Azonium-ammonium tautomerism and inclusion complexation of 4-amino-2', 3-dimethylazobenzene

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The spectral characteristics of 4-amino-2',3-dimethylazobenzene (GBC), 4-aminoazobenzene (AAB) and azobenzene (AB) have been studied in various solvents, varying hydrogen ion concentrations and in β-cyclodextrin (β-CD). The inclusion complexes of GBC, AAB and AB with β-CD have been analysed by UV-visible, fluorometry, FT-IR, 1H NMR, SEM and Cache-DFT methods. The solvent study shows that the azo form is present only in GBC and AAB molecules. No significant spectral difference is observed in GBC indicating that the presence of two methyl groups does not effectively change the spectral behaviour as compared to that of AAB. In acid solutions, unusual red shift is observed in the monocation suggesting that the azonium-ammonium tautomer is present in both molecules. The absorption maximum at ~500 nm, is due to the azonium cation while that at ~320 nm originates from the ammonium cation. In β-CD solutions, the increase in the fluorescence intensity and large bathochromic shift in S1 state indicates that both GBC and AAB form 2:2 inclusion complex, whereas AB forms 1:1 inclusion complex. Also, head-to-head dimer is formed in both the aminoazobenzene compounds.

Keywords: Spectral studies, Tautomerism, Inclusion compounds, Azo compounds, Azobenzenes, Aminoazobenzenes, Cyclodextrins

Azo compounds play an important role not only in analytical chemistry as metal chrome agents but also find application as dyes, acid base indicators as well as histological stains. Moreover, their application as industrial dyes and in biological systems as inhibitors for tumor growth is of great importance. It is well known that azobenzenes containing amino or dialkylamino groups undergo a pronounced color change in solution or in polymer film under protonation. This phenomenon, caused by redistribution of electronic density in protonated dye molecules called as halochromism, is the basis for their use as optical sensors. The relative effect of the colour change in such dyes is associated with the existing ammonium-azonium tautomerism and with the tautomeric ratio, which depends usually on the solvent, temperature, irradiation and existence of additional substituents in the protonated molecule. However, in general it is very difficult to study such types of tautomeric processes because the individual responses of the pure tautomers are unknown.

Furthermore, azo dyes have attracted much attention because of their photo and thermochromic behaviors and there are many reports on the inclusion complexation of β-cyclodextrins (CDs) with azo dyes. The driving force for inclusion of the azo dyes into the CD cavity has been attributed to the hydrophobic interaction between the internal wall of CD and guest molecule and thus the inclusion with CD occurs effectively in aqueous solution.

In the present study, the spectral characteristics of 4-amino-2',3'-dimethyl azobenzene (GBC, Fast garnet GBC base) and 4-aminoazobenzene (AAB) have been studied in solvents of different polarity, varying pH and varying β-CD concentrations, both in the S0 and S1 states and compared with those of azobenzene (AB) with respect to the following: change in spectral characteristics of GBC due to the presence of methyl group; (ii) the increase or decrease in conjugation due to the presence of amino group; (iii) whether H-N=N- will be present as tautomer due to the presence of -N=N- centre in the molecules; (iv) the effect on the dissociation constants for the different prototropic reactions of the respective basic or acidic centre due to the presence of other basic/acidic centre in both S0 and S1 states, and, (v) the increase or decrease in emission of the inclusion complexes.

In addition to the spectral properties of inclusion complexes by UV-visible and fluorescence, the solid inclusion complexes prepared by co-precipitation
Materials and Methods

Absorption spectral measurements were carried out using Hitachi (model U-2001) UV-visible spectrophotometer and fluorescence measurements were made with a Shimadzu RF-5301 spectrofluorometer. The pH values in the range 2.0-12.0 were measured on an Elico pH meter (model LI-120). FT-IR spectra were obtained on Avatar-330 FT-IR spectrometer using KBr pelleting. The range of spectra was from 500-4000 cm$^{-1}$. Microscopic morphological structure measurements were made with a Jeol JSM 5610 LV scanning electron microscope. Bruker Advance DRX 400 MHz superconducting NMR spectrophotometer was used to record $^1$H NMR spectra.

