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Structural Studies of Inclusion Complexes of HP-β-CD and Cetirizine by Combination of ROESY and Molecular Modelling Methods

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Cyclodextrin inclusion complexes (CD ICs) mimics the biological systems in molecular and chiral recognition mechanisms and therefore used as models in understanding molecular and chiral recognition because of their small size as compared to large biological systems. Cross peaks observed in the ROESY spectra of the mixture of cetirizine (CIT) and HP-β-CD demonstrate the portions of the guest encapsulated in the cavity that helped in establishing the probable orientations of complexes. Protons of both chlorobenzene and phenyl rings showed interactions with the cavity protons suggesting the formation of two complexes. Molecular mechanics (MM) and molecular dynamics (MD) studies were performed only from the wider end of the CD cavity because of steric hinderance of hydroxypropyl groups on the narrow side of CD cavity. Using the fusion of 2D ROESY and molecular modelling studies, the final structures of ensembles of the inclusion complexes were established.

Keywords: Cetirizine, HP-β-CD, Inclusion complexes, COSY, ROESY, Molecular mechanics, Molecular dynamics.

INTRODUCTION

Cyclodextrins (CDs) are well known compounds for forming the inclusion complexes (ICs) and demonstrate a wide range of applications because of its characteristic such as stability [1], solubility [2], bioavailability [3], bioactivity [4], *etc.* Cyclodextrin inclusion complexes (CD ICs) are generally known to form with hydrophobic guests,while the resemble biological systems in several ways *i.e.* solubility in aqueous medium, their molecular and chiral recognition. Molecular and chiral recognition plays an important role in biological systems *e.g.* drug-DNA interaction, interaction of proteins and receptors with one of the enantiomers selectively. Understanding the basic mechanisms of these interactions can lead to the discovery of new and efficient drugs, but it is difficult to study these interactions in biological systems like DNA, RNA, proteins because of their large size. Therefore, CD ICs are used by many chemists to understand the molecular and chiral recognition mechanisms and thus their structural studies are of great importance.

These days computational as well as experimental methods are playing an important role in the structure elucidation of CD ICs [5]. The inclusion complexes (ICs) of CDs are under research using different techniques such as FT-IR, XRD [6], NMR [7], *etc.* All these methods generally provide information on the occurrence of complexation while NMR is used largely to show the type of complexation. There are conspicuous changes in chemical shifts of CD cavity protons in NMR spectra upon complexation with guest molecules [8]. Interactions between the guest and CD cavity protons, observed in 2D ROESY spectrum helps in identifying the portions of guest encapsulated in the cavity, along with the information on the possible mode of entry *i.e.* either from the wide or narrow side of cyclodextrin [9].

Ali & Muzaffar [10] have used quantitative ROESY analysis to determine the structures of CD ICs with good atom accuracy. Further, it was shown that the structures obtained from the molecular mechanics (MM), molecular dynamics (MD) and molecular docking in combination with quantitative analysis showed good agreement with those obtained from DFT (B3LYP functional) studies even if ROESY recorded at longer mixing time [5]. The structures of cetirizine (CIT) complexes with α-CD, β-CD and γ-CD have been established by the use of quan-

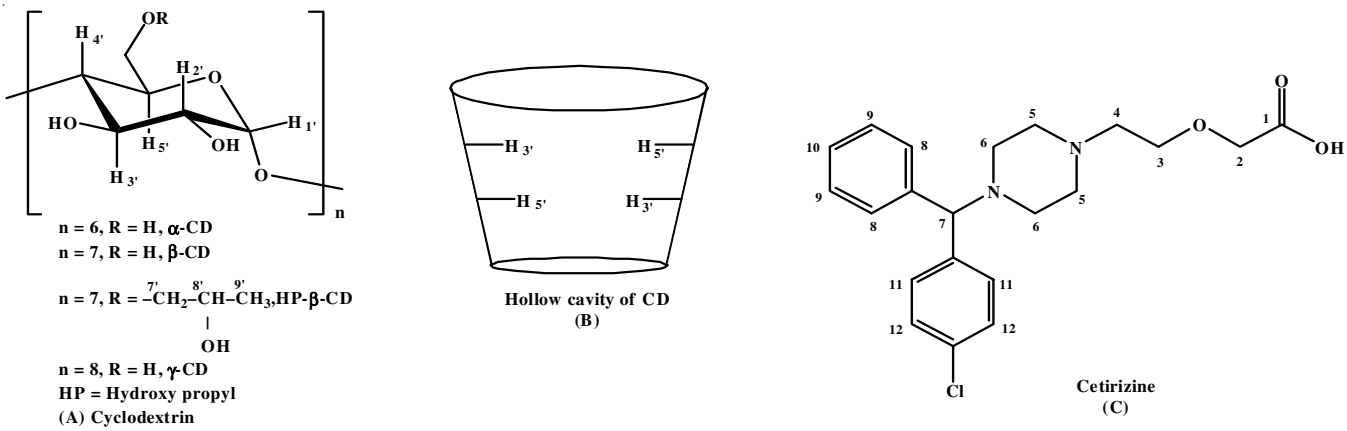

Fig. 1. (A) Structure of CDs, (B) Hollow cavity of CDs, (C) molecular structure of CIT

titative ROESY analysis [11-14]. It was also observed that mostly the minimum energy conformation from MM and MD is in good agreement with experimental ROESY.

