A Proton Magnetic Resonance Study of Fexofenadine/β-Cyclodextrin Inclusion Complexes in Aqueous Solution

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Fexofenadine hydrochloride (FFN), (±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α,α-dimethyl benzeneacetic acid hydrochloride, is a second generation antihistamine that is used to treat allergies.[1] It is a racemate and exists as zwitter ion in aqueous media at physiological pH. It belongs to the group of amine compounds bearing diphenylmethyl functionality. Like other members of this group, the drug is highly hydrophobic and slightly soluble in water.

The study of inclusion complexes of cyclodextrins (CDs) is a subject of great interest.[2-4] CDs are oligosaccharides composed of six to eight glucopyranose units bound by α(1-4) linkages that are commonly named α-, β-, and γ-CD, respectively. β-CD, in particular, has an internal cavity shaped like a truncated cone. The interior of the cavity is relatively hydrophobic while the outer surface is quite hydrophilic because of the presence of numerous hydroxyl groups. CDs can accommodate a variety of guests into its cavity through non-covalent interactions. These complexes serve as models to mimic enzyme activity[5] and to provide understanding of the molecular recognition.[6] Moreover, the physical properties, such as solubility, stability, volatility, sublimation, etc, of the guest molecule are modified upon complexation with CDs, and the resulting inclusion complexes have found numerous practical applications in pharmaceutical sciences and in several other areas of chemistry ranging from analytical to synthetic chemistry.[4-6]

Inclusion complexes of pharmaceutical compounds with CDs have been extensively studied and utilized to improve the solubility,[7] dissolution rate[8] and bioavailability of poorly water-soluble drugs.[9] Other applications of CD complexes of pharmaceuticals include elimination of undesirable drug properties, such as irritation and unpleasant odor or taste. CDs have also been used to stabilize and protect degradation of unstable compounds. In addition, they have shown a potential for improving the stability of light and oxygen sensitive drugs.[10]

Various techniques are used to study the CD inclusion complexes but NMR spectroscopy has been found to be most useful in this type of studies.[11] Evidence for the inclusion of the guest into the CD-cavity is obtained by simple NMR titration experiments. NMR spectra of mixtures of CD and guest molecule are recorded and changes in the chemical shifts of both the host and guest are studied. The formation of the inclusion complex results in upfield shift changes in the CD protons situated inside the cavity, namely H-3' and H-5'. On the other hand, guest protons generally experience downfield shift changes but sometimes upfield shifts are also observed. These shift changes are attributed to the anisotropic ring current effect of the aromatic guests. Moreover, the magnitude of the chemical shift changes for the CD-cavity protons have been shown to be a qualitative measure of the stability of the complex while their ratio, $\Delta\delta_{H\text{-}5'}/\Delta\delta_{H\text{-}3'}$, gives information about the depth of penetration.[12] Also, 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 inference is that $\Delta\delta_{H\text{-}5'} > \Delta\delta_{H\text{-}3'}$ if the guest enters the cavity from narrower side and *vice versa*[13] but there are exceptions and these conclusions can only be drawn when only one complex in formed while in cases where multiple equilibria exist these shift changes can only be used as an evidence for the formation of inclusion complexes. Information regarding the stoichiometry and association and/or dissociation constant of the complex can also be obtained by the treatment of simple ^{1}H NMR titration data.[14]

2D NMR spectroscopy has recently become an important tool for the investigation of the interactions between CDs and guest molecules[11,15] since the NOE cross peaks between the protons that are closer than 4 Å in space are observed in ROESY spectrum. The relative intensities of these cross peaks depend on the spaces between the corresponding protons. The height and the diameter of the β-CD cavity are about 7.9 ± 0.1 Å and 6.0-6.5 Å, respectively.[16] Therefore, while the guest molecule is included into the β-CD cavity, NOE correlation peaks between the protons of the guest and protons of the β-CD cavity (H-3' and H-5') are observed by means of ROESY experiment. According to the relative intensities of these cross peaks, it is possible to estimate the orientation of the guest molecule within the CD cavity.