GBC, AAB, AB and β-CD were obtained from E. Merck and recrystallized from aqueous ethanol. The purity of the compounds was checked by the melting points. All solvents used were of the highest grade (Spectrograde) commercially available. Triply distilled water was used for the preparation of aqueous solutions. Aqueous solutions in the pH range 2.0-12.0 were prepared with appropriate amounts of NaOH and H$_3$PO$_4$. A modified Hammett’s acidity scale ($H_0$) for the aqueous solutions below pH ~2 (using a H$_2$SO$_4$-H$_2$O mixture) and Yagil’s basicity scale ($H_L$) for the aqueous solutions above pH ~12 (using a NaOH-H$_2$O mixture) were employed. The aqueous solutions were prepared just before each measurement. The concentrations of the GBC and AAB solutions were of the order of 2×10$^{-4}$ to 2×10$^{-5}$ M and that of β-CD solution was varied from 1×10$^{-3}$ to 1×10$^{-2}$ M.

The concentration of stock solution of all the azobenzenes was 2×10$^{-3}$ M. The stock solution (0.2 ml) was transferred into 10 ml volumetric flasks. To this, β-CD solution (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2×10$^{-2}$ M) was added. The mixed solution was diluted to 10 ml with triply distilled water or appropriate buffer solution and shaken thoroughly. The final concentration of AB in all the flasks was 4×10$^{-5}$ M. The absorption and fluorescence spectra were recorded or intensities were measured at 30 ± 1°C using 1 cm quartz cell.

Preparation of solid complex of azobenzenes with β-CD

Accurately weighed β-CD (1.2 g) was taken in a 50 ml conical flask and dissolved in 30 ml distilled water. Then, AB was taken in a 50 ml beaker and 20 ml distilled water was added and the contents were stirred on an electromagnetic stirrer until it was dissolved. The β-CD solution was poured into the azobenzene solution slowly. This mixed solution was continuously stirred for 48 h at 50°C. The reaction mixture was refrigerated for 48 h. White crystals were precipitated, which were filtered with G$_4$ sand filter funnel and washed with distilled water. After drying in an oven at 60°C for 12 h, a white powder product was obtained, which was the inclusion complex of the azobenzenes with β-CD.

Results and Discussion

Effect of solvents

The absorption maxima, emission maxima and molar extinction coefficient of GBC, AAB and AB were studied in different solvents. No significant difference was observed in the absorption and emission spectral shape and maxima of GBC and AAB molecules. This indicates that the presence of two methyl groups in GBC molecule does not effectively change the spectral behavior as compared to that of AAB. Similar to that of AB (cyclohexane, $\lambda_{\text{abs}}$ ~314, 218 nm, $\lambda_{\text{flu}}$ ~372 nm; water, $\lambda_{\text{abs}}$ ~320 nm, 228 nm, $\lambda_{\text{flu}}$ ~362 nm), the absorption bands of both amino azobenzene consisted of two peaks in all the solvents (cyclohexane, $\lambda_{\text{abs}}$ ~369, 247 nm, $\lambda_{\text{flu}}$ ~420 nm; water, $\lambda_{\text{abs}}$ ~380, 244 nm, $\lambda_{\text{flu}}$ ~430 nm). In AB, the longer wavelength band (LW) was slightly red shifted whereas the shorter wavelength (SW) band was largely red shifted from cyclohexane to water. On the other hand, in GBC and AAB, the LW band was largely red shifted as compared to the SW band (difference ~55 nm in cyclohexane and ~60 nm in water). In water, red shifted absorption maxima was observed for AB, while a blue shifted absorption maxima was seen for GBC and AAB. This indicates that the positions of both bands are relatively influenced by changing the solvents.

Further, in any one solvent, when compared to 4-hydroxyazobenzene (HAB, cyclohexane, $\lambda_{\text{abs}}$ ~340,
246 nm, $\lambda_{hu} \approx 385$ nm), AAB shows large red shifted absorption and emission maxima. On comparing absorption and fluorescence spectral shifts of AAB and HAB compounds it is seen that the interaction of -OH group with the aromatic ring is less than that of amino group. Examination of the results reveals that the position and/or the molar extinction coefficient of these bands are highly influenced by the nature of polar substituent NH$_2$ group.