In this article, the structures of inclusion complexes (ICs) formed by HP-β-CD and CIT have been determined. Cetirizine (CIT) was chosen because it has multiple binding sites *i.e.* two aromatic rings one phenyl and other one substituted by Cl atom at *p*-position in addition to a pyrazine ring. This molecule helps to study different interactions and binding modes. Here, HP-β-CD is the modified form of β-CD in which hydrogen atom of primary hydroxyl group is replaced by-CH_2-CH(OH)-CH_3 as shown in Fig. 1. The structures of inclusion ICs from ROESY and molecular modelling techniques, molecular mechanics (MM) and molecular dynamics (MD) are also established.

EXPERIMENTAL

All the ^{1}H NMR and 2D NMR (ROESY and COSY) spectra were recorded in D_2O on a JEOL 500 MHz instrument at room temperature. There was no use of external indicator and signal of HDO at 4.8 ppm was used as an internal reference. ^{1}H NMR spectra of pure cetirizine (CIT) and the modified form of β-CD (HP-β-CD) was recorded for comparison with spectra of CIT/HP-β-CD mixture in complexed state. No distinct peaks were observed in NMR time scale of either spectrum for free and complexed CIT, which shows that there is a rapid exchange between complexed and free state. ^{1}H-^{1}H COSY spectra for mixtures (1:1 ratio) of CIT with HP-β-CD was recorded in D_2O at room temperature for assignment of different ^{1}H NMR signals. It was done using gradient selected COSY utilizing 1:1 pulsed field gradient with the repletion time of 1.609 s a relaxation delay of 1.5 s. 2D-ROESY spectra for mixtures (1:1 ratio) of CIT with three cyclodextrins (CDs) was also recorded under spin lock conditions with mixing time of 0.25 s. It was considered that stoichiometric ratio of host to guest in complex formed is 1:1 ratio, since the cavity of CD is large enough to accommodate one aromatic benzene ring [9].

RESULTS AND DISCUSSION

^{1}H NMR spectrum: Generally, it is found that after the accommodation of guest in cyclodextrin (CD) cavity the $H_{3'}$ and $H_{5'}$ protons of CD cavity show highfield shift and the protons of the guest show downfield shift [7]. The upfield chemical shift changes in the $H_{3'}$ and $H_{5'}$ cavity protons, when an aromatic ring of guest is encapsulated into the CD cavity, are inferred because of the anisotropic effect due to aromatic ring in the guest molecule [9]. Many studies have shown that $H_{1'}$, $H_{2'}$, $H_{4'}$, which are present outside the cavity of CD are either relatively unaffected or show negligible chemical shift as compared to $H_{3'}$ and $H_{5'}$ present inside the cavity. Also from previous studies, it was found that the $H_{6'}$ proton, which is on a narrow rim end of cavity shows significant chemical shift changes on the inclusion of guest molecule [15]. In present experiment, ^{1}H NMR spectra of HP-β-CD/CIT mixture (Fig. 2) shows that the chemical shift changes in peaks of $H_{3'}$ and $H_{5'}$ shows that complexation has occurred towards highfield. It can be concluded that inclusion has occurred.

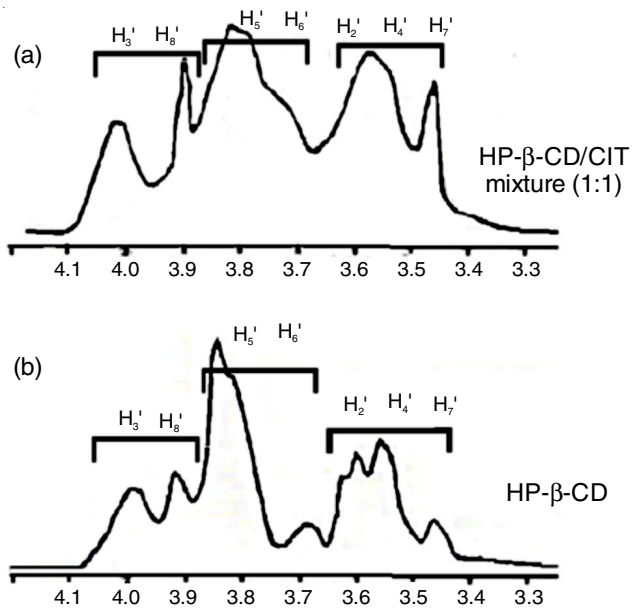

Fig. 2. Partial 500 MHz ^{1}H NMR spectra showing CD region of (a) HP-β-CD/CIT mixture (b) HP-β-CD

