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ORIGINAL ARTICLE

Inclusion complexes of fluconazole with β-cyclodextrin: physicochemical characterization and in vitro evaluation of its formulation

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Abstract Fluconazole (FZ) is a triazole antifungal drug administered orally or intravenously. It is employed for the treatment of mycotic infections. However, the efficacy of FZ is limited with its poor aqueous solubility and low dissolution rate. One of the important pharmaceutical advantages of cyclodextrins is to improve pharmacological efficacy of drugs due to increasing their aqueous solubility. The aim of present study was to prepare an inclusion complex of FZ and β-cyclodextrin (β-CD) to improve the physicochemical and biopharmaceutical properties of FZ. The effects of β-CD on the solubility of FZ were investigated according to the phase solubility technique. Complexes were prepared with 1:1 M ratio by different methods namely, freeze-drying, spray-drying, co-evaporation and kneading. For the characterization of FZ/β-CD complex, FZ amount, practical yield %, thermal, aqueous solubility, XRD, FT-IR and NMR (^{1}H and ^{13}C) analysis were performed. In vitro dissolution from hard cellulose capsules containing FZ/β-CD complexes was compared to pure FZ and its commercial capsules and evaluated by f_1 (difference) and f_2 (similarity) factors. Paddle method defined in USP 31 together with high pressure liquid chromatographic method were used in in vitro dissolution experiments. It was found that solubility enhancement by FZ/β-CD complexes depends on the type of the preparation method. High release of active agent from hard cellulose capsules prepared with β-CD complexes compared to commercial capsules was attributed to the interactions between β-CD and active agent, high energetic amorphous state and inclusion complex formation.

Keywords Fluconazole · β-cyclodextrin · Phase solubility · Inclusion complex · Hard cellulose capsules

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Introduction

Fluconazole (FZ) is a triazole derivative antifungal active agent which is widely used for the treatment of oropharyngeal and esophageal candiasis, serious systemic candidal infections, urinary tract infections, pneumonia and peritonitis. FZ is also used for suppressive therapy and for the acute treatment of cryptococcal meningitis for those with acquired immunodeficiency syndrome [1, 2]. FZ is well absorbed following oral administration, bioavailability from the oral route being 90%. The efficacy of FZ is limited according to its poor aqueous solubility and low dissolution rate. In addition, because of its serious hepatotoxicity reported, FZ has to be used with caution in patients with impaired renal or hepatic function [1].

Many technological methods namely micronization, formation of solvates, adsorbates, complexes, microspheres, and solid dispersions have been used to improve the solubility and dissolution characteristics of poorly water-soluble drugs. 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 [3].

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 [4].

β-cyclodextrin (β-CD) is used many times 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 [5].

The aim of this study was to prepare an inclusion complex of FZ and β-CD to improve the physicochemical and biopharmaceutical properties of FZ. Several methods such as freeze-drying (FD), spray-drying (SD), co-evaporation (CE) and kneading (KN) were used to prepare inclusion complex of FZ/β-CD. The other aim was to prepare hard cellulose capsules with this complexes for investigation in vitro release rate of FZ.

Materials and methods

Materials

FZ was a kind gift from Bilim İlaç, Türkiye. β-CD was purchased from Sigma, USA. All other chemicals and solvents were of analytical grade and used without further purification.

Determination of fluconazole by HPLC

FZ in capsules, phase solubility and dissolution medium was determined by high-performance liquid chromatography (HPLC) which validated, a United States Pharmacopoeia monograph method (USP 31) [6, 7]. The HPLC system (Shimadzu 20-A, Japan) was composed of a pump, a degasser, an autosampler, a column heater and a ultraviolet detector. FZ was separated by a 4.6-mm × 25-cm analytical column that contains 5-µm packing (ACE-C_{18} column) and detected at 223 nm. The column temperature is maintained at 30 °C, and the flow rate is 1 mL per min. The mobile phase was composed of water (0.34% tetrabutylammonium hydrogen sulfate) and acetonitrile 75:25 (v/v) (USP 31). Injection volume was 25 µL. FZ retention time was 5.1 min.

