Characterization of Inclusion Complexes Between Fluconazol and Different Cyclodextrin Derivatives

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The aim of this study is to confirm the formation of inclusion complexes between fluconazoleand different cyclodextrin derivatives. Fluconazole is slightly soluble in water, but its solubility can be further increased by complexation with cyclodextrins. The binary systems between fluconazole and cyclodextrins were prepared in 1:1 molar ratios by physical-mixture method and kneading method. Differential scanning calorimetry (DSC), Fourier transformed-infrared spectroscopy (FT-IR) and molecular modeling methods were used to characterize solid state interactions between fluconazole and cyclodextrins in their binary systems. The analysis suggest the formation of a new solid phase, indicating a molecular interaction between the components. All binary systems showed superior dissolution profiles compared to pure fluconazole.

Keywords: fluconazole; cyclodextrins; differential scanning calorimetry; Fourier transform -infrared spectroscopy; solubility

Fluconazole (2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol) (FCZ), is a first generation triazole derivative antifungal agent. The chemical structure of FCZ is presented in figure 1.

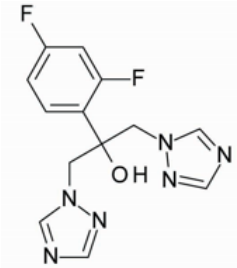

Fig. 1.Chemical structure of FCZ

Structurally FCZ is a bis-triazole, the presence of two weakly basic triazole rings in the molecule conferring sufficient aqueous solubility to balance the lipophilicity of the 2,4-difluorophenyl group. FCZ has a water solubility of approximately 5.55 g/L and an log P value of 0.41, indicating its viability in both aqueous and lipophilic media. FCZ water solubility, makes it suitable for both oral and intravenous administration [1-3].

FCZ is strong and specific inhibitor of the sterol synthesis of fungi, by inhibiting cytochrome P450-mediated 14-alpha-lanesterol demethylation, an essential step in biosynthesis of fungal ergosterol [3]. Administered both orally and intravenously, FCZ is active in a wide variety of fungal infections, its spectrum of activity including most *Candida* species, *Cryptococcus neoformans*, some dimorphic fungi and dermatophytes, among others [1, 4].

Its solubility in water can be further increased by complexation with CDs, which can enhance also the bioavailability of FCZ.

Cyclodextrins (CDs) are crystalline, nonhygroscopicwater-soluble cyclic oligosaccharides derived from starch, composed of α-1,4-linked D-glucopyranose units; having a hydrophilic external surface and a hydrophobic internal cavity, in which they can incorporate different analytes through hydrophobic interactions. The most commonly used form of these ring-shaped molecules are α-, β-, and γ-CDs formed by six, seven and eight glucose units, respectively (fig. 2) [5]. As a consequence of these features CDs can encapsulate a variety of hydrophobic molecules inside their cavity through non-covalent interactions to form inclusion complexes of host-guest type [6-8].

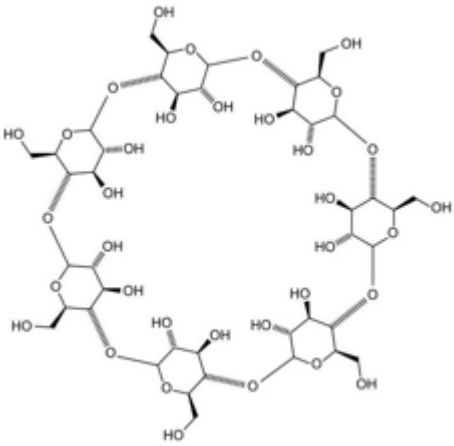

Fig. 2. Chemical structure of native β-CD

Thermoanalytical techniques (differential scanning calorimetry, thermogravimetry) are frequently used in the investigation of the thermal properties of CDs and their inclusion complexes [8,9]. The melting enthalpy is an indication of the amount of guest not involved in the interaction with the CD [10-12].

