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# Inclusion of Paracetamol into β-cyclodextrin nanocavities in solution and in the solid state

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ABSTRACT

We report on steady-state UV–visible absorption and emission characteristics of Paracetamol, drug used as antipyretic agent, in water and within cyclodextrins (CDs): β-CD, 2-hydroxypropyl-β-CD (HP-β-CD) and 2,6-dimethyl-β-CD (Me-β-CD). The results reveal that Paracetamol forms a 1:1 inclusion complex with CD. Upon encapsulation, the emission intensity enhances, indicating a confinement effect of the nanocages on the photophysical behavior of the drug. Due to its methyl groups, the Me-β-CD shows the largest effect for the drug. The observed binding constant showing the following trend: Me-β-CD > HP-β-CD > β-CD. The less complexing effectiveness of HP-β-CD is due to the steric effect of the hydroxypropyl-substituents, which can hamper the inclusion of the guest molecules. The solid state inclusion complex was prepared by co-precipitation method and its characterization was investigated by Fourier transform infrared spectroscopy, $^1$H NMR and X-ray diffractometry. These approaches indicated that Paracetamol was able to form an inclusion complex with CDs, and the inclusion compounds exhibited different spectroscopic features and properties from Paracetamol.



## 1. Introduction

Cyclodextrin (CD) nanocavities are potential candidates for drug delivery systems through the formation of inclusion complexes (Scheme 1) on the basis of noncovalent interaction [1], due to their effect on solubility, dissolution rate, chemical stability and absorption of drugs. The hydrophobic environment of a CD nanocavity can lead to the alteration of physical, chemical, and biological properties of guest molecules [2–8]. As already reported for a large variety of systems, a CD cavity is able to influence both the fate of the reaction intermediates and the deactivation pathways of the excited caged drugs [9,10]. This may lead to a decrease in phototoxicity.

Paracetamol (*N*-acetyl-4-aminophenol, Scheme 2) is a popular antipyretic agent which also possessed analgesic (pain-relieving) properties. In several countries, it is most commonly used by people who are sensitive or allergic to aspirin. Although, it is not effective against inflammation, compared to Aspirin or Ibuprofen; it is well tolerated by most people, including children. Paracetamol rarely causes side effects as long as it is taken as directed. However, prolonged or habitual use of Paracetamol may lead to liver damage or failure.

Recently, the spectroscopy and photophysical properties of Paracetamol was reported in α-, β- and γ-cyclodextrin cavities and micellar medium [11]. The systematic study of some modified β-CD as HP-β-CD and Me-β-CD in solution and in the solid state is also important. Most CD derivatives are highly water-soluble products. Among the chemically modified β-CDs, methylated and hydroxyalkylated CDs have received considerable attention because their physicochemical properties and aqueous solubility are significantly changed and the inclusion behavior is largely magnified, depending on the degree of substitution [12].

In this paper we present the influence of native and modified β-CD on the complexation of Paracetamol by the determination of the corresponding stability constants in the hope of improving some of the pharmaceutical properties of Paracetamol using UV–visible absorption and fluorescence spectroscopy. Solid inclusion complexes containing Paracetamol and cyclodextrins were prepared by co-precipitation method and characterized by Fourier-transform infrared and X-ray diffractometry techniques.

## 2. Experimental

### 2.1. Materials

Paracetamol 99% (Aldrich), diethylether (Sigma–Aldrich), β-CD (Fluka), HP-β-CD (Fluka) and Me-β-CD (Fluka) were analytical

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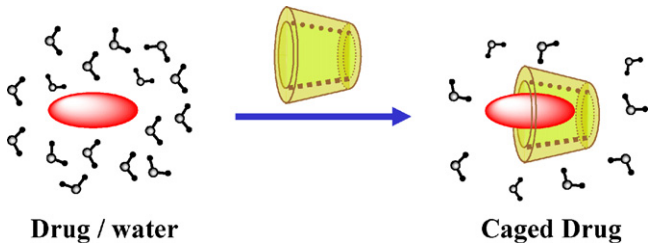
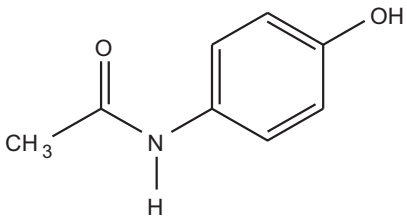



**Scheme 1.** Schematic view of the process of drug cyclodextrin inclusion.




**Scheme 2.** Paracetamol (*N*-acetyl-4-aminophenol).

reagent grades and used without further purification. Double distilled water was used for the preparation of all solutions.

