December 2009 Volume 5 Issue (1-2)

Trade Science Inc.

Macromolecules

An Indian Journal

Full Paper

MMAIJ, 5(1-2), 2009 [1-8]

Ketoconazole binary and ternary solid dispersions in different macromolecular matrices

Mohamed Abbas Ibrahim

Dr Walid Kayyali Chair for Pharmaceutical Industries, Department of Pharmaceutics, Faculty of Pharmacy, King Saud University, Riyadh 11451, (KINGDOM OF SAUDI ARABIA)

E-mail : abbma71@gmail.com

Received: 14th June, 2009 ; Accepted: 24th June, 2009

ABSTRACT

The objective of this study was to enhance the dissolution of Ketoconazole- an imidazole antifungal agent- by solid dispersions consisting of the drug, a polymeric carrier, and a surfactant. Binary and ternary solid dispersions of Ketoconazole were prepared with polyvinylpyrrolidone (PVP 40000) or PEG 6000 and a non-ionic surfactant (Brij 35) using coevaporation technique. Preliminarily, the micellar solubilization of Ketoconazole was studied in aqueous solutions of 0.1 to1% of nonionic surfactants at 37 °C. It was found that Brij 35 exhibited a higher solubilizing capacity on the drug than Tween 80 and Myrj-52 and the drug exhibited a higher distribution coefficient between Brij 35 micelles. A higher dissolution rate of Ketoconazole was obtained when the drug was present in the form of solid dispersion. In addition, increasing the polymer concentration in the binary systems was accompanied by an increase in the drug dissolution rate in terms of dissolution efficiency (DE_{30}) and relative dissolution rate (RDR_{30}) after 30 minutes. The addition of Brij 35 to the carrier matrix led to a vast improvement of the dissolution characteristics. The powder x-ray diffraction and differential scanning calorimetry of binary Ketoconazole-PVP (1:1 and 1: 5) and Ternary Ketoconazole-PVP-Brij (1:1:0.5) solid dispersions indicated absence of drug crystalline form.

KEYWORDS

Ketoconazole;
PVP 40000;
PEG 4000;
Brij-35;
Binary and ternary solid dispersions.

INTRODUCTION

In order to ensure the optimum therapeutic effect of a drug, it is necessary to prepare the proper dosage form for targeted and time release. A large percentage of potential drug candidates suffer from low aqueous solubility and/or low dissolution rate. Amidon et al.[1] classified such drugs in the Biopharmaceutical Classification System (BCS) as class II compounds. The formulation of solid dispersions of BCS II compounds either by coprecipitation of drug and carrier from a common solvent or by co-melting and quench cooling is a popular strategy to reduce the drug particle size and hence increase the dissolution rate.[2]

The enhancement of the drug dosage form formulation is connected with the application of auxiliary substances or with new technological possibilities[3]. Solid dispersions in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and hence possibly bioavailability, of a range

of hydrophobic drugs[4]. Hydrophilic polymers have been commonly used as carriers for preparing solid dispersions. Among them, Polyvinylpyrrolidone (PVP) and Polyethylene glycol (PEG) were widely employed for their high aqueous solubility, high physiological tolerance, and low toxicity[5].

In recent years, the interest in incorporating a surface-active carrier into solid dispersion systems increased greatly and a high improvement in drug dissolution was reported[6,7]. It was reported that a solid dispersion in a mixture of polyethylene glycol and polysorbate 80 could improve the dissolution rate and enhance the bioavailability of LAB687, a poorly water-soluble drug[8]. Okonogi et al.[9] incorporated polysorbate 80 into the binary solid dispersion of ofloxacin with polyethylene glycol of different molecular weights systems. They found that a decreased crystallinity of the solid dispersions obtained revealed that a portion of the drug was in an amorphous state to which the enhanced drug dissolution rate was attributed. Furthermore, Sjökvist et al.[10] prepared ternary solid dispersion systems by incorporating sodium dodecylsulphate (SDS) in griseofulvin-PEG 3000 binary solid dispersion. Due to the incorporation of surfactant, the dissolution rate of grisefulvin was faster than the dispersion without SDS. Moreover, Solid dispersions of griseofulvin were prepared by the melting method with polyethylene glycols (PEGs) of molecular weights 3000, 6000 and 20 000 as carriers, with or without the incorporation of the anionic surfactant sodium dodecyl sulphate (SDS)[11]. When SDS was incorporated in the polyethylene glycols, the solid solubility of griseofulvin increased to 40% w/w in PEG 6000 and to 25% w/w in PEG 3000 and PEG 20 000.

