ELSEVIER

International Journal of Pharmaceutics 193 (1999) 85–95

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Influence of the preparation method on the physicochemical properties of binary systems of econazole with cyclodextrins

P. Mura *, M.T. Faucci, A. Manderioli, G. Bramanti

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9, I-50121 Florence, Italy

Received 30 April 1999; received in revised form 14 September 1999; accepted 14 September 1999

Abstract

Equimolar combinations of econazole, a very poorly water soluble antifungal agent, with β-cyclodextrin and statistically substituted methyl-β-cyclodextrin were investigated for both solid state characterization (differential scanning calorimetry, hot-stage microscopy, infrared spectroscopy, scanning electron microscopy) and dissolution properties (dispersed amount method). The influence of the preparation method (physical mixing, ball-milling, kneading, sealed-heating) on the physicochemical properties of the products was evaluated. Kneading and sealed-heating techniques led to amorphous products in the case of systems with methyl-β-cyclodextrin, whereas crystalline drug was still clearly detectable in all products with β-cyclodextrin. Independently of the preparation technique, all combinations with methyl-β-cyclodextrin yielded better performance than the corresponding ones with β-cyclodextrin. However, the influence of the preparation method was clearly more marked for products with methyl-β-cyclodextrin and made to be possible to better display the different performance of the examined carriers. In fact, the sealed-heated with the β-derivative showed an increase of drug dissolution efficiency of 130% with respect to the corresponding physical mixture, in comparison to the 70% increase obtained from that with β-cyclodextrin. Moreover, whereas the difference in dissolution efficiency values between coground products was only about 8% in favor of the β-derivative, it reached 80 and 90% between sealed-heated and kneaded products, respectively.

Keywords: Econazole; β-Cyclodextrin; Amorphous methyl-β-cyclodextrin; Differential scanning calorimetry; Hot-stage-microscopy; Scanning-electron-microscopy; Dissolution properties

1. Introduction

Cyclodextrins have recently been recognized as useful pharmaceutical excipients, due to their potential to form inclusion complexes with appropriately sized drug molecules (Szejtli, 1988). The resulting complexes generally offer a variety of physicochemical advantages over the free drug, including increased water solubility, enhanced bioavailability, improved stability, reduced side effects, etc. (Duchêne et al., 1987). Previous studies showed that both dissolution properties

* Corresponding author. Tel.: + 39-055-2757292; fax: + 39-055-240776.

E-mail address: mura@farmfi.scifarm.unifi.it (P. Mura)

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00326-9

and consequently microbiological activities of econazole, an antifungal agent with very low water solubility (about 3 μg/ml at 25°C), can be improved by complexation with natural cyclodextrins, particularly with β-cyclodextrins (Bononi, 1988; Mura et al., 1992; Pedersen et al., 1993). However, alkylated and particularly methylated cyclodextrins demonstrated to be often more effective as solubilizing and complexing agents than parent cyclodextrins (Uekama and Irie, 1987; Müller et al., 1988). Furthermore, several techniques have been proposed for complex preparation and the choice of the method should be carefully evaluated, taking into account not only factors such as good yield, simplicity, rapidity, ease of scaling up and low cost but also of the performance of the end product (Duchêne et al., 1987). Therefore, in the present work it was considered worthy of interest to extend our investigations and study in detail the influence of preparation method and of the type of carrier on the physicochemical properties of a series of binary systems of econazole with both crystalline native β-cyclodextrin and its amorphous and highly soluble derivative methyl-β-cyclodextrin, in order to arrive at a rational and careful evaluation of the most suitable econazole-cyclodextrin solid binary system. The systems were prepared by three different methods commonly used for production of solid drug-cyclodextrin complexes (ball-milling, kneading, sealed-heating) and compared with the corresponding physical mixtures. Differential scanning calorimetry (DSC), hot-stage microscopy (HSM), scanning electron microscopy (SEM) and infrared spectroscopy were used to characterize the solid state of various binary systems, whereas their dissolution properties were evaluated according to the dispersed amount method. Drug-carrier interactions in solution were studied by phase-solubility analysis. The results, in terms of both amorphization and solubilization of econazole are discussed. The effect of ageing on dissolution properties of the various preparations was also investigated.

2. Materials and methods

2.1. Materials

Econazole (1[2-(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)-ethyl-1-H-imidazole; kindly donated by Italfarmaco, Genova, Italy), β-cyclodextrin (βCd; Sigma) and methyl-β-cyclodextrin (MeβCd) MS 1.8 (kindly donated by Waker–Chemie GmbH, Germany) were used as received. All other materials and solvents were of analytical reagent grade.

2.2. Preparation of solid binary systems

Equimolar drug-Cd systems were prepared from the individual components previously sieved (75–150 μm): (a) by tumble mixing for 20 min with a turbula mixer (physical mixtures); (b) by ball-milling physical mixtures in a vibrational mill (Retsch, GmbH, Düsseldorf, Germany) for 30 min (ground systems); (c) by wetting physical mixtures in a mortar with the minimum volume of an ethanol-water 1:1 (by volume) mixture and grinding thoroughly with a pestle to obtain a paste which was then dried under vacuum at room temperature up to constant weight (kneaded systems); and (d) by heating physical mixtures in sealed containers at 70°C for 3 h (sealed-heated systems).