In previously published articles, inclusion complexes of azole derivatives with CDs in aqueous solution and in the

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solid phase were studied by solubility methods, spectroscopy (UV, IR), thermal analysis, and X-ray diffractometry, and their modes of interaction were assessed [13,14].

NMR spectroscopic studies were published in order to evaluate the complexation of FCZ with β-CD, the results confirmed the formation of an inclusion complex in aqueous solution [1, 13, 15]. Complexes of FCZ an dbifonazole, miconazole, voriconazole prepared with different CDs by various methods such as kneading, co-evaporation, physical mixing and spray-dried were characterized by FT-IR and DSC studies; the effect of complexation on the dissolution rate of azoles was studied [16-20].

The purpose of this study is to evaluate the possibility of interaction of FCZ through complexation with different types of CDs and the solubility increasing effect of different CD-derivatives. The binary systems between FCZ and different CDs were prepared in one molar ratios 1:1 (FCZ:CD), using several methods (mixing, kneading). The resulted complexes were characterized by means of: thin-layer chromatography (TLC), thermal analyses differential scanning calorimetry (DSC), Fourier transformed infrared spectroscopy (FT-IR), and molecular modelling.

Experimental part

Materials

FCZ was kindly provided by VIM SPECTRUM td. (Corunca, Romania), while the CDs were purchased from Cyclolab Ltd. (Budapest, Hungary): native α-, β-, γ-CD and derivatized randomly-methylated-β-CD (RAMEB) and hydroxypropyl-β-CD (HP-β-CD)were used. All the chemicals were of analytical grade.

Preparation of the binary systems

Physical mixtures (PM): the components were mixed in a mortar and sieved through a 100 μm sieve.

Kneaded products (KP): physical mixtures of FCZ and CDs derivatives were mixed with the same quantity of a 50 % ethanolic solution. The obtained paste was kneaded until the bulk of the solvent had evaporated. After drying at room temperature and then in the oven at 105°C, the KP were pulverized and sieved through a 100 μm sieve.

These methods are simple and provide a high yield. The molecular ratio of the products were 1:1.

Thin-layer chromatography (TLC)

TLC has been successfully used for the analysis of CD inclusion complexes. As a result of inclusion complexation, the Rf value for stable complexes should be lower than Rf values of the active substances.

The TLC system consisted of: CamagLinomat IV semiautomatic sampler (Camag, Switzerland), Camag Normal Development Chamber, Camag UV fluorescence inspection lamp (Camag, Switzerland). As stationary phase we used 20x20 cm pre-coated silicagel GF_{254} HPTLC glass plates (Merck, Germany).

The mobile phase was toluene-chloroform-methanol 1.2:3.0:0.4 (v/v) [24]. Sample of 5μL were applied on the chromatographic plate. The plates were developed over a distance of 15 cm, dried in a stream of hot air, and examined first under UV radiation at 254nm wavelength, finally the plate was sprayed with iodine solution.

Differential scanning calorimetry (DSC)

The temperature and enthalpy measurements were performed using a Mettler Toledo DSC 823e Thermal Analysis system (Schwerzenbach, Switzerland). Approximately 1-2 mg of the active material or binary systems were examined in aluminium pans between 25-400°C in a nitrogen atmosphere (flow rate of 50 mL/min.). The heating rate was 10°C/min.

Fourier-transformed - infrared spectroscopy (FT-IR)

FT-IR analysis provide the detection of inclusion complexation in terms of diffraction and IR spectra patterns of the complex which must be clearly distinct from that resulting by the superimposition of individual diffraction and IR spectra. The IR spectra of FCZ, CD derivatives and their binary systems were recorded using a FT-IR 470 Plus, (AbleJasco, Japan) spectrometer. The resolution was 4 cm^{-1}, the wave number range was 2000-400 cm^{-1} and the scan number was 64. The samples were included in KBr pellets. Analyses were performed at room temperature.

