Yashoda Multidisciplinary Research Journal

# PREPARATION AND EVALUATION OF KETOCONAZOLE/B-CYCLODEXTRIN COMPLEX

By

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**Abstract:**

This study deals with the preparation of ketoconazole (KTZ) /β-cyclodextrin (βCD) inclusion for enhancing the solubility of KTZ. The complexes were prepared by physical mixing and kneading method. The prepared complexes were characterized by ATR-FTIR, DSC and XRPD. They were evaluated for drug content and saturation solubility. The results of ATR-FTIR analysis indicate the greater interaction in between KTZ and βCD in kneaded complex than the physical mixture. DSC and XRPD analysis revealed the formation of inclusion complex and amorphization of the KTZ. The KTZ/βCD kneaded complex exhibited maximum solubility in Tris HCl buffer (pH: 7.4) containing 0.5% SLS than the physical mixture. Thus, the kneaded complex of KTZ with βCD showed promising results than the physical mixture.

*Key words*: ketoconazole, β-cyclodextrin, kneading method, solubility enhancement

## Introduction

Ketoconazole (KTZ) is a synthetic azole antifungal agent. It is administered by topical or oral route and used for the treatment of fungal infections related to the skin, fingernails, gastro-intestinal tract and vagina. It is also used for the treatment of the systemic infections some of which include candidiasis, blastomycosis, histoplasmosis and coccidioidomycosis. However, its poor gastro-intestinal absorption

and slow onset of action restricts its use in severe fungal infections such as fungal meningitis (1). Despite of high permeability, the poor gastro-intestinal absorption of KTZ may be due to its low solubility (0.017mg/mL at 25$^{o}$C) which limits the complete dissolution of the administered dose (2). Also, the bioavailability of KTZ after topical or vaginal administration in healthy subjects is found to be less (1).

In order to improve the solubility of KTZ, various methods have been implemented including solid dispersion (3), nanoparticles (4), liquisolid technique (5) and cyclodextrin complexation (6–9). Cyclodextrins (CDs) are cyclic oligosaccharides which form an inclusion complex with the hydrophobic drugs and enhance their aqueous solubility, bioavailability and stability (10). The three-dimensional structure of CDs makes them suitable for the pharmaceutical applications. CD molecules have a hydrophobic cavity of varying sizes which can accommodate the hydrophobic drugs. A large number of hydroxyl groups are present on the outer rims of the CD molecules imparting hydrophilic nature to the outer portion of the CD (11).

This study deals with the preparation of KTZ/βCD inclusion complex by physical mixing and kneading method followed by their characterization and evaluation of drug content and solubility. The phase solubility studies were not performed as it is well known that KTZ and βCD form complex in 1:1 molar ratio (9). The physical mixing and kneading method are advantageous for the preparation of inclusion complex due to their simplicity and minimum loss of product during processing. The complexes were characterized by ATR-FTIR, DSC and XRD. The saturation solubility of the complexes obtained was compared in order to choose the one with maximum solubility for later study.

## Materials and methods

### *Preparation of ketoconazole/βCD complex*

*Physical mixture*

Ketoconazole and βCD were weighed in an equimolar ratio (1:1). The weighed components were mixed and were pulverized using mortar and pestle for 1 h, followed by sifting through 250μm mesh. A binary mixture comprised ketoconazole/ βCD (1:1) were prepared for comparative study. The prepared mixtures were stored in airtight glass desiccators under vacuum (11).

*Kneading method*

Kneaded complex was prepared according to the method reported by Demirel et al. (2011) (8). A small volume of a water–methanol (50/50 v/v) solution was added to the ketoconazole/βCD physical mixture (1:1 molar ratio) in a mortar and kneaded thoroughly with pestle to obtain homogeneous slurry. The kneading was continued until the solvent was

completely removed. The kneaded mixture was kept in a desiccator overnight to remove the traces of solvent. The product was stored in a desiccator containing calcium chloride, at atmospheric pressure and room temperature.

