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Inclusion compound of Fosinopril with β-cyclodextrin

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Abstract. Solid state interactions of bioactive substance (4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxy-propoxy)-(4-phenyl-butyl)phosphoryl]acetyl]-pyrrolidine-2-carboxylic acid, called Fosinopril), with β-cyclodextrin (β-CD), the so-called inclusion compounds of a bioactive (cardiovascular) drug is obtained by different preparation methods: kneading, co-precipitation and freeze-drying. The so obtained compounds were investigated by FTIR spectroscopy, X-ray diffraction method, and differential scanning calorimetric measurements (DSC) to evidence their formation. X-ray diffraction patterns show that the inclusion compound was obtained for kneaded, co-precipitation and freeze-dried products. The crystalline/amorphous degree for these compounds was also investigated. Molecular modeling (MM+ molecular mechanics) shows the spatial architecture of the inclusion compound in good agreement with FTIR experimental data: the drug is included with the propanoyloxy-propoxy group inside β-cyclodextrin cavity. These findings may constitute a direct contribution to the molecular encapsulation of Fosinopril into β-cyclodextrin, improving Fosinopril stability and bioavailability of the drug, also.

Keywords: FTIR, X-ray powder diffraction, DSC, molecular modeling, inclusion compounds, β-cyclodextrin

1. Introduction

Fosinopril is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of chronic heart failure. It may be used alone or in combination with thiazide diuretics. It is also indicated in the management of heart failure. Fosinopril sodium is a white to off-white crystalline powder. It is quite soluble in water, methanol and ethanol, and slightly soluble in hexane. Bioavailability is approximately 36% following oral administration. The molecular formula of this compound is presented in Fig. 1.

It is known that the solubility in water and the bioavailability of drug is increased when it is incorporated in cyclodextrins (CD) [1]. On the other hand, drug properties depend on the amorphous or crystalline state of it. An amorphous solid-state powder may influence the drug bioavailability.

CDs are natural products obtained by enzymatic reaction of starch. Upon the addition of the CGT-ase enzyme to an aqueous solution of starch, every sixth or seventh of the eight α-1,4-glycosilic linkages is split, reacting with their own non-reducing end. It results a six-, seven- or eight-membered macro-ring. These cyclic maltodextrins are called α-, β- and γ-cyclodextrins, see Fig. 2.

To our knowledge, there are no papers dedicated to the inclusion compounds of β-CD with Fosinopril. Consequently, the aim of the paper was to prepare by different methods and to evidence with some

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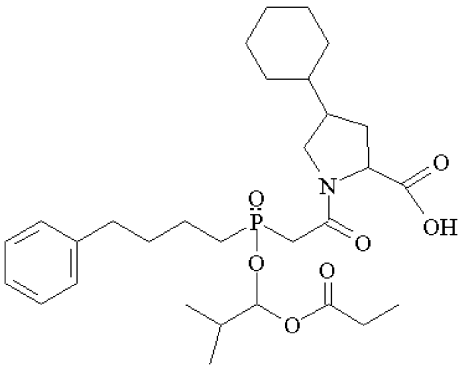

Fig. 1. Fosinopril molecule.

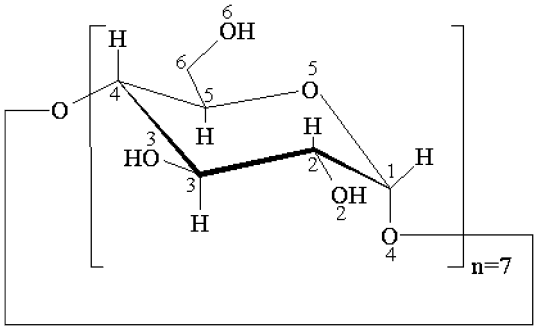

Fig. 2. β-Cyclodextrin (β-CD) molecule.

analytical methods the inclusion compounds of Fosinopril (used as sodium salt) with β-CD. Several experimental methods such as FTIR spectroscopy, X-ray diffraction and DSC together with molecular modeling were employed to confirm [2] inclusion compound formation, to put into evidence the amorphous state and to evaluate their degree of crystallinity. The architecture of these inclusion compounds was proposed, also.

