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Characterization and in vitro dissolution behaviour of ketoconazole/β- and 2-hydroxypropyl-β-cyclodextrin inclusion compounds

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Abstract

The effect of β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin on the solubility of ketoconazole in different media were studied. A type A_L solubility diagram was obtained for ketoconazole and the two cyclodextrins in buffer solution, pH 5 and pH 6. The stability constants between ketoconazole and the two cyclodextrins were calculated from the phase solubility diagrams. Increased ionization of the imidazole derivative decreased the values of the stability constants. The formation of solid inclusion complexes were experimentally prepared by the kneading and spray-drying techniques. In order to confirm solid complex formation, X-ray diffractometry and differential scanning calorimetry were used. It was found that the spray-drying technique could be used to prepare the amorphous state of drug inclusion complexes. The dissolution rates of ketoconazole from the inclusion complex made by spray-drying were faster than the pure drug, kneading systems and the physical mixtures of drug and cyclodextrins. The enhanced dissolution rate of spray-dried products might be attributed to the decreased particle size, the high-energetic amorphous state and inclusion complex formation.

Keywords: Ketoconazole; Cyclodextrins; Inclusion compounds; Dissolution

1. Introduction

Ketoconazole (KET) is a broad-spectrum antifungal imidazole derivative that has been shown to be efficent in oropharyngeal candidiasis, the

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most common infection in AIDS patients. This drug is a weak base with limited water solubility. With pKa values of 2.94 and 6.51 one would expect that KET would dissolve much more completely and rapidly in a solution with low pH than at higher pH. Therefore, the inhibition or neutralization of gastric secretion—common in those patients with AIDS-might be expected to reduce the bioavailability of KET (Van der Meer et al., 1980).

Cyclodextrins are oligosaccharides which have received increasing attention in the pharmaceutical field because of their ability to form inclusion complexes with many lipophilic drugs, thus changing the physicochemical and biopharmaceutical properties of them (Szejtli, 1988; Duchêne and Wouessidjewe, 1990). The complexation between imidazole derivatives and cyclodextrins has already been studied (Pedersen, 1994; De Beule, 1996) and the antimycotic activity of these complexes has been found superior to the activity of drug alone.

The present study was carried out to investigate the thermodynamics of the complexation of KET with two cyclodextrins-β-cyclodextrin (BCD) and hydroxypropyl-β-cyclodextrin (HP-BCD)— by determining the effects of temperature and cyclodextrin concentrations on solubility of KET in aqueous solutions pH 5 and pH 6. Kneading, spray-drying and physical mixing were employed for the preparation of KET systems with BCD and HPBCD and the effect of complexation in the solubility and dissolution rate of KET was evaluated. Furthermore, X-ray diffractometry and differential scanning calorimetry (DSC) were used in the study of interactions between KET with either BCD and HPBCD in solid state.

2. Materials and methods

2.1. Materials

Ketoconazole (*cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1H-imidazol-1-ylmethyl) -1,3 dioxolan-4-yl] piperazine) was supplied by Guinama (Spain); BCD by Cyclolab (Hungary) and HPBCD (d.s. 5.8) by Pharmatec (USA.). All other reagents were of analytical grade.

2.2. Effect of pH on ketoconazole solubility

A back-titration procedure was carried out to evaluate the effect of pH on ketoconazole solubility. Excess KET (25 mg) was added to 5 ml vials containing unbuffered dissolution medium as the pH was increased from 3 to 7.5. The vials were shaken in a water bath at 37°C and after 5 days equilibration period the samples were filtered for spectrophotometric analysis. In the same way, the KET was added to vials containing the same unbuffered solutions with BCD or HPBCD (0.5%).

