Spectroscopic studies of the inclusion compound of lisinopril with β-cyclodextrin

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Abstract. The inclusion compounds of lisinopril with β-cyclodextrin, prepared by different methods: kneading, co-precipitation and freeze-drying were investigated by XRD, FTIR, DSC and solid-state NMR techniques. It was established by these methods that the complexation process was successfully. The degree of crystallinity for the product obtained by kneading was also determined. The kneaded product has a certain crystallinity degree, so it is possible that the inclusion process is less effective in this case. In the case of freeze-dried and co-precipitated products only amorphous phase exists, i.e. the complexation was produced with a higher efficiency.

Keywords: FTIR, X-ray powder diffraction, DSC, 13C NMR, molecular modeling, inclusion compounds, β-cyclodextrin

1. Introduction

Lisinopril (C21H31N3O5, 1-[6-amino-2-(1-carboxy-3-phenyl-propyl)amino-hexanoyl] pyrrolidine-2-carboxylic acid dehydrate) (Fig. 1), an angiotensin-converting enzyme (ACE) inhibitor, is used to treat hypertension, congestive heart failure (CHF), postmyocardial infarction, and diabetic nephropathy or retinopathy.

CDs are cyclic oligosaccharides consisting of six, seven or eight units of α-D-(+)-glucopyranose, referred to as α-, β- and γ-CD respectively [2], see Fig. 2, obtained from starch by enzymatic reaction, that may encapsulate a wide variety of guest molecules (completely, or at least partially) in their hydrophobic cavity.

The encapsulation of the drug in cyclodextrin increases its solubility in water, enhancing the drug delivery quality also [1]. On the other hand the encapsulation can lead to amorphous state which can be substantially more soluble than the corresponding crystalline material [3,4].

The aim of this paper was to obtain an inclusion compound (IC) of lisinopril with β-cyclodextrin (β-CD) and to characterize it by X-ray powder diffraction, DSC, FTIR and solid-state NMR spectroscopy. Molecular modeling technique was employed to obtain the spatial architecture of this supramolecular assembly.

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2. Materials and methods

2.1. Materials

Lisinopril dihydrate (Terapia-Ranbaxy, Romania) was used as given. β-cyclodextrin was obtained from Sigma Aldrich. Double distilled water was used throughout the study.

2.2. Methods

The inclusion compounds were prepared by different methods:

(i) by kneading the amounts of lisinopril with β-CD (molar ratio = 1:1) wetted with double distilled water in an agate mortar;
(ii) by co-precipitation – an amount of lisinopril dissolved in β-CD aqueous solution with a concentration of $1.5 \times 10^{-2}$ mol l$^{-1}$ (molar ratio = 1:1) was stirred for 24 h at room temperature, succeeded by evaporation and drying at 38°C;
(iii) $2 \times 10^{-4}$ mol lisinopril and $2 \times 10^{-3}$ mol β-CD dissolved in 15 ml double distilled water, stirred it at room temperature for 6 h; the solution was frozen and freeze dried by using an ALPHA 1-2LD plus Freeze-Dryer for over 24 h.
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 thermograms were recorded with a Shimadzu DSC-60 differential scanning calorimeter using Shimadzu TA-WS60 and TA60 2.1 version system software for data acquisition. The samples were heated at a speed of 10\(^\circ\)C/min from 30 to 350\(^\circ\)C.

X-ray diffraction measurements were performed with a Bruker D8 Advance X-ray diffractometer using CuK\(_\alpha\) radiation.

Nuclear magnetic resonance (\(^{13}\)C NMR) studies were performed with Bruker Avance 400 spectrometer with a magnetic field of 9.4 T. The samples were analyzed in solid state using CP MAS NMR technique with a spinning frequency of 10 kHz. For the reference of the isotropic chemical shift of \(^{13}\)C resonance, tetramethylsilane (TMS) was used.

Molecular mechanics computations have been carried out with the HyperChem software [5] in order to optimize the geometry of lisinopril molecule and \(\beta\)-CD in vacuum. The \(\beta\)-CD model was taken from CSD Entry with ref. code BCDEXD03 [6]. Lisinopril was obtained from geometrical optimization of the HyperChem software. In the starting model the lisinopril molecule was positioned at the larger side of the \(\beta\)-CD cavity. The well-known MM\(^+\) method was used with the Polak–Ribière conjugate gradient to minimize the energy of the structures of lisinopril molecule and \(\beta\)-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].

### 2.3. Determination of the degree of crystallinity

The determination of degree of crystallinity 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 [8].

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.

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

\[
X_c = \frac{\int_{s_0}^{s_p} s^2 I_c(s) \, ds}{\int_{s_0}^{s_p} s^2 I(s) \, ds},
\]

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 subtractions 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 [9] has shown that the fraction of crystallinity can be determined by using the formula:

\[
X_c = \frac{\int_{s_0}^{s_p} s^2 I_c(s) \, ds}{\int_{s_0}^{s_p} s^2 I(s) \, ds} \frac{\int_{s_0}^{s_p} s^2 \overline{F}^2(s) \, ds}{\int_{s_0}^{s_p} s^2 \overline{F}^2 D(s) \, ds},
\]

where \(\overline{F}^2\) is weighted mean-square atomic-scattering factor:

\[
\overline{F}^2 = \frac{\sum N_i I_i^2}{\sum N_i},
\]
$D$ is a factor which takes into consideration all kinds of displacements of atoms from their ideal positions (thermal motion, lattice imperfections) $D = \exp(-ks^2)$.

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

This method was applied for determination of degree of crystallinity for polymers and we try to using it for inclusion compounds of drugs with $\beta$-cyclodextrin.

