Inclusion complex of 1,2,3-trihydroxybenzene with α- and β-cyclodextrins

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Effect of α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) on the absorption and fluorescence spectra of pyrogallol (THB) has been discussed. The solid inclusion complex of THB with CD has been investigated by UV-visible, fluorimetry, FT-IR, scanning electron microscopy and semiempirical methods. The thermodynamic parameters (ΔG, ΔH and ΔS) values indicate that the inclusion processes are exothermic and spontaneous. β-CD studies reveal that THB forms 1:1 inclusion complex with β-CD. The small formation constant of THB shows that this molecule is not tightly embedded in the β-CD cavity. In β-CD medium, neutral, monoanion and dianion maxima are blue shifted as compared to in aqueous medium. Also, the pKa (pKα*) values in β-CD medium are greater than in aqueous medium. Of the two CDs, β-CD can more readily include THB than α-CD. Dual luminescence observed in α-CD shows that intramolecular hydrogen bonding interactions of THB in α-CD is greater than in β-CD. The study also shows that THB is more tightly embedded in α-CD than in β-CD.

Studies on inclusion complexes of smaller molecules in macromolecular cavities of appropriate size are interesting developments in supramolecular chemistry. Cyclodextrins (CDs) are α-(1,4)-linked glucopyranose rings forming truncated cone type compounds and a wide variety of organic molecules can be complexed in the hydrophobic interior. This property leads to several applications of CDs in different fields of analytical and synthetic chemistry. The spectroscopic and photochemical behaviours of such complexes are of interest and the changes in fluorescence and electronic absorption properties, excimer and exciplex formation in the excited state protolytic equilibria and photoisomerization have been reviewed. Studies on the prototropic reactions have established that the deprotonation rate of CD inclusion complexes either increases (e.g. heterocyclic compounds like carbazole and aminopyrene) or decreases in comparison to the corresponding free molecules as observed for 1- and 2-naphthols. The decrease in the deprotonation rate of excited 2-naphthol has been ascribed to the protection of the probe moiety towards OH attack by the hydrophobic environment within the CD cavity. These results suggest different conformations for 2-naphthol complexed by α and β-CDs, once with the polar –OH group and once with the aromatic moiety of 2-naphthol embedded in the cavity. For substituted CDs, a 1:2 guest/host complex of 1-naphthol has been postulated from absorption spectroscopic studies.

In this study, we report for first time, the absorption and fluorescence characteristics of THB in different α/β-cyclodextrin concentrations. For the past one decade, our laboratory has largely been involved in studying photochemical properties of various fluorophores. Recently, we have studied 4-hydroxy-3,5-dimethoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzoic acid and aminobenzoic acids. These molecules show intramolecular charge transfer emission in the excited singlet state. This stimulated us to carry out a study on THB.

The techniques of UV-visible, fluorimetry, FT-IR, scanning electron microscopy, thermodynamic parameters and semiempirical methods (AM-1) have been used to examine the effects of α/β-CD upon complexation of THB. The stoichiometry and formation constants are determined by use of the data obtained from α/β-CD measurements. The formation constants of the complexes have been estimated in order to predict and understand the factors affecting complexation between α/β-CD and THB molecules in solution.
Materials and Methods

Absorption spectral measurements were carried out with a Hitachi UV-visible spectrophotometer (model U-2001), and fluorescence measurements were made using a Jasco FP-550 spectrofluorimeter. The pH values in the range 2.0-12.0 were measured on Elico pH meter (model LI-10 T). FT-IR spectra were obtained in the range 300-4000 cm⁻¹ with Avatar-330 FT-IR spectrometer using KBr pelleting. Microscopic morphological structure measurements were carried out with a Jeol JSM 5610 LV scanning electron microscope.

THB, α-CD and β-CD were obtained from E-Merck and recrystallized from aqueous ethanol. The purity of the compound was checked by similar fluorescence spectra when excited with different wavelengths. Triply distilled water used for the preparation of aqueous solutions. Buffer solutions in the pH range 2.0-10.0 were prepared by adding the appropriate amount of NaOH and H₃PO₄.

Preparation of cycloextrin solution

Stock solution of THB (0.2 ml, 2×10⁻³ M) was taken in 10 ml standard measuring flasks and CD solutions of varying concentrations (zero i.e., without CD to 1.2×10⁻² mol dm⁻³ CD) were added into these flasks. Then, the mixture of CD/THB solutions were diluted to 10 ml with triply distilled water or appropriate buffer solution and shaken thoroughly. The final concentration of THB in each flask was 4×10⁻⁵ mol dm⁻³. Concentration of CD was varied from zero (without CD) to 1.2×10⁻² mol dm⁻³. The absorption and fluorescence spectra were recorded at 30±1°C using 1 cm quartz cell.

