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Physicochemical properties of antifungal drug–cyclodextrin complexes prepared by supercritical carbon dioxide and by conventional techniques

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ABSTRACT

Antifungal drugs are the most common systemic drugs used for the treatment of oropharyngeal candidiasis, which is the first symptom of HIV infection. However, the efficacy and bioavailability of these drugs have been limited by their poor aqueous solubility and dissolution rate. Therefore, the aim of this study was to investigate the effect of different preparation methods (i.e. kneading, coevaporation, sealed-heating, and a solid inclusion technique using supercritical carbon dioxide carrier (SC CO_2-inclusion)) for obtaining solid inclusion complexes between β-cyclodextrin and three antifungal drugs (itraconazole, econazole, and fluconazole). The physicochemical properties of the different products were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and powder X-ray diffractometry (PXRD). For the complexes prepared by the SC CO_2-inclusion method, the effects of temperature and pressure have also been investigated.

Results suggested the possibility of complex formation between β-cyclodextrin and the three antifungal agents, and indicated that inclusion formation was influenced by the preparation technique. SC CO_2-inclusion method proved to be an effective technique for preparing solid-state inclusion complexes between β-cyclodextrin and antifungal drugs, avoiding the use of organic solvents. Moreover, temperature of the SC CO_2 played a major role in promoting drug–carrier interactions, whereas pressure had limited effects.

1. Introduction

Antifungal drugs have therapeutic effects on patients with fungal diseases such as oropharyngeal candidiasis (OPC), which is the first symptom of HIV infection. Approximately 90% of patients with AIDS develop the disease at some stage. The improved efficacy and safety of some triazole antifungal drugs (e.g. itraconazole, econazole, and fluconazole) make them very popular for the treatment of OPC in HIV-positive patients [1–3]. Itraconazole is highly effective for the treatment of superficial fungal maladies and disseminated infections. Econazole has shown important microbiological activities against fungal infections. The important features of fluconazole are its rapidity of response and its high clinical cure rate in HIV-positive patients [4,5]. However, the poor aqueous solubility and dissolution rate of these drugs have restricted their use for the treatment of OPC. Therefore, it is desirable to enhance the solubility and dissolution rate of these antifungal drugs.

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 [6,7].

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 cyclic oligomers of glucose with cone-like structures, whose exterior surface has hydrophilic properties, while the interior is hydrophobic in nature. This particular characteristic of cyclodextrins allows them to form non-covalent inclusion complexes with various drugs of proper size and polarity leading to changes in their physicochemical and biopharmaceutical properties, which enhance their solubility, dissolution rate, chemical stability and bioavailability and reduce their side effects and toxicity [8–14]. Cyclodextrins are also able to form non-inclusion complexes,

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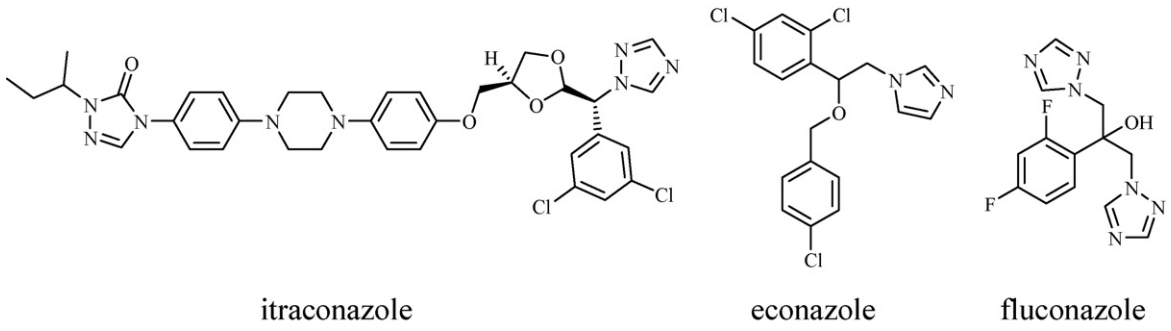

Fig. 1. Chemical structure of itraconazole, econazole, and fluconazole.

aggregates and micelle-like structures, which also effectively solubilize poorly-water soluble drugs [15,16].

