

INVESTIGATING THE POTENTIAL OF POLYETHYLENE GLYCOLS IN SOLUBILIZATION OF IMIDAZOLE DRUGS OF INTEREST

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ABSTRACT

Objective: The study aimed to solubilization of imidazole drugs of interest; ketoconazole (KT) and miconazole nitrate (MN), for enhancement of the water solubility, the dissolution properties and the antimycotic activity of these drugs.

Method: Different solid dispersion methods were used to prepare various dispersion systems of KT and MN using polyethylene glycol 4000 (PEG 4000), polyethylene glycol 6000 (PEG 6000) as water-soluble carriers. The prepared systems were evaluated for their physicochemical and dissolution properties and their antimycotic activities.

Results: The result obtained showed that the crystallinity of the imidazole drugs was almost lost and a greater amount of drug found in an amorphous state. Dissolution studies proved that the prepared systems showed an improvement in the dissolution of their drug comparing by that of their drug intact and this improvement was progressed as the fractional and molecular weights of PEGs increased. Further, the systems prepared by melting method showed higher dissolution profile than their corresponding systems prepared by evaporation method. The results showed that the aqueous solutions of both KT-PEGs and MN-PEGs systems showed superior antimycotic activity to their drug intact.

Conclusion: The solid dispersion of imidazole drugs, KT or MN, with each of PEG 4000 and PEG 6000, using either evaporation or melting method, could be used as a potential drug delivery system for solubilization of imidazole drugs

Keywords: Polyethylene glycol 4000 (PEG 4000), Polyethylene glycol 6000 (PEG 6000), Ketoconazole (KT), Miconazole nitrate (MN), Solid dispersion and dissolution.

INTRODUCTION

Imidazole drugs are a group of antifungal drugs which have broad-spectrum antifungal activities against wide range of fungi that cause many of mycotic infections [1]. The members of this group are structurally related and have similar physicochemical properties and mechanisms of action⁽¹⁾. The members of imidazole group are miconazole (base or nitrate salt), ketoconazole, econazole (base or nitrate salt) and clotrimazole....ect [1]. Although there are a number of imidazole drugs currently available, their efficacy may not be completely achieved in the treatment of human mycoses due to their poor water solubility and limited dissolution properties associated with slow drug absorption leading eventually to inadequate and variable bioavailability [1,2]. Several efforts have been reported to enhance the water solubility and the dissolution properties of some imidazole drugs using cyclodextrin complexation [3,4].

Pharmaceutical carriers, in particular, water-soluble carriers have been received an increasing attention in the pharmaceutical field because of their ability to enhance aqueous solubility, dissolution rate and bioavailability of many poorly water soluble drugs [5]. Polyethylene glycols (PEGs) with molecular weights of 1,500–20,000 are extensively used as water-soluble carriers for preparation of solid dispersions of many poorly water soluble drugs [6]. This extensive use of PEGs is attributed to their numerous advantages including low melting point, rapid solidification rate, low toxicity, low costs and good solubility in water and most of organic solvents [6]. Further, a particular advantage of using of PEGs is their high ability to solubilize many of poorly water soluble drugs [6]. The high solubilization ability of PEGs may be due to different positive effects offered by PEGs including good wettability, local solubilization and particle size reduction [6].

In this study, imidazole drugs of interest; KT and MN, were chosen as model drugs. Biopharmaceutics classification system (BCS) classified both KT and MN as Class II drugs having dissolution-limited bioavailability because of their poor water solubility [7]. The present study reports the potential of PEGs; PEG 4000 and PEG 6000 in solubilization of the imidazole model drugs; KT and MN using solid dispersion approach. Also, the effects of carrier type, carrier weight ratio and dispersion method on the physicochemical and the dissolution properties of the two imidazole model drugs were

investigated. Further, the extent of enhancement in the antimycotic activity of the two imidazole model drugs upon their solubilization by using either PEG 4000 or PEG 6000 was investigated microbiologically by using a cup diffusion method.

MATERIALS AND METHODS

Materials

Ketoconazole (KT) was gifted by Sedeco Pharmaceuticals Company, Cairo, Egypt. Miconazole nitrate (MN), was gifted by Medical Union Pharmaceuticals Company, Ismailia, Egypt. Polyethylene glycols (PEGs); PEG 4000 and PEG 6000, were obtained from Winlab, Harborough. U. K Methanol, ethanol and tween 80, all obtained from El-Nasr Company, Abu-Zabal, Cairo, Egypt. Strains of *Candida albicans* (*C. albicans*) and a modified Sabouraud agar were gifted by Microbiology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.