Ketoconazole (KET) is an imidazole antifungal agent suitable for the treatment of candidiasis and other systemic fungal infections. The major drawback in the therapeutic application and efficacy of KET as oral dosage forms is its very low aqueous solubility because of its hydrophobic structure[12]. The dissolution of KET was enhanced by solid dispersion in different carriers. The dissolution rate of ketoconazole increased when solubilizing excipients were incorporated into the PEG 6000-based solid dispersions[13]. When hydrophilic and lipophilic excipients were combined and incorporated into PEG solid dispersions, a remarkable enhancement of the dissolution rate was observed. An increase in poor buffer pH 5 and 6 solubility of ketoconazole was studied. Two systems were used: binary complexes prepared with β-cyclodextrin and multicomponent systems (β-cyclodextrin and an acid compound) obtained by spray-drying[14]. The solubility of ketoconazole increased significantly with the cyclodextrin complexes. However, enhancement was better from the multicomponent systems.

The objective of this study was to enhance the dissolution of Ketoconazole by dispersing the drug in the matrices of hydrophilic polymers; PVP 40000 and PEG 4000. In addition, the research aimed at preparing ternary solid dispersions of the drug with the aforementioned polymers with the addition of a non-ionic surfactant so as to study the effect of incorporating surfactant on the physicochemical properties of the prepared solid dispersions.

MATERIALS

Ketoconazole (KET) was kindly supplied by Egyptian International Pharmaceutical Industries Co. (E.I.P.I.Co.), Egypt. Polyvinyl Pyrrolidones: PVP 40000, Sigma chemical Co., USA. Polyethyleneglycol 6000 was purchased from Fluka Chemica, Buch, Switzerland. Dicholromethane (DCM), Potassium Dihydrogen Orthophosphate, Disodium Hydrogen Orthophosphate and absolue ethanol (95%) were purchased from El-Nasr Pharmaceutical Co., Egypt. Polyoxyethylene (20) sorbitan monooleate (Tween 80) was purchased from BDH chemical ltd. Co., Poole, England. Polyoxyethylene (40) stearate (Myrj52) and Polyoxyethylene (23) lauryl ether (Brij 35) were purchased from Sigma chemical Co., USA. Other materials and solvents are of reagent or analytical grade, and they were used without further purification.

EXPERIMENTAL

Solubility studies

Three non-ionic surfactants were studied for their solubilizing actions on KET, namely, Tween 80, Myrj52 and Brij35. For each surfactant, aqueous solutions containing different surfactant concentrations (0.25, 0.5 and

1%w/w) were prepared. Excess KET powder was added to screw capped bottles containing 10 ml of each surfactant concentration. The bottles were shaken in a water bath (Shaking water bath, thermostatically controlled, BF21 (SBS Instruments, Germany) at 37°C until equilibrium was reached (24 hr). The content of each bottle was filtered (0.22 pm pore size) and the concentration of KET in the filtered solutions was measured by UV spectrophotometry at 225 nm against a suitable blank (Double beam Spectrophotometer UV. 1601, Shimadzu Co., Japan).

Preparation of KET-polymer binary and ternary solid dispersions

Co-evaporation technique was employed to prepare coprecipitates of KET with PVP 40000 and PEG 6000 binary systems or ternary systems with the surfactant. Briefly, the weighed amounts of KET and the polymer -with or without the surfactant- were dissolved in 50 ml of dichloromethane. The solution was stirred at room temperature for 20 minutes, and the solvent was then removed under vacuum in a rotary evaporator, at a maximum temperature of 40°C. Solid residue was dried in a desiccator for 24 h at room temperature, pulverized and sieved (Sieve shaker, Rx-86-1, Cole-Parmer Instrument Co., USA). Powder samples below 420 μm (40 mesh) were stored in closed containers away from the light and humidity pending the investigations.

Dissolution studies

The drug dissolution experiments on KET binary and ternary systems were carried out by the rotating paddle method (Dissolution test apparatus, SR11 6 Flask, Hanson Co., USA). Details of the experiment in triplicate are provided elsewhere. The experiments were carried out at 37°C and the dissolution medium was 500 mL of phosphate buffer saline (PBS, pH 7.4).

The coevaporate powder was spread over the surface of the dissolution liquid. At pre-set intervals, samples of 5 ml were withdrawn and filtered. The sample was diluted to a suitable volume with the buffer solution and the absorbance was measured at 225 nm. Equal volume of fresh dissolution medium, prewarmed at 37°C, was replaced into the dissolution medium so as to keep the volume of the dissolution medium constant.

Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time[15]. Also, The relative dissolution rate (RDR_{30}) data of the different samples were calculated by determining the amount of KET dissolved from a particular sample and normalizing for the amount of drug dissolved from pure drug sample over the same time interval (30 minutes).

Differential scanning calorimetry (DSC)

DSC scans were recorded for KET binary and ternary systems compared to that of the individual componenets in order to determine the extent of crystallinity of the drug in presence of the studied polymers.

Samples of about 5 mg were accurately weighed and encapsulated into flat-bottomed aluminum pans with crimped-on lids. A scanning rate of 10 °C/min from 30°C to 200°C was used in presence of nitrogen at flow rate of 40 ml/min (T.A. 501 Differential scanning calorimeter, DSC, Shimadzu Co., Japan).

Powder X-ray diffraction (PXRD)

The X-ray diffractograms were obtained using Jeol XR Diffractometer (Jeol, Tokyo, Japan). The radiation source was a copper (λ=1.54184 Å) high-intensity x-ray tube operated at 35 KV and a current of 15 mA. The diffraction patterns were achieved using continuous scan mode with 2θ values ranging from 4-100 at a rate of 4 degrees/minute.

RESULTS

Effect of different non-ionic surfactants on KET aqueous solubility

The solubilization of KET in different non-ionic surfactant solutions at 37°C is shown in TABLE 1. The tested non-ionic surfactants were used above their respective critical micelle concentrations (CMC). The solubility of the drug in the non-ionic surfactant solutions was increased linearly by increasing the surfactant concentrations.

The distribution coefficient (Km) of KET which was expressed by the ratio of the solubilized drug in the micelle of surfactant solution to that present in the aque-

ous phase[16] is listed in TABLE 1. There is a good relationship between the solubility of the KET and the Km value, in the manner of the higher the incorporation of the drug within the surfactant micelle, the higher will be its solubility and higher its Km value. Thus, the order of the Km values was the same order of the solubilizing efficiencies of the investigated nonionic surfactants. In addition, is the solubilizing capacity of the micelles could be calculated from the slope resulting from a plot of total molar solubility of the drug in the micellar solution versus the molar concentration of the micelle[17].

On the solubilization of KET in solutions of these surfactants, it was found that Brij 35 was more efficient as a solubilizer than the other tested surfactants. In addition, it was found that Brij 35 exhibited a higher solubilizing capacity on the drug than Tween 80 and Myrj-52 and the drug exhibited a higher distribution coefficient between Brij 35 micelles, TABLE 1. Therefore, Brij-35 was chosen to be incorporated into the ternary solid dispersions of KET in the polymeric matrices.

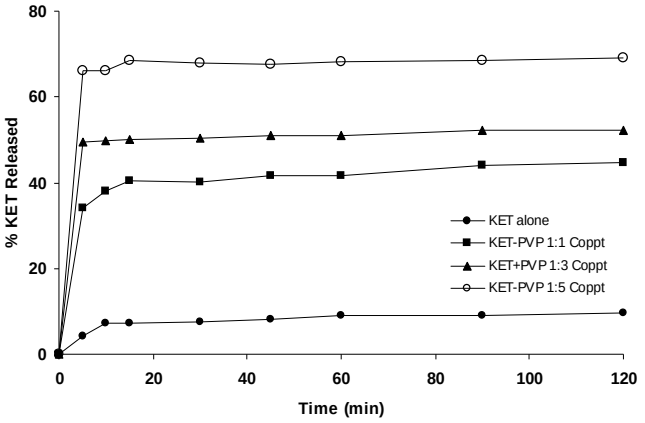

Figure 1 : Dissolution profile of ketoconazole and its binary solid dispersions with PVP 40000.

TABLE 1 : Solubilizing capacity of different surfactants and the distribution coefficient of ketoconazole between the micellar and aqueous phases

Surfactant	Solubilizing Capacity ($K_m S_w$)x 10^3	Distribution Coefficient (K_m) (mM^{-1}) x 10^3
Myrj 52	150.48	267.79
Tween 80	261.56	334.58
Brij 35	289.20	353.17

Dissolution studies

KET-PVP 40000 binary systems

The binary solid dispersions of KET in different polymers were tested in preliminary experiments aimed at choosing a suitable carrier. The dissolution profiles of KET varied depending on the ratio of KET to the dispersing polymer.

