ELSEVIER

International Journal of Pharmaceutics 171 (1998) 111–121

international journal of pharmaceutics

Effect of cyclodextrins on the physicochemical properties and antimycotic activity of clotrimazole

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Received 3 February 1998; accepted 17 April 1998

Abstract

Clotrimazole is a lipophilic drug of an antimycotic action used both locally and systemically. Improvement of solubility and release rate of the clotrimazole is therefore essential for a rapid antimycotic activity. Hence, the effect of cyclodextrins (CDs) on the physicochemical properties and antimycotic activity of clotrimazole was studied. Inclusion complexation of clotrimazole with α-, β- and DM-β-CD was assessed by solubility method, microbiological assay, differential scanning calorimetry (DSC) and powder X-ray diffractometry. The solubility of clotrimazole was increased markedly with DM-β-CD, among the studied CDs, showing A_N-type phase solubility diagram. The antimycotic activity of clotrimazole against *Candida albicans* (*C. albicans*) was improved as measured for the solubilized amounts of clotrimazole taken from the phase solubility diagrams with CDs. DSC curve of coevaporate of clotrimazole with DM-β-CD showed almost no peak in the transition region of clotrimazole which could be attributed to the complex formation. Moreover, powder X-ray diffraction pattern of coevaporate of clotrimazole with DM-β-CD gave new crystalline diffraction pattern which confirmed the DSC results of inclusion complexation between clotrimazole and DM-β-CD. The coevaporates of clotrimazole with CDs showed superior antimycotic activity against *C. albicans* than the physical mixtures and drug alone, respectively. The largest inhibition zone of growth of *C. albicans* was obtained in the case of inclusion complex of clotrimazole with DM-β-CD. The coevaporate of clotrimazole with DM-β-CD showed higher dissolution rate in good correlation with the solubility data, and these reflect the higher antimycotic activity by rapid diffusion through agar medium. Both physical mixture and inclusion complex of clotrimazole with DM-β-CD were formulated as effervescent vaginal tablets; they found to possess an excellent antimycotic activity.

Keywords: Clotrimazole; Cyclodextrins; Dimethyl-β-cyclodextrin; Effervescent vaginal tablets; Inclusion complex; Microbiological assay

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0378-5173/98/$19.00
PII: S0378-5173(98)00163-X

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of α-D-glucopyranose units joined through a 1-4 bond, which are known to form inclusion complexes with many lipophilic drugs; which lead to change the physicochemical and biopharmaceutical properties of the complexed drugs (Szejtili, 1982, 1988; Uekama and Otagiri, 1987). In recent years, CDs research has made remarkable progress and the application of CDs has been extended to various fields. Loftsson and Brewster (1996) reviewed the use of CDs for the solubilization, stabilization and formulation of drugs through the formation of inclusion complexes. The actual and potential pharmaceutical uses of CDs have been published, since the safety of CDs and their new derivatives has been confirmed (Duchêne, 1987, 1991; Frömming and Szejtili, 1994; Rajewski and Stella, 1996; Irie and Uekama, 1997).

Little attention has been paid to the interaction between CDs and antifungal drugs. The stability and in vitro activity of nystatin and its γ-CD complex against *C. albicans* was studied (Van Doorne and Bosch, 1991). The interaction between CDs and various imidazole derivatives were studied (Bononi, 1988; Van Doorne et al., 1988a,b; Pedersen, 1993; Pedersen et al., 1993). Hoshino et al. (1993); found that increase in temperature enhances solubility of griseofulvine in aqueous solution of HP-β-CD. Clotrimzole is a lipophilic broad spectrum antimycotic agent, the preparations of the drug are used both in the topical treatment of dermal infections and to combat vulvovaginal candidiasis (Hoogerheide and Wyka, 1982). The topical applications of clotrimazole preparations into the skin, mouth or the vagina are appropriate to be washed off rapidly. Therefore, the increase in solubility and rapid release of clotrimazole is essential for such preparations.

The aim of this study is to gain insight into the effect of CDs on the physicochemical properties of clotrimazole in the solution and the solid state. In addition, the antimycotic activity of the complexes in liquid and in solid state was evaluated using inhibition zone measurements of *C. albicans*. A trial was done to formulate the physical mixture and inclusion complex of clotrimazole with DM-β-CD into effervescent vaginal tablets.

