J Incl Phenom Macrocycl Chem (2008) 60:85–93
DOI 10.1007/s10847-007-9356-6

ORIGINAL ARTICLE

Influence of the preparation method on the physicochemical properties of econazole-β-cyclodextrin complexes

Ali H. Al-Marzouqi · Ayser Solieman · Ihsan Shehadi · Abdu Adem

Received: 24 May 2007 / Accepted: 25 July 2007 / Published online: 28 August 2007

Abstract Econazole ($C_{18}H_{15}Cl_3N_2O$) is one of the common antifungal agents whose poor aqueous solubility restricts its use for the treatment of oropharyngeal candidiasis, which is the first symptom of HIV infection. Therefore, the aim of the current study was to investigate the effect of different preparation methods (i.e. kneading, coevaporation, sealed-heating, and supercritical carbon dioxide (SC CO_2)) for obtaining solid inclusion complexes between β-cyclodextrin and econazole. 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 method, the effects of temperature and pressure have also been investigated and related to the solubility of econazole in SC CO_2. Results suggested the validity of the SC CO_2 method for preparing solid complexes between cyclodextrins and econazole, avoiding the use of organic solvents and problems of their complete removal. Moreover, temperature played a major role in promoting drug-carrier interactions, whereas pressure had limited effects.

Keywords Cyclodextrin · Econazole · Inclusion complex · Supercritical carbon dioxide

A. H. Al-Marzouqi (✉) · A. Solieman
Department of Chemical & Petroleum Engineering, UAE University, P.O. Box: 17555, Al-Ain, UAE
e-mail: hassana@uaeu.ac.ae

I. Shehadi
Department of Chemistry, UAE University, P.O. Box: 17555, Al-Ain, UAE

A. Adem
Department of Pharmacology and Therapeutics, UAE University, P.O. Box: 17666, Al-Ain, UAE

Introduction

Oropharyngeal candidiasis (OPC) may be the first symptom of HIV infection, and approximately 90% of patients with AIDS develop the disease at some stage. Triazole antifungal drugs (e.g. itraconazole, fluconazole, econazole, ketoconazole) are the most common systemic drugs used for the treatment of OPC in HIV-positive patients. Ketoconazole was the first available oral antifungal drug for the treatment of OPC and showed clinical cure rates in excess of 75% when administered daily for 10–14 days [1]. Use of ketoconazole is limited by the fears of drug–drug interactions and concerns about its gastric absorption and efficacy. Therefore, the newer triazoles (itraconazole, fluconazole, and econazole), with considerably improved efficacy and safety, are very popular, especially for patients with HIV infection and AIDS with moderate to severe OPC [2–4]. The important features of fluconazole are its rapidity of response (usually within 10 days with 50 mg/day) and its high clinical cure rate of 87% in HIV-positive patients [5, 6]. Econazole has showed important microbiological activities against fungal infections. However, the efficacy and bioavailability of these drugs have been limited by their poor aqueous solubility and dissolution rate. Therefore, there is a need for enhancement of the solubility and stability of antifungal drugs.

Pharmaceutical modification of drug molecules by inclusion complexation with cyclodextrins (CDs) has been extensively studied to improve solubility, dissolution rate, chemical stability, absorption, and bioavailability of poorly water-soluble drugs, and reduce side effects and toxicity of drugs [7–9]. Cyclodextrins (oligomers of glucose) have received increasing attention in the pharmaceutical field [10–16]. Because of the particular arrangement of the glucose units, cyclodextrins have cone-like structures,

which make interior of the cone hydrophobic in nature, leading to formation of inclusion complexes with various drugs into their cavity and resulting in improvement in solubility and drug release. Cyclodextrins are able to form, either in solution or in solid state, inclusion complexes with a wide variety of guest molecules, differing in their chemical and physical properties such as size, functionalities or solubility, many of which are very interesting from a pharmaceutical point of view. According to Strickley [17], cyclodextrins interact with some hydrophobic molecules and form a noncovalent inclusion complex that lowers the chemical potential of the molecule in solution and thus enhances the solubility of the molecule. Studies have shown that cyclodextrins are also able to form non-inclusion complexes. Loftsson et al. [18] discussed numerous reported evidence that cyclodextrins and their complexes can self-associate to form aggregates through non-inclusion complexation or micelle-like structures, which also effectively solubilize poorly-water soluble drugs.