Each solid product was sieved and the 75–150-μm granulometric sieve fraction was collected. All systems were stored for 6 months in closed containers at room temperature.

2.3. Thermal analysis

DSC analysis was performed with a Mettler TA4000 apparatus equipped with a DSC 25 cell. Samples were weighed in Al pans (5–8 mg), pierced with a perforated lid and scanned at 10 K min^{-1} in the 30–200°C range, under static air.

HSM analysis was performed using an Olympus BH-2 microscope fitted with a Mettler FP-82 hot-stage. A small amount of sample, placed on the sample stage, was heated in the 30–200°C range at 1 or 5 K min^{-1}.

2.4. SEM

SEM photographs were recorded on a Philips XL-30 scanning microscope. Prior to examination, samples were gold sputter-coated to render them electrically conductive.

2.5. Infrared spectroscopy

Infrared spectra were obtained as Nujol mulls with a Perkin–Elmer Mod. 983 infrared spectrophotometer.

2.6. Dissolution studies

Dissolution rates of econazole from all drug-carrier binary systems, and for econazole alone, were determined in water at 37 ± 0.5°C according to the dispersed amount method, by adding 20 mg of drug or drug-equivalent to 300 ml of water, in a 400-ml beaker. A glass three-blade propeller (19 mm diameter) was immersed in the beaker 25 mm from the bottom and rotated ($f = 100$ min^{-1}). Suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 μm) at the specified times and the drug concentration was determined by second derivative spectroscopy ($^2D_{278\text{-}274}$; Perkin Elmer Mod. Lambda 12). The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated. Each test was repeated three times (coefficient of variation $< 5\%$). Dissolution was characterized through the percent of drug dissolved after 10 min, as index of the rate of dissolution, and the dissolution efficiency (DE), as index of the totality of the process. DE was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time (Khan, 1975). Statistical analysis (Student's t-test) was applied to evaluate the significance of the inter-treatment and inter-carrier differences.

2.7. Phase solubility analysis

Excess amounts of econazole were added to 10 ml of water or Cd aqueous solution (in the 5–100-mmol l^{-1} concentration range) in sealed glass containers electromagnetically stirred at a constant temperature (25 or 37°C) up to equilibrium (3 days). Aliquots were withdrawn and filtered (pore size 0.45 μm), and the drug concentration was spectrophotometrically determined as described above. The experiments were performed in triplicate. Apparent 1:1 stability constants were calculated from the straight-line portion of the phase solubility diagrams (Higuchi and Connors, 1965).

3. Results and discussion

DSC curves of pure components and of the various drug-carrier binary systems are shown in Fig. 1. The thermal curve of econazole ($T_{onset} = 86.9$°C; $T_{peak} = 89.2$°C; $T_{endset} = 92.9$°C; $\Delta H = 80.6$ J/g) indicated its crystalline anhydrous state. Liberation of crystal water from βCd (14.5% as mass fraction) was observed as an endothermal effect peaked at about 130°C, whereas a broader endotherm effect, associated with water loss (7.5% as mass fraction), was recorded for amorphous MeβCd. Thermal curves of all the binary systems of econazole with βCd always showed the typical drug melting endotherm, which progressively reduced its area and shifted to lower temperature passing from physical mixture (86.5°C) to coground (83.5°C), kneaded (81.8°C) and sealed-heated (78.4°C) systems, as a consequence of gradually increasing interactions between the components (Kim et al., 1985). Marked reduction of area, broadening, and down-shift of the peak temperature of drug melting endotherm (79.1°C) were observed in the physical mixture with MeβCd, indicative of a more evident loss of drug crystallinity. In the coground system, the drug endothermal effect further broadened and was almost hidden by the dehydration band of the carrier, and finally it disappeared in the kneaded and sealed-heated systems. This last phenomenon, attributable both to inclusion complex formation and/or drug amorphization (Bettinetti et al., 1989), was however undoubtedly an index of a stronger interaction in the solid state between

econazole and the β-derivative. The thermal behavior of the various binary systems appeared practically unchanged after 6 months storage.

HSM analysis confirmed in all systems with βCd (Fig. 2) the presence of free drug crystals, which appeared to melt between 82 and 88°C. On the contrary, the absence of any change around the drug melting temperature was found in kneaded and sealed-heated systems with MeβCd (Fig. 3), and the fusion of drug crystals was detectable only in the physical mixture and coground system.

From SEM analysis, econazole particles appeared as small crystals (5–10 μm) of homoge-

