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Complexation of Fluvastatin Sodium with β-Cyclodextrin: NMR Spectroscopic Study in Solution

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Abstracts

^{1}H NMR spectroscopic study of fluvastatin sodium (FLU), β-Cyclodextrin (β-CD) and their mixtures confirmed the formation of FLU/β-CD inclusion complex in solution. The stoichiometry of the complex was determined to be 1:1 and the overall binding constant (K_s) was calculated to be 340 M^{-1}. Two dimensional COSY, ROESY and DEPTO experiments were performed for the unambiguous assignment of aromatic proton resonances and it was found that two isomeric forms of FLU are present in solution. It was confirmed with the help of ROESY spectral data that only F-substituted aromatic ring penetrates the β-CD cavity and there is chiral differentiation by the β-CD as one of the isomer binds more strongly, which is indicated by the intensity of correlation peaks. The mode of penetration of the guest into the β-CD cavity was also established and structure of the complex has been proposed.

Introduction

Fluvastatin sodium (FLU) helps in preventing heart disease, angina, stroke and heart attacks by reducing the cholesterol and certain other fatty substances in the blood [1, 2]. It is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and lowers the overall blood cholesterol as well as blood LDL (bad) cholesterol levels, which is responsible for coronary artery diseases. FLU is light sensitive and its solubility in water is 1.91 mg/ml.

Complexation of pharmaceutical compounds with cyclodextrins (CDs) leads to altered physicochemical properties of the guest. Inclusion complexes of pharmaceutical compounds with CDs, therefore, have been extensively studied and utilized to improve the solubility [3], dissolution rate [4] and bioavailability of poorly water-soluble drugs [5] and other desirable properties [6].

CDs are cyclic oligomers of α-D-glucose linked through glycosidic α-1,4-bonds resulting in the formation of doughnut shaped molecules having one rim (narrow) lined with *n* primary hydroxyl groups while the other rim (wider) lined with 2*n* secondary hydroxyl groups. The H-3′, H-5′ and glycosidic oxygen are located inside the cavity which is relatively hydrophobic. The CDs, therefore, act as hosts for a variety of non-polar molecules [7–9].

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NMR spectroscopy is an important tool to study CD inclusion complexes [10]. ^{1}H NMR spectra of mixtures of CD and guest molecule are recorded and changes in the chemical shifts ($\Delta\delta$) of both the host as well as guest are studied. The formation of the inclusion complex is indicated by highfield shift changes in the CD protons situated inside the cavity, namely H-3′ and H-5′ and downfield shift changes in the guest protons. The chemical shift change data can be used for the determination of stoichiometry, binding constant and mode of penetration of the guest into the CD cavity. ROESY [11] spectroscopy is particularly useful in the study of inclusion complexes. NOE correlation peaks observed between the protons of the included part of the guest and β-CD cavity protons give direct evidence for the formation of inclusion complex and provide very useful information regarding the structure of the complex.

We are interested in the study of the inclusion complexes of pharmaceutical compounds with β-CD in solution [12, 13] and report herein our results on the detailed NMR spectroscopic study of β-CD–fluvastatin sodium complexation.

Results and discussion

All the ^{1}H NMR spectra were recorded on a Varian Mercury Plus-300 instrument while COSY, DEPTO and ROESY experiments were performed on a JEOL

α-400 MHz instrument in D_2O at room temperature and the chemical shift values (δ) are reported in ppm. No external indicator was used and HDO signal at 4.800 was used as internal reference throughout this work. ^{1}H NMR spectra for five samples of FLU and β-CD with β-CD/FLU molar ratios ranging from 0.2 to 1.1 were recorded. The concentration of the FLU was kept constant at 1.6×10^{-2} M while that of β-CD was varied. Distinct peaks for bound and free form of the FLU were not observed indicating the rapid exchange of FLU between free and bind state on the NMR time scale.

^{1}H NMR spectra of mixtures of FLU and β-CD displayed highfield shift changes in the H-3′ and H-5′ of the β-CD, located inside the cavity, while insignificant shift changes were observed for other β-CD protons. Expansions of part of the spectra showing β-CD protons, in the presence as well as in the absence of FLU, are shown in Figure 1. These high field shift changes in the β-CD cavity protons can only be explained in term of ring current effect of aromatic ring penetrating the β-CD cavity and thus confirm the formation of FLU/β-CD complex, in analogy to previous studies [10, 14]. This is also supported by concomitant downfield shift changes in the aromatic protons of the FLU in the FLU/β-CD mixtures compared to pure FLU [14].