### 2.2. Apparatus and methods

The absorption and fluorescence spectra were recorded on a Shimadzu 2450 UV–visible spectrophotometer and a Shimadzu 5301 PC spectrofluorometer, respectively. Fourier-transform infrared (FT-IR) spectra were measured with a JASCO spectrometer 4100. The powder X-ray diffraction (XRD) analysis was made on a Rigaku 2550D/max VB/PC X-ray diffractometer using Cu Kα radiation (λ = 0.154056 nm).

NMR spectra were recorded in $D_2O$ on a Varian Mercury VX-300 spectrometer. $^1$H NMR data analysis was carried out using MestReC 4.9.9 software. Chemical shifts have been expressed in parts per million (ppm) relative to HDO signal as a reference at 4.80 ppm. For $^1$H NMR measurements, the concentrations of Paracetamol and β-CD were about $2 \times 10^{-3}$ M and 0.02 M, respectively.

The geometry of the molecule was optimized using AM1 of the MOPAC 97 program.

### 2.3. Preparation of the physical mixture

Appropriate amounts of drug and CD to give 1:1 molar ratio were mixed thoroughly in a mortar by geometric dilution technique [13]. The physical mixture was sieved and stored in airtight container.

### 2.4. Preparation of solid inclusion complexes

The solid inclusion complexes used in this investigation were prepared by the Co-precipitation method. Amounts of the drug and CD with 1:1 molar ratios were accurately weighed. Drug was dissolved in 20 ml of ether and CD was dissolved in 100 ml of water. The two solutions were mixed, agitated for 24 h at 28 °C and then cooled to 2 °C in a refrigerator. The mixture was filtered, washed with ether and dried at 25 °C under vacuum for 24 h.

## 3. Results and discussions

### 3.1. Steady-state absorption and fluorescence spectra

Fig. 1A displays the UV–visible absorption spectra of Paracetamol drug in neutral water and in the presence of Me-β-CD. A very small change in the spectra was detected upon addition of Me-β-CD, suggesting a weak interaction between CD and drug or a small change in the molar absorption coefficient of the drug. Fig. 1B shows the steady-state emission spectra of neutral aqueous solution of Paracetamol and in the presence of different Me-β-CD concentrations, upon excitation at 275 nm. In water, Paracetamol exhibits a band with a maximum at 302 nm. Upon addition of Me-β-CD, the emission intensity is enhanced with a small red shift of the band, suggesting the formation of complex between Paracetamol and Me-β-CD (Scheme 1). For β-CD and HP-β-CD solutions, the increase in the emission intensity is relatively weaker.

The fluorescence band maximum of Paracetamol displays also insignificant shift on going from protic polar water (302 nm) to relatively aprotic non-polar cyclohexane (304 nm; not shown in figure). A similar spectral change was also observed on the absorption spectra of Paracetamol, reflecting non-polar neutral structure of both ground and excited states of Paracetamol.

This result indicates that binding of Paracetamol to CD changes the local solvation environment (water) in a way similar to changing the solvent from protic to aprotic medium. Since Paracetamol has three hydrogen-bonding sites (amino nitrogen, phenolic oxygen as well as carbonyl group, Scheme 2). Upon encapsulation, Paracetamol experiences a reduced H-bonding interaction with water.

