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Fosinopril-cyclodextrin inclusion complexes: phase solubility and physicochemical analysis

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Fosinopril is one of the most hydrophobic substances among the angiotensin-converting enzyme inhibitors, exhibiting low water solubility and poor bioavailability following oral administration. Inclusion complexes between the drug substance and cyclodextrins (CDs) were obtained in order to improve its solubility. The purpose of this study was to investigate the guest-host interaction of fosinopril sodium (FOS) with β-cyclodextrin (β-CD) and its derivative, randomly methylated β-cyclodextrin (RAMEB) in solution by phase solubility diagrams (PSD) and in solid state by using thermal analysis, powder X-ray diffractometry (PXRD) and Fourier transform infrared spectroscopy (FTIR). The phase solubility analysis indicated that the solubility of FOS in simulated gastric fluid was increased in the presence of CDs and revealed for RAMEB an A_L-type diagram, suggesting the formation of a 1:1 inclusion complex, and for β-CD a B_S-type phase diagram. The estimated apparent stability constant ($K_{1:1}$), according to the Higuchi and Connors method, is 3209.99 M^{-1} and 1770.34 M^{-1} for RAMEB and β-CD complexes respectively. The binary systems FOS/CDs were prepared using the kneading method in the molar ratio 1:1. The PXRD patterns and the thermograms indicated a drug amorphization process, higher for FOS/RAMEB binary system and the FTIR analysis suggested that the ester group of FOS is probably enclosed in the CD's cavity. The results of this study confirm the formation of inclusion complexes both in solution and in solid state and suggest that the complexes formation between FOS and CDs could improve the bioavailability of the drug due to the enhancing absorption expected from increased drug solubility.

1. Introduction

Fosinopril, (2*S*,4*S*)-4-cyclohexyl-1-(2-{[2-methyl-1-(propanoyloxy) propoxy](4-phenylbutyl)phosphoryl}acetyl)pyrrolidine-2-carboxylic acid, (Fig.1) a phosphinic acid derivative, is an angiotensin-converting enzyme inhibitor used in the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post myocardial infarction (Brown and Vaughan 1998; Ferrar et al. 2003). Fosinopril is characterized by very high lipophilicity (log P = 4.75), very low aqueous solubility in the unionized form and poor bioavailability folowing oral administration (36%) (Remko 2007).

The absorption rate and bioavailability of poorly water-soluble active substances is determined by the dissolution rate of the drug in the gastrointestinal tract (Mitchel et al. 2003). Several technological methods have been used to enhance the solubility rate of slightly water-soluble drugs and, among them, the cyclodextrin (CD) complexation is of particular interest (Reddy et al. 2004; Yurtdaş et al. 2010; Aramă et al. 2011).

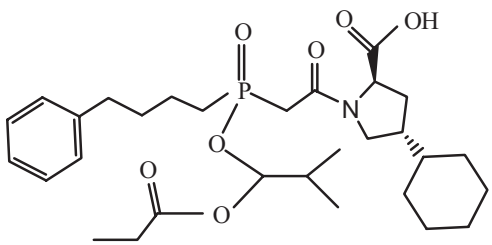


Fig. 1: Fosinopril chemical structure

The CDs are cyclic oligosaccharides consisting of glucopyranose units linked by α-(1,4) bonds, obtained through enzymatic degradation of starch. These are torus shaped molecules with a hydrophilic external surface which makes them water-soluble and a hydrophobic internal cavity which provides a microenvironment for appropriate sized molecules (Loftson and Duchene 2007; Loftson et al. 2005; Del Valle 2004). Due to their particular geometry, the CDs have the capacity of forming inclusion complexes (IC) by encapsulating in their cavity, entirely or partially, a wide variety of molecules, with drug substances among them. As a result of the CDs encapsulation, some physicochemical and biopharmaceutical properties of the included compounds, such as solubility, stability, melting point, chemical reactivity, volatility, unpleasant taste and smell, will be modified (Sreenivasa et al. 2010; Manca et al. 2005; Doile et al. 2008; Onyeji et al. 2007; Onyeji et al. 2009). Among the CDs, β-CD is the most used as a pharmaceutical agent because of its complexing ability, low cost and cavity size suitable for the widest range of drugs (Singh et al. 2002; Rawat et al. 2004; Kacsó et al. 2010; Uekama et al. 1998). However, its low aqueous solubility is a major drawback

Table: Apparent stability constant, slope and correlation coefficient (R^2) obtained from the phase solubility diagrams

Cyclodextrin	Slope	$K_{1:1}$ [M^{-1}]	R^2
β-CD	0.03460	1770.34	0.9735
RAMEB	0.06102	3209.99	0.9716

in its utilization, but this has been overcome by chemical modification resulting in derivatives with enhanced solubility. One of them is RAMEB, a randomly methylated β-cyclodextrin which has been widely used in pharmaceutical fields due to its high water solubility.