^{1}H-^{1}H COSY spectrum: COSY spectrum was used to identify the peaks in ^{1}H NMR. The COSY spectrum for the mixture of CIT/HP-β-CD (1:1 ratio) is shown in Fig. 3. In present study, Since its accommodation within the CD cavity

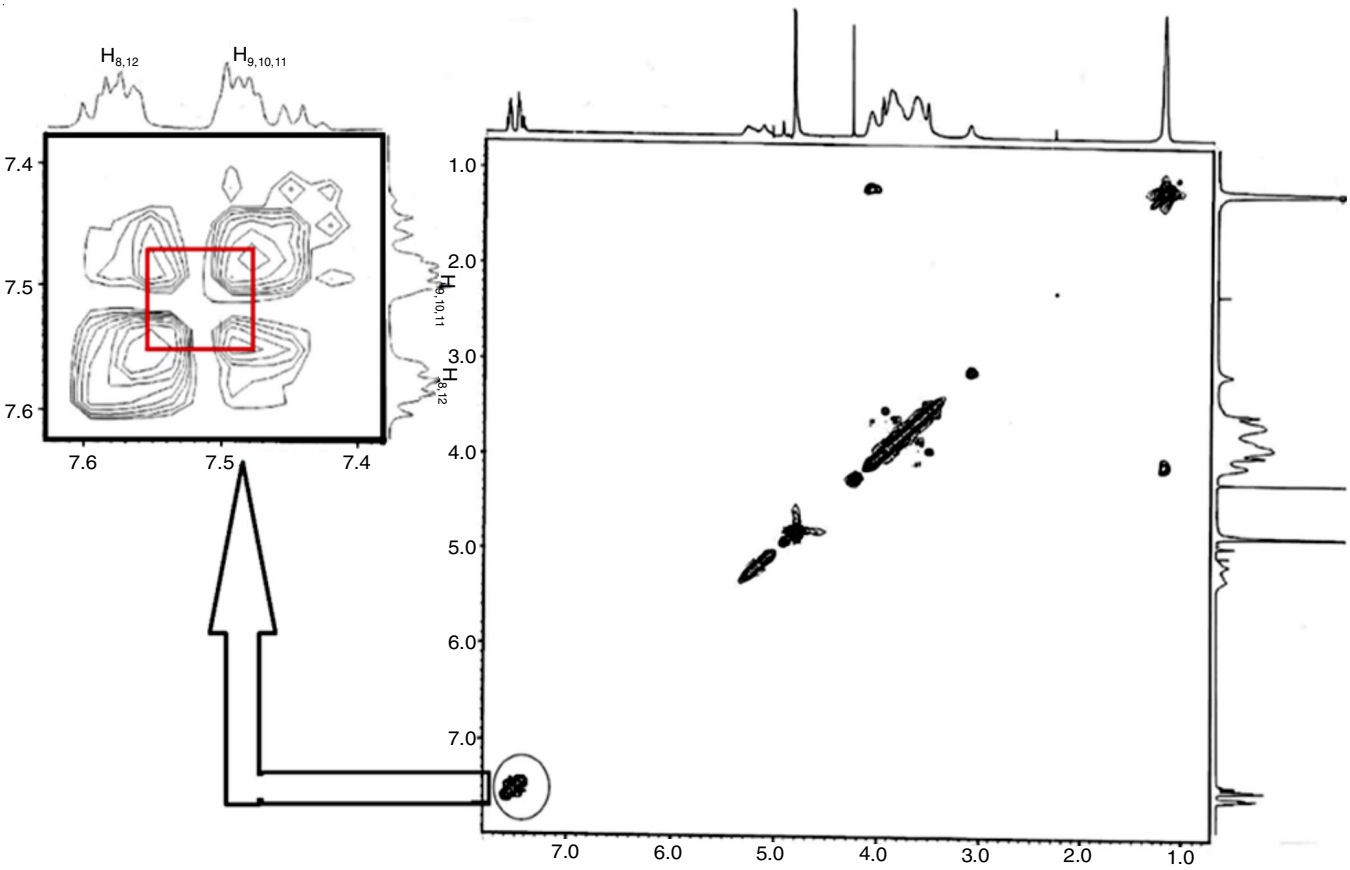

Fig. 3. ^{1}H-^{1}H COSY spectrum of HP-β-CD/CIT mixture (1:1 ratio)

can be accurately predicted using experimental approaches, we are particularly interested in the aromatic region of the guest. The aromatic region of the COSY spectrum has two diagonal and two cross-peaks showing two different types of signals. Furthermore, the chemical shift values showed that the peak towards left is for H_8 and H_{12} while the peak towards right is for H_9, H_{10} and H_{11}.