Solubility studies

Solubility studies were performed according to Higuchi and Connors [8]. Excess amounts of KT or MN were added to 10 ml distilled water containing various concentrations of PEGs; PEG 4000 and PEG 6000. The aqueous systems obtained, were shaken in a water bath at $37 \pm 0.5^\circ\text{C}$ till equilibrium reached. Then, the aqueous systems were filtered and the drug concentration in the filtrate, after appropriate dilutions, was measured spectrophotometrically at λ_{max} 255 nm for KT and at λ_{max} 273 nm for MN. The average of triplicates was reported. The presence of the two investigated PEGs in the solutions does not interfere with the spectrophotometric assay of KT or MN.

Preparation of physical mixtures and solid dispersion systems

The physical mixtures (PM) of KT or MN with each of the two investigated PEGs at drug-carrier weight ratios; 1:1 and 1:5 w/w, were prepared by simply mixing of the appropriate amounts of drug and PEG for 5 minutes in a mortar. The obtained PM were sieved to size 250-90 μm and stored in a dry desiccator till used.

Solid dispersion systems of KT or MN with each of the two investigated PEGs at drug-carrier weight ratios; 1:1, 1:3 and 1:5 w/w were prepared by both evaporation and melting methods. In the

evaporation method, the appropriate PEG was dissolved in an aqueous solution of methanol over a magnetic stirrer and then, a methanolic solution of drug (KT or MN) was added with continuous stirring till obtain a clear solution. The solutions allowed to be evaporated in a vacuum oven at 40 °C till complete drying. The dried residues were scratched, pulverized, and sieved to size 250-90 µm. The evaporate systems (ES) obtained, were stored in a dry desiccator till used. In the melting method, the appropriate PEG was melted in porcelain dish over a thermostatic hot plate and then drug (KT or MN) was gradually added with continuous stirring till obtain a homogenous mass. The masses were left to cool and solidify at room temperature away of moisture. The solidified sheets were scratched, pulverized and sieved to size 250-90 µm. The melt systems (MS) obtained, were stored in a dry desiccator till used.

Infrared spectrometry (IRS)

IRS of KT, MN, PEG 4000, PEG 6000 and some prepared KT-PEGs and MN-PEGs systems were performed according to KBr disk method. The samples were scanned at wave number 4000-400 cm⁻¹ using an empty pellet holder as a reference.

Differential Scanning Calorimetry (DSC)

DSC of KT, MN, PEG 4000, PEG 6000 some prepared KT-PEGs and MN-PEGs systems were carried out using DSC-shimadzu apparatus. The apparatus was adjusted for purging of nitrogen at rate of 40 ml/min, and heating rate at 10°C/min. The instrument was calibrated with indium as a standard.

Drug contents studies

A suitable amount of each of the different prepared solid systems was dissolved in 15 ml methanol. Each volume was completed to 50 ml with distilled water with 0.02% w/v of tween 80 and assayed for its drug concentration spectrophotometrically at λ_{max}. 255 nm for KT and at λ_{max}. 273 nm for MN. The average of triplicates was reported.

Dissolution studies

The dissolution of KT, MN and their different prepared solid systems were carried out in USP XXI-dissolution apparatus II (paddle method). Samples containing 50 mg of drug intact (KT or MN) or its equivalent of the different prepared systems were introduced into 500 ml distilled water with 0.02% w/v of tween 80. The contents of dissolution vessels were stirred at rate of 100 rpm and maintained at a temperature of 37±0.5°C. At certain time intervals, 5-ml samples of the dissolution medium were withdrawn and replaced by fresh 5-ml of the dissolution medium kept at 37±0.5°C. Drug concentrations were determined spectrophotometrically at λ_{max}. 255 nm for KT and at λ_{max}. 273 nm for MN. The average of three determinations was taken.