Bratu et al. (2009) reported that FOS forms inclusion complexes with β-CD, did not evaluate the interaction between the components in solution.

The aim of this work was to investigate the molecular encapsulation of the FOS in CDs (β-CD and RAMEB) cavity. The binary products were prepared by the kneading method in molar ratio 1:1. Physicochemical determination based on thermal analyses, PXRD and FTIR were used to characterize the IC. Phase solubility analyses were performed in order to investigate the interaction between FOS and CDs in solution.

2. Investigations, results and discussion

2.1. Phase solubility studies

Phase solubility diagrams of FOS with CDs at 25 °C were obtained by plotting the apparent equilibrium concentration of the drug against CDs concentrations (Fig. 2). The apparent solubility of FOS increased linearly as a function of RAMEB concentration in the 0–15 mM concentration domain as a result of soluble complex formation in simulated gastric fluid (SGF). The linear relation between FOS solubility in SGF and RAMEB concentration indicates an A_L-type diagram defined by Higuchi and Connors (1965). In the case of β-CD, the phase solubility diagram can be classified as B_S-type according to Higuchi and Connors, displaying a linear increase up to 0.004 M followed by a plateau region, highlighting a limited solubility of the formed inclusion complex. Increase in the β-CD concentration above 0.008 M results in the precipitation of the inclusion complex from the saturated solution. Both the A_L-type diagram and the initial linear segment of the B_S diagram have a slope less than unity, indicating that inclusion complexes in the molar ratio 1:1 between the guest (FOS) and host (β-CD, RAMEB) were obtained. The inclusion complex stability constants ($K_{1:1}$) calculated from the slope of the fitted straight line, corresponding slopes and correlation coefficients are summarized in the Table.

2.2. Thermal analysis

The thermal behavior of FOS, CDs (β-CD, RAMEB) and their kneaded products (KPs) was studied using thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA) in order to evaluate the formation of the complexes. TG, DTA and DTG thermograms of pure substances and inclusion complexes are shown in Figs. 3 A-E.

The DTA thermogram of pure FOS indicates a sharp endothermic peak at 191.6 °C corresponding to the melting point of the drug followed by melt degradation confirmed by TG/DTG curves. The DTA curve of pure β-CD did not show any endothermic peaks in the melting point region of FOS. The thermograms of β-CD alone show a weight loss process between 45–130 °C

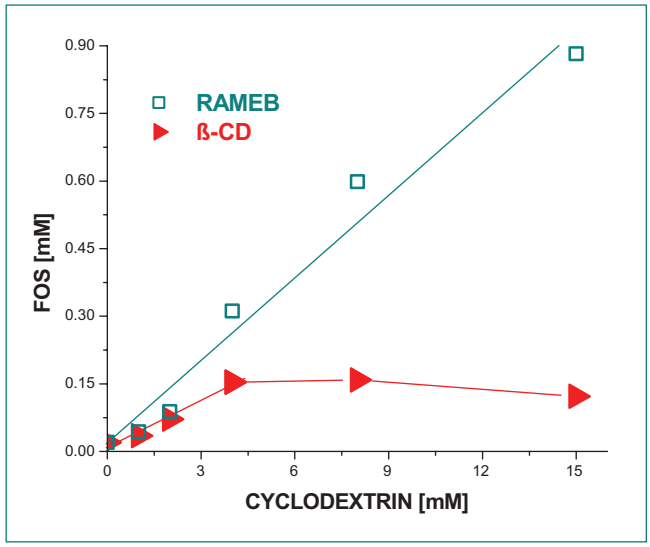


Fig. 2: Phase solubility diagrams of FOS/CD systems in SGF

and a very broad endothermic effect, between 40–130 °C with a maximum at 100.2 °C corresponding to the dehydration process. Broadening and marked reduction in intensity of the endothermic melting peak of FOS were recorded in the thermal DTA curve of the KP FOS/β-CD. Also the FOS melting peak shifted to lower temperature (186.3 °C). Furthermore, the endothermic peak due to the loss of water, characteristic of β-CD exhibited a reduction in its intensity and shifted to lower temperature (65.2 °C) in the binary system. These results indicate the complexation of FOS with β-CD.