Preparation of solid complex of THB with β-CD

Accurately weighted β-CD (0.6 g) was placed into a 50 ml conical flask and 30 ml distilled water was added and the contents were oscillated. THB (0.2 g) was taken in a 250 ml beaker and 20 ml distilled water was added. The contents were placed on an electromagnetic stirrer until THB dissolved. The β-CD solution was poured slowly into the THB solution. The above mixed solution was continuously stirred for 48 hrs at room temperature and then kept in a refrigerator for 48 hrs. The reddish brown powder that precipitated was filtered with a G₂ sand filter funnel, washed with distilled water and dried in an oven at 60°C for 12 h. This is the solid inclusion complex of THB with β-CD.

Results and Discussion

Effect of β-cyclodextrin

Table 1 shows the absorption and fluorescence maxima of THB and 1,2-dihydroxybenzene (DHB) (4×10⁻³ mol dm⁻³) in pH-1 and pH-7 solutions containing different concentrations of β-CD respectively. The absorption spectral change upon the addition of β-CD at pH-7 is similar to that at pH-1. Upon increasing the concentration of β-CD, the absorption maxima is slightly blue shifted in THB (λ₀,λₘₐₓ = 266 to 263 nm) with a gradual increase in absorbance occurring in both solutions. On the other hand, in DHB (λ₀,λₘₐₓ = 276 to 274 nm) and phenol (λ₀,λₘₐₓ = 277 to 274 nm) the absorbance intensity decreased along with blue shifted maxima with increase in β-CD concentration. The above results are due to all the three molecules being transferred from more protic environments (bulk aqueous phase) to less protic environments (cavity of β-CD). At β-CD concentrations higher than 8×10⁻³ mol dm⁻³, the band maxima and the absorbance remain unchanged. This behaviour has been attributed to the enhanced dissolution of the guest molecule through the hydrophobic interaction between guest and non-polar cavity of β-CD. These results indicate that all the three molecules are entrapped in the β-CD to form guest-host complex. For 1:1 complex between β-CD and THB molecule, the following equilibrium can be written:

\[
\text{THB} + \beta\text{-CD} \rightleftharpoons \text{THB...β-CD} \tag{1}
\]

The binding constant (K) and stoichiometric ratios of the inclusion complex of guest can be determined according to the Benesi-Hildebrand relation assuming the formation of a 1:1 THB-β-CD complex (Eq. 2):

\[
\frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon} + \frac{1}{K [\text{THB}]_o \Delta \varepsilon} + \frac{1}{[\beta\text{-CD}]_o} \tag{2}
\]

where \(\Delta A\) is the difference between the absorbance of THB in the presence and absence of β-CD. \(\Delta \varepsilon\) is the difference between the molar absorption coefficient of THB and the inclusion complex, and \([\text{THB}]_o\) and \([\beta\text{-CD}]_o\) are the initial concentrations of THB and β-CD, respectively. Good linear correlation was obtained for a plot of \(1/\Delta A\) as a function of \(1/[\beta\text{-CD}]\) for THB molecule, confirming the formation of a 1:1 inclusion complex. The value of formation constant
calculated from the slope and the intercept of the plot for THB is 78 and 63 $M^{-1}$ at pH-7 and pH-1 respectively. The formation constant of THB is less than that of DHB ($280 M^{-1}$). The formation constants of both molecules are not changed significantly with change of pH, which indicates the strong intra-molecular hydrogen bonded THB/DHB entrapped in the β-CD cavity\textsuperscript{15,16} and shows that THB/DHB is not tightly embedded in the β-CD cavity\textsuperscript{16}.

