Original Article

Comparative evaluation of ketoconazole-β-cyclodextrin systems prepared by coprecipitation and kneading

Maha A. Marzouk*, Alaa A. Kassem, Ahmed M. Samy, Reham I. Amer

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

ABSTRACT: Ketoconazole (KZ), an imidazole antifungal, was formulated into inclusion complexes *via* coprecipitation and kneading with β-cyclodextrin (β-CD) as a carrier in 1:1 and 1:2 drug to carrier ratios. The KZ-β-CD solid complexes were characterized by X-ray diffraction and differential scanning calorimetry (DSC). The diffraction pattern of the pure drug revealed the drug to be highly crystalline in nature, as indicated by numerous distinctive peaks. The lack of numerous distinctive peaks of the drug in KZ-β-CD complexes prepared by the two methods revealed that a large number of the drug molecules were dissolved in a solid-state carrier matrix with an amorphous structure. The thermograms of the KZ-β-CD complexes showed a strong reduction in the intensity and broadening of drug peaks somewhat in both kneading and coprecipitation systems, suggesting that the drug is monomolecularly dispersed in the β-CD cavity. The prepared tablets of KZ-β-CD solid complexes prepared by the two methods were evaluated for their quality control testing, and an *in vitro* release study and the results of quality control complied with pharmacopeial requirements and the release profiles indicated complete drug release after 30 min. The kinetic parameters obtained from release data were analyzed in order to explain the mechanism of drug release and revealed non-Fickian transport. Accelerated stability testing at 35°C, 45°C, and 55° C and at 75% relative humidity was carried out for six months and revealed somewhat stable systems as indicated by a t_{90} of about 2 years for both KZ-β-CD systems. A microbiological *in vitro* assay of KZ from the prepared tablets was performed using *Candida albicans* as a model fungus, and KZ had improved microbiological activity when administered as an inclusion complex with β-CD. The results confirmed the benefit of using CDs as a useful tool to enhance the dissolution and hence bioavailability of poorly water-soluble drugs by forming solubilizing systems when exposed to gastrointestinal fluid.

Keywords: Ketoconazole, β-cyclodextrin inclusion complex, spectroscopic study, tablets, quality control, *in vitro* release, stability, microbiological study

1. Introduction

A prerequisite for drug absorption and clinical success for all drugs given orally in a solid dosage form is drug dissolution within the gastrointestinal tract, which in many cases can be the rate-limiting step in the overall absorption process *in vivo* (*1*). Ketoconazole (KZ) is a dibasic imidazole antifungal synthetic agent developed for the treatment of human mycotic infections and plays an essential role in antifungal chemotherapy (*2*). It is slightly soluble in water, with a molecular weight of 531.44; it must be administered either topically or by mouth (*3*). Since it is a weak base with limited water solubility, an acid medium is required to transform the drug into the soluble hydrochloride salt (*4-6*).

Cyclodextrins (CDs) are oligosaccharides that have received increasing attention in the pharmaceutical field because of their ability to form inclusion complexes with many lipophilic drugs, thus changing the physicochemical and biopharmaceutical properties of those drugs (*7,8*). Complexation between imidazole derivatives and cyclodextrins has already been studied (*5*) and the antimycotic activity of these complexes has been found to be superior to the activity of drug alone.

The objective of this study was to improve the aqueous solubility and dissolution rate of KZ by preparing inclusion complexes with β-cyclodextrin using coprecipitation and kneading. Furthermore, X-ray diffractometry and differential scanning calorimetry (DSC) were used to study interactions between KZ and β-CD in a solid state. The prepared tablets of KZ-β-CD systems were evaluated for their uniformity of weight, thickness, hardness, friability, disintegration time, and drug content uniformity. The *in vitro* release of KZ

**Address correspondence to:*
Dr. Maha Abd El-hamid Marzouk, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
e-mail: ma.marzouk@yahoo.com

tablets was determined and the kinetic parameters were analyzed in order to explain the mechanism of drug release. Accelerated stability testing of KZ at 35°C, 45°C, and 55°C and at 75% relative humidity was carried out for six months, and $t_{1/2}$ and t_{90} were estimated for each formula. A microbiological *in vitro* assay of KZ from the prepared tablets was performed using *Candida albicans* as a model fungus. The inhibition zone diameter in each case was measured, and the obtained value served as a measure of the antifungal activity of KZ.