It has been shown that the preparation technique can affect the properties of the final drug–cyclodextrin products [17]. Conventional methods for the preparation of solid inclusion complexes between cyclodextrins and various drugs include kneading, co-evaporation, sealed-heating, co-grinding, spray-drying and freeze-drying [18,19]. The use of supercritical carbon dioxide (SC CO_2) has been recently proposed for the preparation of various drug–cyclodextrin inclusion complexes for enhanced solubility and dissolution rate [20–29]. SC CO_2 is a non-toxic, non-hazardous, chemically stable, inexpensive, environmentally acceptable solvent and can easily be separated from the products. Moreover, properties of SC CO_2 can be changed from gas-like to liquid-like values by simply adjusting the pressure and temperature.

We have previously shown that inclusion yields for itraconazole–cyclodextrin complexes prepared by SC CO_2 were relatively low, which was probably due to the large molecular structure of itraconazole (Fig. 1) and its low solubility in SC CO_2 [26,27]. Therefore, the aim of the current study was to investigate the effectiveness of SC CO_2-inclusion method for obtaining solid inclusion complexes between β-cyclodextrin and two lower molecular weight antifungal drugs (econazole and fluconazole), by comparing them to itraconazole. Results of the SC CO_2-inclusion method were also compared to traditional techniques (sealed-heating, co-evaporation, and kneading). The products were characterized by differential scanning calorimetry, Fourier transform infrared spectroscopy and powder X-ray diffractometry. For the complexes prepared by the SC CO_2-inclusion method, the effects of variations of experimental conditions (temperature and pressure) were also investigated and the results were related to the solubility of these drugs in SC CO_2.

2. Materials and methods

2.1. Materials

Itraconazole and fluconazole were generously donated by the College of Pharmacy at Oregon State University (USA) and Medpharma (UAE), respectively. Econazole nitrate and β-cyclodextrin were purchased from Sigma Chemical Co. (Milwaukee, WI). All other reagents and solvents were of analytical grade.

2.2. Solubility of drugs in supercritical carbon dioxide

The supercritical fluid apparatus consisted of a 260 ml syringe pump and controller system (ISCO 260D), and an ISCO series 2000 SCF Extraction system (SFX 220) consisting of a dual-chamber extraction module with two 10-ml stainless steel vessels as described earlier [26]. One of the 10-ml stainless steel cells was filled with about 200 mg of pure drug, mixed with glass beads. The cell was pressurized and heated to the desired pressure and temperature and kept for 15 min to reach equilibrium. SC CO_2 (20 ml) was passed through the cell at a low flow rate of 0.5–0.7 ml/min. The solubilized drug was collected in about 10 ml acetonitrile after depressurization of the gas. The lines were flushed with acetonitrile to collect any drug deposited in the lines. The collected sample was diluted to 50 ml and the amount of dissolved drug was determined using a Shimadzu UV–visible spectrophotometer (UV 2450) at 265 nm (for itraconazole), 230 nm (for econazole), and 261 nm (for fluconazole).

2.3. Preparation of inclusion complexes

Drug–β-CD inclusion complexes at 1:2 drug:CD molar ratio were prepared by physical mixing, kneading, co-evaporation, sealed heating and SC CO_2 method as described earlier [24]. Physical mixtures were prepared by gently blending known amounts of drug and β-cyclodextrin powders in a mortar with a spatula. The untreated physical mixture was used in the preparation of products by kneading, sealed-heating and SC CO_2-inclusion methods. However, some samples of the physical mixture were also exposed to 130 °C for 3 h in the 10-ml stainless steel vessel of the SFX system at atmospheric pressure (without CO_2) in order to observe the effect of temperature on the physicochemical properties of the sample.

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 (1:2 drug: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.

In the co-evaporation method, known amounts of β-CD and drug (to obtain the desired molar ratio) were dissolved in bi-distilled water and ethanol, respectively. The two solutions were prepared at 25 °C and 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.

Sealed-heating products were prepared by placing a known amount of drug–β-CD physical mixture in a glass container. Bidistilled water (10 μl) was added to the glass container, which was then sealed using a flame. The sample was kept in an oven at 75 °C for 3 h, after which time the sample was removed and kept in a desiccator overnight to remove traces of water.