2. Materials and methods

The cardiovascular bioactive substance, Fosinopril (4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxy-propoxy)-(4-phenylbutyl)phosphoryl]acetyl]-pyrrolidine-2-carboxylic acid), was obtained from Hetero Drugs (India). β-CD (having $\leqslant$15% in water weight) was purchased from *Cyclolab* (Hungary) and was used without further purification.

A physical mixture (*pm*) of Fosinopril and β-CD (1:1 molar ratio) was obtained by a gentle grinding of the mixture in the agate mortar. The inclusion compound was obtained also by the kneading method (*kn*), using distilled water as the wetting agent. Fosinopril and β-CD were mixed in molar ratio 1:1 and then grounded in an agate mortar. The wetting agent was then added to the mixture and kneaded for at least one hour. The paste thus obtained was dried at 38°C. The inclusion compound was obtained by co-precipitation (*co*) as follows: by mixing the amounts of Fosinopril and β-cyclodextrin (15 mM aqueous solution) in 1:1 molar ratio, stirring for several days, succeeded by evaporation and drying at

38°C. The freeze-dried product (*fd*) was obtained by frozen and dried by immersion in freezer-drier (Alpha 1-2 LD plus) for over 24 h the corresponding 1:1 aqueous solution.

FTIR spectra were obtained with a JASCO 6100 FTIR spectrometer in the 4000–400 cm^{-1} with a resolution of 2 cm^{-1}, using the KBr pellet technique.

DSC scans were recorded on a Shimadzu DSC-60 differential scanning calorimeter and a Shimadzu TA-WS60 and TA60 2.1 version system software was used for data acquisition. Samples were scanned at a speed of 10°C/min from 30 to 350°C.

X-ray diffraction measurements were performed with a Bruker D8 Advance X-ray diffractometer using CuKα radiation. The degree of crystallinity determination is based on the fact that the intensity of the X-ray scattered by a given assemblage of atoms over all angles is independent of their state of order or disorder [3].

In a crystalline sample only sharp diffraction peaks whereas for an amorphous sample one or two halos are obtained. For a mixture of crystalline and amorphous phases we should obtain both, halos and diffraction peaks. The degree of crystallinity can be determined by preparing standard samples with crystalline/amorphous ratios determined, being still difficult to prepare standard samples. Another method for degree of crystallinity determination is based [3] on following consideration.

In a first approximation, the fraction of crystalline material X_c in the specimen is given by:

$$X_c = \frac{\int_{s_0}^{s_p} s^2 I_c \,\mathrm{d}s}{\int_{s_0}^{s_p} s^2 I \,\mathrm{d}s},$$

where s is given as function of diffraction angle $s = \frac{2\sin\theta}{\lambda}$, $I(s)$ is X-ray scatter from the specimen at point s and $I_c(s)$ is X-ray scatter from specimen due crystalline phase. s_0 and s_p being the limits of the integration. The integral of $I(s)$ is calculate after background subtraction and X_c tends to be smaller due to thermal vibration and lattice imperfections.

By taking into consideration the thermal vibration and lattice imperfections Ruland [4] has shown that the fraction of crystalline can be determined by using the formula:

$$X_c = \frac{\int_{s_0}^{s_p} s^2 I_c \,\mathrm{d}s}{\int_{s_0}^{s_p} s^2 I \,\mathrm{d}s} \frac{\int_{s_0}^{s_p} s^2 \overline{f^2} \,\mathrm{d}s}{\int_{s_0}^{s_p} s^2 \overline{f^2} D \,\mathrm{d}s},$$

where $\overline{f^2}$ is weighted mean-square atomic-scattering factor, $\overline{f^2} = \frac{\sum N_i f_i^2}{\sum N_i}$, D is a factor which take into consideration all kinds of displacements of atoms from their ideal positions (thermal motion, lattice imperfections), $D = \exp(-ks^2)$.

The coefficient k is not known from theory; it should be taken so that X_c does not depend on upper limit of integration s_p.

We have built a C++ package in order to calculate integrals for evaluation of X_c. The integrals are calculated for different limits of integration and for k values from $k_{\min}$ to $k_{\max}$ and retain k value for which X_c change as little as it is possible when limit of integration s_p is changed.