2. Materials and Methods

2.1. *Materials*

KZ was kindly supplied by Memphis Company for Pharmaceuticals (Cairo, Egypt). β-CD was obtained from Alexandria Chemical Co. (Alexandria, Egypt). Absolute methyl alcohol, hydrochloric acid, sodium chloride, talc, 70% perchloric acid, and glacial acetic acid were from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). Avicel PH 101 was from Fluka AG (Buchs, Switzerland). Magnesium stearate was from VWR (West Chester, PA, USA). Sodium starch glycolate (Explotab) was kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO; Cairo, Egypt). Acetic anhydride was purchased from Eastern Fine Chemicals Co., Ltd. (Milano, Italy). Commercial KZ tablets (Rameda, 200 mg) were from the Tenth of Ramadan for Pharmaceutical Industries and Diagnostic Reagents (6th of October City, Egypt). Sabouraud's agar consisting of 2% glucose, 1% neopeptone, and 2% agar was from Oxoid Ltd. (Cambridge, UK). A strain of *Candida albicans* was from the American Typing Culture Collection (ATCC).

2.2. *Preparation of KZ-β-CD systems*

Inclusion complexes of KZ and β-CD were prepared by coprecipitation and kneading using drug to carrier molar ratios of 1:1 and 1:2 (*5,9*). The coprecipitation system was prepared using methanol as a solvent; the calculated amount of KZ was dissolved homogeneously in the least amount of methanol and mixed with a solution of carrier in distilled water. The solvents were evaporated at room temperature overnight and then dried at 45°C (controlled hot air ovens; VELP Scientifica, Usmate, Italy). The kneading system was prepared by mixing KZ and β-CD powder with a small amount of water and ethanol mixture (1:1) in a mortar so as to obtain a homogeneous paste. The slurries were kneaded for 30 min and then dried at 45°C. The coprecipitation and kneading systems were pulverized and sieved. The fraction of powder passed through a 250-µm sieve and retained on a 125-µm sieve (ASTM; SIEBTECHNIK GmbH, Mulheim/Ruhr, Germany) was collected and used for further investigation.

2.3. *Characterization of KZ-β-CD systems*

2.3.1. *Solubility study of KZ-β-CD systems in 0.1 N HCl*

The solubility of coprecipitated and kneaded KZ-β-CD systems at 1:1 and 1:2 molar ratios was determined by weighing the known excess of both systems in vials containing 0.1 N HCl. The vials containing samples were shaken for 48 h in a thermostatically controlled shaker (Oscillating thermostatically controlled water bath shaker, Weiss-Gallenkamp, Loughborough, UK) at 37°C. The samples were then filtered through a 0.45-µm membrane filter (Millipore, Billerica, MA, USA), suitably diluted, and then analyzed spectrophotometrically (Spectrophotometer Model 6105 UV/Vis Felsted; Jenway Ltd., UK) for KZ content at the determined λ_{max} while referring to the corresponding calibration curve. Each experiment was done in triplicate and the equilibrium solubility served as the average value.

2.3.2. *Drug content and incorporation efficiency*

The drug content of KZ in the prepared inclusion complexes was estimated according to The United States Pharmacopoeia (USP) XXV (*3*). A weight of 200 mg from each system was dissolved in 40 mL glacial acetic acid, shaken for 10 min, and then filtered. The filtrate was treated with a 0.1 N perchloric acid mixture consisting of 8.5 mL of 70% perchloric acid, 900 mL glacial acetic acid, and 30 mL acetic anhydride; this was then left for 24 h in a cold place and was finally adjusted to 1 liter with glacial acetic acid and the end point was determined potentiometrically (*3,10*). A blank sample was prepared, and the necessary corrections were made (each mL of 0.1 N perchloric acid was equivalent to 26.57 mg of KZ). The mean value of triplicate estimates was used to calculate the amount of KZ in each system. The production yield and the incorporation efficiency were calculated according to the following equations:

Production Yield = [Weight of KZ physical mixtures before preparation]/[Weight of KZ inclusion complex after preparation] × 100

--- Eq. 1

Incorporation efficiency = [Drug incorporated]/[Theoretical drug content] × 100

--- Eq. 2

2.3.3. *Spectroscopic study*

X-Ray diffraction (XRD) – Pure KZ, β-CD and the prepared inclusion complexes were evaluated with an XD-610 X-ray diffractometer (Shimadzu, Kyoto, Japan). The samples were exposed to Cu Ka radiation

(40 kV × 30 mA) at a scan rate of 8 deg./min.

Differential scanning calorimetry (DSC) – Pure KZ, β-CD, and the prepared inclusion complexes were subjected to differential scanning calorimetry (Shimadzu DSC TA-50 ESI, Tokyo, Japan). Samples of about 2 mg were accurately weighed and scanned at a rate of 10°C/min over a 20-300°C temperature range in an inert atmosphere of nitrogen.

