

A study on the inclusion complexation of 3,4,5-trihydroxybenzoic acid with β -cyclodextrin at different pH

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

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Abstract Effect of β -cyclodextrin (β -CD) on the absorption and fluorescence spectra of 3,4,5-trihydroxybenzoic acid (THB) have been studied buffer solutions of different pHs (pH \sim 1, pH \sim 7 and pH \sim 10). The study reveals that in all the above pHs THB forms 1:1 inclusion complex. The hydroxyl groups are present in the interior part of the β -CD cavity and carboxyl group is present in the hydrophilic part of the β -CD cavity. Dual luminescence is observed at pH \sim 1 and pH \sim 7 solutions which shows that intramolecular charge transfer present at these pH. The broad spectral maximum at pH \sim 10 indicates that intramolecular proton transfer is present in THB.

Keywords 3,4,5-Trihydroxybenzoic acid ·
 β -Cyclodextrin · Inclusion complex ·
Intramolecular charge transfer

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that possess internal cavities capable of complexing hydrophobic organic molecules in aqueous solutions [1–3]. These cavities comprises six, seven, or eight glucopyranose units which are designated as α , β , and γ with diameter opening at respectively, the primary hydroxyl and secondary hydroxyl faces of the cyclic sugar network. The interior part of the cavity is lined with ether oxygens and presents a relatively hydrophobic surface to an incoming guest which

enables it to form inclusion complexes with many different (organic, inorganic, neutral, and ionic) molecules. This complexation leads to widespread applications in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis [1–6]. In particular, these systems are considered good models for the study of protein–ligand interactions and enzymatic analysis [7]. Therefore, investigation of the driving forces for the complexation and the structure of inclusion complexes appears to have fundamental importance for the understanding of basic biological functions [7].

The inclusion of organic compounds within the CD hydrophobic cavity and its effect on the properties of organic molecules has been the subject of many investigations [8–15]. The variable cavity diameter of the CDs has been used advantageously to sequester guests on the basis of their size; e.g., simple benzene derivatives fit easily within α -CD while larger aromatics can be accommodated within β -CD [16–22]. It is well documented that depending upon the host (CD) and the size of the guest, different host/guest stoichiometries are possible. Complexation between the CD and wholly or partially included guest molecules results in a number of interesting spectroscopic effects. Turro et al. observed the enhanced emission of the twisted intramolecular charge transfer state (TICT) fluorescence of dimethyl benzonitrile in β -CD [23]. A study of β -CD-indole inclusion complexation has been performed by both absorption and fluorescence lifetime measurements [24]. Warner and co-workers have studied the effect of alcohols and polynuclear aromatic hydrocarbons on the inclusion complexation with CD [25].

In the last few years, we have been examining amino, hydroxyl, aldehyde and COOH group substituted benzenes in various environments [26–37]. It has been observed that the spectroscopy and photophysical characteristics of these

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

molecules are largely influenced by the nature of substituents in the *para* position of the phenyl rings. These are also sensitive to environments [38–41], making them potential probe candidates for microstructures. By taking advantage of environment-dependent spectral shifts of these molecules, we have probed successfully the mean structural properties of the solvents, pH and β -CD [26–37]. The buffer solutions play a major role in the inclusion of guests with CD. In order to clarify the factors which are played a major role in the complexation, this analysis was performed. In this regard we are interested in the orientation of 3,4,5-trihydroxybenzoic acid (THB, gallic acid) in the inclusion complexes. Here we report the formation of the inclusion complexes of THB molecule with β -CD.

Experimental

THB and β -CD were obtained from Sigma-Aldrich. The purity of the compound was checked by similar fluorescence spectra by the excitation at different wavelengths. Triply distilled water was used for the preparation of aqueous solutions. The buffer solutions of different pHs were prepared by adding appropriate amounts of NaOH and H_3PO_4 ; the solutions were prepared just before taking each measurement. The concentration of the THB solutions were the order of 2×10^{-5} M. The concentration of β -CD solution was varied from 1×10^{-3} to 1×10^{-2} M. The stock solution was prepared in methanol and keeping the solution has 2% methanol content.

Absorption spectral measurements were carried out using a Hitachi (model U-2001) UV–Visible spectrophotometer and fluorescence measurements were made using a Shimadzu RF 5301 spectrofluorimeter. The pH values in the range 2.0–12.0 were measured on an Elico pH meter (model LI-120).