The location of the LW band relative to the AB may be assigned to $\pi-\pi^*$ transition involving the entire electronic system of the compounds with a considerable charge transfer character. Such a charge transfer originates mainly from the benzene ring or amino group to the azo group which is characterized by a high electron accepting character. Due to the greater charge transfer effect of the NH$_2$ group with the aromatic ring, a larger red shifted maximum is observed in GBC/AAB than in AB.

In all the solvents, the fluorescence spectra of GBC, AAB and AB molecules show a single emission. The emission spectra of GBC and AAB were excited at 370 nm whereas AB was excited at 315 nm. In GBC and AAB, the emission maximum appeared at around 420-430 nm whereas for AB it was seen at 370 nm. Similar to the absorption spectra, the fluorescence spectra of both GBC and AAB molecules are more red shifted than the spectra of HAB and AB molecules.

In contrast to azonaphthol, azoanilines and azophenols do not exist as a tautomer mixture in solution at room temperature, e.g., azonaphthol molecules are present in both azo (A) and hydrazo form (H) and their relative stability depends on the solvent and/or temperature. The tautomeric behaviour of azonaphthol compounds differs considerably from that of the corresponding azophenols and azoanilines even in polar solvents which exist mainly in azo form at room temperature. Such a difference may be caused by the loss of aromaticity in going from azo to hydrazo form, while in azonaphthol compounds, this effect is compensated by the transfer of aromaticity within the naphthalene fragment; i.e., in GBC/AAB the number of delocalised electrons in the tautomeric phenyl ring is reduced from six to four in the H-form. This is because two of these electrons are engaged in the strong C=O bonds, and thus, the phenyl ring looses much of its aromaticity. In naphthalene compounds, this effect is compensated by the second aromatic ring. Hence, the emission maximum appears around 430 nm because only the azo form exists for both the molecules.

Correlation of solvent shifts with solvent polarity parameters

When a solute is placed in a solvent, the combined effects of general and specific interactions are observed and the separation of these interactions is often difficult. Empirically or theoretically derived solvent parameters like Reichardt’s-Dimroth, $E_{T}(30)$, Biolet-Kawasaki (BK) and Lipert $f(D, n)$ values as accurate registers of solvent polarity have been used by several authors to correlate molecular spectroscopic properties. Among these parameters, BK and $f(D, n)$ take into account the solvent polarity alone, whereas $E_{T}(30)$ incorporates both solvent polarity and hydrogen bonding effects. Correlation of Stokes shift with any one of these parameters gives an idea about the type of interaction between the solute and solvent.

The correlation of Stokes shifts ($\Delta\nu_{ss} \text{ cm}^{-1} = \nu_{\text{max}}^{\text{abs}} - \nu_{\text{max}}^{\text{flu}}$) of GBC, AAB and AB in different solvents with $E_{T}(30)$ and BK is shown in Fig. 1. The increase in Stokes shift from cyclohexane to water is found to be more with the $E_{T}(30)$ than with BK values. Since hydrogen bonding interactions are predominant in the solvatochromic shifts, $E_{T}(30)$ gives better correlation than BK values. Figure 1
shows a dramatic change in the slope in an intermediate polarity region, i.e., the plots are non-linear.

**Effect of pH**

The absorption and fluorescence spectra of GBC, AAB and AB were measured in the pH range from $H_0$ -10 to H. 17. Figures 2 and 3 illustrate the absorption and fluorescence spectra of various prototropic species of the above molecules. The effect of proton concentration on the absorption and fluorescence spectra of both the aminoazobenzene molecules is different to that observed for other aromatic amines.\(^{16-18}\) When the pH is decreased from 7 to $H_0$ -1, no significant spectral shift is observed in AB ($\lambda_{\text{abs}} \sim 317, 228$ nm, $\lambda_{\text{flu}} \sim 370$ nm). Further, as acid concentration is increased from $H_0$ -2, a large red shifted spectrum is observed in both $S_0$ and $S_1$ states ($\lambda_{\text{abs}} \sim 415, 293$ sh, 250 nm, $\lambda_{\text{flu}} \sim 430$ nm), indicating that protonation takes place in the azo group. Even at very high acid concentrations ($H_0$ -10), no further spectral change is noticed in AB molecule indicating dication is not formed in this case. When the pH is increased from 11 to 14, both the amino compounds give a new red shifted spectrum. This is because the formation of monoanion, i.e., deprotonation takes place in the amino group.