2. Materials and methods

2.1. Materials

Clotrimazole was kindly supplied by the Arab drug, Cairo, Egypt. α-CD and β-CD were obtained from Sigma (St. Louis, MO) and they were used without further treatment. Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) (Toshin Chemical, Japan) was used as received. Deionized double distilled water was used throughout this study. All other reagents and solvents were of analytical grade.

C. albicans strain was from our collection, Sabouraud Dextrose Agar was purchased from Oxoid, England; its typical formula (g/l) mycological peptone 10.0; glucose 40.0; Agar 15.0, pH 5.6 ± 0.2 Lot/CH, -B: 340 53683.

2.2. Phase solubility studies

Solubility study was carried out according to the method described by Higuchi and Connors (1965). To 10 ml distilled water containing various concentrations of α-, β-CD or DM-β-CD, 10 mg (or 30 mg in case of DM-β-CD) of clotrimazole were added and were shaked in water bath at 30 ± 0.5°C until equilibrium was reached after approximately 7 days. The suspensions were filtered through 0.45 μm Sartorius cellulose acetate membrane filters. The concentration of the solubilized clotrimazole in the filtered solutions were measured by the double beam UV spectrophotometer (UVIDEC-320, Jasco, Japan) at 261 nm.

The stability constant (K_s) was then determined from the initial straight part of phase solubility diagrams using the equation of Higuchi and Connors (1965) assuming that a 1:1 stoichiometric ratio complex was formed at the initial step.

2.3. Microbiological analysis

The antimycotic activity of some of the solutions used to construct the phase diagrams, between the solubilized clotrimazole and CDs, was measured by a plate microbioassay (agar-cup diffusion method). The agar medium was prepared by dissolving 65 g of Sabouraud dextrose agar powder per litre of distilled water and was sterilized by autoclave at 121°C for 20 min. The indicator strain *C. albicans* was inoculated into liquid Sabouraud's dextrose medium, for 24 h prior to testing, then the *C. albicans* was incubated at 32°C for 24 h. Once an actively growing broth culture or suspension of *C. albicans* is obtained, the turbidity is adjusted to match that of standard 0.5 M McFarland barium sulfate, which indicate to be contain approximately 10^7 cells/ml (Lorian, 1991). The strain of *Candida albicans* was seeded from standardized suspension to a concentration of 10^5 viable cells per ml in agar medium. The seeded agar medium was poured into petri-dishes (9 cm) to a depth of about 4 mm, two wells in each dish were cut using borer, the diameter of well was 6 mm.

25 μl samples of the phase diagram solutions were placed in the wells, each solution was applied twice. The petri-dishes were left for 1 h then incubated at 32°C for 20 h. The inhibition zone diameter of growth of *C. albicans* was measured.

2.4. Preparation of coevaporates of clotrimazole with CDs

Equimolar amounts (1:1 molar ratio) of clotrimazole and CDs were dissolved in acetone and water, respectively and mixed together, clear solution was obtained. Then the solvents were evaporated in a vacuum oven at 50°C until complete drying was obtained as checked by constant weight. The samples were reserved in desiccator until used. Physical mixtures were prepared by simple mixing of the drug and CDs in the same molar ratio as those of coevaporates.

All the samples were sieved. Fractions passing through a 250 μm sieve and retained on a 125 μm sieve were used throughout this study. The autimycotic activity for clotrimazole and its physical mixtures and coevaporates with CDs was measured against *C. albicans* by preparing discs (13 mm diameter), from those samples and put on the seeded agar media.

2.5. Differential scanning calorimetry (DSC)

DSC analysis for the clotrimazole, its physical mixtures and coevaporates with CDs, was carried out using Shimadzu DSC-50 connected with TA-50I. Nitrogen was used as a purge gas 40–50 ml/min, and the scanning speed of 10°C/min, using aluminium sample pan. The sample size was in the range 2–5 mg.

2.6. Powder X-ray diffraction

The powder X-ray diffraction patterns of the samples were measured using Philips 1710 X-ray diffractometry (Netherlands-Endhoven) under the following conditions: target Cu, filter Ni, voltage 40 Kv, current 30 mA, scanning speed 2°/min, chart speed 40 mm/min and count range 1000, CPS. The detector was a proportional counter, 1.7 Kv detector voltage.

2.7. Dissolution test

The dissolution test was measured for clotrimazole alone, its physical mixtures and coevaporates with α-, β- and DM-β-CD, using a six-vessel dissolution apparatus (Erweka, DT-D6, Germany). Each sample equivalent to 10 mg clotrimazole was sprinkled on the surface of the dissolution medium (500 ml of distilled water) maintained at 37 ± 0.5°C and stirred at 55 rpm. At each time interval, 10 ml of the solution was withdrawn and replaced by an equal volume of the same dissolution medium kept at 37°C. The concentration of dissolved clotrimazole was determined spectrophotometrically. Each experiment was performed at least three times and the mean was calculated in each case.