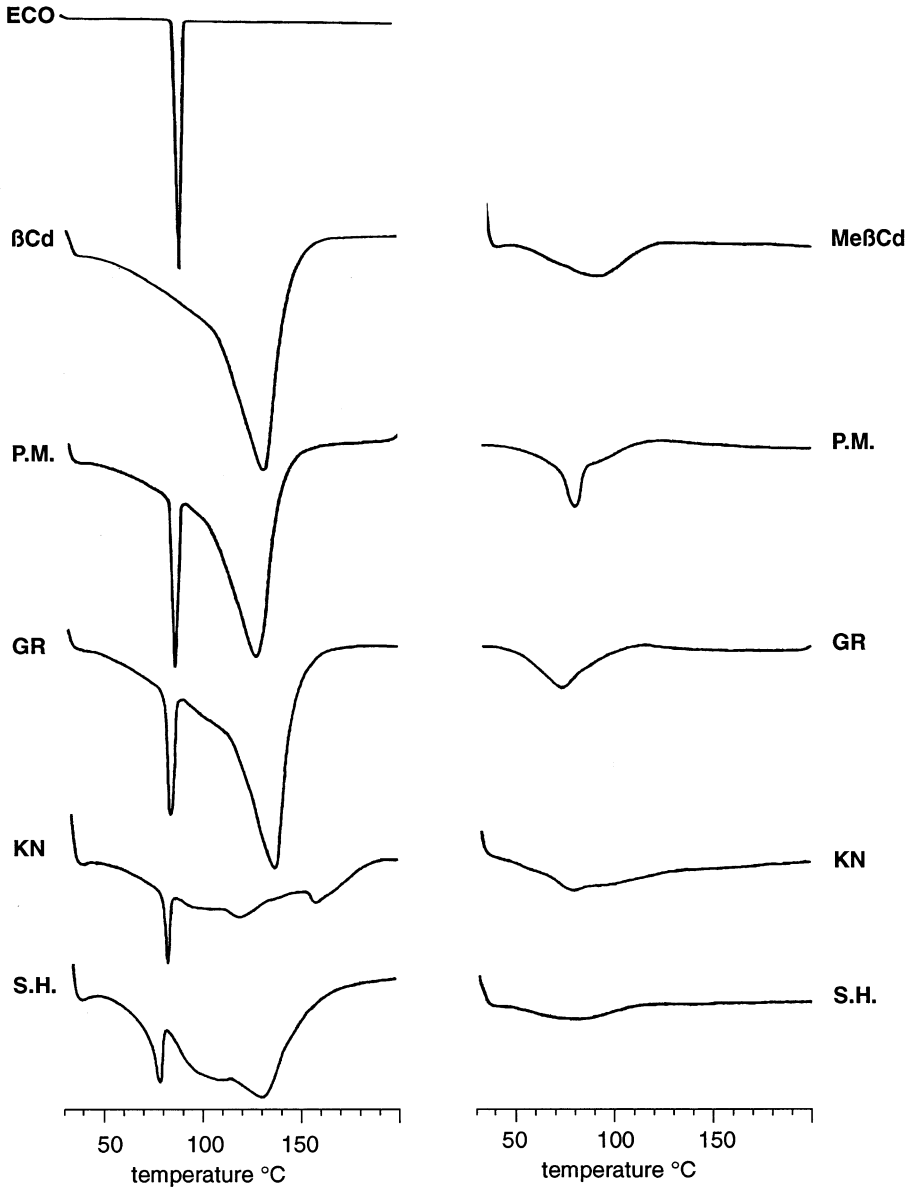

Fig. 1. DSC curves of econazole (ECO), βCd and MeβCd and the corresponding drug-carrier equimolar physical mixtures (PM), coground (GR) kneaded (KN) and sealed-heated (SH) products.