In both $S_0$ and $S_1$ states, the protonation spectral shifts of GBC and AAB molecules follow a similar

![Fig. 2](image1.png)  
**Fig. 2**—Absorption spectra of different prototropic species of GBC, AAB and AB at 303 K. [Conc. 2 × 10⁻⁵ M. 1, dication; 2, monocation; 3, neutral; 4, monoanion].

![Fig. 3](image2.png)  
**Fig. 3**—Fluorescence spectra of different prototropic species of GBC, AAB and AB at 303 K. [Conc. 2×10⁻⁵ M. 1, dication; 2, monocation; 3, neutral].
trend in acid and base environment. With an increase in the acid concentrations from pH ~7 to H~0 -1, the absorbance and emission maxima are regularly red shifted from λabs ~380, 245 nm to 500, 300, 230 nm and λflu ~430 nm to 515 nm showing that protonation takes place in the azo group. The regular red shift shows that tautomerism takes place between the amino and azo nitrogen atoms. This assumption is based on the following reasons: (i) it is well known that if protonation takes place in the amino group, a blue shifted spectrum should be noticed, and, (ii) the protonation will block the amino group lone pair electrons and thus the absorption spectrum should resemble that of AB. In both azoanilines, further increase in the acidity leads to a second protonation although the first process is not fully complete (Fig. 2). The absorption and fluorescence maxima of GBC and AAB are largely blue shifted (λabs ~ 426, 215 nm) and resemble that of AB molecule indicating formation of dication, i.e., the second protonation occurs in the NH2 group. This is further confirmed by the resemblance of the fluorescence behaviour of the latter species to that of AB molecule. When the basicity is increased above pH~12, the emission intensity is quenched, indicating formation of a non-fluorescent monoanion.

In acidic solutions, three absorption spectral maxima are observed for GBC/AAB. The various forms, unprotonated (B-band, neutral), monoprotonated (C-band, azonium) and diprotonated (A-band, ammonium) structures are shown in Scheme 1. Even though, the tautomeric equilibrium depends on acid concentration19, the substituent effect and environmental (solvent polarity) effects in the investigated compounds may be explained with the charge transfer model 1, where the amino substituted azobenzene chromophore system is classified as a donor (D), acceptor (A) system. The azonium structure can be presented within the CT model 2. The ammonium-azonium structures19 can be represented within the CT models 1 and 2. The donor fragment in CT model 1 and the azonium system in CT model 2 acts as an acceptor; i.e., structural changes in this system result in different effects in the absorption bands of each configuration.

Based on the above results, it may be concluded that on protonation, there exists a tautomeric equilibrium between the ammonium (AM) and azonium (AZ) forms19. Thus, the C-band at 500 nm is assigned to the azonium cation, in which the β-azo nitrogen atom is protonated, which is responsible for

Scheme 1
the large red shift. Also, the A-band at 320 nm is associated with the protonated terminal nitrogen of the NH₂ group (ammonium form) and this protonation prevents mesomeric interaction of the terminal lone pair with the π-electronic system.

It is worth noting that the donor fragments in the azo form (CT model 1) acts as an acceptor in the azonium system (CT model 2) which explains how structural changes in a particular fragment can lead to different effects in the absorption bands. The introduction of a stronger electron donor (NH₂) substituent instead of hydrogen atom into the AB molecule (unprotonated AAB) causes a bathochromic shift of about 60 nm (320 to 380 nm), whereas the introduction of the -CH₃ group in AAB (i.e., GBC) does not lead to any significant difference in the spectral maxima in accordance with CT model 2. The position of the A-band at 320-330 nm associated with the ammonium tautomeric form closely corresponds with that of the compound without a protonated amino group. The C-band of the azonium tautomeric form also has vibronic structure (Fig. 2) and the electronic transition is associated with a charge transfer from the anilino fragment (D) to the quinoneimine fragment (A). It is also of interest to note that the adsorption spectra shows that the protonation of GBC/AAB compounds proceeds with relative increase of ammonium and azonium forms.