2.3. Phase solubility studies and thermodynamic parameters of complexation

Solubility measurements were carried out according to the method described by Higuchi and Connors (1965). To 5 ml phosphate buffer solutions, pH 5 and 6, containing various concentrations of BCD ($0–4.4 \cdot 10^{-3}$ M) and HPBCD ($0–3.4 \cdot 10^{-3}$ M), 15 mg of KET were added. The vials containing these suspensions were placed in a water bath at 25, 30 and 37°C with shaking. After equilibrium had been reached (7 days), the contents of each vial were filtered (0.22 μm pore size) and the concentration of the antimicrobial agent in the filtered solutions was measured by UV spectrophotometry at 225 nm (pH 5: $\epsilon_{1\%\ 1\ cm} = 445.57$; pH 6: $\epsilon_{1\%\ 1\ cm} = 428.42$). The presence of BCD or HPBCD did not interfere with the spectrophotometric assay. The 1:1 stability constants were calculated from the initial straight-line portions of the phase-solubility diagrams as

$$K_{1:1} = \text{slope}/S_0(1 - \text{slope})$$

where S_0 is the solubility of the pure drug (Higuchi and Connors, 1965).

Apparent values of enthalpy and entropy for the stability constants of the cyclodextrin complexes were determined from linear plots of ln $K_{1:1}$ vs. $1/T$ (figures not shown), according to the integrated form of the Van't Hoff equation (Martin et al., 1993).

2.4. Preparation of the physical mixtures

The physical mixtures of an appropiate amount of KET/BCD and KET/HPBCD in a 1:1 molar ratio were obtained by pulverizing and there after mixing the solids in a Turbula T2C mixer (5 min at 30 rpm).

2.5. Preparation of solid inclusion complexes

The solid complexes of KET with BCD and HPBCD—1:1, M:M– were prepared using kneading and spray-dried methods.

(1) Kneading: KET, BCD and HPBCD powders (50–80 μm fraction) in the 1:1 drug/CD molar ratio were mixed in a Turbula T2C mixer. The drug and the cyclodextrins were tritured with a mortar and pestle, adding a 1:1 in volume water-ethanol solution. The slurries were kneaded for 30 min and then dried at 45°C for 2 days. The yield of the kneading process was 95%.

(2) Spray drying: KET and cyclodextrins were dissolved in ethanol and water, respectively. Then, both solutions were mixed before spray-drying. Spray-drying was carried out in a Büchi 190 apparatus (flow rate 4 ml min^{-1}, inlet temperature 85°C, atomizing air pressure 3 kg cm^{-2}). The yield of the spray-drying process was 50%.

2.6. Characterization of the mixtures and the inclusion complexes

Thermal analysis was carried out in a Shimadzu DSC-50 system with a differential scanning calorimeter equipped with a computerized data station (scanning rate 10°C min^{-1}).

Powder X-ray diffraction patterns were obtained with a Philips X-ray diffractometer (PW 1710 BASED) using Cu-K_α radiation.

2.7. Dissolution studies

In vitro dissolution studies of pure drug, physical mixtures and the inclusion complexes prepared were carried out according to Nogami et al. (1969) in phosphate buffer solutions of pH 5 and pH 6 as dissolution medium. Powdered samples containing 30 mg of KET or its equivalent in complexed or physically mixed form (50–80 μm fraction), were placed in 100 ml of the dissolution medium in a cell at 37°C for 180 min and shaken at 500 rpm. At predetermined time intervals, samples were taken for spectrophotometric determination of KET concentration following filtration and, if necessary, dilution. All samples were analyzed in triplicate.

Dissolution efficiencies after 180 min (DE_{180}) were calculated according to the method of Khan (1975). The effects of drug form on dissolution efficiency at each pH were investigated by one-way analysis of variance with the Scheffé test for multiple comparisons.

3. Results and discussion

3.1. Effect of pH on ketoconazole solubility

In all cases, the back-titration procedure demonstrated a gradual decrease in the solubility of KET up until a pH 4, after this the decline was rapid (Fig. 1). This was found in full accordance with Carlson et al. (1983). When the drug was added to an aqueous solution containing cyclodextrins its solubility increased in all pH ranges, and this effect was higher with BCD.