In continuation of our work on the NMR studies of inclusion complexes of pharmaceutical compounds with β-CD,[17,18] we report herein our results on the detailed study of the complexation between fexofenadine hydrochloride (FFN) and β-CD in aqueous solution using high resolution NMR spectroscopy.

Results and Discussion

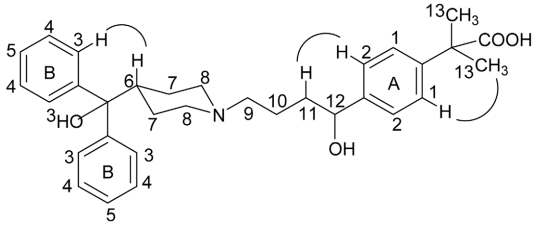
All the NMR spectra were recorded on an Inova 500

instrument in D_2O at 25 °C. The chemical shift values, reported in δ (ppm), were calculated with reference to HDO peak at 4.76. The concentration of the guest was kept constant at 3.7×10^{-3} M while the concentration of the β-CD was varied from 2.5×10^{-3} to 9.1×10^{-3} M. The [β-CD]/[FFN] molar ratios were calculated by direct integration of appropriate signals. All the spectra consist of one set of concentration dependent resonances for each proton or group of equivalent protons, indicating a fast reversible exchange between free and complexed drug on the NMR time scale.

An examination of the β-CD proton signals in the ^{1}H NMR spectra of mixtures of FFN and β-CD revealed significant upfield shift changes in the H-3' and H-5' proton resonances, which are positioned inside the β-CD cavity, compared to pure β-CD. This clearly indicates the formation of FFN/β-CD complex in solution, in analogy to previous reports. Figure 1 shows the expansions of the ^{1}H NMR spectral regions containing β-CD proton signals, in the absence as well as in the presence of FFN.

To have an insight into the detailed structure of the complex, the investigation of the chemical shift changes in the signals for the guest protons in the presence of β-CD is necessary and thus the unambiguous resonance assignment of the guest protons is required. The assignment of resonances of guest protons was made with help of COSY and ROESY spectral data. The signal appearing in the lowest field as a doublet at 7.482 was assigned to H-3 as it showed correlation peaks with H-6. The cross peaks between H-2 and H-11 and between H-1 and CH_3 support the resonance assignment for the *p*-substituted aromatic ring protons. The H-1 appeared relatively downfield compared to H-2 signal.

In the presence of β-CD, significant shift changes were observed in the signals for protons of all the three aromatic rings of FFN (Table 1) confirming the penetration of all the aromatic rings into the β-CD cavity driven by hydrophobic interactions. The signals for H-1 and H-2 of *p*-substituted ring and H-4 of ring B exhibited upfield shifts while H-3 of aromatic ring B showed downfield shift. Moreover, the H-3 signal showed splitting in the presence of β-CD suggesting that both the B rings are not identical which is because the guest has a chiral center. The magnitude of the $\Delta\delta_{H\text{-}3}$ was greater than $\Delta\delta_{H\text{-}1}$ which means that the entry of the phenyl ring into the β-CD cavity is favored compared to *p*-substituted ring. The involvement of all the aromatic rings in



Fexofenadine (FFN)

Table 1. ^{1}H NMR chemical shift change data of studied protons of FFN, in the presence of β-CD

[β-CD]/[FFN]	H-1	H-2, 4	H-3	H-5	CH_3-13
2.46	−0.060	−0.023	0.109	−0.003	0.068
1.76	−0.058	−0.028	0.089	−0.004	0.038
1.27	−0.042	−0.014	0.060	0.001	0.053
1.15	−0.040	−0.011	0.052	0.000	0.052
1.12	−0.044	−0.017	0.047	−0.005	0.039
0.68	−0.030	−0.010	0.026	−0.004	0.030

