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Itraconazole Nanosuspension Meant for Oral Use: Development of Formulation, Characterization and *In Vitro* Comparison with Some Marketed Formulations

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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 Glycerol 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 (1:3.0:50) 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 294nm, 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 [1, 2]. 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 [3]. 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, 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 [4, 5]. 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 [6-8]. With this technique, the drug, dispersed in water, is grounded by shear forces to particles with a mean diameter in the nanometer range (100-1000nm). 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 microparticulate 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 [9].

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 [10-11]. 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 pharmacoconomics.

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).

Materials 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 (1:3.0:50) 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

Where, X₁ – Concentration of stabilizer (Poloxamer 407) (% w/v)

X₂ - % 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, SM 2000. 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 100X and 200X magnification using Jeol Scanning Electron Microscope (Jeol, JSM-840 SEM 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 calorimeter between 30-300°C at a heating rate of 10°C/min with Nitrogen supply at 50.0ml/min.

X-ray Diffraction pattern (XRD)

The study was carried out at Punjab University, Chandigarh, 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 500ml of 0.1N HCl as dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$ at 100rpm speed for pure drug, lyophilized itraconazole nanosuspension formulation and marketed formulation (Canditral capsule). Samples (5ml) were withdrawn at regular intervals of 5min for 60mins and replaced with fresh dissolution medium. Samples were filtered through 0.2 μ filter paper and assayed spectrophotometrically on SHIMADZU UV-VISIBLE spectrophotometer UV-1601 at 255.0nm 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 10mg lyophilized powder (weigh equivalent to 1.25mg of drug), dissolved in 0.4ml Tetra hydrofuran in 50ml dry volumetric flask then volume was made up using 0.1N HCl. From the above 4ml solution was taken to 10ml dry volumetric flask, and volume was made up with 0.1N HCl. The absorbance at 255.0nm wavelength was taken using SHIMADZU UV-VISIBLE spectrophotometer UV-1601 and the drug content was calculated accordingly [12].

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 (0.4mm to 0.7mm and 1.2 mm to 1.7mm) of zirconium oxide beads and keeping the drug: surfactant: milling media volume (1:3.0:50) as constant. Lowest 317 nm mean particle size was achieved at 24h stirring at 50:50 ratios of zirconium oxide beads, further stirring up to 28h lead to increased particle size.

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

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

As shown in Table 3, effect of ratio of different size of zirconium oxide beads from 0.4 nm to 0.7nm and 1.2nm to 1.7nm on particle size was optimized by keeping the drug: surfactant: milling media volume (1:3.0:50) as constant and stirred for 24h. Lowest particle size 315nm 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 (0.4mm to 0.7mm)	Big Size (1.2mm to 1.7mm)	
TSB1	0	100	1.142 μ m
TSB2	25	75	0.674 μ m
TSB3	50	50	0.315μm
TSB4	75	25	0.865 μ m
TSB5	100	0	1.315 μ m

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

Batch No.	Conc. of drug (% w/v)	Conc. 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 μ m	0.512	0.754 μ m	0.523
ITZ2	1	2.5	50	0.516 μ m	0.419	0.523 μ m	0.428
ITZ3	1	2.5	60	0.696 μ m	0.489	0.707 μ m	0.508
ITZ4	1	3.0	40	0.379 μ m	0.376	0.392 μ m	0.389
ITZ5	1	3.0	50	0.283μm	0.307	0.294μm	0.318
ITZ6	1	3.0	60	0.324 μ m	0.453	0.358 μ m	0.461
ITZ7	1	3.5	40	0.501 μ m	0.392	0.515 μ m	0.402
ITZ8	1	3.5	50	0.438 μ m	0.543	0.448 μ m	0.554
ITZ9	1	3.5	60	0.492 μ m	0.465	0.505 μ m	0.481

As shown in Table 4, the optimized formulation showed mean particle size of 283nm 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 294nm with Polydispersity index 0.318, so there was no significant change of lyophilization process was observed in particle size & size distribution.

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 lead to increase in mean particle diameter of Itraconazole nanosuspension.