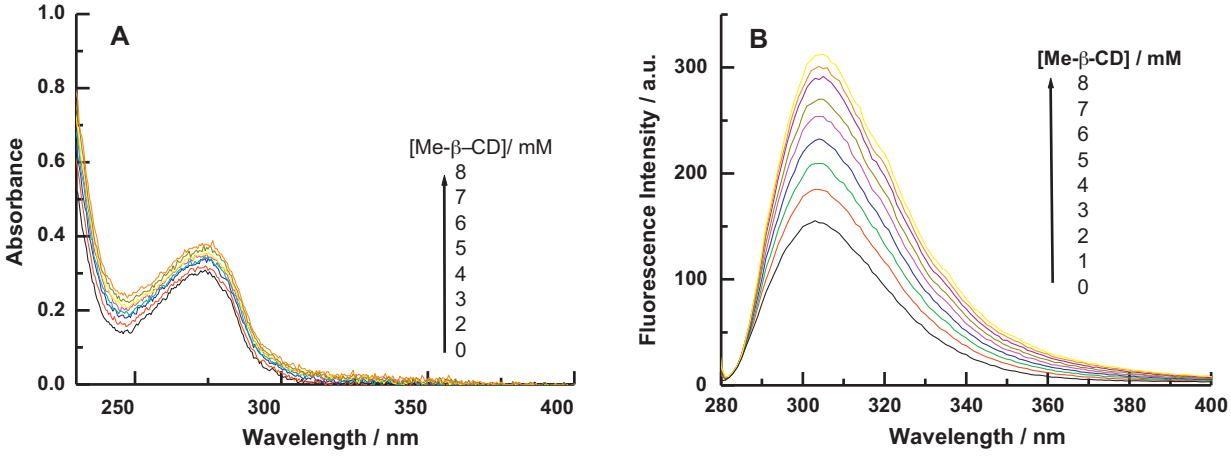



**Fig. 1.** (A) Absorption and (B) fluorescence spectra of Paracetamol ($10^{-4}$ M) in water in absence and presence of Me-β-CD at 298 K.

### 3.2. Association constants

The equilibrium constant $K$ assuming the formation of a 1:1 Paracetamol/CD can be calculated based on the following Benesi–Hildebrand [14] plots using fluorescence data of Fig. 1A:

$$\frac{1}{I - I_0} = \frac{1}{I_1 - I_0} + \frac{1}{K(I_1 - I_0)} \frac{1}{[CD]_0} \quad (1)$$

where $[CD]_0$ represents the initial concentration of CD, $I_0$ and $I$ are the fluorescence intensities in the absence and presence of CD, respectively, and $I_1$ is the limiting intensity of fluorescence.

A plot of $1/(I - I_0)$ versus $1/[CD]_0$ give a straight line, indicating the formation of 1:1 inclusion complex between Paracetamol and CD. The $K$ values were obtained from the slope and intercept of the plots. The Benesi–Hildebrand plots (Fig. 2) show excellent linear regression ($r = 0.99$), supporting the assumed 1:1 Paracetamol/Me-$\beta$-CD inclusion complex. From the plot, $K$ is evaluated. We obtained $K$ values were $1.48 \times 10^2\ M^{-1}$, $1.89 \times 10^2\ M^{-1}$ and $2.10 \times 10^2\ M^{-1}$ for the Paracetamol complexes with $\beta$-CD, HP-$\beta$-CD and Me-$\beta$-CD, respectively.

The Benesi–Hildebrand plots assuming 1:2 stoichiometry can be represented by Eq. (2):

$$\frac{1}{I - I_0} = \frac{1}{I_1 - I_0} + \frac{1}{K_2(I_1 - I_0)} \frac{1}{([CD]_0)^2} \quad (2)$$

where $K_2$ represents the association constant of 1:2 complex. The plots of $1/(I - I_0)$ versus $1/([CD])^2$ according to Eq. (2) are curved as shown in Fig. 2B, confirming thereby the previous result of a 1:1 stoichiometric ratio.