Peeters et al. [19] studied the phase solubility of itraconazole as a function of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and showed that HP-β-CD significantly improves solubility of itraconazole in aqueous solutions. Van Hees et al. [20, 21] used supercritical carbon dioxide (SC CO_2) to produce piroxicam-β-CD and miconazole-cyclodextrin inclusion complexes and compared their results with conventional methods of physical mixing, spray drying and freeze drying. SC CO_2 is a nontoxic, nonhazardous, chemically stable, inexpensive, environmentally acceptable solvent and can easily be separated from the products. Properties of supercritical fluids (SCFs) can be changed from gas-like to liquid-like values by simply adjusting the pressure and temperature. Because of these special characteristics, supercritical fluids have received increasing attention in the pharmaceutical field and for the preparation of drug-cyclodextrin complexes for enhanced solubility and dissolution rate [20–31]. Charoenchaitrakool et al. [32] showed that ibuprofen-methyl-β-CD complexes prepared by passing ibuprofen-laden CO_2 through a methyl-β-CD packed bed can enhance dissolution profiles due to the amorphous character and improved wettability of the product. Recently, Türk et al. [33] developed a controlled particle deposition process to prepare ibuprofen-β-CD complexes using SC CO_2, which resulted in higher dissolution rates than the physical mixture of ibuprofen and β-CD. We have previously shown that itraconazole-cyclodextrin inclusion complexes prepared by SC CO_2 can improve the solubility of itraconazole in aqueous solutions [28, 29]. However, inclusion yield of itraconazole-cyclodextrin complex prepared by SC CO_2 was relatively low, which was probably due to the large molecular weight of itraconazole and its low solubility in SC CO_2. Therefore, the present work investigates another drug from the same triazole group (econazole)(Fig. 1) to test the SC CO_2 technique with a lower molecular weight antifungal drug. Although econazole-cyclodextrin inclusion complexes have been prepared by conventional methods [12], [16], [34], [35], the use of SC CO_2 for the formation of econazole-cyclodextrin systems have not been reported in the literature. In the current study, cyclodextrin-econazole inclusion complexes are prepared using SC CO_2, as well as conventional methods (i.e. kneading, co-evaporation, sealed-heating, physical mixing). Thermal analyses of the prepared inclusion complexes are performed.

Experimental

Materials and reagents

Econazole nitrate and β-cyclodextrin were purchased from Sigma Chemical Co. (Milwaukee, WI). All solutions were prepared using deionized water (specific conductance <2 μS cm^{-1}) and used within 24 h. Bidistilled water was purified by using a Millipore super Q system and was also degassed prior to the preparation of the solutions.

Supercritical fluid apparatus

The SCF experimental apparatus, which was used for the preparation of inclusion complexes has been described in earlier publications [28], [29]. The experimental apparatus consisted of a 260-mL capacity 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. Temperature and pressure within the vessels were measured and could be independently adjusted.

Solubility of econazole in supercritical carbon dioxide

The 10-mL stainless steel cell was filled with about 200 mg of pure econazole, mixed with glass beads. The cell was

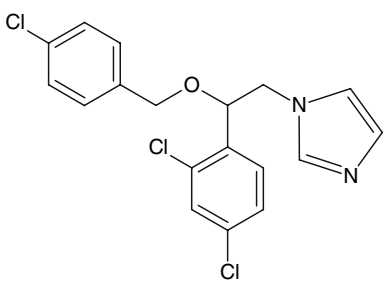

Fig. 1 Chemical structure of econazole

pressurized and heated to the desired pressure and temperature and kept for 15 min to reach equilibrium. 20 mL of SC CO_2 was passed through the cell at a flow rate of 0.5–0.7 mL/min. The solubilized econazole was collected in about 10 mL acetonitrile after depressurization of the gas. The lines were flushed with acetonitrile to collect any econazole deposited in the lines. The collected sample was diluted to 50 mL and the amount of dissolved econazole was determined using a Shimadzu UV-visible spectrophotometer (UV 2450) at 230 nm.

Preparation of physical mixtures

All the physical mixtures were prepared by gently grinding the drug and cyclodextrin powders in a mortar with a pestle to form a desired molar ratio of 1:2 (0.125 mmole drug:0.25 mmole β-cyclodextrin).

Preparation of inclusion complexes using kneading method

A small volume of water-ethanol (50/50 v/v) was added to a known amount of the physical mixture consisting of drug and β-cyclodextrin at a desired molar ratio. The resultant mixture was kneaded thoroughly with a pestle to obtain a homogeneous slurry and continued until the solvent was completely removed. The sample was kept in a desiccator overnight to remove traces of solvent.

Preparation of inclusion complexes using co-evaporation method

A known amount of β-cyclodextrin was dissolved in bidistilled water at 25 °C and a known amount of drug (giving the desired drug to β-cyclodextrin molar ratio) was dissolved in ethanol at the same temperature. The two solutions were added together after the powders were completely dissolved. The solvents were then removed from the mixture using a rotary evaporator at 75 °C and 210 rpm and the sample was kept in a desiccator overnight to remove traces of solvents.

Preparation of inclusion complexes using sealed heating method

A known amount of the physical mixture consisting of drug and β-cyclodextrin at a desired molar ratio was placed 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 the sample was removed and kept in a desiccator overnight to remove traces of water.

Preparation of inclusion complexes using supercritical fluid method

The 10-mL cell was filled with a physical mixture of econazole-β-cyclodextrin at a drug to β-cyclodextrin molar ratio of 1:2 (0.125 mmole drug:0.25 mmole β-cyclodextrin). The system was pressurized and heated to the desired pressure and temperature and left in a static mode for 3 h. At the end of the process, the pressure in the cell was dropped to atmospheric pressure within 15 min. The contents of the cell were ground and homogenized in a mortar.