2.8. Ageing

Samples of the physical mixture and coevaporate of clotrimazole with DM-β-CD were stored

for 6 months in tightly closed containers at room temperature. TLC, the dissolution profiles and antimycotic activity of these samples were investigated and compared to those of fresh samples.

2.9. Thin-layer chromatography (TLC)

Equal volumes of alcoholic solutions of the test samples, fresh and aged, containing the same drug content were spotted on silica gel F_{254} plates (Merck). The plates were developed using a solvent system of ether equilibrated with ammonia vapor and the spots were visualized by spraying with Dragendorff reagent ($R_f = 0.4$).

2.10. Formulation of clotrimazole effervescent vaginal tablets

The complex of clotrimazole with DM-β-CD was chosen as the best, to be formulated in effervescent vaginal tablets and compared with the drug alone and its physical mixture with DM-β-CD. The nystatin effervescent vaginal tablets was prepared and reported by Safwat et al. (1994). The reported effervescent base is composed of sodium bicarbonate, anhydrous citric acid and boric acid as a lubricant and florite as carrier for nystatin. In this study, the effervescent base is composed of sodium bicarbonate, anhydrous citric acid and boric acid and mixed with clotrimazole alone or its physical mixture or its coevaporate of DM-β-CD. The effervescent mixture (100 mg) which equivalent to 10 mg clotrimazole potency was compressed as disc (13 mm in diameter) using Riken Power (Riken Seiki) and these discs were put on seeded agar medium to measure the antimycotic activity of these new clotrimazole effervescent tablets. Moreover, the effervescent vaginal tablets of clotrimazole systems were prepared by direct compression technique using the Korsch tabletting machine (Berlin, Germany) equipped with two punches (8 mm flat). The machine settings were adjusted to produce tablets of 200 mg weight, and the compression of effervescent tablets was done under controlled conditions of humidity and light. Physical properties of prepared tablets such as uniformity of weight, disintegration time (British Pharmacopoeia, 1993), hardness and friability, were also evaluated using Erweka hardness tester and Erweka friabiliator, respectively.

3. Results and discussion

The complexing capacity of each CD; α-, β- and DM-β-CDs with clotrimazole was quantified using the solubility method, in water at 30°C. The equilibrium phase solubility diagram for clotrimazole with DM-β-CD is presented in Fig. 1. Table 1 shows the types of solubility curves, which classified as A_N, A_L, and A_N-type phase solubility diagrams with α-, β- and DM-β-CD, respectively. The apparent stability constants (K) for clotrimazole with α-, β- and DM-β-CD were calculated as 255, 268 and 9101 M^{-1}, respectively. As shown in Fig. 1, the solubility of clortimazole increased with the increasing concentration of DM-β-CD with negative deviation from linearity at higher concentration of ligand. Also from Table 1, the solubility of clotrimazole increased by 3.35, 3.75 and 91.4 folds with α-, β- and DM-β-CD, respectively at 0.01 M CDs as evaluated by S_t/S_o ratio. This solubility enhancement is considered to be

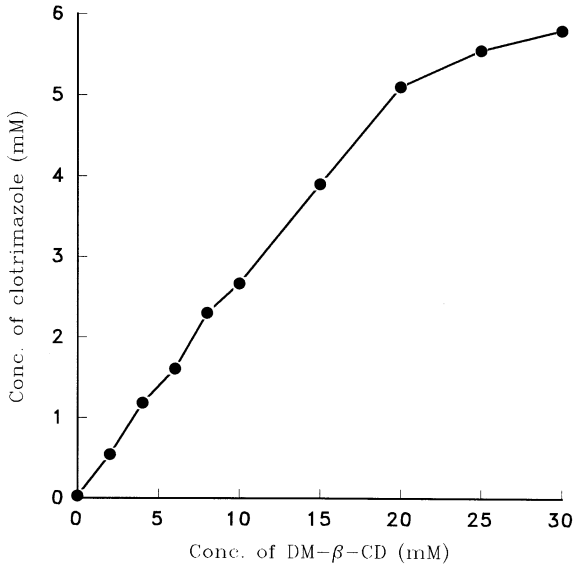

Fig. 1. Phase solubility diagram of clotrimazole with DM-β-CD in water at 30°C.