Results and discussion

Table 1 and Figs. 1, 2, 3, 4, 5, 6 depict the absorption and fluorescence spectra of THB (2×10^{-5} M) at pH ~ 1 , pH ~ 7 and pH ~ 10 phosphate buffer solution containing different concentrations of β -CD. THB exists as carboxylic anion at pH ~ 7 ($\lambda_{\text{abs}} \sim 260$ nm), dianion (carboxylic and hydroxyl anion) at pH ~ 10 ($\lambda_{\text{abs}} \sim 340, 262$ nm), and hence neutral THB spectrum was recorded for the solution at pH ~ 1 ($\lambda_{\text{abs}} \sim 270$ nm). In the absence and presence of β -CD solutions the absorption spectral shape of THB at pH ~ 1 (Fig. 1) are similar to pH ~ 7 (Fig. 2) but differ from pH ~ 10 (Fig. 3). The inset figures from 1 to 6 depict the changes of both absorbance and fluorescence intensities

were observed as a function of the concentration of β -CD added. Upon increasing the β -CD concentration at pH ~ 1 the molar absorbance coefficient decreased at the same wavelength ($\lambda_{\text{abs}} \sim 271$ nm). However, at pH ~ 7 ($\lambda_{\text{abs}} \sim 260$ to 270 nm) and pH ~ 10 ($\lambda_{\text{abs}} \sim 340, 262$ nm to 342, 279 nm) the absorbance is decreased and the absorption maximum is red shifted in β -CD than that of aqueous medium. It should be noted that at higher β -CD concentrations, the absorption maxima and the spectral shape of both pHs (pH ~ 1 and pH ~ 7) are same which suggests that similar inclusion complexes are formed.

The effect of β -CD on the fluorescence spectra of THB is more pronounced than the corresponding effect on the absorption spectra. Figures 4, 5 and 6 depict the emission spectra of THB at pH ~ 1 , pH ~ 7 and pH ~ 10 containing different concentrations of β -CD. With increasing the β -CD concentration the fluorescence intensities increased at pH ~ 1 and pH ~ 10 whereas decreased at pH ~ 7 (inset Figs. 4, 5, 6). In aqueous solutions, the fluorescence maxima of all the three buffer solutions are different from each other. In the β -CD free aqueous solution single emission maximum is observed in these pHs (pH ~ 1 : $\lambda_{\text{flu}} \sim 366$ nm; pH ~ 7 : $\lambda_{\text{flu}} \sim 342$ nm and pH ~ 10 : $\lambda_{\text{flu}} \sim 449$ nm). However, on increasing the β -CD concentration the typical dual emission can be seen at pH ~ 1 ($\lambda_{\text{flu}} \sim 366, 450$ nm) and pH ~ 7 ($\lambda_{\text{flu}} \sim 365, 450$ nm). An addition of β -CD at pH ~ 1 , both the shorter wavelength (SW: $\lambda_{\text{flu}} \sim 365$ nm) and the longer wavelength (LW: $\lambda_{\text{flu}} \sim 450$ nm) emission intensity gradually enhanced at the same wavelength. In contrast at pH ~ 7 , the SW emission maximum is red shifted regularly ($\lambda_{\text{flu}} \sim 342$ to 365 nm) and the emission intensity is decreased whereas the LW ($\lambda_{\text{flu}} \sim 450$ nm) emission intensity gradually increases at the same wavelength. More interestingly, like in absorption maxima, with higher β -CD concentrations, the emission maxima and the spectral shape of both pHs (pH ~ 1 and pH ~ 7) are the same. At pH ~ 10 the dual fluorescence is not observed, however, the emission intensity is increased at 445 nm, while the emission intensity decreased at 520 nm.

The presence of isosbestic point in the absorption spectra suggest that in all the pHs THB forms 1:1 inclusion complex is formed [8–14]. However, the absorption and emission maxima at pH ~ 1 and pH ~ 7 are different from the pH ~ 10 indicating at least two kinds of inclusion complex exists in this system. The formation constant for the THB- β -CD inclusion complex has been determined by analyzing the changes in the intensity of the absorption and fluorescence maxima with β -CD concentration. The binding constant of THB with β -CD was determined by using the Benesi–Hildebrand relation [42]. In the case of inclusion complex formed between THB and β -CD, the equilibrium can be written as follows:

Table 1 Absorption and fluorescence maxima (nm) of THB in different β -CD concentrations

No.	Concentration of β -CD (M)	pH ~ 1			pH ~ 7			pH ~ 10		
		λ_{abs}	log ϵ	λ_{flu}	λ_{abs}	log ϵ	λ_{flu}	λ_{abs}	log ϵ	λ_{flu}
1	Water (without β -CD)	271.0	3.93	367	260.8	3.92	342	340s		449
		216.0	4.37		213.0	4.43		262	3.79	
2	0.001	271.0	3.90	366	268.2	3.92	346	340s		446
		216.4	4.36	450	214.8	4.43	450	269	3.72	
								217	3.92	
3	0.002	271.0	3.88	365	268.8	3.91	352	340s		443
		216.0	4.34	450	215.6	4.43	450	275	3.30	
								219	3.83	
4	0.004	270.2	3.88	365	269.6	3.91	360	340 s		443
		216.2	4.34	450	215.8	4.39	450	278	3.55	
								217	3.79	
5	0.006	270.6	3.88	366	270.0	3.90	363	342		444
		216.8	4.33	450	216.4	4.38	450	278	3.62	
								219	3.78	
6	0.008	270.2	3.87	365	270.0	3.89	365	342		443
		217.2	4.33	450	216.6	4.43	450	279	3.63	
								219	3.77	
7	0.010	270.4	3.84	365	270.1	3.88	365	342		443
		217.4	4.29	450	216.6	4.36	450	279	3.71	
								219	3.76	
8	Excitation wavelength	270			260			280		
9	Binding constant (M^{-1})	410		615	435		1482	301		380
10	ΔG ($kJ\ mole^{-1}$)	-15.2		-16.2	-15.3		-18.4	-14.4		-15.0

