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ORIGINAL ARTICLE

Inclusion complexes of ketoconazole with beta-cyclodextrin: physicochemical characterization and in vitro dissolution behaviour of its vaginal suppositories

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Abstract Ketoconazole (KZ) is an imidazole antifungal agent which is administered topically and also orally. KZ is practically insoluble in water. Vaginal candidiasis is a common condition and up to 75% of all women have at least one episode of this infection during their lifetime. The aim of study was to prepare KZ/β-cyclodextrin (β-CD) complex to improve the physicochemical properties of KZ and to investigate the possibility of preparing vaginal suppositories with the complexes. A linear increase in KZ solubility as a function of β-CD concentration was verified using the phase-solubility diagram. The resulting diagram was classified as A_L-type, is generally related to the formation of a soluble complex. Complexes were prepared in a 1:1 molar ratio by different methods, namely freeze-drying, spray-drying, co-evaporation and kneading. Characterization of the complexes prepared was performed by practical yield %, aqueous solubility, active agent amount analyses, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffractometry (PXRD) and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Characterization studies provided additional evidences of complexation. The paddle method defined in USP31 was used in in vitro dissolution experiments on the prepared vaginal suppositories. It was found that solubility enhancement by preparing KZ/β-CD complexes depends on the type of the preparation method. Dissolution of KZ from complexes was found to be faster than the active agent and the commercial suppositories. This result may be attributed to the interactions between β-CD and active agent, high energetic amorphous state and decrease in the interfacial tension between insoluble active agent and dissolution media.

Keywords Ketoconazole · β-Cyclodextrin · Phase-solubility studies · Inclusion complexes · Vaginal suppositories

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Introduction

Ketoconazole (KZ) is an imidazole antifungal administered topically or by mouth. It is given by mouth in chronic mucocutaneous candidiasis, in fungal infections of the gastro-intestinal tract, in dermatophyte infections of the skin and fingernails not responding to topical treatment, and in systemic infections including blastomycosis, candidiasis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis. It has been recommended that because of its erratic absorption and slow therapeutic response, KZ should not be used for the treatment of life-threatening fungal infections including fungal meningitis or for severe infections in immunocompromised patients [1]. Vaginal candidiasis is a common condition and up to 75% of all women have at least one episode of this infection during their lifetime. Most patients with *Candida vaginitis* respond to topical treatment with nystatin or imidazoles [2].

KZ is an aqueous solubility of 0.017 mg mL^{-1} at 25 °C [3]. The absorption of KZ from the gastro-intestinal tract is variable and systemic absorption following topical or vaginal application in healthy subjects is minimal [1]. Due to its dissolution and absorption properties, KZ is classified

in the Biopharmaceutics Classification Scheme as a class II drug, since it has a high permeability, but a solubility in aqueous media which is insufficient for the whole dose to be dissolved in the gastro-intestinal fluids under normal conditions [4]. Depending on several factors, an oral dose of KZ has a wide range of bioavailability, with between 37 and 97% reaching the blood [5].

Many technological methods of enhancing the solubility and dissolution characteristics of poorly water-soluble drugs have been reported in the literature, such as micronization, formation of solvates, adsorbates, complexes, microspheres, and solid dispersions. However, conventional methods used to prepare these systems suffer from serious limitations on their applicability in the market, often involving physical instabilities of the solid dispersions on storage, problems of grinding or difficulties in removing the toxic organic solvent [6].

Among the various approaches that have been used to improve the solubility and dissolution rate of drugs, complexation with cyclodextrins is one of the most promising ones. Cyclodextrins are crystalline, nonhygroscopic, cyclic oligosaccharides derived from starch. Due to arrangement of hydroxyl groups within the molecule the internal surface of the cavity is hydrophobic while the outside of the torus is hydrophilic. This arrangement permits the cyclodextrin to accommodate a guest molecule within the cavity so forming an inclusion complex. Cyclodextrins may thus be used to form inclusion complexes with a variety of drug molecules resulting primarily in improvements to dissolution and bioavailability due to enhanced solubility and improved chemical and physical stability [7].

β-CD appears to have the most use in pharmaceutical industry of all the natural cyclodextrins because of its cavity size, efficiency of drug complexation, availability in pure form, and relatively low cost. The lipophilic cavity of the β-CD molecule provides a microenvironment into which an appropriately sized nonpolar drug molecule, or more often nonpolar moieties of the drug molecule, can enter to form inclusion complexes. No covalent bonds are formed or broken during formation of the drug-cyclodextrin complex. In aqueous solutions, free drug molecules are in dynamic equilibrium with those bound within the cyclodextrin [8].