Scanning electron microscopy (SEM)

SEM micrographs clearly showed the great differences between pure itraconazole (Fig. 1) and optimized nanosuspension formulation (Fig. 2). The particles of itraconazole were found to be large and especially irregular (Fig. 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. [13]

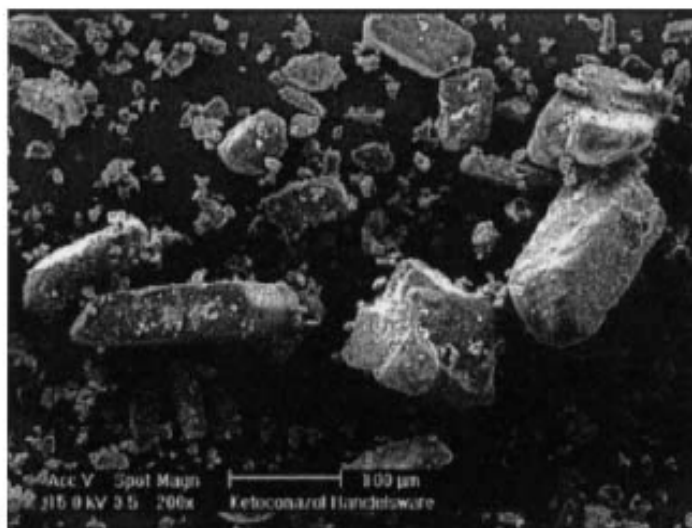


Figure 1. Photomicrograph of scanning electron micrographs of pure itraconazole powder

Differential Scanning Calorimetry (DSC)

DSC was performed to investigate the effect of surfactant on the inner structure of itraconazole nanosuspension. Figures 3 and figure 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 (14).

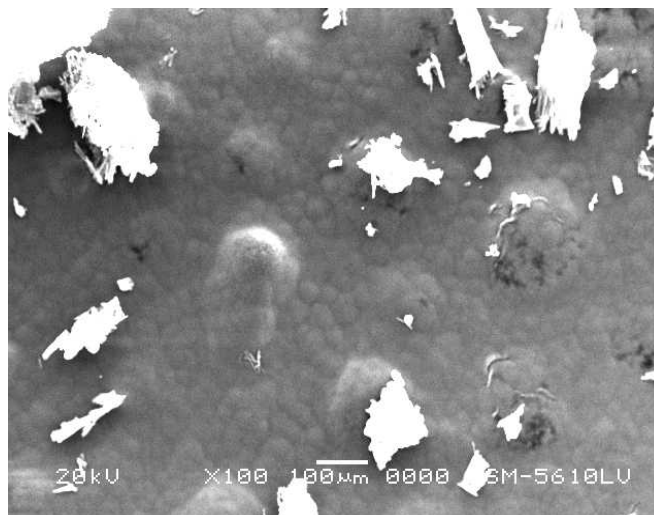


Figure 2. Photomicrograph of scanning electron micrographs of itraconazole nano suspension formulation

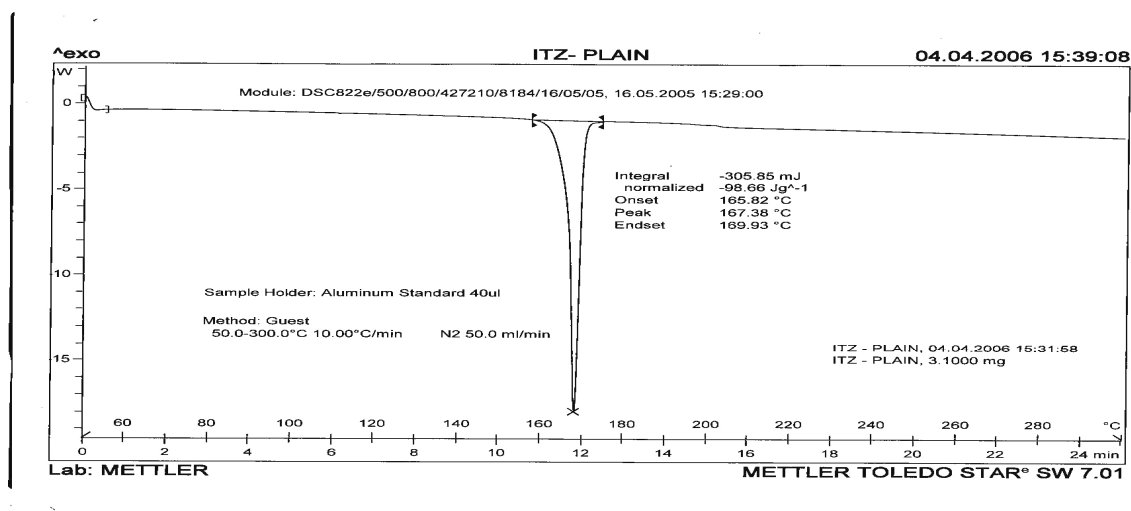


Figure 3. DSC thermograms of Bulk Itraconazole powder

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 (15). 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 [14].