Molecular mechanics computations have been carried out with the HyperChem software [5] to optimize the geometry of Fosinopril molecule and β-CD in vacuum. The β-CD model was taken from

CSD Entry with ref. code BCDEXD03 [6]. Fosinopril geometry was obtained with HyperChem software. In the starting model the Fosinopril molecule was positioned at the larger side of the β-CD cavity. The well-known MM+ method was used with the Polak–Ribière conjugate gradient to minimize the energy of the structures of Fosinopril molecule and β-CD jointly until a RMS gradient lower than 0.015 kcal mol^{-1} $Å^{-1}$ was obtained. Details of the algorithms are given elsewhere [7].

3. Results and discussion

3.1. X-ray diffraction

3.1.1. Inclusion compound of Fosinopril with β-CD

The powder diffraction patterns of Fosinopril (bottom), β-CD (middle) and of inclusion compound (top) are shown in the Fig. 3.

To evidence the inclusion compound formation between Fosinopril and β-CD the X-ray diffraction patterns of β-CD and of Fosinopril were compared with the diffraction pattern of the inclusion complex Fosinopril-β-CD. From this figure on can see that the inclusion compound pattern is different as compared with those of β-CD and of Fosinopril. The X-ray diffraction pattern of Fosinopril-β-CD inclusion compound showed a large amount of amorphous phase, see Fig. 4.

Based on the previously developed model the degree of crystallinity can be determined. We obtained a value of 27% for the degree of crystallinity in the case of kneaded product. For the inclusion compounds

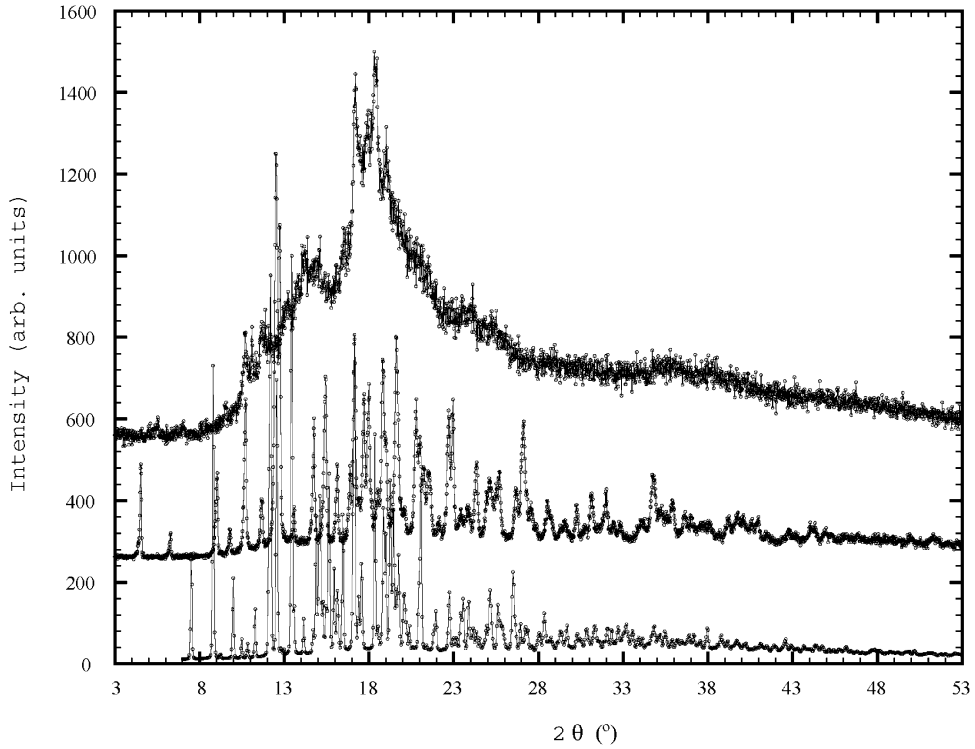

Fig. 3. X-ray diffraction patterns of Fosinopril (bottom), β-CD (middle) and inclusion compound (top).