Phase solubility studies

The effect of β-CD on the solubility of FZ was investigated according to the phase solubility technique [8]. Excess amounts of FZ (150 mg) were added to either distilled water or 10 mL of aqueous solutions containing increasing concentrations of the β-CD (0.0–20.0 mM). The suspensions obtained were shaken at room temperature (25 ± 2 °C) in a horizontal shaker (Heidolph, Vibramax 100, Germany) for 4 days that is considered sufficient to reach the equilibrium according to a preliminary study. After equilibrium was attained aliquots were withdrawn, filtered through a 0.45 µm membrane filter (Sartorius, Minisart®) and assayed with HPLC. FZ concentration in the filtrate was analyzed by HPLC at 223 nm ($n = 3$). The phase solubility diagram at this temperature was obtained by plotting the amount of dissolved FZ (mM) versus the amount of β-CD added (mM).

Preparation of solid inclusion complex

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

Freeze-drying

The required amount of FZ and aqueous solution of β-CD (20.0 mM) were mixed (1:1 FZ: β-CD molar ratio) and stirred at 25 °C for 4 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 48 h by lyophilization (Leybold Heraeus Lyovac GT-2, Germany) for the solid inclusion complex to be collected [9].

Spray-drying

Equimolar quantities of FZ and β-CD were dissolved in ethanol and distilled water, respectively. 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} [10].

Co-evaporation

Co-evaporated products were obtained by dissolving known amounts of β-CD in distilled water at 25 °C and drug (1:1 FZ:β-CD molar ratio) in ethanol at the same temperature. The solutions were added together after the powders were completely dissolved. The solvents were then removed using a rotary evaporator at 75 °C and 210 rpm, which took about 3–4 h. The sample was kept in a desiccator overnight to remove traces of solvents [11].

Kneading

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 FZ: 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 [11].

All the formulations obtained were kept in a dessicator with $CaCl_2$ at atmospheric pressure and room temperature until analysis and fill into hard cellulose capsules. Practical yields of solid complexes were calculated based on the amounts of material used (Table 1).

Characterization of solid inclusion complex

Total FZ content, aqueous solubility, thermal (differential scanning calorimetry, DSC), X-ray diffractometry (XRD), Fourier transform–infrared Spectroscopy (FT–IR) and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) analysis were performed for the characterization of FZ/β-CD inclusion complex.

Total FZ content

In order to find out the percentage of the FZ in solid complexes, 10 mg complex was dissolved in 5 mL of mobile phase. The solutions were analyzed by HPLC and the amount of FZ was calculated by using regression equation ($n = 3$).

Determination of the aqueous solubility of FZ

To investigate of increase on aqueous solubility of FZ after the complexation, excess amounts of prepared complexes were added to water (pH 6.8). 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 ($n = 3$).

Differantial scanning calorimetry (DSC)

Thermal analysis of the samples (FZ, β-CD, physical mixture of 1:1 FZ/β-CD molar ratio and inclusion complex powders) were carried out with a DSC 60 (Shimadzu, Japan). The samples were heated from 50 to 200 °C at a heating rate of 10 °C min^{-1}. All the DSC measurements were made in nitrogen atmosphere and the flow rate was 50 mL min^{-1}.

Powder X-ray diffractometry (PXRD)

Powder X-ray diffractometry of the samples (FZ, β-CD and inclusion complex powders) was performed with an X-ray diffractometer (XRD-Rikagu D/Max-3C, Japan) 2θ over a 2°–40° range at a scan rate of 2 min^{-1}. The pattern was collected with 40 kV of tube voltage and 20 mA of the current in step scan mode.

Fourier transform–infrared spectrometry (FT–IR)

The FT–IR spectra were recorded from 4,000 to 400 cm^{-1}, using a Perkin Elmer spectrophotometer model (Perkin Elmer Spectrum 2000, UK) on samples prepared as KBr discs.

^{1}H-and ^{13}C-Nuclear magnetic resonance (^{1}H-and ^{13}C-NMR)

^{1}H and ^{13}C-NMR spectra were recorded at room temperature on a Ultra Shielded Bruker Avance 500 MHz NMR spectrometer (Germany) in D_2O.