Differential scanning calorimetry (DSC)

Thermal characteristics of the individual components or econazole-β-CD products were determined 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 20 °C to 185 °C. Indium and Zinc were used as standards.

Fourier transform infrared spectroscopy (FTIR)

IR spectra of individual econazole, β-CD, and drug-CD binary systems were obtained as Nujol dispersion using a Perkin-Elmer Mod. 1600 FTIR spectrophotometer in the 4,000–400 cm^{-1} wave number range.

Powder X-ray diffractometry (PXRD)

The powder X-ray diffraction patterns of individual econazole, β-cyclodextrin, and 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 2–70° range at a scan rate of 1°/min.

Results and discussions

Solubility of econazole in supercritical carbon dioxide

The solubility of econazole in SC CO_2 was found to be low (maximum of 160.64 μg/g of CO_2), but could vary over a wide range depending on temperature and pressure

(Table 1). The lowest solubility was 8.97 μg/g of CO_2 obtained at the lowest temperature and pressure (75 °C and 10 MPa) studied, while the maximum solubility (160.64 μg/g of CO_2) was obtained at the highest temperature and pressure (130 °C and 45 MPa). At a constant temperature, higher pressure leads to greater solubility of econazole in SC CO_2. For example, raising the pressure from 10 to 45 MPa increases the solubility of econazole by 3.4 times (from 8.97 to 30.09 μg/g of CO_2) at 75 °C and by 3.8 times (from 12.19 to 46.35 μg/g of CO_2) at 100 °C. This increase of solubility becomes more important at 130 °C (20.20 to 160.64 μg/g of CO_2 or about 8 times increase in solubility when the pressure is increased from 10 to 45 MPa). This makes sense since an increase in pressure leads to an increase in density and solubility.

The influence of temperature on the solubility of econazole in SC CO_2 was similar to that of pressure. At all pressures, when the temperature was increased, the solubility increased although the density of SC CO_2 decreases with temperature. Moreover, the increase of solubility became more important at higher pressures. For example, increasing the temperature from 75 °C to 130 °C increased the solubility of econazole by 2.25 times (from 8.97 to 20.20 μg/g of CO_2) at 10 MPa, by 4.75 times (from 17.46 to 82.94 μg/g of CO_2) at 25 MPa, by 5.0 times (from 22.41 to 112.05 μg/g of CO_2) at 35 MPa and by 5.34 times (from 30.09 to 160.64 μg/g of CO_2) at 45 MPa. This is because higher temperatures increase the volatility of econazole and thus improve its 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. Density of SC CO_2 decreases with increasing temperature, reducing the solvating power of CO_2 and thus reducing the solubility of econazole. The density and volatility effects are two competing factors in affecting the solubility when the temperature is changed. At all the conditions reported in this study, the volatility effect was dominant, leading to higher solubilities at higher temperatures.

Table 1 Solubility of econazole in supercritical carbon dioxide ($n = 3$)

Temperature (°C)	Pressure (MPa)	CO_2 Density (g/cm^3)	Solubility of econazole (μg/g of CO_2)
75	10	0.233	8.97 ± 0.9
	25	0.712	17.46 ± 1.5
	35	0.808	22.41 ± 2.1
	45	0.867	30.09 ± 2.7
100	10	0.189	12.19 ± 1.1
	25	0.588	25.98 ± 2.4
	35	0.715	37.74 ± 3.1
	45	0.790	46.35 ± 3.6
130	10	0.159	20.20 ± 1.9
	25	0.471	82.94 ± 7.3
	35	0.613	112.05 ± 9.2
	45	0.703	160.64 ± 13.9

Analysis of inclusion complexes

Different analytical techniques, such as DSC, FTIR and PXRD, were used to characterize and compare the physicochemical properties of the solid complexes prepared between econazole and β-CD, in order to investigate and compare the potential and effectiveness of the different preparation methods. For the SC CO_2 method, the influence of varying experimental conditions such as temperature (75, 100 or 130 °C) and pressure (10 or 45 MPa) on the complex formation was also investigated.

DSC Analysis

DSC curves for pure econazole nitrate, pure β-CD and econazole/β-CD (1:2 mole-mole) products obtained by physical mixing, co-evaporation, kneading, sealed heating, and SC CO_2 method are shown in Fig. 2. Pure econazole nitrate showed a sharp melting endotherm at 164.6 °C indicative of its anhydrous and crystalline state, while pure β-CD exhibited a broad endothermal effect, ranging between 50 °C and 150 °C corresponding to its dehydration. The DSC curve for the untreated physical mixture consisted of the superimposition of the thermal profiles of econazole and β-CD, with no significant changes in the drug melting peak, indicative of no drug-cyclodextrin interactions. Similar results were obtained for the physical mixtures exposed to 100 or 130 °C for 3 h, except for a small decrease in the intensity of the drug peak (for the physical mixture exposed to 100 °C) and disappearance of the broad endothermal effect corresponding to the dehydration of β-CD (for the physical mixture exposed to 130 °C). DSC curve for the product obtained by sealed heating did not show significant differences from that of the untreated physical mixture, suggesting no significant drug-CD interactions. The product obtained by co-evaporation method showed some endothermal effect corresponding to the β-CD dehydration and a small reduction in intensity of the drug peak, suggesting a small degree of drug-CD interactions. However, kneading method resulted in the complete disappearance of the drug peak, suggesting complex formation and/or sample amorphization. As it can be seen in Fig. 2, temperature had a significant effect on the thermal behavior of the products obtained by the SC CO_2 method. DSC curves of the econazole-β-CD samples