It is readily seen that the magnitude of inclusion equilibrium constant increases in the order Me-$\beta$-CD > HP-$\beta$-CD > $\beta$-CD. For Me-$\beta$-CD, the $K$ value is almost larger than that for $\beta$-CD and HP-$\beta$-CD. A significant increase in the stability constants of many drugs in the presence of Me-$\beta$-CD in comparison with $\beta$-CD has been reported [4,12–16]. Both $\beta$-CD and Me-$\beta$-CD have the same cavity diameter. However, the unsubstituted $\beta$-CD has height (~8 Å), therefore part of the drug could still be outside the nanocage (vide infra). Due to the methyl groups, the height of Me-$\beta$-CD cavity is longer (~11 Å) [17]. The hydrophobic part of Me-$\beta$-CD provided by the methyl groups, at the 2- and 6-positions increases the hydrophobicity of the cavity [17,18] and leads to a greater efficiency towards the drug compared to that with $\beta$-CD and enhances more penetration of the drug inside the cavity of Me-$\beta$-CD.

However, the observed better performance of Me-$\beta$-CD compared to HP-$\beta$-CD has also been attributed to the presence of the methyl groups that increased the hydrophobic region of the macro molecule by capping the edge of the cavity and expanding the location of substrate binding, without causing any structural hindrance to the drug inclusion [19]. On the contrary, some effect of steric blocking, due to the presence of the hydroxypropyl-substituents, which can hamper the inclusion of the guest molecules, could explain the less complexing effectiveness exhibited by HP-$\beta$-CD.

It should be noted that the hydrogen-bonding ability of the alcoholic hydrogens in $\beta$-CD is low compared to that of HP-$\beta$-CD because, in the former, the secondary alcoholic –OH groups at the 2- and 3-positions of the adjacent glucopyranose rings are engaged in intramolecular hydrogen-bonding with each other. In the case of Me-$\beta$-CD, however, this intramolecular hydrogen-bonding is destroyed due to substitution of the alcoholic proton at the 2-position with a methyl group. This structure enhances the intermolecular hydrogen-bonding ability of the alcoholic –OH group at the 3-position with water. Although the methylation makes the host molecule incapable of forming intramolecular hydrogen bonds, the stability constant with Me-$\beta$-CD is higher than that with $\beta$-CD, therefore the role of the hydrogen binding seems to be less important [20].

However, for HP-$\beta$-CD the hydroxyl groups and the hydroxypropyl groups on the exterior of the molecule can interact with water to provide the increased aqueous solubility of HP-$\beta$-CD and complexes made with it. These groups may also account for the stronger hydrogen bonding between Paracetamol and HP-$\beta$-CD than the $\beta$-CD. On the other hand, hydrophobic bonding may be another important contribution to the interactions between Paracetamol and HP-$\beta$-CD. This would account for the higher value of association constant for Paracetamol/HP-$\beta$-CD complex than for Paracetamol/$\beta$-CD complex.

The results suggest that the size of CD cavity plays a role in the formation and stability of the formed complex. The dimensions of Paracetamol geometry of the ground-state were optimized by using AM1 (MOPAC 97 program). This calculation revealed that the molecular length of Paracetamol (distance between atoms 15 and 17) is ~8 Å, and its width (distance between atoms 18 and 19) is ~5 Å, Scheme 3. The internal diameter of the $\beta$-CD is approximately 6–6.5 Å and its height is 7.8 Å [21,22]. Due to the methyl groups, the height of Me-$\beta$-CD cavity is longer (~11 Å) [17]. The resulting structures show that the Paracetamol drug is included in the cavities with the aliphatic part and the stability constant value of drug–cyclodextrin complexes is a useful index of the binding strength of the complex. This conclusion has been confirmed by using $^1$H NMR spectroscopy as shown in next section.

From the above results we concluded that the drug is well fitted and deeply included into the deeper Me-$\beta$-CD cavity than that of $\beta$-CD because many methyl groups are located at both ends of the cavity and the whole Paracetamol molecule, is buried in the host.