***Characterization of ketoconazole/βCD complex***

*ATR-FTIR analysis*

The infrared spectra of ketoconazole, βCD, ketoconazole/βCD physical mixture and ketoconazole/βCD kneaded complex obtained using ATR-FTIR spectrophotometer (Shimadzu, IRAffinity, Japan). The samples to be analyzed were transferred to the ATR compartment. The spectra were obtained for the range of 600-4000$cm^{-1}$ at an average of 25scans and resolution of 4$cm^{-1}$.

*DSC analysis*

Differential scanning calorimetry (DSC) analysis of ketoconazole, βCD, ketoconazole/βCD physical mixture and ketoconazole/β-CD kneaded complex was performed using thermogravimetric analyzer (SDT Q600 V20.9 Build 20, TA instruments, Waters, USA). Samples were heated from 30$^{o}$C–300$^{o}$C at the rate of 10$^{o}$C/min, under nitrogen atmosphere (flow rate: 10 ml/min).

*XRPD analysis*

X-ray powder diffraction (XRPD) patterns of ketoconazole, βCD, ketoconazole/βCD physical mixture and ketoconazole/βCD kneaded complex were recorded using X-ray diffractometer (PW 1729, Philips, The Netherlands) with a copper target, voltage 30 kV, current 30 mA, respectively.

***Determination of saturation solubility***

The saturation solubility studies were conducted for pure ketoconazole, physical mixture and kneaded complex in Tris HCl buffer (pH 7.4) containing 0.5% sodium lauryl sulphate (SLS), according to the method reported by Higuchi and Connors (12). Excess of pure ketoconazole, physical mixture, and kneaded complexes were added to the vials containing 20ml buffer. The vials were shaken for 72h using rotary shaker at 37°C. After the complete equilibration, the supernatant solutions were collected carefully and filtered using membrane filter (0.45 μm). The concentration of ketoconazole in filtered solution was determined using UV-visible spectrophotometer at 225nm.

***Determination of drug content***

The physical mixture and kneaded complex equivalent to 10mg of drug was accurately weighed and added into 100ml volumetric flask. Fifty ml of methanol was added to these flasks. The solution so obtained was stirred for 60

Yashoda Multidisciplinary Research Journal

minutes, till the entire drug leached out. The solution was filtered and 1 ml withdrawn from this solution and was added into 10ml volumetric flask. The volume was made to 10ml with methanol. Drug content was estimated spectrophotometrically at 245 nm using methanol as blank.