A higher dissolution rate of KET was obtained when the drug was present in the form of solid dispersion in the PVP matrices, although the amount of the drug dissolved depended considerably on the concentration of carrier. Figure 1 displays the dissolution profile of KET from its-PVP solid dispersions at different drug: polymers ratios. Increasing the polymer concentration in these binary systems was accompanied by an increase in the dissolution drug rate. In terms of dissolution efficiency after 30 minutes (DE_{30}), the values were 16.21, 22.37 and 27.94 for 1:1, 1:3 and 1: 5 KET-PVP ratios, respectively, compared to 2.97 for the original KET, TABLE 2. In addition and the relative dissolution rates at 30 minutes (RDR_{30}) from such binary systems were 5.26, 6.29 and 8.63, respectively.

TABLE 2 : Dissolution efficiency at 30 minutes ($DE_{30\%}$) and relative dissolution rate at 30 minutes (RDR_{30}) of ketoconazole from its-PVP binary and ternary systems.

System	$DE_{30\%}$	RDR_{30}
KET alone	2.97	–
KET-PVP 40000 Binary Systems		
KET: PVP (1:1)	16.21	5.26
KET: PVP (1:3)	22.37	6.29
KET: PVP (1:5)	27.94	8.63
KZ-PVP 40000 Ternary Systems		
KET: PVP: Brij 35 (1:1: 0.1)	22.34	6.54
KET: PVP: Brij 35 (1:1: 0.2)	26.25	8.81
KET: PVP: Brij 35 (1:1: 0.5)	28.28	9.22
KET-PEG 6000 Binary Systems		
KET: PEG (1:1)	3.09	1.03
KET: PEG (1:3)	4.60	1.45
KET: PEG (1:5)	11.56	3.60
KET-PEG 6000 Ternary Systems		
KET: PEG: Brij 35 (1:1: 0.1)	5.29	1.96
KET: PEG: Brij 35 (1:1: 0.2)	6.65	2.34
KET: PEG: Brij 35 (1:1: 0.5)	8.32	3.45

KET-PVP 40000 ternary systems

The comparison made between the binary KET/PVP (1: 1) solid dispersion with the ternary solid dispersions showed that addition of Brij 35 to the

carrier matrix led to a vast improvement of the dissolution characteristics, Figure 2. The DE_{30} values of KET-PVP-Brij35 ternary systems have been improved to be 22.34, 26.25 and 28.28 for KET-PVP-Brij 1: 1: 0.1, 1: 1: 0.2 and 1: 1: 0.5, respectively, compared to DE_{30} value of 16.21 for KET-PVP 1:1 binary system, TABLE 2.

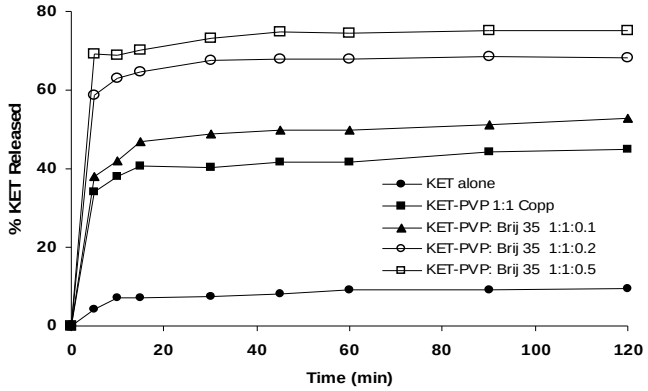

Figure 2 : Dissolution profile of ketoconazole and its ternary solid dispersions with PVP 40000 and Brij 35.

KET-PEG 6000 binary systems

The dissolution profiles of KET from its-PEG 6000 binary solid dispersions compared to the untreated drug are shown in Figure 3. It is clearly evident that PEG did not improve the dissolution rate of the drug as PVP did. This could be seen from the lower DE_{30} values of KET-PEG binary solid dispersions (3.09, 4.6 and 11.65 for 1: 1, 1: 3 and 1: 5 binary systems, respectively, TABLE 2). However, the results referred to a slight enhancement of the drug dissolution by increasing the polymer concentration, especially with a drug: polymer ratio 1: 5.

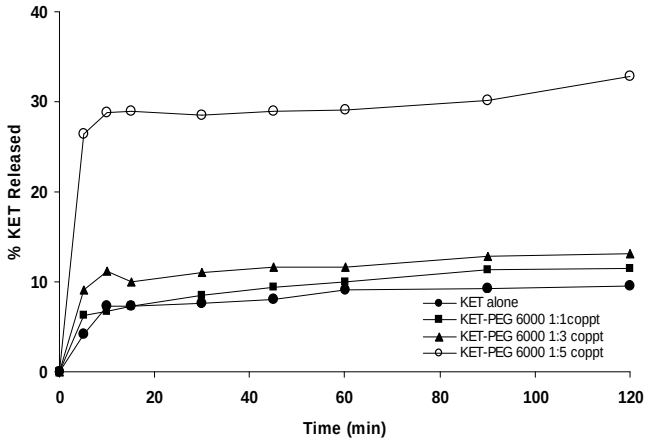

Figure 3 : Dissolution profile of ketoconazole and its binary solid dispersions with PEG 6000.