In a one study, increase in poor buffer pH 5 and 6 solubility of KZ was studied. Two systems were used: binary complexes prepared with β-CD and multicomponent systems (β-CD and an acid compound), obtained by spray-drying. This confers improved drug solubility in both media studied [9]. In this study, attemps were made for the preparation inclusion complex of KZ using β-CD and several methods namely, freeze-drying, spray-drying, co-evaporation and kneading to improve the physicochemical properties of KZ. Concerning the pharmaceutical interest, vaginal suppositories with this complexes were prepared and evaluated.

Materials and methods

Materials

KZ was generously donated by the Bilim İlaç (Türkiye). β-CD, polyethylene glycol 6000, 1000 and 400 were purchased from Sigma (Germany), Merck (Germany), Merck (Germany) and Fluka (Germany), respectively. All other chemicals and solvents were of analytical reagent grade. Water was purified and deionized by the Millipore Elix® 5 UV system (USA).

High performance liquid chromatographic (HPLC) measurement

Since the method of HPLC (Shimadzu 20-A, Japan) was used to determine the amount of KZ loaded in the inclusion complexes in the phase-solubility studies and in the dissolution tests of vaginal suppositories, this method was validated [10]. The liquid chromatograph is equipped with a 223 nm detector, a 4.6 mm × 25 cm analytical column that contains 5 µm packing (ACE-C_{18} column). The column temperature is maintained at 30 °C, and the flow rate is 1 mL per minute. The mobile phase was composed of water (0.34% tetrabutylammonium hydrogen sulfate) and acetonitrile 75:25 (v/v) [11]. Injection volume was 25 µL. KZ retention time was 7.6 min.

Phase solubility studies

The solubility studies were carried out, according to the method previously reported by Higuchi and Connors [12], by using a vibrated horizontal shaker (Heidolph Vibramax 100, Germany). Excess amounts of drug (150 mg) were added to 10 mL of an aqueous solution containing various concentrations of β-CD [0–10 mM]. The suspensions were vigorously shaken at 25 ± 2 °C for 3 days. This time is considered sufficient to reach the equilibrium according to a preliminar study. After equilibration, the suspensions were filtered through (Sartorius Minisart®) 0.45 µm membrane filters, appropriately diluted and KZ concentration in the filtrate was analyzed by HPLC at 223 nm. Each experiment was carried out in triplicate. The phase-solubility diagram at this temperature was obtained by plotting the amount of dissolved KZ (mM) versus the amount of β-CD added (mM). The stability constant $K_{1:1}$ calculated from the equation: $K_{1:1} = \text{slope}/S_o(1\text{-slope})$ where slope is the value obtained from linear adjust of data and S_o is the solubility of KZ in the absence of β-CD [12].

Preparation of solid inclusion complex

The preparation of KZ/β-CD solid inclusion complex was performed by different techniques, which are described below in detail. The 1:1 molar ratio was based on the previous solubility studies.

Freeze-drying (FD)

KZ and β-CD were mixed in water in the molar proportion 1:1 and stirred at 25 °C for 3 days protected from light with vibrated shaker. After this period the solid residue was separated by centrifugation at 15,000 rpm for 15 min and the upper liquid layer was filtered over 0.45 μm membrane. The resulting solution was frozen at −18 °C for 24 h, and then dried by lyophilization (Leybold Heraeus Lyovac GT-2, Germany) for the solid inclusion complex to be collected [13]. The same procedure was carried out without KZ for preparing placebo formulation (PLA FD).

Spray-drying (SD)

KZ was dissolved in ethanol, β-CD was dissolved in distilled water with the molar proportion 1:1. The two solutions were mixed and atomized into the drying chamber. The spray-dryer (Büchi, 190, Switzerland) with a standard nozzle (0.7 mm diameter) was operated under the following conditions: inlet temperature, 105 ± 1 °C; outlet temperature, 58 ± 1 °C; drying air flow rate, 7 mL min^{-1}.

Co-evaporation (CE)

Co-evaporated products were obtained by dissolving known amounts of β-CD in distilled water at 25 °C and drug (giving the desired drug: β-CD molar ratio) in ethanol at the same temperature. The solutions were added together after the powders were completely dissolved. Then, the solvents were removed using a rotary evaporator at 75 °C and 210 rpm, under the vacuum which took about 3–4 h. The sample was kept in a desiccator overnight to remove traces of solvents [14].