Microbiological studies

The solubilization effects of the two investigated PEGs on the antimycotic activity of the imidazole model drugs; KT and MN, were investigated by using a cup diffusion method. The aqueous solutions of KT-PEGs and MN-PEGs at the same various concentrations employed in solubility studies were used in the microbiological studies. The studies were carried out in a similar manner described by Pederson et al. [9]. A modified Sabouraud agar medium was freshly prepared and sterilized by autoclaving at 120°C for 1 hr. The indicator strains of *C. albicans* was grown for approximately for 48 hrs at 31°C, in a dish containing the Sabouraud agar medium. The strain of *C. albicans* was seeded to a concentration of 10⁵ yeast cells per ml in the agar medium at 40-50°C. The seeded agar medium was poured in lots of 35ml into 14 cm-Petri dishes and six wells in each dish were cut, each 6 mm in diameter. 50 ul sample of each of the used solubility diagram solutions were placed in each wells according to randomization scheme. The dishes were incubated at 32°C for 18 hrs before the diameters of inhibition zones were measured. The results reported are inhibition zone diameter minus well diameter (6mm). Each of the used solutions was applied twice and the average of the results of two applications was taken.

RESULTS AND DISCUSSION

Solubility studies

The solubility diagrams of KT or MN with each of the two investigated PEGs; PEG 4000 and PEG 6000 are shown in Figs. (1-2). It can be seen that the water solubility of KT or MN is increased linearly as a function of the investigated PEGs concentrations and the features of an A₁-type phase solubility diagrams were obtained. Enhancement of the water solubility of KT or MN in the presence of the two investigated PEGs may be due to molecular interactions of KT or MN with PEGs in the aqueous state related to hydrogen bonding formation [10] and also, may be due to good wettability and local solubilization properties of the two investigated PEGs creating more energetically favorable environment around drug particles [6,11]. The data listed in table (1) showed that the number of folds increase in drug water solubility in presence of 12% w/v of PEG 4000 and PEG 6000 are about 9 and 10 times, respectively for KT and about 13 and 15 times, respectively for MN.

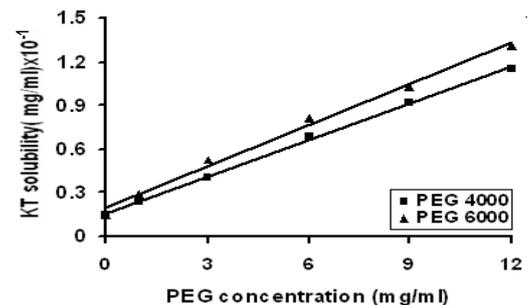


Fig. 1: Phase solubility diagram of KT-PEGs systems in distilled water at 37°C.

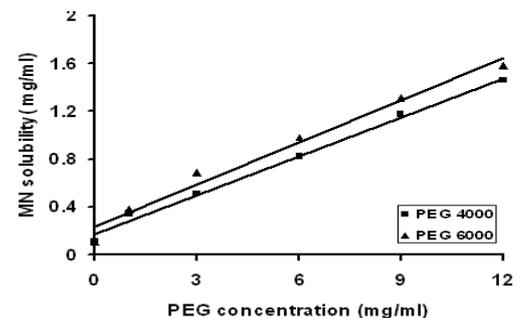


Fig. 2: Phase solubility diagram of MN-PEGs systems in distilled water at 37°C.

Table 1: The physico-chemical characteristics of KT-PEGs and MN-PEGs systems in distilled water at 37°C

Systems types	Intrinsic solubility (mg/ml)	No. of folds increase at PEG concentration 12% w/v
KT-PEG 4000	0.015	9
KT-PEG 6000	0.015	10
MN-PEG 4000	0.11	13
MN-PEG 6000	0.11	15

Infrared spectrometry (IRS)

IRS of KT, MN, PEG 4000 and some their prepared systems are displayed in Fig. (3). IRS of the model drugs displayed -C-O-C- stretching bands between 1000 and 1100 cm⁻¹ for both KT and MN intact, C=O stretching bands between 1640 and 16100 cm⁻¹ for KT intact and C=N stretching bands between 1300 and 1500 cm⁻¹ for MN intact. IRS of PM of KT or MN with PEG 4000 are simply super-

imposition of IR spectra of the components forming PM under test and no marked changes can be reported. While, IRS of the corresponding ES and MS showed certain changes in the intensity and the position of some characteristic absorbance bands of their

drug (KT or MN). These changes may be attributed to hydrogen bond formation between KT or MN and PEG 4000 [10,11]. Similar observations were displayed by IRS of KT, MN and some of their different prepared systems with PEG 6000 as shown in Fig. (4).