Negative values indicate upfield shift changes

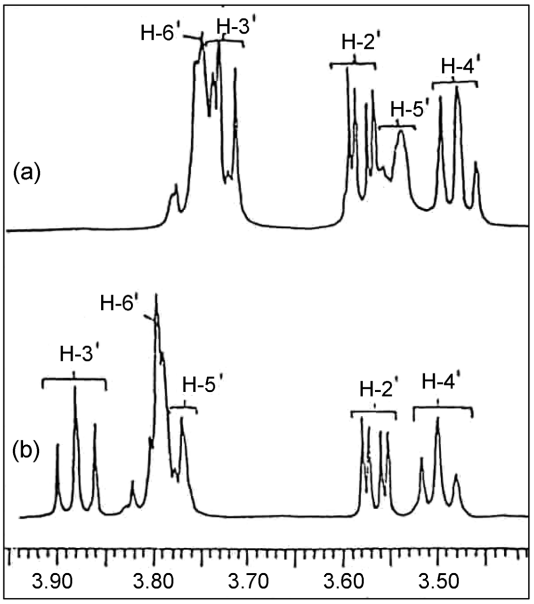


Figure 1. Part of the 500 MHz ^{1}H NMR spectra showing β-CD protons of the (a) 1 : 1.15 FFN/β-CD mixture in comparison to (b) pure β-CD.

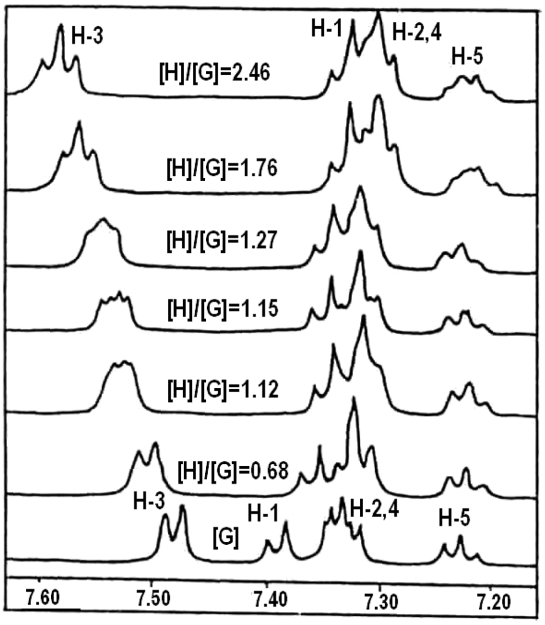


Figure 2. A part of ^{1}H NMR spectrum (500 MHz) showing aromatic protons of fexofenadine hydrochloride (FFN) in the absence as well as in the presence of varying amounts of β-CD.

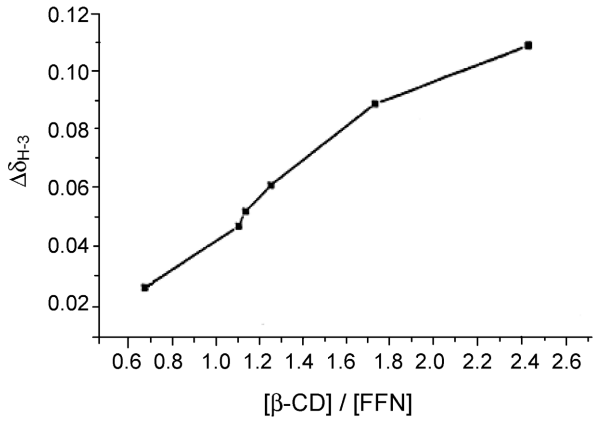


Figure 3. Mole ratio diagram of the chemical shift changes of FFN proton during titration with β-CD in D_2O. The concentration of the FFN was kept constant at 3.7×10^{-3} M.

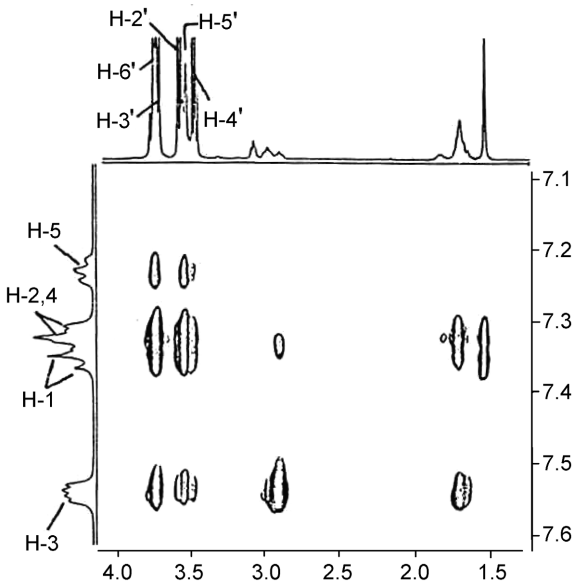


Figure 4. Expansion of the 500 MHz ROESY spectrum recorded with *Tm*=300 ms showing cross peaks of methyl and all the three aromatic ring protons with β-CD protons.