KET-PEG 6000 ternary systems

The incorporation of Brij-35 into KET-PEG 6000 binary systems did not remarkably enhance the drug dissolution rate. The RDR_{30} recorded for KET-PEG-Brij ternary systems at 1: 1: 0.1, 1: 1: 0.2 and 1: 1: 0.5 were 1.96, 2.34 and 3.45, respectively, TABLE 2 and Figure 4.

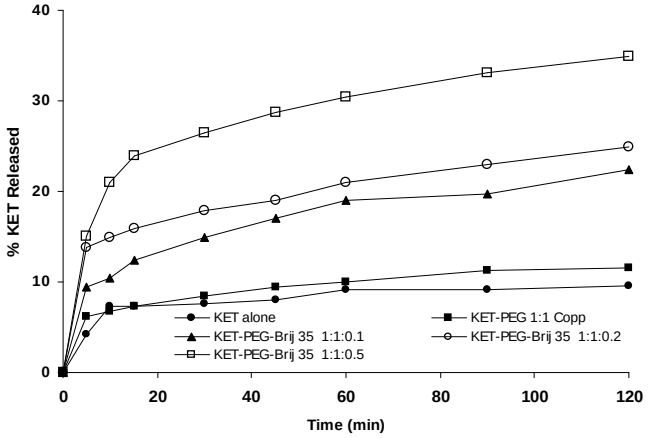

Figure 4 : Dissolution profile of ketoconazole and its ternary solid dispersions with PEG 6000 and Brij 35.

Differential scanning calorimerty

According to the dissolution rate studies, the best results were obtained with 1:5 molar ratio of drug: polymer, prepared by the solvent method for binary solid dispersions. In addition, the ratio 1: 1: 0.5 of ternary solid dispersions exhibited the highest dissolution rates. For this reason XRD and DSC studies were conducted. The thermal behavior of pure components and of some selected drug-PVP dispersed mixtures are depicted in Figure 5. DSC curve of the pure drug showed a sharp endothermic peak at 150^0C that indicated the crystalline anhydrous state of KET. In contrast, the large endotherm over the temperature range 50 to 100 ^{0}C associated with water loss, shown by PVP K-30 was typical of amorphous hydrated substance[18].

The thermal behavior of KET-PVP binary systems demonstrated the presence of intense solid state changes of the drug in the polymer matrices. In fact, the melting endotherm of KET fully disappeared in ratios of 1:1 and 1:5, Figure 5A, revealing total drug amorphization[19]. The amorphizing power of PVP toward the drug was also confirmed from the results of XRD analysis. Similarly, the DSC tracing of KET-PVP-

Brij 35 (1:1:0.5) ternary system showed complete disappearance of the drug melting endotherm confirming the transformation of KET to an amorphous form in the ternary system, Figure 5B.

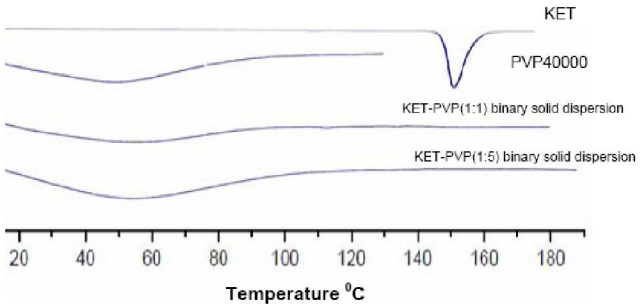

Figure 5A : DSC curves of ketoconazole and binary systems with PVP 40000.

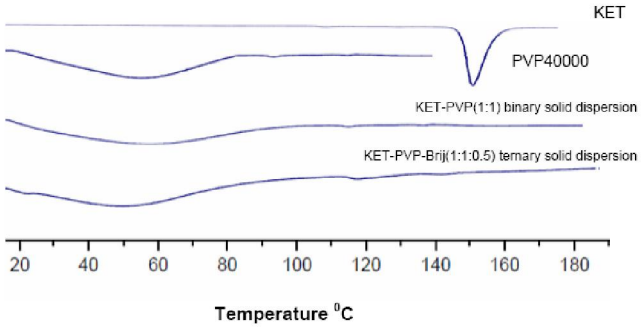

Figure 5B: DSC curves of ketoconazole and binary and ternary systems with PVP 40000 and Brij 35.

