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Physicochemical characterization of zofenopril inclusion complex with 2-hydroxypropyl-*β*-cyclodextrin

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Abstract: Zofenopril calcium (ZOF) is one of the newest angiotensin-converting enzyme (ACE) inhibitors, highly lipophilic and with low water solubility. This research investigates the interaction between ZOF and a chemically modified derivative of *β*-cyclodextrin, 2-hydroxypropyl-*β*-cyclodextrin (HPBCD), in order to prove the formation of an inclusion complex with an enhanced water solubility profile of ZOF. In this research, for the first time, the physicochemical characterization and the solubility profile of an inclusion complex between ZOF and HPBCD are reported. Different spectroscopic techniques (UV absorption spectrometry, powder X-ray diffraction, attenuated total reflectance Fourier transform IR spectroscopy) were applied in order to prove the formation of the ZOF/HPBCD inclusion complex, both in water and in the solid state, backed by thermal analysis (TGA/DTG/HF). The obtained results confirmed that the physicochemical properties of the ZOF/HPBCD binary system, prepared using the kneading method, are different in comparison to both with the parent substances and the corresponding physical mixture, thus suggesting that an inclusion complex was formed. After the formation of the inclusion complex with HPBCD, the solubility test indicated that the water solubility of ZOF was increased 5-fold.

Key words: Job's plot; ATR-FTIR; PXRD; thermal analysis; solubility.

INTRODUCTION

Zofenopril calcium (ZOF), chemically named calcium (2*S*,4*S*)-1-[(2*S*)-3-(benzoylsulfanyl)-2-methylpropanoyl]-4-(phenylsulfanyl)pyrrolidine-2-carboxylate, is a sulfhydryl-containing ACE inhibitor (Fig. 1A). It is a selective cardiac ACE

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inhibitor, being an effective antihypertensive agent and a valuable therapeutic option in congestive heart failure and in acute myocardial infarction.[1–3]

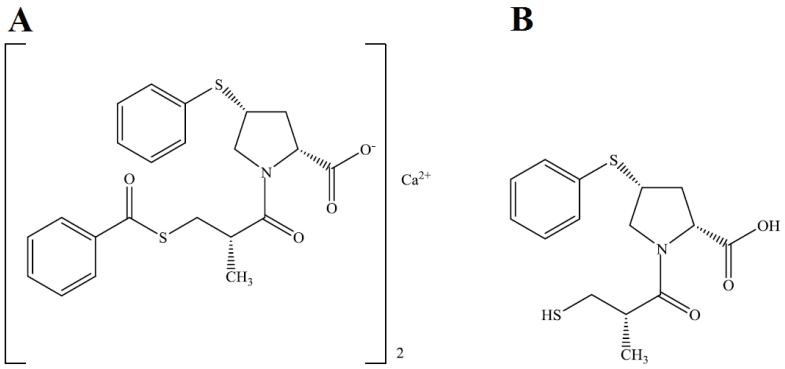

Fig. 1. The chemical structures of: A) zofenopril calcium and B) its active form, zofenoprilat.

ZOF is an ester prodrug of the active acid form, namely zofenoprilat (Fig. 1B), which contains a sulfhydryl group.[4] This potent ACE inhibitor also possesses remarkable antioxidant and cardioprotective properties.[5,6] The major inconvenience is the low water solubility of the calcium salt of zofenopril (0.3 mg ml^{-1} reported by Subissi *et al.*[1]), this form being utilized in the oral dosage forms. On the other hand, zofenopril calcium exhibits the tendency of forming numerous polymorph forms,[7] resulting in its decreasing water solubility.

