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

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ABSTRACT

Background and the purpose of the study: Itraconazole is a poorly water soluble drug which results in its insufficient bioavailability. The purpose of the present study was to formulate Itraconazole in a nanosuspension to increase the aqueous solubility and to improve its formulation related parameters, dissolution and hence oral bioavailability.

Methods: 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. Effects of various process parameters like, stirring time and the ratio of the beads were optimized by keeping drug:surfactant:milling media (1:3.0:50) as a constant initially and then optimized process parameters were used to optimize formulation parameters by 32 factorial designs. The optimized nanosuspension was lyophilized using mannitol (1:1 ratio) as a cryoprotectant. Nanosuspension was characterized by particle size and size distribution, drug content, scanning electron microscopy, differential scanning calorimetry and X-ray diffraction techniques.

Results: Optimized nanosuspension showed spherical shape with surface oriented surfactant molecules and a mean particle diameter of 294 nm. There was no significant change in crystalline nature after formulation and it was found to be chemically stable with high drug content.

Conclusion: The in vitro dissolution profile of the optimized formulation compared to the pure drug and marketed formulation (Canditral Capsule) by using 0.1N Hydrochloric acid as release medium showed higher drug release.

Keywords: Nanosuspension, Itraconazole, Pearl milling technique

INTRODUCTION

Itraconazole is an orally active triazole antymycotic 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 weak basic drug which is soluble in lipids (n-Octanol/Water partition, 5.66 at pH of 8.1) and a pKa of 3.7 (3). Itraconazole is ionized only at a low pH, such as gastric juice and as a result on oral administration, the gastric acidity is required for adequate dissolution. The bioavailability of itraconazole is known to be increased after a meal in comparison to the fasting state. Since the bioavailability of poorly water-soluble drugs can be influenced by interactions with food or by the physicochemical conditions of the gastrointestinal (GI) tract, oral preparation of itraconazole is commonly prescribed to be administered according to a fixed dosing schedule, especially, to be taken immediately after meals. The oral bioavailability of itraconazole is maximal when it is taken with a full meal.

Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency are the most important and common problems with this drug. Major efforts have been made for the development of customized drug carriers to overcome the disappointing in vivo fates of the drug (4, 5). 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 dissolution rate of poorly water soluble-drugs often becomes a rate-limiting step in their absorption from GI tract (6, 7). 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...
Poloxamer 407 was purchased from BASF (Germany). Glycerol and Mannitol were purchased from S.d fine chemicals (India).

Preparation of 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. Zirconium oxide beads were used in the preparation of nanosuspension due to their low cost and easy availability for lab scale production of nanosuspension in comparison to silver beads.

Various parameters like the 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 optimize concentration of Poloxamer 407 and volume of milling media using $3^2$ factorial designs to achieve minimum particle size (Table 1). The stirring was continued for 24 hrs at 750 rpm for the preparation of optimized nanosuspension formulation. 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).

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

| Batch. No. | Time (hrs) | 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                     |

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MATERIAL AND METHODS

Materials
Itraconazole was a gift from Intas pharmaceutical limited (India). Zirconium oxide beads were gifted from Sun Pharmaceutical Industries Ltd. (India).

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Table 3. Effect of Ratio of beads on particle size of itraconazole nanosuspension.

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

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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                                            | 0.747µm                                        | 0.512               | 0.754µm                                        | 0.523               |
| ITZ2      | 1                      | 2.5                                      | 50                                            | 0.516µm                                        | 0.419               | 0.523µm                                        | 0.428               |
| ITZ3      | 1                      | 2.5                                      | 60                                            | 0.696µm                                        | 0.489               | 0.707µm                                        | 0.508               |
| ITZ4      | 1                      | 3.0                                      | 40                                            | 0.379µm                                        | 0.376               | 0.392µm                                        | 0.389               |
| ITZ5      | 1                      | 3.0                                      | 50                                            | 0.283µm                                        | 0.307               | 0.294µm                                        | 0.318               |
| ITZ6      | 1                      | 3.0                                      | 60                                            | 0.324µm                                        | 0.453               | 0.358µm                                        | 0.461               |
| ITZ7      | 1                      | 3.5                                      | 40                                            | 0.501µm                                        | 0.392               | 0.515µm                                        | 0.402               |
| ITZ8      | 1                      | 3.5                                      | 50                                            | 0.438µm                                        | 0.543               | 0.448µm                                        | 0.554               |
| ITZ9      | 1                      | 3.5                                      | 60                                            | 0.492µm                                        | 0.465               | 0.505µm                                        | 0.481               |

Particle size and Size distribution
The mean particle diameter 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, and stirred at 2000 rpm with magnet 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 triplicates.