**Results and discussion**

***Characterization of KTZ/βCD complex***

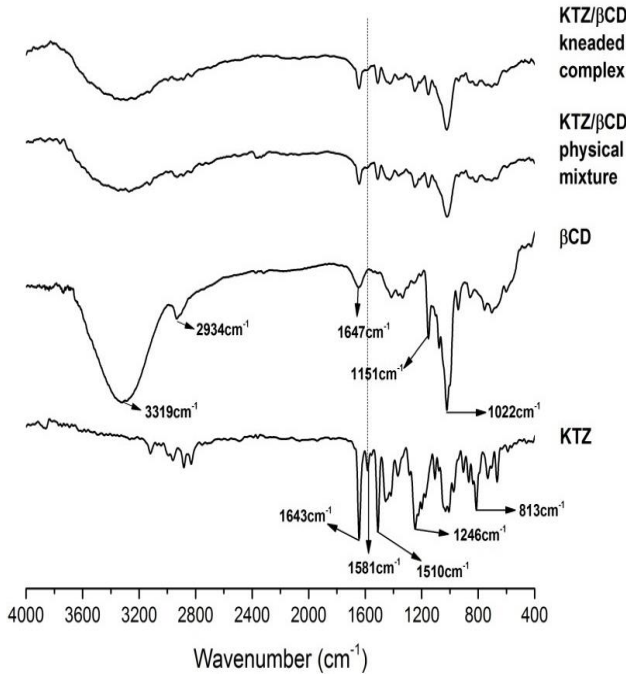



**Figure 1.** ATR-FTIR spectra of KTZ, βCD, KTZ/βCD physical mixture and KTZ/βCD kneaded complex

The ATR-FTIR spectrum of KTZ, βCD, KTZ/βCD physical mixture and KTZ/βCD kneaded complex is shown in Fig. 1. KTZ showed characteristic peaks at 1643cm$^{-1}$ (C=O stretching), 1581cm$^{-1}$ (C=C aromatic symmetrical stretching), 1510cm$^{-1}$ (C=C aromatic asymmetrical stretching), 1246cm$^{-1}$ (C-O stretching of cyclic ether) and 813cm$^{-1}$ (C-Cl stretching). The spectrum of βCD shows sharp peaks at 3319cm$^{-1}$ (O-H stretching), 2934cm$^{-1}$ (C-H stretching), 1647cm$^{-1}$ (H-O-H bending), 1151cm$^{-1}$ and 1022cm$^{-1}$ (C-O stretching). In the spectra of KTZ/βCD physical mixture and KTZ/βCD kneaded complex, the intensity of the peaks of KTZ was found to be reduced. This reduction in the peak intensity was found to be more in case of KTZ/βCD kneaded complex than the physical mixture. The peak of KTZ at 1581cm$^{-1}$ showed marked reduction in its intensity and shifted to 1591cm$^{-1}$ in the physical mixture as well as kneaded complex indicating inclusion of the aromatic ring of KTZ in the cavity of βCD. The remaining peaks of KTZ and βCD overlapped with each other. The results of ATR-FTIR analysis indicate the greater interaction in between KTZ and βCD in kneaded complex than the physical mixture. This may be due to the formation of better inclusion complex by kneading method.

Yashoda Multidisciplinary Research Journal

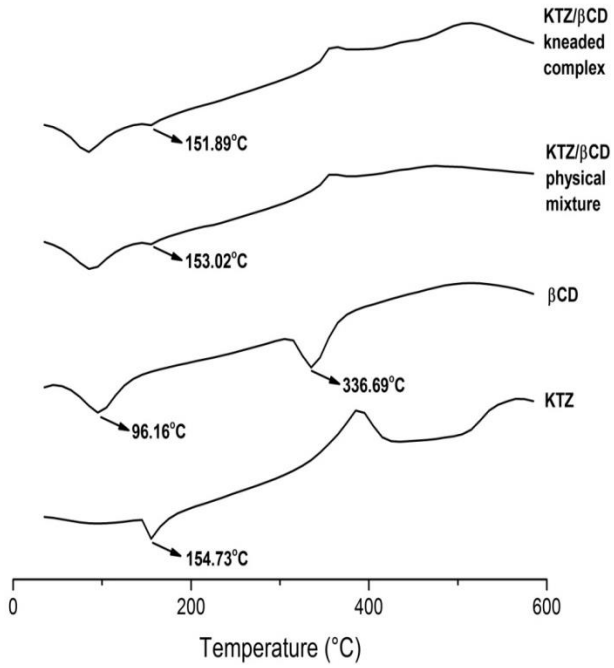
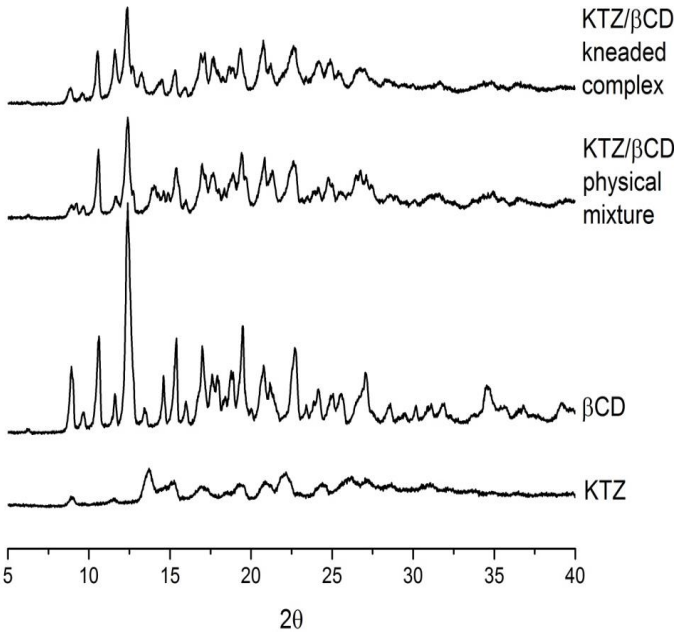



