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# Itraconazole nanosuspension for oral delivery: Formulation, characterization and *in vitro* comparison with marketed formulation

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## Abstract

Itraconazole was formulated in a nanosuspension to increase the aqueous solubility and to improve its formulation related parameters, dissolution and hence oral bioavailability. Itraconazole nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media, poloxamer 407 as a stabilizer and gycerol as a wetting agent. Effect of various process parameters like, stirring time and ratio of the beads were optimized by keeping Drug:surfactant:milling media (1:3:50) as a constant initially then optimized process parameters were used to optimize formulation parameters by $3^2$ factorial designs. The optimized nanosuspension was lyophilized using mannitol (1:1 ratio) as a cryoprotectant. Characterization of nanosuspension was performed by particle size and size distribution, drug content, scanning electron microscopy, differential scanning calorimetry and X-ray diffraction technique. Optimized nanosuspension showed a mean particle diameter of 294 nm, spherical shape with surface oriented surfactant molecules, which were stabilized formulation, high drug content, no chemical instability and no significant change in crystalline nature after formulation also. The *in vitro* dissolution profile of optimized formulation compared to pure drug and marketed formulation (Canditral Capsule) using 0.1N hydrochloric acid as dissolution medium showed higher drug release compared to the pure drug and marketed formulation.

**Key words:** Nanosuspension, Itraconazole, Pearl milling technique.

## Introduction

Itraconazole is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species including *Cryptococcus, Candida, Aspergillus, Blastomyces* and *Histoplasma capsulatum* var. capsulatum (Saag et al. 1988, Odds et al. 2000). It is a weakly base drug with lipid solubility (n-octanol/water partition log 5.66 at PH 8.1) and a pKa of 3.7 (Fromtling 1987). Itraconazole is ionized only at a low pH, such as gastric juice. Therefore, on oral administration, the gastric acidity is needed for adequate dissolution. The bioavailability of itraconazole is known to be increased after a meal as compared with that found in the fasting state. Since the bioavailability of poorly water-soluble drug can be influenced by interactions with food or by the physicochemical conditions in gastrointestinal (GI) tract, oral preparation of itraconazole is commonly prescribed to be administered according to a fixed dosing schedule, especially,

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to be immediately taken after meal. The oral bioavailability of itraconazole is maximal when Sporanox® capsule are taken with a full meal.

The dissolution rate of poorly water soluble drugs often becomes a rate-limiting step in their absorption from GI tract (Maeda et al. 1979, Chiba et al. 1991). Various solubilization methods have been used to increase the drug solubility and dissolution properties, including the use of surfactant, water-soluble carriers, polymeric conjugates, and solid dispersion.

Preparation of drugs in form of nanosuspensions has shown to be a more cost-effective and technically simpler alternative, particularly for poorly soluble drugs, and yield a physically more stable product than liposome dispersions (Westesen and Siekmann 1995, Liversidge et al. 1996, Muller and Peters 1997). With this technique, the drug, dispersed in water, is grounded by shear forces to particles with a mean diameter in the nanometer range (100-1000 nm). The fineness of the dispersed particles causes them to dissolve more quickly owing to their higher dissolution pressure and leads to an increased saturation solubility. This may enhance the bioavailability of drugs compared with other microparticular systems. If the dissolution velocity of the drug particles is low enough *in vivo*, the drug nanosuspensions will have the passive targeting advantages of colloidal drug carriers (Muller 1991).

Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency are major and common problems. Major efforts have been spent for the development of customized drug carriers to overcome the disappointing *in vivo* fates of the drug (Amidon et al. 1995, Barratt et al. 2000). Hence, there is a growing need for a unique strategy that can tackle the formulation related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics.

The aim of this study was, to employ the nanosuspension technique to produce itraconazole nanoparticles for oral administration, thereby avoiding the use of harmful additives and enabling to enhance the saturation solubility, dissolution and oral absorption of itraconazole. The optimized nanosuspension formulation was evaluated for *in vitro* dissolution profile in comparison to pure drug and marketed formulation (Canditral Capsule).

## Material and Methods

*Materials*

Itraconazole was gifted from Intas pharmaceutical limited, India. Zirconium oxide beads were gifted from sun Pharmaceutical Industries Ltd, India. Poloxamer 407 was purchased from BASF, Germany. Glycerol and Mannitol were purchased from S.D fine chemicals, India.