complexation points to the existence of multiple equilibria in solution.[19,20] Part of the spectra showing aromatic protons of the FFN, in the absence as well as in the presence of β-CD, are shown in Figure 2.

The stoichiometry of the complex was determined by mole ratio method, though Job's plot is considered better, because of poor solubility of FFN in D_2O. Figure 3 illustrates plot of $\Delta\delta$ vs mole ratio ([β-CD]/[FFN]) for H-3 of rings B. The $\Delta\delta$ values increase in a linear fashion till [β-CD]/[FFN]=1 suggesting 1 : 1 stoichiometry but since $\Delta\delta$ does not become constant and still keeps increasing even when [β-CD]/[FFN]>1 implies that the stoichiometry is not simply 1 : 1 but a combination of 1 : 1 and 1 : 2 (guest/host) complexes. The formation of 1 : 2 complex is favored when [β-CD]/[FFN]>1.

ROESY spectrum was recorded for a mixture of FFN and β-CD having molar ratio equal to 1.15 in D_2O to ascertain

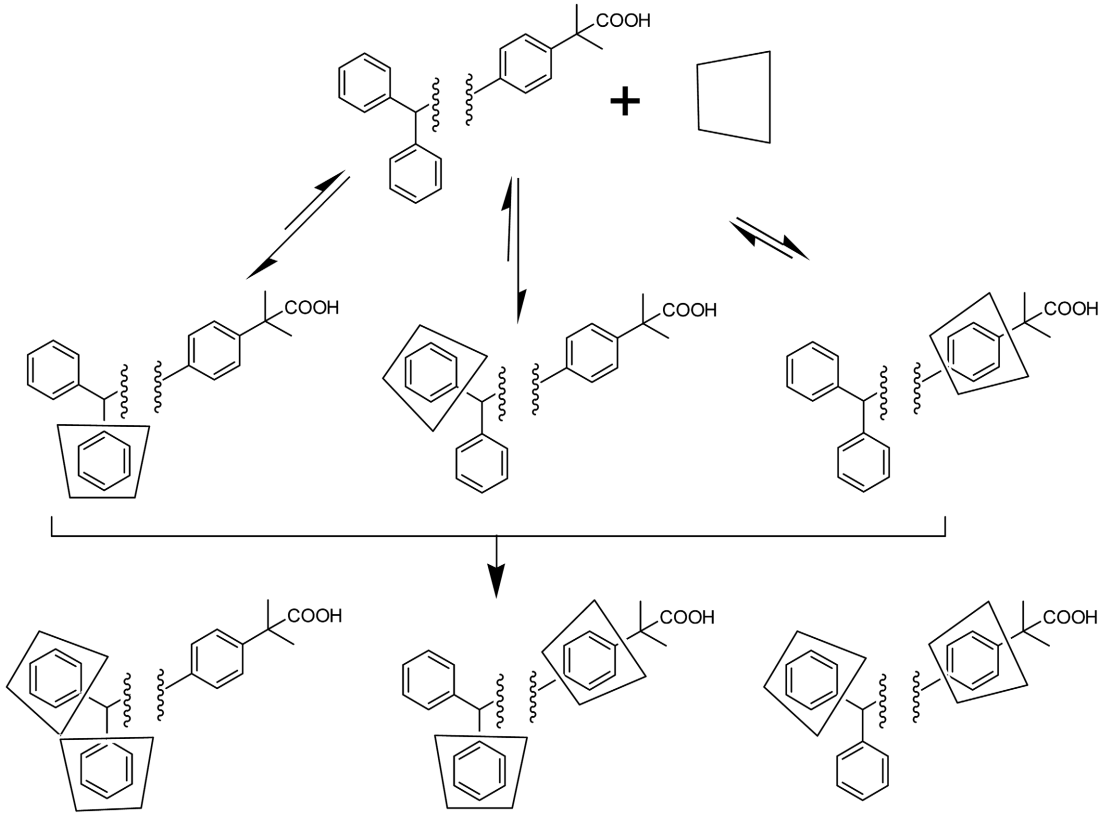


Figure 5. Schematic representation of all the possible inclusion complexes formed in D_2O between FFN and β-CD.

the existence of multiple equilibria in solution and to have an insight into the mode of penetration and orientation of the aromatic ring into the β-CD cavity. As shown in the Figure 4, the ROESY spectrum displayed correlation peaks between protons of all the aromatic ring protons with H-3' and H-5' of β-CD situated inside the cavity thus confirming beyond doubt the involvement of all the aromatic rings in the complexation. The cross peaks between H-3 of FFN and H-2',4' of β-CD, situated near wider rim clearly indicate that phenyl ring penetrates the cavity from wider rim side while *p*-substituted ring penetrates from narrower rim side since H-1 is showing cross peak with H-2',4' of β-CD. Figure 5 shows the proposed structures for all the possible FFN/β-CD inclusion complexes present in solution.

Conclusion