^{1}H-^{1}H ROESY spectrum: ROESY, a 2D NMR spectroscopy, has been used widely in structure elucidation of ICs formed by different types of cyclodextrins (CDs) [15,16]. This spectroscopy uses through-space interaction between host and guest protons. One of the most important parameters for observation of cross correlation is mixing time. For longer mixing times in large molecules more ROEs are observed but COSY and TOCSY type interactions also appear due to coherence transfer between scalar-coupled spin. ROESY can also be recorded in a short time (few minutes) with mixing time of 500 ms or more but contains COSY and TOCSY type artefacts. However, if ROESY cross peaks are not interfered by these artefacts valuable information can be obtained from ROESY spectra.

In ROESY spectrum (Fig. 4) of CIT/HP-β-CD mixture having equimolar ratio of host and guest two peaks were observed above the diagonal showing the interaction of the aromatic protons of CIT with the cavity protons of CD *i.e.* $H_{3'}$ and $H_{5'}$. This type of interaction clearly shows that complexation of both the chlorophenyl and phenyl rings has been occurred within HP-β-CD cavity.

Molecular modelling: To further investigate the complexation of CIT with HP-β-CD molecular modelling calculations were carried out in CS Chem3D Pro. These studies were performed by utilizing Allinger's force field [17] at room temperature and in vapour phase [18]. The structures of the two ICs *i.e.* chlorophenyl/HP-β-CD and phenyl/HP-β-CD were observed from ROESY spectrum were generated by minimizing their geometries to RMS gradient of 0.1 kcal/mol. In MM and MD calculations the host molecule *i.e.* HP-β-CD was kept static because CDs adopt more symmetrical geometry in its complexed formed. The guest molecule *i.e.* CIT was allowed to move to explore the geometry of the inclusion complexes.

Molecular mechanics (MM): Molecular mechanics studies are performed to get local minima and in these studies a guest molecule is placed manually in the cavity of a host. The guest molecule is then minimized to its low energy by exploring the inner surface of the host. This energy minimization was done by inserting phenyl as well as chlorophenyl rings of the CIT perpendicular to the diameter of the CD cavity using two different modes *i.e.*, mode 1 and mode 2 (Fig. 5). Three different depths inside the CD cavity were studied to complete the energy minimization processes. The MM studies were done only from wider end of CD (WS). The three different depths are named as W1, W2 and W3 as shown in Fig. 6.

As observed from ROESY spectrum, MM2 studies were performed for both ICs of CIT/HP-β-CD *i.e.* phenyl/HP-β-CD and chlorophenyl/HP-β-CD. The above defined modes were used in MM2 studies. The conformation of chlorophenyl/HP-