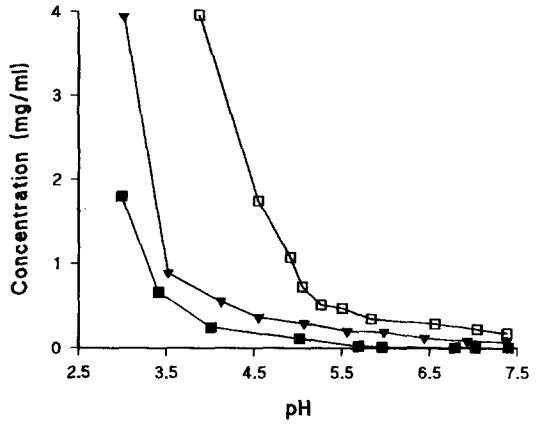

Fig. 1. pH-solubility profiles of KET in unbuffered dissolution medium: (■) KET; (□) KET-BCD; (▼) KET-HPBCD.

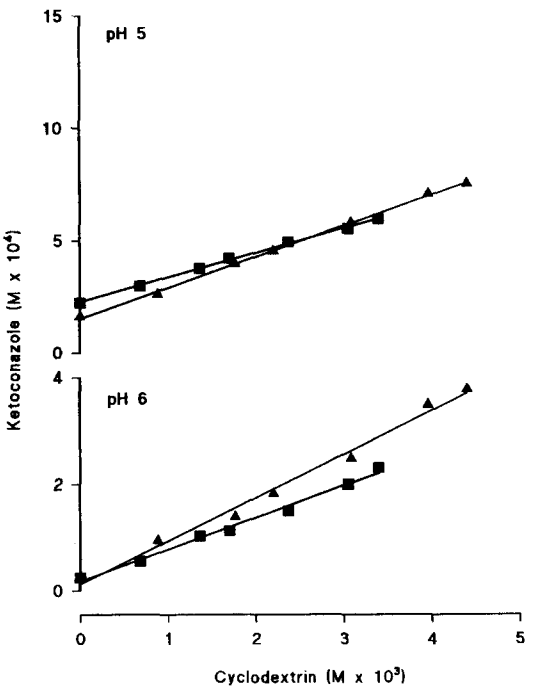

Fig. 2. Phase solubility diagrams of KET and both cyclodextrin derivatives in phosphate buffer pH 5 and 6, at 37°C. (▲) BCD; (■) HPBCD.

3.2. Phase solubility diagrams

The phase solubility diagrams for KET with BCD and HPBCD obtained at 37°C in both phosphate buffer solutions are shown in Fig. 2. It was found that the solubility of KET increased linearly as a function of BCD and HPBCD concentrations and showed the features of an A_L type following Higuchi and Connors (1965) classification. Solubility diagrams obtained at 25 and 30°C were likewise type A_L (data not shown).

An attempt to calculate the stability constants from the A_L phase diagrams was made by assuming that only 1:1 (M:M) complexes were formed. Additionally, the thermodynamic parameters of the complexes obtained from the temperature dependence of the disassociation constant were calculated (Table 1). The values of the stability constants for KET-BCD system are higher than for the KET-HPBCD system—except at 25°C in pH 6—which means a better interaction with BCD. Moreover, these values were higher at pH 6 due to the lower ionization of the drug (Otero-Espinar et al., 1989).

The formation of the complexes with both cyclodextrins is associated with an apparent unfavorable enthalpy change (except with HPBCD at pH 5) and a favorable entropy change. In these cases, the negative entropy change implies that hydrophobic interactions are predominant in the interaction of the KET-BCD and KET-HPBCD in solutions (Bender and Komiyama, 1978).

3.3. Characterization of solid complexes

The X-ray diffractometry patterns (Figs. 3 and 4) of the physical mixtures and kneaded system of KET-BCD and KET-HPBCD are approximately the superposition of the patterns of the raw materials. On the other hand, the amorphous character of the spray-dried inclusion complexes is obvious, compared with the physical mixtures or kneaded products and they have a completely different pattern in which it is no longer possible to distinguish the characteristic peaks of KET.

