

## COMPLEXATION OF ENALAPRIL MALEATE WITH $\beta$ -CYCLODEXTRIN: NMR SPECTROSCOPIC STUDY IN SOLUTION

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A detailed NMR ( $^1\text{H}$ , COSY, ROESY) spectroscopic study of complexation of enalapril maleate with  $\beta$ -cyclodextrin was carried out. The  $^1\text{H}$  NMR spectrum of enalapril maleate confirmed the existence of cis-trans equilibrium in solution, possibly due to hindered rotation along the amide bond. The cis-trans ratio remained almost the same in the presence of  $\beta$ -cyclodextrin but in one case it was found significantly different which suggests a catalytic role of  $\beta$ -cyclodextrin in the isomerization.  $^1\text{H}$  NMR titration studies confirmed the formation of an enalapril- $\beta$ -cyclodextrin inclusion complex as evidenced by chemical shift variations in the proton resonances of both the host and the guest. The stoichiometry of the complex was determined to be 2:1 (guest: host). The mode of penetration of the guest into the  $\beta$ -cyclodextrin cavity as well as the structure of the complex were established using ROESY spectroscopy.

Keywords: enalapril maleate;  $\beta$ -cyclodextrin; NMR spectroscopy.

### INTRODUCTION

Enalapril maleate and its analogs are highly effective antihypertensive agents which act as angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors prevent conversion of angiotensin I to II and act to widen the blood vessels resulting in easier pumping of blood by the heart. They are, therefore, used for the treatment of hypertension, congestive heart failure and to alleviate strain on heart damaged by heart attack<sup>1,2</sup>. The solubility of enalapril maleate in water is poor but it is highly soluble in methanol.

The study of inclusion complex of pharmaceuticals with cyclodextrins (CDs) is a subject of great interest. Such studies involve structure determination of the complex besides the energetics of the complex formation. CDs are doughnut shaped molecules composed of 6, 7 or 8  $\alpha$ -D-glucose units, linked through  $\alpha$ -1,4 bonds, called  $\alpha$ ,  $\beta$  and  $\gamma$ -CD, respectively. The primary hydroxyl groups are located at the narrow rim while wider rim is lined with secondary hydroxyl groups. The exterior of the CDs is fairly hydrophilic due to the presence of hydroxyl groups while CD cavity is relatively hydrophobic. By virtue of their shape and hydrophobic nature of cavity, CDs accommodate a variety of hydrophobic molecules, or part of it, inside their cavity through non-covalent interactions to form inclusion complexes<sup>3</sup>.

Inclusion of the guest into the CD cavity results in altered physico-chemical properties of the guest like water solubility, bioavailability, stability and volatility etc<sup>4</sup>. The majority of drug molecules are poorly water soluble and their biological absorption is, consequently, slow and far from being complete. Most drug molecules are ideal guests for CDs because their polarity, size and structure enable them to penetrate into the cavity. Moreover, the inclusion complexes of pharmaceutical compounds with CDs have several desirable properties. This is the reason why major portion of CD related studies is dedicated to pharmaceutical

applications. A variety of techniques are used, for characterization of inclusion complexes in terms of geometry and configurational preferences, of which solid state and high resolution NMR spectroscopy are most important<sup>5</sup>.

The formation of inclusion complex results in the shift changes in resonances of both the CDs and the guest protons<sup>6</sup>. The magnitude of  $\Delta\delta$  is a critical function of position of proton in the molecule, size of CD cavity as well as host/guest ratio. In general, the inclusion of an aromatic ligand into the CD cavity causes major upfield shifts in the H-3' and H-5' of CD, situated inside the cavity, which is attributed to the ring current effect of the aromatic ring. The protons located outside cavity show negligible or trivial changes. Contrary to the upfield shift changes experienced by the CD protons, situated inside the cavity as a result of complexation, all the guest protons, and not only those included inside the CD cavity, generally show downfield shift changes upon complexation. This has been attributed, besides the shielding effect of the cavity, to other factors like changes in the microenvironment or conformational changes<sup>7</sup>. The preferred mode of penetration of guest into the cavity is from wider rim side but complexes in which the entry of the guest takes place from narrower rim side are also known<sup>8</sup>.

Simple  $^1\text{H}$  NMR titration studies between CDs and the guest confirmed the formation of inclusion complex. Moreover, valuable information regarding mode of penetration of guest into CD cavity, i.e. from narrower or wider rim side, stoichiometry and stability of the complex can be obtained by the treatment of  $^1\text{H}$  NMR titration data<sup>5</sup>. Advanced 2D NMR techniques like NOESY and ROESY prove very helpful in unambiguous assignment of structure of the complex.