*Preparation of Nanosuspension*

In order to produce the nanosuspension, itraconazole powder (1% w/v) was dispersed in an aqueous solution containing glycerol (2.2% w/v) and different ratio of poloxamer 407 in 20 mL vial. The resulting coarse pre-dispersion was comminuted using zirconium oxide beads (milling media) on a magnetic stirrer. The various parameters like effect of stirring time and ratio of different size of zirconium oxide beads were optimized by keeping the drug: surfactant: milling media volume (1:3:50) as constant initially, then the optimized conditions of stirring time and ratio of different size of zirconium oxide beads were used throughout the study to optimized concentration of poloxamer 407 and volume of milling media using $3^2$ factorial designs to achieve minimum particle size (Table 1). The optimized formulation was lyophilized using mannitol as a cryoprotectant (1:1 ratio). Lyophilized nanosuspension was used for further study.

**Table 1.** $3^2$ factorial design lay out for preparation of Itraconazole nanosuspension

| Batch No. | $X_1$ | $X_2$ |
|---|---|---|
| ITZ1 | 2.5 | 40 |
| ITZ2 | 2.5 | 50 |
| ITZ3 | 2.5 | 60 |
| ITZ4 | 3.0 | 40 |
| ITZ5 | 3.0 | 50 |
| ITZ6 | 3.0 | 60 |
| ITZ7 | 3.5 | 40 |
| ITZ8 | 3.5 | 50 |
| ITZ9 | 3.5 | 60 |

$X_1$ Concentration of stabilizer (Poloxamer 407) (% w/v); $X_2$ % v/v of milling media (Zirconium oxide beads)

*Particle size and Size distribution*

The mean particle size and size distribution of the prepared nanosuspension was measured by laser diffraction technique using Malvern particle size analyzer, MS 2000 (Malvern Ins., UK). Nanosuspension was added to the sample dispersion unit, containing stirrer and stirred at 2000 rpm in order to reduce the interparticulate aggregation, and laser obscuration range was maintained between 10-20%. The average particle size was measured after performing the experiment in triplicate.

*Scanning Electron Microscopy (SEM)*

The lyophilized powder nanosuspension formulation was kept in the sampling unit as a thin film and then photographs were taken at x100 and x200 magnification using Jeol scanning electron microscope (Jeol, JSM-840, Japan).

*Differential Scanning Calorimetry (DSC)*

The DSC thermograms of bulk itraconazole powder and lyophilized nanosuspension formulation were taken on a Mettler Toledo Star SW 7.01 DSC differential scanning colorimeter between 30-300°C at a heating rate of 10°C/min with nitrogen supply at 50 mL/min.

*X-ray Diffraction pattern (XRD)*

The study was carried out at Punjab University, Chandigadh, India. The XRD thermograms of bulk itraconazole powder and lyophilized nanosuspension formulation were carried on Philips PW 1710 X-ray generator (Philips, Amedo, The Netherlands).

*In vitro dissolution profile*

*In vitro* dissolution study was performed using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 500 mL of 0.1 N HCl as dissolution medium maintained at 37 ± 0.5°C at 100 rpm speed for pure drug, lyophilized itraconazole nanosuspension formulation and marketed formulation (Canditral capsule). Samples (5mL) were withdrawn at regular intervals of 5 min for 60 min and replaced with fresh dissolution medium. Samples were filtered through 0.2μ filter paper and assayed spectrophotometrically on UV-visible spectrophotometer (Shimadzu UV-1601, Japan) at 255.0 nm wavelength. Dissolution for each formulation was performed in triplicate and mean of absorbance was used to calculate cumulative percent of drug release.

*Drug Content*

Assay was carried out by taking 10 mg lyophilized powder (weigh equivalent to 1.25 mg of drug), dissolved in 0.4 mL tetrahydrofuran in 50 mL dry volumetric flask then volume was made up using 0.1 N HCl. From the above 4mL

solution was taken to 10 mL dry volumetric flask, and volume was made up with 0.1N HCl. The absorbance at 255 nm wavelength was taken using UV-visible spectrophotometer (UV-1601, Shimadzu, Japan) and the drug content was calculated accordingly (Guo et al. 2001).