Kneading (KN)

Kneaded products were obtained by adding a small volume of a water–ethanol (50/50, v/v) solution to the drug-β-CD physical mixture (1:1 drug:β-CD molar ratio) and kneading the resultant mixture thoroughly with a pestle to obtain a homogeneous slurry, and continuing until the solvent was completely removed. The sample was kept in a desiccator overnight to remove traces of solvent [14].

All the formulations obtained were kept in a dessicator with $CaCl_2$ at atmospheric pressure and room temperature until analysis and use in vaginal suppository formulations. Percent practical yields of solid complexes were calculated with this equation: practical yield (%) = (practical mass of solid complex/theoretical mass of drug and carrier)×100.

Characterization of solid inclusion complex

To determine whether the four methods used in this study resulted in an inclusion complexation or a physical mixture of the KZ with β-CD, examinations were conducted using aqueous solubility, FT-IR, thermal, XRD, NMR (^1H and ^{13}C) and active agent analysis. To investigate the increase of aqueous solubility of KZ after the complexation, excess amounts of prepared complexes were added to water. The suspensions were shaken at 25 °C for 15 min. After, the suspensions were filtered through 0.45 μm membrane filters and analyzed by HPLC ($n = 3$). Separately to investigate the effect of β-CD on aqueous solubility of KZ, physical mixture (1:1 drug: β-CD molar ratio) was prepared by gently mixing with simulated turbula shaker. After this, study of aqueous solubility of KZ was carried out for this physical mixture ($n = 3$). All FT-IR spectra were carried out in a Perkin Elmer spectrophotometer model (Perkin Elmer Spectrum 2000, UK) in KBr pellets. The spectra were obtained at the region of 4000–400 cm^{-1}. Thermal analysis of the individual components or KZ/β-CD combinations were performed using a differential scanning calorimeter (DSC 60, Shimadzu, Japan) with a nitrogen flow rate of 50 mL min^{-1} and a heating rate of 10 °C min^{-1} from 50 to 200 °C. Aluminum was used as standard. The powder X-ray diffraction patterns of individual KZ, β-CD and drug/cyclodextrin combinations were determined using the X-ray diffractometer (XRD-Rikagu D/Max-3C, Japan), with voltage 40 kV, current 20 mA and 2θ over a 2–40° range at a scan rate of 2°min^{-1}. ^1H and ^{13}C-NMR spectra were recorded at 25 °C on a Ultra Shield CP MAS NMR (Germany), in D_2O (deuterium) and $(CD_3)_2CO$ (deuteroaceton). To find out the percentage of the active agent in solid complexes, about 10 mg complexes were dissolved in 5 mL of mobile phase. The solutions were analyzed by HPLC and the amount of KZ was calculated by using regression equation. The experiment was carried out in triplicate for each complex.

Preparation of vaginal suppositories contained solid KZ/β-CD complexes

Vaginal suppositories were prepared by fusion method. The suppositories containing pure KZ were prepared using three different bases as PEG 6000, 1000 and 400 with their different ratios in preliminary in vitro experiments. The vaginal suppositories were prepared by using solid KZ/β-

CD complexes with PEG 6000 and 400 (1:1 mass ratio) which was decided as the most suitable ratio base according to the good results of the preliminary in vitro experiments. For example, each suppository of K–KN–VSup formulation was contained 1237.58 mg K–KN complex, 1853.71 mg PEG 6000 and 1853.71 mg PEG 400. The each solid KZ/β-CD complexes were mixed thoroughly in the melted suppository base separately, and the resultant mixture was poured into moulds and allowed to solidify at 25 °C.

Each formulation with prepared solid complexes contained 400 mg of KZ (except K–FD complex contained formulation) which is equal to the amount in the commercial conventional vaginal suppository (Ketoral®, Bilim İlaç, Türkiye) (Table 1).

In vitro KZ release from vaginal suppositories

The dissolution behaviour of KZ from vaginal suppository formulations which are prepared with pure KZ and solid KZ/β-CD complexes was determined using the USP31 dissolution apparatus II in 1000 mL [15] simulated vaginal fluid (SVF, pH 4.2) as the dissolution medium. This simulant given as compound and weight (g), is as follows: NaCl 3.51; KOH 1.40; $Ca(OH)_2$ 0.222; bovine serum albumin 0.018; lactic acid 2.00; acetic acid 1.00; glycerol 0.16; urea 0.4; glucose 5.0 and water to 1000 mL [16]. Once these compounds are combined, the mixture is adjusted to a pH of 4.2 using HCl. The rate of stirring was 50 rpm [17]. The medium temperature was maintained at 37 ± 0.5 °C. At each sampling interval, 2 mL of the dissolution medium was withdrawn and replaced by an equal volume of fresh SVF. The samples were filtered and assayed for KZ using HPLC ($n = 3$). The same procedure was applied to the commercial vaginal suppository (Ketoral®, Bilim İlaç, Türkiye) of KZ. All samples contained 400 mg KZ (only K–FD–VSup contained 100 mg KZ) which is claimed for the commercial vaginal suppository.