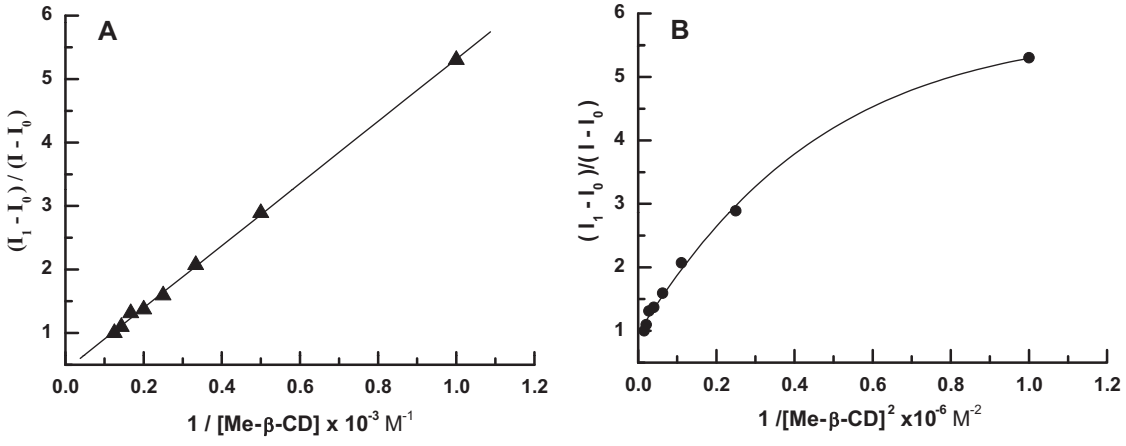
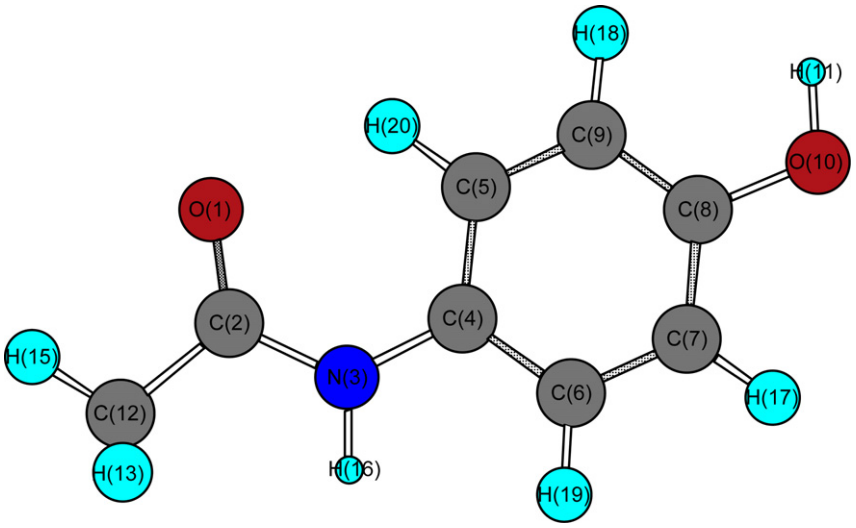



**Fig. 2.** Benesi–Hildebrand plot of data from Fig. 1 for (A) 1:1 and (B) 1:2 inclusion complex.




**Scheme 3.** Optimized geometry of the ground-state Paracetamol by using AM1.

This may explain the higher stability constant of Paracetamol with Me-$\beta$-CD in comparison with $\beta$-CD.

### 3.3. Solid state inclusion complex

Fig. 3 shows the X-ray diffraction patterns of Paracetamol, $\beta$-CD, the physical mixture and inclusion complex. The diffraction pattern of physical mixture (1:1) is simply the superposition of signals of the two components, while that of inclusion mixture is different and its peaks change apparently contrasted with those of Paracetamol and $\beta$-CD. A diffraction pattern that is clearly distinct and not a superposition of each of the components of the binary system are indicative of formation of a true inclusion complex. The diffraction pattern of Paracetamol shows intense and sharp peaks indicating the crystalline nature of the inclusion complex.

On the other hand, the X-ray diffractogram of HP-$\beta$-CD physical mixture corresponded to superimposition of the individual components, whereas that of complex consisted fundamentally of a single very broad band, Fig. 3B. These results suggest the amorphous nature of the caged drug in the nanocavity [23].

To confirm our observations on the inclusion complex, we performed series of FTIR measurements on the drug with different CDs. No significant variations were observed in the absorption spectra corresponding to the physical mixtures which resulted from the overlapping of the simple spectra of the drug and CD. On the other hand, a shift and attenuation in the absorption band corresponding to the carbonyl of drug were observed when the complex is formed.