## Results and Discussion

*Influence of various parameters on particle size and size distribution*

As shown in Table 2, effect of stirring time on particle size was optimized by keeping 50:50 ratio of different diameter (0.4 mm to 0.7 mm and 1.2 mm to 1.7 mm) of zirconium oxide beads and keeping the drug: surfactant: milling media volume (1:3:50) as constant. Lowest 317 nm mean particle size was achieved at 24 h stirring at 50:50 ratios of zirconium oxide beads, further stirring up to 28 h lead to increased particle size.

**Table 2.** Effect of stirring time on particle size of itraconazole nanosuspension

| Batch. No. | Time (h) | Mean particle size [D(4,3)] |
|---|---|---|
| IT1 | Initial (5min.) | 176.49μm |
| IT2 | 2 | 3.060μm |
| IT3 | 4 | 2.328μm |
| IT4 | 6 | 1.824μm |
| IT5 | 8 | 1.368μm |
| IT6 | 10 | 1.309μm |
| IT7 | 12 | 1.225μm |
| **IT8** | **24** | **0.317μm** |
| IT9 | 26 | 0.552μm |
| IT10 | 28 | 0.678μm |

As shown in Table 3, effect of ratio of different size of zirconium oxide beads from 0.4 nm to 0.7 nm and 1.2 nm to 1.7 nm on particle size was optimized by keeping the drug: surfactant: milling media volume (1:3:50) as constant and stirred for 24 h. Lowest particle size 315 nm was observed at 50: 50 ratio of different size of zirconium oxide beads.

**Table 3.** Effect of ratio of beads on particle size of itraconazole nanosuspension

| Batch. No. | Ratio of beads (Zirconium oxide) | | Mean particle size [D(4,3)] |
|---|---|---|---|
| | Small Size (0.4 mm to 0.7 mm ) | Big Size (1.2 mm to 1.7 mm) | |
| TSB1 | 0 | 100 | 1.142 μm |
| TSB2 | 25 | 75 | 0.674 μm |
| TSB3 | 50 | 50 | 0.315 μm |
| TSB4 | 75 | 25 | 0.865 μm |
| TSB5 | 100 | 0 | 1.315 μm |

As shown in Table 4, the optimized formulation showed mean particle size of 283 nm with polydispersity index 0.307 (before lyophilization), with 3.0% w/v of poloxamer 407 used as a stabilizer and 50% v/v of milling media. After lyophilization a mean particle diameter was found to be 294 nm with polydispersity index 0.318, so there was no significant change of lyophilization process was observed in particle size and size distribution.

**Table 4.** Optimization of formulation parameters for the preparation of itraconazole nanosuspension

| Batch No. | Conc. of drug (% w/v) | Conc. of stabilizer (Poloxamer 407) (% w/v) | % v/v of Milling Media (Zirconium oxide beads) | Particle size before Lyophilization [d(4,3)] | Polydispersity index | Particle size after Lyophilization [d(4,3)] | Polydispersity index |
|---|---|---|---|---|---|---|---|
| ITZ1 | 1 | 2.5 | 40 | 0.747 µm | 0.512 | 0.754 µm | 0.523 |
| ITZ2 | 1 | 2.5 | 50 | 0.516 µm | 0.419 | 0.523 µm | 0.428 |
| ITZ3 | 1 | 2.5 | 60 | 0.696 µm | 0.489 | 0.707 µm | 0.508 |
| ITZ4 | 1 | 3.0 | 40 | 0.379 µm | 0.376 | 0.392 µm | 0.389 |
| ITZ5 | 1 | 3.0 | 50 | 0.283 µm | 0.307 | 0.294 µm | 0.318 |
| ITZ6 | 1 | 3.0 | 60 | 0.324 µm | 0.453 | 0.358 µm | 0.461 |
| ITZ7 | 1 | 3.5 | 40 | 0.501 µm | 0.392 | 0.515 µm | 0.402 |
| ITZ8 | 1 | 3.5 | 50 | 0.438 µm | 0.543 | 0.448 µm | 0.554 |
| ITZ9 | 1 | 3.5 | 60 | 0.492 µm | 0.465 | 0.505 µm | 0.481 |

No significant changes in particle size and polydispersity index demonstrate formation of stable non-flocculated nanosuspension of itraconazole developed in this investigation (Table 4). Lowest mean particle size was achieved after 24h stirring with milling media ratio of 0.4-0.7mm and 1.2-1.7mm diameter of beads 50:50. Increased in the media volume lead to slight increased in mean particle diameter but did not lead to significant change by increasing the concentration of stabilizer. Further stirring led to increase in mean particle diameter of itraconazole nanosuspension.