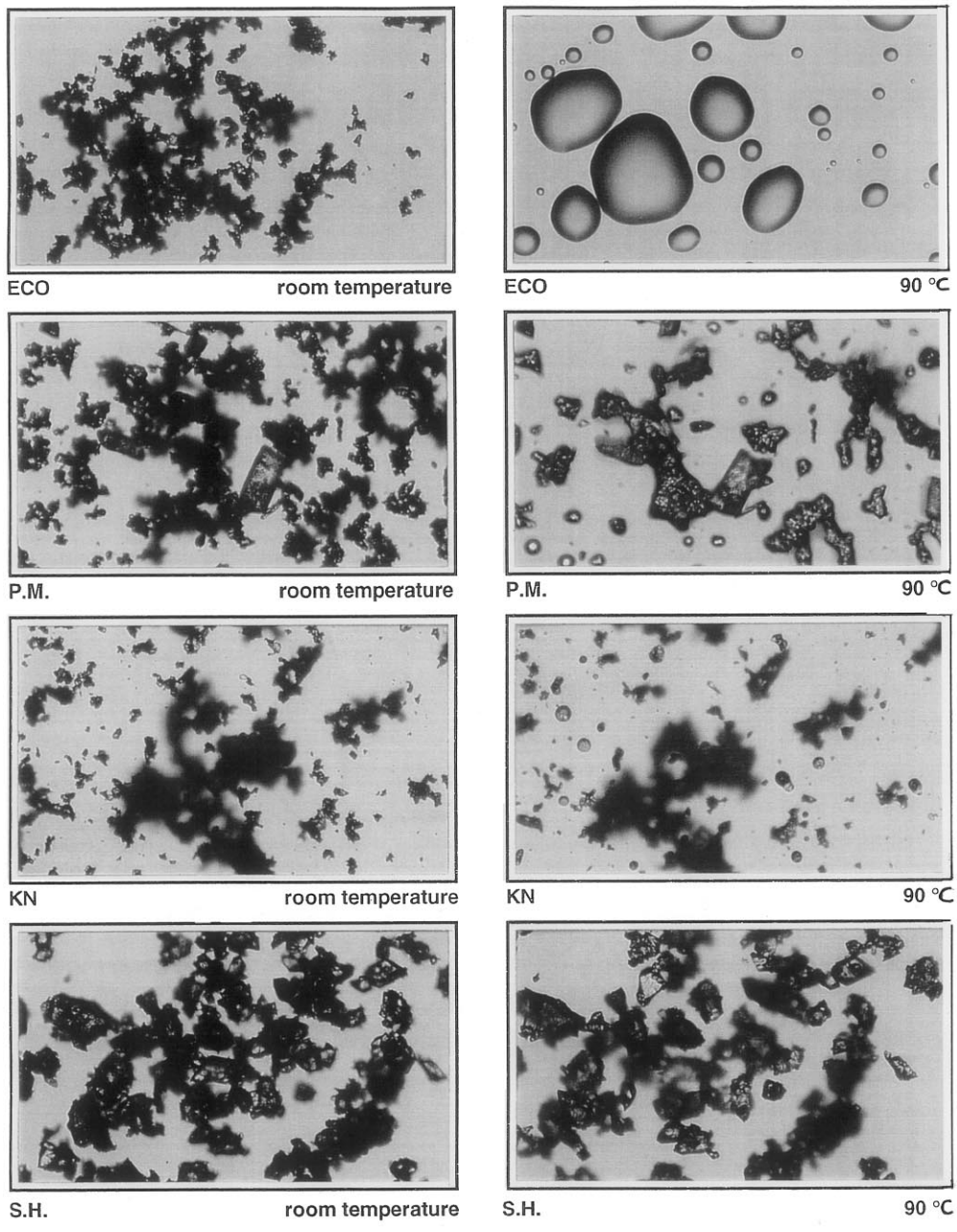

Fig. 2. Photomicrographs of crystals of econazole (ECO) and equimolar physical mixtures (PM), kneaded (KN) and sealed-heated (SH) products with βCd taken during HSM analysis.

neous morphology, with a tendency to self-agglomerate (Fig. 4), whereas βCd consisted of large crystalline particles of rather irregular size, and MeβCd was seen as amorphous spherical or pieces of spherical particles. In keeping with the findings of thermal analysis, in all solid systems with βCd it was possible to distinguish the presence of drug crystals, which appeared mixed with

Cds crystals or more or less finely dispersed on the Cd surface, depending on the preparation method. The formation of a very fine dispersion of microcrystalline drug adhered on the carrier surface was observed in physical mixtures and even more in coground systems with MeβCd, whereas the original morphology of both drug and Cd appeared clearly changed in kneaded and sealed-heated products, where only amorphous pieces of irregular size were present and it was no longer possible to differentiate the two components.