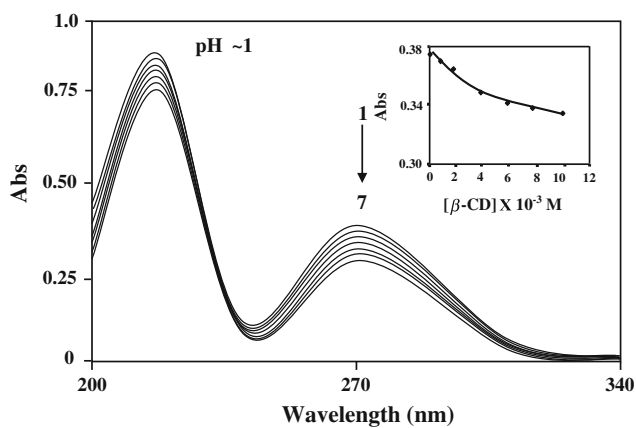


Fig. 1 Absorbance spectra of THB in different β -CD concentrations with pH ~ 1 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset Abs vs. $[\beta$ -CD]

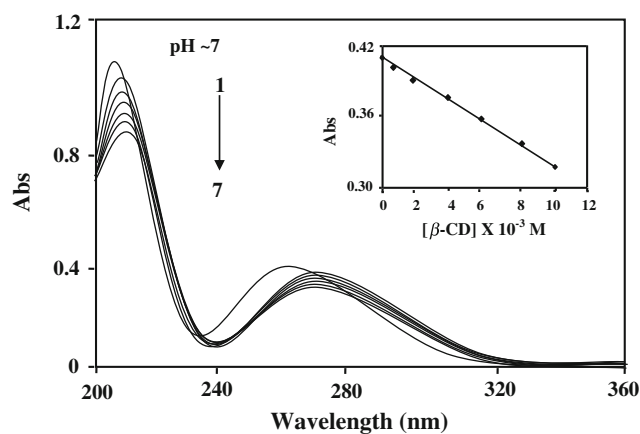


Fig. 2 Absorbance spectra of THB in different β -CD concentrations with pH ~ 7 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset Abs vs. $[\beta$ -CD]



The association constant (K) was calculated based on the following equation:

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

$$\frac{1}{I - I_o} = \frac{1}{I' - I_o} + \frac{1}{K(I' - I_o)[\beta\text{-CD}]^2} \quad (3)$$

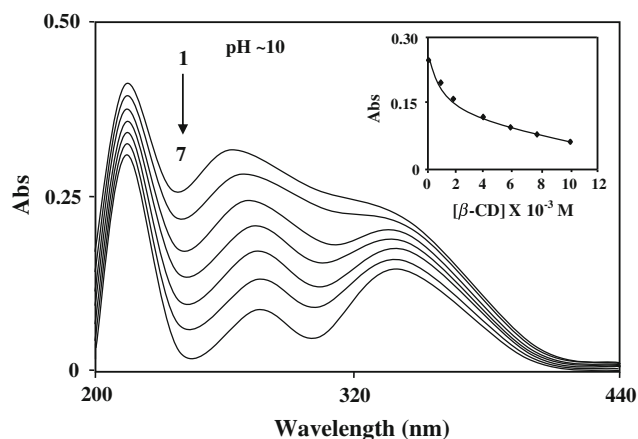


Fig. 3 Absorbance spectra of THB in different β -CD concentrations with pH ~ 10 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset Abs vs. $[\beta\text{-CD}]$

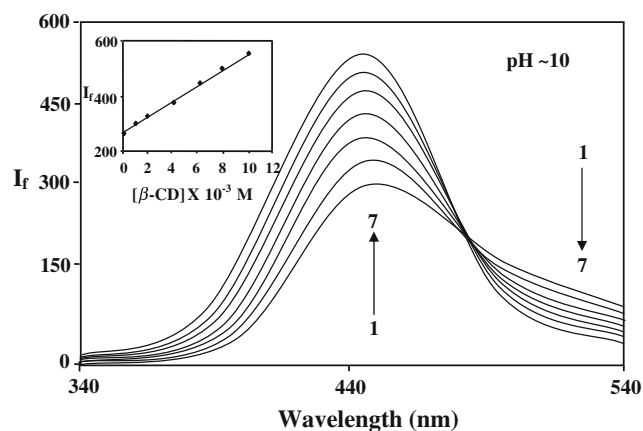


Fig. 6 Fluorescence spectra of THB in different β -CD concentrations with pH ~ 10 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset I_f vs. $[\beta\text{-CD}]$