Molecular modelling studies

Molecular modelling studies were performed using the Hyperchem 8.0 software. In order to find the most stable conformation of the complexes, molecular mechanics simulations were applied. Energy minimization was performed using the force field method with the Polak-Ribiere algorithm (0.01 kcal/moleA gradient). The initial distance between the host and guest molecule was set at about 5 Å; the energy minimization of the complex was conducted until the conformation with the lowest energy was found. The binding energy (E_{bond}) was calculated using the formula (eq. 1):

$$E_{bond} = (E_{FLC} + E_{CD}) - E_{compl} \qquad (1)$$

where E_{FLC} and E_{CD} are the energy of the guest and host molecules and E_{compl} shows the energy of the complex after energy minimization.

Dissolution studies

In vitro dissolution studies of FCZ (SR 8-PLUS Hanson-Research, USA), physical mixtures and kneaded products complexes were performed by adding the solid systems, equivalent to 10 mg of FCZ, to a 900 mL phosphate buffer at *p*H 7.0 thermostated at 37 ± 0.5 °C, and stirred at 100 rpm. At fixed time intervals, samples were withdrawn with a filter-syringe (0.45 μm) and assayed spectrophotometrically using a UV-Vis spectrophotometer (Shimadzu UV-1601, Japan) at 261 nm. The volume of the dissolution media was kept constant during the experiment.

Results and discussions

Thin-layer chromatography (TLC)

The chromatograms of FCZ, CDs, *in situ* CD – FCZ mixtures and binary complexes of FCZ with β-CD, HP-β-CD and RAMEB prepared by physical mixing and kneading are presented in figure 3a, figure 3b and figure 3c respectively..

Examined in UV light, the chromatograms exhibits a fluorescent spot in case of FCZ, with a Rf of 0.25, while for the CD derivatives there isn't any fluorescence spot noticed on the plate. When pulverized with iodine solution, the CDs are revealed as a yellow citrine spots on the start line and no reaction appears for FCZ. For the *in situ* mixture, two spots can be seen, one for FCZ and one at the start line for the CD, visible only after applying the iodine solution. In case of the products obtained by physical-mixture and kneading methods, only one intensely fluorescent spot appears in UV light which turns yellow citrine when pulverized with iodine solution.

The results obtained with the *in situ* mixture acts, exclude the possibility of complex formation on the chromatographic plate, while in the case of binary products

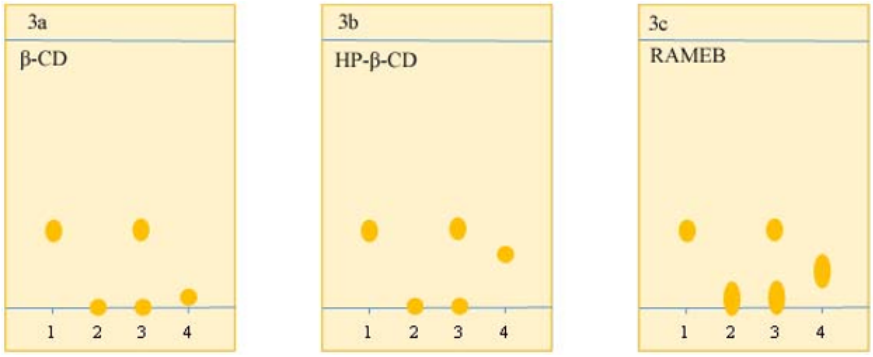

Fig. 3a, 3b, 3c TLC of FCZ, CDs, and complexes (spot 1. 0.5% FCZ in ethanol, spot 2. 0.5% CD in distilled water, spot 3. *in situ* mixture of FCZ and CD solution, spot 4. 0.5% water solutions of the products obtained by physical-mixture methods and kneading method)

the complexes have a different chromatographic behaviour that the active substances, the Rf value of complexes being smaller than that of the guest molecule.