In the SC CO_2-inclusion method, a 10-ml stainless steel cell was filled with a physical mixture of drug–β-CD. The system was then pressurized and heated up to the desired pressure and temperature. After keeping the system in a static mode for 3 h, the pressure in the cell was reduced to atmospheric pressure within 15 min and the contents of the cell were ground and homogenized in a mortar.

2.4. Differential scanning calorimetry (DSC)

Thermal analysis of the individual components or drug–β-CD combinations was performed using a differential scanning calorimeter (DSC Q100, Thermal Analysis) with a nitrogen flow rate of 40 ml/min and a heating rate of 10 °C/min from 50 to 200 °C. Indium and zinc were used as standards.

2.5. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of individual drugs, β-CD, and selected drug–CD binary systems were obtained as Nujol dispersion using a PerkinElmer Mod. 1600 FTIR spectrophotometer in the 4000–400 cm^{-1} wave number range.

2.6. Powder X-ray diffractometry (PXRD)

The powder X-ray diffraction patterns of individual drug, and selected drug–cyclodextrin combinations were determined using a Philips X-ray diffractometer (PW/1840), with Ni filter, Cu Kα radiation, voltage 40 kV, current 40 mA, and 2θ over a 2–38° range at a scan rate of 1°/min.

3. Results and discussion

3.1. Solubility of antifungal drugs in supercritical carbon dioxide

The solubility of itraconazole, econazole, and fluconazole in SC CO_2 at 45 MPa and different temperatures (50, 100, and 130 °C) are tabulated in Table 1. Results show that solubility of fluconazole was more than two orders of magnitude higher than that of itraconazole and econazole at all conditions. Among the three drugs, itraconazole had the lowest solubility in SC CO_2 at all conditions. The maximum solubility (36,929.99 μg/g of CO_2 for fluconazole, 160.64 μg/g of CO_2 for econazole, and 96.86 μg/g of CO_2 for itraconazole) was obtained at the highest temperature (130 °C) for the three drugs. At a constant pressure of 45 MPa, higher temperature led to greater solubility of all drugs in SC CO_2 although the density of SC CO_2 decreases with temperature. For example, raising the temperature from 50 to 100 °C increased the solubility by 1.8 times (from 20.66 to 37.69 μg/g of CO_2) for itraconazole, by 2.0 times (from 22.87 to 46.35 μg/g of CO_2) for econazole and by 3.4 times (from 3910.25 to 13,351.85 μg/g of CO_2) for fluconazole. Moreover, the increase in solubility became more important for itraconazole and econazole when the temperature was raised from 100 to 130 °C (2.6 times increase for itraconazole and 3.5 times increase for econazole). For the same increase in temperature, the solubility of fluconazole in SC CO_2 increased by 2.8 times. The increase in solubility with temperature is attributed to the effect of temperature on the volatility of these drugs; the higher the temperature, the higher the volatility of these molecules and therefore, the higher the solubility. Solubility of solutes in SC CO_2 is also affected by the density of SC CO_2, which depends on the temperature in an opposite way than the volatility effect. As the temperature increases, the density of SC CO_2 decreases, causing a reduction in the solvating power of CO_2, which leads to a decrease in the solubility of these drugs in SC CO_2. The density and volatility effects compete in the way they affect the solubility when the temperature is changed. At all the conditions reported here, the volatility effect was dominant, leading to higher solubilities at higher temperatures for all the three drugs.

Table 1
Solubility of itraconazole, econazole, and fluconazole in supercritical carbon dioxide (μg/g of CO_2) at 45 MPa ($n = 3$).

Temperature (°C)	Itraconazole	Econazole	Fluconazole
50	20.66 ± 1.7	22.87 ± 2.2	3,910.25 ± 134.9
100	37.69 ± 2.8	46.35 ± 3.6	13,351.85 ± 1,084.2
130	96.86 ± 8.6	160.64 ± 13.9	36,929.99 ± 2,906.4