The thermal behavior of cyclodextrin inclusion compounds was studied using DSC in order to confirm the formation of the solid complexes. When guest molecules are incorporated in the cyclodextrin cavity or in the crystal lattice, their

Table 1
Apparent stability constant ($K_{1:1}$) and thermodynamic parameters for the complexation of KET with BCD and HPBCD in buffer pH Sand 6 at 25, 30 and 37°C

		T[a] (°C)	$K_{1:1}$ (M^{-1})	ΔG (kcal/mol)
BCD	pH 5	25	827.14	−3.95
		30	756.76	−4.08
		37	1051.94	−4.27
	pH 6	25	1271.12	−4.52
		30	8826.99	−4.99
		37	6957.29	−5.64
HPBCD	pH 5	25	626.97	−3.88
		30	678.61	−3.89
		37	536.26	−3.92
	pH 6	25	3471.90	−4.31
		30	2079.05	−4.61
		37	1434.41	−5.03

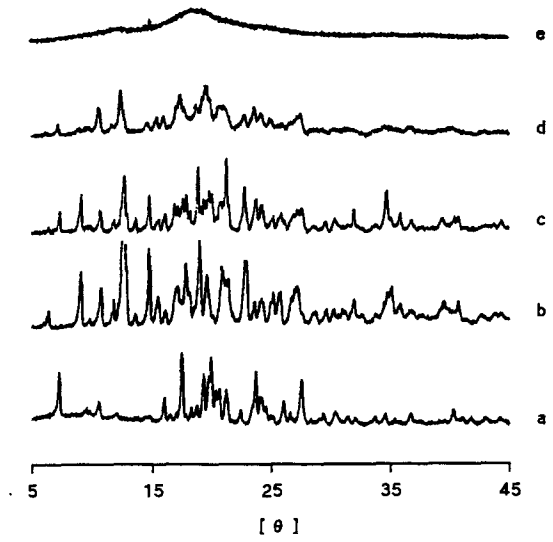

Fig. 3. Powder X-ray diffraction patterns of the different KET-BCD systems: (a) KET; (b) BCD; (c) physical mixture; (d) kneaded complex; (e) spray-dried complex.

melting, boiling and sublimation points usually shift to a different temperature or disappear within the temperature range where the cyclodextrin lattice is decomposed (Cabral Marques et al.,

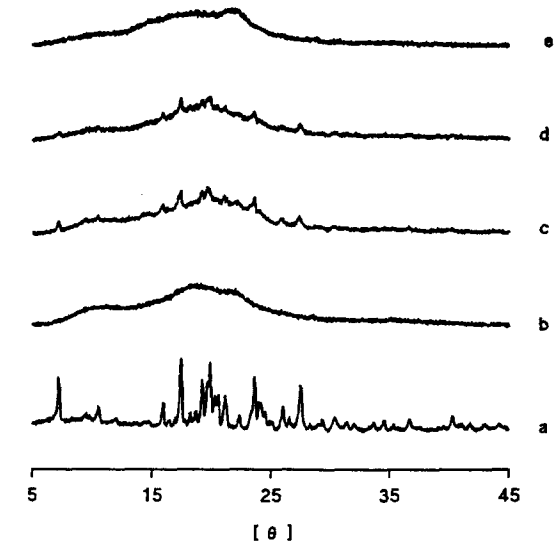

Fig. 4. Powder X-ray diffraction patterns of the different KET-HPBCD systems: (a) KET; (b) BCD; (c) physical mixture; (d) kneaded complex; (e) spray-dried complex.

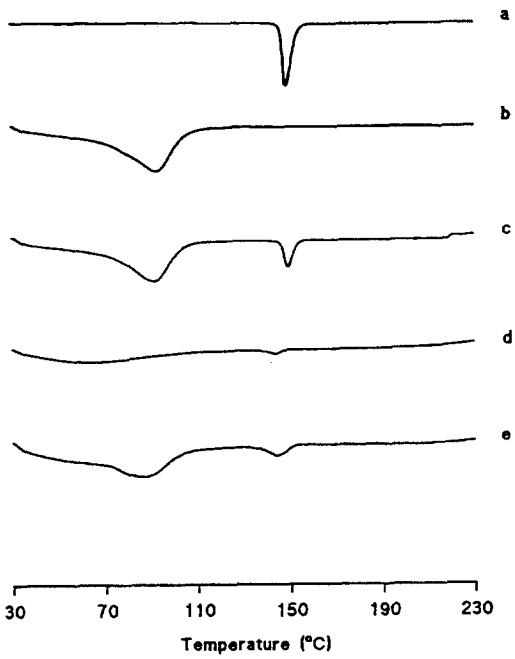

Fig. 5. Differential scanning calorimetry of the different KET-BCD systems: (a) KET; (b) BCD; (c) physical mixture; (d) spray-dried complex; (e) kneaded complex.