^{1}H NMR titration studies of fexofenadine hydrochloride (FFN) with β-cyclodextrin (β-CD) in D_2O confirmed the presence of several inclusion complexes involving all the three aromatic rings. The structures for all the possible 1 : 1 and 1 : 2 complexes have been proposed taking into account the stoichiometry and ROESY spectral data. The mode of penetration has been clearly established.

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References

1. Simpson, K.; Jarvis, B. *Drugs* **2000**, *59*, 301.
2. Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer Verlag: New York, 1978; pp 1.
3. Saenger, W. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344.
4. Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, 1988; pp 450.
5. Vogtle, F. *Supramolecular Chemistry: An Introduction*; John Wiley & Sons Ltd: New York, 1991.
6. Wenz, G. *Angew. Chem. Int. Ed. Engl*. **1994**, *33*, 803.
7. Esclusa-Diaz, M. T.; Gayo-Otero, M.; Perez-Marcos, M. B.; Villa-Jato, J. L. *Int. J. Pharm*. **1996,** *142*, 183.
8. Obaidat, A. A.; Matalgah, S. M.; Najib, N. M. *Acta Pharm*. **2002**, *52*, 9.
9. Torres-Labandeira, J. J.; Blanco-Mendez, J.; Villa-Jato, J. L. *STP Pharm. Sci*. **1994**, *4*, 235.
10. Szejtli, J. *Med. Res. Rev.* **1994**, *14*, 353.
11. Schneider, H.-J.; Hacket, F.; Rudiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755 and references cited therein.
12. Rekharsky, M. V.; Goldberg, R. N.; Schwarz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Am. Chem. Soc*. **1995**, *117*, 8830.
13. Nakajima, T.; Sunagawa, M.; Hirohashi, T.; Fujioka, F. *Chem. Pharm. Bull. Jpn*. **1984**, *32*, 400.
14. Qi, Z. H.; Mak, V.; Diaz, L.; Grant, D. M.; Chang, C. *J. Org. Chem*. **1991**, *56*, 1537.
15. Neuhaus, D.; Williamson, M. In *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers: New York, 1989; p 1.
16. Szejtli, J. *Chem. Rev*. **1998**, *98*, 1743.
17. Ali, S. M.; Maheshwari, A.; Asmat, F. *Pharmazie* **2004**, *59*, 653.
18. Ali, S. M.; Asmat, F.; Maheshwari, A. *IL Farmaco* **2004**, *59*, 835.
19. Fronza, G.; Mele, A.; Redenti, E.; Ventura, P. *J. Pharm. Sci.* **1992**, *81*, 1162.
20. Yoshida, N. J. *Chem. Soc. Perk. Trans. 2* **1995**, 2249.