Table 1 Properties of FZ/β-CD solid complexes ($n = 3$)

Code of solid complex	Preparation method	Practical yield (% ± SD)	Aqueous solubility ± SD (mg mL^{-1})	Total FZ content ± SD (mg)
FZ–FD	Freeze drying	69.69 ± 6.24	8.359 ± 0.03	25.47 ± 0.74
FZ–SD	Spray drying	34.25 ± 7.80	7.806 ± 0.06	22.22 ± 0.26
FZ–CE	Co-evaporation	93.94 ± 0.92	7.883 ± 0.52	24.53 ± 0.79
FZ–KN	Kneading	94.12 ± 0.22	8.270 ± 0.07	21.98 ± 0.74

Preparation of hard cellulose capsules contained solid FZ/β-CD complexes

Solid FZ/β-CD complexes containing 50 mg of FZ were manually filled into hard cellulose capsules of Number 0.

In vitro FZ release from capsules

The dissolution studies of hard cellulose capsules prepared with FZ/β-CD complexes were performed according to the USP 31 paddle method (Aymes, Türkiye) in 500 mL degassed distilled water as the disolution medium. The stirring speed was 50 rpm and the temperature was maintained at 37 ± 0.5 °C [12]. At each sampling interval, 2 mL of the dissolution medium was withdrawn and replaced by an equal volume of fresh medium. The samples were filtered and analyzed by HPLC for FZ ($n = 6$). The same procedure was applied to the commercial hard gelatine capsules (Fluzole®, Umut İlaç, Türkiye) of FZ. All capsules contained 50 mg of FZ which is claimed for the same as commercial hard gelatine capsules content. Dissolution profiles of commercial preparate and the four formulations are presented in Fig. 5. Difference and similarity factors were calculated to evaluate the difference of the commercial hard gelatine capsule of FZ between hard cellulose capsules (HCC) prepared with solid FZ/β-CD complexes.

Results and discussion

Different techniques, such as DSC, aqueous solubility, PXRD, FT-IR and NMR (^{1}H and ^{13}C), were used to characterize and compare the physicohemical properties of the solid complexes prepared between FZ and β-CD, in order to investigate and compare the potential and effectiveness of the different preparation methods.

Phase-solubility

Phase solubility diagram of the FZ/β-CD system (Fig. 1) was verified that the solubility of FZ increased linearly with increasing β-CD concentration at 25 °C.

These linear phase diagram is classified as A_L-type [8] and are considered indicative of the formation of soluble complexes between the substrate (FZ) and the ligand (β-CD). This type of diagram indicates that the solubility of FZ increased linearly with the increase of β-CD concentration [13].

Validation of analytical method

Results of the validation studies were as follows; calibration curves of FZ were linear in the concentration range of

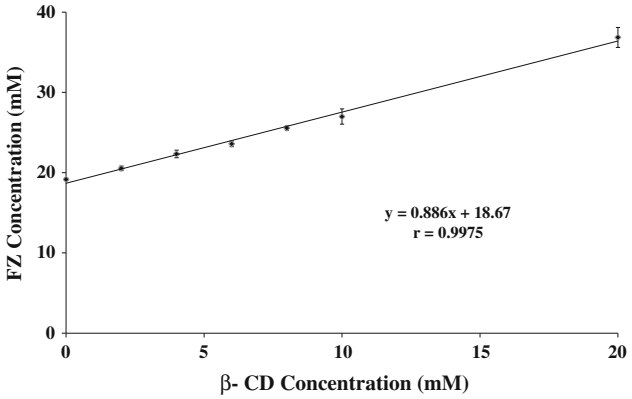

Fig. 1 Phase solubility diagram for FZ/β-CD guest–host system

10–1,000 μg mL^{-1} (R = 0.9997). Limits of detection and quantification were 1.898 and 5.751 μg mL^{-1}, respectively. Recovery was greater than 98%. Intra- and inter-day relative standard deviation was less than 0.756 and 0.803%, respectively. Therefore HPLC method for FZ was determined to be reliable, linear, precise, accurate and selective [7].

Determination of the aqueous solubility of FZ

The aqueous solubility of FZ as a function of different preparation methods of FZ/β-CD complexes are presented in Table 1. When the solubility of free FZ is 5.55 mg mL^{-1} in water, the solubility of FZ/β-CD complexes increases to 7.806 ± 0.06–8.359 ± 0.03 mg mL^{-1}. As it is shown in these results, also it has been extensively reported in the literature, that β-CD molecules are able to increase solubility of the guest molecule by complexation [11, 14, 15].