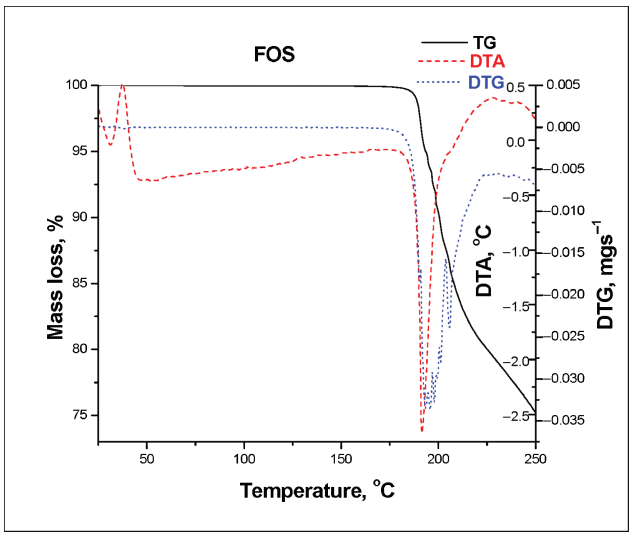
The DTA thermogram of RAMEB presents a broad endothermic phenomenon between 40–80 °C with a maximum around 53 °C due to the loss of crystal water. The thermal curves of the kneaded system FOS/RAMEB reveal the disappearance of the endothermic melting peak of FOS and the shift to lower temperature of the endothermic peak corresponding to the dehydration of RAMEB (41.4 °C). On the other hand, there can be noticed a decrease in the thermal stability of FOS in the inclusion complex with RAMEB confirmed by TG/DTG curves in comparison with the drug alone; the degradation process of the binary system begins at a lower temperature than that of FOS alone. This phenomenon may be a consequence of the amorphous inclusion complex formation (Doile et al. 2008).

The thermal analysis has been considered as an important tool for the study of the interaction between CDs and drug substances. The melting point of the guest molecules witch are embedded in CD cavity generally shifts to a different temperature and decreases its intensity or disappears (Doile et al. 2008; Liu and Zhu 2006; Mura et al. 2003). The disappearance and/or the aria reduction and the shift toward a lower temperature of the melting peak of FOS in the KPs indicate a molecular interaction between the guest and host molecules. The results reported herein endorse the hypothesis of the existence of an inclusion complex between the two components.

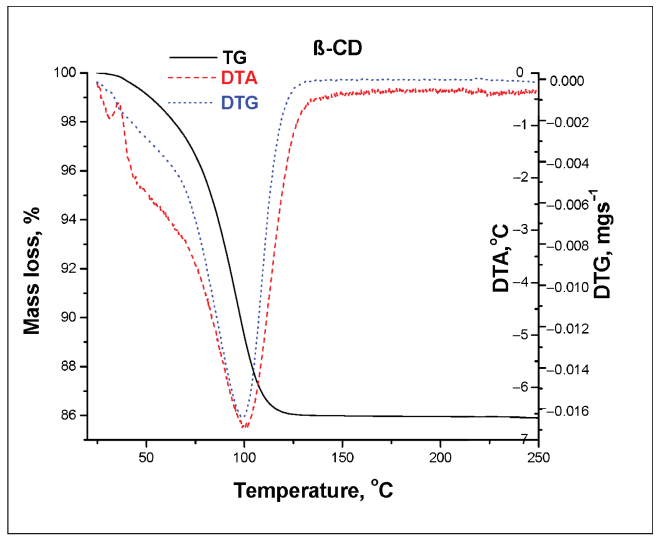
2.3. Powder X-ray diffractometry

PXRD patterns of FOS, CDs (β-CD, RAMEB) and their inclusion complex are presented in Figs. 4 A-B.