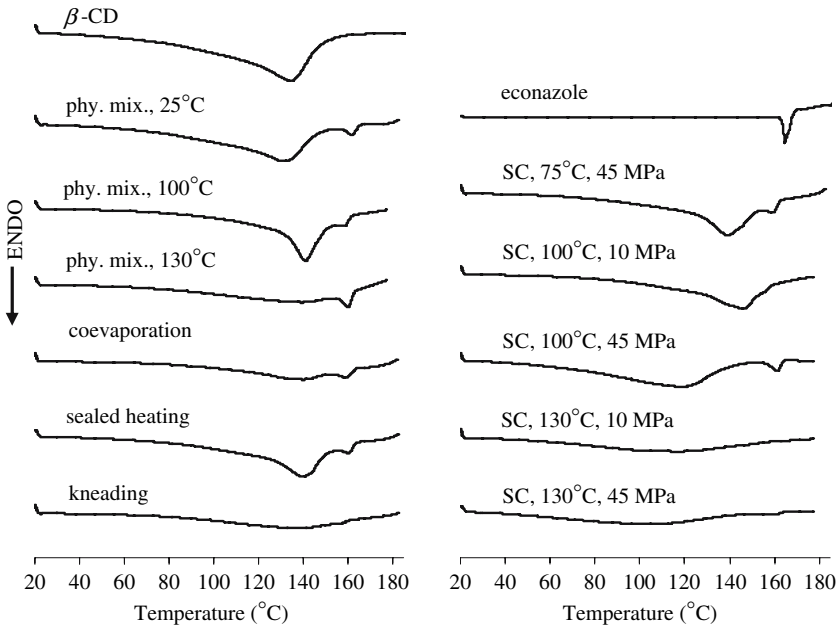

Fig. 2 DSC curves of pure econazole, pure β-CD, and econazole/β-CD (1:2 mole-mole) complexes prepared by physical mixing, kneading, co-evaporation, sealed heating and SC CO_2 method

treated with SC CO_2 at 45 MPa and 75 or 100 °C were similar to that of the untreated physical mixture (except for broadening and a shift of the β-CD dehydration to lower temperature in the product treated at 100 °C), indicating no drug-CD interactions. However, the products treated with SC CO_2 at 100 °C and 10 MPa or at 130 °C and both pressures (10 or 45 MPa) resulted in complete disappearance of the drug peak. These results show that certain SC conditions lead to inclusion 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 method.

FTIR Analysis

FTIR spectra of pure econazole nitrate, pure β-CD, and econazole/β-CD (1:2 mole-mole) products obtained by physical mixing, kneading, co-evaporation, sealed heating, and SC CO_2 method are presented in Fig. 3. Characteristic bands of pure econazole are observed at 1585, 1548, 828, 804, and 638 cm^{-1}. The FTIR spectra of econazole-β-CD product prepared by physical mixing at 25 °C can be considered as the sum of econazole and β-CD spectra, thus confirming the absence of any interactions between the components as indicated by DSC results. No significant modifications were observed for the physical mixture exposed to 100 °C for 3 h (except for a shift of the band at 1,646 cm^{-1} – 1,641 cm^{-1}), suggesting that the simple thermal treatment at this temperature did not promote any drug-CD interaction. However, the FTIR spectra of physical mixture after 3-h exposure to 130 °C showed significant differences with respect to physical mixtures prepared at lower temperatures (25 °C and 100 °C), indicating some drug-CD interactions resulted from a simple thermal treatment at the higher temperature of 130 °C. The changes observed in the FTIR spectra of physical mixture exposed to 130 °C include shift of peaks, reduction in intensity up to almost complete disappearance of peaks, or augmentation in intensity of peaks. For example, the band at 1,585 cm^{-1} shifted to 1,586 cm^{-1} and augmented in intensity as compared to physical mixtures exposed to 25 or 100 °C, the band at 1,646 cm^{-1} reduced in intensity, the band at 1,384 cm^{-1} almost completely disappeared while the band at 1,415 cm^{-1} augmented in intensity. Therefore, temperature is an important factor, which can promote interactions between econazole and β-CD even in the simple thermal treatment of the physical mixture.

