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*Original Research Article*

# Fabrication of ketoconazole nanosuspention, by pearl milling technique and evaluation, characterization of nanocrystals prepared

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## ABSTRACT

In the present study, ketoconazole nanosuspentoin were prepared in order to increase solubility by pearl milling technique via solution include Tween 60 and polyvinylpyrrolidone K30 (PVP K30). The Ketoconazole and the resulting ketoconazole nanosuspention were characterized by Fourier-Transform InfraRed spectroscopy (FT-IR) and Differential scanning calorimetry (DSC) analysis. The Atomic force microscopy (AFM) analysis indicated the formation of homogenous and spherical nanosuspention with the final average particle size of around 113.7 nm. Finally the solubility test has shown that ketoconazole nanosuspention solubility has increased.

***Keywords*:** Ketoconazole, nanosuspention, pearl milling, poor solubility, nanodrug

## INTRODUCTION

Many drugs show poor solubility in water [1]. The solubility of drugs is important for their bioavailability [2]. A new approach has been recently used in order to enhance the water solubility of poorly water-soluble drugs. It can be stated that salt formation of drugs, co-solvents, surfactants, complexing agents and reduced drug size are examples of this method [3,4]. The particle size reduction to the nanometer range resulted in the improvement of their dissolution rate and bioavailability by increasing their surface area. One of the best methods for reducing drug size is the wet-milling technique [5]. In



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this technique, ketoconazole nanocrystals can be prepared by using small and hard beads. The stabilizer is used to prevent aggregation of particles. Therefore, using the polymer and surfactants as a stabilizer is necessary [6]. The stabilizer is able to be adsorbed on the surface of drug particles and prevent ionic interactions [7]. The first oral antifungal drug, ketoconazole is a synthetic antifungal drug to treat fungal infections. However, according to the Biopharmaceutical Classification System, ketoconazole is classified under class-II drugs [8-10]. In this research, we prepared ketoconazole nanosuspensions using a wet-milling method and studied the features of ketoconazole nanosuspensions using atomic force microscopy (AFM), differential scanning calorimetry (DSC), Fourier-transform infrared (FTIR) spectroscopy and dynamic light scattering (DLS).

## MATERIALS AND METHODS

### *Materials*

Polyvinylpyrrolidone K30 (PVP K30) was purchased from Fluka (Germany). Tween 60 and Ketoconazole were obtained from Sigma-Aldrich (located in St. Louis, MO, USA). All other materials used in this study were of analytical grade.

### *Preparation of ketoconazole nanosuspensions*

The stabilizer solution was first prepared by Tween 60 in purified water. Following that, the PVP K30 was added under mechanical agitation. Pure ketoconazole was dispersed inside the stabilizer solution and was maintained under mechanical agitation. As soon as a uniform suspension was prepared, it was placed inside a glass tube. Glass beads were used for preparation (2 mm in diameter) or all of them were placed in a glass tube. In order to prepare NS, the suspension was milled by using glass beads. The quantitative relation between the grinding media and the suspension was (1:1, v/v). An IKA® orbital shaker (situated in Vibrax VXR Basic, Germany) was used to agitate the tubes with 1500 rpm at room temperature for one hour and 15 minutes. Afterward, an ultrasonic bath (Eurosonic 4D, Italy) was applied in order to sonicate the milled suspension at 37°C for 30 min. The stabilizers were used in various concentrations to prepare the finest ketoconazole nanosuspensions. Table 1 illustrates the details of the formulation design.

### *Differential Scanning Calorimetry*

The ketoconazole nanosuspensions were characterized by Differential scanning calorimetry (DSC )(Mettler Toleido, Switzerland). Approximately, 3 mg of dried ketoconazole nanosuspensions were placed

in aluminum pans for further analysis. The measurements were carried out at temperatures from 20°C to 250°C at a scan rate of 20°C/min.

### *Atomic Force Microscopy*

The AFM analysis by NanoWizard system (JPK Instruments AG, Berlin, Germany) was used to characterize the morphology. A drop of adulterated NS was positioned on a mica surface and was dried at room temperature. A silicon nitride cantilever with a spring constant of 40 $nm^{-1}$ was used to perform AFM imaging in tapping mode in the air at room temperature.