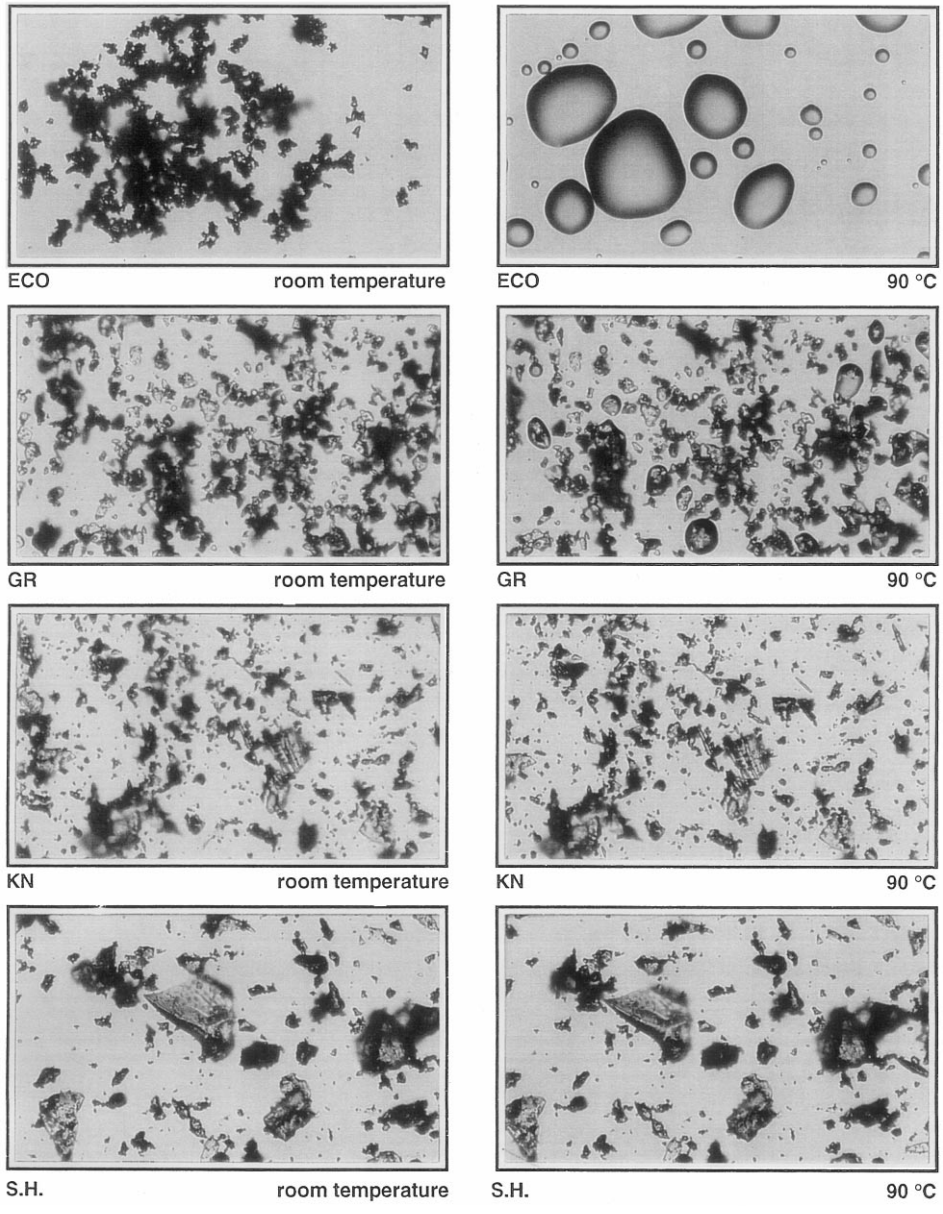

Fig. 3. Photomicrographs of crystals of econazole (ECO) and equimolar coground (GR), kneaded (KN) and sealed-heated (SH) products with MeβCd taken during HSM analysis.

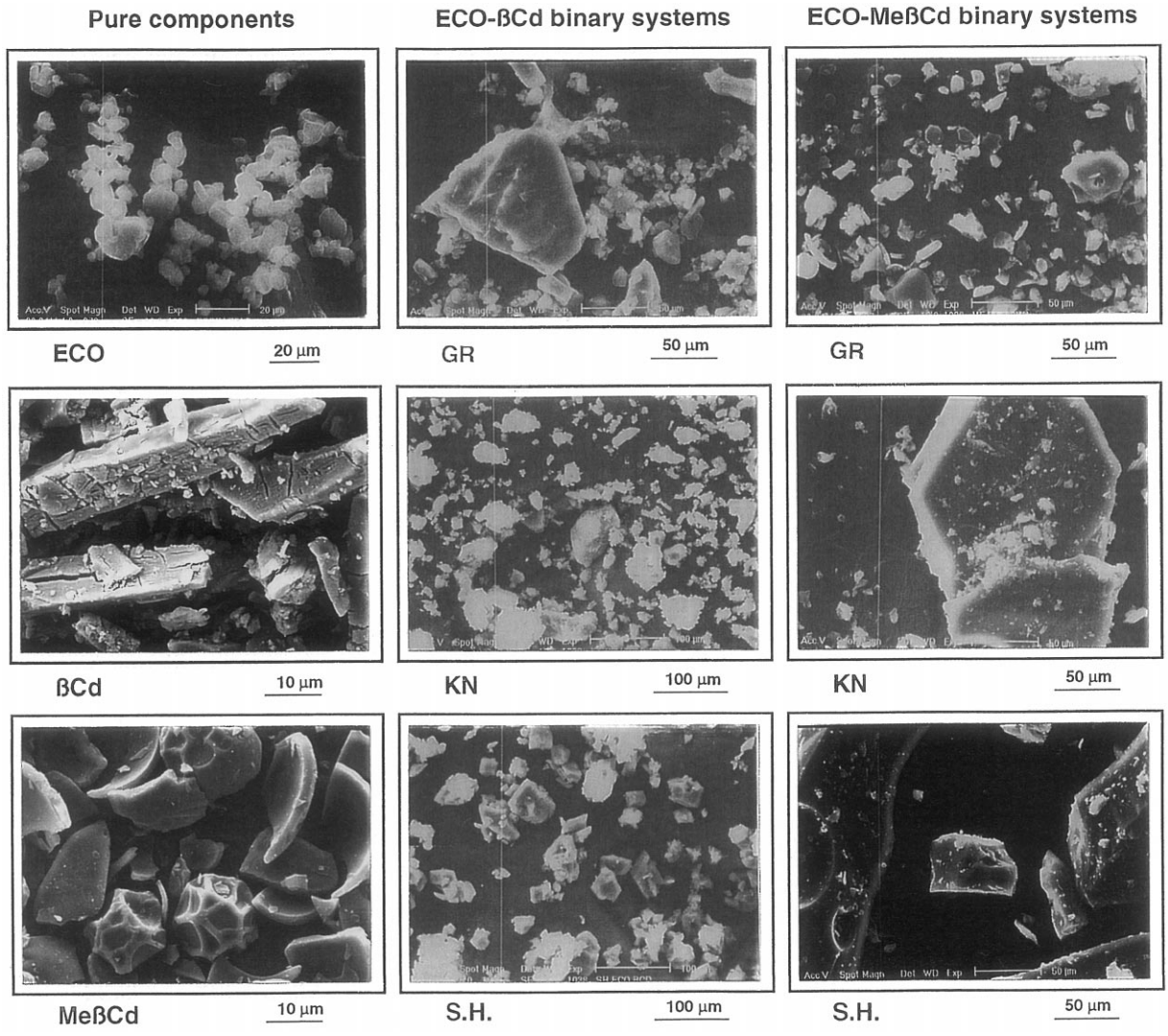

Fig. 4. Scanning electron micrographs of econazole (ECO), βCd and MeβCd and the corresponding drug-carrier equimolar coground (GR), kneaded (KN) and sealed-heated (SH) products.