The FTIR spectra of econazole-β-CD products obtained by sealed heating, co-evaporation, kneading, and SC CO_2 methods showed some differences with respect to those of the untreated physical mixture, indicating some interactions and/or amorphization with different degrees in different products, in agreement with the results obtained by DSC analysis. The FTIR spectra of econazole-β-CD products obtained by sealed heating was similar to that of the physical mixture exposed to 25 °C with a small reduction in intensity of the band at 1,384 cm^{-1}, suggesting no significant drug-CD interactions in the sealed heating product, which is in agreement with DSC results. The product obtained by co-evaporation resulted in 1 cm^{-1} shifts of the bands at 1,646 and 828 cm^{-1} to higher values, 1 cm^{-1} shift of the band at 804 cm^{-1} to a lower value, and a decrease in intensity of the band at 1,384 cm^{-1}, suggesting week drug-CD interactions in this product, in accordance with DSC analysis. The FTIR spectra of the

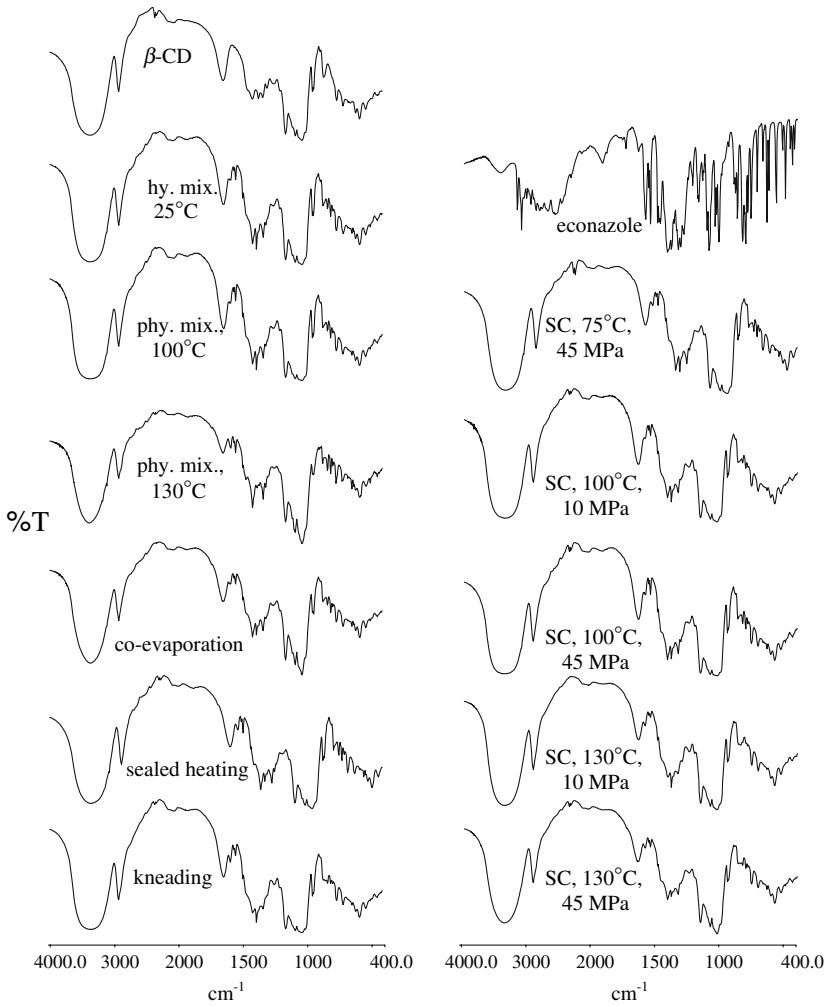

Fig. 3 FTIR spectra of pure econazole, pure β-CD, and econazole/β-CD (1:2 mole-mole) complexes prepared by physical mixing, kneading, co-evaporation, sealed heating, and SC CO_2

product obtained by kneading method exhibits shifts of bands at 1,646 cm^{-1}, decrease in intensity of bands at 1,415, 866 and 804 cm^{-1}, and augmentation in intensity of bands at 1,589 and 1,384 cm^{-1}, thus suggesting strong drug-CD interactions and/or amorphization as indicated by DSC analysis.

Only slight differences can be detected for the products obtained by SC CO_2 at 75 °C and 100 °C (both pressures) as compared to the physical mixtures exposed to 25 °C and 100 °C. However, FTIR spectra of the products obtained by SC CO_2 at 130 °C were substantially different from that of physical mixture exposed to the same temperature or lower temperatures (25 °C and 100 °C). In particular, the band at 1,646 cm^{-1} was shifted to 1,647 or 1,641 cm^{-1} in the products treated with SC CO_2 at 130 °C and 45 or 10 MPa, respectively. Moreover, the band at 1,415 cm^{-1} shifted to 1,413 cm^{-1} and showed a lower intensity in the sample treated at the lower pressure (10 MPa). Also, bands at 1,548, 828 and 804 cm^{-1} were significantly reduced in intensity and the intensity of the band at 1,384 cm^{-1} was increased for this sample as compared to the physical mixture exposed to the same temperature of 130 °C. For the product treated with SC CO_2 at 130 °C and the higher pressure (45 MPa), the bands at 1,548 and 867 cm^{-1} were reduced in intensity as compared to the physical mixture exposed to 130 °C. These results show that the effect of varying pressure (10 or 45 MPa) on the products obtained by SC CO_2 method is significant. Therefore, the changes in the thermal behavior observed for the samples treated with SC CO_2 at 130 °C cannot be due to the simple thermal treatment, but, on the contrary, they have to be attributed to the formation of an inclusion complex and/or amorphization of the sample resulting from a combined effect of both temperature and SC CO_2 as also indicated in our previous study [26].