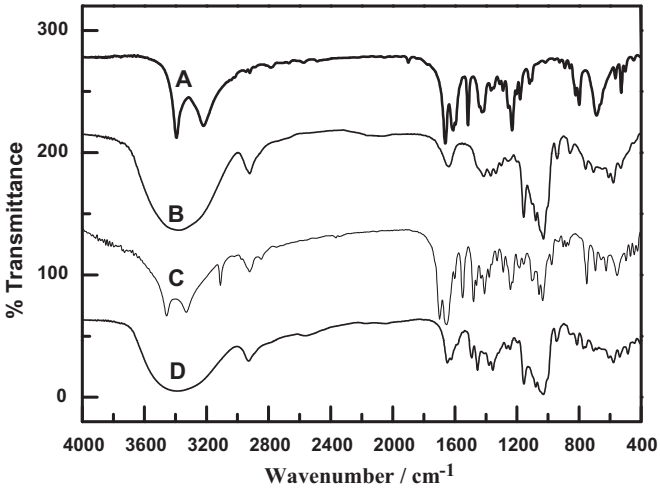



**Fig. 4.** FT-IR of (A) Paracetamol, (B) $\beta$-CD, (C) their physical mixture and (D) their inclusion complex.

In Fig. 4 we show the FTIR spectrum of the inclusion complex drug/$\beta$-CD as representatives. The spectrum shows specific absorp-

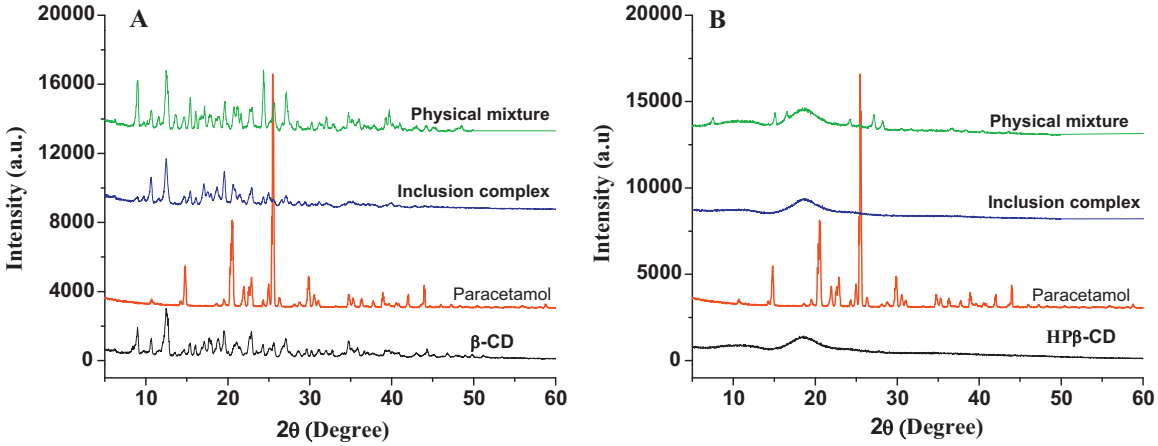



**Fig. 3.** X-ray diffraction patterns of Paracetamol: (A) $\beta$-CD and (B) HP-$\beta$-CD, their physical mixture and inclusion complexes.

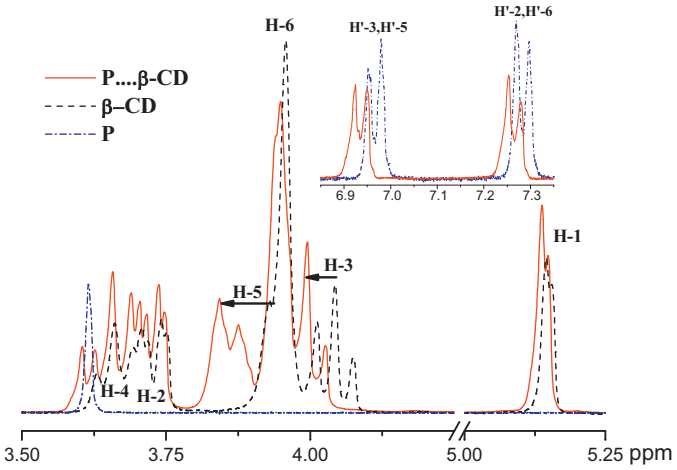



**Fig. 5.** $^1$H NMR spectra of $\beta$-cyclodextrin ($\beta$-CD), Paracetamol (P) and their mixture (P. $\beta$-CD) in $D_2O$.