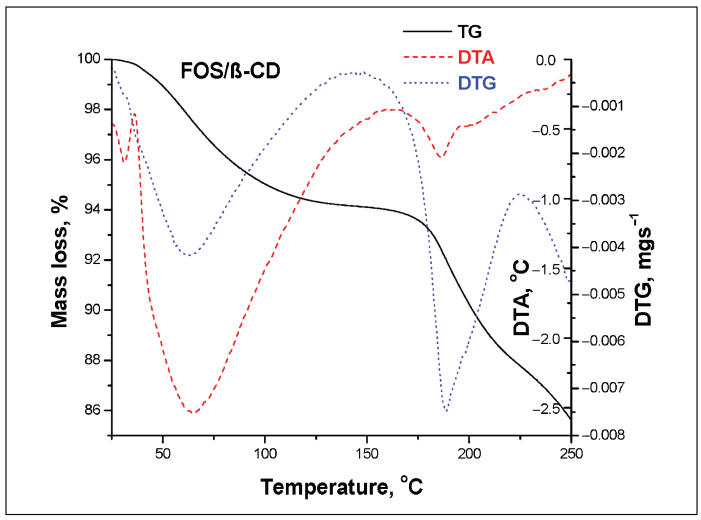
The PXRD pattern of FOS contains a number of sharp and intense peaks indicating its crystalline nature, while the diffraction pattern of β-CD also shows several characteristic peaks. The diffractogram of the KP FOS/β-CD presents considerable diminution of the characteristic diffraction peaks of FOS suggesting that the new compound is less crystalline than the drug



(A)



(B)



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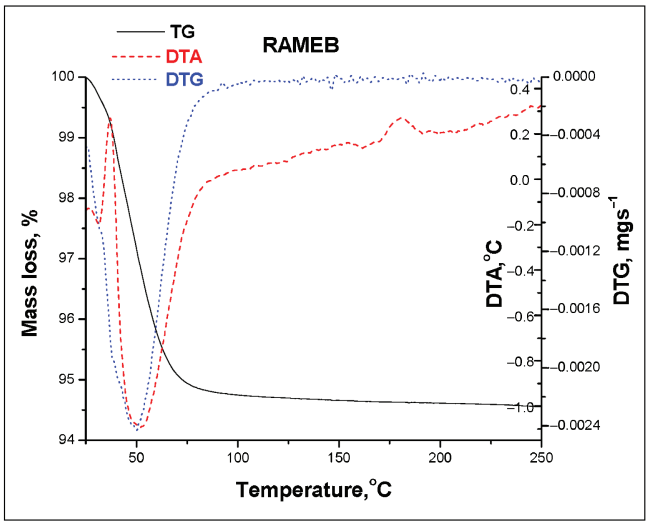