The carbonyl stretching region of Infrared spectra of econazole and its different systems with Cds are presented in Fig. 5. No significant shifts or reduction of intensity of the characteristic carbonyl-stretching band of econazole (1564 cm^{-1}) were observed in any drug-Cd combination, either with βCd or with MeβCd, indicating that no important hydrogen bonding should be involved in the drug-carrier interactions.

The mean dissolution curves of econazole from various binary systems with Cds are presented in Fig. 6, together with a comprehensive picture of the relative increase in initial econazole concentrations in the dissolution medium (obtained by the ratio between the amount of drug dissolved after 5 min from a given drug-Cd preparation and the corresponding value of drug alone). The results in terms of dissolution efficiency and percent of active ingredient dissolved at 10 min are collected in

Table 1. It is evident at a glance that all the systems with Cds exhibited better dissolution properties than drug alone. Statistically significant differences ($P \approx 0.005$) in terms of both dissolution efficiency and percent of dissolved drug were found between all the econazole-MeβCd systems and the corresponding ones with native βCd, reflecting the stronger interaction of econazole with the βCd-derivative previously found in solid state studies. The greater ability of MeβCd in

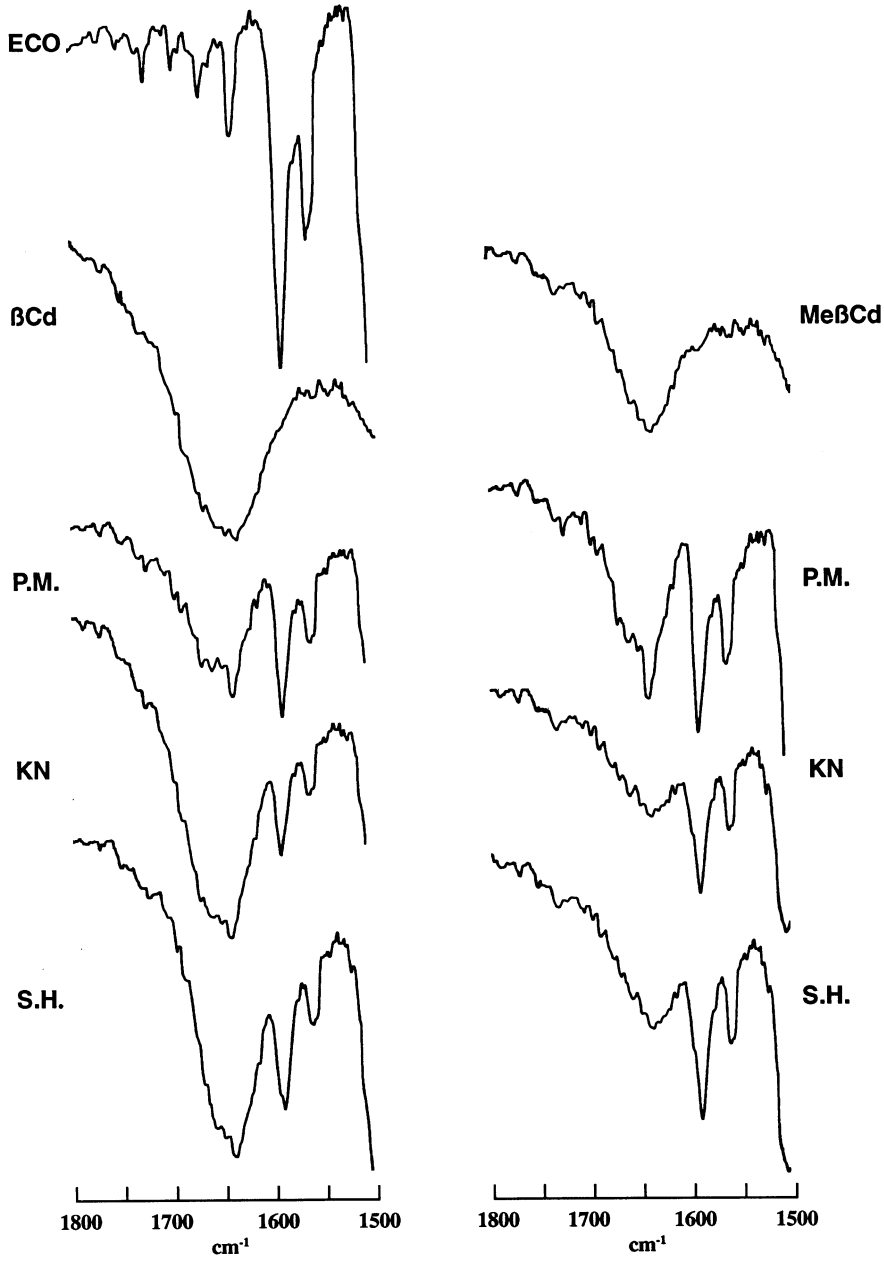

Fig. 5. IR spectra of econazole (ECO), cyclodextrins (βCd, MeβCd) and equimolar drug-carrier physical mixtures (PM), kneaded (KN), coground (GR) and sealed-heated (SH) products.