The protonation of the amino nitrogen by removing the π-electrons of nitrogen from the aromatic resonance system should result in a dicationic species (Scheme 2) which is iso π-electronic with the protonated azobenzene cation. The absorbance of the latter (424 nm) is in fact very close to the absorbance of the observed diprotonated species (426 nm). This can be taken as an evidence for the second conjugate acid of AAB (str. 2, Scheme 2).

There is also a possibility that a second protonation occurs on the azo group, to give structure 2 (Scheme 2). However in that case, structure 2 should be an important contributor by analogy with monoprotonated GBC/AAB. Consequently, one would expect the diprotonated GBC/AAB to have its absorption maximum largely unchanged from the monoprotonated form, which is not the case herein. A noteworthy point is that azobenzene itself apparently does not undergo a second protonation even in 100% H₂SO₄, which suggests that protonation of both nitrogen in the azo group is relatively unfavorable.

In order to obtain pKₐ values for all the corresponding equilibrium (i.e., dication-monocation, monocation-neutral, neutral-monoanion), the absorbance and emission intensity data when plotted against H₀ / pH /H⁻ give typical sigmoid curve. From the inflexion point, the pKₐ (dication-monocation, monocation-neutral) values of −3.6 and 0.8 are obtained respectively.

**Effect of β-CD**

The absorption and emission spectra of GBC, AAB and AB in pH ~7 aqueous solutions containing different concentrations of β-CD were recorded. With an increase of β-CD concentrations, no spectral shift is observed in the absorption spectra (GBC/AAB: λₐ₅₈ ~380 nm and AB: λₐ₅₈ ~317 nm). However, the molar extinction coefficient increases with increase in β-CD concentration. The above results indicate that the GBC/AAB molecules are entrapped in the β-CD cavity to form inclusion complex. The absorbance of the solutions recorded after 12 hours remains constant indicating that the molecules are present in β-CD solution without decomposing on keeping due to because the formation of inclusion complex. This behaviour may be attributed to the enhanced dissolution of the guest molecule through the hydrophobic interaction between guest and non-polar cavity of β-CD. In GBC/AAB, several isosbestic points are present indicating a simple 1:1 inclusion equilibrium. In general, the existence of an isosbestic point in the absorption spectra is indicative of the formation of well defined 1:1 complex. The formation constant of inclusion complex of AAB and β-CD is small compared to that of GBC with β-CD. This is probably because the AAB molecule is not tightly encapsulated into the cavity, whereas due to the presence of methyl groups in GBC, it may get tightly encapsulated into the β-CD cavity.
The fluorescence characteristics of GBC and AAB (in the absence of β-CD solutions) undergo drastic changes in the presence of β-CD. The emission spectra monitored at 370 nm excitation wavelength is given in Fig. 4. In β-CD medium, the fluorescence spectra of azo molecules are more sensitive than the absorption spectra. As the β-CD concentration is increased, the fluorescence intensity of AB (370 nm) increases significantly at the same emission wavelength. However, in GBC and AAB, the fluorescence maximum is red shifted and the emission intensity increases along with β-CD concentration. Such changes in both molecules caused by the introduction of β-CD are indicative of the formation of an inclusion complex. The above results (the emission intensity greatly enhanced along with red shift in GBC/AAB) indicate that the inclusion process of GBC/AAB is different from that of AB molecule. The considerable increase in the fluorescence intensity compared with absorbance shows that the quantum yields of azo molecules increase in the presence of β-CD.