Table 1
Type of phase solubility diagrams, stability constant (M^{-1}) and increase of solubility of clotrimazole with α-, β- and DM-β-CD in water at 30°C

CDs	Type of diagram	Stability constant (K) (M^{-1})	Increase of solubility (S_t/iS_o)[a]
With α-CD	A_N	255	3.35
With β-CD	A_L	268	3.75
With DM-β-CD	A_N	9101	91.40

[a] S_t = solubility of clotrimazole in 0.01 M α-, β- and DM-β-CD solutions at 30°C; S_o = water solubility of clotrimazole at 30°C.

mainly due to the formation of inclusion complexes (Higuchi and Connors, 1965). The solubility and stability constant obtained with DM-β-CD were much greater than those obtained with the parent β-CD and α-CD. This may be attributed to the different physicochemical properties of the derivative DM-β-CD, such as its highest aqueous solubility and adequate cavity size as compared with natural CDs (Uekama, 1985). The types of phase solubility diagrams of clotrimazole with α-, β- and DM-β-CD are in agreement with that reported figure by Van Doorne et al. (1988b), but the largest increase in solubility was observed with DM-β-CD in this study, in contrast to that observed with β-CD by Van Doorne et al. (1988b). The experimental conditions, such as solvent, temperature, the range of CD concentration and the total amount of clotrimazole used may affect on the solubilized amount obtained with DM-β-CD. It is worth to note that, the stability constant of inclusion complex of clotrimazole with DM-β-CD has the highest value and also largest antimycotic activity as well be described in this study.

The antimycotic activity of clotrimazole in solution was measured on the basic of inhibition zone size of *C. albicans* using cup agar-diffusion method. The saturated solutions of clotrimazole containing various CDs concentrations were taken from the samples solutions which are used for construction of phase solubility diagrams of clotrimazole with CDs. The inhibition zone sizes were plotted versus CD concentration as shown in Fig. 2. The curve of clotrimazole with DM-β-CD showed the largest inhibition zone size as compared with β-CD and α-CD. These data are consistent with the solubility diagrams, since the greatest solubility was observed for DM-β-CD which also gave the greatest inhibition zone size. β-CD at concentration of 18 mg/ml did not affect the growth of the test organism, while DM-β-CD and α-CD at concentrations 570 and 150 mg/ml, respectively, gave rise to a small inhibition zone (13 and 11 mm, respectively) in their own right against *C. albicans*. The connection between inhibition zone size and CD concentration; can be attributed to the increased solubilized amount of clotrimazole by increasing CD concentrations which resulted from the soluble inclusion complex formed, consequently improved the diffusion into

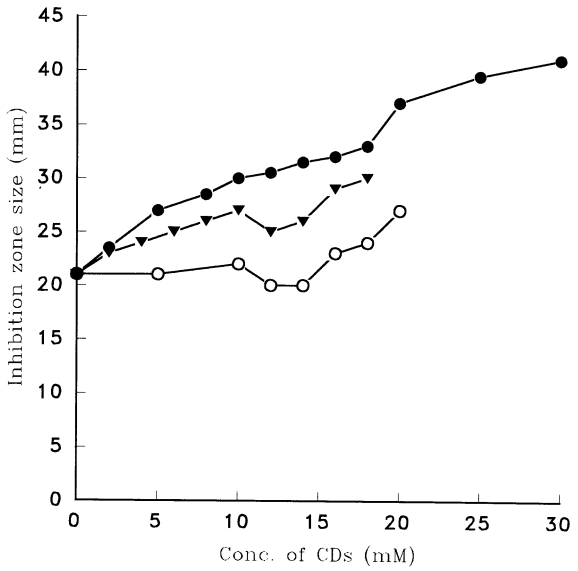

Fig. 2. Effect of various CDs concentration on inhibition zone size of clotrimazole against *C. albicans* in water. ○, α-CD; ▽, β-CD; ●, DM-β-CD.