Differential scanning calorimetry (DSC)

Differences in the thermal behaviour of FCZ, CDs, and the corresponding inclusion complexes were evident. As shown in figure 4, FCZ exhibits a characteristic endothermic fusion peak at 141.73°C corresponding to the FCZ melting point. Furthermore, α-, β-, γ-CD, HP- β-CD and RAMEB show broad endothermic events in the range from 30 to 95°C, which are related to the loss of adsorbed water, and small endo- or exo- effects at 210-325°C due to thermal degradation. DSC thermograms of the physical mixture for FCZ and α-, β and γ-CD, HP- β-CD and RAMEB show the existence of the endothermic peak of FCZ indicating interactions between the CDs and FCZ. The FCZ peak in the physical mixture with CDs decreases, indicating a more intense interaction of FCZ with CDs. This endothermic peak is still present in kneading products, as FCZ-β-CD and FCZ-γ-CD complexes, show an endothermic peak at 131,21°C - 133,35, whereas there is a new endothermic peak in the range of 140 - 160 C that possibly shows complex formation.

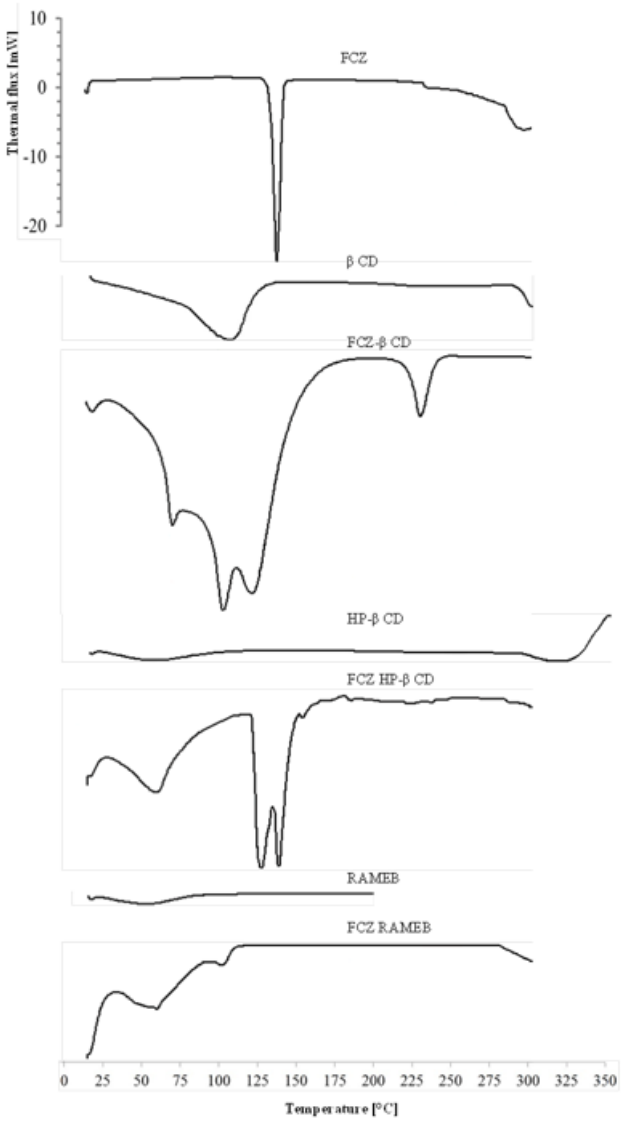

Fig. 4. DSC thermograms of FCZ, CDs, and complexes

In the binary systems of FCZ-RAMEB and FCZ-HP-β-CD we observed the disappearance of the FCZ endothermic peak, indicating a more intense interaction of FCZ with RAMEB andHP- β-CD. The FCZ peak in the kneading products increases at 152,2 - 158,9°C indicating a more intense interaction of FCZ with the CDs.

The absence of the characteristic peak of the analyte is a strong evidence of the inclusion of the analyte into the CD cavity. This could be attributed to the formation of an amorphous solid dispersion, molecular encapsulation of the analyte into the CD cavity, or both.

Fourier-transformed - infrared spectroscopy (FT-IR)

In order to investigate the vibrational changes upon host-guestinteractionbetween FCZ and CDs, FTIR spectroscopy was used. FTIR technique is useful to identify which is the vibrational mode of the analyte and suggesting the interactions between these molecules in solid state [25].