For 1:1 complex between β-CD and guest molecule (GBC, AAB and AB) the following equilibrium exists, \( AB + \beta-CD \rightarrow AB-\beta-CD \). The formation constant \( K \) and stoichiometric ratios of the inclusion complexes of azobenzene compounds can be determined according to the Benesi-Hildebrand relationship assuming the formation of a 1:1 host-guest complex. A good linear correlation is obtained between a plot of \( 1/\Delta A \) (\( \Delta A \) is the difference between the absorbance of azo molecules in the presence and absence of β-CD) and \( 1/\beta-CD \) confirming the formation of a 1:1 inclusion complex (slope, \( GBC \approx 0.0015; AAB \approx 0.0019; AB \approx 0.0021 \)). The association constant, \( K \), for the inclusion complexation as obtained from the slope and intercept was found to be \( GBC \approx 644 \text{ M}^{-1}; AAB \approx 523 \text{ M}^{-1} \) and \( AB \approx 475 \text{ M}^{-1} \). The dependence of concentration of β-CD on fluorescence was also analyzed by the Benesi Hildebrand plot. The plot of \( 1/I-I_0 \) versus \( 1/\beta-CD \) also shows excellent linear regression further supporting the formation of the 1:1 inclusion complex. The \( K \) values obtained from the slope and intercept was \( GBC \approx 855 \text{ M}^{-1}; AAB \approx 624 \text{ M}^{-1} \) and \( AB \approx 548 \text{ M}^{-1} \).

The \( K \) value for GBC is higher than that of AAB and AB, which can be attributed to the hydrophobic interaction between the phenyl ring having the methyl group and the internal wall of β-CD. Also, the high \( K \) value of GBC shows that the fragment of phenyl group having the methyl substituent is too large to pass through the β-CD cavity. The \( K \) values also suggest the steric hindrance of the methyl group prevent deep penetration into the β-CD cavity and the fitness of the phenyl ring of azo molecule in the β-CD cavity is better than that of the others (Scheme 3). The small binding constant for AAB/AB implies that the phenyl ring is not tightly embedded in the β-CD cavity. Comparison of \( K \) value for GBC with those of AAB and AB indicates that the molecular disposition of amino azobenzene derivatives in inclusion complex is similar to that of HAB\(^{26}\). Further; it is well known that hydrophobicity is the driving force of formation.

![Fig. 4—Fluorescence spectra of GBC, AAB and AB in different β-CD concentrations (M): [1, 0; 2, 0.001; 3, 0.002; 4, 0.004; 5, 0.006; 6, 0.008; 7, 0.01].](image-url)
of inclusion complexes. Since phenyl group is more hydrophobic than aniline part, the phenyl ring may be included in the β-CD cavity. Also, in hydrophilic environments, the amino group is located outside the β-CD cavity (in hydrophilic environments).

The ∆G values (GBC: \( S_0 \approx -17.75, S_1 \approx -17.96 \); AAB: \( S_0 \approx -15.87, S_1 \approx -16.27 \) and AB: \( S_0 \approx -15.53, S_1 \approx -15.88 \) kJ/ mol) are negative, which suggest that the inclusion process proceeds simultaneously at 303 K. The negative ∆G values under the experimental conditions indicate that the inclusion process is an exothermic and enthalpy controlled process. The hydrophobic interaction between the internal wall of β-CD and guest molecules is an important factor for the stability of inclusion complexes.

In the case of GBC and AAB, it may safely be considered that the difference in the magnitude of the hydrophobic interaction is related to the contact area of the guest molecule and the internal wall of β-CD.

**Inclusion complexes**

According to the study of Yu *et al.* on β-CD: azobenzene complexes, we can deduce that the azobenzene molecule is included longitudinally in the β-CD cavity. In aqueous β-CD solutions, the red shifted emission spectrum for GBC and AAB (420 to 455 nm) may be due to a higher conjugated system occurring between two benzene rings, i.e., the two benzene rings are located almost in the same plane. Further, like HAB molecule, GBC and AAB molecules exhibit similar emission maximum at 455 nm suggesting that both benzene rings are located similarly.

The increase in fluorescence intensity of GBC/AAB molecules (420-455 nm) along with a large bathochromic shift in β-CD as compared to that in aqueous solution may be explained as follows: GBC/AAB molecules are embedded in two β-CD cavities in different directions to form the head-to-head dimer arrangement while the amino group of azobenzene molecules points to the primary side of the β-CD cavity. This unusual dimerisation behavior is attributed to the cooperative interactions of eight hydrogen bonds between the secondary hydroxyl groups of two adjacent β-CD units as well as the π-π* interaction between the benzene rings. Two GBC or AAB molecules are embedded into two β-CD cavities in two different directions with the amino group of the azo molecules pointing to the primary side of the β-CD cavity (2:2 stoichiometry between GBC or AAB with β-CD are formed). Moreover, two β-CD/AAB complexes are connected by H-bonds (from the hydroxyl groups) with the two benzene rings in a head-to-head arrangement to form a dimer unit.