According to the biopharmaceutics classification system (BCS), a drug that has low water solubility and high permeability is assigned to Class II. Therefore, the formulation effects and the biological parameters can hamper the dissolution profile, this type of drugs presenting variable absorption.[8] Based on the low water solubility and its good permeability, ZOF is categorized as Class II under the BCS framework. The polymorphism is a critical aspect for BCS Class II compounds.[9] Considering these outlooks, the employment of inclusion complexation using cyclodextrin becomes a useful approach.[10]

The cyclodetrins are a class of cyclic oligosaccharides made up of six, seven or eight α-D-glucopyranose units, named α-, β-, and γ-cyclodextrin, respectively. Their particular cavity structure is of biomedical and pharmaceutical interest, because they are able to form inclusion complexes with drugs (or lipophilic moieties) with improved solubility, stability or other physicochemical properties.[11,12] The rendered amorphous solid state following the complexation with cyclodextrins[11,13] has a potential benefit for oral tablets containing ZOF, because of the increased water solubility and the opportunity of transferring ZOF from Class II into Class I of BCS (*i.e.*, into a highly soluble and highly permeable drug).[8]

Hitherto, there have been a few studies on ZOF inclusion complexes with native β-cyclodextrin,[14] but no scientific report on ZOF inclusion complex with

HPBCD. In this study, the formation of the inclusion complexes between ZOF and HPBCD because of ZOF molecular encapsulation within cyclodextrin cavity was investigated. Thus, spectroscopic techniques and TGA/DTG/HF thermal analysis were applied as the most facile and reliable methods for the study of the host–guest interaction (*e.g.*, HPBCD and ZOF).

EXPERIMENTAL

Materials

Zofenopril calcium was a gift sample from Berlin-Chemie Menarini (Berlin, Germany). 2-Hydroxypropyl-*β*-cyclodextrin (average formula weight 1309.3, *DS* = 3) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). The substances were used as received. All other chemicals and reagents were of analytical grade. All experiments were performed using ultrapure water.

Stoichiometry determination using the Job's plot and Benesi–Hildebrand method

The spectrophotometric measurements were performed using a Spectronic Unicam – UV 300 UV-Visible double beam spectrophotometer with 1 cm matched quartz cells.

The stoichiometry of the inclusion complex was determined by application of the Job's method of continuous variation.[15,16] Thus, equimolar 4×10^{-4} M solutions of ZOF and HPBCD were mixed in volumetric flasks to a standard volume of 10 ml (1:9; 2:8, *etc*.), varying the mole ratio while the total concentration of the species was kept constant. An analogous set of ZOF solutions was prepared using ultrapure water as references. After stirring for 24 h at the room temperature, the absorbance for all solutions was measured. The differences between the absorbance values in the presence (A) and in the absence (A_0) of HPBCD were calculated as $\Delta A = A - A_0$. The ΔA (ZOF) values were plotted against the mole fractions, R, where R = [ZOF]/([ZOF] + [HPBCD]).

The Benesi–Hildebrand method was performed by measuring the ZOF absorbance values under complexation-free conditions and in the presence of increasing concentrations of HPBCD. Hence, the concentration of ZOF was kept constant at 0.02 mM and the HPBCD concentration was varied from 0 to 4.5 mM. All the absorption spectra were collected in the UV spectral range of 220–320 nm using 1 cm quartz cells.

Binary systems preparation

The accurate weight of HPBCD for a 1:1 mole ratio ZOF:HPBCD was triturated with an appropriate quantity of water at ambient temperature for 10 min, up to homogenization. Then ZOF was slowly added to the paste. While grinding, a small quantity of water was blended with the mixture in order to assist the dissolution of the drug. The paste was kneaded for 1 h. During this process, an appropriate quantity of water was added in order to maintain a suitable paste consistency. The paste rendered by this process was dried in the oven at 40 °C for 24 h. Then, the dried kneaded product, named ZOF/HPBCD KP, was pulverized and passed through a 75-μm size sieve.

The aforementioned kneaded product was compared with the corresponding physical mixture. Hence, the stoichiometric quantities of ZOF and HPBCD corresponding to a 1:1 mole ratio were gently mixed in a mortar at ambient temperature for 10 min, in order to obtain a homogenous blend. Thereby, a 1:1 mole ratio physical mixture consisting of ZOF and HPBCD at the same ratio as for the inclusion complex was obtained (ZOF/HPBCD PM).