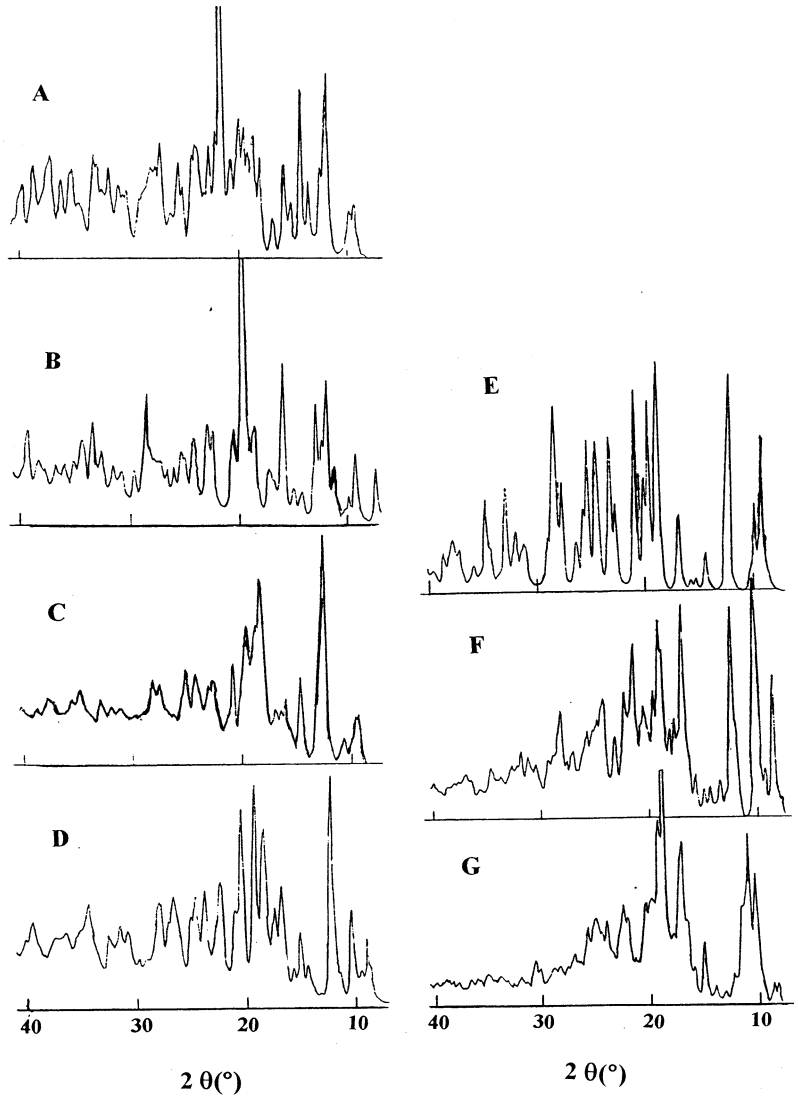

Fig. 3. Powder X-ray diffraction patterns of clotrimazole with CDs systems in 1:1 molar ratio. A, physical mixture of clotrimazole with α-CD; B, coevaporate of clotrimazole with α-CD; C, physical mixture of clotrimazole with β-CD; D, coevaporate of clotrimazole with β-CD; E, clotrimazole alone; F, physical mixture of clotrimazole with DM-β-CD; G, coevaporate of clotrimazole with DM-β-CD.

agar medium. Pedersen (1993) reported that HP-β-CD (500 mg/ml) gave rise to a small inhibitory zone in its own right (8.47 mm) when *C. albicans* was used as test organism.

The data of Figs. 1 and 2 are considered as an evidence of increased antimycotic activity of clotrimazole by inclusion complexation with DM-β-CD in solution and can be applied in formulation of clotrimazole in solution dosage form.

Fig. 3 shows the powder X-ray diffraction patterns of physical mixture and coevaporate of clotrimazole with α-, β- and DM-β-CD in 1:1 molar ratio. The diffraction pattern of the physical mixtures of clotrimazole with these CDs were simply a superposition of those of the two components in each mixture. The diffraction pattern of coevaporate of clotrimazole with α-CD showed crystalline peaks similar to that of its physical