The FT-IR spectra of FCZ (fig.5a) reveal numerous absorption bands in the fingerprint region. The FTIR spectrum of FCZ exhibits characteristic peaks at 3071cm^{-1}attributed to the C-H stretching aromatic ring, 3117cm^{-1} due to stretching triazole ring, 3408 cm^{-1} due to -NH stretching, 3063 cm^{-1} due to C-H stretching, 1584 cm^{-1} due to C-N cm^{-1} stretching, 1619cm^{-1} due to C=C stretching aromatic ring, 1506cm^{-1} due to triazole ring stretching, 1419cm^{-1} due to triazole ring stretching, 1105cm^{-1} due to C-F stretching and 1085 cm^{-1} due to C-OH stretching.

A broad IR band centred at 3190 cm^{-1} is attributed to the hydrogen bonded -OH stretching mode of the tertiary alcohol group. The three weak IR absorption maxima at 1898, 1844 and 1766 cm^{-1} are characteristic of a 1,2,4-trisubstituted benzene ring.

The FT-IR spectra of β-CD (fig.5b) reveal numerous absorption bands in the fingerprint region. In the spectrum of β-CD there is a wide absorption band in the 1200-1000 cm^{-1} area, attributed to the glucopyranosic ring. Another broad and strong absorption band in the 3000 cm^{-1} domain is attributed to -OH stretching. For the binary systems, the 1600-600 cm^{-1} domain was chosen to highlight the modification of spectra due to complexation, CDs mask the characteristic peaks of groups that are included in their cavity.

Between the complexed drug and the IR spectrum of FCZ there is a significant difference between the number

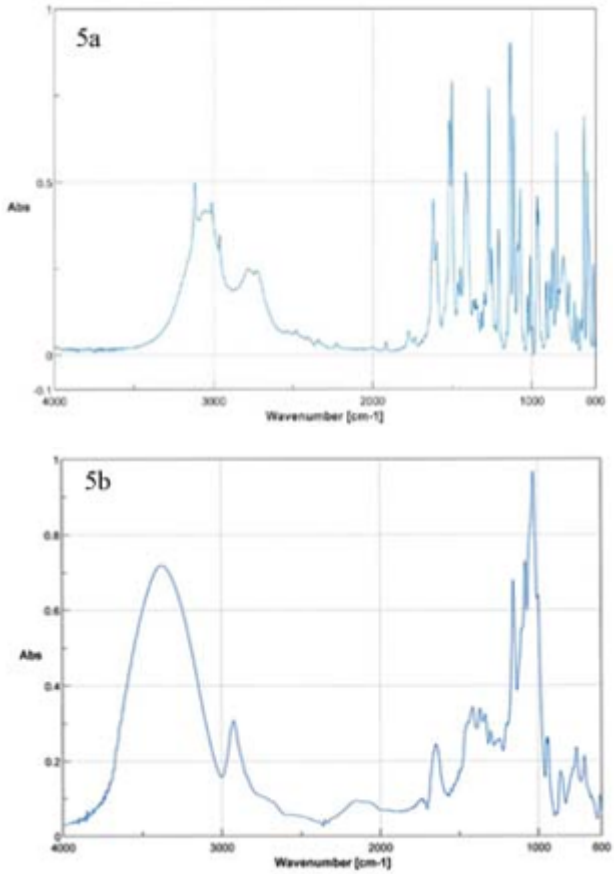

Fig. 5 FT-IR spectra of FCZ (5a) and β-CD (5b)

and location of the peaks. The 3117 cm^{-1} peak characteristic of the triazole ring of FCZ disappears, suggesting a covalent, strong interaction between the guest and the host molecules.

