK_a (pK_a^*) values of both AP molecules in β -CD medium are not different from aqueous medium (Table 3).

FTIR spectral studies

The FT-IR spectra of DAP, AP, β -CD and the solid inclusion complex are also studied. Both molecules are examined in KBr pellet, displays one absorption band at 3392 cm^{-1} in DAP and 3353 cm^{-1} in 2AP. Both bands represent the $-\text{NH}_2$ stretching frequencies and it moves in the inclusion complex to 3385 cm^{-1} and 3362 cm^{-1} respectively. The dimer frequencies of both molecules appear around 3207 cm^{-1} is lost in the inclusion complex. The pyridinic ring stretching of both molecules at $1636\text{--}1565 \text{ cm}^{-1}$ is moved in the inclusion complex to $1630\text{--}1488 \text{ cm}^{-1}$. The N-H bending frequencies at 1595 cm^{-1} in DAP is moved in the inclusion complex to 1568 cm^{-1} . The C-N stretching frequencies at 1304 cm^{-1} in DAP and 1323 cm^{-1} in 2AP are moved in the inclusion complex to 1310 cm^{-1} and 1325 cm^{-1} . The C=C stretching vibration of

Fig. 8 Scanning electron microscope photographs (pt. coated): (a) & (b) β -CD, (c) 2AP, (d) 2AP- β -CD complex, (e) DAP, (f) DAP- β -CD complex



both molecules (DAP=1451 cm^{-1} , 2AP=1442 cm^{-1}) are moved in the inclusion complex to 1445 cm^{-1} . The C=N stretching vibrations of both molecules (DAP=1467 cm^{-1} and 2AP=1487 cm^{-1}) is also shifted in the inclusion complex to 1488 cm^{-1} and 1490 cm^{-1} respectively. The C-H out of plane bending vibrations at 781 cm^{-1} is moves in the inclusion complex to 746 cm^{-1} . Moreover, the absorption intensity of most of the frequencies in the inclusion complex is significantly weaker (10–50%) than the pure molecule. The above results indicate that the amino group is present in hydrophilic part and the pyridine ring is encapsulated in the β -CD cavity.

^1H NMR spectral shifts

Proton nuclear magnetic resonance (^1H NMR) spectroscopy has proved to be a powerful tool in the study of inclusion complexes [47–49]. ^1H NMR spectroscopy provides an effective means of assessing the dynamic interaction site of β -CD with that of the guest molecules. The basis of information gained from NMR spectroscopy is located in this shifts, loss of resolution and broadening of signals observed for the host and guest protons [49].

The resonance assignments of the protons of β -CD are well established [47, 48]. The chemical shift of β -CD protons (consist of six types of protons) reported by different authors [47–49] are very close to those reported in this work. Even though, only limited information can be obtained from the ^1H NMR data, the observation of slight down field shift of the guest protons in the presence of β -CD is consistent with the inclusion of each guest into the β -CD cavity. The H-3 and H-5 protons are located in the interior of the β -CDs cavity, and it is, therefore likely that the interaction of the host with the β -CD inside the cavity will affect the chemical shifts of the H-3 and H-5 protons. A minor shift is observed for the resonance of H-1, H-2 and H-4 located on the exterior of β -CD [47, 48]. In particular, the resonance of the protons of β -CD located within or near the cavity showed remarkably largely down field shift in the inclusion complex. The addition of DAP or 2AP into the β -CD results in a downfield chemical shift for the AP protons in DMSO are given below: **DAP (inclusion complex)** ppm: NH_2 \sim 5.54 (5.58), ^3H , ^5H \sim 5.901 (5.905), ^4H \sim 7.220 (7.225), **2AP (inclusion complex)** values: NH_2 \sim 4.63 (4.68), ^3H \sim 6.469 (6.472), ^4H \sim 7.379 (7.383), ^5H \sim 6.605 (6.608), ^6H \sim 8.049(8.054). In both APs, the amino and C-H are downfield shift in the complex, which suggest that both AP molecules are shielded largely in the complex and it must be penetrate deeply into the cavity. As can be seen from the above values, the chemical shifts for the inclusion complex was different from the free compound. A small downfield shift on both APs are

observed in pyridine ring suggest both molecules completely encapsulated in to the β -CD cavity.

Microscopic morphological observation

Firstly, we observed powdered form of DAP, 2AP and β -CD by scanning electron microscope and then we also observed powdered form of inclusion complex (Fig. 8). These pictures clearly elucidated the difference of APs and their inclusion complex. As seen from the SEM figures (i) β -CD is present in platted form, (ii) APs are present in colloidal form and (iii) the inclusion complex is also present in colloidal form. Modification of these structures can be assumed as a proof of the formation of a new inclusion complex. The colloidal structure of pure APs and their inclusion complex again supported the absence of solid inclusion complex.

Conclusions

From the above study we find out the following conclusions: i) the solvent studies shows more red shift is observed in both absorption and emission maxima in DAP than 2AP molecule indicating that addition of amino group in 2AP molecule is effectively increase the resonance interaction in pyridine ring, ii) the regular red shift observed in pH solutions shows IPT interactions are present in both molecules, iii) in pH \sim 7, a red shift observed in β CD concentrations indicates pyridine ring present in to the β CD cavity (1:1 inclusion complex formed) and iv) in pH \sim 1, a blue shift is noticed in 2AP suggests amino group present in to the β CD cavity.

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