Thermal analysis

DSC curves for pure FZ, pure β-CD, physical mixture (FZ–PM) and FZ/β-CD complexes obtained by different preparation methods are shown in Fig. 2. Pure β-CD exhibited a broad endothermal effect, ranging between 50 and 170 °C corresponding to its dehydration. Pure FZ showed sharp melting endotherm at 142.66 °C [16, 17]. Physical mixture showed the complete disappearance of the FZ endothermic peak, indicating hidden by the dehydration band of the carrier. Characterization by DSC showed the disappearance of the drug fusion peak at 142.66 °C in the case of FZ–SD, FZ–CE and FZ–KN complexes. This endothermic peak is still present in FZ–FD system whereas there is a new endothermic peak at the range of 140–160 °C that possibly shows complex formation [18]. The exothermic peak was observed for the spray-dried product. The peak in thermogram of FZ–SD may be due to the degradation.

Disappearance or decrease in intensity of the drug endothermic peak might be related to possible drug–CD

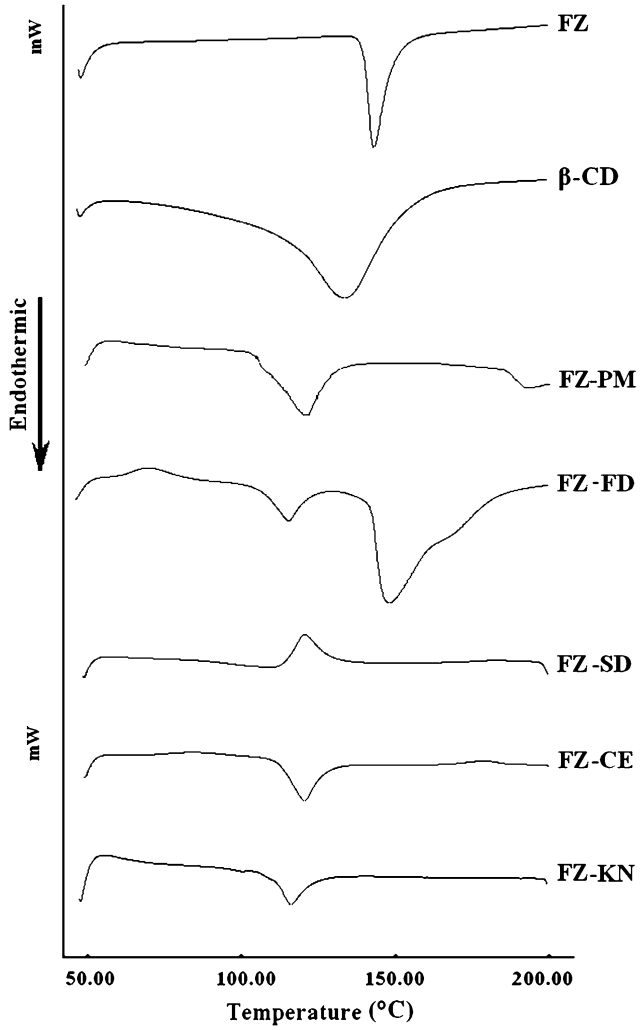

Fig. 2 DSC curves of pure FZ, pure β-CD, physical mixture and FZ/β-CD complexes

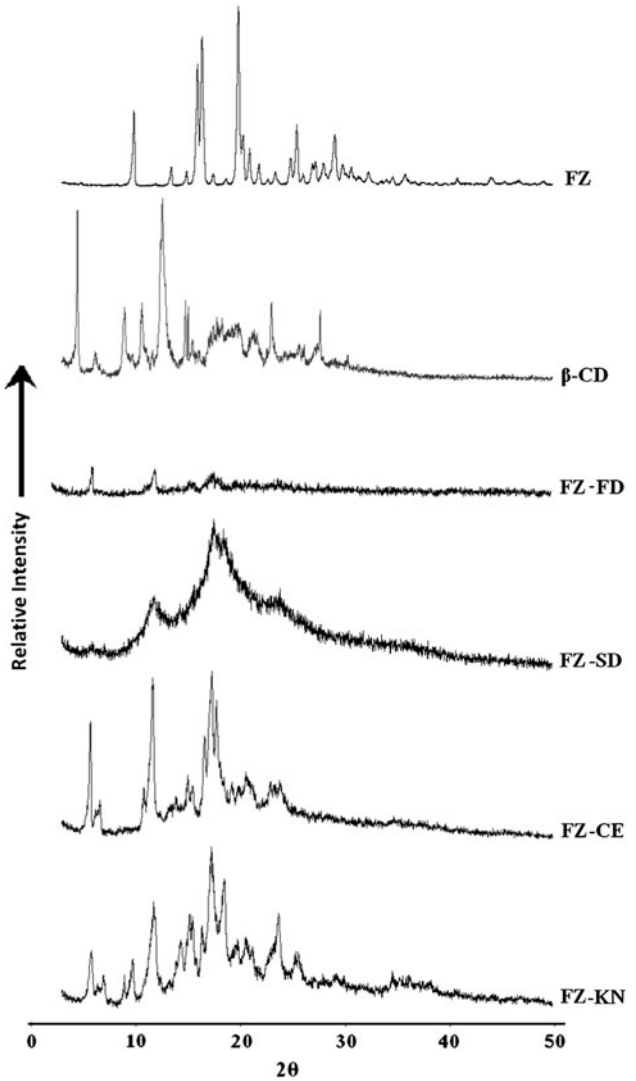

Fig. 3 PXRD patterns of pure FZ, pure β-CD and FZ/β-CD complexes

interactions or loss of drug crystallinity [18, 19]. The absence of the characteristic peak of drug is strong evidence of the inclusion of the drug into the cyclodextrin cavity.