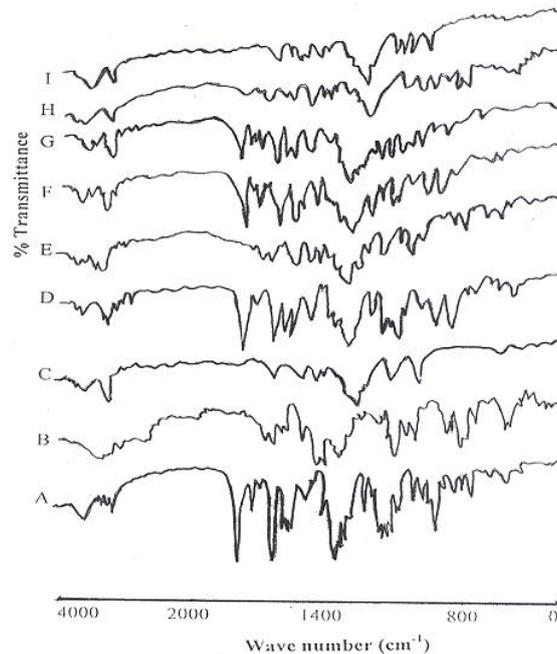


Fig. 3: IRS of KT, MN and some their systems with PEG 4000. Key: a) KT intact, b) MN intact, c) PEG 4000 alone, d) KT-PEG 4000 PM (1:1), e) MN-PEG 4000 PM(1:1), f) KT-PEG 4000 ES (1:1), g) MN-PEG 4000 ES (1:1), h) KT-PEG 4000 MS (1:5), i) MN-PEG 4000 MS (1:5).

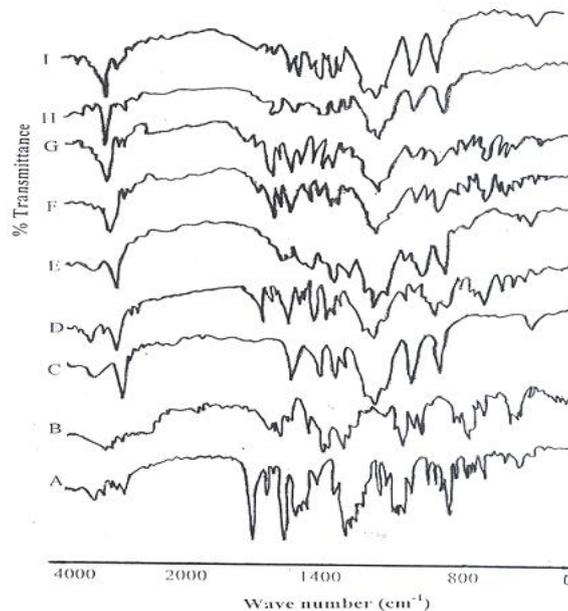


Fig. 4: IRS of KT, MN and some their systems with PEG 6000. Key: a) KT intact, b) MN intact, c) PEG 6000 alone, d) KT-PEG 6000 PM (1:1), e) MN-PEG 6000 PM(1:1), f) KT-PEG 6000 ES (1:1), g) MN-PEG 6000 ES (1:1), h) KT-PEG 6000 MS (1:5), i) MN-PEG 6000 MS (1:5).

Differential Scanning Calorimetry (DSC)

DSC studies demonstrated that DSC patterns of KT, MN and some their different prepared systems with PEG 4000 are exactly similar to that with PEG 6000, so, DSC patterns of KT, MN and some their different prepared systems with PEG 6000 are only shown, Fig. (5). As shown in this figure, DSC patterns of KT, MN and PEG 6000 showed a sharp endothermic peak at 149.6, 183.9 and 62.5 °C respectively, related to their melting points. Further, DSC patterns of

PM of both KT-PEG 6000 and MN-PEG 6000, prepared at drug-carrier ratio 1:1 w/w, exhibited a shallow curvature at the position of endothermic peak of their drug, while, ES and MS of KT-PEG 6000 as well as ES and MS of MN-PEG 6000, prepared at drug-carrier ratios 1:1 and 1:5 w/w, displayed absence of the endothermic peak of their drug (KT or MN) completely. These findings may be due to the dissolution of drug in the molten PEGs during running the DSC studies [11-13], or may be due to the dispersion of drug within PEG 6000 matrix in an amorphous state [14,15].