2.4. *Formulation of KZ tablets*

Tablets of coprecipitated and kneaded KZ-β-CD solid complexes at a molar ratio of 1:2 (*9*) and containing 200 mg of KZ were prepared by direct compression using a tablet compression machine with concave single punches (Erweka, Frankfurt, Germany). The additives used are shown in Table 1 and were talc, magnesium stearate, sodium starch glycolate (Explotab), and Avicel PH 101 as a diluent to yield a tablet of 1,000 mg final weight about 12 mm in diameter. Plain tablets with no inclusion complex and containing 200 mg KZ were also prepared.

2.5. *Evaluation of KZ tablets*

Coprecipitated and kneaded KZ-β-CD tablets at a molar ratio of 1:2 and containing 200 mg of KZ were evaluated for the following parameters. Weight uniformity was determined by a USP XXV procedure (*3*). For drug content determination, random samples of 10 tablets from each system were powdered and treated similarly, as mentioned previously (*10*). Tablet thickness, hardness, disintegration, and friability were determined according to USP XXV (*3*). The experiments were done 6 times in each case and the mean values and standard deviations were calculated.

2.6. In vitro release of KZ tablets

The *in vitro* release of KZ-β-CD systems was investigated by a USP rotating basket method using a USP-Standard Apparatus I Model DA-6D (Veego, Bombay, India). The dissolution media consisted of 900 mL of simulated gastric fluid (pH 1.2) maintained at 37 ± 0.5°C (*11*). Each tablet from the coprecipitated and kneaded KZ-β-CD systems at a molar ratio of 1:2 and containing 200 mg of KZ was held in the basket, which was positioned 2.5 cm from the bottom of the vessel and rotated at 100 rpm. At specified time intervals, 5 mL of sample was withdrawn from each of the six dissolution media and filtered off. This volume was replenished with a similar volume of fresh dissolution medium maintained at the same temperature. The content of KZ was assayed spectrophotometrically at 269 nm against a blank. The same procedure was conducted for the pure KZ tablets containing (200 mg KZ). The experiment was run in triplicate, maintaining sink conditions. The mean percentage of KZ released was calculated.

The dissolution data were fitted to an equation determined by Ritger and Peppas (*12*) as follows:

$$M_t/M_\infty = Kt^n \qquad \text{--- Eq. 3}$$

where M_t/M_∞ is the fraction of drug released at time t, K is the kinetic constant of the system, and n is the exponent characteristic of the release.

2.7. *Stability study*

Accelerated stability studies at 35°C, 45°C, and 55°C ± 1.0°C with thermostatically controlled hot air incubators were carried out for six months on the coprecipitated and kneaded KZ-β-CD tablets at a molar ratio of 1:2 and containing 200 mg of KZ. Relative humidity (RH) was maintained at 75% using saturated solutions of sodium chloride. Adequate samples from each system at each elevated temperature were taken at time intervals of 0, 14, 30, 45, 60, 90, 120, and 180 days. These samples were evaluated according to the method of assay previously mentioned in drug content. The method was carried out in triplicate for each system and the mean value was estimated. Zero-, first-, and second-order kinetics were used to choose the suitable order for the stability study and the relevant kinetic parameters were determined.

2.8. *Microbiological study of KZ tablets*

Coprecipitated and kneaded KZ-β-CD tablets at a molar ratio of 1:2 and commercial KZ tablets were used in this study.

2.8.1. *Preparation of a KZ reference and sample solutions*

An accurately weighed 15 mg KZ reference standard was transferred to a 50-mL volumetric flask and dissolved in methanol (300 µg/mL). An aliquot of this solution (3

Table 1. Formulae of KZ tablets tested

Formula	Inclusion complex wt. (mg)	Magnesium stearate (mg)	Talc (mg)	Explotab (mg)	Avicel (mg)	Total wt. (mg)
Plain KZ*	–	10	10	20	760	1,000
KZ:β-CD 1:2 (Coprecipitated)	950	10	10	20	10	1,000
KZ:β-CD 1:2 (Kneaded)	770	10	10	20	190	1,000

* Plain KZ was 200 mg in weight.

mL) was transferred to a 25-mL volumetric flask and was diluted with 0.1 N HCl solution (pH 1.2) to obtain a solution of concentration (36 µg/mL). Aliquots of 50, 100, and 150 µL from this solution, corresponding to respective drug concentrations of 1.8, 3.6, and 5.4 µg/µL, were used in the assay. Adequate tablets from each formula were weighed, finely powdered, and an amount of powder equivalent to 15 mg of KZ was treated as mentioned previously. Resulting mixtures were then used in the assay as sample solutions.