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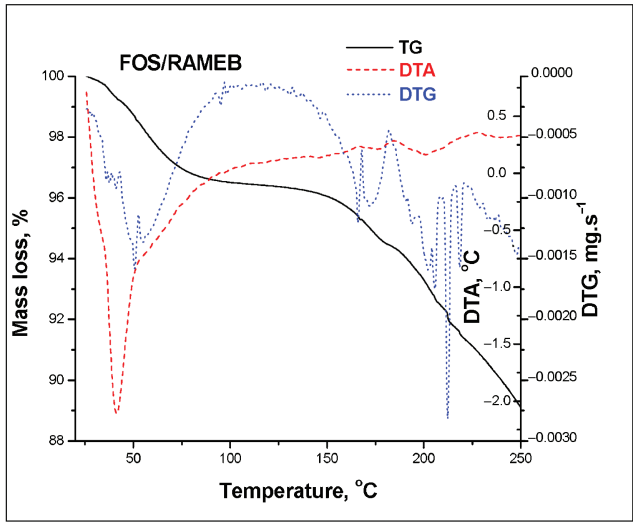
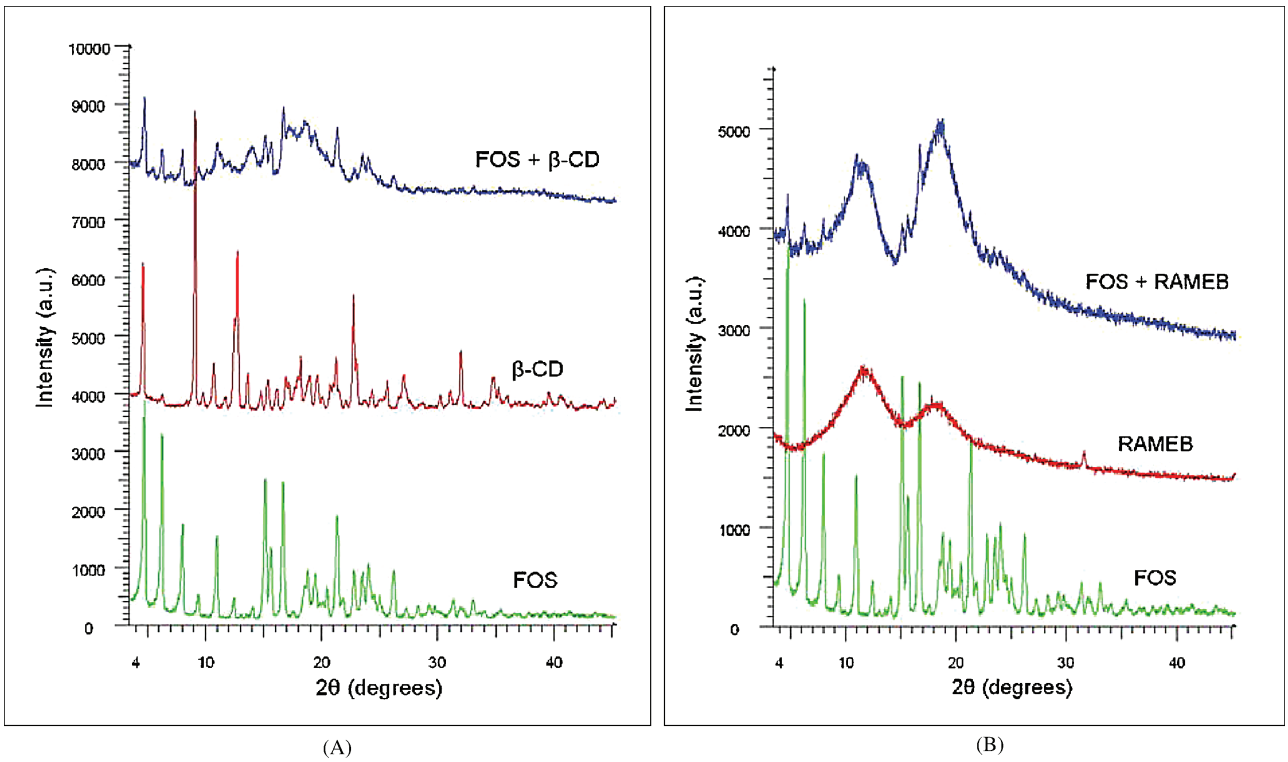


(E)

Fig. 3: TG, DTA and DTG thermograms of: FOS (A); β-CD (B); inclusion complex FOS/βCD (C); RAMEB (D); inclusion complex FOS/RAMEB (E)

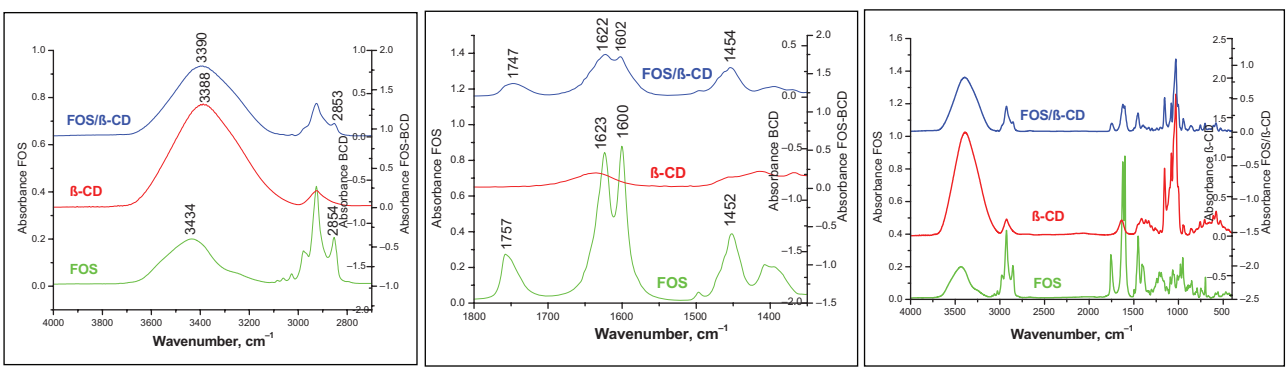
alone. The reduction in crystallinity attributed to KP suggests that FOS and β-CD form an inclusion complex in solid state, demonstrating that a new solid phase is formed.