The fluorescence spectrum of the THB molecule undergoes a large blue shift on increasing the β-CD concentration (Table I, Fig. 1). The fluorescence peak of the three molecules are blue shifted as compared to that observed in the absence of β-CD (THB=$\lambda_{\text{em}}$ ~355 nm, DHB = $\lambda_{\text{em}}$ ~340 nm and phenol = $\lambda_{\text{em}}$ ~310 to 305 nm). The spectral blue shift of THB in β-CD suggests that –OH groups are located within the non polar cavity of the β-CD. This conclusion is based on the following: The large rim of β-CD contains 12 secondary hydroxyl groups and thus provides an environment qualitatively similar to poly-hydroxy alcohols. The blue shift observed in the fluorescence spectrum of THB in β-CD is not consistent with that observed in methanol (355 nm) but agrees with that in cyclohexane (340 nm). This is because the interior β-CD cavity is less polar than the aqueous phase. In the less polar environment of β-CD, the dipole-dipole interactions between the environment in β-CD and the THB will be lowered. The emission intensity and bandwidth of all the three molecules are also gradually increased from water to 0.01 $M$ β-CD concentrations. The enhancement of the fluorescence intensity in pH-7 is greater than that in pH= 1. Usually, the accommodation of a fluorescent guest by β-CD enhances the fluorescence intensity for the formation of an inclusion complex\textsuperscript{15,16}. Hence, the increase in the fluorescence intensity and bandwidth of the THB molecule recorded in β-CD indicates the presence of 1:1 inclusion complex of these molecules. The complexation is completed at $8 \times 10^{-3} \text{mol dm}^{-3}$ β-CD concentration. There is no significant change in the fluorescence intensity of both solutions on further addition of β-CD. The β-CD dependence of THB fluorescence can be analysed by the linear Benesi-Hildebrand\textsuperscript{14} plots obtained in the present study. The formation constant, $K$, value determined from the slope and the intercept of the Benesi-Hildebrand plots

![Fluorescence spectra of THB in β-CD solutions of varying concentrations (mol dm$^{-3}$).](image)

**Table 1 – Absorption and fluorescence maxima (nm) of THB and DHB at different concentrations of β-CD**

<table>
<thead>
<tr>
<th>No.</th>
<th>Conc. of β-CD (M)</th>
<th>pH=1</th>
<th>THB</th>
<th>pH=7</th>
<th>DHB</th>
<th>pH=1</th>
<th>DHB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{abs}}$</td>
<td>$\log \varepsilon$</td>
<td>$\lambda_{\text{em}}$</td>
<td>$\log \varepsilon$</td>
<td>$\lambda_{\text{abs}}$</td>
<td>$\log \varepsilon$</td>
</tr>
<tr>
<td>1</td>
<td>0 (without β-CD)</td>
<td>266.2</td>
<td>3.50</td>
<td>355</td>
<td>265.5</td>
<td>3.55</td>
<td>352</td>
</tr>
<tr>
<td>2</td>
<td>0.002</td>
<td>264.6</td>
<td>3.54</td>
<td>340</td>
<td>265.5</td>
<td>3.63</td>
<td>340</td>
</tr>
<tr>
<td>3</td>
<td>0.012</td>
<td>263.4</td>
<td>3.89</td>
<td>340</td>
<td>263.5</td>
<td>3.89</td>
<td>340</td>
</tr>
<tr>
<td>4</td>
<td>Formation constant, $K$, ($M^{-1}$)</td>
<td>63</td>
<td>148</td>
<td>78</td>
<td>227</td>
<td>260</td>
<td>380</td>
</tr>
<tr>
<td>5</td>
<td>Δ$G$ (kJ mol$^{-1}$)</td>
<td>-10.4</td>
<td>-12.6</td>
<td>-11.0</td>
<td>-13.7</td>
<td>-14.0</td>
<td>-15.0</td>
</tr>
<tr>
<td>6</td>
<td>Δ$H$ (kJ mol$^{-1}$)</td>
<td>-110.75</td>
<td>-110.75</td>
<td>-66.3</td>
<td>-66.3</td>
<td>-66.3</td>
<td>-66.3</td>
</tr>
<tr>
<td>7</td>
<td>Δ$S$ (J mol$^{-1}$K$^{-1}$)</td>
<td>-0.32</td>
<td>-0.31</td>
<td>-0.22</td>
<td>-0.31</td>
<td>-0.27</td>
<td>-0.27</td>
</tr>
</tbody>
</table>
in emission is greater than that obtained from the absorption, because the change in emission intensity upon addition of β-CD is significantly greater than the absorption. Further, the quantitative comparison of binding constants of the two complexes (THB and DHB) suggests that β-CD provides a better site to accommodate a deep inclusion of DHB in the β-CD cavity than THB.