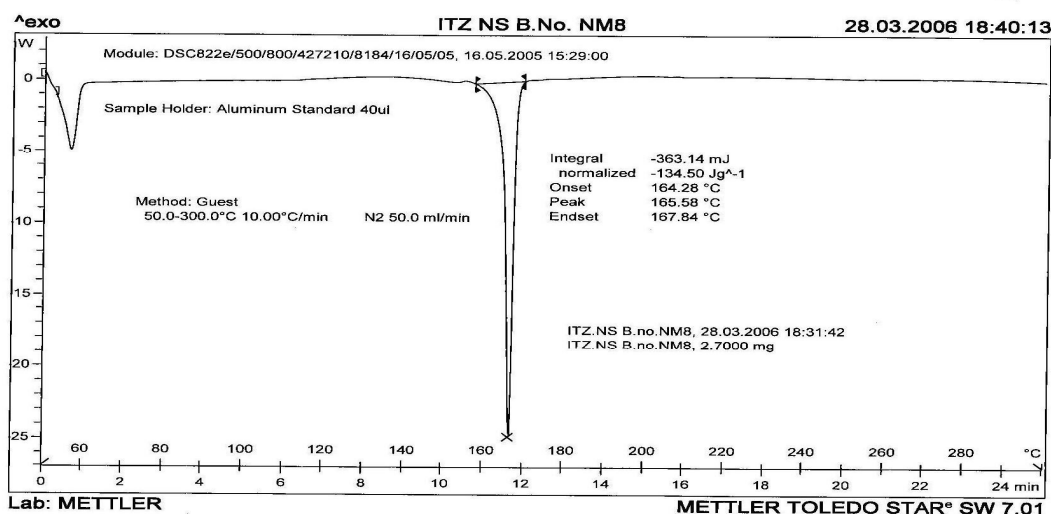


Figure 4. DSC thermograms of Itraconazole nanosuspension formulation

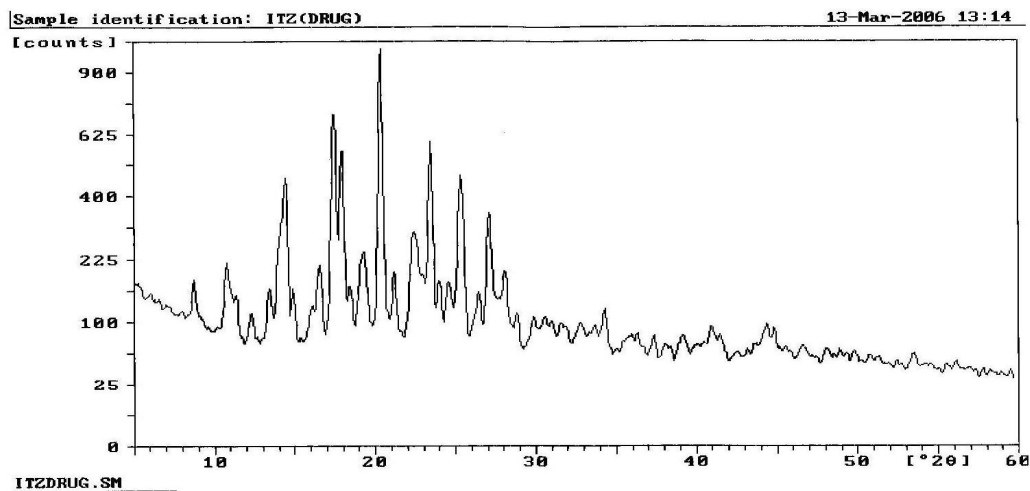


Figure 5. XRD thermograms of bulk Itraconazole powder

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 10mins compared to the 10% and 17% from pure drug and marketed formulation (Canditral capsule) respectively (Fig 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.