Results and discussion

Phase-solubility

Figure 1 shows the aqueous phase-solubility of KZ in different concentrations of the β-CD at 25 °C. The solubility of KZ is increased with the increase in β-CD concentrations. These linear phase diagram is classified as A_L-type [12] and is considered indicative of the formation of soluble complexes between the substrate (the KZ) and the ligand (the β-CD). This type of diagram indicates that the solubility of KZ increased linearly with the increase of β-CD concentration, depending on the aqueous solubility of the β-CD [18]. The $K_{1:1}$ value found was 3706 M^{-1}.

Validation of analytical method

Results of the validation studies are as follows; calibration curves of KZ were linear in the concentration range of 10–1000 μg mL^{-1} ($r = 0.9996$). Limits of detection and quantification were 3 and 9 μg mL^{-1}, respectively. Recovery was greater than 98%. Intra- and inter-day relative standard deviation was less than 3.3 and 2.1%, respectively. HPLC method for KZ was determined to be reliable, linear, precise, accurate and selective [10].

Determination of the aqueous solubility of KZ

It has been extensively reported in the literature, that β-CD molecules are able to increase the guest molecule solubility [19]. In the present work, the aqueous solubility of KZ as a function of different preparation methods of KZ/β-CD complexes is presented in Table 1. As concerns solubility studies at 25 °C, an increase of KZ solubility was observed in the presence of β-CD. The results given in Table 1 show that the solubility of KZ in a K–FD complex is increased to, 2.58 mg mL^{-1} while solubility of free KZ had not obtained in water and solubility of free KZ in physical mixture is 0.30 ± 0.01 (mg mL^{-1} ± SD) ($n = 3$). The KZ solubility increase was strictly related to the magnitude of

Table 1 Properties of KZ/β-CD solid complexes and vaginal suppository formulations prepared with complexes ($n = 3$)

Code of solid complex	Preparation method	Practical yield ± SD (%)	Aqueous solubility ± SD (mg mL^{-1})	Amount of KZ in complex ± SD (%)	Suppository code	Amount of KZ in suppository KZ ± SD (mg)
					KZ–VSup	385.14 ± 144.86
K–FD	Freeze-drying	59.06 ± 6.98	2.58 ± 0.15	5.33 ± 0.05	K–FD–VSup	100.72 ± 2.74
K–SD	Spray-drying	51.00[a]	2.42 ± 0.10	32.55[a]		
K–CE	Co-evaporation	96.30 ± 4.54	1.18 ± 0.19	30.60 ± 6.8	K–CE–VSup	423.02 ± 20.56
K–KN	Kneading	93.47 ± 2.84	0.68 ± 0.01	34.37 ± 7.79	K–KN–VSup	414.62 ± 38.31

[a] Unique batch

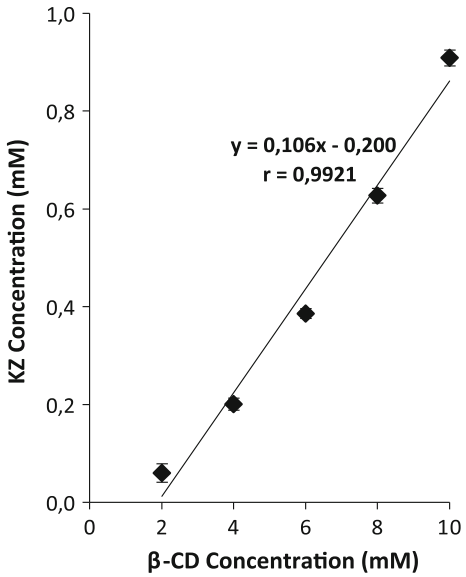

Fig. 1 Phase-solubility diagram for the KZ/β-CD system (mean ± SD, $n = 3$)