### *Fourier-transform Infrared*

The spectrum of FTIR was used to characterize dried ketoconazole nanosuspensions, ketoconazole, and PVPK30. This sample was diluted with potassium bromide and made into pellets using a FTIR spectrometer (Bruker, Tensor 27, Ettlingen, Germany).

### *Determination of solubility for ketoconazole and ketoconazole nanosuspension*

The aqueous solubility of ketoconazole and ketoconazole nanosuspensions were determined by the shake-flask method. Briefly, both samples were suspended in 7 ml of phosphate buffer pH 7.5, and the suspensions were incubated at 37°C. The solutions were withdrawn and filtered through a (0.22) μm Whatman filter. The concentration of ketoconazole nanosuspensions was determined by UV/Visible Spectrophotometer (Genesys, USA) at (240) nm.

## RESULTS

Several formulations of ketoconazole nanosuspensions were prepared with the different concentrations of PVP and Tween as stabilizers, which are presented in Table 1. As can be seen, the concentration of drug was fixed (0.5%) and the concentration of stabilizers was different. The best concentration was F1, obtained by 0.5 % w/v ketoconazole, 0.5 % w/v PVP K30, 2 % w/v and Tween 60, particle size and polydispersity index (PDI) of F1 were 114.1±3.2 (nm) and 0.372±0.04, respectively.

As shown in Figure 1, AFM image of the best ketoconazole nanosuspensions was nearly homogenous and spherical in shape and their dimension was 113.7 ± 40 nm in length.

**Table 1.** Formulation design of ketoconazole nanosuspensions using a different concentration of stabilizers

| Formulation | KET | PVP | Tween 60 | Mean particle size | PDI |
|---|---|---|---|---|---|
| **F1** | 0.5 | 0.5 | 2 | 114.1±3.2 | 0.372± 0.04 |
| **F2** | 0.5 | 0.5 | 5 | 428.4± 4.3 | 0.47± 0.06 |
| **F3** | 0.5 | 0.5 | 10 | 469± 7.2 | 0.72± 0.03 |

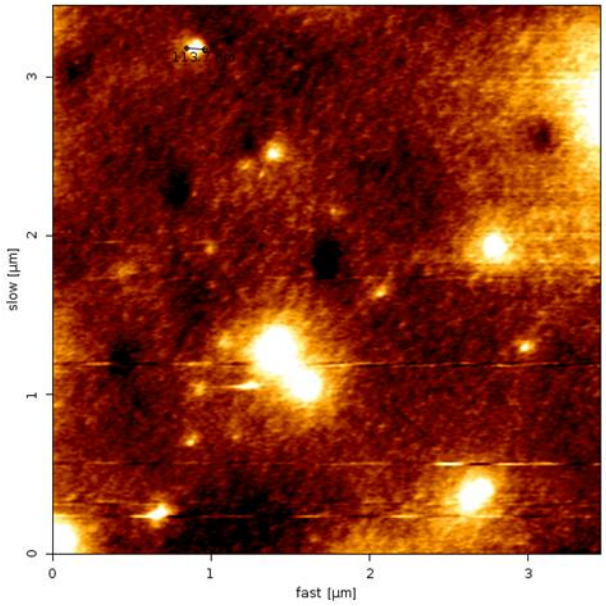


**Figure 1.** Atomic force mocroscopy image of the finest ketoconazol. The cincenteration of KET,PVP,Tween 60,were 0.5%, 0.5 % and 2 respectivly.

### *Differential Scanning Calorimetry*

The DSC thermograph of pure ketoconazole and the finest ketoconazole nanosuspensions (F2) respectively are shown in figures 2 and 3. Figure 2 displays a typical endothermic peak at 53 °C and 152 °C which corresponds to its melting point and the best ketoconazole nanosuspensions (F2) indicated an endothermic peak at 164.14°C.

### *Fourier-Transform Infrared*

The spectra of pure ketoconazole and the best ketoconazole nanosuspensions were investigated in the range of 400-4000 $cm^{-1}$

using FTIR shown in Figure 4,5,6 and 7. The spectrum of the dried finest ketoconazole nanosuspensions (F1) showed no significant difference with the ketoconazole spectra in the whole area of ketoconazole absorption bands.