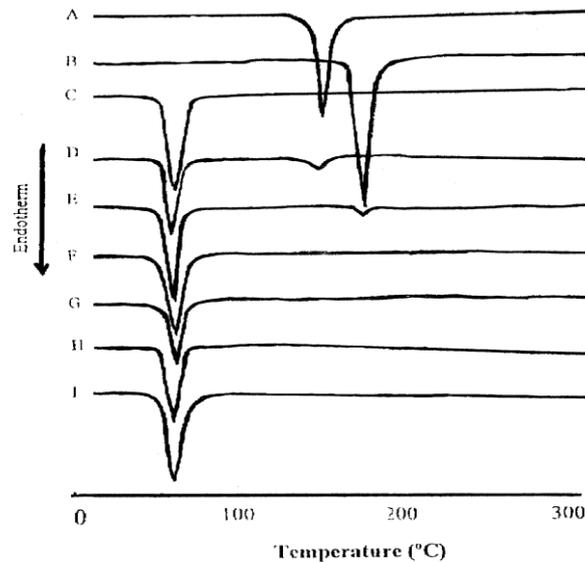


Fig. 5: DSC of KT, MN and some their systems with PEG 6000. Key: a) KT intact, b) MN intact, c) PEG 6000 alone, d) KT-PEG 6000 PM (1:1), e) MN-PEG 6000 PM(1:1), f) KT-PEG 6000 ES (1:1), g) MN-PEG 6000 ES (1:1), h) KT-PEG 6000 MS (1:5), i) MN-PEG 6000 MS (1:5).

The obtained DSC data demonstrated that values of fusion heats (ΔH) of the different prepared solid systems could be arranged in the following order: Drug intact (KT or MN) >>> PM >> ES > MS. The lower ΔH values, the greater loss of drug crystallinity and the more amount of drug found in an amorphous state upon dispersion into the investigated PEGs matrices [15].

Drug contents studies

The results of drug contents studies shown in table (2) demonstrated that the percent values of the actual drug contents ranged from 80.6 to 87.8% w/w ($\pm 2.02\%$) of their theoretical values for different prepared MN solid systems.

Dissolution studies

The dissolution profiles of KT, MN and their different prepared solid systems with PEG 4000 and PEG 6000 are displayed in Figs. (6-9). It can be seen that the dissolution profile of KT or MN was improved by dispersion in the matrices of the two investigated PEGs using both evaporation and melting method as technical dispersion methods [16,17]. PM of KT or MN with either of the two investigated PEGs, at drug-carrier ratio 1:5 w/w, showed an acceptable improvement in the dissolution profile of their drug (KT or MN) due to the good wettability and the local solubilizing effects of PEGs under investigation [6]. On the other side, ES and MS of KT or MN with either of the two investigated PEGs, at drug-carrier ratios 1:1, 1:3 and 1:5 w/w, displayed a good improvement in the dissolution profile of their drug (KT or MN) which found to be progressed as the

fractional and molecular weights of PEGs under investigation increased. The results obtained are in agreement with that of previously reported [10-15].

Further, it has been found that both KT-PEGs and MN-PEGs dispersion systems that prepared by melting method at the different used drug-carrier ratios, showed higher dissolution profile than their corresponding systems that prepared by evaporation method[11]. This may be attributed to the more loss of the drug crystallinity that offered by melting method rather than evaporation method as evidenced by DSC studies. The results obtained are in agreement with that of previously reported [11].

Microbiological studies

The microbiologic studies displayed that the plot of the determined inhibition zone measurements against the various concentrations of the investigated PEGs, are consistent with their solubility diagrams, as shown in Figs. (10-11). Where it was found that as the concentrations of either of the investigated PEGs increase, inhibition zone measurements increase and the highest inhibition zone measurement was obtained by the aqueous solutions of KT or MN in PEG 6000 at concentration of 12% w/v, which showed the higher solubilization power as proved by solubility studies. This is attributed to the increase of the amount of KT or MN solubilized by the increase of the concentrations of either of the investigated PEGs and to the good wettability effects of the investigated PEGs, resulting in a good improvement in the diffusion of KT or MN into the agar medium. However, it is worth to note that the investigated PEGs have no effect on the growth of test organisms in their own rights.

Table 2: Percent values of the actual drug contents of different prepared KT-PEGs and MN-PEGs systems.