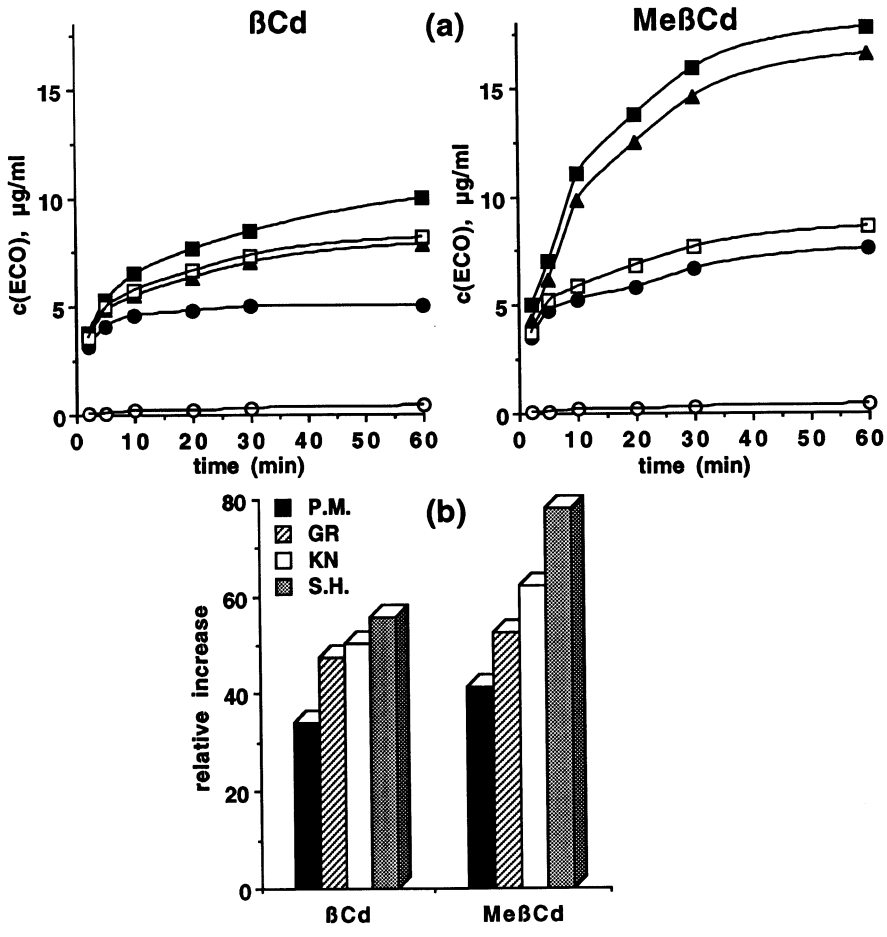

Fig. 6. (a) Dissolution curves of econazole (ECO) alone (○) and from physical mixtures (●), coground (□), kneaded (▲) and sealed-heated (■) products with βCd and MeβCd; (b) ratios between the amount of ECO dissolved from physical mixtures (PM), coground (GR), kneaded (KN) and sealed-heated (SH) products with βCd and MeβCd and that from drug alone at $t = 5$ min.

econazole amorphization could partially explain the better dissolution properties of the drug from systems with the βCd-derivative. As for the influence of the preparation method, an analogous trend was observed with both Cds: the greatest improvement of the drug dissolution properties was obtained with sealed-heated products, followed in the order by kneaded and coground ones and finally by physical mixtures. The increased dissolution rate and dissolution efficiency of physical mixtures (about 17 and 23 times higher than the pure drug for blends with βCd and MeβCd, respectively) is attributable both to improvement in drug wettability and to formation of readily soluble complexes in the dissolution medium (Corrigan and Stanley, 1982). Further improvement obtained with coground, kneaded and sealed-heated products could be explained by both the more intimate contact between drug and carrier and the decrease of drug crystallinity, as well as a phenomenon of at least partial drug inclusion complexation. On the other hand, the non-significance ($P > 0.1$) of the difference in dissolution efficiency between coground and kneaded systems with βCd and the poor increase (about 15%) obtained with the corresponding sealed-heated products, confirmed less interactions and complexation with this Cd, almost independently

Table 1
DE at 60 min and percent of drug dissolved at 10 min (DP) of econazole (ECO) from physical mixtures (PM), coground (GR), kneaded (KN) and sealed-heated (SH) products with βCd or MeβCd

Sample		DE	DP
ECO		0.4	0.3
ECO-βCd	PM	6.90	6.5
	GR	9.76	8.2
	KN	10.2	8.6
	SH	11.9	9.8
ECO-MeβCd	PM	9.2	7.7
	GR	10.6	8.8
	KN	19.4	14.8
	SH	21.2	16.6

of the preparation technique used. On the contrary, the influence of the preparation method was clearly more marked in the case of products with MeβCd, where kneaded and sealed-heated products showed an increase in dissolution efficiency of 80 or 110%, and 100 or 130%, respectively, in comparison to the corresponding coground and physical mixture. The best performance of these products seemed to confirm that drug inclusion complexation occurred substantially only in such systems, thus allowing to obtain the highest dissolution improvement.