The X-Ray diffraction pattern of RAMEB has two broad peaks and many undefined, diffused peaks with low intensities, reflecting the amorphous nature of cyclodextrin. The FOS/RAMEB binary system exhibits a diffraction pattern with marked reduction in the intensity of major peaks of FOS and many undefined and diffused peaks, similar to that of pure RAMEB, indicating the amorphous nature of the compound. This behavior is an evidence for the formation of the inclusion complex.



(A) (B)

Fig. 4: Powder X-Ray diffractograms of FOS, *β*-CD and their binary system (A) and FOS, RAMEB and their binary compound (B)



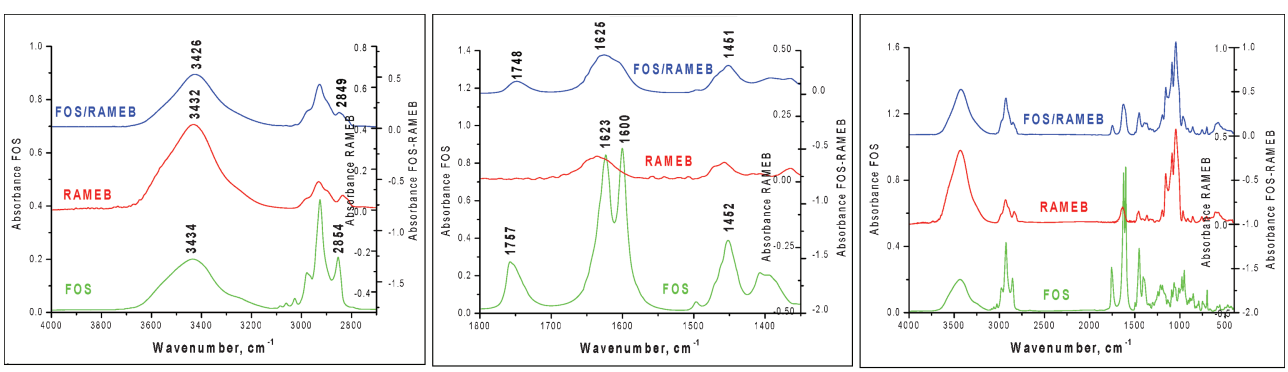
(A) (B) (C)

Fig. 5: FTIR spectra of FOS, *β*-CD and their inclusion complex: 4000–2700 cm^{-1} spectral region (A), 1800–1350 cm^{-1} spectral region (B) and 4000–400 cm^{-1} spectral region (C)

2.4. Fourier transform infrared spectroscopy

The FTIR spectra of FOS, β-CD and their binary products are depicted in Fig. 5 and those of FOS, RAMEB and their binary compounds in Fig. 6 The IR spectra of the KPs were compared with those of the pure substances in order to investigate the inclusion complexes formation. Changes in the characteristic bands of the pure compounds reveal an interaction between them with the formation of a new compound with different spectroscopic bands.

The characteristic bands of FOS are observed at 2925 and 2854 cm^{-1} (aliphating C-H stretching), 1757 cm^{-1} (ester



(A) (B) (C)

Fig. 6: FTIR spectra of FOS, RAMEB and their inclusion complex: 4000–2700 cm^{-1} spectral region (A), 1800–1350 cm^{-1} spectral region (B) and 4000–400 cm^{-1} spectral region (C)

group vibration), 1623 cm^{-1} (C = O stretching from carboxyl group), 1600 cm^{-1} and 1452 cm^{-1} (stretching vibration of the C = C from the aromatic ring). β-CD exhibits a broad absorption band and a peak in the 3000–3700 cm^{-1} region assigned to O-H stretching vibration, a shorter band between 1560–1730 cm^{-1} and a large region which displays distinct peaks in the 900–1500 cm^{-1} spectral region. Comparison of FTIR spectrum of KP FOS/β-CD with those of the pure compounds showed different degrees of changes. It can be seen that in the IR spectrum of the binary compound, the drug characteristic peaks either have reduced their intensity or have disappeared. Furthermore, most of the characteristic peaks of FOS have slightly changed their frequency; the ester group vibration band shifted from 1757 cm^{-1} (pure drug) to 1747 cm^{-1} and the band assigned to O-H stretching vibration in β-CD shifted from 3388 cm^{-1} to 3390 cm^{-1} in KP.

