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ORIGINAL ARTICLE

^{1}H NMR spectroscopic investigation of β-cyclodextrin inclusion compounds with parecoxib

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Abstract The inclusion complexes of β-CD and parecoxib [PRB] in aqueous solution were investigated using ^{1}H NMR spectroscopic study revealed the existence of four different equilibria for 1:1 inclusion complexes in which both the aromatic rings of the guest are tightly held by the host cavity. The NMR spectra of the PRB studied in the presence of β-CD are fully assigned and interpreted by means of COSY spectrum for the unambiguous assignment of guest aromatic ring protons. The parallel interpretation of β-CD chemical shift changes and dipolar contacts, with the aid of 2D ROESY, allows the mode of binding to be established for four possible structures of 1:1 PRB-β-CD inclusion complexes.

Keywords β-cyclodextrin · Parecoxib · Host-guest interaction · NMR spectroscopy

Introduction

Traditionally oral non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat mild to moderate acute pain. This is problematic, however, when the patients are unable to take oral medication or are nauseous and vomiting. Parecoxib (parecoxib sodium, PRB), a non-steroidal anti-inflammatory drug, is the first selective cyclooxygenase-2 inhibitor administered as an intramuscular or intravenous injection for short-term management of post-operative pain. It is an attractive alternative to parenteral ketorolac because of the fewer gastrointestinal events [1].

The solubility of poorly water-soluble drugs can be altered in many ways, such as modification of drug crystal forms or addition of co-solvents, surfactants and cyclodextrins (CDs), etc. CDs are of great interest in the area of pharmaceuticals because complexation of a pharmaceutical compound with CDs results in, besides improved water solubility [2], various altered desirable properties like increased stability [3], dissolution rate [4] and bioavailability [5] and reduced volatility and masking of some undesirable properties of the drug like unpleasant odor and taste [6]. That stimulated a great deal of research towards the synthesis and characterization of CD inclusion complexes.

CDs can be visualized as toroidal, hollow, truncated cones, containing a relatively hydrophobic central cavity and hydrophilic outer surface. Due to their characteristic architecture, CDs have an ability to recognize and embrace a variety of molecules into their cavity to form host-guest complexes [7]. The hydrophobic guest or the hydrophobic part of the guest molecule is held by the CD-cavity by non-covalent interactions while the polar part of the guest protrudes outside the cavity. The guest generally enters the cavity from wider side of the rim and the size and shape of the host cavity, in combination with the size of the guest, are major determinants for the formation of the CD-inclusion complexes [7, 8].

A variety of techniques are available to observe host/guest interactions depending on the applications envisaged for the complex [9]. NMR spectroscopy is, however, one of the most reliable techniques for studying the CD inclusion complexes [10]. Evidence for incorporation of guest into the CD-hydrophobic cavity is easily obtained by simple ^{1}H NMR experiments. ^{1}H NMR spectra of mixtures of CD and

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guest molecule exhibit chemical shift changes for both CD as well as guest protons [11]. Changes in the chemical shift of CD cavity protons, H-3′ and H-5′, are indicative of internalization of the guest or part of the guest molecule into the CD cavity thus confirming the formation of an inclusion complex [12, 13]. A deeper insight into the stereochemical features of the inclusion complex can be obtained by means of 2D ROESY spectroscopy. The observability of intermolecular NOEs between inner cavity protons (H-3′ and H-5′) of β-CD and guest molecules give a direct evidence that the guest has entered inside the cavity of the CD [14] since NOE cross peaks are observed between the protons that are closer than 0.4 nm in space in ROESY spectrum. The relative intensities of these cross peaks depend on the spaces between the corresponding protons.

In continuation of our work on the preparation and characterization of CD inclusion complexes of pharmaceutical compounds in aqueous solution [15, 16], we report here our results on the complexation behavior of parecoxib with β-CD using ^{1}H NMR (^{1}H, COSY and ROESY) spectroscopy.

Experimental

^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument while COSY and ROESY experiments were performed on a Bruker 800 MHz instrument for a mixture of parecoxib and β-CD ([β-CD]/[PRB] = 0.625). All the spectra were recorded in D_2O at room temperature without adding any external reference and the chemical shift values (δ) are reported in ppm. HDO proton signal at 4.790 was used as an internal reference throughout this work. To determine the stoichiometry and the overall binding constant, ^{1}H NMR spectra of four mixtures of parecoxib and β-CD, were recorded by keeping the concentration of PRB constant at 8.4 mM while the concentration of β-CD was varied from 3.9 to 11.7 mM. The molar ratios ([β-CD]/[PRB]), ranging from 0.42–1.15 were calculated by direct integration of appropriate signals. No distinct peaks were observed for bound and free form of PRB and β-CD indicating a fast equilibrium on the NMR time scale.