*Scanning electron microscopy (SEM)*

SEM micrographs clearly showed the great differences between pure itraconazole (Figure 1) and optimized nanosuspension formulation (Figure 2). The particles of itraconazole were found to be large and especially irregular (Figure 1). While after formulation, particles disappeared and drug became small and uniform which was crucial for intravenous safety. However, the nanocrystals seem to be more rounded, perhaps because the particles were coated with a surfactant layer. In the suspension solution, the surfactant used to stabilize the particles would adsorb to surface of the crystals by hydrophobic interaction. Therefore, after lyophilization, the solidification of surfactants formed an amorphous layer on the surface of inner crystals (Muller and Jacobs 2002).

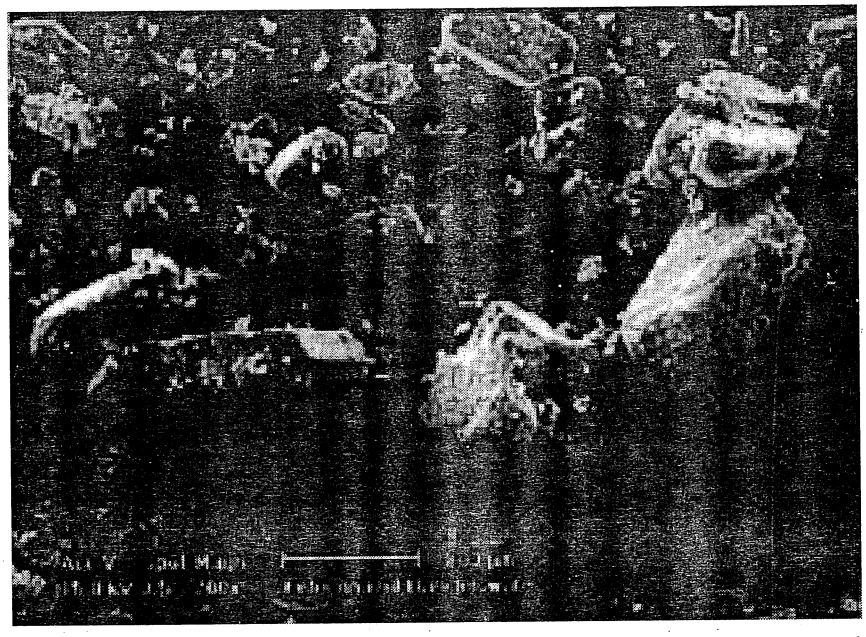

**Figure 1.** Photomicrograph of scanning electron micrographs of pure itraconazole powder (magnification x200)

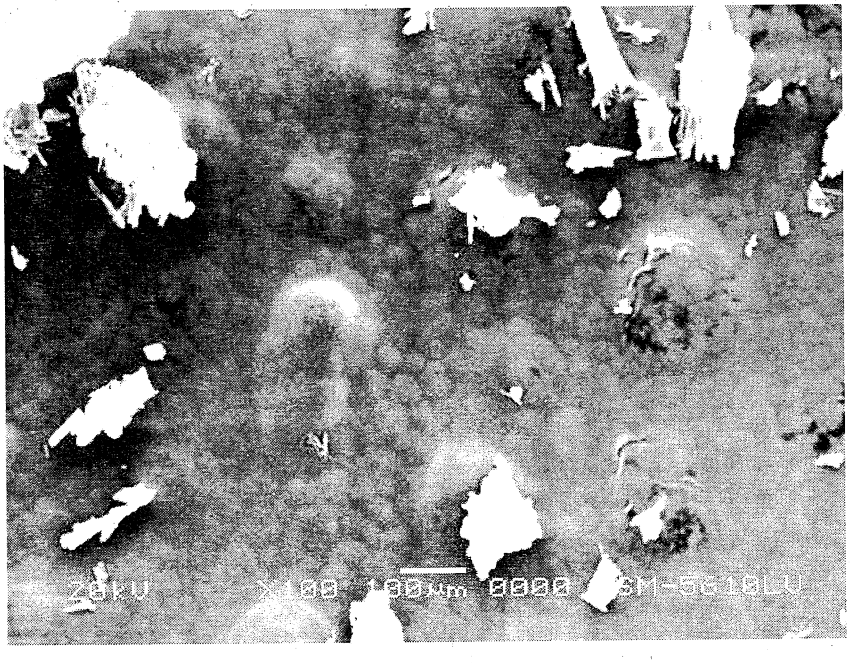

**Figure 2.** Photomicrograph of scanning electron micrographs of itraconazole nanosuspension formulation (magnification x100)

*Differential Scanning Calorimetry (DSC)*

DSC was performed to investigate the effect of surfactant on the inner structure of itraconazole nanosuspension. Figures 3 and 4 show DSC thermograph of pure itraconazole powder and optimized nanosuspension formulation respectively. Pure itraconazole powder showed melting exotherm at 168.38°C

which reveals its melting point and the exotherm of it in formulation was observed at 165.58°C. From thermograms, it was concluded that the drug and the surfactant do not interact with each other (Teeranachaideekul et al. 2008).