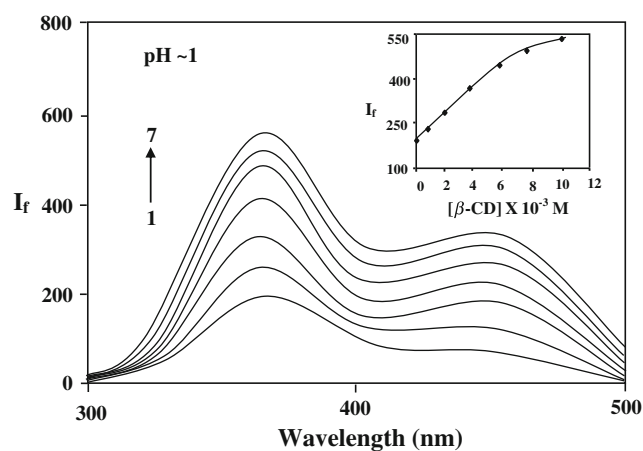


Fig. 4 Fluorescence spectra of THB in different β -CD concentrations with pH ~ 1 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset I_f vs. $[\beta\text{-CD}]$

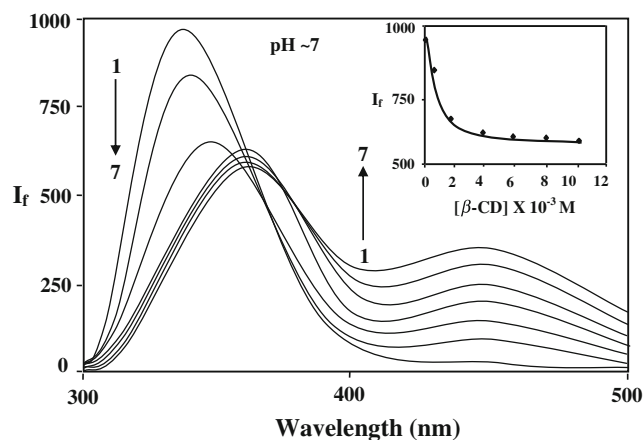


Fig. 5 Fluorescence spectra of THB in different β -CD concentrations with pH ~ 7 (M): 1 0, 2 0.001, 3 0.002, 4 0.004, 5 0.006, 6 0.008 and 7 0.01. Inset I_f vs. $[\beta\text{-CD}]$

where $[\beta\text{-CD}]_0$ represents the initial concentration of β -CD, I_0 and I are the absorbance and fluorescence intensities in the absence and presence of β -CD respectively, and I' is the limiting intensity of absorption/fluorescence. The Eqs. 2 and 3 are used to determine the 1:1 and 1:2 inclusion complex respectively. The K values were obtained from the slope and the intercept of the plots shown in Figs. 7 and 8. According to Eqs. 2 and 3, a plot of $1/I - I_0$ versus $1/[\beta\text{-CD}]^2$ (1:2 inclusion complex for both absorption and fluorescence) gives an upward curves as shown in Figs. 7 and 8. However, a plot of $1/I - I_0$ versus $1/[\beta\text{-CD}]$ (1:1 inclusion complex) reveals a linear relationship. This analysis reflects the formation of 1:1 inclusion complex between THB and β -CD complex.

The free energy change was calculated from the formation constant (K):

$$\Delta G = -RT \ln K \quad (4)$$

The values of thermodynamic parameter ΔG for the formation of the inclusion complex of THB with β -CD is given in Table 1. As can be seen from the Table 1 ΔG is negative which suggests that the inclusion proceeded simultaneously at 303 K. The experimental results indicate that the inclusion reaction of β -CD with THB is an exothermic process.

Several driving forces have been postulated for the inclusion complexation of CD with guest compounds [43, 44]: (1) van der Waals forces; (2) hydrophobic interactions; (3) hydrogen bonding; (4) release of distortional energy of CD by binding guest; and (5) extrusion of 'high energy water' from the cavity of CD upon inclusion complex formation. Tabushi [45] proposed a thermodynamic model for the process of CD inclusion complex formation. Based on the thermodynamic parameter (ΔG) calculated for the inclusion of THB, we conclude that the hydrogen bonding