The absorption spectrum of the complex of FCZ with β-CD shows that the absorption bands of primary and secondary -OH groups flanking the narrower and wider rims of CD do not appear or shift in the complex. This may indicate that the free deformation of these groups is somewhat hindered in the complex. The absorption band around 3280 cm^{-1} is, according to literature data, the range of water vibrations. The fact that this band appears only in the case of pure CD suggestsindirectly that the molecule entering the cavity during the complex formation displaces the previously contained water molecules in the cavity. The disappearance of characteristic frequencies of imidazole ring from the absorption spectra of the complexes in range of 1000-650 cm^{-1} indicates that FCZ fittedthe CDcavity. Together with the imidazole ring the aliphatic carbon atom bonded to N of the imidazole was also encapsulated (fig. 6).

Changes in infrared spectra such as the disappearance or intensity of characteristic bands, the emergence of new bands, demonstrate the interaction between FCZ and CDs. From the spectra it appears that the strongest interaction with RAMEB followed by HP-β-CD.

Molecular modelling studies

Spatial arrangement of the inclusion complexes (front view and side view) are shown in figure 7 According to eq. 1 the highest value of energy difference indicates the most stable complex; in this context the RAMEB-FCZ complex shows the highest binding energy.

The increasing order of the binding energies is: β-CD (1.23 kcal/mole) < HP-β-CD (9.88 kcal/mole) < RAMEB (15.36 kcal/mole). In the case of complexes of β-CD and RAMEB a complete inclusion of the FCZ can be observed; complexation with HP-β-CD involves encapsulation of one of the triazole groups in the CD cavity.

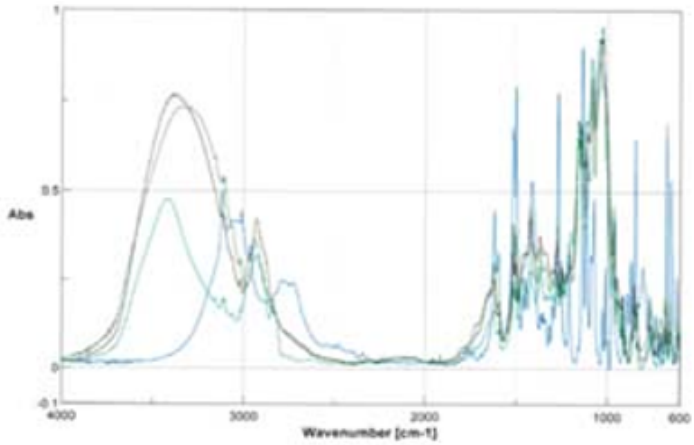

Fig.6. FT-IR spectra of FCZ KP complexes with β-CD, HP-β-CD, RAMEB (FCZ - blue, β-CD - green, HP-β-CD – red, RAMEB - light green)

Dissolution studies

CDs play a very important role in formulation of poorly water soluble drugs by improving the apparent drug solubility and dissolution through inclusion complexation or solid dispersion.