More interestingly, two adjacent head-to-head β-CD-AAB dimers (Scheme 3) adopt different orientations, which consequently result in the formation of longer wavelength emission maxima. Further, water molecules are located on the exterior part of the β-CD cavity and both benzene rings are included in the β-CD cavity which indicates the presence of stronger hydrophobic interaction between the guests and β-CD cavity. On the other hand, the strong H-bond network formed by the -OH groups of the β-CD and amino group of azo benzene further enhance the longer wavelength emission. In AAB/β-CD dimer, two amino groups of the AAB molecule participate in the formation of the inter dimer hydrogen bond leading to the bathochromic shift.

Let us now consider other possible types of inclusion complex also: (i) if azo molecule is partially (i.e. the benzene ring) entrapped in the β-CD cavity,
like in AB molecule in β-CD solutions. If this type of complex is formed, the emission maxima of GBC/AAB should increase at the same wavelength, and, (ii) if the other part of azo molecule (i.e., the aniline ring) is included within the β-CD cavity, the monocation absorption and emission maxima (i.e., protonation of amino group) should be blue shifted as compared to the aqueous medium.\textsuperscript{23-25} Values of prototropic equilibrium and absorption and fluorescence maxima indicate that neutral maximum of GBC/AAB in β-CD is largely red shifted as compared to aqueous medium. Monocation maximum also follows similar trend both in aqueous and β-CD medium. It has been reported, that when amino group is entrapped in the β-CD cavity, the monocation absorption maximum is blue shifted in β-CD as compared to aqueous medium.\textsuperscript{20-25} Further, the large red shift in the $S_1$ state supports the presence of amino group in a more polar environment than water. The question may arise why in β-CD solutions is the fluorescence intensity is greatly enhanced along with red shift. This may be because viscosity is increased along with β-CD concentrations\textsuperscript{21, 22} and also that in β-CD solutions of higher concentrations, due to high viscosity the dimer interaction is increased which greatly enhances the fluorescence intensity along with red shift. This confirms that the environment around the amino group in β-CD is slightly different from the bulk aqueous medium.

This is further supported by semiempirical quantum calculations (DFT/Cache 7.5 PC-model program) which also provide some useful information about the inclusion complexation geometry of guest molecules. To determine the dimensions of GBC, AAB, AB and β-CD, geometry of these molecules (in the ground state) was optimized by using the DFT/Cache program. This method provides acceptable approximations to give results, which are quite close to the experimental findings. The internal diameter of the β-CD was found to be approximately 6.65 Å and its height ~ 7.8 Å (Scheme 4). Considering the shape and dimensions of β-CD, GBC/AAB molecules can not completely get encapsulated with in the β-CD cavity, because in AAB the H-H and C-C distances are greater than the inside diameter of the β-CD cavity (7.8 Å) which may be responsible for the formation of different types of inclusion complexes in the β-CD.

**Solid inclusion complexes studies**

The powder forms of GBC, AAB, AB and β-CD and their inclusion compounds were investigated by

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**Scheme 4**

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**GBC**

*Final heat of formation:*

72.2260 kcal (302.19357 kJ)

**Bond distances (Å)**

- $H_3 - H_{11} = 12.238$
- $H_3 - H_{10} = 12.092$
- $H_3 - N_3 = 11.671$
- $H_3 - H_{13} = 10.460$
- $H_3 - H_{11} = 11.015$
- $C_1 - N_3 = 10.578$
- $H_3 - H_{10} = 11.573$
- $H_3 - H_{15} = 9.555$
- $H_3 - H_{15} = 8.865$
- $H_3 - H_{15} = 8.042$

**AAB**

*Final heat of formation:*

87.91265 kcal (367.82651 kJ)

**Bond distances (Å)**

- $H_3 - H_{10} = 12.122$
- $H_3 - N_3 = 11.717$
- $C_1 - C_7 = 3.639$
- $N_1 - N_3 = 6.456$
- $C_1 - C_7 = 5.822$
- $C_3 - C_1 = 1.431$
- $C_3 - H_{10} = 11.156$
- $N_1 - H_4 = 0.994$
- $C_3 - N_3 = 10.959$
- $C_3 - H_{10} = 9.314$
scanning electron microscopy (Fig. 5). SEM images clearly show the difference in each case. β-CD has sheeted/plated structure, whereas GBC, AAB and AB have stone rock structure. The structures of their inclusion complex are different from those of the pure compounds and β-CD. Modification of crystals and powder can be assumed as a proof of the formation of new inclusion complex.