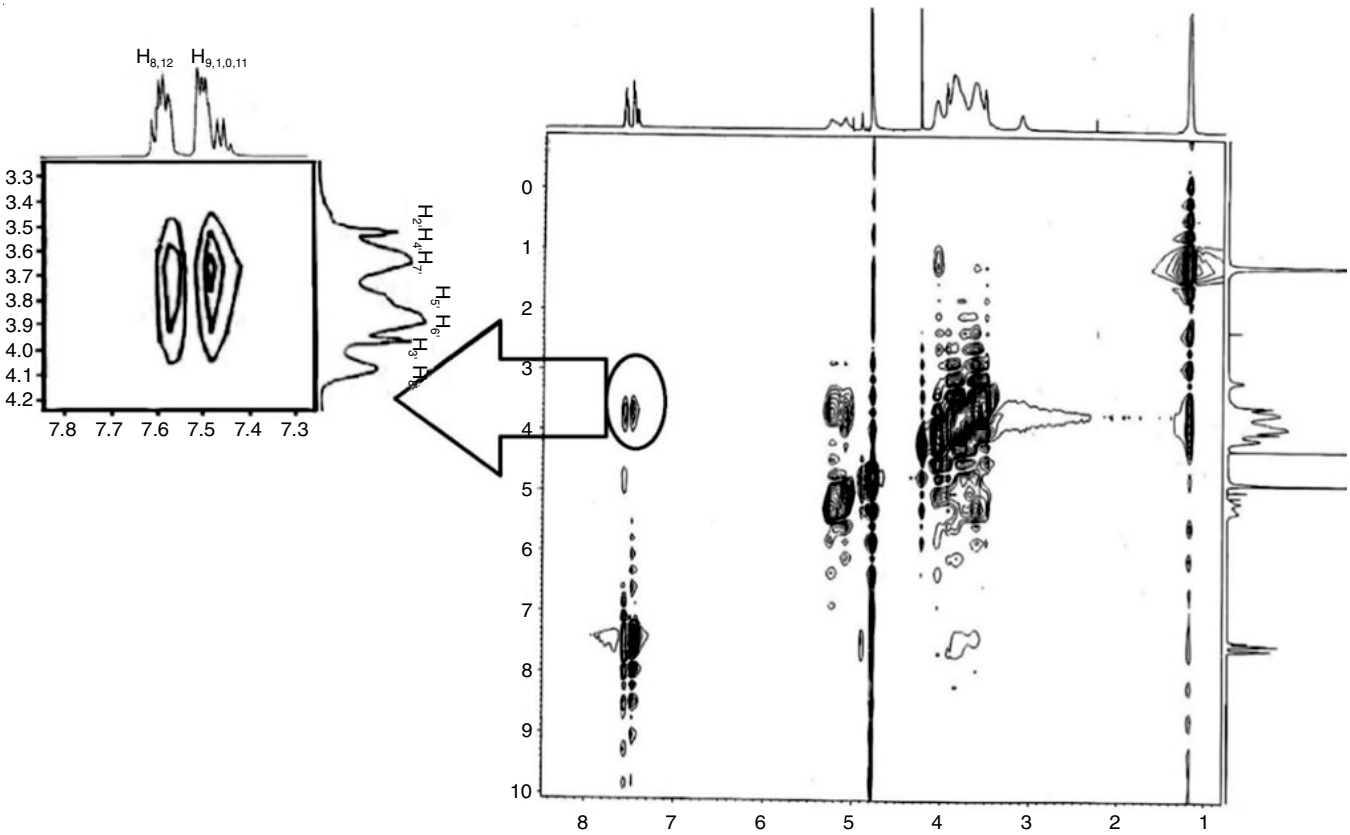

Fig. 4. ^{1}H-^{1}H ROESY spectrum of HP-β-CD/CIT mixture (1:1 ratio)

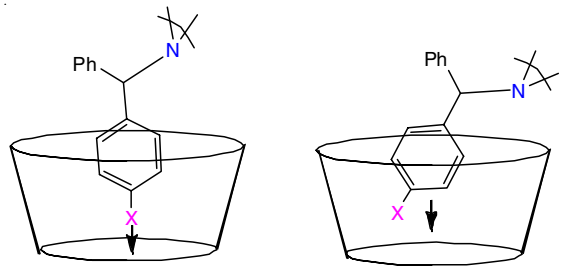

Fig. 5. Two different modes of MM study

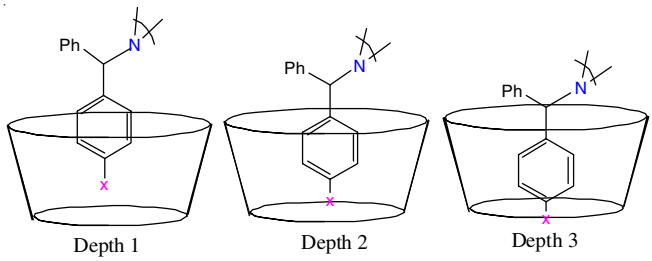

Fig. 6. Three different depths *i.e.*, W1, W2 and W3 at which MM2 studies were performed

β-CD complex with minimum energy was found when molecule was placed in mode 1 and depth W3 (bottom) followed by energy minimization to 135.473 kcal/mol. Similarly, the minimum energy conformation of phenyl/HP-β-CD complex was found at W3 (bottom) and mode 1 having steric energy 135.805 kcal/mol, where a little tilted conformation of phenyl ring was obtained after energy minimization. Fig. 7a-b show the side and top views for the two complexes obtained after MM studies. The steric energy data obtained after MM studies by utilizing different modes and depths are shown in Table-1.

Molecular dynamics (MD): In CIT/HP-β-CD mixture, MD study was done for both chlorophenyl as well as the phenyl ring. The chlorophenyl ring was placed at the surface of CD cavity in suitable conformation as shown in Fig. 8 (frame 1) to initiate MD studies. The chlorophenyl ring moved to the bottom of cavity in 3210 fs and then showed backward movement towards the cavity centre after 3870 fs. The minimum

TABLE-1
STERIC ENERGY (kcal/mol) OF (a) CHLOROPHENYL/HP-β-CD (b) PHENYL/HP-β-CD COMPLEXES BY MOLECULAR MECHANICS STUDIES

Modes	(a) Chlorophenyl ring			(b) Phenyl ring		
	WS			WS		
	W1	W2	W3	W1	W2	W3
Mode 4	155.265	143.652	135.473	146.498	140.489	135.805
Mode 5	154.34	142.681	142.732	154.514	143.472	136.596