The fact that the solubility of fluconazole in SC CO_2 was significantly larger than that of itraconazole and econazole suggests that fluconazole should more favorably exist in the SC CO_2 phase at the system pressure and temperature as compared to the other two antifungal drugs. As the pressure of the system is decreased the solubility of these drugs decrease drastically, forcing the drug to leave the CO_2 phase. Therefore, in the presence of a CD, fluconazole leaving the CO_2 phase might enter the CD cavity to a greater extent than itraconazole and econazole since larger amount of fluconazole is dissolved in the solvent (SC CO_2) as compared to the other two drugs. Another factor that might positively affect the inclusion formation between the antifungal drugs and β-CD is the melting point depression of the drug at the system pressure. The melting point of fluconazole (139.2 °C) is lower than that of itraconazole (165.2 °C) and econazole (164.6 °C). Therefore, it is more likely that fluconazole would melt under SC CO_2 condition (130 °C and 45 MPa) due to melting point depression at high pressures, resulting in a more amorphous and/or inclusion formation when the pressure is dropped and CO_2 is allowed to leave the sample. The higher solubility of fluconazole and the possibility of fluconazole to be melted at the SC CO_2 conditions may lead to higher extent of interaction between the drug and β-CD as compared to itraconazole and econazole, which can be verified by DSC, FTIR and PXRD analysis.

3.2. Differential scanning calorimetry analysis

DSC curves for pure β-CD, pure drug (itraconazole, econazole, and fluconazole), and drug–β-CD (1:2 mol:mol) products obtained by physical mixing (exposed to 130 °C for 3 h), sealed heating, co-evaporation, kneading, and SC CO_2-inclusion method are shown in Fig. 2. Pure β-CD exhibited a broad endothermal effect, ranging between 50 and 150 °C corresponding to its dehydration. Pure itraconazole, econazole, and fluconazole showed sharp melting endotherms at 165.2, 164.6, and 139.2 °C, respectively. Disappearance or decrease in intensity of the drug endothermic peak might be related to possible drug–CD interactions and/or loss of drug crystallinity. The DSC curve for the itraconazole–β-CD physical mixture exposed to 130 °C consisted of the sum of those for the pure components, indicating the absence of interactions between itraconazole and β-CD. For the econazole–β-CD physical mixtures exposed to 130 °C, a small decrease in the intensity of the drug peak was observed, indicating a small degree of interaction between econazole and β-CD. However, the drug peak disappeared for the physical mixture between fluconazole and β-CD exposed to the same temperature, indicating strong interactions between fluconazole and β-CD with possible formation of inclusion complex or amorphization.

The endothermic peak corresponding to pure itraconazole, although reduced in size, was observed for all samples, indicating an incomplete inclusion of the drug in the cyclodextrin cavity. Comparing the itraconazole peak size for the products prepared by different methods, it seems that inclusion yield was almost the same for the samples prepared by sealed heating, co-evaporation, kneading and SC CO_2-inclusion (at 130 °C and both pressures). However, the larger drug peak size for the sample prepared by SC CO_2-inclusion at the lower temperature (100 °C) indicates smaller inclusion yield as compared to the samples prepared at 130 °C, suggesting that temperature is an important factor in promoting interactions between itraconazole and β-CD. This is probably due