The thermograms of the PEG-based binary solid dispersions showed the characteristic peak of the carrier matrix around 60°C. The characteristic endothermic peak of the drug appeared at its original position, but reduced in intensity, shifted to lower temperatures and lost its sharpened distinct appearance in case of KET-PEG 6000 (1:1) binary solid dispersion, Figure 6A. On the other hand, the exothermic peak of the drug completely disappeared at a drug: polymer ratio 1: 5, Figure 6A.

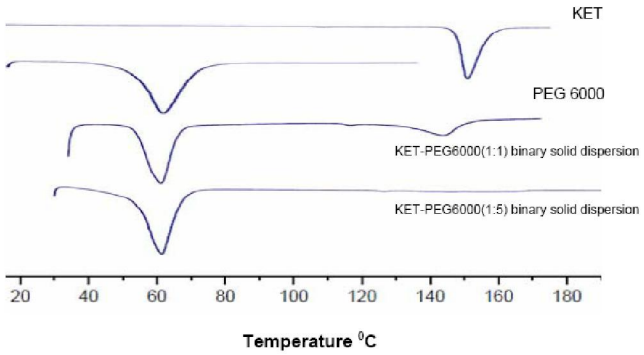

Figure 6A: DSC curves of ketoconazole and binary systems with PEG 6000.

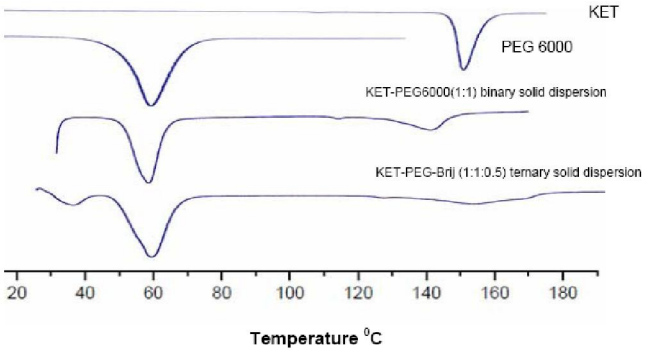

Figure 6B : DSC curves of Ketoconazole and binary and Ternary systems with PEG 6000 and Brij 35.

Powder X-ray diffraction (PXRD)

The powder XRD patterns of KET binary (1: 5) and ternary (1: 1: 0.5) solid dispersions with PVP 40000 and Brij 35 are shown in Figure 7A and B, respectively. Intact KET exhibited the internal crystalline characteristics and showed identical sharp XRD peaks at various 2θ, while PVP and Brij 35 do not show any characteristic peaks within the observed range of (2θ). The XRD peaks of intact KET crystals were observed at the same 2θ values in both binary and ternary physical mixtures. This indicated that the crystallinity of KET did not change in the physical mixtures.

The diffractogram of KET-PVP (1: 5) solid dispersion is more like that of PVP indicating absence of the drug crystalline form. The PXRD results are well correlated with the DSC data, Figure 7A. Also, the x-ray diffraction spectra of KET-PVP-Brij35 (1: 1: 0.5) solid dispersion system compared to the corresponding physical mixture is shown in Figure 7B. The characteristic diffraction peaks of the drug completely disappeared in such ternary solid dispersion compared to that in case of the corresponding physical mixture.

The powder XRD patterns of PEG-based binary and ternary solid dispersion systems at a drug: polymer ratio 1: 5 and drug: polymer: brij ratio of 1: 1: 0.5 compared to the physical mixtures of the same composition are illustrated in Figure 8A and B. PEG 6000 revealed

two distinct peaks at 19 and 23 2θ, characteristic of its crystallinity[20]. The X-ray diffractometry of the binary and ternary solid dispersions and physical mixtures of KET-PEG and KET-PEG-Brij are approximately the superposition of the patterns of the raw materials.

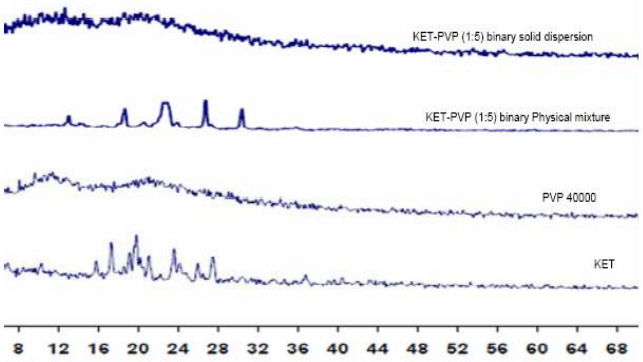

Figure 7A : PXRD spectra of ketoconazole and binary systems with PVP 40000.