Results and discussion

^{1}H NMR spectrum of pure parecoxib exhibited a pair of interacting doublets at 7.721 (J = 8.5 Hz) and 7.274 (J = 9.0 Hz), each integrating for two protons, which were assigned to H-3, 5 and H-4, 6 of p-substituted aromatic ring, respectively. The signal for H-10 of phenyl ring observed as a multiplet at 7.403 (1H, m, H-10) while signals for remaining phenyl ring protons appeared merged in the region 7.303–7.400. A singlet at 2.387, for three protons, was due to methyl group of the heterocyclic ring (H-7). The ethyl group protons were observed as a quartet at 2.089 (2H, J = 7.5 Hz, CH_2) and a triplet at 0.911 (3H, J = 8.0 Hz, CH_3). Distinction between the signals for two aromatic rings of PRB could be determined with the help of COSY experiment. Figure 1 showing a pair of cross connections for two separate doublets at 7.332 and 7.859 were confirmed for disubstituted aromatic ring. Whereas, the remaining cross peaks were determined for phenyl ring protons but due to some ambiguity their assignments could not be made (Fig. 1).

Prominent changes in the nature and position of signals for most of the protons of parecoxib were observed in the presence of β-CD, which increased with the increasing concentration of β-CD. The signals for the protons of both the phenyl and p-substituted aromatic rings exhibited downfield shift changes. The chemical shift changes for aliphatic protons were also observed but these were found not to be affected by the change in the concentration of β-CD. Part of spectra exhibiting aromatic proton signals of parecoxib, in the absence as well as in the presence of β-CD, are shown in Fig. 2, while chemical shift change ($\Delta\delta$) data for parecoxib protons is given in Table 1.

All the β-CD protons displayed chemical shift changes in the presence of parecoxib. The Fig. 3 shows the spectral region containing β-CD protons for pure β-CD as well as mixture of β-CD and PRB. Significant chemical shift

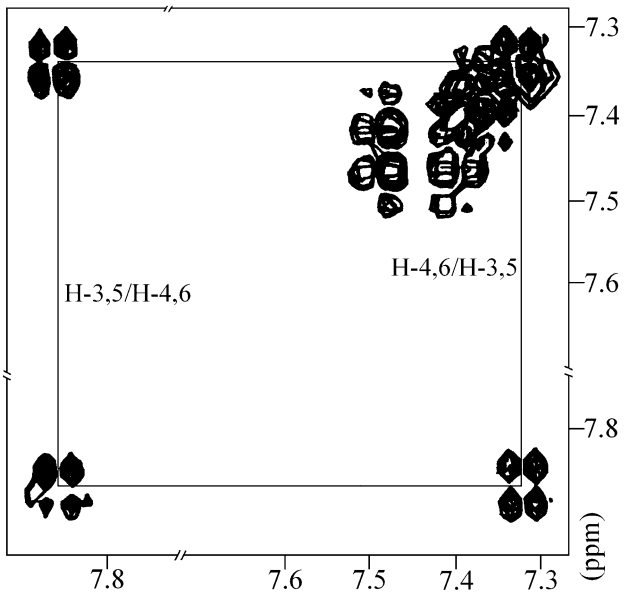


Fig. 1 A partial 800 MHz COSY spectrum of parecoxib-β-CD ([β-CD]/[PRB] = 0.625) inclusion complex in D_2O at 25 °C showing distinction between the cross peaks of two aromatic ring protons of the parecoxib