### *Solubility for ketoconazole and ketoconazole nanosuspensions*

The solubility of the pure drug in buffer was 1.0 ± 0.01 µg/ml while the solubility of pure ketoconazole from the dried best ketoconazole nanosuspensions (F2) was 6.4 ± 0.04 µg/ml. Hence, the solubility of ketoconazole from the dried finest ketoconazole nanosuspensions (F2) was almost eight times higher than the ketoconazole.

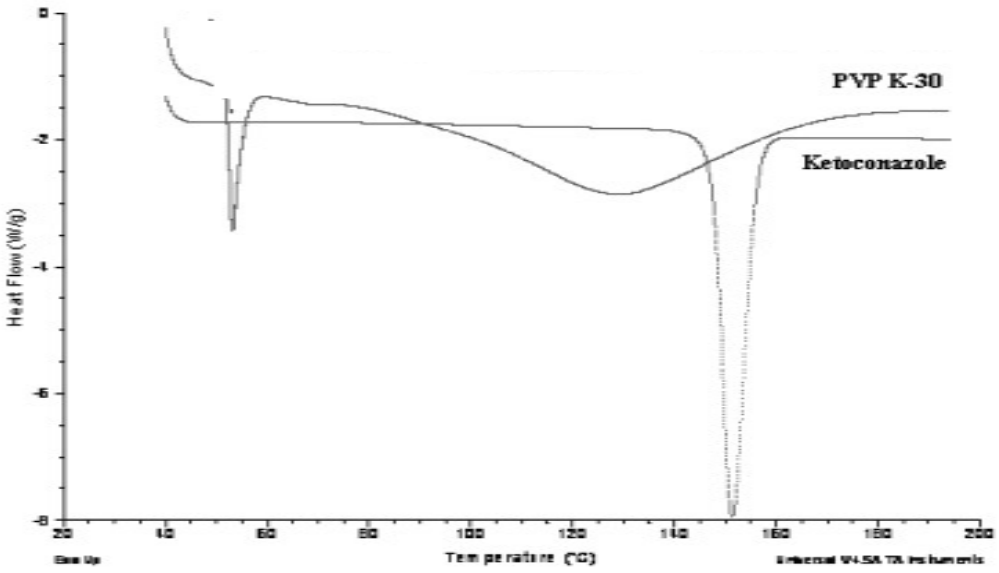


**Figure 2.** DSC of pvp k30 and Ketokonazol

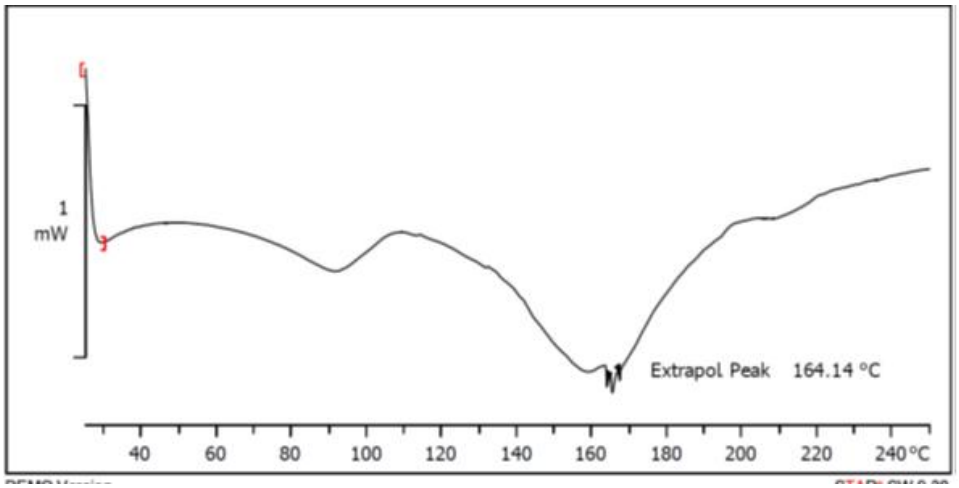


**Figure 3**. DSC of Ketoconazolenanosuspentoin

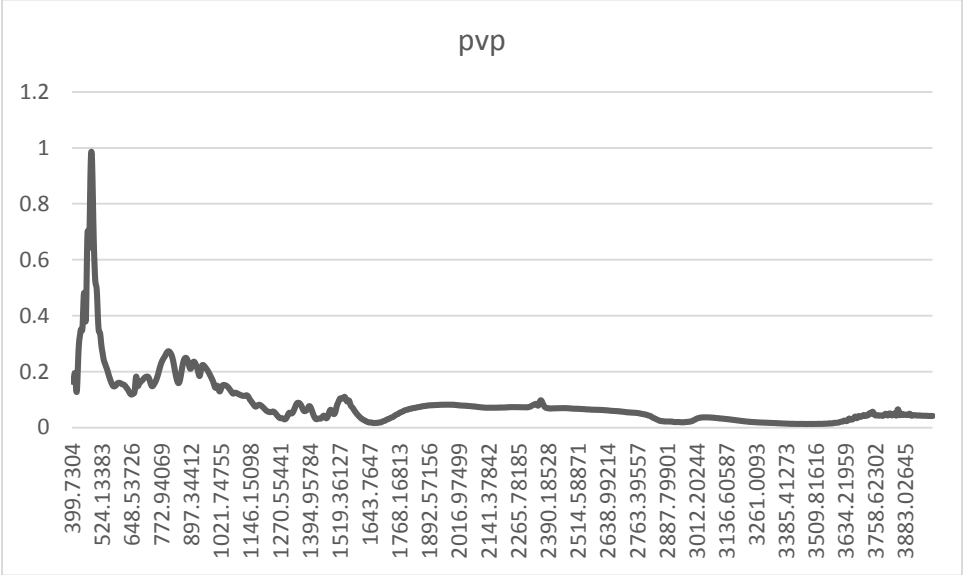


**Figure 4**. Fourier transform infrared spectra of pvp

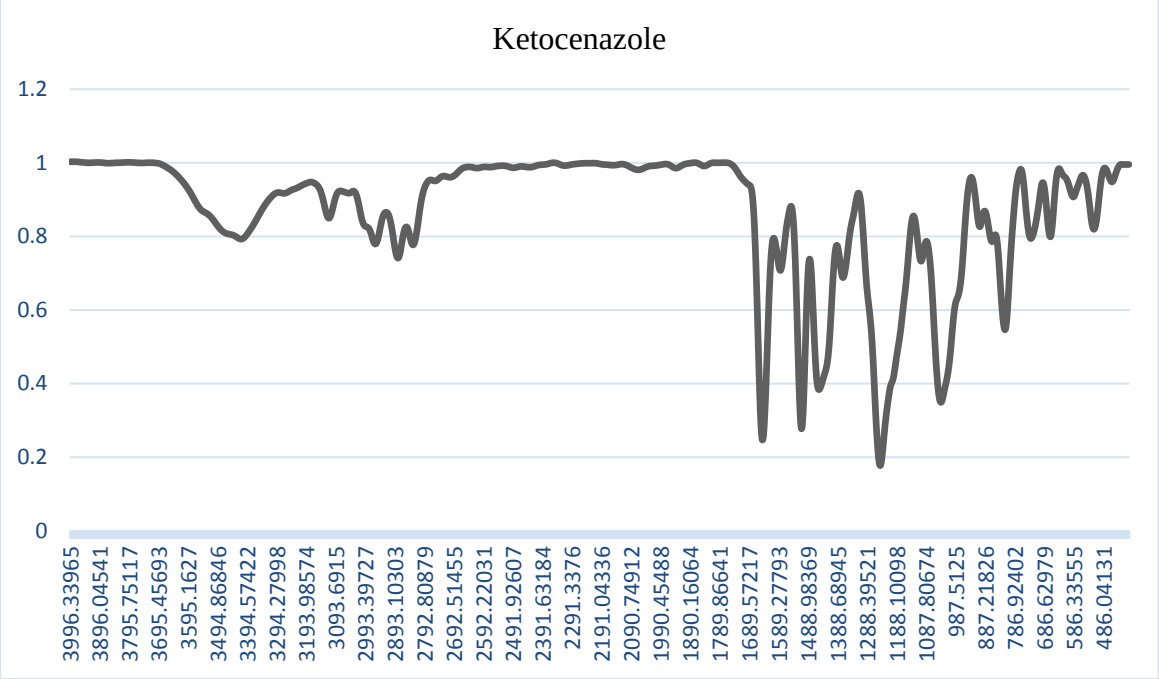


**Figure 5**. Fourier transform infrared spectra of Ketocenazol

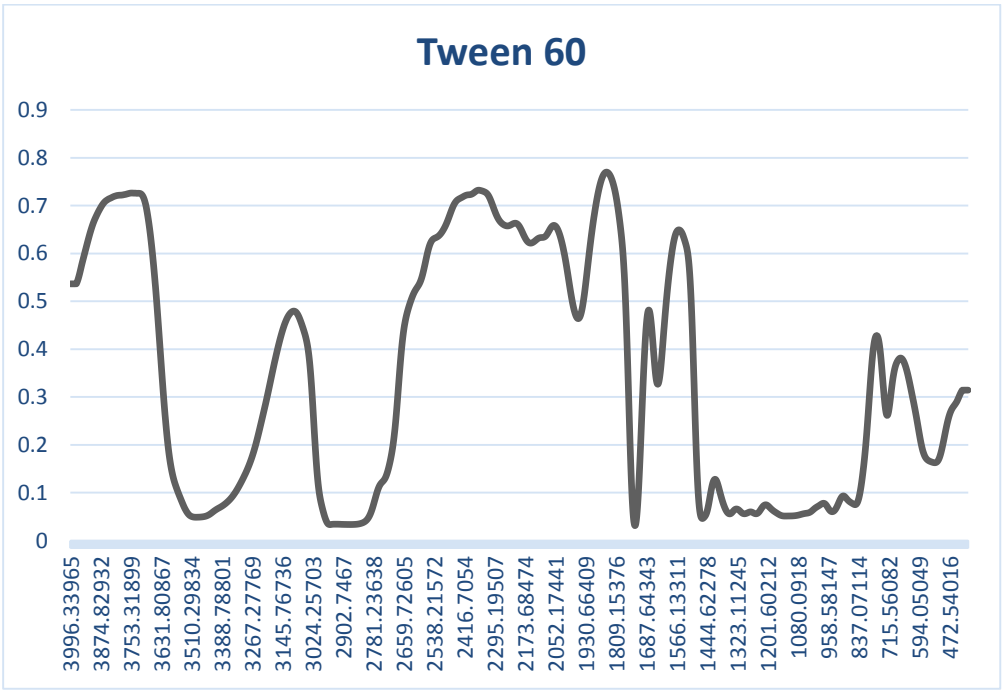