PXRD analysis

Figure 3 shows the PXRD patterns of pure FZ, pure β-CD and their corresponding 1:1 mol:mol systems obtained by the different preparation methods. Characteristic peaks observed in the XRD patterns showed high crystallinity of pure FZ [16] and also showed that β-CD comply with the standard data file. The diffraction pattern of FZ powder revealed several sharp high intensity peaks at diffraction angles (2θ) of 9.60°, 16.00°, 16.44°, 19.90°, 25.54° and 29.16°. The diffraction peaks of FZ were completely disappeared in FZ–FD, FZ–SD and FZ–CE complexes that show formation of amorphous state or FZ/β-CD complexes.

These results may be attributed to an interaction between FZ and β-CD, in the FD, SD and CE complexes, suggesting the presence of a new solid phase with lower crystalline state than the drug, where a possible complexation of FZ and β-CD cavity was contemplated, corroborating the DSC observations [20].

FT–IR analysis

Fourier transform–infrared spectrometry spectra of pure FZ, pure β-CD and FZ/β-CD complexes obtained by different preparation methods are shown in Fig. 4. In order to investigate the vibrational changes upon host:guest interaction between FZ and β-CD, FT–IR spectroscopy was used. Fourier transform–infrared spectroscopy technique is useful to identify which vibrational mode of drug and β-CD are being disturbed during the inclusion process,