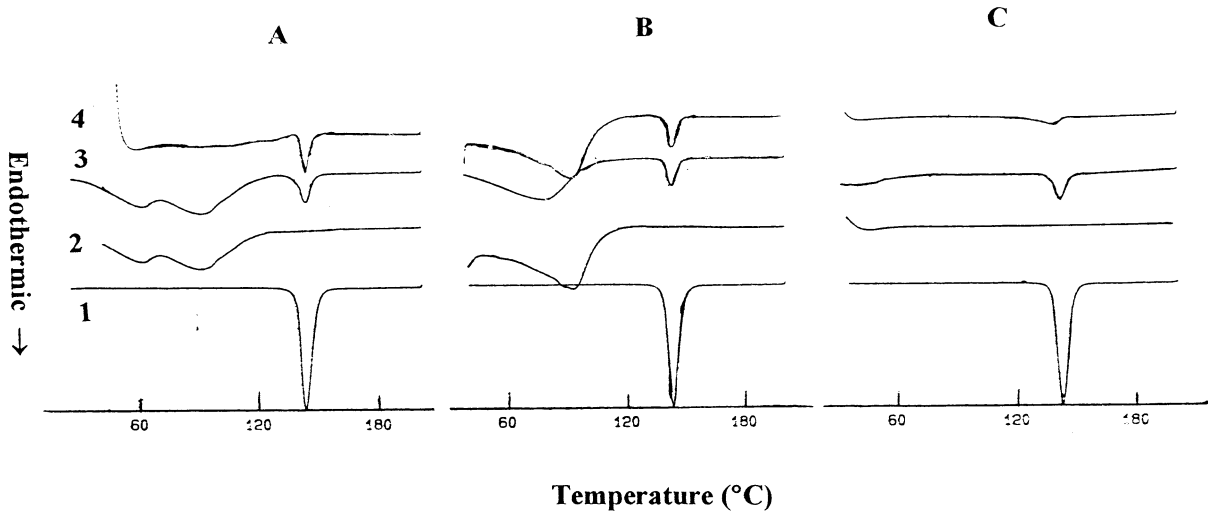

Fig. 4. DSC curves of clotrimazole with α-CD [A], β-CD [B] and DM-β-CD [C] systems. 1-drug; 2-CD; 3-physical mixture; 4-coevaporate.

mixture but with two new crystalline peaks at $2\theta = 16$ and 19.8° (curve B). This may be attributed to that some molecules of drug was included in the α-CD cavity or dispersed in hydrogen bonding network formed by α-CD molecules and another part of drug molecules was presented as crystals embedded in the α-CD matrix or in a crystalline state similar to that of intact drug crystals (Oguchi et al., 1989). Curve D shows the X-ray diffraction pattern of coevaporate of clotrimazole with β-CD which is similar to its physical mixture except two small crystalline peaks at $2\theta = 17$ and 17.7° was appeared; this result can be explained as the same reason with α-CD. The diffraction pattern of coevaporate of clotrimazole with DM-β-CD (curve G) gave a crystalline diffraction peaks at $2\theta = 10.7$, 11.3 and 19.2° which were quite different from the diffraction peaks of both components, suggesting the formation of the crystalline inclusion compound.

Fig. 4 shows the DSC curves of clotrimazole with α-, β- and DM-β-CD systems in 1:1 molar ratio. DSC curve of clotrimazole crystals (curve 1) showed an endothermic peak at 143.6°C due to the melting of clotrimazole. DSC curves of α-, β- and DM-β-CD did not show an endothermic peak in the melting region of the clotrimazole (curves 2A–C, respectively). On the other hand, an endothermic peak at 88°C was observed on DSC curve of β-CD and two endothermic peaks at 60 and 89°C were observed on DSC curve of α-CD, which were due to the evaporation of the adsorbed water. Curves 3A–3C shows DSC curves of the physical mixtures of clotrimazole with α-, β- and DM-β-CD respectively, an endothermic peak at 143°C was detected on each curve which is due to the fusion of clotrimazole. DSC curves (curves 4A and B) of coevaporates of clotrimazole with α- and β-CD showed an endothermic peak at 143.3 and 143.6°C, respectively which is due to the melting of clotrimazole. Indeed, the heat of fusion (ΔH) of coevaporates of α- and β-CD was decreased from -88 J/g for the drug to -17 and -15.5 J/g, respectively, which can be ascribed to the highly energetic crystals of clotrimazole resulting from the dispersion of these fine crystals in the matrix of CD. Curve 4C shows the DSC curve of coevaporate of clotrimazole with DM-β-CD, a very small endothermic peak at 140°C was recorded which due to fusion of very small amount of excess fine crystals of clotrimazole. This reduction of melting peak of drug in that case is considered significantly to prove the formation of crystalline