PXRD Analysis

Powder X-ray diffractometry was used to further investigate the differences in the econazole-β-CD products prepared by the different methods. Figure 4 shows the X-

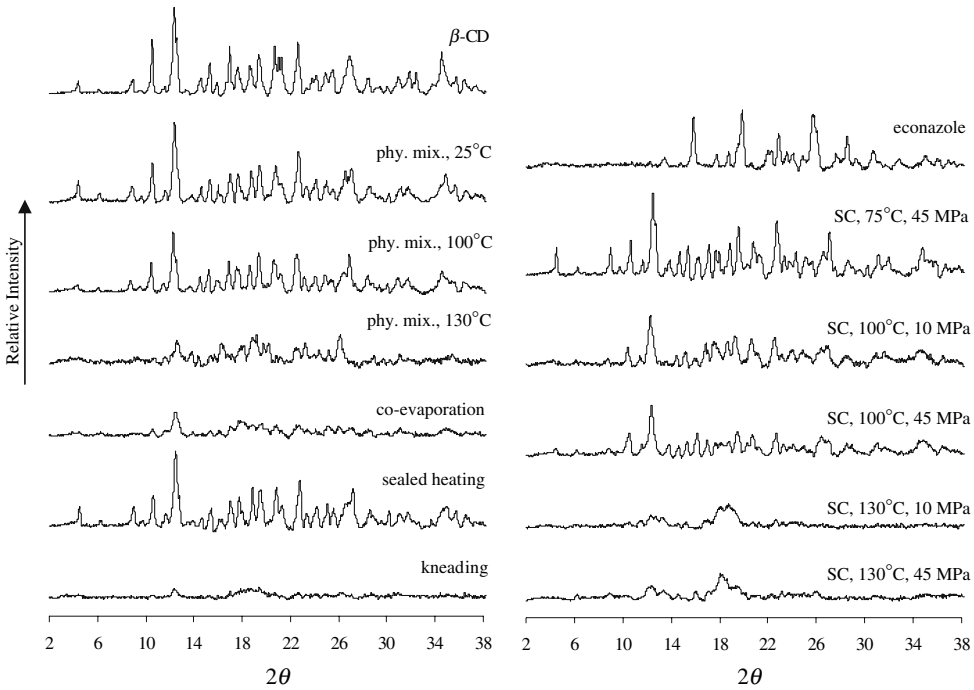

Fig. 4 PXRD patterns of pure econazole, pure β-CD, and econazole/β-CD (1:2 mole-mole) complexes prepared by physical mixing, kneading, co-evaporation, sealed heating, and SC CO_2

ray powder diffraction patterns of pure econazole, pure β-CD, and their 1:2 mol:mol systems obtained by physical mixing, kneading, co-evaporation, sealed-heating, and SC CO_2 method. The diffraction pattern of both econazole and β-CD displayed several sharp peaks, indicative of their crystalline nature. A crystalline pattern, given by the sum of the spectra of pure components, was obtained for the physical mixtures exposed to 25 °C and 100 °C with small difference in peak size for the sample exposed to 100 °C, suggesting no drug-CD interaction as indicated by DSC and FTIR analysis. Although the crystallinity nature of the physical mixture exposed to 130 °C was maintained, significant changes were observed in the PXRD pattern of this sample as compared to physical mixtures exposed to 25 °C and 100 °C (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 physical mixtures exposed to the lower temperatures (25 °C and 100 °C). 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. 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 patterns of the products obtained by SC CO_2 at 75 °C and 100 °C (both pressures: 10 and 45 MPa) were similar to those obtained for the physical mixtures exposed to 25 °C and 100 °C with small difference in peak size for the sample treated by SC CO_2 at 100 °C, suggesting no drug-CD interaction. However, PXRD patterns for the products obtained by SC CO_2 at 130 °C (both pressures) were significantly different from those of physical mixture exposed to the same temperature or lower temperatures (25 °C and 100 °C), showing a diffuse pattern with a very few low-intensity peaks, suggesting drug amorphization and/or complexation. The PXRD patterns also show that the different pressures (10 or 45 MPa) result in slightly different products obtained by SC CO_2 method at 130 °C. These results confirm that the thermal behavior observed for the samples treated with SC CO_2 at 130 °C are due to the combined effect of both temperature and SC CO_2 as indicated by FTIR analysis.