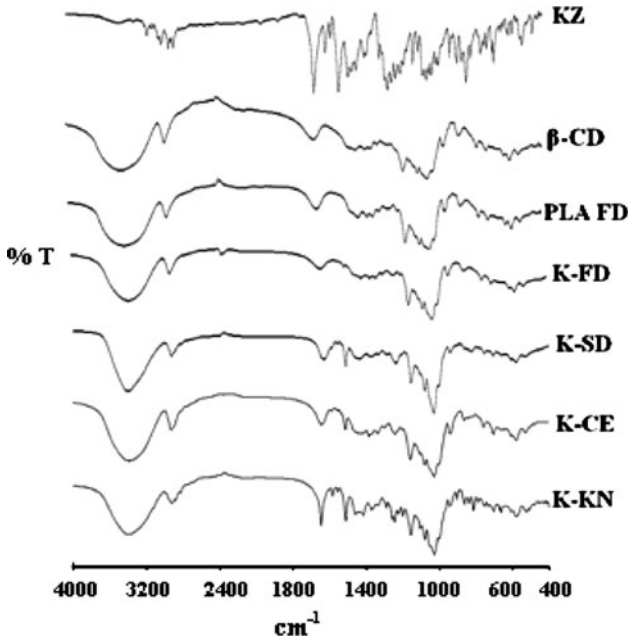

Fig. 2 FT-IR spectra of pure KZ, pure β-CD and KZ/β-CD complexes

the interaction of the drug with β-CD, thus confirming the higher affinity of KZ for the apolar cavity of β-CD. The significant enhancement of the solubility that occured with freeze-dried and spray-dried has been attributed to: complexation in the solid state and to the high energetic amorphous state/reduction of the crystallinity following complexation [20].

FT-IR analysis

In order to investigate the vibrational changes upon host:-guest interaction between KZ and β-CD, FT-IR spectroscopy was used. FT-IR spectrocopy technique is useful to identify which vibrational mode of drug and β-CD is being disturbed during the inclusion process, suggesting the interactions between these molecules in solid state [21].

More evidence of complex formation was obtained by FT-IR spectroscopic investigation of the bands of the functional groups of KZ involved in the complexation. The infrared spectrum of KZ shows the presence of characteristic peaks at 1646, 1585, 1512, 1458, 1291, 1244 and 1106 cm^{-1} (Fig. 2). Compare FT-IR spectra of KZ, β-CD and complex of KZ/β-CD with prepared freeze-drying method, characteristic peaks of KZ in 1458, 1291, 1244 and 1106 cm^{-1} disappeared. Also C–N group appearing in 1646, 1585 and 1512 cm^{-1} disappeared in K–FD, so we can deduce that C–N in KZ was included into cavity of β-CD [22].

When the FT-IR spectra of the product of kneading was examined, the principal peaks of KZ were observed to be present although with lower intensity. FT-IR spectra of the products of spray-drying, and co-evaporating were examined, the peaks in 1512 and 1244 cm^{-1} were observed to be present with lower intensity, while the peaks in 1106, 1219, 1458, 1585 and 1646 cm^{-1} were disappeared. These IR spectra changes could be evidence of the alteration as a consequence of the drug interaction with β-CD.

DSC analysis

Thermal analysis has been reported as a method to characterize cyclodextrin complexes. When guest molecules are incorporated in the CD cavity, their melting, boiling or sublimation points usually shift to a different temperature or disappear within the temperature range where the CD is dehydrated or decomposed. The absence of the characteristic peak of drug is strong evidence of the inclusion of the drug into the cyclodextrin cavity.

The KZ thermal curve is typical for crystalline anhydrous substance and is all characterized by a sharp endothermic effect (peak temperature at 154 °C), assigned to its melting (Fig. 3). The β-CD displays a wide and strong endothermic effect in the 70–160 °C interval, which may be ascribed to dehydration or fusion. In the freeze-dried systems, the drug endothermal effect further broadened and was almost hidden by the dehydration band of the carrier, and finally it disappeared in the spray-dried system. This last phenomenon, attributable both to inclusion complex formation and/or drug amorphization [23].