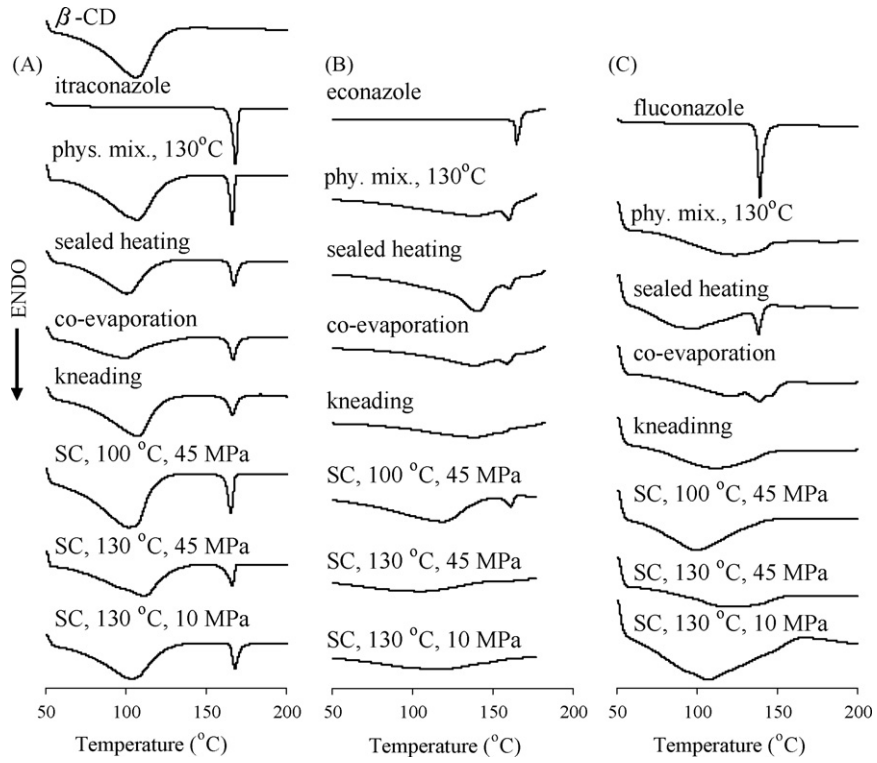

Fig. 2. DSC curves of pure drug (itraconazole, econazole, and fluconazole), pure β-CD, and drug–β-CD (1:2 mol:mol) systems prepared by physical mixing, sealed heating, co-evaporation, kneading, and SC CO_2 at different temperatures and pressures. (A) Itraconazole system, (B) econazole system, and (C) fluconazole system.

to the higher itraconazole solubility in SC CO_2 at the higher temperature (Table 1).

The DSC curve for the econazole–β-CD samples prepared by sealed heating, co-evaporation and SC CO_2-inclusion at 100 °C and 45 MPa showed a small reduction in intensity of the drug peak, suggesting a small degree of drug–CD interaction. However, products prepared by kneading and SC CO_2-inclusion (at 130 °C and both pressures) resulted in the complete disappearance of the drug peak, suggesting complex formation and/or sample amorphization. Therefore, temperature and pressure are critical factors to promote interactions between econazole and β-CD using SC CO_2-inclusion method.

For the fluconazole–β-CD samples the sealed heating and co-evaporation methods resulted in partial inclusion formation or amorphization, whereas the kneading and all SC CO_2-inclusion conditions reported here produced complete inclusion or amorphization of the samples. Complete disappearance of drug peak was not observed for the itraconazole and econazole samples prepared by SC CO_2-inclusion at 100 °C and 45 MPa, however, fluconazole–β-CD samples prepared at these conditions resulted in complete disappearance of the drug peak, suggesting stronger drug–CD interactions in the case of fluconazole as compared to itraconazole and econazole. This could be due to the presence of two pyrole rings in fluconazole, which enhance the chance for inclusion formation as compared to econazole and itraconazole with only one pyrole ring. Moreover, the larger molecular structure and lower solubility of itraconazole are attributed to its smaller interaction with β-CD in comparison with the other two drugs.

3.3. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra of pure β-CD, pure drug (itraconazole, econazole, and fluconazole), and drug–β-CD (1:2 mol:mol) products obtained by physical mixing (exposed to 130 °C for 3 h), kneading, co-evaporation, sealed heating, and SC CO_2-inclusion method are presented in Fig. 3. The characteristic bands of pure itraconazole (1699, 1511, 1452, 1273, 1229, and 825 cm^{-1}), pure econazole (1585, 1548, 828, 804, and 638 cm^{-1}), and pure fluconazole (1621, 1503, 1417, 1272, 1137, and 968 cm^{-1}) were determined and compared to the drug–β-CD products prepared by different methods. Changes in the FTIR spectra such as shift of characteristic bands, disappearance or reduction in intensity, and appearance of new bands might be related to possible drug–CD interactions and/or amorphization of the product. Characteristic bands that have been modified are indicated by arrows in Fig. 3. The FTIR spectra of itraconazole–β-CD physical mixture exposed to 130 °C can be considered as the result of the sum of the pure components, indicating the absence of interactions between itraconazole and β-CD. For econazole–β-CD physical mixture exposed to the same temperature small modifications (i.e. shift of the band at 1585 to 1586 cm^{-1}) were observed, indicating minor drug–CD interactions due to the simple thermal treatment at 130 °C. The effect of temperature was more prominent for the fluconazole–β-CD physical mixture exposed to 130 °C (i.e. shift of bands at 1503 to 1502 cm^{-1}, at 1272 to 1278 cm^{-1}, at 968 to 967 cm^{-1}), suggesting strong interactions between fluconazole and β-CD. Therefore, temperature is an important factor, which can promote a high degree of interaction between fluconazole and β-CD and a weak interaction between econazole and β-CD even in the simple thermal treatment of the physical mixture. These results are in agreement with the results obtained by DSC analysis (Fig. 2) showing more interactions in physical mixtures of fluconazole–β-CD as compared to econazole–β-CD and itraconazole–β-CD.