2.8.2. *Microbiological assay of KZ tablets (Cylinder-plate method)*

Microorganism and inoculum-cultures of *Candida albicans* ATCC were cultivated on 2% Sabouraud's agar (*13*) and maintained in test tubes in a refrigerator at 4 ± 2°C until use. The microorganism was suspended in 0.9% NaCl; the concentration in the obtained suspension was fit to 25 ± 2% of transmittance at 580 nm using a spectrophotometer analyzer and a 10 mm diameter test tube as an absorption cell against 0.9% NaCl as a blank (*14*). For the biological assay of KZ, 1 mL of this suspension was added to 60 mL of 2% Sabouraud's agar using sterile plates; the media was allowed to harden and was used as an inoculated layer. In each plate, three cups were made using a Wasserman tube; each cup was carefully filled with a known quantity of the sample under sterile conditions. The plates were incubated at 37°C for 24 h. The resulting zone diameters of inhibition of growth were then measured in mm. Each determination was done in triplicate and the average zone diameter was calculated (*15*).

3. Results and Discussion

3.1. *Characterization of KZ-β-CD systems*

Solubility of KZ-β-CD is summarized in Table 2. The solubility of pure KZ was found to be greatly enhanced by its incorporation in inclusion complex with β-CD systems prepared by either coprecipitation or kneading. An inclusion complex with a 1:2 drug to β-CD molar ratio had better solubility than one with a 1:1 drug to β-CD molar ratio, and this result agreed with the results of Choi *et al*. (*9*).

Table 2. Solubility of KZ-β-CD systems in 0.1 N HCl

Method of preparation	Drug to β-CD molar ratios	Solubility (mg/mL)
Pure drug	–	0.096
Coprecipitation	1:1	17.9
	1:2	18.3
Kneading	1:1	18.1
	1:2	19.8

The KZ inclusion complex prepared by coprecipitation and kneading using a molar ratio of 1:2 resulted in good production yield of about 99% in relation to the theoretical maximum and also higher incorporation efficiency (Table 3), which was in accordance with the findings of Bergamasco *et al*. (*16*).

3.2. *Spectroscopic study*

Figure 1 shows the X-ray diffraction pattern of a KZ-inclusion complex system (1:1 molar ratio) in comparison to that of pure KZ. The diffraction pattern of KZ showed that the drug has a high degree of crystallinity because of the presence of numerous distinct peaks. The most characteristic peaks at a 2θ-diffraction angle were located at 17.5, 18.52, 19.9, 20.95, 23.5, and 28.6 degrees. The change in the X-ray diffraction pattern of the solid complex, represented by the reduction in intensity of peaks of drug, in the kneaded and coprecipitated products suggested an interaction between drug and the CD used and it also revealed that a portion of KZ was in an amorphous state (*5,17,18*).

Figure 2 shows the DSC thermogram of a KZ-inclusion complex system (1:1 molar ratio) in comparison to that of pure KZ, which is characterized by one sharp endothermic peak at about 152.16°C, indicating that the drug had a melting point like that shown in Figure 2A. Figure 2B shows the DSC thermogram of pure β-CD, which had a shallow and broad endothermic peak at about 105.52°C with the potential to be extended because of the release of water from the molecule (*17*). The DSC thermogram of the inclusion complex of the drug with β-CD is represented in Figure 2C. The characteristic thermal peak of the drug appeared at a lower temperature than 147.73°C and decreased substantially in intensity and broadened somewhat with the kneaded and coprecipitated products (*17*). These results suggest that the drug is monomolecularly dispersed in the β-CD cavity (*19*).

3.3. *Evaluation of KZ tablets*

Solubility in 0.1 N HCl, production yield, and

Table 3. KZ content and incorporation efficiency of KZ-β-CD systems

Method of preparation	Drug to β-CD molar ratio	Production yield (%)	Theoretical drug content (mg)	Actual drug content (mg)	Incorporation efficiency (%)
Coprecipitation	1:1	97.0	200	208.6	104.3
	1:2	99.9	200	211.8	105.9
Kneading	1:1	95.6	200	197.8	98.9
	1:2	98.9	200	213.4	106.7

incorporation efficiency were higher in KZ-β-CD systems prepared by coprecipitation and kneading using a 1:2 molar ratio than in those using a 1:1 molar ratio (*9,16*). Therefore, the 1:2 KZ-β-CD systems were chosen for tablet formulation and further studies.

Table 4 shows the quality control results for KZ tablets. The uniformity of weight of KZ tablets manufactured by direct compression revealed that the weight of the tested tablets complied with the pharmacopeial requirements (*3*). The uniformity of drug content of KZ tablets revealed that the average drug content of KZ tablets ranged from 98.1% to 99.0%, with a standard deviation ranging from 1.00 to 1.80 (Table 4), which complies with the pharmacopeial requirements (*3*).

The uniformity of thickness, although unofficial, can be considered as an additional control to the tablet dimension and increased reproducibility. The average thickness value of KZ tablets ranged from 8.25 mm to 8.54 mm, with standard deviations ranging from 0.07 to 0.14 (Table 4). These results indicate that all of the prepared KZ tablets prepared by different techniques had acceptable limits of thickness uniformity.