Scanning Electron Microscopy (SEM)
The lyophilized powder for 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 colorimeter between 30-300°C at a heating rate of 10°C/min with Nitrogen supply at 50.0 ml/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 500 ml of 0.1N HCl and 900 ml phosphate buffer solution (PBS) of pH 6.8 as dissolution mediums maintained at 37 ± 0.5°C and 100 rpm 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 SHIMADZU UV-VISIBLE spectrophotometer UV-1601 at 255.0 nm wavelength. Dissolution for each formulation was performed in triplicates and mean of absorbance was used to calculate cumulative percent of drug release (12).
Drug Content
Assay was carried out by taking 10 mg of lyophilized powder (weigh equivalent to 1.25 mg of drug), dissolved in 0.4 ml of tetrahydrofuran in 50 ml dry volumetric flask and then volume was made up using 0.1 N HCl. Then 4 ml of the solution was taken to 10 ml dry volumetric flask, and volume adjusted with 0.1 N HCl. The absorbance at 255.0 nm wavelength was taken using SHIMADZU UV-VISIBLE spectrophotometer UV-1601 and the drug content was calculated accordingly (13).

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 (1:3.0:50) constant. Lowest 317 nm mean particle size was achieved after 24 hrs stirring of 50:50 ratios of zirconium oxide beads. Further stirring up to 28 hrs may lead to increased particle size due to increased surface free energy.

As shown in table 3, the 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 (1:3.0:50) constant and stirring for 24 hrs. Lowest particle size of 315 nm was observed at 50:50 resulting ratio of different size of zirconium oxide beads. When the ratios of different size of zirconium oxide beads were different than 50:50, resulting nanosuspensions had higher particle size (Table 3). One possible explanation is that at this ratio beads were closely packed and lead to reduced void space.
Figure 5. XRD thermograms of bulk Itraconazole powder.

Figure 6. XRD thermograms of itraconazole nanosuspension formulation.

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

Figure 8. Dissolution profile for nanosuspension formulation (circle), pure drug (square), and marketed formulation (triangle) [mean ± SD (n=3)] in PBS pH 6.8.
between various size beads. At different ratios other than this, the void spaces were found to be higher and attrition between drug particles and beads were at maximum.

As shown in table 4, the optimized formulation showed mean particle size of 283 nm with Polydispersity index of 0.307 (before lyophilization), with 3.0 % w/v of poloxamer 407 which was 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 in lyophilization process there was no significant change in particle size and size distribution.

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

**Scanning electron microscopy (SEM)**

SEM micrographs clearly showed 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). However after formulation, particles disappeared and drug became small and uniform. The nanocrystals seem to be more rounded, perhaps because the particles were coated with a surfactant layer. In the suspension solution, the surfactant which was used to stabilize the particles could be adsorbed to surface of the crystals by hydrophobic interaction. Therefore, after lyophilization of surfactants an amorphous layer formed on the surface of inner crystals. (14)

**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 corresponds to its melting point and its exotherm 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 (15).

**X-Ray Diffraction pattern (XRD)**

X-Ray diffraction was 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 physical hardness of the active ingredient (16). Figures 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 crystallanity of nanosuspension formulation was not affected significantly (15).

**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 amount of 10% and 17% for pure drug and marketed formulation (Canditral capsule) respectively (Figure 7) in 0.1N HCl (pH 1.2) while in PBS (pH 6.8) it was 92% as compared to 13% and 20% for pure drug and marketed formulation (Canditral capsule) respectively (Figure 8). The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for six fold increase in dissolution rate.

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

**CONCLUSION**

From the results of this study it may be 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 for treatment 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. Consequently nanosuspensions represent a promising alternative to current delivery systems aiming to improve the biopharmaceutic performance of drugs with low water solubility.

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