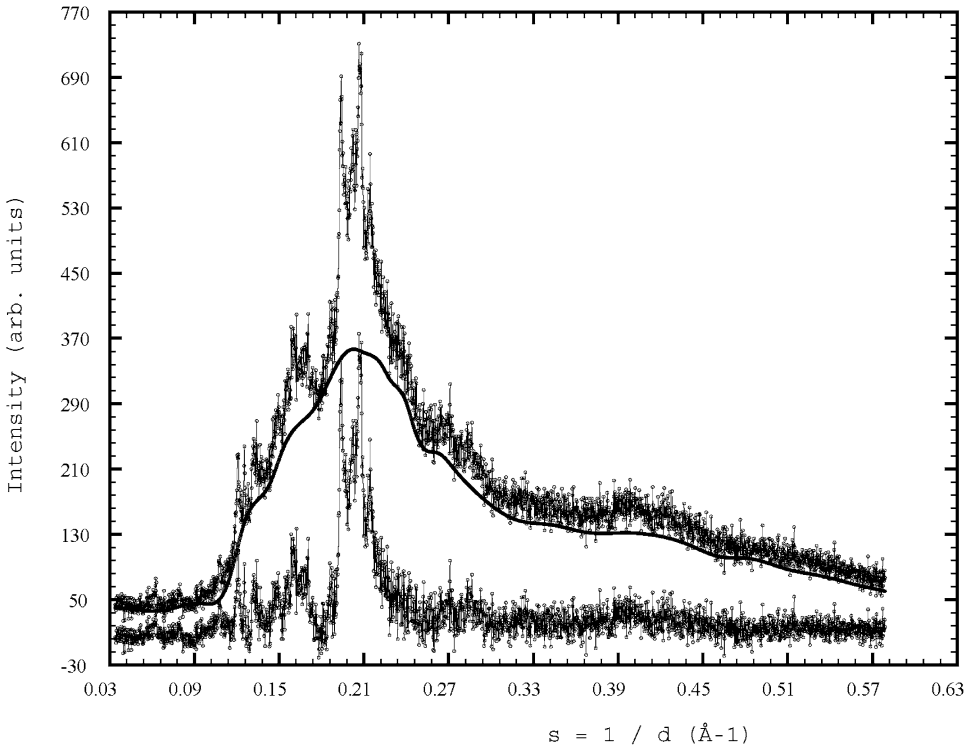

Fig. 4. The determination of the degree of crystallinity for Fosinopril β-CD inclusion compound: the experimental diffraction pattern (top), crystalline component (bottom) and the amorphous one (middle).

obtained by co-precipitation or freeze-drying procedures the crystallization degree is not changed significantly.

3.2. FTIR spectroscopy

FTIR spectrum of the 1:1 physical mixture (*pm*), see Fig. 5, contains the absorption bands of each component, so no inclusion compound was obtained in this case.

In the 4000–2000 cm^{-1} spectral region the O–H stretching vibration are located at 3382 cm^{-1} for *pm* and for β-CD whereas for *kn* this band is shifted to 3398 cm^{-1} and to 3388 cm^{-1} for *fd* and *co* products. The expulsion of the CD intracavity molecular water can explain these spectral differences [8,9]. In the 1800–1550 cm^{-1} spectral region, see Fig. 6, one identify the carboxyl group vibration (located at 1623 cm^{-1}) and the ester group vibration (located at 1760 cm^{-1}).

The last band is shifted upon complexation at 1750 cm^{-1} for *kn* whereas for *co* and *fd* products this band is shifted to 1746 cm^{-1}. One concludes that the hydrogen bonds are implied severely [10] in the inclusion compound formation. By analyzing both spectral regions, see Figs 5 and 6, one observes differences on comparing Fosinopril FTIR spectrum and those of the inclusion compounds obtained by various methods. The inclusion of the propanoyloxy-propoxy group explains these spectral differences.

3.3. DSC

The curves for the β-cyclodextrin reveal a wide band (range approx. 74–118°C) that corresponds to loss by evaporation of the water molecules existing as residual humidity ($t < 100$°C) as well as those