The FTIR spectra of itraconazole–β-CD products obtained by sealed heating, co-evaporation, kneading and SC CO_2-inclusion were either identical to or a little different from the corresponding pure molecules, indicating no or minor drug–CD interactions as evident from the DSC analysis. For econazole–β-CD products obtained by sealed heating, co-evaporation, kneading, and SC CO_2-

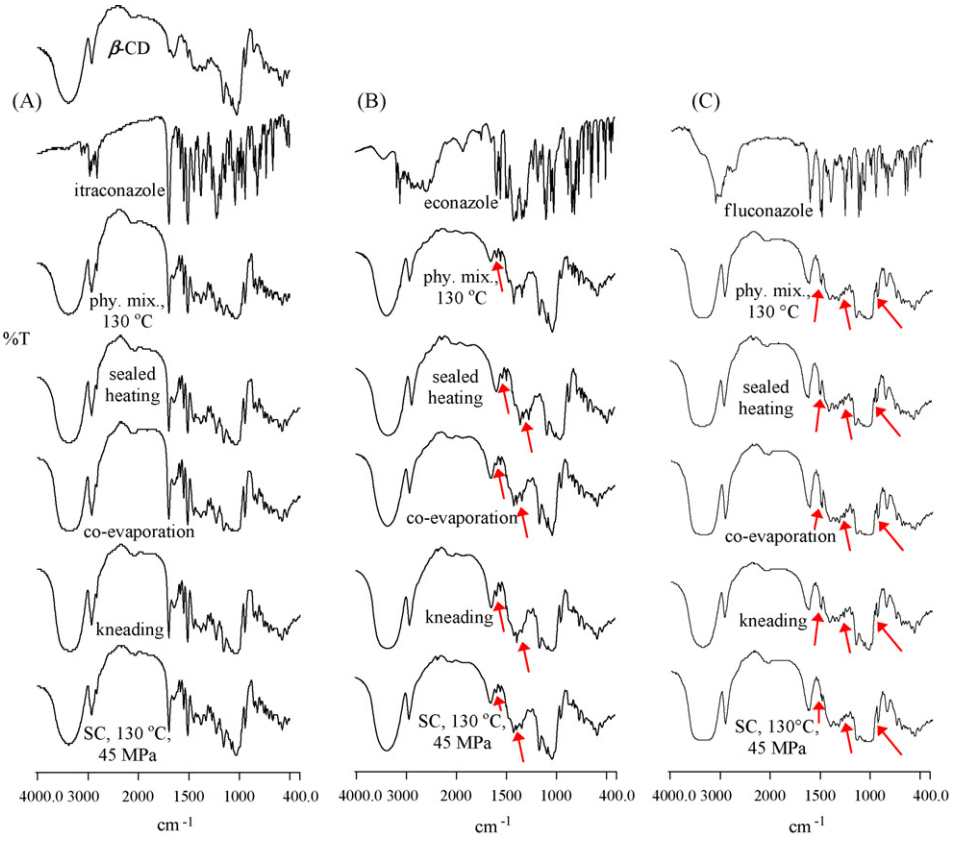

Fig. 3. FTIR spectra of pure drug (itraconazole, econazole, and fluconazole), pure β-CD, and drug–β-CD (1:2 mol:mol) systems prepared by physical mixing, sealed heating, co-evaporation, kneading, and SC CO_2 at 130 °C.