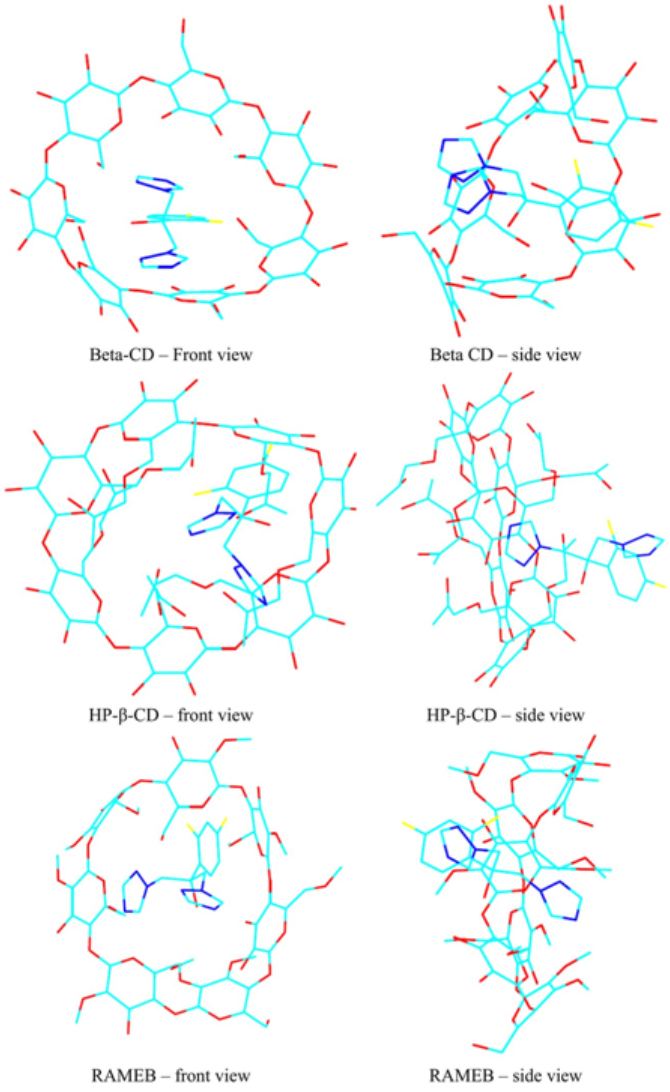

Fig. 7.The spatial arrangement of the complexes

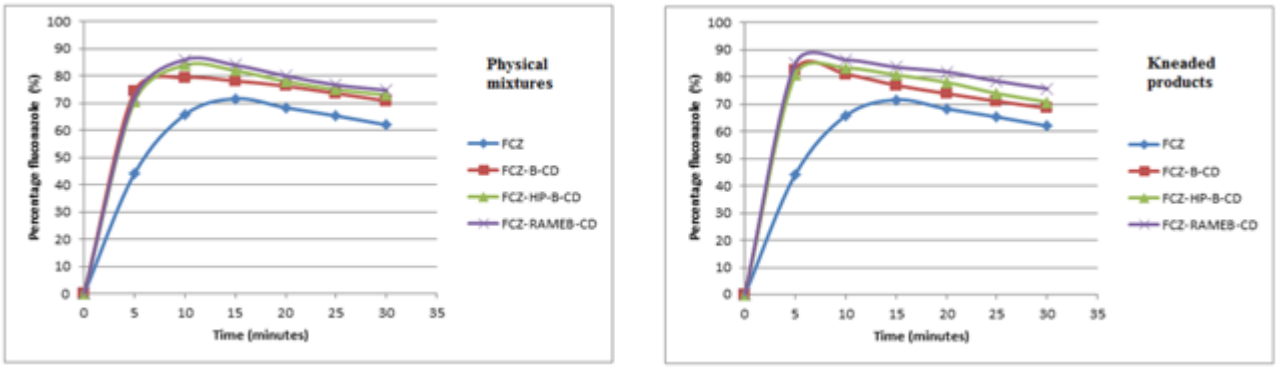

Fig. 8. Comparative dissolution profile of FCZ and the products of CDs

Comparing the dissolution profile of FCZ with the release curves of complexes with CDs, it can also be observed that the kneaded products dissolve faster and the amount of dissolved material is higher, which suggests that the inclusion complex formation is better for a kneaded product.

The increase in the dissolution profiles depend on the nature of the CD derivative, the FCZ concentration in the products and on the processing method. The various CD derivatives increased the solubility of FCZ to different extents, RAMEB proving to be the most effective CD derivative for the complexation.

Conclusions

In TLC there was a significant diminution of the Rf values of the complexes compared with the active substances which can be interpreted as the formation of inclusion complexes. DSC analysis sustains the hypothesis of partial inclusion complexes formation between FCZ and CDs. The thermo behaviour depends both on the preparation method used and the composition of the product. Changes in IR spectra such as the disappearance or change in intensity of characteristic bands, the emergence of new bands, demonstrate the interaction between FCZ and CDs. The increase in the dissolution characteristics depend on the nature of the CD derivative, on the processing method. The various CD derivatives increased the solubility of FCZ to different extents.

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