ATR-FTIR analysis

The FTIR spectra were recorded using a Bruker Vertex 70 spectrometer equipped with a platinum ATR unit, type Bruker Diamond A225/Q. Each spectrum represents 64 co-added scans, achieved at a resolution of 2 cm^{-1}, in the 4000–400 cm^{-1} wavenumber range.

PXRD analysis

X-Ray diffraction studies of the pure substances (ZOF and HPBCD) and of their binary systems (the corresponding PM and KP) were performed using a Bruker D8 Advance powder X-ray diffractometer, in the range of 5–45° angular domain (2θ), with CuK radiation generated at 40 mA, 40 kV and a Ni filter.

Thermal analysis

The pure substances and the binary systems were characterized by performing thermal analysis. A Perkin-Elmer Diamond simultaneous TGA/DTA instrument was used. The samples were placed in aluminum crucibles. The DTA curves (in μV) were changed with the heat flow (HF) curves (in mW) in order to determine the heat effects. The thermal behavior of the substances were studied under an air atmosphere, at a flow rate of 100 mL min^{-1}, under non-isothermal conditions by increasing the ambient temperature up to 350 °C at a constant heating rate of 10 °C min^{-1}.

Standard curve for ZOF and the solubility profile of the kneaded product

A set of ZOF aqueous solutions was prepared with concentrations ranging between 9–70 μg ml^{-1}. The absorbance was recorded at 248 nm at 25 °C in order to represent absorbance (A) as a function of concentration (c / μg ml^{-1}) to obtain the standard curve for ZOF.

The water solubility of ZOF within its inclusion complex was determined as follows: an excess amount of kneaded product was placed in 2 mL of distilled water, to obtain a saturated solution. The mixture was shaken for 24 h at 25 °C. The insoluble substance was removed by filtration using a 0.45-μm cellulose acetate filter. The clear supernatant was properly diluted and its absorbance was measured at 248 nm at 25 °C. The residue dosing was calculated by means of the standard ZOF curve.

RESULTS AND DISCUSSION

Stoichiometry determination

According to the Job's method,[15] if a physical parameter directly related to the concentration (*i.e.*, absorbance) of the complex can be measured for a series of samples with continuously varying mole fraction of its components, then the maximum concentration of the complex will be obtained when the mole ratio R corresponds to the complexation stoichiometry. The maximum variation in absorbance ΔA was consequently observed for the mole ratio R = 0.5 (Fig. 2A), thus indicating that the ZOF/HPBCD stoichiometry is 1:1.

Additional investigations using the Benesi–Hildebrand method were performed, in order to verify the stoichiometry. A good linear relationship by double-reciprocal plotting of $1/\Delta A$ *vs.* 1/[HPBCD] (R^2 = 0.9996) was accomplished (as presented in Fig. 2B) using the Benesi–Hildebrand Equation:[17,18]

$$\frac{1}{\Delta A} = \frac{1}{[\mathrm{ZOF}][\mathrm{HPBCD}]\Delta\varepsilon K_\mathrm{S}} + \frac{1}{\Delta\varepsilon[\mathrm{ZOF}]} \quad (1)$$

The results highlighted the 1:1 stoichiometry of the ZOF/HPBCD binary system under these experimental conditions.