Effect of α-cyclodextrin

The absorption and emission spectra of THB in aqueous solution as a function of α-CD were also studied (Table 2). As in β-CD, no significant change is observed in the α-CD medium (λ<sub>abs</sub> 265, 208 nm). With an increase in α-CD concentration, the absorbance of both the THB bands increases slightly. The formation constant for the THB : α-CD complex formation determined by Benesi-Hildebrand relation indicates formation of a 1:1 complex between α-CD and THB. A plot of 1/ΔA versus 1/(α-CD) and 1/H<sub>0</sub> versus 1/(α-CD) is linear. The formation constant for THB with α-CD, (K = 131 M<sup>-1</sup>), is greater than that with β-CD (K = 78 M<sup>-1</sup>), (K<sub>eq</sub>: α-CD = 295 M<sup>-1</sup>, β-CD = 227 M<sup>-1</sup>). The higher K value in α-CD suggests that THB is more tightly embedded in α-CD than in β-CD. The THB formation constants are very sensitive to α-CD and β-CD complexation, indicating that selective inclusion complex is formed with α-CD and β-CD. Of the two CDs, it should be noted that β-CD can more readily include THB than α-CD, because the β-CD inner cavity size (5.6Å) is greater than α-CD (4.7Å).

The fluorescence spectra of aqueous THB solutions as a function of α-CD concentration are presented in Fig. 2. The fluorescence characteristics of THB in α-CD are entirely different from those in β-CD. In β-CD, the intensity of fluorescence only increases whereas in α-CD, two effects are observed: firstly, a dual luminescence is observed in α-CD at 345 and 420 nm. Secondly, fluorescence intensity is increased along with α-CD concentrations. From Figs 1 and 2, it is clear that the fluorescence enhancement in α-CD is greater than that in β-CD. This suggests the formation of an inclusion complex between THB and α-CD.

Both the emission bands (longer wavelength LW 420 nm, shorter wavelength SW 345 nm) are slightly red shifted at higher α-CD concentrations. The full width at half of maximum (FWHM) increases with increase in α-CD concentration, indicating that IHB interactions are greater in α-CD than in β-CD solution. The increase in FWHM observed in both bands clearly establishes that the THB is present in the interior part of the α-CD cavity; it is well known that the interior part of CDs is less polar than the outer part and the IHB interactions are larger in non-polar part than in the polar part. The larger enhancement in fluorescence and greater FWHM in α-CD indicates that THB is more tightly embedded in α-CD than in β-CD. This is because the inner and outer cavity size of α-CD is smaller than β-CD, and the THB molecule is more tightly embedded in the α-CD cavity.

The thermodynamic parameters, ΔG, ΔH and ΔS, for the binding of the guest molecule to α/β-CD are given in Tables 1 and 2. As can be seen from the tables, ΔG is negative, which suggests that the inclusion process proceeded simultaneously at 303 K. ΔH and ΔS are also negative in the experimental temperature range which indicates that the inclusion process is an exothermic and enthalpy controlled process. The negative enthalpy change is due to the
van der Waal's interaction, while the negative entropy change (ΔS) is due to the steric barrier caused by molecular geometrical shape and the limitation of β-CD cavity to freedom of shift and rotation of guest molecule. The experimental results indicate that the inclusion reaction of CD with THB was an exothermic reaction accompanied with negative ΔS. In this case, the effects of enthalpy and entropy changes are opposite. The study indicates that changes in ΔH are largely compensated by changes in ΔS.

### Prototropic reactions in β-CD medium

To know the effect of β-CD on the prototropic equilibrium between neutral and monoanion of THB, the pH dependent changes in absorption and fluorescence spectra of THB molecule in aqueous solution containing β-CD (8×10⁻³ mol dm⁻³) have been recorded in the Hₒ/pH range of -1.0-pH -11. The absorbance and fluorescence maxima of various Hₒ/pH ranges are given in Table 3. The pKₐ value of trianion could not be measured because deprotonation of β-CD-OH takes place around pKₐ-12. On comparison with aqueous medium, the absorption and fluorescence maxima of neutral and monoanion are blue shifted in β-CD. The blue shifts in λₐₐbs and λₐₐₐ of the THB/DHB are attributable to the inclusion of the THB/DHB molecule into the hydrophobic cavity of β-CD. The ground and excited singlet state pKₐ and pKₐ* values of the THB/DHB molecules in β-CD medium are greater than in aqueous medium (Table 4). This is because the formation of the THB:β-CD complex results in the protonation of anionic THB and DHB¹⁰, leading to more neutral form being produced which permits greater hydrophobic stabilization in the interior of the β-CD cavity. The neutral/anionic form of guest in β-CD cavity favours a closed conformer by IHB.¹⁵ The increase in the bandwidth of the fluorescence spectrum recorded in β-CD medium further supports the presence of IHB. Furthermore, the 448, 336, 292 nm monoanionic absorption maxima of THB become significantly broad and slightly blue shifted (441, 324, 258 nm) as the concentration of β-CD increases. Even in β-CD free solution, the absorption band of THB was found to be composed of three different bands
(monoanion $\lambda_{abs}=448, 336, 292$ nm; $\lambda_{em}=$non-fluorescent, dianion, $\lambda_{abs}=429, 338, 292$ nm, $\lambda_{flu}=480, 415, 360$ nm) indicating that different conformers exist in aqueous solution. The closed conformer can be tautomerized by the IPT and the $\lambda_{abs}=429$ nm, $\lambda_{flu}=336$ nm and $\lambda_{flu}=415$ nm are due to the other tautomer, and the $\lambda_{em}=292$, $\lambda_{flu}=365$ nm is due to the open conformer of the dianion.