The FT-IR spectra of GBC, AAB, AB and the solid inclusion complex were investigated. In the spectra of GBC and AAB the CH stretching vibrations in the regions 3198, 3121 cm\(^{-1}\) and 3205, 3049, 3030, 2924 cm\(^{-1}\) were shifted in the inclusion complex to 2924 cm\(^{-1}\) and 2926 cm\(^{-1}\) respectively. The C=C stretching vibrations at 1620 cm\(^{-1}\) and azo stretching vibrations at 1594 cm\(^{-1}\) were shifted slightly in the inclusion complex to longer frequencies. In GBC and AAB, the amino stretch appearing at 3489, 3361 cm\(^{-1}\) and 3474, 3378 cm\(^{-1}\) were merged and shifted in the inclusion complex to 3376 cm\(^{-1}\) and 3380 cm\(^{-1}\) respectively. In GBC and AAB, the ring deformation stretch at 582, 548 cm\(^{-1}\) was shifted in the inclusion complex to 578 cm\(^{-1}\). In GBC the methyl stretch at 2920 cm\(^{-1}\) was shifted to 2924 cm\(^{-1}\) in the inclusion complex. In GBC and AAB the amino group deformation appearing at 1619, 1595 cm\(^{-1}\) and 1620, 1593 cm\(^{-1}\) were shifted in the inclusion complex to 1630 and 1599 cm\(^{-1}\) respectively. The azo group stretching frequency intensities at 1500-1396 cm\(^{-1}\) and 1502-1414 cm\(^{-1}\) were strongly affected in the inclusion complex indicating azo group is included in to the β-CD cavity.

The resonance assignments of the protons of β-CD are well established\(^{29}\) and consist of six types of protons. The chemical shift of β-CD protons reported by different authors\(^{29}\) are very close to those reported

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Fig. 5—SEM images of the studied compounds. [a, b, β-CD; c, GBC; d, GBC-β-CD complex; e, AAB; f, AAB-β-CD complex; g, AB; h, AB-β-CD complex].
in this work. 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. In particular, the resonance of the protons of β-CD located within or near the cavity showed remarkably largely upfield shift (0.18 ppm) in the inclusion complex.

Even though only limited information can be obtained from the 1H NMR data, the observation of slight upfield 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 addition of GBC/AAB into the β-CD results in a downfield chemical shift (δ; shifts from pure to complex GBC: 4H or NH₂ ~ -0.004, 3H or CH₃ ~ -0.132, 2’H or CH₂ ~ -0.029; AAB: 4H or NH₂ ~ -0.002, 3H or CH₃ ~ -0.042, 2’H or CH₂ ~ -0.022; AB: 4H or NH₂ ~ -0.003, 3H or CH₃ ~ -0.003, 2’H or CH₂ ~ 0.002) for the GBC/AAB protons. A small downfield shift on GBC/AAB was observed in phenyl ring suggesting this part is encapsulated in the β-CD cavity and the other anilino ring is present in the outer part of the β-CD cavity.

Conclusions

The solvent study shows that only the azo form is present in both the aminoazobenzene compounds. On comparison of GBC and AAB, no significant difference is noticed in the absorption and an emission spectral shift, indicating that the presence of two methyl groups in GBC does not effectively change the spectral behaviour. In acid solutions, unusual red shift observed in monocations suggests that the azonium-ammonium tautomer is present in both the molecules. In β-CD solutions, the increase in the fluorescence intensity and a large bathochromic shift in S₁ state indicates that GBC/AAB forms 2:2 inclusion complex whereas AB forms 1:1 inclusion complex. head-to-head dimer is formed in both amino azobenzene compounds.

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References