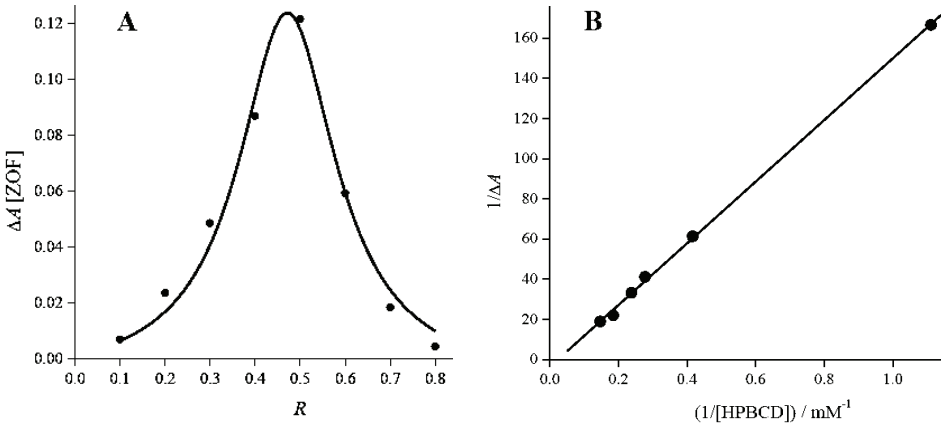

Fig. 2. A) Job's plot for different mole ratios of ZOF and HPBCD from absorbance measurements and B) Benesi–Hildebrand linear plot for 1/Δ*A vs.* 1/[HPBCD].

ATR-FTIR analysis

FTIR spectroscopy is an important method regarding an evaluation of the interactions between the components of a mixture. The changes in the position or intensity, as well as the disappearance or appearance of absorption bands are irrefutable proofs for an interaction between a host-cyclodextrin and a drug--guest. The major benefits of the ATR-FTIR technique in comparison with the KBr pellets technique are the small amount of the samples, the rapidity and accuracy, and the lack of the extra interactions induced by the mechanical press, as occurs in the formulation of a KBr pellet.

The ATR-FTIR spectra of the pure ZOF and HPBCD, along with their physical mixture and kneaded product are presented in Fig. 3. The characteristic absorbance peaks of ZOF and HPBCD, as well as the changes which appear after the physical mixing and the kneading process, are summarized in Table I.

The ATR-FTIR spectrum of pure ZOF, as shown in Fig. 3A, is characterized by the presence of peaks for the proline group (at 1470 cm^{-1}),[19] the carboxyl groups (the anti-symmetric and symmetric stretching C=O vibrations at 1659 and 1612 cm^{-1}, respectively) and the aromatic ring (the C_{ar}–H stretching vibration arises at 3061 cm^{-1}, the skeletal vibration of the aromatic ring appears at 1582 cm^{-1}, and the C_{ar}–H and C_{ar}–C_{ar} bending vibrations appear at 772 and 746 cm^{-1}, respectively).[20]

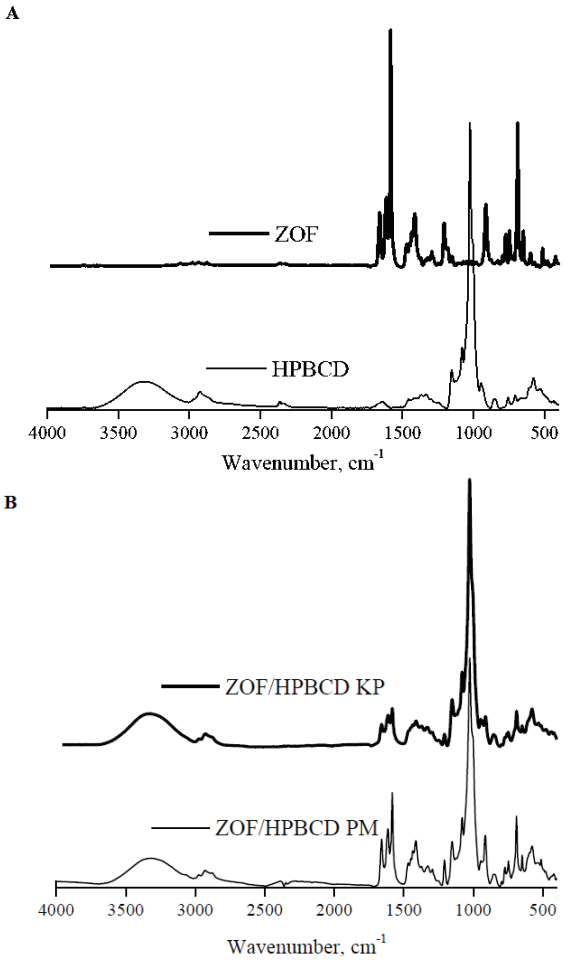

Fig. 3. ATR-FTIR spectra of: A) ZOF and HPBCD and B) their binary systems in the 4000–400 cm^{-1} spectral range.