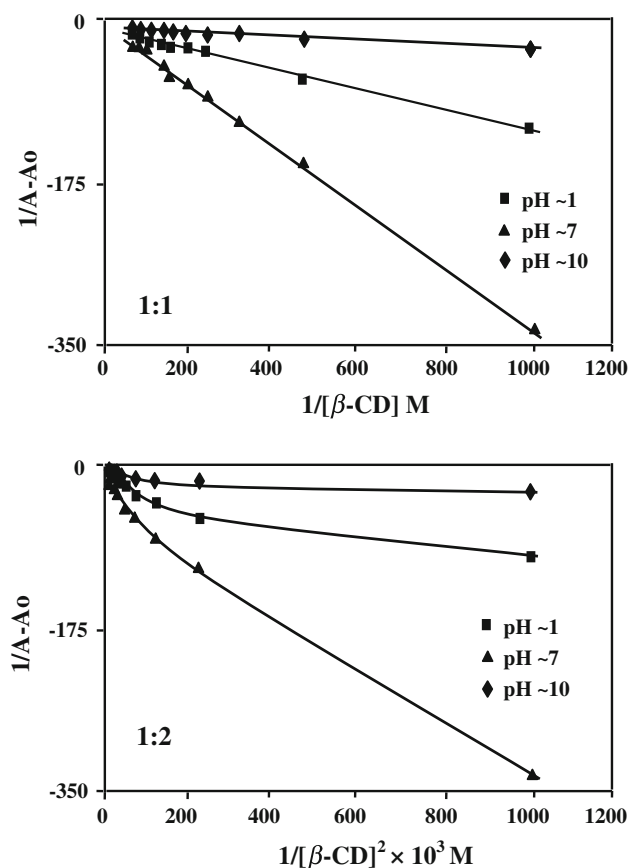


Fig. 7 Absorption spectra of Benesi-Hildebrand plot for the complexation of THB with β -CD: Plot of $1/A - A_0$ vs. $1/[\beta\text{-CD}]$ and $1/\Delta A$ vs. $1/[\beta\text{-CD}]^2$

interaction, van der Waals interaction, and breaking of the water cluster around this polar THB compound are mainly dominate the driving force for the inclusion complex formation.

It is well known that the strength of interaction is also dependent on the size of the CD cavity and size of the substituent in the inclusion complex [46]. This means that the interaction is more sensitive to the size of substituents and the CD in the complexation. The CDs are truncated, right-cylindrical, cone-shaped molecules, 7.8 Å heights with a central cavity. The diameters of the narrower and wider rim of the cavity for β -CD are 5.8 and 6.5 Å, respectively [47]. It is well known that the van der Waals force including the dipole-induced dipole interactions [48, 49] are proportional to the distance between the THB and the wall of the CD cavity and to the polarizabilities of the two components. It is thus a short range interaction. Therefore, the neutral THB may embedded deeper in the β -CD cavity than its anion. The phenyl moiety may achieve a maximum contact area [50] with the internal surface of the cavity of the β -CD, hence, the interaction of the phenyl ring with β -CD would play an important role.

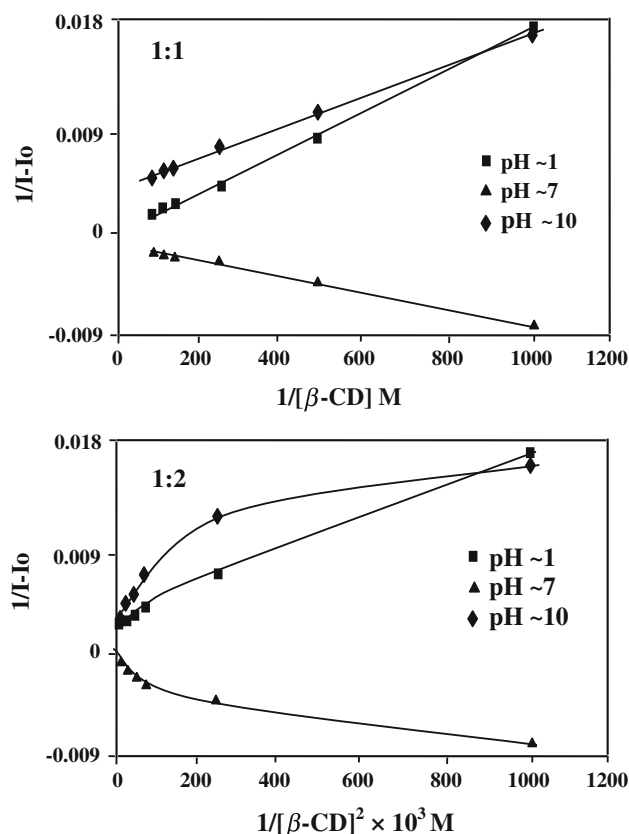


Fig. 8 Fluorescence spectra of Benesi-Hildebrand plot for the complexation of THB with β -CD: Plot of $1/I - I_0$ vs. $1/[\beta\text{-CD}]$. $1/I - I_0$ vs. $1/[\beta\text{-CD}]^2$

In general, the inclusion of CDs with guest compounds is also affected by hydrophobic and electronic interactions [51–54]. Since CDs have a permanent dipole [55–57], the primary hydroxyl end is positive and the secondary hydroxyl end is negative in the glucose units of CDs. The stability of binding by hydrophobic interaction is partly the result of van der Waals force but is mainly due to the effects of entropy produced on the water molecules [58]. In aqueous solution, a hydrophobic guest compound is restricted by the water shell formed by the hydrogen bonding network [59]. It has a strong tendency to break down the water cluster and penetrate the non-polar cavity of CD. This process is an exothermic due to entropic gain [58, 59]. The association constants for the inclusion of β -CD with guest compounds were observed to be proportional to the substituent hydrophobic constant of the guest.