inclusion methods, some differences with respect to those of the original molecules were observed. This indicates some interactions and/or amorphization with different degrees in different products, which are in agreement with the results obtained by DSC analysis. Econazole–β-CD products obtained by sealed heating showed no significant drug–CD interactions and the product obtained by co-evaporation resulted in a weak drug–CD interaction. However, strong interactions (shifts, decrease in intensity, and augmentation in intensity of some bands) were noticed in the products prepared by kneading and SC CO_2-inclusion (at 130 °C and 45 MPa), suggesting inclusion formation and/or amorphization as also indicated by DSC analysis. The spectra of fluconazole–β-CD products prepared by sealed heating, co-evaporation and kneading methods showed shifts at several bands (i.e. 1503, 1272, and 968 cm^{-1}) and disappearance of the band at 1137 cm^{-1}, indicative of some interaction between fluconazole and β-CD. For the fluconazole–β-CD product prepared by SC CO_2-inclusion at 130 °C and 45 MPa, in addition to the shifts at several bands (i.e. 1503 to 1502 cm^{-1}, 1272 to 1277 cm^{-1}), the band at 1621 and 968 cm^{-1} completely disappeared and new bands at 1641 and 1632 cm^{-1} were observed. These results suggest stronger interactions in the products prepared by SC CO_2-inclusion as compared to other methods.

3.4. Powder X-ray diffractometry (PXRD) analysis

Fig. 4 shows the PXRD patterns of pure drug (itraconazole, econazole, and fluconazole), pure β-CD, and their corresponding 1:2 mol:mol systems obtained by the different preparation methods. The crystalline nature of a sample is displayed by sharp peaks in the PXRD patterns. Decrease in the crystallinity (reductions in peak intensity), shifts and disappearance of peaks, appearance of new diffraction peaks, or a complete diffuse pattern might be related to possible drug amorphization and/or complexation. The PXRD results show crystalline state for all itraconazole–β-CD samples, indicating the absence of an amorphous state. The itraconazole–β-CD physical mixture exposed to 130 °C for 3 h showed a similar PXRD pattern to that of the respective individual components, but with some changes in the size of several peaks in the binary sample. However, the itraconazole–β-CD sample treated with SC CO_2 at 130 °C and 45 MPa showed a different PXRD pattern with fewer and smaller peaks than the other samples. Therefore, PXRD analysis confirm DSC results that the thermal events observed for the samples treated with SC CO_2 may be attributed to the partial complexation of the drug in the cyclodextrin cavity.

The diffraction pattern of pure econazole also displayed several sharp peaks, indicative of its crystalline nature. Although the crystallinity nature of the econazole–β-CD physical mixture exposed to 130 °C was maintained, significant changes were observed in the PXRD pattern of this sample as compared to the pure components (disappearance of many peaks, reduction or augmentation in intensity of some peaks, and appearance of new diffraction peaks). These results concur with FTIR analysis that temperature is an important factor, which can promote drug–CD interactions even in the simple thermal treatment of the physical mixture at 130 °C. The sealed-heated product resulted in a crystalline pattern similar to that of the pure components, the co-evaporated product showed reduced intensity of drug and CD peaks, and the crystallinity loss was most pronounced for the product prepared by kneading method, suggesting an almost complete drug amorphization and/or complexation in agreement with DSC and FTIR analysis. The PXRD pattern for the product obtained by SC CO_2-inclusion at 130 °C and 45 MPa was significantly different from that of physical mixture