TABLE I. Wavenumbers, cm^{-1}, and peak assignments from the ATR-FTIR spectra of ZOF, HPBCD, ZOF/HPBCD PM and ZOF/HPBCD KP

ZOF	HPBCD	ZOF/HPBCD PM	ZOF/HPBCD KP
	3325, ν(O–H)	3319	3321
3061, $\nu(C_{ar}–H)$			
2978, ν(C–H) from CH_2 + CH_3		2974	2970
2936, ν(C–H) from CH_2 + CH_3	2924, ν(C–H) from CH_2	2928	2926
1659, $\nu_{asym}(C=O)$ from carboxylate		1659	1659
1612, $\nu_{sym}(C=O)$ from carboxylate		1614	1614

TABLE I. Continued

ZOF	HPBCD	ZOF/HPBCD PM	ZOF/HPBCD KP
1582, skeletal vibration aromatic ring		1584	1584
1470, proline group vibration		1468	
	1456, δ(C–H) from CH_2 + CH_3	1448	1448
1435, 1412, δ(C–H) from CH_2 and CH_3			
	1364, δ(C–H) from CH_3	1371	1371
	1331, coupled δ(O–C–H), δ(C–O–H), δ(H–C–H) 1152, δ(C–O–C)	1327	1329
	1024, ν(C–C–O) from C–OH	1024	1026
	947, skeletal vibration involving *α*-1,4 linkage	945	945
	851, δ(C–C–H), (C–O), (C–C) from anomeric vibration	854	854
772, δ(C_{ar}–H) from the phenyl ring		772	770
746, δ(C_{ar}–C_{ar}) from the phenyl ring		746	748
513, γ(aromatic ring)		513	511

The O–H stretching vibrations from HPBCD (see Fig. 3A) exhibit a broad band with a maximum value at 3325 cm^{-1}, and characteristic peaks that correspond to C–H, C–O–H, O–C–H, and C–O–C bending vibrations in the 1000––1500 cm^{-1} spectral range. These data are in agreement with previously reported results.[18,21,22]

The IR spectrum of the inclusion complex prepared by kneading, shown in Fig. 3B, revealed some differences in comparison with the corresponding physical mixture and the parent substances. Almost all the characteristic peaks of ZOF are intact in the physical mixture spectrum, but they are shifted (or absent) in the ZOF/HPBCD KP spectrum, thus indicating inclusion interaction between the two components. On analyzing the spectral data, it was found that the ZOF C_{ar}–H stretching vibration is present neither in ZOF/HPBCD PM, nor in the ZOF/HPBCD KP, which indicate that one from the two ZOF aromatic rings is entrapped within the HPBCD cavity. The C=O stretching vibrations and aromatic ring skeletal vibration of ZOF are still present in the spectra of the binary systems, albeit shifted and attenuated in ZOF/HPBCD KP, as a consequence of the drug–cyclodextrin interaction when the kneading method was employed. Simultaneously, the proline group vibration, which was identified at 1470 cm^{-1} in the

ZOF spectrum, was shifted to 1468 cm^{-1} in the ZOF/HPBCD PM spectrum, and was no longer present in the ZOF/HPBCD KP spectrum, thus suggesting that an inclusion complex was formed and the 4-(phenylsulfanyl)pyrrolidine was enclosed in the cavity of the cyclodextrin.

PXRD analysis

PXRD is a fast analytical method that is applied for an assessment of the crystalline nature of a sample. This technique allowed the observation of sharp peaks attenuation of the crystalline substance (*i.e.*, ZOF) due to the interaction by inclusion with the amorphous cyclodextrin (HPBCD).

The PXRD patterns of the native ZOF and HPBCD and of their physical mixture and kneaded product are depicted in Fig. 4.