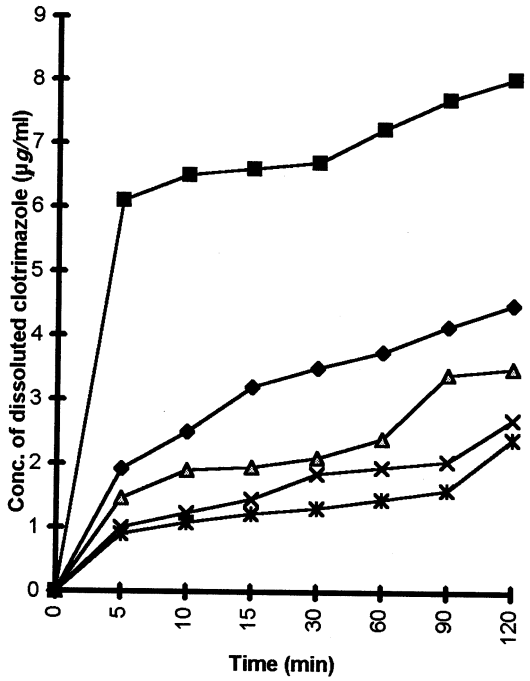

Fig. 5. Dissolution profiles of clotrimazole with CDs systems in water at 37°C.●, Clotrimazole crystals; ○, Coevaporate of clotrimazole with DM-β-CD, ▽ Coevaporate of clotrimazole with β-CD, ▽ Coevaporate of clotrimazole with α-CD.

inclusion complex between the clotrimazole and DM-β-CD by coevaporation method, in agreement with the data of powder X-ray diffraction pattern (Fig. 3G).

Fig. 5 shows the dissolution profiles of clotrimazole and its coevaporates with α-, β- and DM-β-CD in water at 37 ± 0.5°C. Table 2 explains the relative dissolution rate (RDR) of

Table 2
Relative dissolution rate (RDR) for different systems of clotrimazole in water at 37°C

System	RDR after			
	5 min	15 min	60 min	120 min
Clotrimazole	1.00	1.00	1.00	1.00
Coevaporate with α-CD	1.21	1.12	1.32	1.07
Coevaporate with β-CD	1.64	1.57	1.70	1.46
Coevaporate with DM-β-CD	6.88	5.32	5.04	3.34

Table 3
Effect of various CDs on the antimycotic activity of different systems of clotrimazole in solid state

System of drug[a]	Size of inhibition zone(mm)
Clotrimazole	26
PM of clortimazole with DM-β-CD[b]	51
Coevaporate of clotrimazole with DM-β-CD	55
PM of clotrimazole with β-CD	40
Coevaporate of clotrimazole with β-CD	45
PM of clotrimazole with α-CD	38
Coevaporate of clotrmiazole with α-CD	43

[a] From each system, solid disc was prepared, which equivalent to 10 mg clotrimazole, and each disc was put in a single agar plate.
[b] PM, physical mixture.

clotrimazole which represent the ratio between the amount of dissolved drug from the coevaporate to that of drug alone at the same time interval. As shown in Fig. 5 and Table 2, the dissolution rate of clotrimazole was improved greatly in the case of coevaporate of DM-β-CD than that of β-, α-CD and drug alone. This enhancement of dissolution rate of coevaporate of clotrimazole with DM-β-CD is considered due to the formation of inclusion complex between the drug and DM-β-CD though this inclusion complex is in crystalline form.

Table 3 shows the antimycotic activity of clotrimazole with α-, β- and DM-β-CD different systems in the solid state. Clotrimazole alone or its physical mixtures or coevaporates with CDs was prepared in the disc form (13 mm in diameter) and each disc contains or equivalent to 10 mg clotrimazole and put on the agar medium. The size of the inhibition zone of growth of *C. albicans* was used as a measure for the release of the clotrimazole from these preparations, and to assess whether the physical mixtures or coevaporates have antimycotic activity as compared with the drug alone. From the Table 3, it is clear that the coevaporate of clotrimazole with DM-β-CD showed the largest inhibition zone size (55 mm)

among the all systems used. This can be attributed to the formation of inclusion complex between the drug and DM-β-CD as confirmed by DSC and X-ray diffraction data, in addition to the enhanced dissolution rate of this inclusion complex as compared to the drug alone (Fig. 5). The size of inhibition zone of these studied preparations can be arranged in the following order: coevaporate of DM-β-CD > physical mixture of DM-β-CD > coevaporate of β-CD > physical mixture of β-CD > coevaporate of α-CD > physical mixture of α-CD > clotrimazole alone. A pile of clotrimazole powder (25 mg) was put on agar medium and it was found that the inhibition zone size was 27 mm which equal to that resulted from the disc of 10 mg drug. This result indicates that the solubility and diffusion rate into agar medium are the rate limiting step for antimycotic action of clotrimazole, since the measured inhibition zone size is independent on the size of the sample of clotrimazole and on the form of the sample (powder or disc).