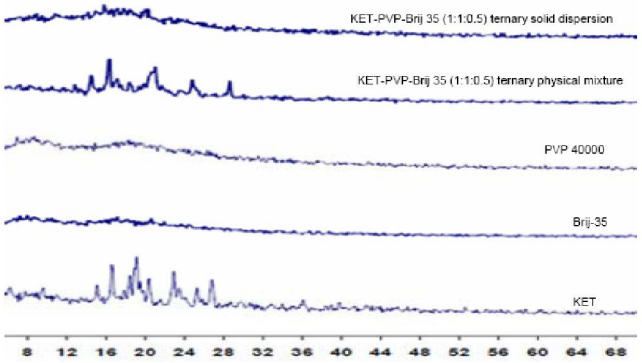

Figure 7B : PXRD spectra of ketoconazole and binary and ternary systems with PVP 40000 and Brij 35.

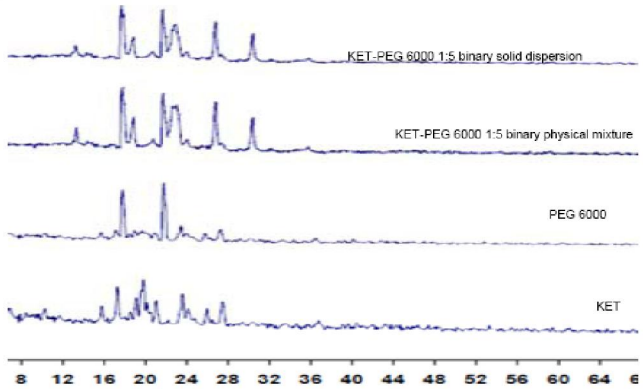

Figure 8A : PXRD spectra of ketoconazole and binary systems with PEG 6000.

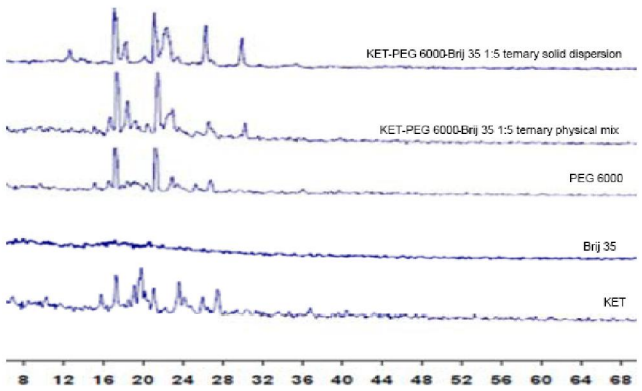

Figure 8B : PXRD spectra of ketoconazole and binary and ternary systems with PEG 6000 and Brij 3

DISCUSSION

According to Chiou and Riegelman[21], several factors could contribute the enhanced drug dissolution performance from drug-polymer dispersed mixtures. These factors are particle size reduction of the drug, improved wettability, and loss of crystallinity occurring during the coprecipitation are considered the principal factors responsible for the enhanced dissolution behavior of the drug. Furthermore, according to Van den Mooter et al.[22], PVP was found to be effective in prevention of such crystallization on the condition that the drug was formulated in solid dispersions since physical mixing with the polymer led to crystallization. They concluded therefore that the physical mechanism of the protective effect of PVP in the case of amorphous ketoconazole is not the consequence of drug–polymer interactions, but mainly due to the polymer anti-plasticizing effect, thereby increasing the viscosity of the binary system and decreasing the diffusion of drug molecules necessary to form a lattice.

When the proportion of PVP 40000 in the solid dispersion is increased, the rate of KET dissolution also increases, the greatest value corresponding to the KET solid dispersion. The explanation is that this solid dispersion was a homogeneous dispersion of the drug in the polymer matrix is produced[23]. In addition, Van der Waals interaction forces between drug molecules are decreased in these mixed crystals, and as a consequence the dissolution of the drug from this solid dispersion is faster than from the pure drug. Moreover, In the case of binary and ternary solid dispersions, the improved KET dissolution rate might be attributed to the prohibi-

tion of crystallization of the drug caused by PVP[24] as indicated from the DSC and PXRD data.

In the latter case, the addition of a surfactant to the solid dispersion during preparation will decrease the interfacial tension between drug particles and the dissolution medium, and enhance the dissolution rate of the drug from the ternary system[25,26].