**Figure 6.** Fourier transform infrared spectra of Tween 60

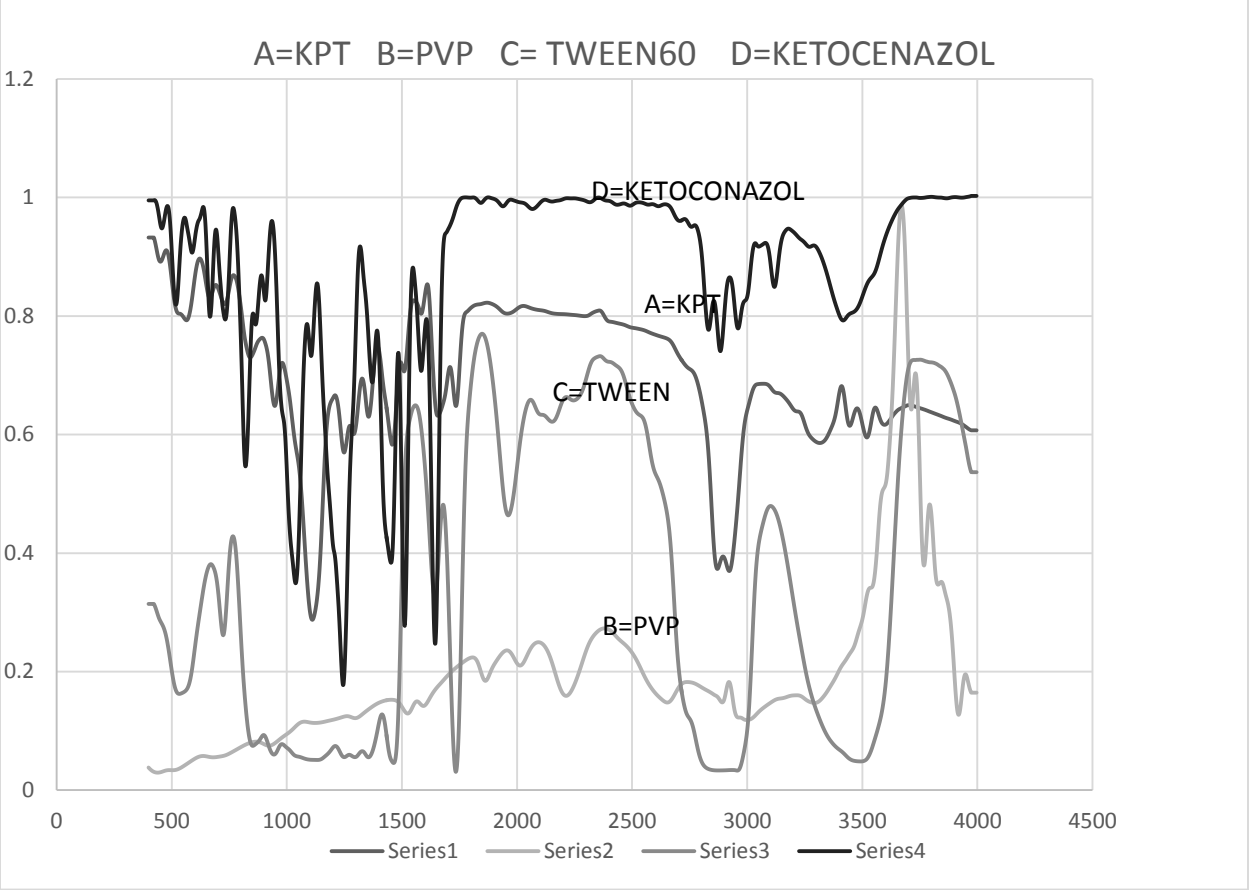


**Figure 7.** Fourier transform infrared spectra of A=Ketoconazol nanosuspension)- Tween 60-PVP (KPT)- B=PVP C=Tween D= Ketoconazol

## DISCUSSION

During the last decade, several techniques have been presented for formulating poorly water-soluble drugs. The advantage of this method is improving the solubility and bioavailability of these drugs [11-13] and the disadvantage of this method is that it is time-consuming [14-15].

Several articles have investigated this subject using different methods [14-16]. Wet-milling technology is one of the new methods for formulating poorly water-soluble drugs. In this method, the decreased size of the drug could improve solubility by using a jet mill before wet milling. This process could reduce the processing time of wet milling [17-18]. In the present study, we could prepare the best ketoconazole nanosuspensions by wet milling method