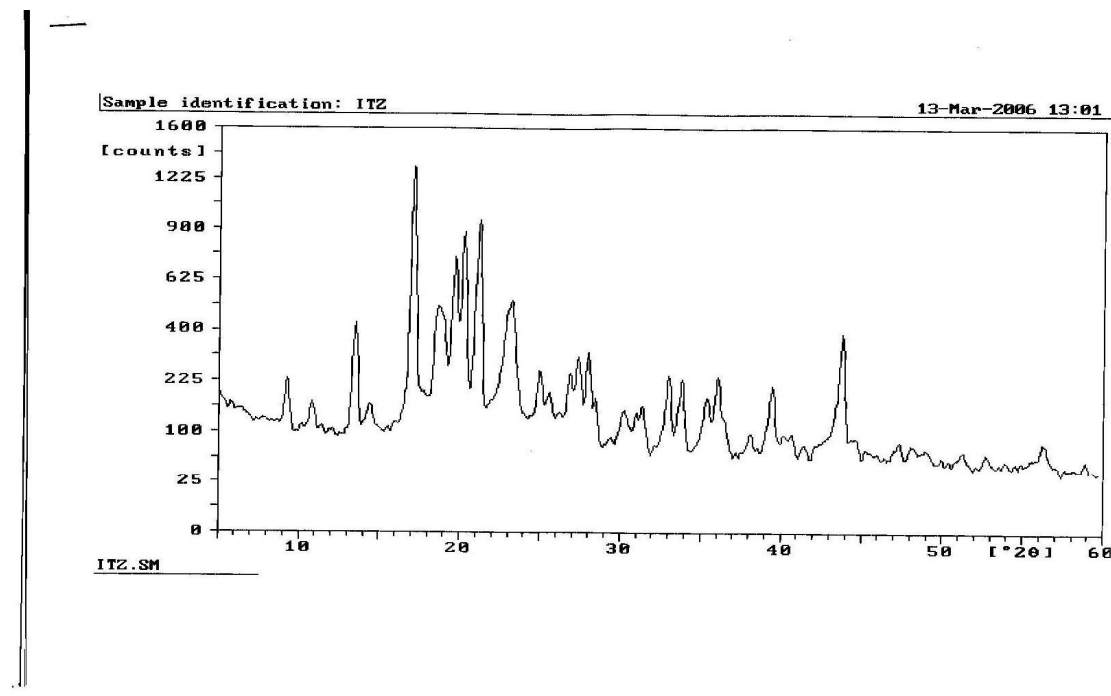


Figure 6. XRD thermograms of itraconazole nanosuspension formulation

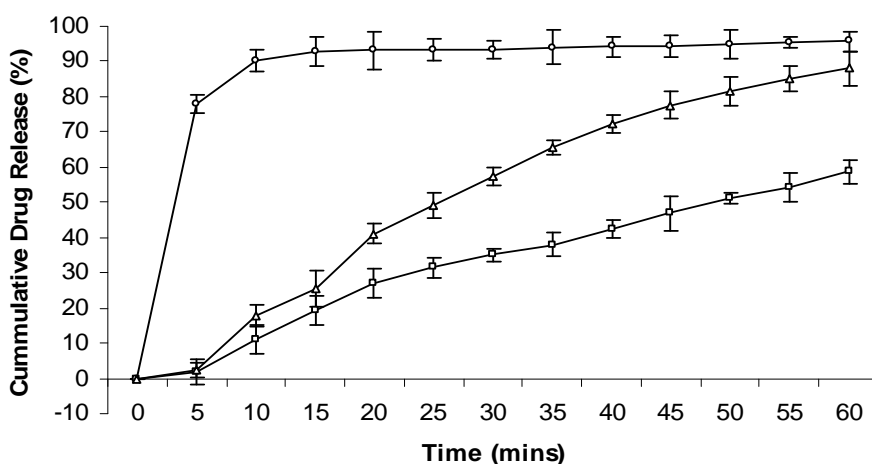


Figure 7. Dissolution profile for nanosuspension formulation (circle), pure drug (square), and marketed formulation (triangle) [mean \pm SD (n=3)] in 0.1N HCl

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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