The mechanical properties of the investigated tablets were evaluated by testing their hardness and friability. The average hardness values ranged from 8.82 kg to 9.66 kg, with standard deviation values between 0.76 and 0.85 (Table 4). The results of the friability study of different KZ tablets indicated average percent loss of weight ranging from 0.30% to 0.87%, with a standard deviation ranging from 0.01 to 0.08. A maximum weight loss of not more than 1% of the weight of the tablets being tested was considered acceptable for most products.

According to USP XXV (*3*), complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus is a soft mass having no palpably film core. The mean value of the disintegration time for KZ tablets ranged from 2.33 to 5.33 min, with a standard deviation of 0.57 min (Table 4). The aforementioned tests led to the conclusion that all of the prepared KZ tablets complied with the pharmacopeial requirements and demonstrated good inter-batch uniformity.

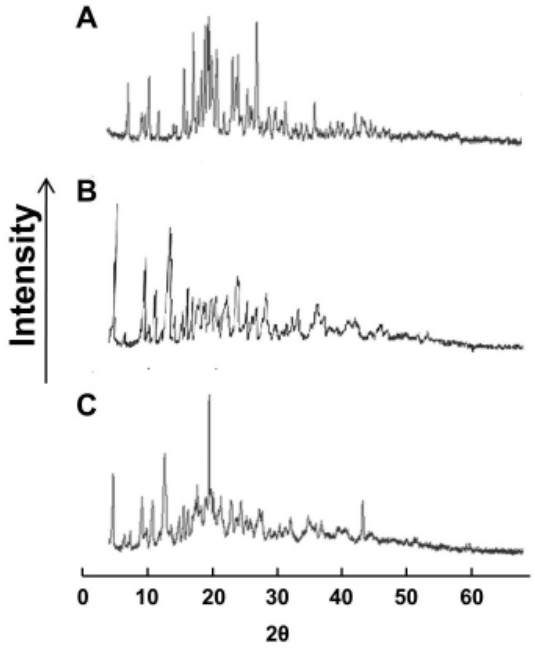

Figure 1. X-Ray Diffraction of (A) Pure KZ, (B) β-cyclodextrin, and (C) KZ-β-CD systems.

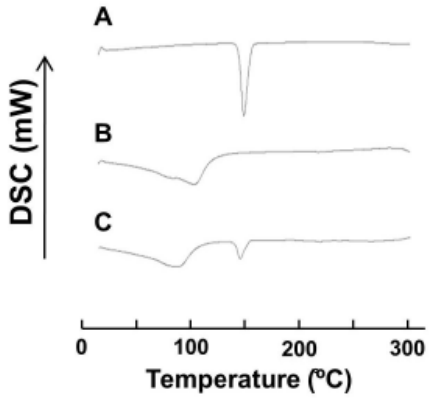

Figure 2. DSC thermograms of pure KZ (A), β-cyclodextrin (B), and KZ-β-CD systems (C).

3.4. *In vitro release of KZ tablets*

The *in vitro* release of KZ tablets containing a β-CD 1:2 molar ratio prepared by coprecipitation and kneading in 0.1 N HCl are shown in Figure 3. Apparent was the fact that the both the coprecipitated and kneaded KZ tablets had maximum drug release (100%) after 30 min, while the plain KZ had release of only 69.6% after the same amount of time. The enhanced dissolution rate might be attributed to the decreased particle size, indicating the high level of energy of drug inclusion complexes.

Table 4. Quality control tests for KZ tablets

Formula	Weight (g)	Drug content (%)	Thickness (mm)	Hardness (kg)	Weight loss (%)	Disintegration time (min)
Coprecipitated KZ-β-CD	0.994 ± 0.006	98.1 ± 1.80	8.25 ± 0.07	9.66 ± 0.76	0.30 ± 0.01	2.33 ± 0.57
Kneaded KZ-β-CD	1.008 ± 0.018	99.0 ± 1.00	8.54 ± 0.14	8.82 ± 0.85	0.87 ± 0.09	5.33 ± 0.57

Data are shown as mean ± S.D.

The medium of 0.1 N HCl has been reported to be one of the most commonly used in such studies to imitate physiological gastric fluid (*11*).

To analyze the release mechanism of the drug from these tablets with hydrophilic matrices, the release data obtained were fit to a simple power equation (*12*) as follows:

$$M_t/M_\infty = Kt^n$$

where M_t/M_∞ is the fraction of drug released at time t, K denotes the constant incorporating structural and geometrical characteristics of the drug/carrier system, n is the diffusion exponent related to the mechanism of the drug release, and M_∞ is the amount of drug incorporated in the tablet, *i.e.*, 200 mg. The values of K and n were estimated by linear regression of log (M_t/M_∞) on log t where log K is the intercept and n is the slope of the straight line.

$$\text{Log } M_t/M_\infty = \log K + n \log t$$

Kinetic analysis of the *in vitro* release data for KZ from all of the tablets revealed that the n value fell between 0.5 and 1, indicating non-Fickian (anomalous) transport (where release is controlled by a combination of diffusion and polymer relaxation), and this finding was in accordance with the findings of Karasulu *et al*. (*13*).