However, in THB the hydrogen bonding interactions play major roles in the inclusion complexation of β -CD. In THB, the inclusion process is determined by the carboxylic and hydroxyl anions. The ‘K’ value is a reasonable measure of hydrogen bonding and the change in hydrogen bonding of THB is caused only by the hydrogen ion

concentrations. Since the hydroxyl substituent locates near the wider rim of the CD cavity and carboxyl group locates narrower range of the CD cavity, the 'K' values are proportional to the hydrogen bonding interactions. The difference in the slope in Figs. 7 and 8 for THB and β -CD complexes indicates that the interactions of hydrogen atoms, especially pH \sim 1 and pH \sim 7 with β -CD are much stronger than at pH \sim 10. This is because of THB interaction is approximate to the hydrogen bonding contact but in pH \sim 10 they are somewhat weak, since the hydroxyl anion are far from the internal surface of the β -CD cavity in the inclusion complexes. Thus for THB, the association constant with pH \sim 1 is greater than with pH \sim 10 (Table 1).

When compared to 4-hydroxy-3,5-dimethoxybenzoic acid (HDMB) [29] no significant shift is observed in the THB absorption maximum whereas emission maximum is red shifted in all solvents (HDMB: cyclohexane $\approx \lambda_{\text{abs}} \sim 269$ nm, $\lambda_{\text{emi}} \sim 322$ nm, methanol $\approx \lambda_{\text{abs}} \sim 272$ nm, $\lambda_{\text{emi}} \sim 348$ nm, water (pH \sim 2) $\approx \lambda_{\text{abs}} \sim 272$ nm, $\lambda_{\text{emi}} \sim 352$ nm, THB: cyclohexane $\approx \lambda_{\text{abs}} \sim 269$ nm, $\lambda_{\text{emi}} \sim 353$ nm, methanol $\approx \lambda_{\text{abs}} \sim 272$ nm, $\lambda_{\text{emi}} \sim 362$ nm, water (pH \sim 2) $\approx \lambda_{\text{abs}} \sim 272$ nm, $\lambda_{\text{emi}} \sim 365$ nm). This blue shift is due to steric effect of methoxy groups in HDMB; i.e., the presence of two methoxy groups at *ortho* position (with respect to OH group) decrease the OH group interactions with aromatic ring [27–30].

An analysis of solvatochromic shifts of both THB and HDMB reveals that the absorption maxima are red shifted from cyclohexane to water. The high molar extinction coefficients ($\sim 10^4$ cm $^{-1}$) implies that this band is attributed to the (π , π^*) transition of benzene ring and not to the (n , π^*) transition of the carbonyl group [60–63]. The fluorescence spectrum is regularly red shifted as the polarity and proton donor capacity of the solvent increases. Moreover, the spectral shifts observed in the absorption spectrum in protic and aprotic solvents are consistent with the characteristic behaviour of hydroxyl groups [28–30].

Another remarkable aspect of the fluorescence emission of THB and HDMB is the effect of solvent polarity. In the presence of highly polar solvents (like H $_2$ O), the emission spectrum widens and losing gradually its emission intensities. This is due to presence of dipolar structure of this molecule (Fig. 9). The emission in water is strongly pH dependent and the Stokes shift reaching its maximum value in acidic aqueous solutions. The broad Stokes shifted emission at acidic pH is common in molecules having an electron withdrawing group such as COOH, attached to an aromatic nucleus [29–34, 60–63]. However, the nature of such an emission is not always easy to ascertain, since it can be the result of several causes, including dimer formation in the ground states (or other kinds of aggregates) excimer formation (or) charge transfer processes.

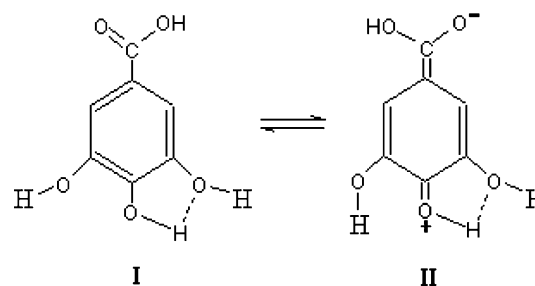


Fig. 9 Dipolar structure of THB

In both molecules, the appearance of broad band in alkaline pH ruled out the possibility of THB and HDMB forming dimers in the ground state (linked by H-bonding). This is because dimers cannot be formed if the carboxylate group is ionized. Further, the low concentrations currently used (2×10^{-5} M) also ruled out this possibility. Even in very low concentration (as low as 2×10^{-6} M) the broad emission can be detected. In both molecules the hydroxyl group is the donor and COOH is the acceptor. In an acidic medium, the carboxyl group becomes more conjugated with the aromatic π -system, a situation where there is a marked charge separation within the molecule (Fig. 9). The large Stokes shift in polar and non-polar solvents indicates a primarily dipolar interaction between the solute and the solvent molecules.