**Figure 2.** DSC thermograms of KTZ, βCD, KTZ/βCD physical mixture and KTZ/βCD kneaded complex

Figure 2 displays the DSC thermograms of KTZ, βCD, KTZ/βCD physical mixture and KTZ/βCD kneaded complex. The thermogram of KTZ showed a sharp endothermic peak at 154.73°C corresponding to the melting of KTZ. A broad peak was observed at 96.16°C in the thermogram of βCD which can be ascribed to the desolvation of water molecules present in the βCD cavity. Besides, a relatively sharp peak was observed at 336.69°C corresponding to the melting point of βCD. The KTZ/βCD physical mixture and kneaded complex showed reduction in the depth of the broad endotherm at 96.16°C. This may be due to displacement of water in the cavity of βCD by the drug molecules during formation of complex. Moreover, the reduction in depth was greater in case of the kneaded complex than the physical mixture.

It is reported that inclusion of the guest molecules into CD cavity leads to shifting or disappearance of their melting, boiling, and sublimation points (13). The melting endotherm of KTZ showed marked decrease in the thermograms of physical mixture and the kneaded complex. It also shifted from 154.73°C to 153.02°C in case of physical mixture and from 154.73°C to 151.89°C in case of kneaded




complex.

Yashoda Multidisciplinary Research Journal

**Figure 3.** X-ray diffractograms of KTZ, βCD, KTZ/βCD physical mixture and KTZ/βCD kneaded complex

XRD analysis was performed to confirm the reduction in crystallinity of the prepared complexes. The diffraction patterns of KTZ, βCD, physical mixture and kneaded complex is shown in Fig. 3. The spectrum of pure KTZ exhibited sharp peaks at 13.71°, 22.15°, and 26.17° indicating its crystalline nature. The high intensity of the peaks of βCD than the KTZ indicates that βCD was more crystalline than KTZ. The intensity of the sharp peaks of KTZ was found to be noticeably reduced in the diffractogram of kneaded complex indicating reduction in the crystallinity of KTZ in the kneaded complex to a greater extent than the physical mixture.

***Saturation solubility and drug content***

The saturation solubility data for KTZ, KTZ/βCD physical mixture and kneaded complex is displayed Table 1. The physical mixture and kneaded complex showed significant enhancement ($P < 0.05$) in solubility of KTZ. The kneaded complex showed better solubility enhancement of KTZ than the physical mixture. This may be attributed to the reduction in the crystallinity of the KTZ/βCD kneaded complex to a greater extent than the physical mixture.

The drug content of the KTZ/βCD physical mixture and kneaded complex is also given in Table 1. The drug content in the physical mixture and the kneaded complex was found to be above 90% which indicates a minimum loss of product.

**Table 1.** Saturation solubility and drug content of the pure drug, KTZ/βCD physical mixture and kneaded complex

| System | Solubility in Tris HCl buffer containing 0.5% SLS (μg/mL) (mean±SD) | Drug content (%) (mean±SD) |
|---|---|---|
| **KTZ** | 523.96±2.13 | - |
| **KTZ/βCD physical mixture** | 1086.24±1.19 | 92.63±1.24 |
| **KTZ/βCD kneaded complex** | 1493.32±1.51 | 93.11±0.88 |

KTZ: ketoconazole; βCD: β-cyclodextrin; SD: standard deviation

**Conclusions**

KTZ/βCD complexes were prepared by physical mixing and kneading method. The ATR-FTIR, DSC and XRD analysis revealed the inclusion of KTZ within the βCD cavity and

reduction in the crystallinity of the complexes. The KTZ/βCD kneaded complex exhibited maximum solubility in Tris HCl buffer (pH: 7.4) containing 0.5% SLS than the physical mixture. The methods chosen for the preparation of the inclusion complexes gave high product yield.

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