Changes are also noticed in the FOS/RAMEB binary product spectrum compared with the pure substances spectra: the ester group vibration band shifted from 1757 cm^{-1} (pure drug) to 1548 cm^{-1}, the FOS absorption band from 1600 cm^{-1} has disappeared in the KP and the O-H stretching vibration band in RAMEB shifted from 3423 cm^{-1} to 3425 cm^{-1} in KP. These results support the hypothesis of inclusion complex formation when the kneading method was used and suggest that the ester group is probably enclosed in the β-CD cavity as previously reported by Bratu et al. (2009).

2.5. Conclusions

The present study reveals that FOS can form inclusion complexes with β-CD and RAMEB in the stoichiometric ratio of 1:1. Phase solubility profile indicates that the solubility of FOS in SGF was significantly increased in the presence of CDs, resulting in an A_L-type phase diagram for RAMEB and a B_S-type diagram for β-CD. The results obtained herein by different characterization techniques clearly show that the physicochemical properties of the binary products were different in relation to the drug, indicating that the kneading method leads to formation of solid state complexes between FOS and CDs (β-CD and RAMEB).

3. Experimental

3.1. Materials

Fosinopril sodium was a gift sample from Terapia-Ranbaxy (Cluj-Napoca, Romania). Randomly methylated β-cyclodextrin was purchased from Cyclolab R&D Ltd. (Budapest, Hungary) and β-cyclodextrin was obtained from Fluka Chemie GmbH (Germany). The substances were used as received. All other chemicals and reagents were of analytical grade.

3.2. Methods

3.2.1. Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (Higuchi and Connors 1965) in SGF (without enzyme). Excess amounts of FOS (10 mg) were added to 6 ml of CD solution in SGF (β-CD and RAMEB in concentration of 0–15 mM). Suspensions were vigorously shaken at 20 °C for 5 days. After the equilibrium was reached (5 days), the samples were filtered using a 0.20 μm nylon disk filter and suitably diluted. The concentration of FOS in filtered solutions was measured by UV-spectrophotometry at 210 nm against blanks prepared in the same concentration of CDs in SGF in order to cancel any absorbance that may be exhibited by the CDs and the components of SGF. The solubility experiments were realized in triplicate. The apparent stability constants (K_c) were calculated from the phase solubility diagrams using the following equation (Higuchi and Connors 1965): $K_c = Slope/S_0 (1 - Slope)$.

The slope was obtained from the initial straight-line portion of the phase solubility diagrams and S_0 is the solubility of FOS in SGF in absence of CDs.

3.2.2. Preparation of solid binary systems

The binary systems FOS/CDs were prepared using the kneading method. The amounts of guest substance and CDs were weighed according to molar ratio 1:1. The resulted mixture was pulverized in a mortar and triturated with a small amount of ethanol-water solution (50:50, w/w) in order to obtain a homogenous paste. The thick slurry was then kneaded for 1 h and during the process few drops of solvent were added to maintain a suitable consistency. After drying at room temperature, the product was also dried in oven at 105 °C for 1 h. The dried complexes were pulverized into a fine powder and sieved (100 μm).

3.2.3. Thermal analysis

FOS, CDs and their binary systems were analyzed by TG, DTG and DTA using a TGA/SDTA 851-LF 1100 METTLER apparatus. The thermal behaviour of the substances was studied under a nitrogen flow of 50 $ml \cdot min^{-1}$ in the temperature range of 25–250 °C with heating rate of 5 $°C \cdot min^{-1}$. Samples with mass of about 35–50 mg were packed in platinum crucibles of 150 μl.

3.2.4. Powder X-ray diffractometry

X-ray diffraction patterns of the pure compounds (FOS, β-CD, RAMEB) and the binary systems were recorded at room temperature using a Bruker D8 Advance powder X-ray diffractometer in the $2\theta = 3–45°$ angular domain using CuKα radiation (40 kV, 40 mA) and a Ni filter.

3.2.5. Fourier transform infrared spectroscopy

The IR spectra of FOS, CDs and their inclusion complexes were recorded using a JASCO FT/IR -670 PLUS spectrometer at room temperature, the samples being included in KBr pellet.

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