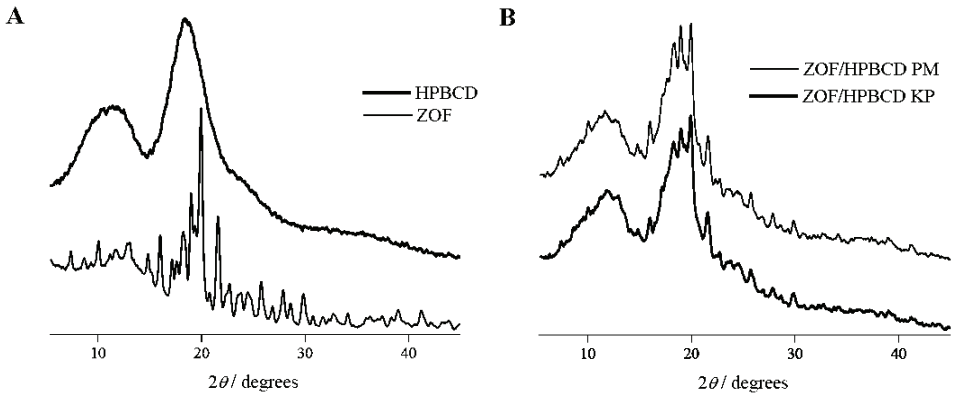

Fig. 4. PXRD patterns of: A) the native substances and B) their binary systems.

The PXRD spectrum of ZOF, presented in Fig 4A, emphasizes its highly crystalline nature by its characteristic diffraction peaks at 2θ 9.72, 13.74, 14.48, 16.00, 17.70, 18.42, 18.52, 19.00, 19.98, 20.56, 22.34 and 24.60°.[22] The XRD pattern of HPBCD (Fig. 4A) had two broad peaks and many diffuse peaks of low intensity, reflecting the amorphous nature of this cyclodextrin. The XRD spectrum of ZOF/HPBCD PM (Fig. 4B) almost overlapped with the individual patterns. In contrast, the XRD pattern of the kneaded product (Fig. 4B) revealed that the crystallinity of ZOF was drastically reduced, as inferred by the mitigated intensities of the characteristic sharp peaks (*i.e.*, at 2θ 16.00, 17.70, 19.00, 19.98 and 21.93°). This was the result of modifications in the environment of ZOF and HPBCD when inclusion complexation occurred.[18]

Thermal analysis

Thermal analysis techniques led to valuable outcomes regarding the formation of cyclodextrin inclusion complexes. The thermal behavior of the studied products under non-isothermal conditions is presented in Fig. 5.