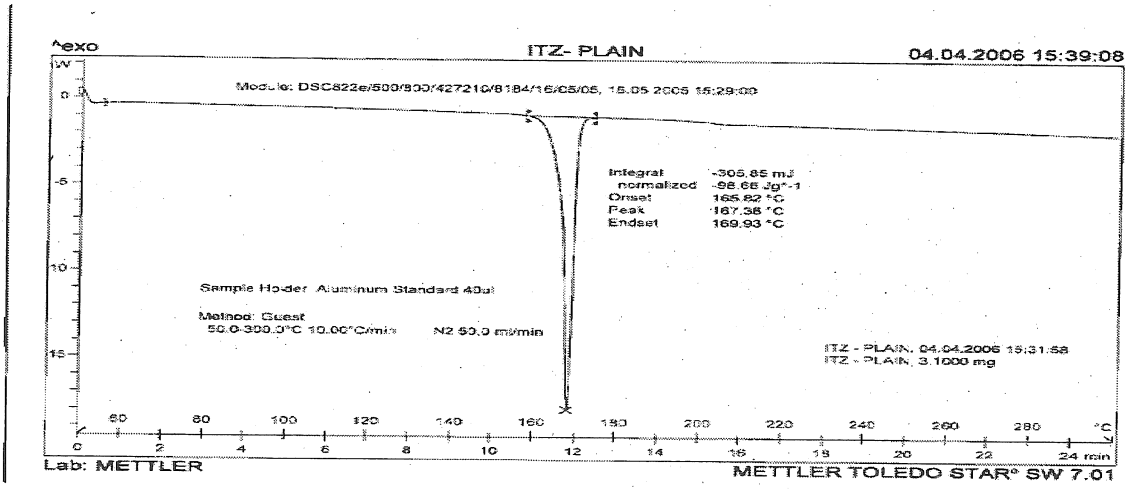


**Figure 3.** DSC thermograms of bulk itraconazole powder

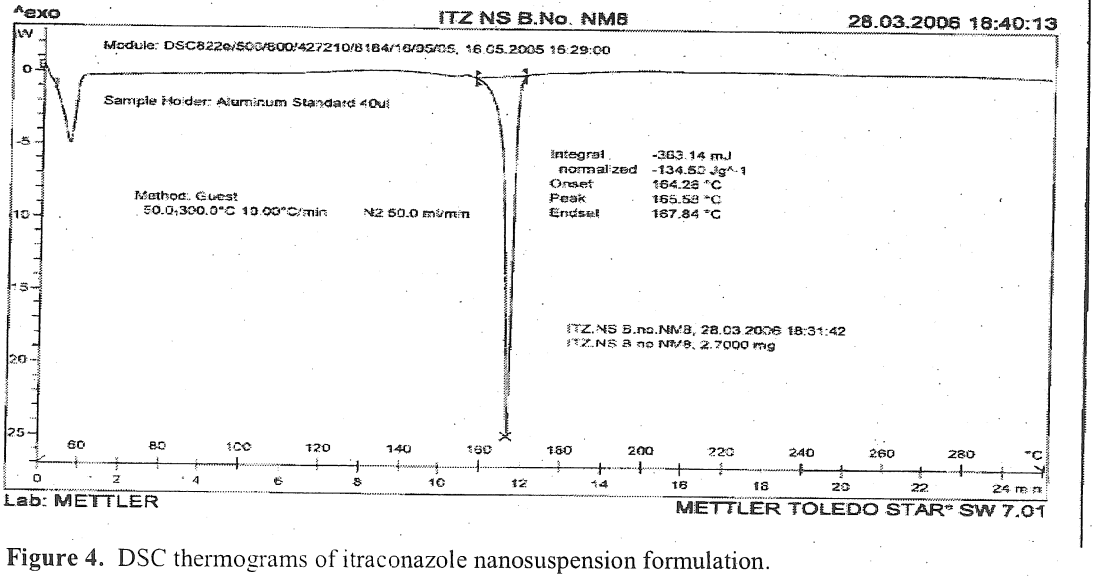


**Figure 4.** DSC thermograms of itraconazole nanosuspension formulation.

*X-Ray Diffraction pattern (XRD)*

X-Ray diffraction has been used to analyze potential changes in the inner structure of itraconazole nanocrystal during the formulation. The extent of such changes depends on the chemical nature and on physical hardness of the active ingredient (Muller et al. 2001). Figure 5 and 6 show XRD thermograph of pure itraconazole powder, poloxamer 407, mannitol and itraconazole nanosuspension formulation respectively. The obtained patterns reveal that the drug crystallinity of nanosuspension formulation was not affected significantly (Teeranachaideekul et al. 2008).