1990). The DSC curves of the KET-BCD and KET-HPBCD are shown in Figs. 5 and 6. The thermograms showed an endothermic peak for the KET at 149°C and for the physical mixture and the kneaded systems this peak shows a slight change. The fact that the peak of these systems changed relative to that of the pure drug showed that there was a weak interaction (Erden and Celebi, 1988). Besides, the KET fusion appeared to be more definite in the case of the physical mixture than for the kneaded complex. This may be explained by a better dispersion of the KET microcrystals in the BCD in the case of the complex. Evidence that the spray-dried complexes are true inclusion compounds and not a simple physical mixture was based upon the fact that the endothermic peak due to the phase transition profile of the KET was not observed for the preparation which was thought to contain the inclusion compounds. The disappearance of the endothermic peak may be attributed to the inclusion of the drug in the BCD and HPBCD cavity (Lin et al., 1991; Esclusa-Díaz et al., 1994).

These results indicate that the complexes obtained by the kneading method did not seem to be a true inclusion. However, the encapsulation of the drug within the BCD and HPBCD can be achieved by the spray-drying process.

3.4. Dissolution studies

The solubility results carried out in buffer solution pH 5 show that the presence of cyclodextrins leads to an improvement in the solubility of KET. As may be seen in Fig. 7, the KET dissolution rate from the kneaded systems and spray-dried complexes is clearly increased compared with that of the physical mixture and KET alone.

For the KET-HPBCD systems, at 5 min, nearly 80% of KET is dissolved from the spray-dried complex compared with 65% from the kneaded compound or 30% from the physical mixture. For the KET-BCD systems, at the same time, only 50% of KET is solubilized from the spray-dried complex, and from the kneaded system (30%) and physical mixture (17%) the percentages are lower.

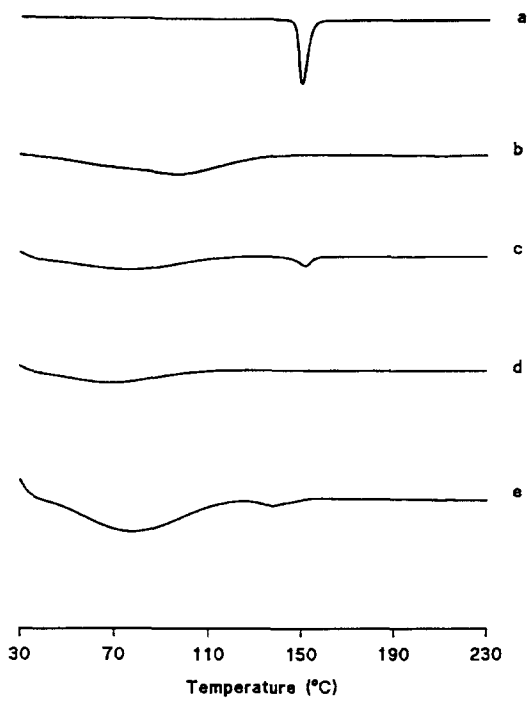

Fig. 6. Differential scanning calorimetry of the different KET-HPBCD systems: (a) KET; (b) HPBCD; (c) physical mixture; (d) spray-dried complex; (e) kneaded complex.