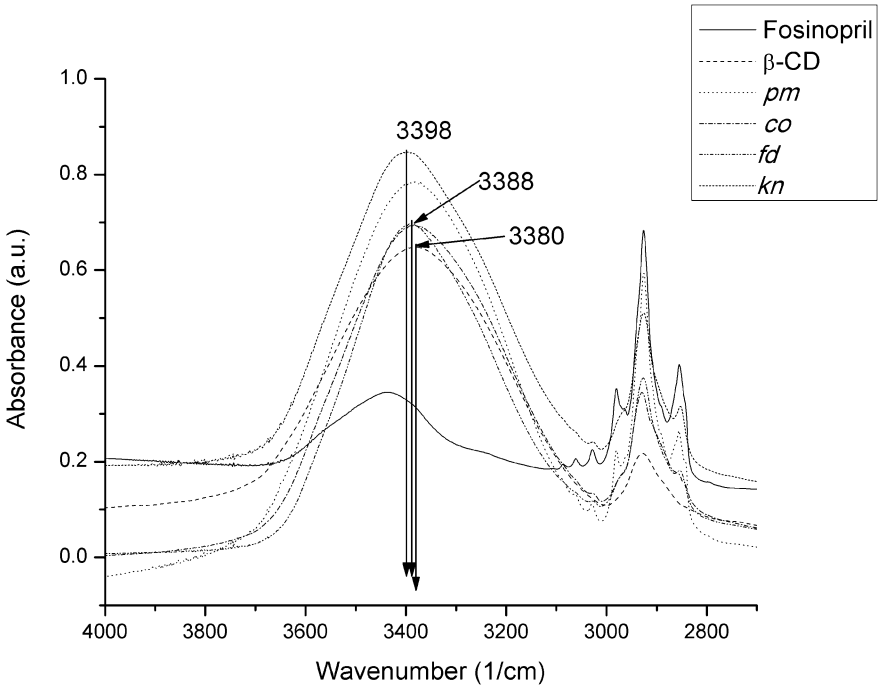

Fig. 5. FTIR spectra of Fosinopril, β-CD, their physical mixtures (*pm*) and the corresponding inclusion compounds obtained by co-precipitation (*co*), kneading (*kn*) and freeze-drying (*fd*) procedures, 4000–2700 cm^{-1} spectral region.

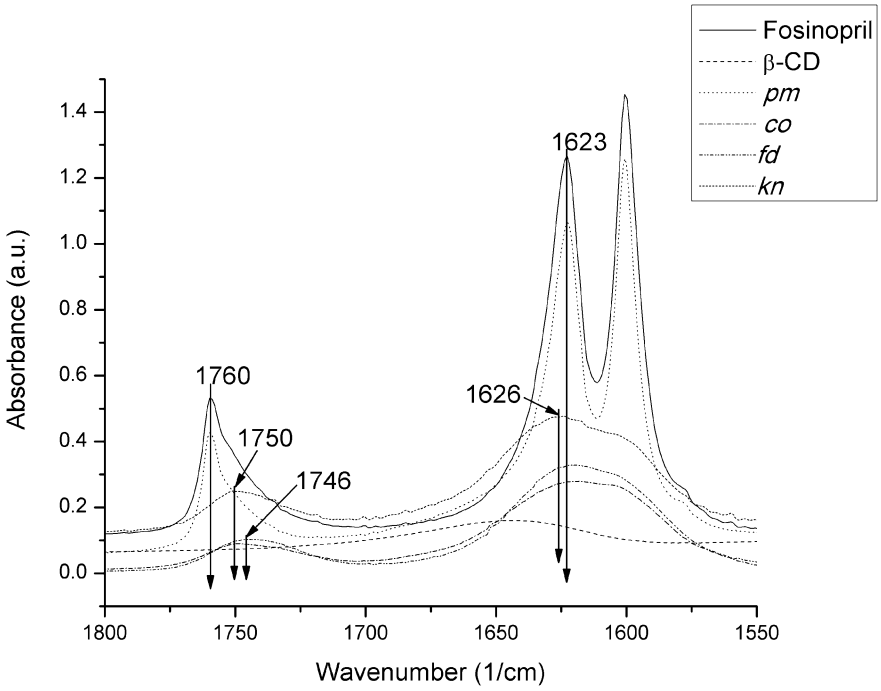

Fig. 6. FTIR spectra of Fosinopril, β-CD, their physical mixtures (*pm*) and the corresponding inclusion compounds obtained by co-precipitation (*co*), kneading (*kn*) and freeze-drying (*fd*) procedures, 1800–1550 cm^{-1} spectral region.