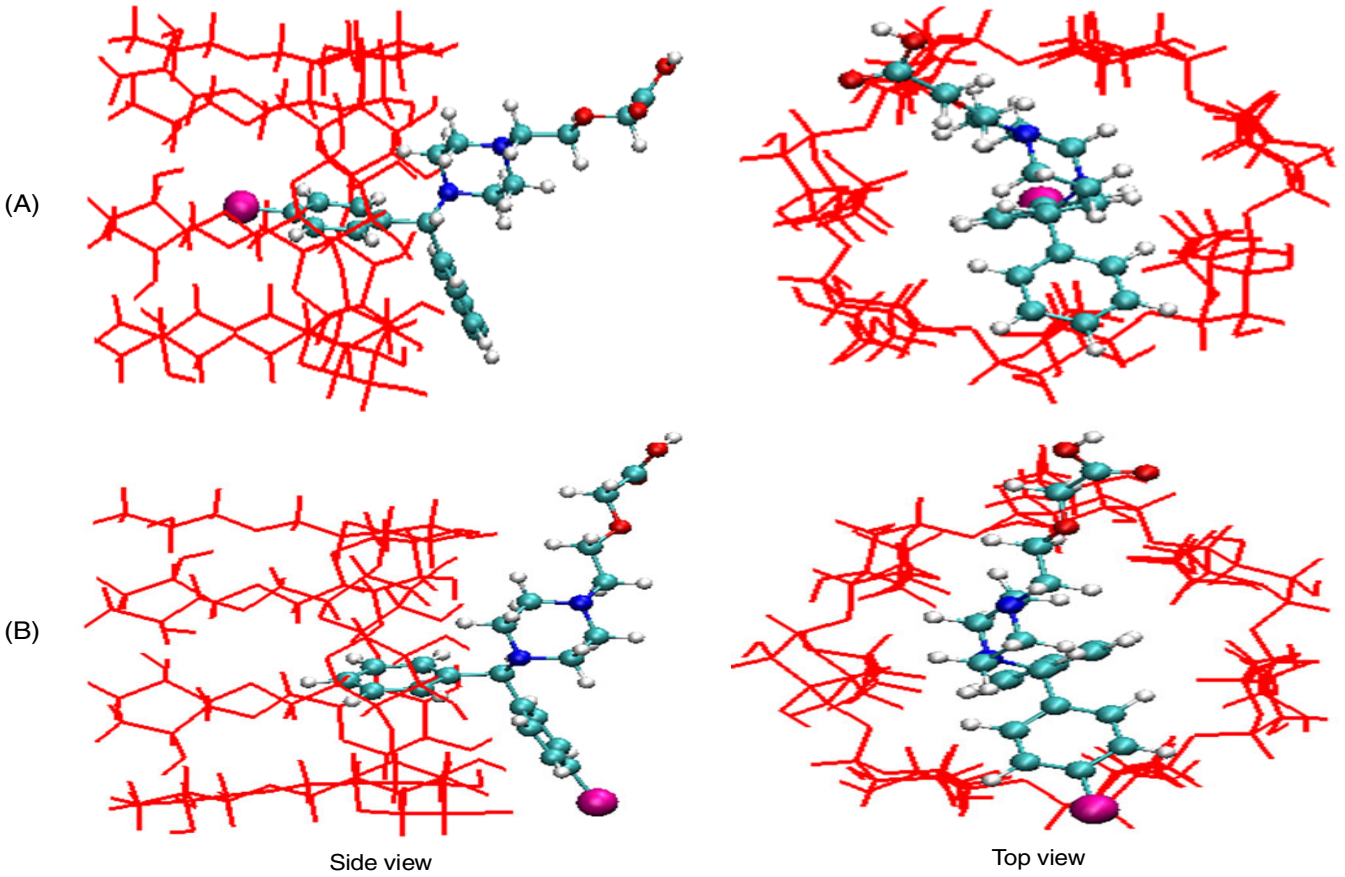

Fig. 7. Least energy conformations of (A) chlorophenyl/HP-β-CD and (B) phenyl/HP-β-CD complexes obtained from MM studies