In inclusion complexes, depending upon relative affinity of the guest for the host, $pK_a$ is known to change as a result of complexation. This is due to both $-OH$ groups in DHB present in the non-polar part of the $\beta$-CD cavity. But in THB, monoanion is red shifted as compared to the neutral species, because THB $-OH$ groups are partially present in the hydrophilic part of the $\beta$-CD cavity. The blue shift in the absorption and fluorescence of neutral, monoanion of THB and DHB in $\beta$-CD medium substantiates this. Similar results are also observed in naphthols. To substantiate this, the ground state geometry of THB was optimized using the semiempirical quantum mechanical calculations, AM-1 method (MOPAC-6.0 version). The vertical distance between $H_2- H_8$ is 4.332 Å and $H_1- H_6$ is 6.25 Å, whereas the horizontal distance between $O_1- O_3$ is 4.81 Å. The internal diameter of the $\beta$-CD is approximately 6.5 Å and its height is 7.8 Å. The shape and dimensions of $\beta$-CD and THB indicates that the THB and DHB molecules are completely encapsulated in the $\beta$-CD cavity (Scheme 1). Since the length of THB/DHB is less than that of the upper/lower rim of $\beta$-CD, the OH groups attached benzene ring may be present inside the $\beta$-CD cavity. Under no circumstance can the THB/DHB be encapsulated partially in the $\beta$-CD cavity.

According to AM-1 calculations, THB mainly exists as shown in Scheme 1, which facilitates the intermolecular H-bonding and consequently the formation constant of the monoanion, $pK_a$ value in $\beta$-CD medium is slightly greater than in aqueous medium, because it is still possible that some water molecules still remain inside the $\beta$-CD cavity due to the loose geometry for this complex. This would allow the formation of H-bonds between the probe and water molecules which would give rise to the

![Scheme 1](image-url)
observed broad fluorescence emission. On the other hand, DHB is located deep inside the β-CD cavity as shown in Scheme I and the intermolecular H-bonding would be inhibited. Under this circumstance, the monoanion and dianion maxima of DHB should be blue shifted as compared to the neutral species. Our results clearly indicate that the monoanion and dianion maxima of THB are slightly blue shifted in β-CD than in aqueous medium but red shifted as compared to the neutral species. The ground and excited state $pK_a$ ($pK_a^+$) values for the neutral-monoanion equilibrium in β-CD is greater than in aqueous medium, whereas monoanion-dianion $pK_a$ ($pK_a^+$) values in β-CD are closer to those in aqueous medium. These findings reveal that both molecules are completely included in the β-CD cavity.

FT-IR spectra of THB, β-CD and the solid inclusion complex of THB with β-CD were studied. When compared to THB (3375 cm$^{-1}$), the OH stretching frequency of the inclusion complex is largely red shifted (3391 cm$^{-1}$). The OH bending (1321 cm$^{-1}$), C−O stretching (1245 cm$^{-1}$) and C=C stretching (1620 cm$^{-1}$) frequencies of the THB molecule are also slightly red shifted in the inclusion complex (1326 cm$^{-1}$, 1248 cm$^{-1}$ and 1626 cm$^{-1}$ respectively). Further, the absorption intensity of the inclusion complex is also significantly weaker (up to 30%) as compared to that of the THB molecule. The above findings confirm that the THB molecule is included into the cavity of β-CD.

SEM photographs of powdered form of THB, β-CD and the inclusion complex (Fig. 3) clearly elucidate the difference between the three. β-CD shows plated structure, while THB shows stick structure. The complex is present in colloidal form. Modification of crystals and powder can be assumed as a proof of the formation of a new inclusion complex.

The above study shows that THB forms 1:1 inclusion complex with α-CD and β-CD. The FT-IR, SEM and AM-1 studies confirm that THB forms an inclusion complex with β-CD. The study also shows that the THB is more tightly encapsulated in the α-CD than in β-CD. Prototropic reactions in β-CD show both DHB and THB molecules to be encapsulated in the cavity.

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References