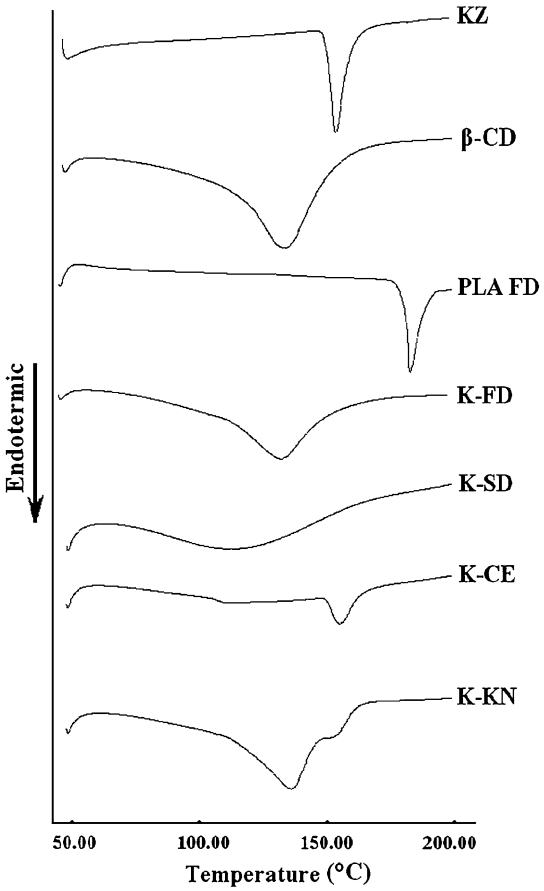

Fig. 3 DSC curves of pure KZ, pure β-CD and KZ/β-CD complexes

The DSC curve of the K–KN is similar to the one of β-CD, however, a weak endothermic effect can be observed at about 155 °C, considering as a small quantity of free KZ. The intensity of these endothermic peaks is smaller than those observed on the DSC thermograms of KZ and cyclodextrin components before complexation. This lower intensity can possibly be attributed to the sum of the individual components and can also be indicative of the formation of an inclusion complex. Characterization by DSC showed the disappearance of the drug fusion peak at 154 °C in the case of K–FD, K–SD and K–KN complexes, whereas this endothermic peak was still present in K–CE system. Marked reduction of area, broadening, and downshift of the peak temperature of drug melting endoterm ($\sim$155 °C) were observed in the K–CE, is indicative of the more evident loss of drug cristallinity. Accordingly, no or at most partial complex formation is expected.

PXRD analysis

Characteristic peaks observed in the XRD patterns showed high crystallinity of pure KZ [24] and also showed that β-CD complies with the standard data file. The diffraction

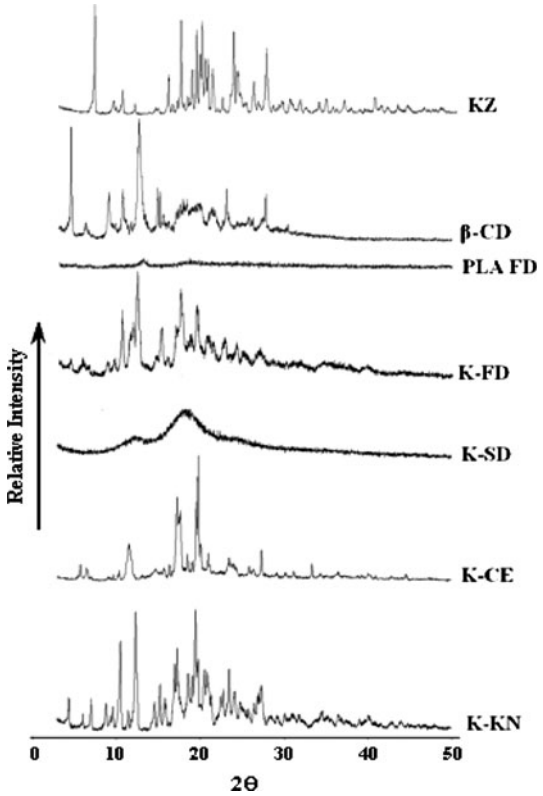

Fig. 4 PXRD patterns of pure KZ, pure β-CD and KZ/β-CD complexes

pattern of KZ powder revealed several sharp high intensity peaks at diffraction angles (2θ) of 15.96°, 17.42°, 20.56°, 21.14°, 23.62° and 27.48° (Fig. 4).

The freeze-drying and spray-drying preparation procedure led to formation of amorphous samples that were investigated to evaluate the formation of KZ/β-CD complexes.

The freeze-dried products exhibited a decrease in the diffraction peaks. This indicates that these products were markedly less crystalline than the individual components, implying the formation of a new structure. It has been reported previously that formation of complexes is accompanied by a decrease in the relative crystallinity of the dispersions [25]. Spray-drying usually yields amorphous products [20, 26]. Complete KZ amorphization was also detected in the spray-dried product as a result of the preparation technique. Nevertheless, having in account the results of DSC analysis and the complete drug amorphization in the spray-dried product, it can be assumed that the formation of some new solid phases may be credited to the formation of inclusion complexes in which KZ is at least partially entrapped in the CD cavity [27, 28].