tion peaks at 3402 $cm^{-1}$ (OH stretching H-bonded), 2929 $cm^{-1}$ (OH stretching), 1648 $cm^{-1}$ (OH bending), 1353 $cm^{-1}$ (OH deformation), 1243 $cm^{-1}$ (CH bending), 1160 $cm^{-1}$ (COC stretching and OH bending), 1079 and 1027 $cm^{-1}$ (COC stretching) characteristic for $\beta$-CD. The band at 3388 $cm^{-1}$ from pure drug shifted to 3402 $cm^{-1}$ in drug/$\beta$-CD inclusion complex due to the intermolecular hydrogen bonding.

The chemical shift of $\beta$-CD protons reported by different authors was very close to those in this work ±0.05 ppm [7,8 and references therein]. It is well known that the H-3 and H-5 protons are located in the interior of the $\beta$-CD's cavity and it is, therefore, likely that the inclusion of Paracetamol with $\beta$-CD will specifically affect the chemical shifts of these two protons. The addition of Paracetamol to $\beta$-CD causes an upfield shift of $\beta$-CD spectrum (Fig. 5). The $^1$H NMR spectrum of Paracetamol–$\beta$-CD shows that the signals due to H-1, H-2 and H-4 are upfield shifted by about 0.007, 0.005 and 0.02 ppm, respectively, with similar amount of shifts as observed for other systems [7,8]. The signal due to H-3 is upfield shifted by about 0.05 ppm while the unresolved band becomes resolved with a maximum for H-6 is slightly shifted by less than 0.009 ppm while that for H-5 is upfield shifted by about 0.07 ppm. That assignment for H-5 protons is based on the fact that H-3 and H-5 are located in the interior of the cavity and therefore is expected to suffer similar amount of shielding. The upfield shift observed for H-3 and H-5 protons confirm the inclusion inside the cavity.

The $^1$H NMR spectrum of Paracetamol is shown in Fig. 5 and reveals that Paracetamol has three types of protons: two doublets for (H′-2 and H′-6) at 7.27 ppm and (H′-3 and H′-5) at 6.95 ppm and singlet of $CH_3$ group is found at 3.61 ppm (Fig. 5). The chemical shift values given by the Chem-Draw version 8 NMR calculations show that (H′-2 and H′-6) > (H′-3 and H′-5). The upfield chemical shift observed for H′-2 and H′-6 doublets is about 0.02 while that for H′-3 and H′-5 is about 0.03. Methyl group protons overlapped with H-2 and H-4 $\beta$-CD protons with approximate upfield shift of 0.01 ppm. Therefore, we can conclude that the upfield shift of Paracetamol protons together with $\beta$-CD protons confirm tight inclusion of Paracetamol inside $\beta$-CD cavity.

## 4. Conclusions

The Paracetamol molecule forms inclusion complexes with CDs ($\beta$-CD, Me-$\beta$-CD and HP-$\beta$-CD). The stoichiometry of the complexes is 1:1. The stability constants determined by spectrofluorometric method. The stability constant with Me-$\beta$-CD is higher than with $\beta$-CD and HP-$\beta$-CD owing to the extension of the hydrophobic depth of the cavity. The difference in the stability constants between HP-$\beta$-CD and $\beta$-CD may be ascribed to the stronger hydrogen bonding between Paracetamol and HP-$\beta$-CD than the $\beta$-CD. Solid inclusion complexes with $\beta$-CD or HP-$\beta$-CD and drug can be obtained by co-precipitation and their formation has been probed by using FTIR $^1$H NMR and X-ray diffraction techniques.

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