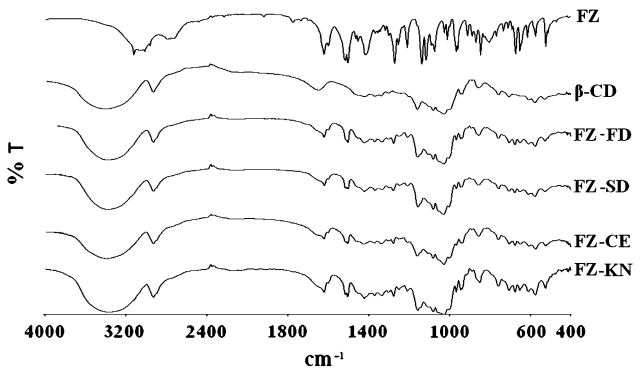

Fig. 4 FT–IR spectra of pure FZ, pure β-CD and FZ/β-CD complexes

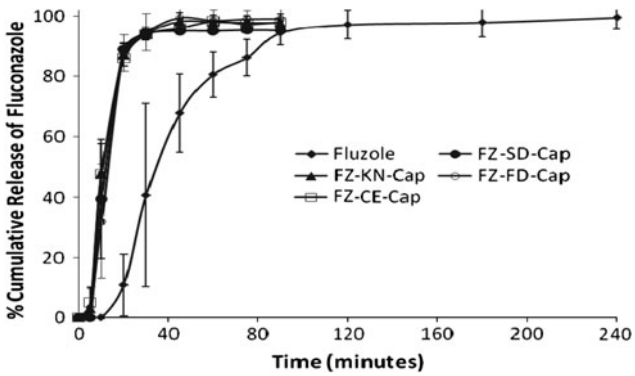

Fig. 5 Dissolution profiles of hard cellulose capsules containing FZ/β-CD complexes and Fluzole®

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 FZ involved in the complexation. The infrared spectrum of FZ shows the presence of characteristic peaks at 1621, 1417, 1272, 1137 and 968 cm^{-1} [11]. Generally, all FZ/β-CD comlexes were observed reduced intensity of characteristic FZ peaks compared to pure FZ. Among complexes, FZ–KN has more intense characteristic FZ peaks. Changes in the FT–IR spectra such as shift of characteristic bands, disappearance or reduction in intensity and appearance of new bands might be releated to possible drug–CD interactions or amorphization of product.

NMR analysis

Nuclear magnetic resonance spectroscopy is the most effective method for studying space conformation of β-CD inclusion complex. Therefore, prior to the decision for formation of an inclusion complex, the complexation of FZ with β-CD have been characterized by ^{1}H NMR and ^{13}C NMR. The formation of inclusion comlexes of FZ with β-CD was indicated by downfield shifts observed for all the resonances of the ring protons of FZ in our complexes. An upfield or downfield shift of some of drug and CD protons are evidence for formation of complex [22]. ^{1}H NMR spectra show characteristic signals for FZ and β-CD. After preparation of complexes FZ–FD, FZ–SD, FZ–CE and FZ–KN, chemical shifts observed in β-CD ^{1}H NMR signals. As a result, increase in chemical shift means the molecular structure has electron rich functional groups.

Dissolution behaviour of hard capsules

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 [23]. 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 and molecular dispersion [24].

Dissolution patterns of the hard cellulose capsules containing FZ/β-CD complexes and commercial hard gelatine capsules of FZ (Fluzole®) in distilled water were determined using the USP 31 paddle dissolution method, as shown in Fig. 5. Capsule shell materials (gelatine and cellulose) were dissolved at maximum four minutes and capsule contents were dispersed in dissolution medium ($n = 3$). Therefore capsule material dont affect dissolution of drug, The comparison of commercial hard gelatine capsule of FZ and HCC prepared with solid FZ/β-CD complexes were evaluated according to difference and similarity factors. It is evident that the dissolution rate of the capsules which are prepared FZ/β-CD complexes were faster than that of the commercial hard gelatine capsules of pure drug. This might be due to the high energetic amorphous state of complexes, 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 [23]. 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 [25].

It was found that solubility enhancement by FZ/β-CD complexes depends on the type of the preparation method. High release of active agent from capsules prepared with β-CD complexes compared to commercial capsules was attributed to the interactions between β-CD and active agent, high energetic amorphous state and inclusion complex formation.

Conclusion

FZ was encapsulated by β-CD, forming an inclusion complex and the ratio of 1:1 the complex was valued by the several methods namely, KN, CE, SD and FD. Among complexes, FZ–KN has more intense characteristic FZ peaks in PXRD and FT–IR analysis. These characterization results showed that the inclusion process was occured by FD, SD and CE methods but not by KN. Its structure was confirmed by DSC, XRD, FT–IR, ^{1}H NMR and ^{13}C NMR, which all verified the inclusion complex formation between β-CD and FZ. Furthermore, solubility and dissolution studies suggest that the β-CD is suitable of FZ, since it possessed formation complex and acceptable solubility and dissolution profiles. FZ/β-CD complex may be usefull for different pharmaceutical formulations.

As a conclusion, evaluation of FZ/β-CD complexes showed the increase in solubility and release of FZ from capsules might be indicated that adverse effects may be decreased via β-CD complexation.

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