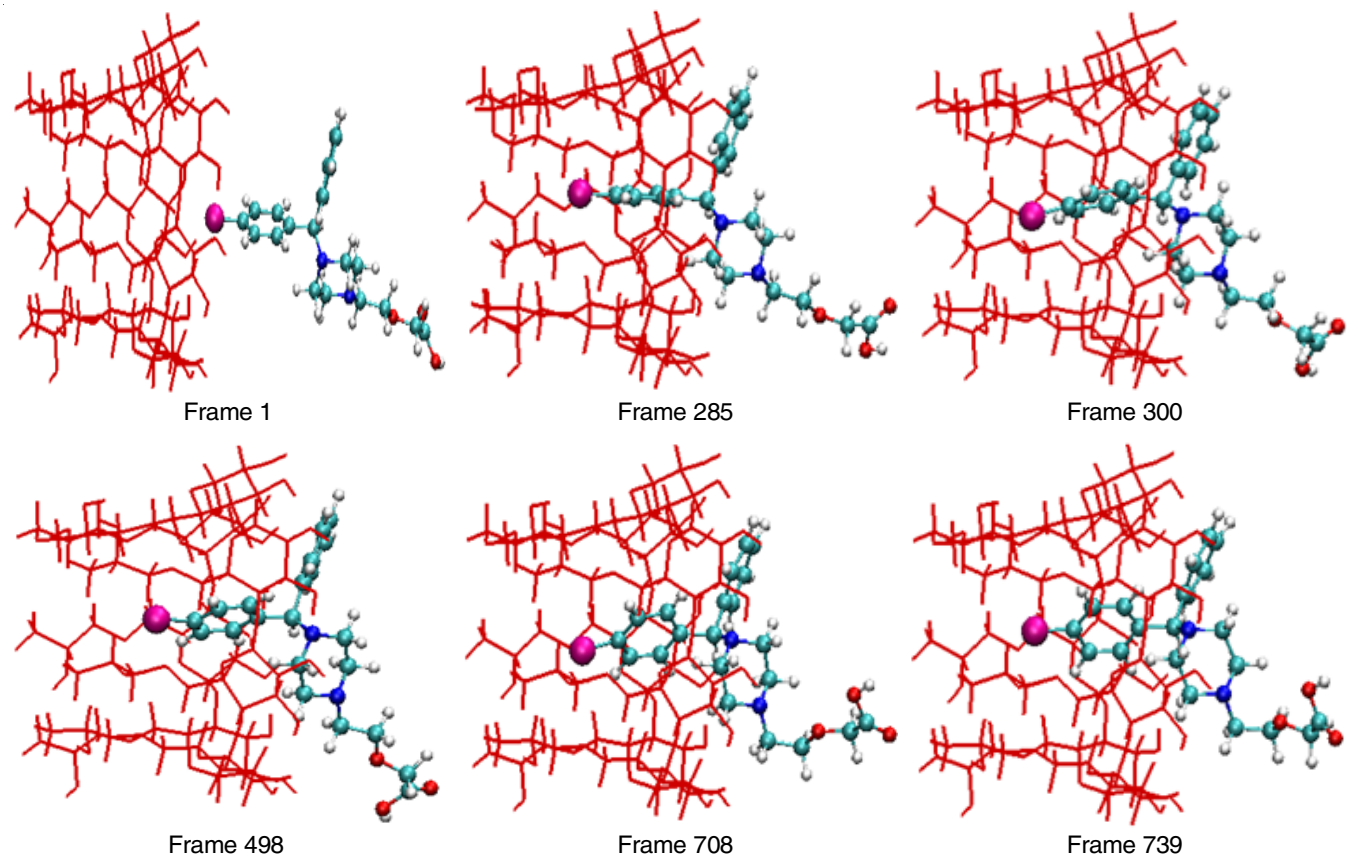

Fig. 8. Initial and some pivotal snapshots from MD trajectory of HP-β-CD/chlorophenyl ring of CIT inclusion simulation

energy frame was observed at 2817 fs of the MD simulation. After 3870 fs, the ring showed movement in all possible orientation from the surface to bottom showing five more minima after 4983, 7091, 7402, 9315 and 9754 fs having energy 137.12, 136.54, 135.16 and 135.80 kcal/mol, respectively. The frame 281 with energy 134.94 kcal/mol is the minimum energy frame. The energy-time plot for this MD simulation is shown in Fig. 9, while some of the minimum energy frames are attached in Fig. 8.

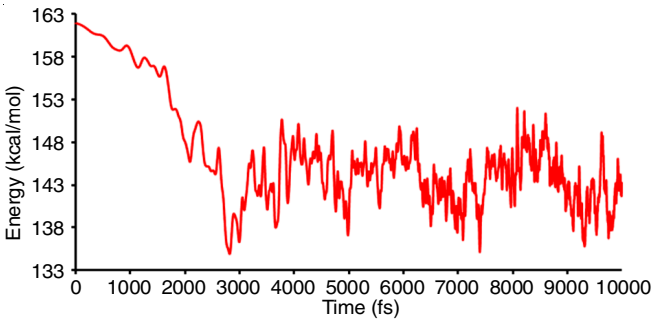

Fig. 9. Energy-time plot of molecular dynamics (MD) run for complex between HP-β-CD and chlorophenyl ring

The molecular dynamics (MD) for phenyl ring was performed by placing the phenyl ring outside the cavity as shown in frame 1 of Fig. 10. The trajectory showed the motion of guest towards the bottom of cavity till 1920 fs followed by movement between centre and bottom showing six minima at 3477, 4935, 5701, 6466, 9006 and 9190 fs with energy 134.80, 134.58, 134.55, 132.95, 134.28 and 134.24 kcal/mol, respectively. Of all these frames, frame 646 is the minimum energy frame 133.00 kcal/mol. The energy-time plot for this MD simulation is shown in

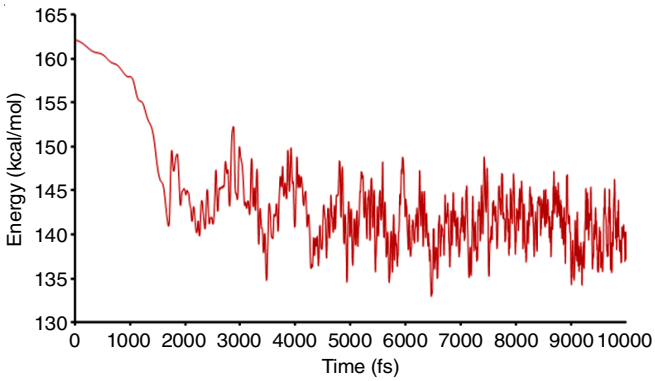

Fig. 11. Energy-time plot for MD simulation study (HP-β-CD complexation with the phenyl ring of CIT)

Fig. 11 with initial and some of the minimum energy frames are shown in Fig. 10.