In continuation of our work on the NMR studies of inclusion complexes of pharmaceutical compounds with  $\beta$ -CD<sup>9,10</sup>, we report herein our results on the study of complexation of enalapril maleate with  $\beta$ -CD in solution by NMR spectroscopy.

### EXPERIMENTAL PART

Enalapril maleate was obtained from Nebula Health Care Ltd, India, while  $\beta$ -CD was obtained from Cerestar, Belgium, and these

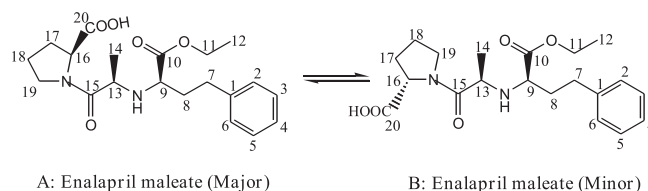
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were used without further purification.  $^1\text{H}$  NMR spectra were recorded on a JEOL 400 MHz instrument in  $\text{D}_2\text{O}$  at  $25^\circ\text{C}$ . The  $^1\text{H}$  NMR measurements were carried out with 4 s relaxation delay, 0.73275969 Hz resolution and 16384 data points. 2D ROESY measurements were made with the experimental conditions as follows: scans 32, acquisition time 0.1470 s, pulse delay 2.3530 s and data points 512. The concentration of  $\beta$ -CD was kept constant at 10 mM while the concentration of enalapril maleate was varied from 11.4 to 52.6 mM. The H/G molar ratios were calculated by direct integration of appropriate signals. The chemical shift values, reported in  $\delta$  (ppm), were calculated with reference to HDO peak at 4.7846.

## RESULTS AND DISCUSSION

Two diastereomeric forms, (SSS)- and (RSS)-, of enalapril maleate, chemically known as 1-[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl-L-proline maleate, are known of which only (SSS)-diastereomer is used as drug<sup>11</sup>.  $^1\text{H}$  NMR spectra of enalapril maleate (SSS-diastereomer), in the absence as well as in the presence of  $\beta$ -CD, showed that two geometrical isomers are in equilibrium in solution. Cis-trans isomerism is possible in the case of enalapril maleate due to hindered rotation along amide bond<sup>12</sup>. The spectral assignment for the major (A) and minor (B) isomers was made with the help of COSY spectrum. The chemical shift data of the studied protons for pure enalapril maleate and enalapril maleate- $\beta$ -CD mixtures is given in Table 1. The signals for the protons of three methylenic groups, for two isomers, at C-8, 17, 18 were found resonating as multiplets centered at 2.2700 ( $3\text{H}_A+4\text{H}_B$ ), 1.9600 ( $3\text{H}_A+1\text{H}_B$ ) and 1.7200 ( $1\text{H}_B$ ). The signal for  $\text{H}_A$ -19 was observed resonating at 3.5328

in the case of pure enalapril maleate. In the presence of  $\beta$ -CD, this signal merged with  $\beta$ -CD protons and, therefore, its chemical shift changes could not be determined. The structures for the two isomers could be established by comparative study of the H-13, 14 and 16 signals. The major isomer was assigned structure (A) since H-13 and 14, being in close proximity of oxygen of -COOH group should appear downfield compared to signals for the same protons of the minor isomer (B) which is in good agreement with earlier observation<sup>13</sup>. As expected, the signal for H-16 for major isomer (A) appears relatively upfield compared to that of minor isomer.



As expected, changes in the shapes as well as chemical shifts of enalapril maleate protons were observed in the presence of  $\beta$ -CD. All the protons, except H-11, exhibited downfield shift changes. The highfield shift in the H-11 signal may be due to hydrogen bonding between the hydrogen of the rim hydroxyl and  $-\text{COOC}_2\text{H}_5$  group. The signal for H-11, which appeared as a quartet at 4.2321 in the spectrum of pure enalapril maleate, became complex in the presence of  $\beta$ -CD making it difficult to calculate chemical shift changes. The  $\Delta\delta$  values for all the studied protons of the enalapril maleate are given in Table 1.