In solutions of pH \sim 1 and pH \sim 7, a typical dual fluorescence can be seen even though the emission quantum yield of the longer wavelength (LW) is extremely low. The weak LW emission has been ascribed to the stabilization of the highly polar ICT state by strong dipole–dipole interaction with water (Figs. 4, 5) and consequent rapid non-radiative transition to the ground state [18–21]. Upon addition of β -CD, both ICT emission is gradually enhanced, while the SW emission is increased at pH \sim 1 and decreased at pH \sim 7. It is also noteworthy that at both pHs the ICT emission intensity is same and the maximum increased in the same wavelength. The fluorescence intensity ratio of the ICT band to the normal band (I_a/I_b) is constant at pH \sim 1, whereas at pH \sim 7 the I_a/I_b ratio decreases as the concentration of β -CD increases. In other words, as the concentration of β -CD increases, I_a/I_b ratio at pH \sim 7 decreases in contrast to that of the pH \sim 1. At pH \sim 10, ICT emission is not observed and the spectral change is different from that at other pHs suggesting the structural geometry of the THB/ β -CD inclusion complex at pH \sim 10 is different from that in other buffer solutions. In other words, the hydroxyl anion dramatically affected the ICT formation for THB.

On increasing the β -CD concentrations at pH \sim 1, the absorbance decreased at the same wavelength suggesting that THB molecule is deeply encapsulated in the β -CD cavity. Since dipole–dipole interaction between THB and

β -CD is lowered in the less polar environment (hydrophobic part) so decrease in the absorbance is noticed [8–14]. Both the absorption and emission maxima are regularly red shift at pH ~ 7 and pH ~ 10 , indicating protonation takes place in the carboxylate anion. This because the large rim of β -CD containing 14 secondary hydroxyl groups provides an environment qualitatively similar to polyhydroxy alcohols [2–5]. Further, it is well known that β -CD is a very good proton donor [8–13] and this may provide proton to the carboxylic anion. More interestingly, at higher β -CD concentrations, the absorption and emission maxima and the spectral shape of THB at pH ~ 1 and pH ~ 7 are same, indicating formation of similar inclusion complexes. Moreover, it is well known that substituents of aromatic rings (THB) capable to form the hydrogen bonding with the hydroxyl groups of the β -CD edges. The energy involved in such hydrogen bond interaction is responsible for the higher/lower binding constants compared to those of the substituted/unsubstituted molecules. The higher formation constants at pH ~ 1 and pH ~ 7 imply that at both pHs THB is more tightly embedded in the β -CD cavity than at pH ~ 10 .

The question may arise, why ICT emission is not observed at pH ~ 10 buffer? This is because at this pH the presence of hydroxyl anion prevents the formation of ICT emission. The presence of broad and LW emission in β -CD-free aqueous solution suggests that the intramolecular proton transfer (IPT) is present at pH ~ 10 . The decrease in full width at half a maximum (FWHM) with increasing β -CD concentration gives the further supports to this prediction. With increasing the β -CD concentration, decreasing the FWHM and increasing emission intensity confirms IPT is present at pH ~ 10 . Similar results on 1,2,3-trihydroxy benzene also support this idea [28]. We have earlier reported that if carboxyl or hydroxyl groups are deeply entrapped in the β -CD cavity, the absorption and emission maxima should be blue shifted as compared to that of aqueous medium because the interior part of the β -CD cavity provides a non-polar environment like cyclohexane. Further, one should expect if hydroxyl group is present in the hydrophilic part of the β -CD cavity, protonation should take place in the hydroxyl anion. In β -CD, if protonation takes place in the hydroxyl anion, blue shift should be observed and the absorption and emission maximum should be similar to that of pH ~ 7 medium because under this environment carboxylic anion is present. The present results indicates that in β -CD solutions the monoanion absorption and emission maxima are red shifted as compared to that in the aqueous medium while the dianion emission maximum is blue shifted. The above findings are confirm that the THB hydroxyl groups are entrapped in the interior part of the β -CD cavity and COOH group is present in the hydrophilic part of the β -CD cavity. In other words,

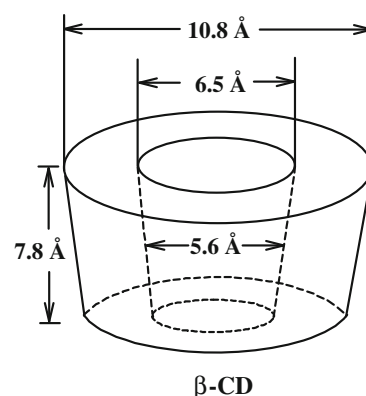
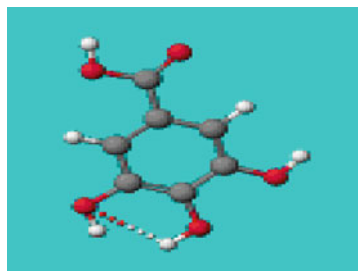
both the carboxyl and hydroxyl groups can be hydrogen bonded with water, but the orientations of those two groups can be different with regard to facing water molecule and forming different patterns of β -CD inclusion complexes.