The crystallinity loss was most pronounced for the product prepared by freeze-drying and spray-drying, suggesting an almost complete drug amorphization and/or complexation in aggrement with DSC and FT-IR analysis.

However, these techniques (DSC, FT-IR and XRD) can not provide a clear answer about the type of complex formed (inclusion or adsorption) or the structural conformation of the molecules involved. This information can only be provided by high resolution nuclear magnetic resonance spectroscopy, since this technique allows a clear distinction between inclusion and other possible external interaction processes by observing guest and host molecules simultaneously and is capable to differentiate the part of the guest molecule involved in the interaction with the CD cavity [27, 28].

XRD patterns of the K–CE was examined, the all principal peaks of KZ were observed to be present despite the lower intensity. There is an intense increase in characteristic peak only at 17.32° 2θ value. We can conclude that the most crystalline complex was the one obtained by the evaporation method [29]. A crystalline pattern, given by the sum of the spectra of pure KZ, was obtained for the co-evaporated product, suggesting no drug–CD interaction as indicated by DSC and FT-IR analysis.

In a similar study, the characteristic peaks of econazole and β-CD, although significantly reduced in intensity, were still detectable in the product obtained by co-evaporation, indicating that the drug maintained a residual crystallinity in this product and only partially interacted with β-CD [30].

XRD patterns of the K–KN was examined, the several principal peaks of KZ were disappeared though the existance of sharp, high and intense two peaks at diffraction angles (2θ) of 17.32° and 20.60°. The presence of KZ peaks in the diffractogram of KZ/β-CD, kneaded system could suggest the presence of the free crystalline drug, despite the reduction in number and intensities, as a similar to literature [31].

NMR analysis

NMR (nuclear magnetic resonance) is the most effective method for studying space conformation of β-CD inclusion. Therefore prior to the decision for formation of an inclusion complex, the complexation of the drug molecules with β-CD have been characterized by ^{1}H NMR and ^{13}C NMR. The formation of an inclusion complex is evidenced by an up- or downfield shift of some of the drug protons and of the CD protons [32].

Spectra of the ^{1}H NMR and ^{13}C NMR were examined, the all principal peaks of KZ were observed (Figs. 5, 6). Peaks in 3–5 ppm intervals are belong to –CH and –CH_2 groups and peak at 2.8 ppm belongs to –CH_3 in the spectrum of KZ of ^{1}H NMR analyses.

Twenty-four carbon peaks are observed in the ^{13}C NMR spectrum of KZ through same structure in the benzene ring, although KZ have 26 carbons. ^{1}H NMR spectroscopy of

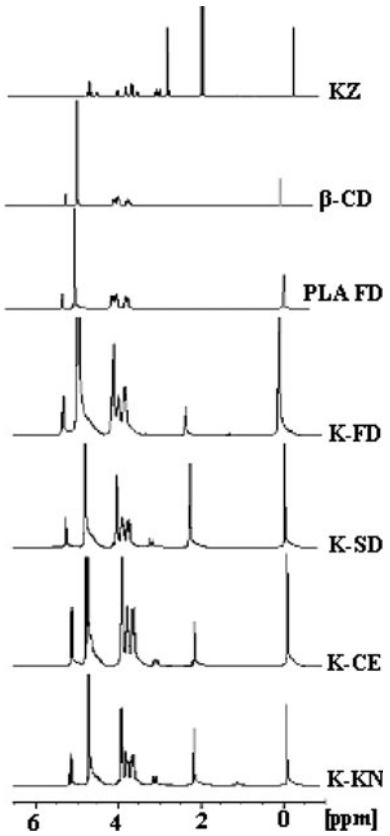

Fig. 5 ^{1}H NMR spectra of pure KZ, pure β-CD and KZ/β-CD complexes

freeze-dried products, when complexed with the β-CD, demonstrated an upfield shift (0.082 ppm) in K–FD. These shifts suggests that the aromatic groups (benzene ring or diclorophenil structure) of the KZ were interacting with the β-CD [33].

Dissolution behaviour of vaginal suppositories

The cyclodextrin has been playing 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 [34]. The increased solubility and dissolution rates can be determined from phase-solubility diagram and drug dissolution kinetics, respectively. In general, it can be concluded that the increased dissolution rate of CD-entrapped drug molecules is a result of various factors: an increased solubility, an improved wettability, molecular dispersion and large area available for dissolution [35].