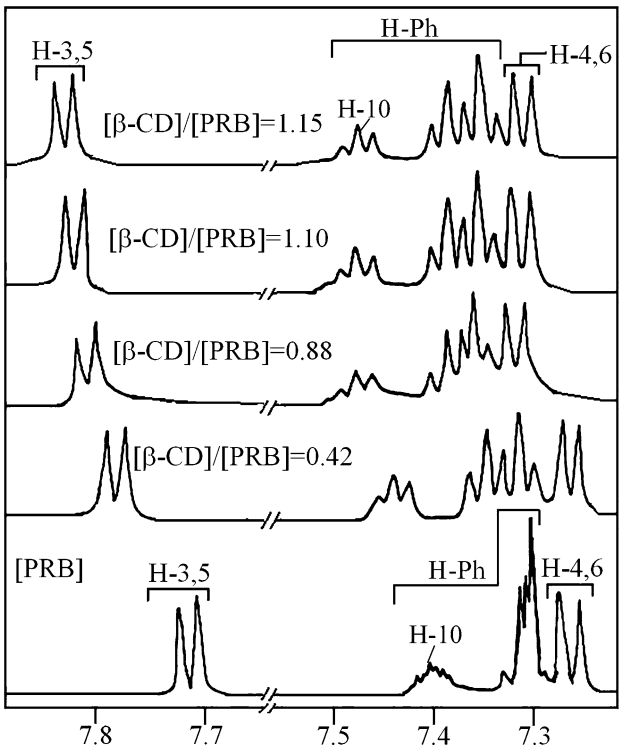


Fig. 2 Partial 400 MHz ^{1}H NMR spectra for parecoxib in the absence and presence of β-cyclodextrin, in D_2O at 25 °C

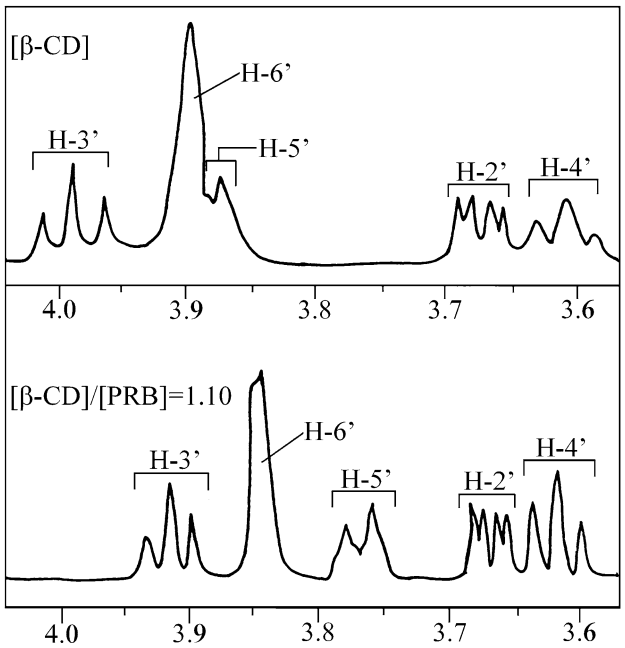


Fig. 3 A part of ^{1}H NMR spectra of β-cyclodextrin (400 MHz), showing the changes in chemical shift for β-cyclodextrin protons on interaction with parecoxib

changes for the protons positioned inside the cavity, namely H-3′ and H-5′, and the protons located near the narrow rim (H-6′) were observed in the presence of PRB. The protons situated near the wider rim, H-2′ and H-4′, showed only insignificant chemical shift changes in the presence of PRB. Moreover, the chemical shift change for H-5′ was more pronounced compared to H-3′.

β-CD is a truncated right cylindrical cone shaped molecule, 7.9×10^{-8} cm high, with a hollow tapered cavity whose top and bottom dimensions are 6.5×10^{-8} and 6.8×10^{-8} cm [7]. On internalization of a guest, β-CD cavity protons show upfield chemical shift changes. On the other hand, all the guest protons generally experience downfield shift changes. These shift changes are attributed to the anisotropic ring current effect of the aromatic guest. Moreover, information regarding the mode of penetration of the guest into the cavity, i.e. from narrower or wider rim side, can be obtained from these shift changes. A typical interference is that $\Delta\delta_{H\text{-}5'} > \Delta\delta_{H\text{-}3'}$ if the guest enters the cavity from narrower side and vice versa [11] but there are exceptions and these conclusions can only be drawn when only one complex is formed while in cases where multiple equilibria exist these shift changes can only be used as an evidence for the formation of inclusion complexes. The observed shift changes in the β-CD cavity protons, and the concomitant chemical shift changes in the PRB proton signals, in the spectra of mixtures of β-CD and PRB, compared to pure components, is a clear indication of inclusion of some part of the guest into the β-CD cavity and thus confirming the formation of β-CD-PRB inclusion complex/es.