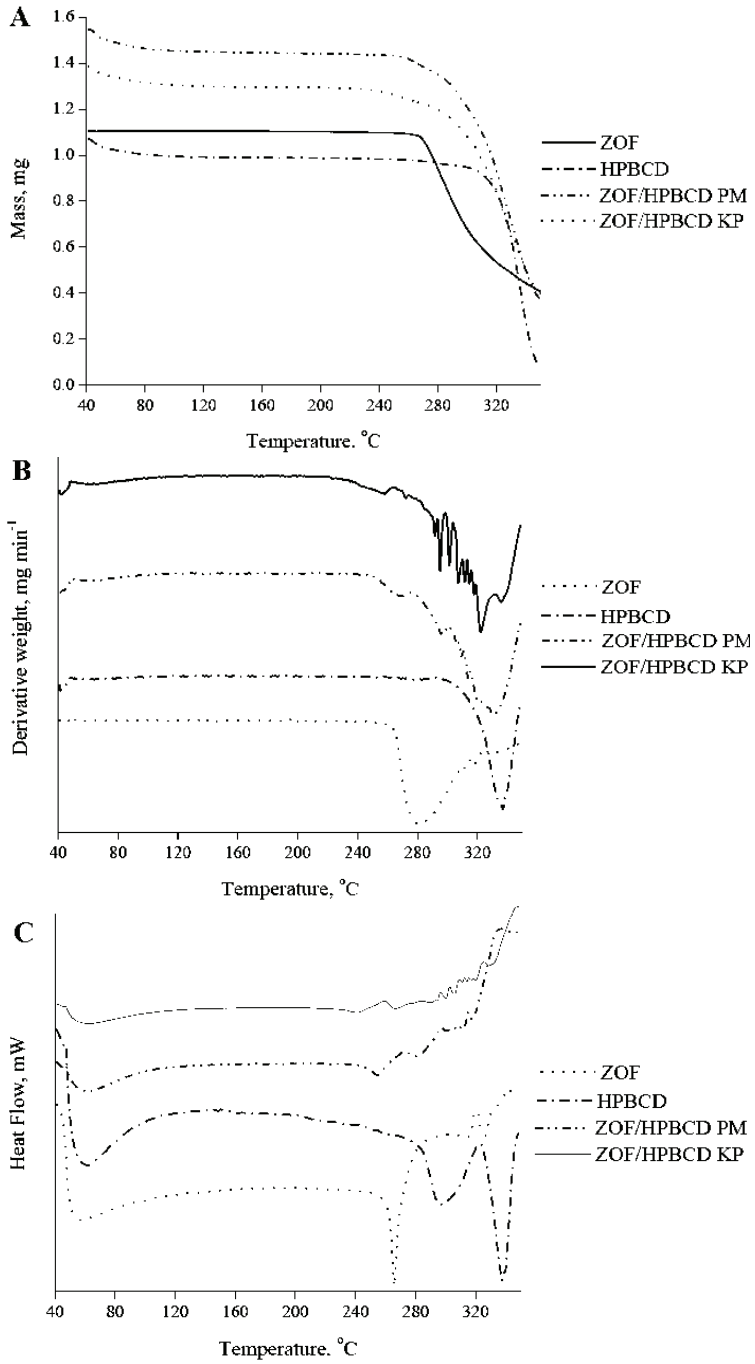

Fig. 5. A) Thermogravimetric, B) derivative thermogravimetric and C) heat flow curves of ZOF, HPBCD, ZOF/HPBCD PM and ZOF/HPBCD KP.

The TG diagrams obtained for native compounds, as well as for their binary systems, are presented in Fig. 5A. ZOF exhibited thermal stability up to 250 °C, followed by decomposition processes. The pure HPBCD and the studied binary systems exhibit a small mass loss corresponding to their dehydration, which occurred between 50 and 130 °C. The water loss of HPBCD, as well as of the binary systems, was also revealed by small and broad endothermic peaks, as indicated by the DTG curves in Fig. 5B.

The melting peak of ZOF appeared at 265 °C,[23] as given by the HF diagram (Fig. 5C). The endothermic process was followed by thermal degradation – a process that was confirmed by the TG curve (Fig. 5A). The broad endothermic HF peak also indicated HPBCD dehydration within the same temperature interval; the melting point of HPBCD had a higher value than that of ZOF (336 °C), proven by both the HF and DTG curves, which is consistent with the previously reported value.[24] The HF curve, corresponding to ZOF/HPBCD PM, indicated that the ZOF melting peak was shifted to 254.5 °C. The endothermic peak of ZOF melting process from ZOF/HPBCD KP was reduced in comparison with that from the physical mixture and was shifted to a lower temperature of 240.6 °C. Hence, a decrease in the thermal stability of the kneaded product was observed, which was the consequence of ZOF amorphization through the formation of the inclusion complex.[25] Similar results were also reported for the inclusion process of other substances.[26,16]

By corroborating the DTG and HF results, a different thermal pattern of ZOF was observed within the kneaded product, because the melting point of the ZOF guest molecule, which is entrapped within HPBCD cavity, was shifted to a different, lower temperature and therefore the peak intensity was decreased. These results indicate a molecular interaction between ZOF and HPBCD, endorsing the hypothesis of the existence of an inclusion complex between the two components when the kneading method was employed.

Solubility

The water solubility appraisal of the ZOF/HPBCD inclusion complex prepared by kneading was the milestone in our experimental efforts. Hence, the saturation shake-flask method[27] was employed for measuring the water solubility at 25 °C of ZOF in ZOF/HPBCD KP using the standard curve of ZOF in water, at 25 °C (Fig. 6).

The obtained standard curve of ZOF was characterized by the equation $A = 0.02909c + 0.01022$ ($R = 0.9999$), where A stands for absorbance, measured at 248 nm, and c is the concentration in μg ml^{-1}.