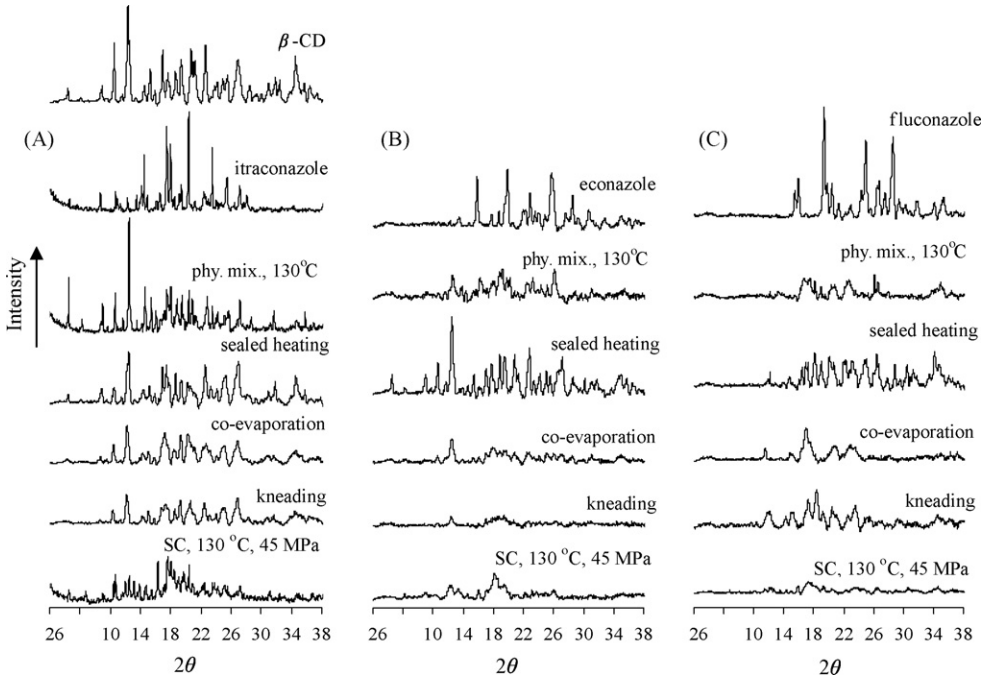

Fig. 4. PXRD patterns of pure drug (itraconazole, econazole, and fluconazole), pure β-CD, and drug–β-CD (1:2 mol:mol) systems prepared by physical mixing, sealed heating, co-evaporation, kneading, and SC CO_2 at 130 °C and 45 MPa. (A) Itraconazole system, (B) econazole system, and (C) fluconazole system.

exposed to the same temperature, showing a diffuse pattern with a very few low-intensity peaks, suggesting drug amorphization and/or complexation. The amorphization of the product might be due to CO_2 at SC conditions getting into the drug–CD sample and leaving the sample during depressurization step.

The PXRD pattern of pure fluconazole also showed a crystalline state by several sharp peaks. The crystallinity of the fluconazole–β-CD physical mixture exposed to 130 °C for 3 h was reduced, indicating some degree of amorphization and/or complexation due to the simple thermal treatment, which is in agreement with DSC and FTIR analysis. The sealed-heated product was also crystalline with some changes in the PXRD pattern as compared to the pure components (complete disappearance, reduction or augmentation in intensity of some peaks, and appearance of new diffraction peaks), indicative of some interaction between fluconazole and β-CD. The co-evaporated and kneaded products also showed reduced crystallinity, but the crystallinity loss was most prominent for the product prepared by SC CO_2-inclusion at 130 °C and 45 MPa, suggesting an almost complete drug amorphization and/or complexation in agreement with DSC and FTIR analysis.

4. Conclusions

Solubility of itraconazole, econazole, and fluconazole in SC CO_2 was measured at 45 MPa and found to increase with temperature while having the rank order: fluconazole > econazole > itraconazole. Solid systems of itraconazole, econazole, and fluconazole with β-CD in the 1:2 mol:mol ratio were prepared by SC CO_2-inclusion method and compared to products obtained using different techniques such as physical mixing, sealed heating, co-evaporation, and kneading. DSC, FTIR, and PXRD analysis suggest only partial complexation or amorphization for itraconazole–β-CD products while complete complexation or amorphization was observed for econazole and fluconazole samples prepared by some preparation methods. This could be due to the smaller molecular size of econazole and fluconazole and therefore better fit into the CD cavity as compared to itraconazole. Additionally, the presence of two pyrole rings in fluconazole is believed to increase the chance for inclusion formation and result in observed stronger drug–CD interaction as compared to econazole and itraconazole.

Different degrees of modification were observed in the analyses of products prepared by various methods, suggesting the possibility of drug–CD interactions of different strengths, which may give rise to different degrees of inclusion formation and/or amorphization of the sample. Nevertheless, for the three drugs studied here, products obtained by the SC CO_2-inclusion method were among the ones showing the highest interaction between the drug and the CD. Therefore, a solid inclusion method using supercritical CO_2 carrier proved to be a novel and useful complexation method for antifungal drugs into β-CD. Moreover, since this method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques, which require the use of organic solvents. Pharmacokinetic studies of the inclusion complexes obtained by different methods are being investigated in both *in vitro* and *in vivo*. Appropriate mechanisms of the inclusion complexes are also being developed.

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