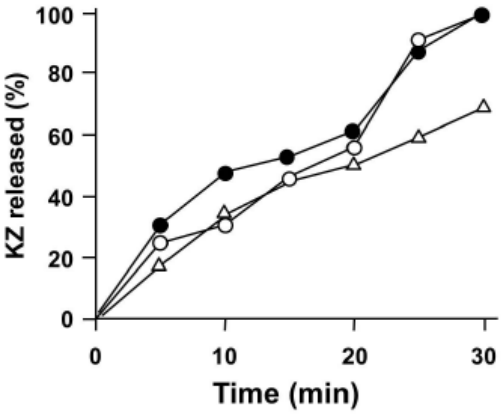

Figure 3. *In vitro* release of coprecipitated and kneaded KZ-β-CD tablets and plain KZ tablets in 0.1 N HCl. Open circles, coprecipitated KZ-β-CD; Closed circles, kneaded KZ-β-CD; Open triangles, plain KZ.

3.5. *Stability study*

Accelerated stability testing could be of value in rating the stability of the tested KZ tablets for scale up studies. The effect of storage of the formulated KZ tablets at three elevated temperatures (35°C, 45°C, and 55°C) on drug chemical stability was studied. Correlation coefficient (*r*) values were determined according to zero-, first-, and second-order equations using the % of drug remaining after specified time intervals over a period of 6 months. The degradation of KZ was found to be a zero-order reaction based on the mean values of the correlation coefficient (*r*). The decomposition rate constants K_{35}, K_{45}, and K_{55} were determined at each temperature using Arrhenius' equation. The energy of activation (E_a) and the decomposition reaction rate constant at room temperature (K_{20}) were determined. $t_{1/2}$ and t_{90} were also estimated for each formula. Figures 4A-4C show the accelerated stability testing of KZ tablets at the three elevated temperatures of 35°C, 45°C, and 55°C, respectively.

Stability results led to the conclusion that the tablets of KZ-β-CD systems at a 1:2 molar ratio prepared by coprecipitation and kneading yielded good stability results as their t_{90} was about 2 years (Table 5).

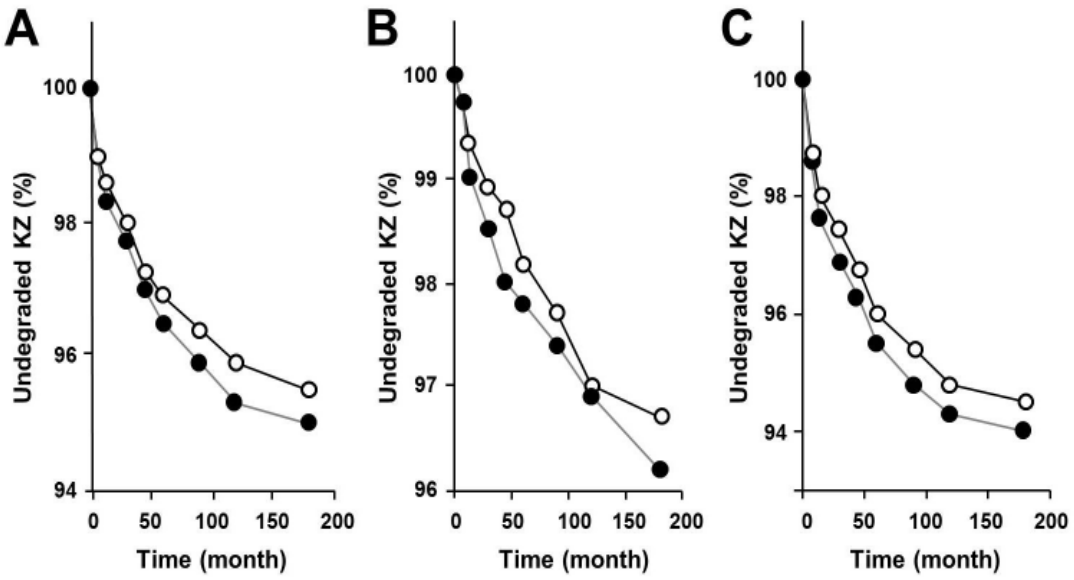

Figure 4. Time course of percent undegraded KZ tablets at various temperatures. (A) 35°C; **(B)** 45°C; **(C)** 55°C. Open circles, coprecipitated KZ-β-CD; Closed circles, kneaded KZ-β-CD.