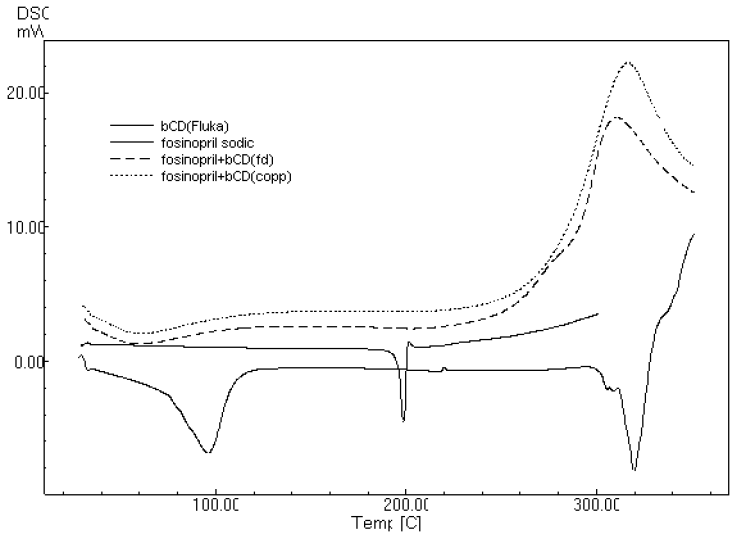

Fig. 7. DSC thermograms of Fosinopril, β-CD, their physical mixture (*pm*) and the corresponding inclusion compounds obtained by co-precipitation (*co*) and freeze-drying (*fd*) procedures.

included in the cavity ($t > 100°$C) [11]. From 290°C onwards there is a new endotherm succeeded of the exotherm, corresponding to the melting, respectively the degradation of the β-cyclodextrin.

DSC curve of Fosinopril, see Fig. 7, presents on endothermic peak at 198.54°C corresponding to the melting point of the drug. In the case of the inclusion compounds of Fosinopril with β-cyclodextrin, obtained by co-precipitation and freeze-drying methods, are observed the decreasing of dehydration endotherm peak of cyclodextrin, as well as a disappearance of the melting peak of the Fosinopril in the inclusion compounds, from 260°C beginning the process of decomposition.

3.4. Molecular modelling

Molecular modeling (Fig. 8) shows the spatial architecture of the inclusion compound in good agreement with FTIR data.

The drug is included with the propanoyloxy-propoxy part inside β-cyclodextrin cavity, the phenylbutyl part being located above the wide rim of β-CD and the 4-cyclohexyl-2-carboxyl-pyrrolidine part above the narrower rim of β-CD. The results of the molecular mechanics calculations must be taken with caution, because there are numerous local minima in the potential energy surface of the investigated molecules. One must also take into account the fact that the simulated system is not real. The calculations were done for the system in a vacuum by neglecting the influence of the water molecules and the Na ions of the Fosinopril sodium salt. However, the obtained results are in good agreement with our hypotheses. Supplementary efforts are necessary to establish the nature [8] of the forces implied in the inclusion process.

4. Conclusions

X-ray diffraction certifies the Fosinopril-β-CD inclusion compound formation having a higher amorphous phase content. A method based on X-ray powder diffraction data was implemented to determine the degree of crystallinity of the inclusion compounds.

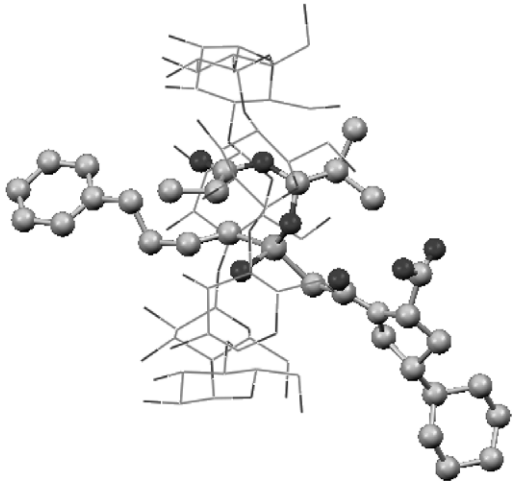

Fig. 8. The structure of the β-CD-Fosinopril complex obtained with the HyperChem software.

DSC measurements confirm the inclusion by disappearance both of the peaks of the Fosinopril in the inclusion compounds' thermograms.

FTIR experiments show that the propanoyloxy-propoxy group is included inside CD cavity during the inclusion process, the hydrogen bonds being implied severely in the inclusion compound formation.

Molecular modeling shows that the drug is included with the propanoyloxy-propoxy part inside β-cyclodextrin cavity, the phenyl butyl part being located above the wide rim of β-CD and the 4-cyclohexyl-2-carboxyl-pyrrolidine part above the narrower rim of β-CD in agreement with FTIR data.

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