The water solubility of the ZOF/HPBCD KP was estimated by preparing a concentrated solution.[28,29] The UV spectrophotometric measurements were achieved after adequate dilution, indicating that the water solubility of the included ZOF was 1.510±0.005 mg mL^{-1} (an average value of five experimental

determinations). This result emphasizes that the water solubility of ZOF was increased five-fold in comparison with the free ZOF (0.3 mg mL^{-1}), due to the solubilizing effect of HPBCD.

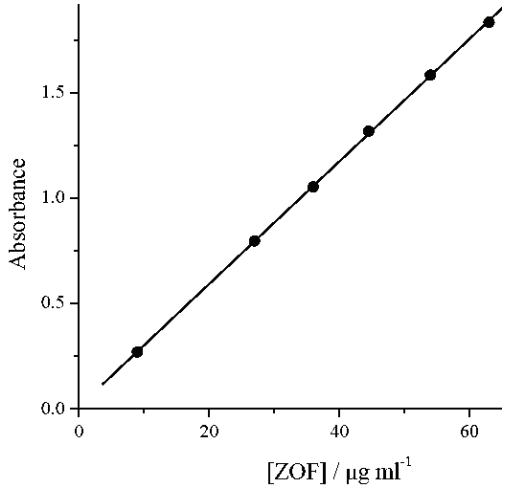

Fig. 6. Standard curve of ZOF (water, 25 °C).

A standard control experiment was further performed by dissolving the ZOF/ /HPBCD kneaded complex (59.2 mg), thus obtaining a clear solution that was equivalent to 1.51 mg of ZOF. This indicates that the solubility of the ZOF/ /HPBCD inclusion complex was adequate for tablet dosage form.[28,29]

CONCLUSIONS

This is the first work to report the physicochemical characterization of an inclusion complex between ZOF and HPBCD. Simultaneously, it brings forth the role of cyclodextrins, and HPBCD in particular, in improving the water solubility of the calcium salt of zofenopril. The formation of the 1:1 inclusion complex between ZOF and HPBCD, prepared by the kneading method, was proven by ATR-FTIR spectroscopy, PXRD and thermal analysis. The ZOF/HPBCD KP exhibited lower crystallinity and enhanced water solubility in comparison with free ZOF.

ИЗВОД

ФИЗИЧКОХЕМИЈСКА КАРАКТЕРИЗАЦИЈА ИНКЛУЗИОНОГ КОМПЛЕКСА КОЈИ НАСТАЈЕ ИНТЕРАКЦИЈОМ ИЗМЕЂУ ЗОФЕНОПРИЛА И ХИДРОКСИПРОПИЛ-*β*-ЦИКЛОДЕКСТРИНА

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Калцијум-зофеноприл (ZOF) је један од најновијих инхибитора ангиотензин- конвертирајућег ензима (ACE), који је високо липофилан и слабо растворан у води. У циљу

потврде формирања инклузионог комплекса који има већу растворљивост у односу на ZOF, испитивана је интеракција између ZOF и хемијски модификованог *β*-циклодекстрина (2-хидроксипропил-*β*-циклодекстрин, HPBCD). По први пут у овом раду дате су физичкохемијске карактеристике и описана је растворљивост комплекса који настаје инклузијом између ZOF и HPBCD. Формирање инклузионог комплекса ZOF/HPBCD, како у чврстом стању тако и у воденом раствору, потврђено је применом различитих спектроскопских техника (UV апсорпциона спектрофотометрија, дифракција рендгенских зрака са прахова, IR спектроскопија са Фуријеовом трансформацијом). Добијени резултати ових испитивања су додатно потврђени на основу термалне анализе (TG/DTG/HF). На основу добијених резултата потврђено је да су физичкохемијске карактеристике бинарног ZOF/HPBCD система различите у односу на полазне супстанце, као и њихову смешу, што недвосмислено указује на формирање инклузионог комплекса. Нађено је да овај инклузиони комплекс има пет пута већу растворљивост у води у односу на полазни ZOF.

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