The determination of stoichiometry of the complex is the first step for any structural studies of the inclusion

Table 1 ^{1}H NMR (400 MHz) chemical shift (δ, ppm) data for parecoxib and chemical shift change ($\Delta\delta$) values for the studied protons of parecoxib in the presence of β-cyclodextrin in D_2O at 25 °C

Protons	[PRB]	[β-CD]/[PRB] = 1.15	[β-CD]/[PRB] = 1.10	[β-CD]/[PRB] = 0.88	[β-CD]/[PRB] = 0.42
H-3,5	7.721	0.116	0.110	0.100	0.076
H-10	7.403	0.071	0.071	0.068	0.034
H-4,6	7.274	0.043	0.046	0.050	0.003
H-7	2.387	0.048	0.049	0.049	0.023
H-2	2.089	0.063	0.060	0.062	0.067
H-1	0.911	0.058	0.055	0.056	0.060

complexes, which was achieved by Hanna-Ashbaugh method [17–19].

$$1/\triangle\delta \;=\; 1/(K_a \triangle\delta_{max}[H]_0) \;+\; 1/\triangle\delta_{max}$$

In the Hanna-Ashbaugh equation $\Delta\delta$ is the observed chemical shift difference for a given $[H]_0$ concentration, $\Delta\delta_{max}$ is the chemical shift difference between a pure sample of complex and the free component, K_a is the binding constant and $[H]_0$ is the initial concentration of the host. ^{1}H NMR spectra of several mixtures of PRB and β-CD were recorded by keeping the concentration of PRB constant and varying the concentration of β-CD. The ^{1}H NMR chemical shift change data was obtained for PRB. The double reciprocal plot was obtained for H-3, 5 by plotting $\Delta\delta_{H\text{-}3,5}$ against $[H]_0$ in the form of $1/\Delta\delta$ versus $1/[H]_0$ which gave a straight line, with a slope $1/K_a\Delta\delta_{max}$ = 26.50938 mM ppm^{-1} and intercept $1/\Delta\delta_{max}$ = 6.30737 ppm^{-1} (Fig. 4), confirming the 1:1 stoichiometry for the PRB-β-CD complex. The overall binding constant (K_a) of the complex/es was determined to be 238 M^{-1}. The binding constant was also calculated by Scott's method which comes out to be 252 M^{-1}.

To ascertain the involvement of two aromatic rings in the complexation, a ROESY experiment was performed on a mixture of β-CD and PRB. A set of cross peaks connects both the H-5′ and H-3′ of β-CD to both the aromatic ring protons (Fig. 5), taking into account the 1:1 stoichiometry of the complex, indicates that four different equilibria may exist in solution (Scheme 1). The presence of strong cross peaks between β-CD inner cavity protons with both the guest aromatic ring protons confirmed that both the rings are involved in complexation. The presence of multiple equilibria for PRB-β-CD inclusion complexes in solution has been suggested due to the significant intermolecular

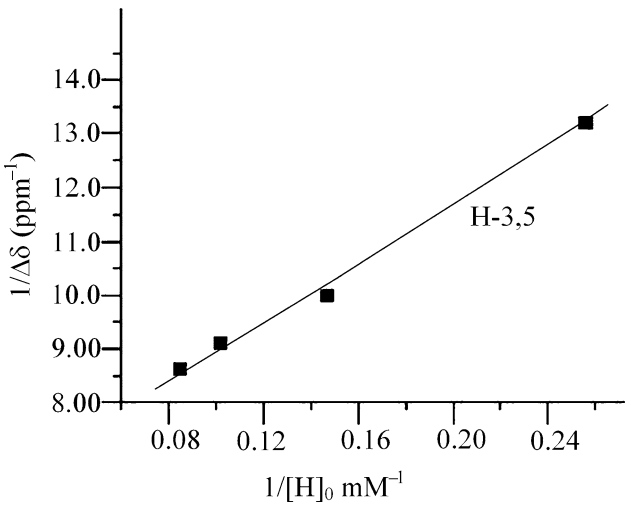


Fig. 4 Illustration of the Benesi-Hildebrand data treatment gives K_a = 238 M^{-1} for 1:1 parecoxib-β-CD complex