using Tween60 and PVP K30 as stabilizers after 24 h milling time. Sonication was used when the wet milling process decreased the milling .time from 24 h to 75 min.

According to our findings shown in Table 1, the best ketoconazole nanosuspensions (F1) indicated a mean particle size of 114.1 ± 3.2 nm with a PDI of 0.372 ± 0.04. The zeta potential of the finest ketoconazole nanosuspensions (F1) was approximately -20 mV which is adequate for electrostatic stabilization of ketoconazole nanosuspensions [19]. The result of size is confirmed by DLS analysis results. We investigated the effect of stabilizers on the ketoconazole structure by using the DSC technique. After comparing it to DSC thermographs, no significant differences were found between ketoconazole and the best ketoconazole nanosuspensions curves. Investigating FTIR showed no difference between pure ketoconazole spectra and ketoconazole nanosuspensions. In this study, we observed that the ketoconazole nanosuspensions solubility increased up to eight times due to the formation of ketoconazole nanocrystals.

## CONCLUSION

In this study, nanosuspension (NS) of a poorly soluble drugs, ketoconazole, was prepared easily using a combination of pearl milling method and sonication. This NS could be used for preparation of a promising new drug formulation of ketoconazole. Solubility study in water shows that ketoconazole NS gives higher ketoconazole solubility compared to the pure ketoconazole. Consequently, ketoconazole NS could represent a promising alternative drug delivery system to improve the bioavailability of ketoconazole.

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