**Table 1.**  $^1\text{H}$  NMR (400 MHz) Chemical shift ( $\delta$ , ppm) data for Enalapril maleate (G) and chemical shift change ( $\Delta\delta$ ) values for the studied protons of Enalapril maleate in the presence of  $\beta$ -cyclodextrin (H) in  $\text{D}_2\text{O}$

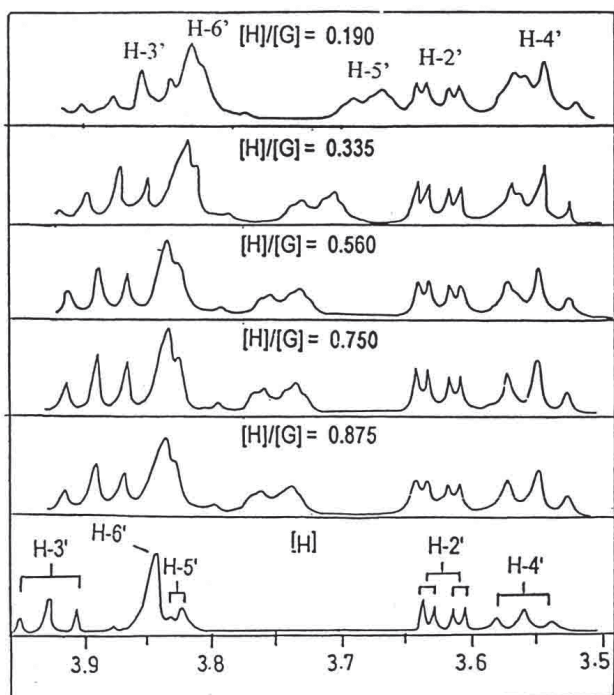
Proton	[G]	[H]/[G]=0.875	[H]/[G]=0.750	[H]/[G]=0.560	[H]/[G]=0.335	[H]/[G]=0.190
$\text{H}_A$ -12	1.2490	0.0092	0.0110	0.0110	0.0128	0.0092
$\text{H}_B$ -12	1.2490	0.0183	0.0202	0.0202	0.0202	0.0147
$\text{H}_B$ -14	1.4910	0.0302	0.0284	0.0247	0.0229	0.0174
$\text{H}_A$ -14	1.5368	0.0275	0.0257	0.0232	0.0211	0.0156
$\text{H}_{A,B}$ -7	2.7600	0.0700	0.0620	0.0500	0.0450	0.0350
$\text{H}_B$ -19	3.4000	0.0300	0.0200	0.0150	0.0100	0.0020
$\text{H}_B$ -9	3.8700	0.0530	0.0476	0.0421	0.0312	0.0310
$\text{H}_A$ -9	3.9100	0.0608	0.0517	0.0462	0.0388	0.0278
$\text{H}_B$ -13	4.0380	#	0.0290	0.0250	0.0220	0.0170
$\text{H}_A$ -13	4.2620	0.0360	0.0333	0.0296	0.0280	0.0220
$\text{H}_A$ -16	4.3800	0.0436	0.0300	0.0280	0.0262	0.0230
$\text{H}_B$ -16	4.4200	0.0130	0.0100	0.0080	0.0050	0.0015
$\text{H}_{A,B}$ -2,4,6	7.2550	0.0250	0.0200	0.0150	0.0120	0.0080
$\text{H}_{A,B}$ -3,5	7.3360	0.0140	0.0140	0.0100	0.0090	0.0040

#could not be determined.

**Table 2.**  $^1\text{H}$  NMR (400 MHz) chemical shift change ( $\Delta\delta$ ) values for  $\beta$ -cyclodextrin protons in the presence of Enalapril maleate in  $\text{D}_2\text{O}$

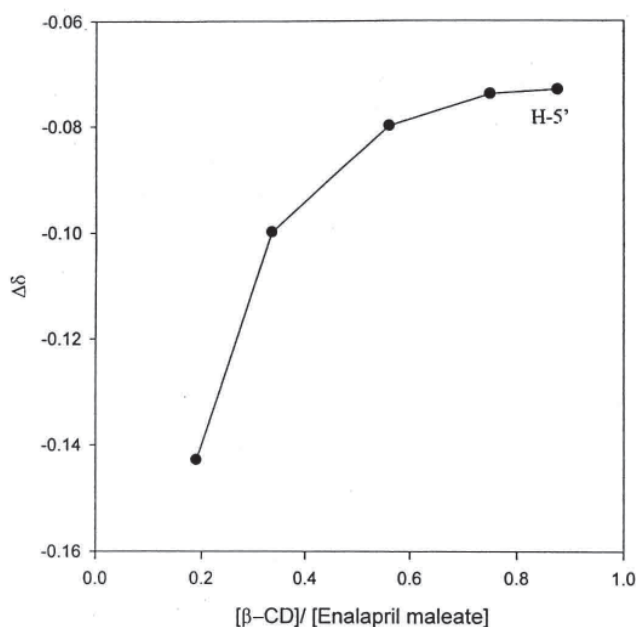
A/B#	[H]/[G]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
73:27	0.875	-0.0055	0.0100	-0.0402	-0.0019	-0.0730	-0.0073
60:40	0.750	-0.0073	0.0064	-0.0403	-0.0037	-0.0738	-0.0091
61:39	0.560	-0.0091	0.0046	-0.0439	-0.0055	-0.0798	-0.0128
59:41	0.335	-0.0128	0.0046	-0.0567	-0.0092	-0.0998	-0.0183
64:36	0.190	-0.0201	0.0037	-0.0751	-0.0147	-0.1428	-0.0311