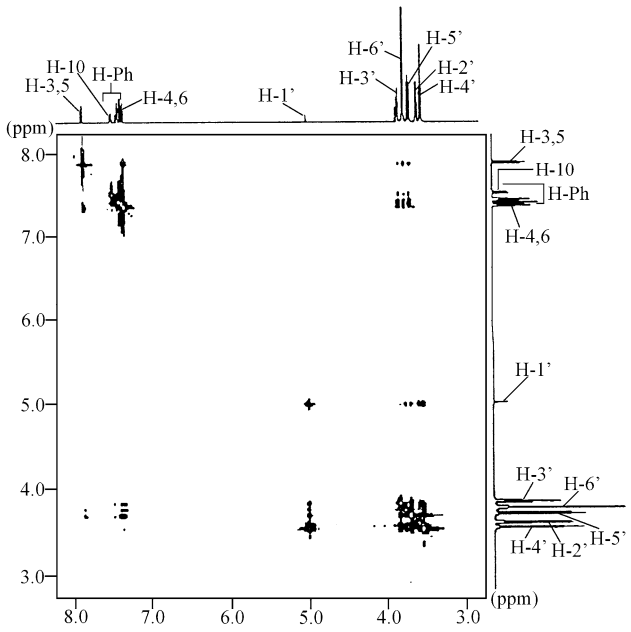
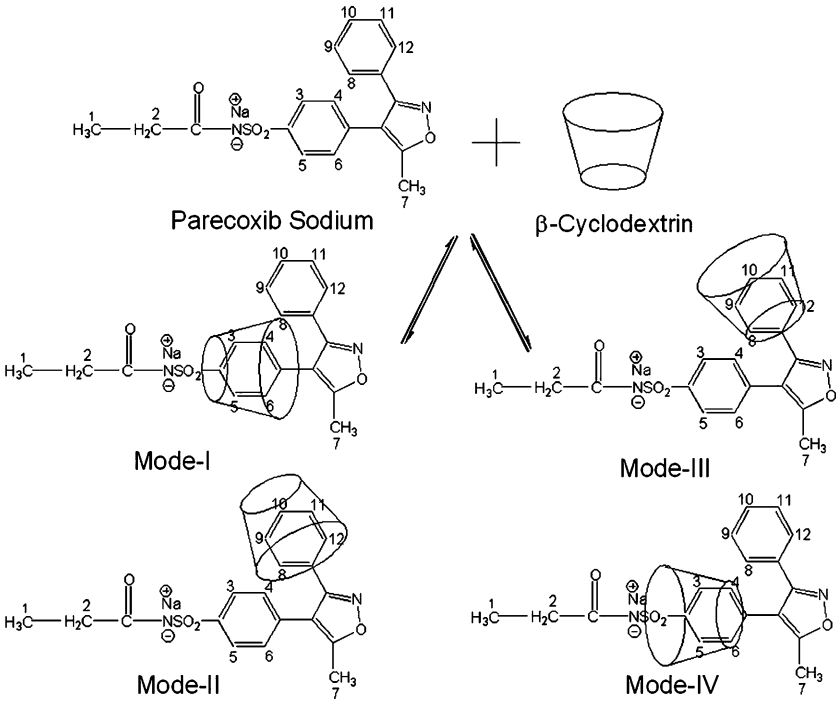


Fig. 5 A partial 800 MHz 2D ROESY spectrum of parecoxib-β-CD ([β-CD]/[PRB] = 0.625) inclusion complex in D_2O at 25 °C with mixing time of 500 ms, indicating the dipolar contacts between protons of the parecoxib and protons of β-CD

interactions between both the aromatic rings with β-CD inner cavity protons (H-3′, H-5′ and H-6′) (Fig. 5). Several topologies may arise for each aromatic ring because entry may occur through either the wider or narrower rim of β-CD and penetration may be either shallow or deep. NOE peaks confirm four binding Modes of PRB with host cavity in which the mono- and di-substituted guest aromatic rings may enter either from primary or secondary hydroxyl side and penetrate deep. Therefore, four possible modes of inclusion equilibria have been suggested on the basis of our findings i.e. cross peaks with all of β-CD cavity protons. A schematic representation of the inclusion equilibria is showing plausible structure of all the four 1:1 PRB-β-CD inclusion complexes (Scheme 1).

Conclusions

The detailed ^{1}H NMR spectroscopy study of parecoxib in the presence as well absence of β-CD in solution confirmed multi-equilibria as the formation of four 1:1 inclusion complex, resulting by the penetration of both the aromatic ring into the β-CD cavity from narrow as well as secondary rim side, as evidenced by ROESY spectrum. The complexes including aromatic ring of the guest and CDs are generally formed by the penetration of the guest from wider rim side [10] but there are cases reported where guest enters the cavity from both the sides [20, 21], which has been found in



Scheme 1 Supposed representation of all the four possible mode of penetrations for 1:1 inclusion complexes formed between PRB and β-CD system in solution

the case of parecoxib-β-CD complexes. The association constant (K_a) of the complex was determined to be 238 M^{-1}.

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