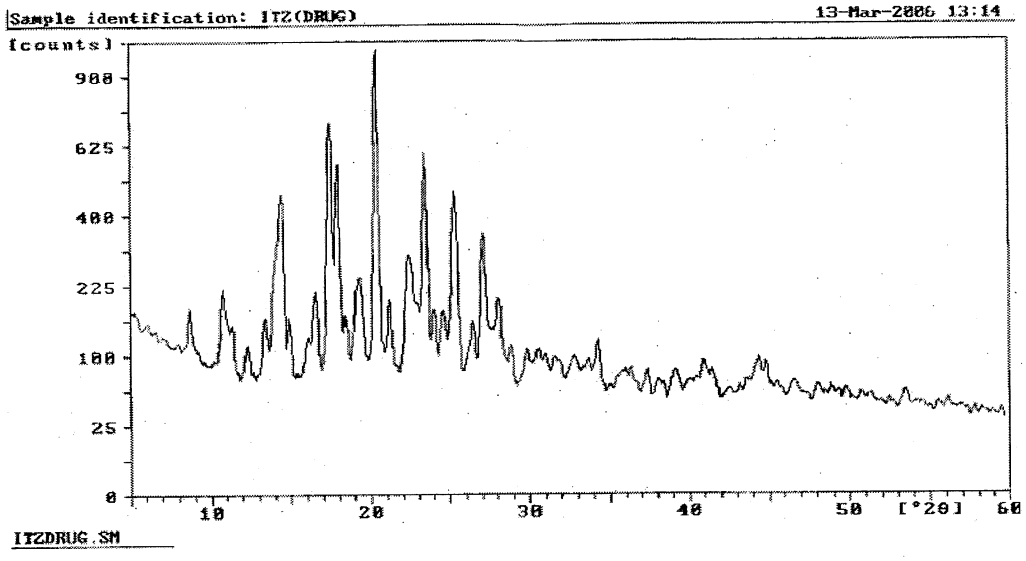


**Figure 5.** XRD thermograms of bulk itraconazole powder

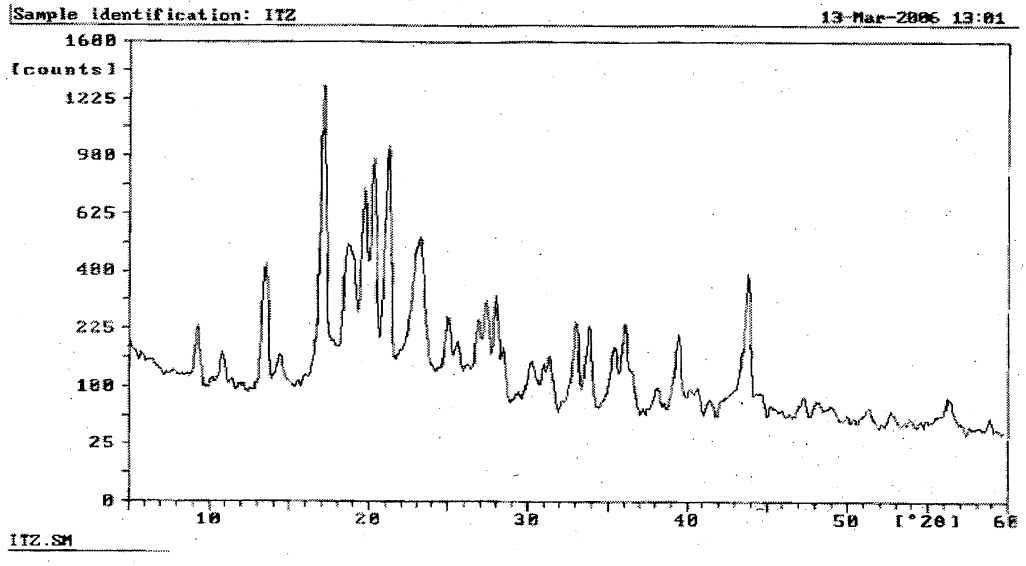


**Figure 6.** XRD thermograms of itraconazole nanosuspension formulation.

*In vitro dissolution study*

Dissolution studies were performed for pure drug, marketed formulation (Canditral capsule) and optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 90% within 10 min compared to the 10% and 17% from pure drug and marketed formulation (Canditral

capsule) respectively (Figure 7). The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for the six fold increase in dissolution rate.

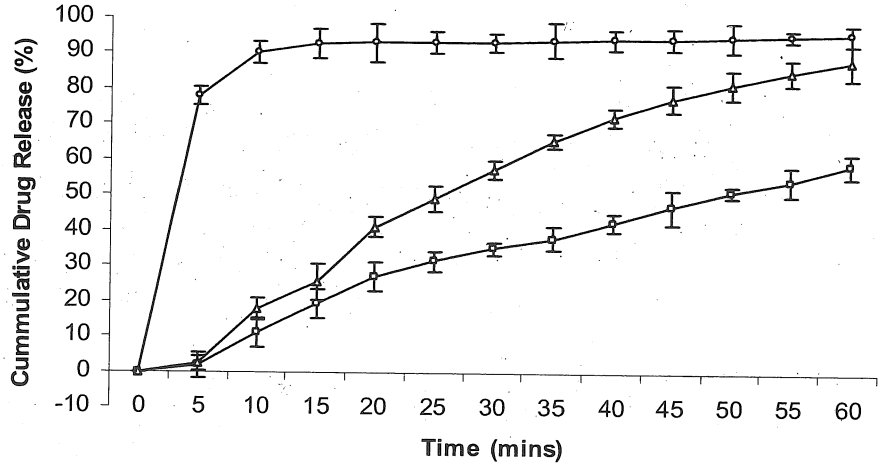


**Figure 7**. Dissolution profile for nanosuspension formulation (circle), pure drug (square), and marketed formulation (triangle) [mean ± SD (n=3)] in 0.1N HCl

The drug content in the formulation was found to be 99.25% w/w of the amount of drug theoretically added. In the formulation no step was involved which can cause the drug loss hence the high amount of drug content was obtained.

## Conclusion

It has been concluded that nanocrystalline suspensions of poorly soluble drugs such as itraconazole are easy to prepare and to lyophilize for extended storage and represent a promising new drug formulation for oral drug delivery of fungal infection. Dissolution study in 0.1N HCl shows that nanosuspension formulation gives higher drug release compared to the pure drug and marketed formulation. Nanosuspensions consequently represent a promising alternative to current delivery systems aiming to improve the biopharmaceutic performance of drugs with low water solubility.

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