To clearly establish whether both or either of the two rings is involved in complexation, an unambiguous resonance assignment of FLU protons was required. A cursory examination of the aromatic region of the FLU spectra, in the presence as well as in the absence of β-CD, points to the presence of two isomeric forms of FLU. The COSY, ROESY and DEPTO experiments were performed on a mixture of FLU/β-CD, which proved very helpful in resonance assignment of the two isomers. Only the signals for the F-substituted aromatic ring appeared separately for the two isomers. The doublet at 7.5634 ($J = 6.88$ Hz) was assigned to the H-1 of one of the isomers. This signal showed cross peak, in the COSY spectrum, with the multiplet at 7.1048 suggesting that H-2 of one isomer is merged with H-3, 5 signal. It was confirmed by the DEPTO experiments that signals for the protons of the two aromatic rings are merged in the multiplet at 7.1048. The doublet at 7.3876 ($J = 6.88$ Hz) and triplet at 6.9742 ($J = 6.88$ Hz) were assignable to H-1 and H-2, respectively, of the other isomer. The cross peaks between signals at 6.9742 and 7.1048, observed in COSY spectrum, may arise due to the interaction between H-2 and H-3. Expansions of the COSY (Figure 2) and DEPTO (Figure 3) spectra of a FLU/β-CD mixture, showing aromatic regions, support

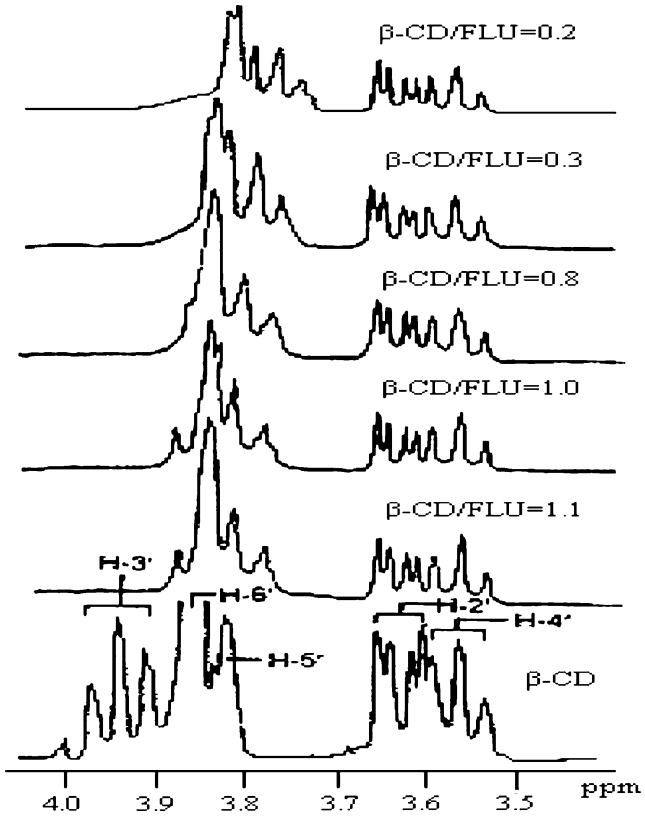

Figure 1. Partial ^{1}H NMR spectra (300 MHz) showing upfield shift of the β-CD protons with increasing amount of FLU.

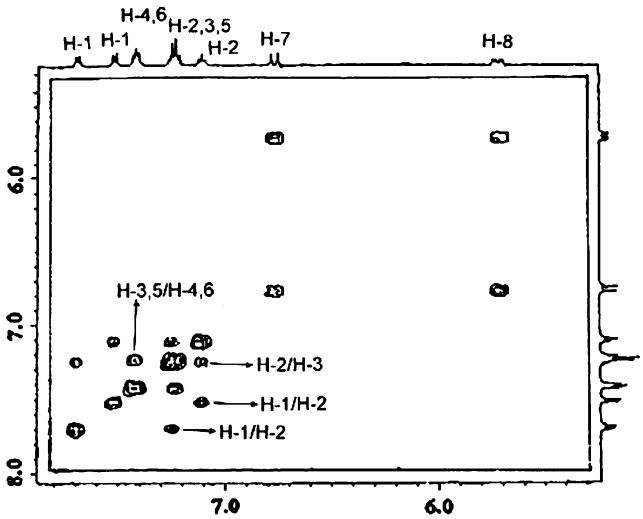

Figure 2. Part of the COSY spectrum (400 MHz) of a mixture of FLU and β-CD showing interaction of aromatic protons.