Conclusions

Solubility of econazole in SC CO_2 was measured and found to vary with both temperature and pressure. Drug-cyclodextrin formulations were prepared by complexation of econazole into β-CD using physical mixing, kneading, co-evaporation, sealed heating, and SC CO_2. The formation of inclusion complex in the SC CO_2 method was verified by

DSC, FTIR, and PXRD analysis and compared to those obtained by conventional methods. Results of these analyses indicated more drug-cyclodextrin interactions in the samples prepared by kneading and SC CO_2 (at 130 °C) methods compared to the products obtained by physical mixing, co-evaporation, and sealed heating methods. Moreover, the thermal behavior observed for the samples treated with SC CO_2 at 130 °C were attributed to the combined effect of both temperature and SC CO_2. Efficacy, safety and pharmacokinetics of the new inclusion complexes obtained by SC CO_2 are being investigated in both in vitro and in vivo. Appropriate mechanisms of the inclusion complexes are also being developed. This study has the potential to prepare solvent-free formulations for econazole, thus reducing the side effects compared to conventional formulations.

Acknowledgements The authors are grateful to the Research Affairs at the United Arab Emirates University for the financial support of this project (contract no. 01-02-7-12/04) and to Ali Dowaidar and Baboucarr Jobe for their assistance with analysis of the samples.

References

1. Hughes, W.T., Bartley, D.L., Patterson, G.G., Tufenkeji, H.: Ketoconazole and candidiasis: a controlled study. J. Infect. Dis. **147**, 1060–1063 (1983)
2. Murray, P.A., Koletar, S.L., Mallegol, I., Wu, J., Moskovitz, B.L.: Itraconazole oral solution versus clotrimazole troches for the treatment of oropharyngeal candidiasis in immunocompromised patients. Clin. Ther. **19**, 471–480 (1997)
3. Hay, R.J.: Overview of studies of fluconazole in oropharyngeal candidiasis. Rev. Infect. Dis. **12**(suppl. 3), S334–S337 (1990)
4. Meunier, F., Aoun, M., Gerard, M.: Therapy of oropharyngeal candidiasis in the immunocompromised host: a randomized double-blind study of fluconazole vs ketoconazole. Rev. Infect. Dis. **12**(suppl. 3), S364–S368 (1990)
5. Darouiche, R.O.: Oropharyngeal and esophageal candidiasis in immunocompromised patients: treatment issues. Clin. Infect. Dis. **26**, 259–274 (1998)
6. Pons, V., Greenspan, D., Lozada-Nur, F., McPhail, L., Gallant, J.E., Tunkel, A., Johnson, C.C., McCarty, J., Panzer, H., Levenstein, M., Barranco, A., Green, S.: Oropharyngeal candidiasis in patients with AIDS: randomized comparison of fluconazole versus nystatin oral suspensions. Clin. Infect. Dis. **24**, 1204–1207 (1997)
7. Szejtli, J.: Cyclodextrins in drug formulations: Part II. Pharm. Technol. Int. **Aug.**, 24–38 (1991)
8. Duchene, D., Wouessidjewe, D.: Physicochemical characteristics and pharmaceutical uses of cyclodextrin derivatives, Part II. Pharm. Technol. **Aug.**, 22–30 (1990)
9. Nambu, N., Kikuchi, K., Kikuchi, T., Takahashi, Y., Ueda, H., Nagai, T.: Influence of inclusion of nonsteroidal antiinflammatory drugs with β-cylcodextrin on the irritation to stomach of rats upon oral administration. Chem. Pharm. Bull. **26**(12), 3609–3612 (1978)
10. Dhanaraju, M.D., Kumaran, K.S., Baskaran, T., Moorthy, M.S.R.: Enhancement of bioavailability of Griseofulvin by its complexation with β-cyclodextrin. Drug Dev. Ind. Pharm. **24**(6), 583–587 (1998)
11. Hostetler, J.S., Hanson, L.H., Stevens, D.A.: Effect of cyclodextrin on the pharmacology of antifungal oral azoles. Antimicrob. Agents Chemother. **36**(2), 477–480 (1992)
12. Jacobsen, J., Bjerregaard, S., Pedersen, M.: Cyclodextrin inclusion complexes of antimycotics intended to act in the oral cavity-drug supersaturation, toxicity on TR146 cells and release from a delivery system. Eur. J. Pharm. Biopharm. **48**(3), 217–224 (1999)
13. Lee, B., Lee, J.: Enhancement of solubility and dissolution rate of poorly water-soluble Naproxen by complexation with 2-hydroxypropyl-β-cyclodextrin. Arch. Pharm. Res. **18**(1), 22–26 (1995)
14. Lee, S.Y., Chun, I.K.: Design of new parenteral aqueous formulations of fluconazole by the use of modified cyclodextrins. Yakhak Hoechi. **45**(4), 357–365 (2001)
15. Mura, P., Adragna, E., Rabasco, A.M., Moyano, J.R., Perez-Martinez, J.I., Arias, M.J., Gines, J.M.: Effect of the host cavity size and the preparation method on the physicochemical properties of Ibuproxam-cyclodextrin systems. Drug Dev. Ind. Pharm. **25**(3), 279–287 (1999)
16. Mura, P., Faucci, M.T., Manderioli, A., Bramanti, G.: Influence of the preparation method on the physicochemical properties of binary systems of econazole with cyclodextrins. Int. J. Pharm. **193**(1), 85–95 (1999)
17. Strickley, R.: Solubilizing excipients in oral and injectable formulations. Pharm. Res. **21**, 201–230 (2004)
18. Loftsson, T., Masson, M., Brewster, M.: Self-association of cyclodextrins and cyclodextrin complexes. J. Pharm. Sci. **93**, 1091–1099 (2004)
19. Peeters, J., Neeskens, P., Tollenaere, J., Remoortere, P.V., Brewstrer, M.E.: Characterization of the interaction of 2-hydroxypropyl-β-cyclodextrin with Itraconazole at pH 2, 4, and 7. J. Pharm. Sci. **91**(6), 1414–1422 (2002)
20. Van Hees, T., Piel, G., Evrard, B., Otte, X., Thunus, L., Delattre, L.: Application of supercritical carbon dioxide for the preparation of a Piroxicam-β-cyclodextrin inclusion compound. Pharm. Res. **16**(12), 1864–1870 (1999)
21. Van Hees, T., Barillaro, V., Piel, G., Bertholet, P., De Hassonville, S., Evrard, B., Delattre, L.: Application of supercritical carbon dioxide for the preparation of drug-cyclodextrin inclusion compounds. J. Incl. Phenom. Macro. **44**, 271–274 (2002)
22. Bandi, N., Wei, W., Roberts, C.B., Kotra, L.P., Kompella, U.B.: Preparation of budesonide–and indomethacin–hydroxypropyl-β-cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process. Eur. J. Pharm. Sci. **23**, 159–168 (2004)
23. Fabing, I., Leboeuf, F., Jung, J., Perrut, M.: Method for making very fine particles consisting of a principle inserted in a host-molecule. Patents FR 2 815 540- WO 0232462- EP 1 330 266, 2000
24. Perrut, M., Jung, J., Leboeuf, F.: Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes, Part II: preparation of composite particles. Int. J. Pharm. **288**, 11–16 (2005)
25. Rodier, E., Lochard, H., Sauceau, M., Letourneau, J.-J., Freiss, B., Fages, J.: A three step supercritical process to improve the dissolution rate of Eflucimibe. Eur. J. Pharm. Sci. **26**, 184–193 (2005)
26. Al-Marzouqi, A.H., Jobe, B., Dowaidar, A., Maestrelli, F., Mura, P.: Evaluation of supercritical fluid technology as preparative technique of benzocaine-cyclodextrin complexes-Comparison with conventional methods. J. Pharm. Biomed. Anal. **43**, 566–574 (2007)
27. Al-Marzouqi, A.H., Jobe, B., Corti, G., Cirri, M., Mura, P.: Physicochemical characterization of drug-cyclodextrin