#cis / trans ratio of enalapril maleate. Negative values indicate high field shift.



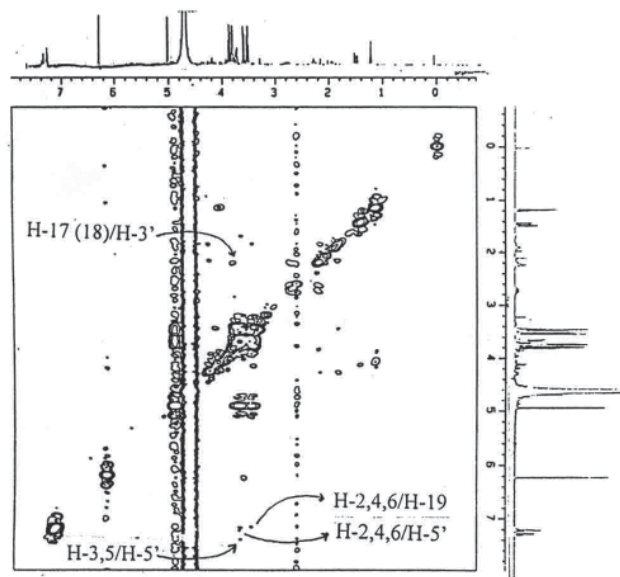
**Figure 1.** Partial  $^1\text{H}$  NMR (400 MHz) spectra showing signals for the protons of  $\beta$ -CD [H] in the absence as well as in the presence of varying amounts of enalapril maleate [G]. Note the large upfield shifts of H-3' and H-5' in sharp contrast to the small shift changes observed for H-2', H-4' and H-6'

$^1\text{H}$  NMR spectra of  $\beta$ -CD in the presence of enalapril maleate displayed significant highfield shift changes in the signal for H-3' and H-5' while the peaks for other protons showed negligible  $\Delta\delta$ . The  $\Delta\delta$  data for all the  $\beta$ -CD protons compared to pure  $\beta$ -CD has been given in Table 2. The progressive upfield shift of H-3' and H-5' resonances in the presence of varying amounts of enalapril maleate are shown in Figure 1 which shows the part of the  $^1\text{H}$  NMR spectra containing  $\beta$ -CD signals.



**Figure 2.** Mole ratio plot of chemical shift change for H-5' ( $\Delta\delta_{\text{H-5'}}$ ) of  $\beta$ -CD during titration with enalapril maleate

The upfield shifts observed for the signals of H-3' and H-5', situated inside the  $\beta$ -CD cavity, and concomitant downfield shift changes for enalapril maleate protons in the  $^1\text{H}$  NMR spectra of mixtures of enalapril maleate and  $\beta$ -CD are a clear indication of complexation between enalapril maleate and  $\beta$ -CD, in analogy to previous studies<sup>5,6</sup>. Furthermore, the magnitude of shift changes of these  $\beta$ -CD protons increased with the increase in the concentration of enalapril maleate. The stoichiometry of the complex was determined by mole ratio method<sup>14</sup>. The plot  $\Delta\delta_{\text{H-5'}}$  vs H/G (Figure 2) shows flattening of the slope of the curve at a molar ratio of around 0.5 suggesting a 1:2 stoichiometry for the  $\beta$ -CD-enalapril maleate complex.



