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 supercritical carbon dioxide (SC CO₂)) for obtaining solid inclusion complexes between β-cyclodextrin and three antifungal drugs (itraconazole, econazole, fluconazole). The physico-chemical 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 CO₂ 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 CO₂ method proved to be an effective technique for preparing solid systems between β-cyclodextrin and antifungal drugs, avoiding the use of organic solvents. Moreover, temperature of the SC CO₂ played a major role in promoting drug-carrier interactions, whereas pressure had limited effects.

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 [1-5]. 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.

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 [6-10]. 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 [10]. The use of supercritical carbon dioxide (SC CO₂) has been recently proposed for the preparation of various drug-cyclodextrin inclusion complexes for enhanced solubility and dissolution rate [11-16]. The aim of the current study was to investigate the effectiveness of SC CO₂ method for obtaining solid inclusion complexes between β-cyclodextrin and three antifungal drugs (itraconazole, econazole and fluconazole). Results of the SC CO₂ method were also compared to traditional techniques (sealed-heating, co-evaporation and kneading).

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

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 CO₂ method as described earlier [13]. Physical mixtures were prepared by gently blending known amounts of drug and β-CD powders. 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 and kneading the resultant mixture thoroughly with a pestle until the solvent was completely removed. In the co-evaporation method, known amounts of β-CD and drug were dissolved in bidistilled water and ethanol, respectively. The two solutions were then mixed and the solvents were removed using a rotary evaporator at 75 °C and 210 rpm. Sealed heating products were prepared by placing a known amount of drug-β-CD physical mixture in a glass container. 10 μl bidistilled water 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. The supercritical fluid apparatus has been described earlier [14]. A 10-ml stainless steel cells 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 hours, the pressure in the cell was reduced to atmospheric pressure within 15 minutes and the contents of the cell were ground and homogenized in a mortar.

Analysis of the prepared samples
Thermal analysis of the individual components or drug-β-CD combinations were 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. FTIR spectra were obtained as Nujol dispersion using a Perkin-Elmer Mod. 1600 FTIR spectrophotometer in the 4000-400 cm⁻¹ wave number range. The powder X-ray diffraction patterns 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.
Results and discussions

DSC curves for pure $\beta$-CD, pure drug, and drug-$\beta$-CD (1:2 mole-mole) products obtained by physical mixing (exposed to 130 °C for three hours), sealed heating, co-evaporation, kneading, and SC CO$_2$ method are shown in Figure 1. Pure $\beta$-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. The DSC curve for the itraconazole-$\beta$-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 $\beta$-CD. For the econazole-$\beta$-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 $\beta$-CD. However, the drug peak disappeared for the physical mixture between fluconazole and $\beta$-CD exposed to the same temperature, indicating strong interactions between fluconazole and $\beta$-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$_2$ (at 130 °C and both pressures). However, the larger drug peak size for the sample prepared by SC CO$_2$ 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 $\beta$-CD. DSC curve for the econazole-$\beta$-CD samples prepared by sealed heating, co-evaporation and SC CO$_2$ 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$_2$ (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 CO₂ 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 CO₂ 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 CO₂ 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.

FTIR spectra of pure β-CD, pure drug, and drug/β-CD (1:2 mole-mole) products obtained by physical mixing (exposed to 130 °C for three hours), kneading, co-evaporation, sealed heating, and SC CO₂ method are presented in Figure 2. 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. 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 cm⁻¹ to 1586 cm⁻¹) was 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 cm⁻¹ to 1502 cm⁻¹, at 1272 cm⁻¹ to 1278 cm⁻¹, at 968 cm⁻¹ 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 (Figure 1) 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 CO₂ 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 CO₂ methods, some differences with respect to those of the original molecules were observed, indicating some interactions and/or amorphization with different degrees in different products, in agreement with the results obtained by DSC analysis. Econazole-β-CD products obtained by sealed heating showed no significant drug-CD interactions, the product obtained by co-evaporation resulted in a
week drug-CD interaction, while strong interactions (shifts, decrease in intensity, and augmentation in intensity of some bands) were noticed in the products prepared by kneading and SC CO₂ (at 130 °C and 45 MPa), thus 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, 968 cm⁻¹) and disappearance of the band at 1137 cm⁻¹, indicative of some interaction between fluconazole and β-CD. However, for the fluconazole-β-CD product prepared by SC CO₂ 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, suggesting stronger interactions in the products prepared by SC CO₂ as compared to other methods.

Figure 2. FTIR spectra of pure drugs, pure β-CD, and drug-β-CD (1:2 mol-mol) systems prepared by different methods.

Figure 3 shows the PXRD patterns of pure drug (itraconazole and econazole), pure β-CD, and their corresponding 1:2 mol-mol systems obtained by the different preparation methods. 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 three hours 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 CO₂ 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 CO₂ may be attributed to the partial complexation of the drug in the cyclodextrin cavity.

Figure 3. PXRD patterns of pure drugs, pure β-CD, and drug-β-CD (1:2 mol-mol) systems prepared by different methods.

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), indicating 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 as observed by FTIR analysis. 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 CO₂ at 130 °C and 45 MPa was significantly different from that of physical mixture exposed to the same temperature, showing a diffuse pattern with a very few low-intensity peaks, suggesting drug amorphization and/or complexation.

Conclusions
Solid systems of itraconazole, econazole and fluconazole with β-CD in the 1:2 mol:mol ratio were prepared by different techniques. 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 was 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 CO₂ method were among the ones showing the highest interaction between the drug and the CD. Therefore, SC CO₂ proved to be a novel and useful complexation method for antifungal drugs into β-CD.

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References