Discs were prepared from α-, β- and DM-β-CD alone in amounts equivalent to that present when mixed with clotrimazole, no inhibition zones were observed when put on agar medium. However, only DM-β-CD gave rise of small inhibition zone size of 18 mm when 200 mg disc of DM-β-CD was used.

It is worth to note that the physical mixtures of clotrimazole with α-, β- and DM-β-CD gave significant difference of inhibition zone size than the drug alone, which can be attributed to the increased solubility of clotrimazole in the presence of CDs and formation of complex in the agar medium (Van Doorne et al., 1988b). From DSC curves of these physical mixtures of clotrimazole with CDs, the heat of fusion (ΔH) was decreased from -88 J/g to -20, -16.5 and -15.5 J/g for physical mixture of α-, β- and DM-β-CD, respectively. It can be imagine or postulate that there is a relationship between the smaller heat of fusion (ΔH) of clotrimazole in the physical mixture with CDs and the larger size of inhibition zones as compared with the clotrimazole alone. This imagination can be explained by that the molecules of drug in these physical mixtures became energetic and absorbed small amount of heat to melt than the drug alone which absorbed larger amount of heat to melt, consequently the molecules of clotrimazole from physical mixture dissolved and diffused rapidly in agar medium giving large inhibition zone size.

After ageing of physical mixture and coevaporate of clotrimazole with DM-β-CD for 6 months at room temperatures, only one spot was detected on the TLC plate with R_F value = 0.4 indicating the stability of clotrimazole in these systems. The dissolution rate and antimycotic activity were not changed as compared with fresh samples. From TLC data, it was revealed that no chemical degradation for the clotrimazole occurred in these systems.

From these results it is evident that CDs may enhance the release of clotrimazole from topical preparations or pessaries formulated with incorporation of physical mixture or complex of the drug with CDs. Economically, it is simple to prepare physical mixture or coevaporate of clotrimazole with CDs and then formulated into suitable dosage form.

Table 4 summarizes the physical characteristics of different formulations of effervescent clotrimazole vaginal tablets. Uniformity of weight and disintegration time of prepared tablets were found to comply with the requirements of British Pharmacopoeia (1993). All effervescent tablets showed an acceptable values of hardness an friability (% loss is less than 1.5%), the diameter of tablets is 8 mm. The antimycotic activity of these effervescent tablets was measured by size of inhibition zones and they exhibited larger inhibition zone sizes for tablets prepared from physical mixture and inclusion complex of clotrimazole with DM-β-CD. From these results it was concluded that the prepared effervescent vaginal tablets have good mechanical properties which facilitate their handling and durability and are promising for excellent antimycotic activity of clotrimazole in this suggested formulation.

Table 4
Physicochemical properties of different formulations of clotrimazole effervescent vaginal tablets

Formula	Weight (g) mean (c.v.%)	Hardness (kg)	Friability loss (%)	Disintegration time (min)	[a] Antimycotic activity(mm)
Formula 1	0.1920 (4.1)	3.15	0.75	3.0	33
Formula 2	0.1998 (2.2)	4.35	0.62	2.0	55
Formula 3	0.2043 (1.9)	4.65	0.47	1.5	70

Formula 1: Tablets contain 10% clotrimazole alone.
Formula 2: Tablets contain 48.6% of physical mixture with DM-β-CD which equivalent to 10% of clotrimazole.
Formula 3:Tablets contian 48.6% of inclusion complex with DM-β-CD which is equivalent to 10% of clotrimazole.
[a] Antimycotic activity was measured by inhibition zone size of *C. albicans* for each disc (100 mg) prepared from each formula which is equivalent to 10 mg clotrimazole, exactly.

4. Conclusion

The solubility, dissolution rate, and antimycotic activity of clotrimazole was improved by inclusion complexation with CDs either in the solution or in the solid state. DM-β-CD with clotrimazole solubility diagram revealed that DM-β-CD gave the highest solubility and highest stability constant among the studied CDs. Also, the solid inclusion complex of clotrimazole with DM-β-CD, which prepared by co-evaporation method and confirmed by X-ray diffraction and DSC data, showed enhancement of release and antimycotic activity of clotrimazole. Clotrimazole can be formulated into effervescent vaginal tablets using its physical mixture or inclusion complex with DM-β-CD to obtain rapid release and rapid onset of antimycotic action against Candidiasis using small dose of clotrimazole.

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