complexes prepared by supercritical carbon dioxide and by conventional techniques. J. Incl. Phenom. Macro. **57**, 223–231 (2007)
28. Al-Marzouqi, A.H., Shehatta, I., Jobe, B., Dowaidar, A.: Phase solubility and inclusion complex of itraconazole with β-cyclodextrin using supercritical carbon dioxide. J. pharm. Sci. **95**(2), 292–304 (2006)
29. Shehatta, I., Al-Marzouqi, A.H., Jobe, B., Dowaidar, A.: Enhancement of aqueous solubility of itraconazole by complexation with cyclodextrins using supercritical carbon dioxide. Can. J. Chem. **83**(10), 1833–1838 (2005)
30. Hassan, A., Tang, Y., Ayres, J.: Itraconazole formation using supercritical carbon dioxide. Drug Dev. Ind. Pharm. **30**(10), 1029–1035 (2004)
31. Kiran, E., Brennecke, J.: Supercritical Fluid Engineering Science, ACS Symposium Series 514. American Chemical Society, Washington, D.C. (1993)
32. Charoenchaitrakool, M., Dehghani, F., Foster, N.R.: Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl-β-cyclodextrin. Int. J. Pharm. **239**, 103–112 (2002)
33. Türk, M., Upper, G., Steurenthaler, M., Hussein, Kh., Wahl, M.A.: Complex formation of ibuprofen and β-cyclodextrin by controlled particle deposition (CPD) using SC-CO2. J. Supercrit. Fluids **39**, 435–443 (2007)
34. Pedersen, M., Edelsten, M., Nielsen, V.F., Scarpellini, A., Skytte, S., Slot, C.: Formation and antimycotic effect of cyclodextrin inclusion complexes of econazole and miconazole. Int. J. Pharm. **90**, 247–254 (1993)
35. Pedersen, M., Bjerregaard, S., Jacobsen, J., Larsen, A.R., Sørensen, A.M.: An econazole β-cyclodextrin inclusion complex: an unusual dissolution rate, supersaturation, and biological efficacy example. Int. J. Pharm. **165**, 57–68 (1998)