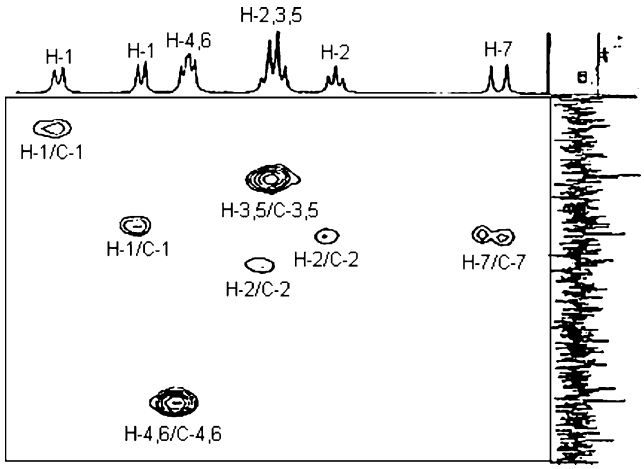

Figure 3. Part of DEPTO spectrum showing that H-2 is merged with H-3,5.

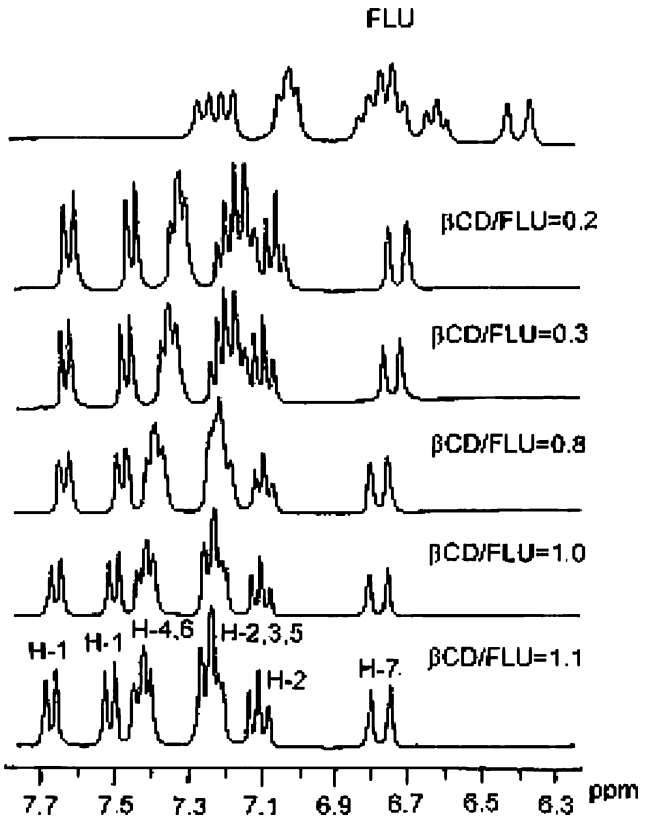

Figure 4. Partial ^{1}H NMR (300 MHz) spectra showing downfield shift of aromatic protons in the presence of β-CD.

the assignment of FLU aromatic proton resonances beyond doubt.

In the presence of β-CD, all the aromatic protons significantly shifted downfield but the shift for the signals of the F-substituted aromatic ring was more pronounced (Figure 4). These observations clearly indicate that atleast F-substituted aromatic ring is inserted into the β-CD cavity but whether other ring is also involved in complexation is not clear because, generally, all the protons of the guest, and not only the part that enters

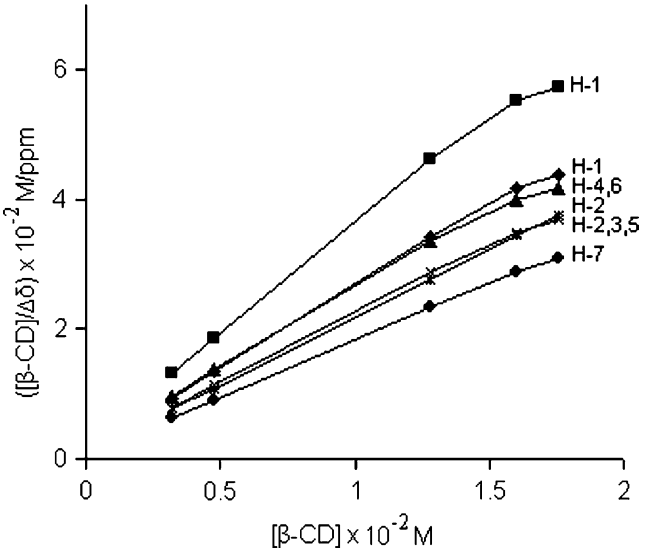

Figure 5. Scott's plot showing 1:1 stoichiometry of the β-CD/FLU complex.