Systems	Drug-Carrier Ratios		
	1:1	1:3	1:5
KT-PEG 4000 PM	81.2 (± 3.11)	--	82.4 (± 2.23)
KT-PEG 4000 ES	83.5 (± 2.60)	85.7 (± 1.99)	86.3 (± 1.68)
KT-PEG 4000 MS	82.8 (± 1.98)	86.4 (± 1.25)	87.6 (± 1.37)
KT-PEG 6000 PM	81.6 (± 2.95)	--	83.1 (± 3.36)
KT-PEG 6000 ES	84.4 (± 1.89)	84.9 (± 2.01)	86.5 (± 1.93)
KT-PEG 6000 MS	85.1 (± 1.33)	87.1 (± 1.51)	87.8 (± 1.53)
MN-PEG 4000 PM	80.6 (± 2.79)	--	81.8 (± 2.23)
MN-PEG 4000 ES	84.1 (± 2.33)	84.6 (± 1.80)	85.6 (± 1.47)
MN-PEG 4000 MS	83.6 (± 1.57)	86.4 (± 1.27)	87.1 (± 1.57)
MN-PEG 6000 PM	82.2 (± 2.85)	--	83.4 (± 2.28)
MN-PEG 6000 ES	86.1 (± 1.93)	83.9 (± 2.31)	85.5 (± 1.71)
MN-PEG 6000 MS	85.7 (± 1.31)	86.2 (± 1.41)	87.3 (± 1.54)

Where: PM= physical mixture, ES=evaporate systems, MS=melt systems.

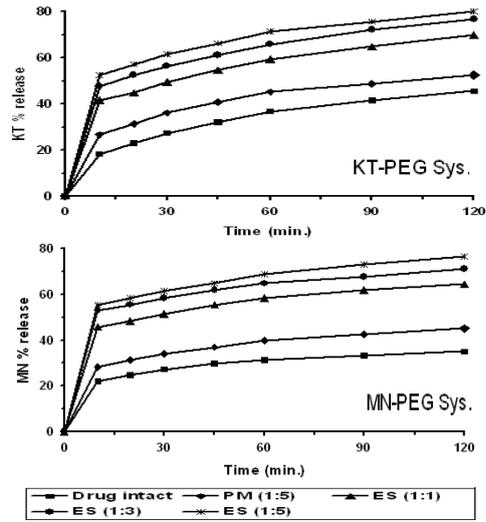


Fig. 6: Dissolution profiles of the model drugs (KT & MN) from their ES with PEG 4000 in distilled water containing 0.02% w/v of tween 80.

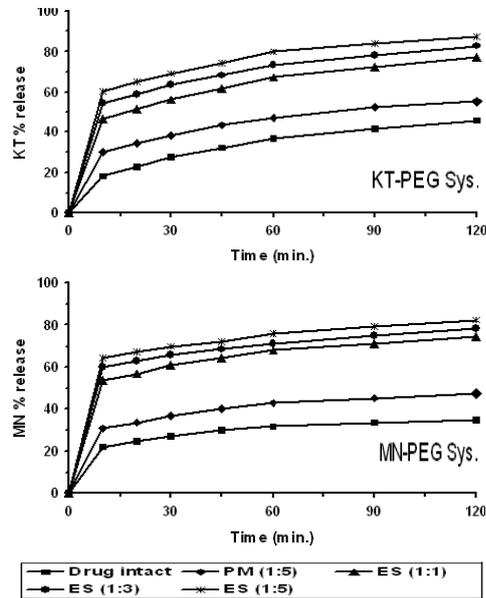


Fig. 7: Dissolution profiles of the model drugs (KT & MN) from their ES with PEG 6000 in distilled water containing 0.02% w/v of tween 80.

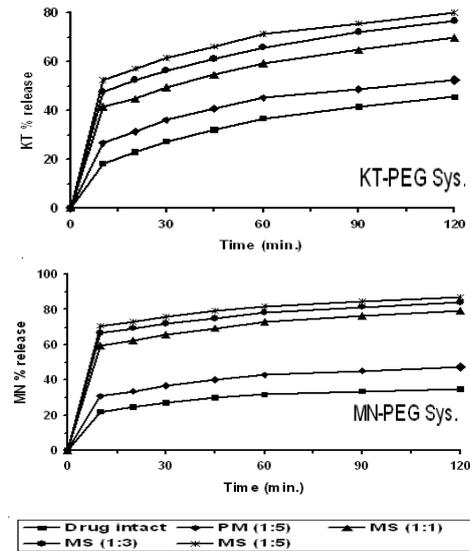


Fig. 7: Dissolution profiles of the model drugs (KT & MN) from their MS with PEG 4000 in distilled water containing 0.02% w/v of tween 80.