Table 5. Kinetic parameters for accelerated stability testing of KZ tablets

Method of preparation	K_{35} (days) $^{-1}$	K_{45} (days) $^{-1}$	K_{55} (days) $^{-1}$	E_a (Cal/mol)	K_{20} (days) $^{-1}$	t_{90} (year)	$t_{1/2}$ (year)
Coprecipitated KZ-β-CD	0.017	0.020	0.024	3,072.126	0.01329	2.06	10.30
Kneaded KZ-β-CD	0.018	0.022	0.025	3,857.295	0.01326	2.07	10.32

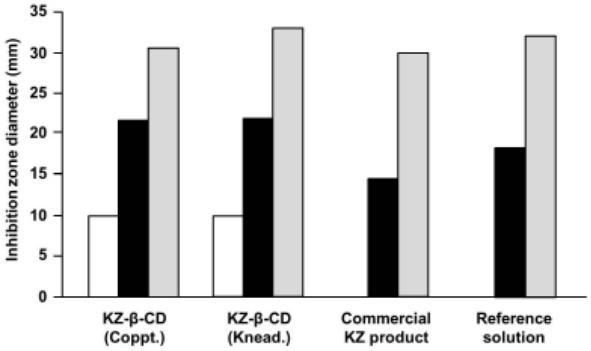

Figure 5. Histogram of the mean values of the inhibition zones diameters of KZ tablets, a commercial product, and a reference solution at different concentrations. Open column, 1.8 µg/µL of KZ; Closed column, 3.6 µg/µL of KZ; Gray column, 5.4 µg/µL of KZ. Coppt. and Knead. represent coprecipitated and kneaded, respectively.

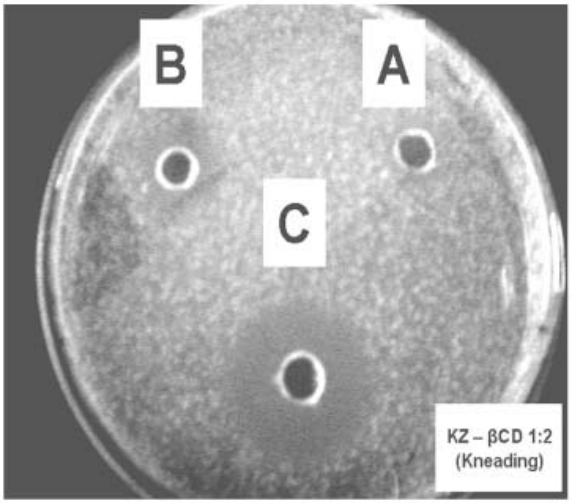

Figure 6. Inhibition zones of kneaded KZ-β-CD 1:2. (A) 1.8 µg/µL; **(B)** 3.6 µg/µL; and **(C)** 5.4 µg/µL.

3.6. *Microbiological study of KZ tablets*

The antifungal activity of KZ-β-CD tablets and a commercial product was studied using *Candida albicans* as a standard fungus. The cylinder plate method (*14*) was used for this study. Figure 5 shows the microbiological data from coprecipitated and kneaded KZ tablets, a KZ commercial product, and a reference KZ sample. The antifungal activity of all of the investigated formulations was determined by measuring the inhibition zone diameter in mm produced by the addition of three volumes (50, 100, and 150 µL) of KZ that were equivalent to three concentrations (1.8, 3.6, and 5.4 µg/µL), respectively. Apparent was the fact that the commercial KZ product and the reference sample had no inhibition zone at the lower concentration (1.8 µg/µL) while the kneaded and coprecipitated KZ tablets prepared at a β-CD 1:2 ratio displayed an increase in the inhibition zone diameter with increasing concentrations ranging from 1.8 to 5.4 µg/µL.

The data obtained, represented by inhibition zone diameters in mm, were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer post-tests for multiple comparison at $p < 0.05$. The results showed that both KZ-β-CD inclusion complex tablets prepared by coprecipitation and kneading did not differ significantly at 3.6 µg/µL while the reference and commercial formulations differed significantly from both KZ-β-CD systems, especially at low concentrations. The antifungal activity of KZ-β-CD formulations, represented by the inhibition zone diameter of 5.4 µg/µL, were, in descending order, as follows: kneaded KZ-β-CD > coprecipitated KZ-β-CD. Therefore, KZ prepared by kneading as an inclusion complex with a β-CD 1:2 drug/carrier molar ratio yielded the best microbiological results with *Candida albicans* as a fungal model, as shown in Figure 6. The aforementioned results indicate that the KZ-β-CD system tablets have more potent antifungal activity, even at lower concentrations, which may be due to increased drug solubility.