Conclusion

The comparison of ^{1}H NMR spectrum of pure cyclodextrin (CD) with ^{1}H NMR spectrum of CIT/HP-β-CD mixture showed that complexation has occurred. Then the peaks were assigned by COSY spectrum. ROESY analysis confirmed the formation two inclusion complexes, phenyl/HP-β-CD and chloro-phenyl/HP-β-CD, between CIT and HP-β-CD. Keeping ROESY interactions in mind the structures of inclusion complexes formed were established using molecular mechanics (MM) and molecular dynamics (MD) studies. The structures established by MD studies were found in close agreement with structures established by MM studies. The chlorophenyl ring showed shallow penetration into CD cavity whereas phenyl ring in cetirizine (CIT) showed a tilted orientation inside the CD cavity.

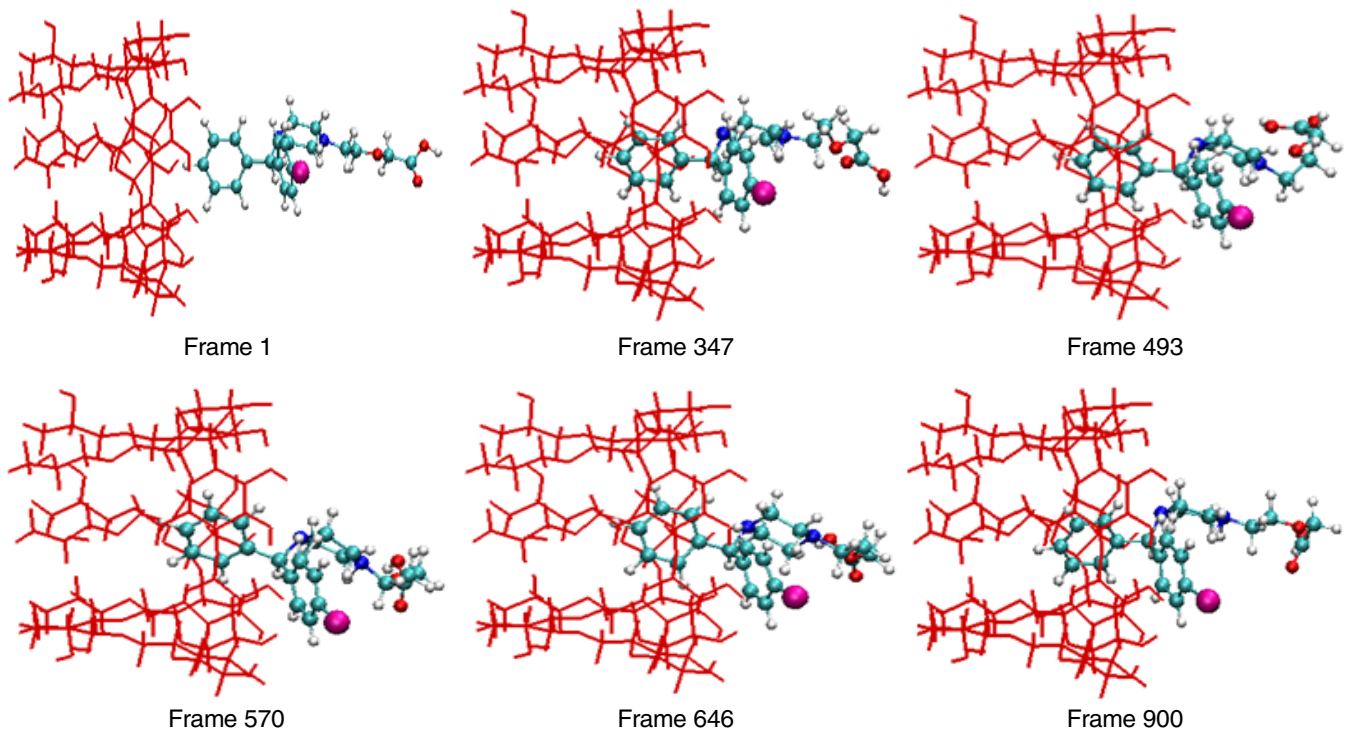

Fig. 10. Initial and some pivotal snapshots from MD trajectory of HP-β-CD/phenyl ring of CIT inclusion simulation

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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