3. Results and discussion

3.1. FTIR

In the 1800–1400 cm$^{-1}$ spectral region the FTIR spectra of the IC are quite similar, the following changes can be observed (see Fig. 3):

- 1658, 1611 (the vibrations characteristic to carboxyl group), 1578 cm$^{-1}$ (NH bending group vibration) and 1546 cm$^{-1}$ for pure lisinopril compound are shifted to 1637 and 1592 cm$^{-1}$ for all IC products. These frequency shifts can be explained probably by breaking the hydrogen bonds in the case of NH groups and respectively by the formation of hydrogen bonds in the case of carboxyl groups. Consequently, FTIR studies established clearly the functional groups implied in the inclusion process.

In the 4000–2500 cm$^{-1}$ spectral region, see Fig. 4, the most important contributions are due to the O–H stretching vibrations of primary (3504 cm$^{-1}$ [9]), secondary OH groups of the cyclodextrin and of water molecules present inside CD cavity, also. The different shape of this massif (corresponding to ICs’

![FTIR spectra of lisinopril, β-CD, their physical mixture and the IC obtained by kneading (kn), co-evaporation (co), freeze-drying (fd) or combination of these methods, 1800–1400 cm$^{-1}$ spectral region.](image-url)
obtained with various preparation methods), especially in the higher frequency side reveal the important role of the intra- and intermolecular H-bridges [10] and of the water molecules’ expulsion during the inclusion process, also.

3.2. X-ray powder diffraction

In Fig. 5 are shown X-ray powder diffraction patterns for β-cyclodextrin, lisinopril and inclusion compounds obtained by kneading, co-precipitation and freeze drying. One can see that powder diffraction for inclusion compound is totally different vis a vis β-cyclodextrin and pure lisinopril. Therefore, inclusion compounds were formed by all preparation methods.

The inclusion compound obtained by kneading contains both amorphous and crystalline phases. The degree of crystallinity, determined in according with the method described previously, is 38% (Fig. 6). The kneaded product has a certain crystallinity degree. In this case the inclusion process is less effective.

The X-ray pattern diffractions corresponding to samples obtained by co-precipitation and freeze drying are very alike and they contain, in the first approximation, only amorphous phase. In the case of freeze-dried and co-precipitated products only amorphous phase exists, i.e. the complexation was produced with a higher efficiency.

If we consider also the crystalline phase, the crystallite dimensions should be around 25 Å which reduces the dimensions of the elementary cell. Consequently, we can consider that the samples contain only amorphous phase.

3.3. DSC

DSC reveals some information on solid-state interactions between drug and cyclodextrin. The DSC thermograms of pure components and of lisinopril–β-cyclodextrin inclusion compounds are presented in
Fig. 5. X-ray pattern diffractions for $\beta$-cyclodextrin, lisinopril and their inclusion compounds obtained by kneading, co-precipitation and freeze drying methods.

Fig. 6. Determination of crystallinity degree for lisinopril–$\beta$-cyclodextrin inclusion compound obtained by kneading.
Fig. 7. DSC thermograms of lisinopril, β-cyclodextrin and their inclusion compounds obtained by co-precipitation and freeze-drying methods.

Fig. 7. The curve for the β-cyclodextrin revealed a wide band (range approx. 74–118°C) that corresponds to the loss by evaporation of the water molecules existing as residual humidity (t < 100°C) as well as those 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.

The DSC curve of lisinopril, presents three endothermic peaks at 83.5, 105.2 and 179.7°C corresponding to the loss of first water molecule, then the second one of the initially dehydrate form and to the melting of the drug, respectively. In the case of the inclusion compounds of lisinopril with β-cyclodextrin obtained by co-precipitation and freeze-drying methods, the strong decreasing of dehydration endothermic peak of cyclodextrin is observed, as well as a disappearance of the dehydration and melting peak of the lisinopril in the inclusion compounds. From 260°C the process of decomposition begins.

3.4. $^{13}$C NMR

Solid-state $^{13}$C CP-MAS NMR spectroscopy has been used to investigate highly crystalline, partially crystalline and amorphous materials. The amorphous domains have broad resonances, while the crystalline domains have narrower resonances [12].

In Fig. 8 are shown the $^{13}$C NMR spectra for lisinopril, β-cyclodextrin and their inclusion compound obtained by kneading. One can see that the $^{13}$C NMR spectrum of inclusion compound differs from lisinopril and β-cyclodextrin spectra by broadening of resonance peaks and their shift. The broadening of resonance peak put into evidence the existence of amorphous phase in agreement with the results obtained by X-ray powder diffraction.
Fig. 8. $^{13}$C NMR spectra of lisinopril, β-cyclodextrin and their inclusion compound obtained by kneading method.

Fig. 9. Molecular modeling of the investigated IC by MM$^+$ molecular mechanics.

3.5. Molecular modeling

Molecular modeling by molecular mechanics establishes (see Fig. 9) the geometry of the inclusion compound.
As a result of this process, it was established that lisinopril is included inside CD cavity with the pyrrolidine-2-carboxylic ring.

4. Conclusions

The lisinopril inclusion compounds with β-cyclodextrin were obtained by kneading, co-precipitation and freeze-drying methods. FTIR, DSC, XRPD and NMR techniques put into evidence the inclusion compound formation. The kneaded product contains both amorphous and crystalline phases. The crystallinity degree was determined as being 38%. The co-precipitated and freeze-dried inclusion compounds are completely amorphous. Molecular modeling confirms the results obtained by FTIR spectroscopy: the inclusion of the lisinopril molecule inside cyclodextrin cavity take place with the pyrrolidine-2-carboxylic part of the lisinopril molecule.

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