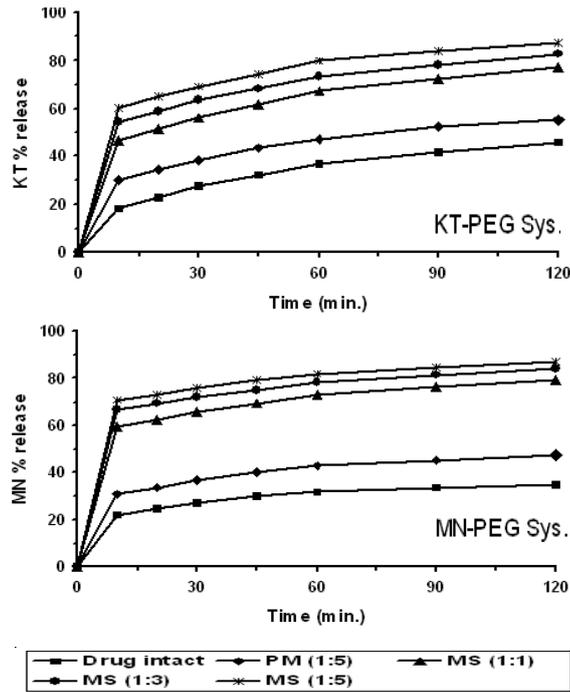


Fig. 9: Dissolution profiles of the model drugs (KT & MN) from their MS with PEG 6000 in distilled water containing 0.02% w/v of tween 80.

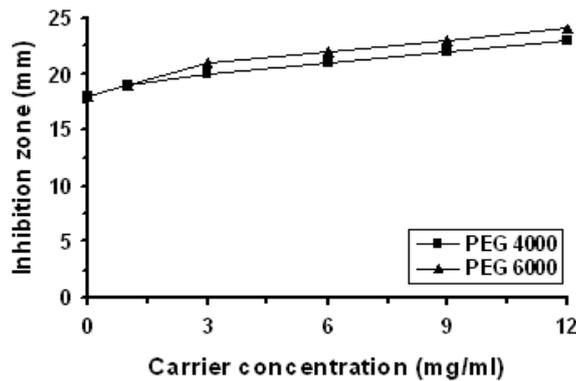


Fig. 10: Inhibition zone measurements of KT as a function of various concentrations of PEGs using *C. albicans* as a test organism.

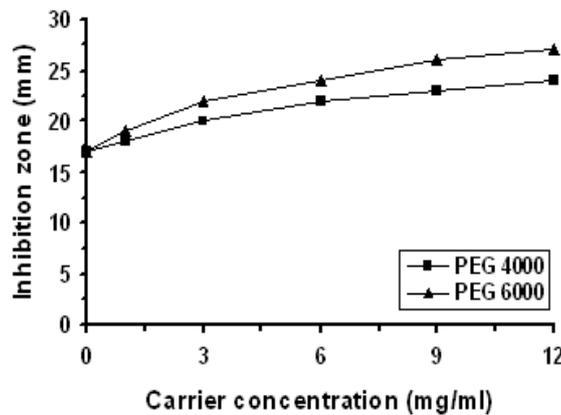


Fig. 10: Inhibition zone measurements of MN as a function of various concentrations of PEGs using *C. albicans* as a test organism.

CONCLUSION

The solid dispersion of imidazole model drugs, KT or MN, with each of PEG 4000 and PEG 6000, at the different used drug-carrier ratios using both evaporation and melting methods, was succeeded in improvement of the dissolution profiles of either KT and MN and a

higher dissolution profile was obtained as the fractional and molecular weights of the investigated PEGs increased. DSC studies demonstrated that the crystallinity of KT or MN was highly lost and a greater amount of these drugs found in an amorphous state upon using melting method rather than evaporation one. In accordance with the results of DSC studies, KT-PEGs and MN-PEGs dispersion

systems that prepared by melting method at the different used drug-carrier ratios, showed higher dissolution profile than their corresponding systems that prepared by evaporation method. The results of microbiologic investigation showed that the aqueous solutions of both KT-PEGs and MN-PEGs systems showed superior antimycotic activity to their drug intact.

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