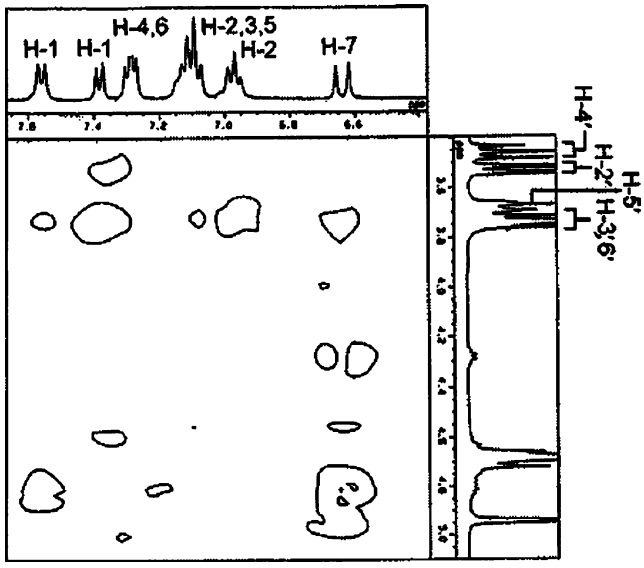

Figure 6. Expanded region of ROESY spectrum of β-CD/FLU mixture showing cross peaks of F-substituted aromatic ring protons with β-CD cavity protons.

the β-CD cavity, show downfield shift changes upon complexation with CDs [15].

The stoichiometry of the complex was determined using Scott's modification [16] of Benesi-Hildebrand equation. In Scott's equation

$$[CD]_t/\Delta\delta_{obs} = [CD]_t/\Delta\delta_c + 1/K_a\Delta\delta_c$$

$[CD]_t$ is the molar concentration of the CDs, $\Delta\delta_{obs}$ is the observed chemical shift difference for a given $[CD]_t$ concentration, $\Delta\delta_c$ is the chemical shift difference between a pure sample of complex and the free component at the saturation. The plot of chemical shift changes ($\Delta\delta$) for the FLU protons against [β-CD] in the form of [β-CD]/$\Delta\delta$ *versus* [β-CD] gave excellent linear fit (Figure 5) confirmimg 1:1 stoichiometry for the complex. The slope of the plot of [CD] is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K_a\Delta\delta_c$, allowing the estimation of K_a. The binding constant (K_a) was determined to be 340 M^{-1}.

In order to clearly establish the structure of the complex, ROESY experiment was performed on a

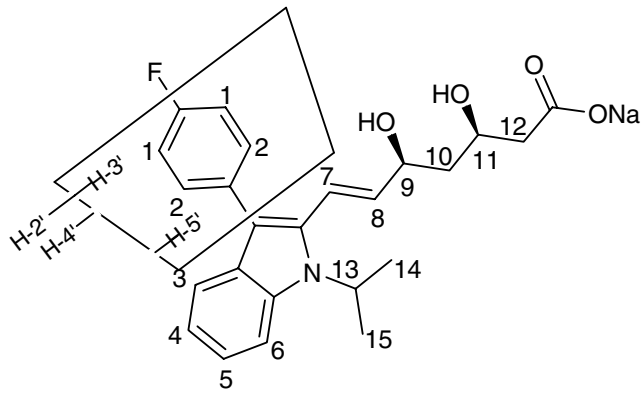

Figure 7. Plausible structure of the 1:1 β-CD/Fluvastatin sodium complex.

FLU/β-CD mixture under spin lock conditions. The result is displayed in Figure 6; a set of cross peaks connects H-3′ and H-5′ resonances of β-CD to the signals for the F-substituted aromatic ring. The signals for the other aromatic ring did not show any cross peaks with β-CD cavity protons confirming beyond doubt the penetration of only F-substituted aromatic ring into the β-CD cavity. The intensity of the cross peaks for the two isomers was not identical suggesting chiral differentiation by the β-CD cavity [17]. Moreover, the H-1 of the F-substituted aromatic ring also showed cross connection peaks with H-2′ and 4′ protons, which are situated near the wider rim. This implies that aromatic ring penetrates deep from the narrower rim side [18] and H-1 is located near wider rim in the complex. The plausible structure for the FLU/β-CD complex is shown in Figure 7.

In conclusion, the detailed NMR spectroscopic study of FLU in solution in the presence of β-CD confirmed the formation of 1:1 inclusion complex resulting by the penetration of the F-substituted aromatic ring into the β-CD cavity from narrower rim side. FLU is a mixture of two isomers and β-CD seems to play a role in their chiral differentiation by favourably binding to one of the isomers. The structure for the β-CD–fluvastatin sodium complex has been proposed.

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