4. Conclusion

The present results confirmed the benefit of using CDs as a useful tool to enhance the dissolution and hence the bioavailability of poorly water-soluble drugs by forming solubilizing systems when exposed to gastrointestinal fluid. The KZ-β-CD system tablets allowed better quality control and had better *in vitro* release and stability values in addition to more potent antifungal activity at low concentrations.

References

1. Remon JP, Belparie F, Van Severen R, Braeckman P. Interaction of antacids with antiarrhythmics. V. Effect of aluminium hydroxide and magnesium oxide on the bioavailability of quinidine, procainamide and propranolol in dogs. Arzneim Forsch. 1983; 33:117-120.
2. Wang L, Tang X. A novel ketoconazole bioadhesive effervescent tablet for vaginal delivery: Design, *in-vitro* and '*in-vivo*' evaluation. Int J pharm. 2008; 350:181-187.
3. USP XXV. The United States Pharmacopoeia, NF 20, US. Pharmacopieal Convention, Inc., Rockville, MD, USA, 2002; p. 1148.

4. Carlson JA, Mann HJ, Canafax DM. Effect of pH on disintegration and dissolution of ketoconazole tablets. Am J Hosp Pharm. 1983; 40:1334-1336.
5. Esclusa-Diaz MT, Guimaraens-Méndez M, Pérez-Marcos MB, Vila-Jato JL, Torres-Labandeira JJ. Characterization and *in vitro* dissolution behaviour of ketoconazole/β- and 2-hydroxypropyl-β-cyclodextrin inclusion compounds. Int J Pharm. 1996; 143:203-210.
6. Heo MY, Piao ZZ, Kim TW, Cao QR, Kim A, Lee BJ. Effect of solubilizing and microemulsifying excipients in polyethylene glycol 6000 solid dispersion on enhanced dissolution and bioavailability of ketoconazole. Arch Pharm Res. 2005; 28:604-611.
7. Jambhekar S, Casella R, Maher T. The physicochemical characterization and bioavailability of indomethacin from β-cyclodextrin, hydroxylethyl-β-cyclodextrin, and hydroxypropyl-β-cyclodextrin complexes. Int J Pharm. 2004; 270:149-166.
8. Nagarsenker MS, Joshi MS. Celecoxib-cyclodextrin systems: Characterization and evaluation of *in vitro* and *in vivo* advantage. Drug Dev Ind Pharm. 2005; 31:169-178.
9. Choi HG, Lee BJ, Han JH, Lee MK, Park KM, Yong CS, Rhee JD, Kim YB, Kim CK. Terfenadine-β-cyclodextrin inclusion complex with antihistaminic activity enhancement. Drug Dev Ind Pharm. 2001; 27:857-862.
10. Svehla G. In: Vogel's Qualitative Inorganic Analysis. 7th ed., Thomson Press India Ltd., Mumbai, India, 1996; p. 207.
11. Lindahl A, Ungell AL, Kunston L, Lennernäs H. Characterization of fluids from stomach and proximal jejunum in men and women. Pharm Res. 1997; 14:497-502.
12. Karasulu HY, Hilmioğlu S, Metin DY, Güneri T. Efficacy of a new ketoconazole bioadhesive vaginal tablet on *Candida albicans*. Farmaco. 2004; 59:163-167.
13. Ritger PL, Peppas NA. A simple equation for description of solute release. II. Fickian and anomalous release from swellable device. J Control Release. 1987; 5:37-42.
14. Vaucher LC, Breier AR, Schapoval EE. Microbiological assay for the determination of telithromycin in tablets. J AOAC Int. 2006; 89:1398-1402.
15. Vanitha J, Saravana Kumar A, Ganesh M, Saravanan VS. Comparative analysis of climbazole in pharmaceutical formulation. Asian J Research Chem. 2008; 1:43-45.
16. Bergamasco Rde C, Zanin GM, de Moraes FF. Sulfluramid volatility reduction by beta-cyclodextrin. J Agric Food Chem. 2005; 53:1139-1143.
17. Tayade PT, Vavia PR. Inclusion complexes of ketoprofen with β-cyclodextrins: Oral pharmacokinetics of ketoprofen in human. Indian J Pharm Sci. 2006; 68:164-170.
18. Abdelkader H, Abdallah OY, Salem HS. Comparison of the effect of tromethamine and polyvinylpyrrolidone on dissolution properties and analgesic effect of nimesulide. AAPS PharmSciTech. 2007; 8:E65.
19. Choi HG, Kim DD, Jun HW, Yoo BK, Yong CS. Improvement of dissolution and bioavailability of nitrendipine by inclusion in hydroxypropyl-β-cyclodextrin. Drug Dev Ind Pharm. 2003; 29:1085-1094.

(*Received July 26, 2010; Revised August 7, 2010; Accepted August 7, 2010****)***