On the basis of the different orientations of THB, it is clear that as long as the carboxyl group is present in the hydrophilic part, the magnitude of the contribution of the fast relaxations process to form the ICT state in the β -CD inclusion complex increases. The smaller size and presence of different anionic species of THB is responsible for the formation different inclusion complexes in the β -CD solutions. As shown in Fig. 10 one of the two different inclusion complexes could be the type I complex (pH ~ 1 and pH ~ 7), in which the COOH group is located in the narrower part and the other could be the type II complex in which OH group is more deeply located in the wider part of the cavity. At pH ~ 1 type I complex is formed whereas at pH ~ 7 and in low β -CD concentrations type II complex is more favorable. However, at pH ~ 7 and high β -CD concentrations because of the protonation in the carboxyl group type I complex is favourable. Supporting this absorption and normal maxima are red shifted with increasing β -CD concentration. Whereas at pH ~ 1 THB is deeply encapsulated, hence absorbance is decreased and emission intensity is increased at the same wavelength. As the β -CD concentration is increased, at pH ~ 10 a small portion of type II complex is formed.

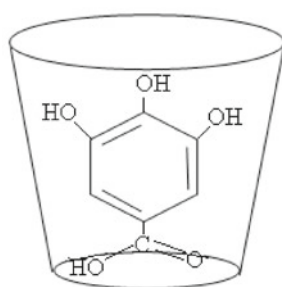
Considering that TICT emission was observed in benzoic acid derivatives [11–14, 31–33], it can be concluded that the restriction or the increased viscosity in the β -CD cavity does not affect the formation the ICT state. The enhancement of the ICT emission of β -CD solution should be due to the reduced polarity of the β -CD environment as proposed by Nag et al. [18, 19]. In the less polar environment the ICT state and the Franck–Condon state is increased. This is supported by the formation of ICT state of THB upon complexation with β -CD. The increased energy gap causes a decrease in the non-radiative transitions from the ICT state so that the ICT emission is enhanced. Such an increase in the energy gap is attributable to the reduced dipole–dipole interaction of the ICT state in the β -CD cavity.

The difference in the inclusion complex is also responsible for the different association constants. The large association constant at pH ~ 1 and pH ~ 7 implies that at the pHs, THB is more deeply embedded in the β -CD cavity than at pH ~ 10 . The gradual enhancement of ICT emission with increasing β -CD concentration is consistent with this speculation. In other words, at pH ~ 1 and pH ~ 7 , THB is more deeply entrapped in the non-polar β -CD cavity than pH ~ 10 . Thus, the enhancement of the ICT emission in both pH ~ 1 and pH ~ 7 solutions indicates that the energy barrier is not affected by the

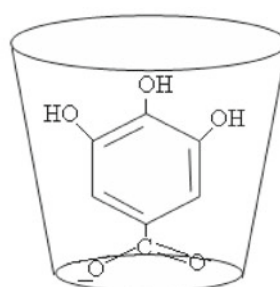
Fig. 10 Proposed inclusion complex structure of THB



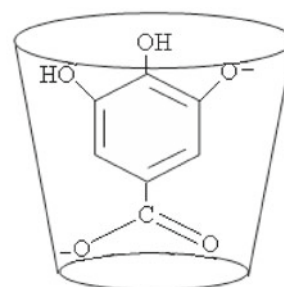
THB: bond distance in Å
PM3 method $\Delta H = -194.167$ kcal/mol
 IHB = 2.485 $H_7-H'_4 = 7.61$ $H_3-H_5 = 5.66$
 $H_3-H_7 = 7.37$ $H_3-H_5 = 6.11$ $H_4-H_7 = 8.12$
 $H_5-H_7 = 7.25$



pH ~1



pH ~7



pH ~10

entrapment of THB in the non-polar β -CD cavity. From this observation, it may be concluded that the energy barrier for the formation the ICT state is governed by the hydrogen bonding between the carboxyl group and water. As long as the H-bonding between the carboxyl group and water is effective, the energy barrier for the ICT is not changed. As discussed above the orientation of THB inclusion complex at pH ~1 and pH ~7 is different from that in pH ~10.

This is further supported by using semiempirical quantum calculations. PC-model program was used to find the initial geometry of the inclusion complex. This program helped us to draw the structure of the inclusion complex. The ground state geometry of THB and β -CD were optimized using PM3 method. The internal diameters of the β -CD is approximately 6.5 Å and its height is 7.8 Å. Considering the shape and dimensions of β -CD (Fig. 10) THB can be completely embedded in the β -CD cavity. The vertical distance of OH–OH is 5.58 Å and that of H_4 – O_2 is 6.22 Å, and is less than the inside β -CD cavity value (6.5 Å). Since the length of THB is lower than that of upper/lower rim of β -CD, the carboxyl and hydroxyl groups attached to benzene ring may be present inside the β -CD cavity as shown in Fig. 10. These finding reveals that THB molecule is embedded in the β -CD cavity.

Conclusions

The present study shows that THB forms 1:1 inclusion complex in all the pH buffer solutions. The entrapment of THB in the β -CD causes ICT emission at pH ~1 and pH ~7. The ratio of ICT emission to the normal emission of THB at pH ~7 increases as the concentration of β -CD is increased, whereas this ratio is constant at pH ~1. At pH ~10, the presence of hydroxyl anion and IPT prevents ICT emission. It is proposed that hydrogen bonding interactions of the electron withdrawing group with water plays an important role in controlling the ICT process in aqueous β -CD solutions.

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