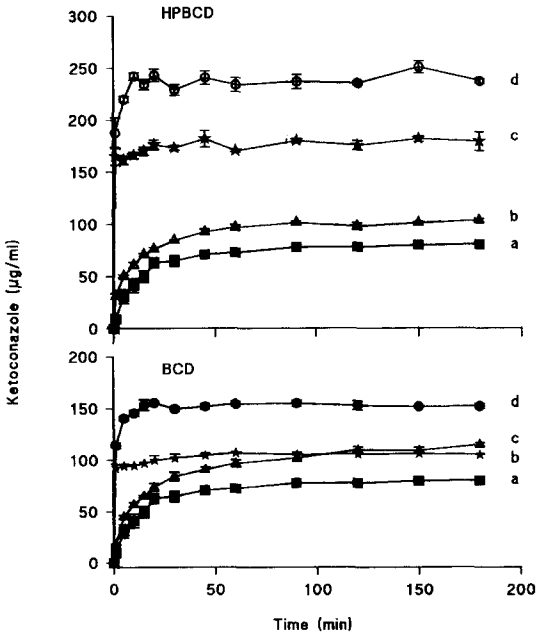

Fig. 7. Dissolution profiles of KET and its inclusion complexes at pH 5 phosphate buffer: (a) KET; (b) physical mixture; (c) kneaded complex; (d) spray-dried complex.

The faster dissolution of KET results with the HPBCD complex. However, it is important to point out that 100% dissolution is not reached at the end of the assay. Analysis of variance indicates that the factor 'formulation' has a significant effect on 0–180 min dissolution efficiency in all cases: $F_{(3,8)} = 606.3$, $P < 0.01$ for KET-BCD and $F_{(3,8)} = 999.9$, $P < 0.01$ for KET-HPBCD. The Scheffé test indicates that there are significant differences between all the formulations.

The dissolution profiles of the KET-HPBCD and KET-BCD systems at pH 6 are shown in Fig. 8. The inclusion complexes, as well as physical mixtures, significantly increased the solubility of KET in the pH 6 phosphate buffer. At this pH, the analysis of variance indicates that the factor 'formulation' has a significant effect on 180-min dissolution efficiency too: $F_{(3,8)} = 442.0$, $P < 0.01$ for KET-BCD and $F_{(3,8)} = 999.9$, $P < 0.01$ for KET-HPBCD.

It is important to note the dissolution behavior of the drug from the KET-HPBCD spray-dried system in this medium. The drug dissolved very rapidly in the first minute and then decreased gradually due to the recrystallization of KET in this medium (Lin et al., 1991). It is evidence that the dissolution rates of the spray-dried products are faster than those of the kneaded system, physical mixture and pure drug. This behavior may be attributed to the decreased particle size, the high energetic amorphous state and inclusion complex formation. On the other hand, the kneading complex obtained with BCD shows approximately the same dissolution behavior than the physical mixture, which is in complete accordance with the physical characterization.

To conclude, the solid inclusion complexes of KET with both BCD and HPBCD can be obtained by the spray-dried method. Kneading seems to be of interest only if one needs to obtain a simple increase in solubility and dissolution rate, without requiring the formation of true inclusion compounds. An important factor is the cyclodextrin employed, in fact, complexes obtained with HPBCD exhibit a better solubility and dissolution rate compared to the BCD complexes. In spite of the higher stability constant found for BCD, the effect on the solubility of KET is higher with HPBCD. This effect is probably due to the higher water solubility and the lower hydrophobicity of the HPBCD.

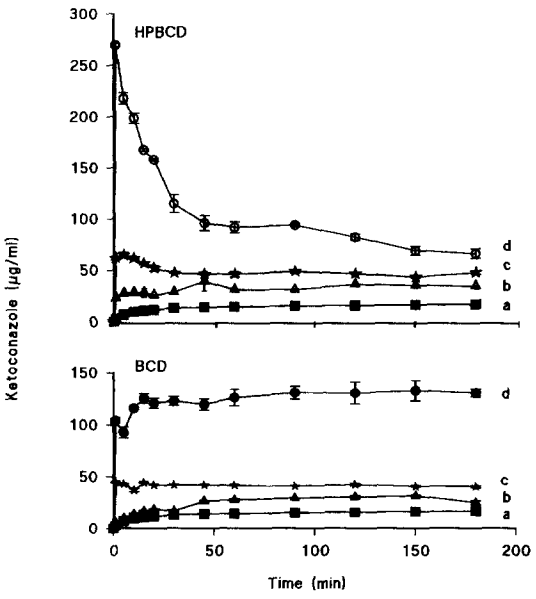

Fig. 8. Dissolution profiles of KET and its inclusion complexes at pH 6 phosphate buffer: (a) KET; (b) physical mixture; (c) kneaded complex; (d) spray-dried complex.

Acknowledgements

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