Dissolution patterns of the vaginal suppositories made from the KZ/β-CD complexes, pure drug and commercial vaginal suppository of KZ in SVF were determined using the USP 31 paddle dissolution method, as shown in Fig. 7. It is evident that the dissolution rate of the vaginal suppositories

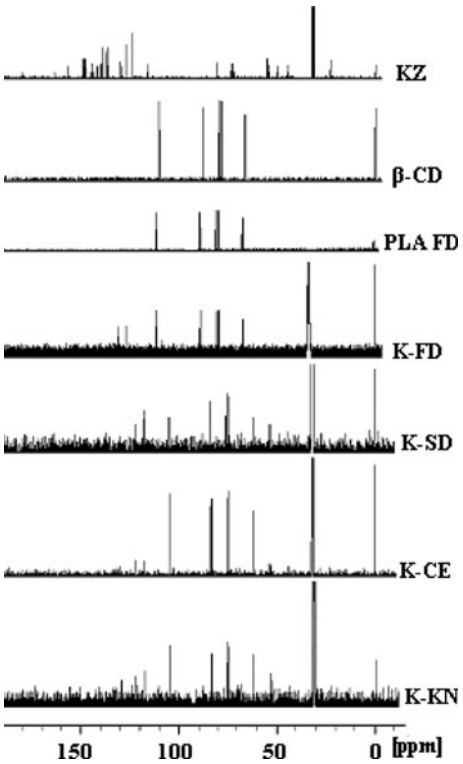

Fig. 6 ^{13}C NMR spectra of pure KZ, pure β-CD and KZ/β-CD complexes

which are prepared freeze-dried and kneaded complexes of drug with β-CD were faster than that of the vaginal suppository of pure drug and commercial preparation. This might be due to the high energetic amorphous state of freeze-dried and kneaded products, resulting in a faster dissolution rate, because the reduction of drug crystallinity on complexation or solid dispersion with cyclodextrins also contributes to the cyclodextrin increased apparent drug solubility and dissolution rate [36]. Furthermore, β-CD has surfactant-like properties which can reduce the interfacial tension between water-insoluble drug and the dissolution medium, leading to a higher dissolution rate [20].

Thus, the enhanced dissolution rate of the vaginal suppositories which are prepared freeze-dried and kneaded complexes of drug with β-CD might be due to decrease in crystallinity and formation of an inclusion complex.

Conclusion

KZ was encapsulated by β-CD, forming an inclusion complex and the ratio of 1:1 the complex was valued by the several methods namely, kneading, co-evaporation, spray-drying and freeze-drying. The results showed that the inclusion process was occured all the complexation methods except co-evaporation. Its structure was confirmed by

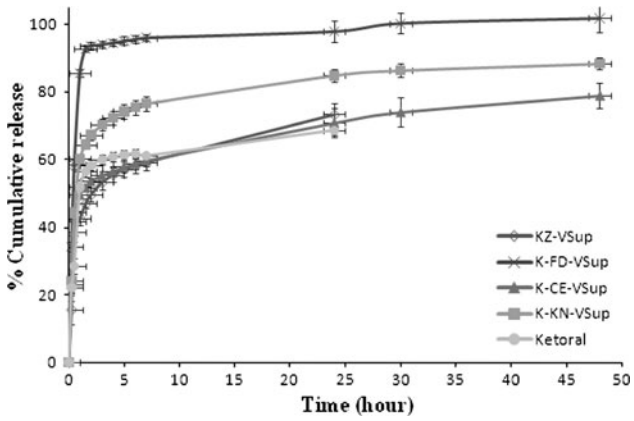

Fig. 7 Dissolution profiles of vaginal suppositories containing KZ/β-CD complexes and Ketoral®

DSC, XRD, FT-IR, ^{1}H NMR and ^{13}C NMR, which all verified the inclusion complex formation between β-CD and KZ. Furthermore, solubility and dissolution studies suggest that the β-CD is the most suitable vehicle for the KZ, since the aqueous solubility of KZ, which is less than 0.017 mg mL^{-1} for the pure KZ [3] increases to 0.303 mg mL^{-1} for the physical mixture KZ and β-CD, and reaches 2.580 mg mL^{-1} for the inclusion complexes prepared by freeze-drying that was possessed the highest formation complex, acceptable solubility and dissolution profiles. The present results indicate potential use of β-CD to improve the solubility of KZ if the inclusion complex is prepared by the freeze-drying.

As a conclusion, the overall evaluation of KZ/β-CD complexes developed showed that bioavailability problems can be overcome by increasing the aqueous solubility and the release.

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