The previous results showed that PEG 6000 did not improve the dissolution rate of KET as PVP did. Clearly, PEG 6000 acts as a disaggregant in these systems; the electrostatic charges that keep drug particles united together are reduced and the drug can dissolve in the dissolution medium[26].

CONCLUSION

The incorporation of a non-ionic surfactant (Brij-35) into the binary solid dispersions of KET with the tested polymers was found to have a pronounced effect on enhancing the in vitro drug dissolution rate. In addition, the presence of the surfactant, in addition to enhancing drug dissolution rate, might present an advantage that the amounts of polymer required to produced the desirable solid dispersion were reduced to 20% when compared to that used in binary solid dispersions.

REFERENCES

[1] G.L.Amidon, H.Lennernäs, V.P.Shah, J.R.Crison; Pharm.Res., **12**, 413 **(1995)**.
[2] A.T.M.Serajuddin; J.Pharm.Sci., **88**, 1058 **(1999)**.
[3] R.Jachowicz, E.Nu¨rnberg, B.Pieszczek, B.Kluczykowska, A.Maciejewska; Int.J.Pharm., **206**, 13 **(2000)**.
[4] D.Q.M.Craig; Int.J.Pharm., **231**, 131 **(2002)**.
[5] L.Liu, X.Wang; AAPS PharmSciTech., **8**, E1 **(2007)**.
[6] S.Janssens, S.Nagels, H.N.de Armas, W.D'Autry, A.Van Schepdael, G.Van den Mooter; Eur.J.Pharm.Biopharm., **69**, 158 **(2008)**.
[7] M.Wulff, M.Alden, D.Q.M.Craig; Int.J.Pharm., **142**, 189 **(1996)**.
[8] R.Dannenfelser, H.He, Y.Joshi, S.Bateman, A.T.M.Serajuddin; J.Pharm.Sci., **93**, 1165 **(2004)**.
[9] S.Okonogi, S.Puttipipatkhachorn; AAPS Pharm. Sci.Tech, **7**, E1 **(2006)**.
[10] E.Sjökvist, Ch.Nyström, M.Alden, N.Caramelham; Int.J.Pharm., **79**, 123 **(1992)**.
[11] M.Wulff, M.Aldēn; Thermochim.Acta, **256**, 151 **(1995)**.
[12] M.T.Esclusa-Diaz, M.Gayo-Otero, M.B.P6rez-Marcos, J.L.Vila-Jato, J.J.Torres-Labandeira; Int.J.Pharm., **142**,183 **(1996)**.
[13] M.-Y.Heo, Z.-Z.Piao, T.-W.Kim, Q.-R.Cao, A.Kim, B.-J.Lee; Arch Pharm.Res., **28**, 604 **(2005)**.
[14] M.T.Esclusa-Díaz, M.Gayo-Otero, M.B.Pérez-Marcos, J.L.Vila-Jato, J.J.Torres-Labandeira; Int.J.Pharm., **142**, 183 **(1996)**.
[15] K.A.Khan; J.Pharm.Pharmacol., **27**, 48 **(1975)**.
[16] M.O.Ahmed; Eur.J.Pharm.Biopharm., **51**, 221 **(2001)**.
[17] J.T.H.Ong, E.Manoukian; Pharm.Res., **5**, 704 **(1988)**.
[18] N.Zerrouk, N.Mennini, M.Francesca, C.Chemtob, P.Mura; Eur.J.Pharm.Biopharm., **57**, 93 **(2004)**.
[19] H.Abdelkader, O.Y.Abdallah, H.S.Salem; AAPS PharmSciTech, **8**, E1 **(2007)**.
[20] Y.Özkan, N.Doðanay, N.Dikmen, A.Işimer; Farmaco, **55**, 433 **(2000)**.
[21] W.L.Chiou, S.Riegelman; J.Pharm.Sci., **60**, 1281 **(1971)**.
[22] G.Van den Mooter, M.Wuyts, N.Blaton, R.Busson, P.Grobet, P.Augustijns, R.Kinget; Eur.J.Pharm.Sci., **12**, 261 **(2001)**.
[23] M.D.Veiga, M.J.Bernad, C.Escobar; Int.J.Pharm., **89**, 119 **(1993)**.
[24] C.Leuner, J.Dressman; Eur.J.Pharm.Biopharm., **50**, 54 **(2000)**.
[25] L.Liu, X.Wang; AAPS PharmSciTech, **8**, E1 **(2007)**.
[26] M.D.Veiga, C.Escobar, M.J.Bernad; Int.J.Pharm., **93**, 215 **(1993)**.