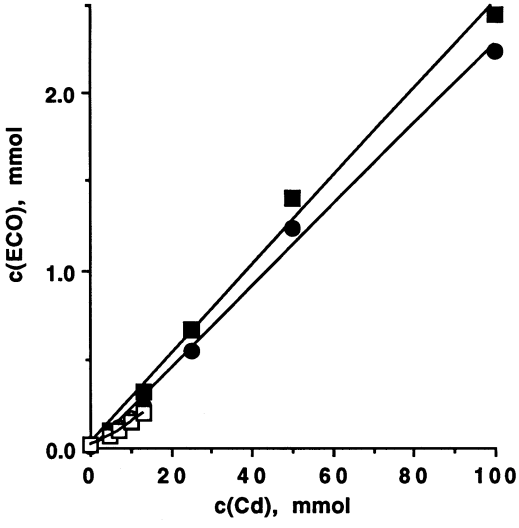

Fig. 7. Phase-solubility diagrams of econazole (ECO) with βCd (open symbols) and MeβCd (closed symbols) at 25°C (○, ●) and 37°C (□, ■).

Table 2
Apparent stability constants of complexes of econazole with βCd or MeβCd

Cyclodextrin	Solubilizing efficiency[a]	Apparent stability constant $K_{1:1}$ (M^{-1})	
		25°C	37°C
βCd	23	1710	697
MeβCd	224	2340	1276

[a] Ratio between drug solubility in the presence of $1 \cdot 10^{-2}$ M of MeβCd or the highest solubility of βCd and in pure water at 25°C.

Upon ageing of all systems for 6 months at room temperature, no significant ($P > 1$) reductions in drug dissolution efficiencies were observed in comparison to the respective freshly prepared products.

Phase solubility analysis showed that the solubility of econazole linearly increased by increasing the concentration of both cyclodextrins, giving A_L type solubility diagrams (Higuchi and Connors, 1965) and indicating the formation of 1:1 complexes (Fig. 7). The values of the apparent stability constants (Table 2) confirmed the best complexation properties of MeβCd towards econazole as well as its greater solubilizing efficiency reflected the faster drug release rates found from its combinations with MeβCd.

4. Conclusions

Both the solid state characteristics and the dissolution properties of ECO-Cd products were influenced by the preparation procedure, regardless of whether the actual inclusion formation can be stated or not. MeβCd proved to be more active in performing solid-state interaction, i.e. amorphization, of crystalline econazole and showed the highest complexing properties towards the drug. However, in regard to the drug dissolution properties, it was the different type of preparative method of drug-Cd solid systems, which made it possible to better point out the different performance of the examined carriers. In fact all combi-

nations with MeβCd exhibited better performance than the corresponding with βCd. However it should be pointed out that, interestingly, the difference in dissolution efficiency values between coground products was only about 8% in favor of the β-derivative; on the contrary, it increased up to 80 and 90% between sealed-heated and kneaded products, respectively, thus justifying a possible commercial use of MeβCd, despite is higher cost. Therefore, in addition to the type of Cd employed, the selection of the most suitable preparation technique was a decisive factor to optimize the performance, and particularly the dissolution properties of the final solid product.

Acknowledgements

Financial support from M.U.R.S.T. is gratefully acknowledged.

References

Bettinetti, G.P., Mura, P., Liguori, A., Bramanti, G., Giordano, F., 1989. Solubilization and interaction of naproxen with cyclodextrins in aqueous solution and in the solid state. Farmaco 44, 195–213.

Bononi, L.J., 1988. β-Cyclodextrin complexes having anti-mycotic activity. European Patent Application, 288.019.

Corrigan, O.I., Stanley, T., 1982. Mechanism of drug dissolution rate enhancement from β-cyclodextrin-drug systems. J. Pharm. Pharmacol. 34, 621–626.

Duchêne, D., Glomot, F., Vaution, C., 1987. Pharmaceutical applications of cyclodextrins. In: Duchêne, D. (Ed.), Cyclodextrins and Their Industrial Uses. Editions De Santé, Paris, Cap. 9.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instr. 4, 117–212.

Khan, K.A., 1975. The concept of dissolution efficiency. J. Pharm. Pharmacol. 27, 48–49.

Kim, K.H., Frank, M.J., Henderson, N.L., 1985. Application of differential scanning calorimetry to the study of solid drug dispersion. J. Pharm. Sci. 74, 283–289.

Müller, B.W., Brauns, U., Backensfeld, T., 1988. Cyclodextrin derivates for solubilization, stabilization and absorption of drugs. In: Huber, D., Szejtli, T. (Eds.), Proc. 4th Int. Symp. Cyclodex., Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 369–382.

Mura, P., Liguori, A., Bramanti, G., Campisi, E., Faggi, E., 1992. Improvement of dissolution properties and microbiological activity of miconazole and econazole by cyclodextrin complexation. Eur. J. Pharm. Biopharm. 3, 119–123.

Pedersen, M., Edelsten, M., Nielsen, V.F., Scarpellini, A., Skytte, S., Slot, C., 1993. Formation and antimycotic effect of cyclodextrin inclusion complexes of econazole and miconazole. Int. J. Pharm. 90, 247–254.

Szejtli, J., 1988. Cyclodextrin Technology. Kluwer, Dordrecht.

Uekama, K., Irie, T., 1987. Pharmaceutical applications of methylated cyclodextrin derivatives. In: Duchêne, D. (Ed.), Cyclodextrins and Their Industrial Uses. Editions De Santé, Paris, Cap. 10.