**Figure 3.** 2D ROESY spectrum (400 MHz) of mixture of  $\beta$ -CD [H] and enalapril maleate [G] ( $[\text{H}]/[\text{G}]=0.807$ ). The marked cross peaks support structure for 1:2  $\beta$ -CD enalapril maleate complex

ROESY<sup>15</sup> spectrum (Figure 3) was recorded for a mixture of enalapril maleate and  $\beta$ -CD ( $\text{H}/\text{G}=0.807$ ) to study the mode of penetration of two guest molecules into a  $\beta$ -CD cavity. Intermolecular cross peaks observed between the aromatic protons of the guest and H-5' of  $\beta$ -CD and no cross peaks observed between the aromatic protons and H-3' confirm the shallow penetration of the aromatic ring into the  $\beta$ -CD cavity from narrower rim side<sup>13</sup>. Moreover, Cross peak between H-19 and H-2, 4, 6 of enalapril maleate confirmed that aromatic ring of one guest molecule and 5-membered ring of another molecule share the same  $\beta$ -CD cavity which is in agreement with the 1:2 host:guest stoichiometry of the complex. Cross peak between H-17 (18) and H-3' was also observed which confirms that 5-membered ring enters the  $\beta$ -CD cavity from wider rim side. Figure 4 shows an expanded region of the ROESY spectrum containing cross peaks between the  $\beta$ -CD and enalapril maleate protons. The proposed structure for the 1:2  $\beta$ -CD-enalapril maleate complex is shown in Figure 5.

It is important to mention here that though the cis-trans ratio for enalapril maleate, as determined from the integration of 14- $\text{CH}_3$  doublet, remained almost unchanged, in the presence of  $\beta$ -CD, in four samples. However, there was a significant change observed in one of the sample (Table 2). This can only be explained in terms of catalysis by the  $\beta$ -CD. However, this needs further investigation.

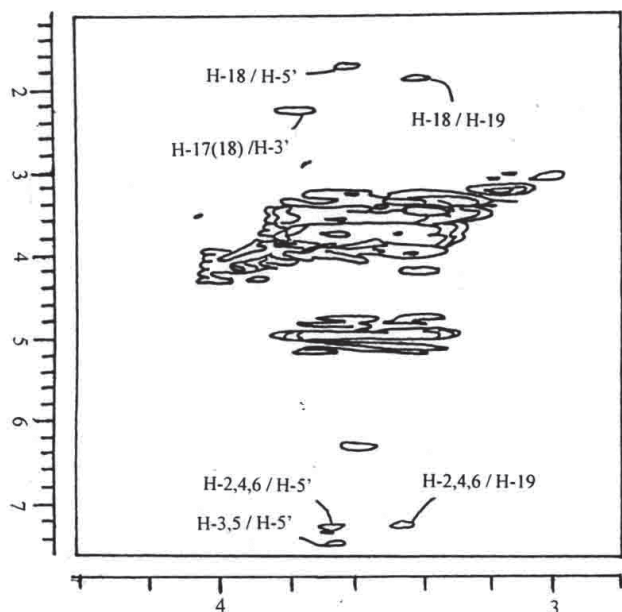


Figure 4. Expansion of part 2D ROESY spectrum showing crosspeaks between enalapril maleate and  $\beta$ -CD

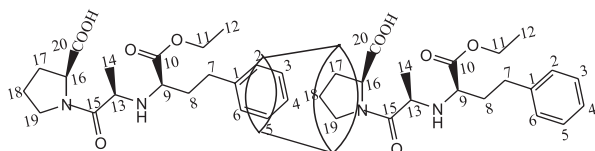


Figure 5. Proposed structure for 1:2  $\beta$ -cyclodextrin-enalapril maleate complex

## CONCLUSION

Enalapril maleate, which exists in two geometrical forms in solution, forms a 1: 2 host-guest inclusion complex with  $\beta$ -CD in the concentration range studied. The aromatic ring of one guest

molecule enters the  $\beta$ -CD cavity from narrower rim side while 5-membered ring penetrates through wider rim side as evidenced by ROESY spectrum. The structure for the complex has been proposed.

## ACKNOWLEDGEMENT

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- <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  1.30 (t, J=7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.54 (minor), 1.59 (major, d, J=6.5 Hz, Ala-CH<sub>3</sub>), 1.75 (minor), 2.02 (major, m, Pro  $\gamma$ -CH<sub>2</sub>), 2.02 (m, Pro  $\beta$ -H), 2.33 (m, Pro  $\beta$ -H, PhCH<sub>2</sub>CH<sub>2</sub>), 2.82 (m, PhCH<sub>2</sub>), 3.48 (minor), 3.60 (major, Pro  $\delta$ -CH<sub>2</sub>), 3.96 (m, PhCH<sub>2</sub>CH<sub>2</sub>CH), 4.09 (minor), 4.33 (major, m, q, J = 6.5 Hz, Ala  $\alpha$ -H), 4.27 (q, J = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.44 (m, Pro  $\alpha$ -H), 6.37 (s, CH=CH), 7.39 (m, ArH<sub>2-6</sub>). The rotameric distribution is indicated by *minor/major* terminology.
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