Formulation and Characterization of Itraconazole Oral Nanosuspension: Methyl Cellulose as Promising Stabilizer

Nehal Daebis¹, Ossama Y Abdallah², Magda El-Massik¹, and Hamdy Abdelkader³*

¹Department of Pharmaceutics, Faculty of Pharmacy and Drug Manufacturing, Pharos University, Alexandria, Egypt
²Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
³Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia, Egypt

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*Corresponding author: Hamdy Abdelkader, Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia, Egypt, Tel: 002-0127-511-9494; Fax: 002-0862-369-075; E-mail: hamdy2002m@yahoo.com, h.abdelkader@mu.edu.eg

Abstract

Itraconazole (ITZ), a broad spectrum antifungal, is used orally for the treatment of wide range of systemic fungal diseases; it’s extremely poor water solubility mainly causes erratic absorption and low bioavailability. This study aimed to prepare ITZ oral nanosuspensions with enhanced stability and dissolution rate for paediatrics and geriatrics who finds difficulty in swallowing. A simple and scalable, acid-base precipitation method was adopted. Methylcellulose (MC) was investigated as a new stabilizer among other polymeric and surfactant stabilizers including poloxamer 188 (P188), poloxamer 407 (P407), sodium lauryl sulphate (SLS), polyvinyl pyrrolidone K25 (PVP) and hydroxypropylmethylcellulose E15 (HPMC) at different concentrations. The results revealed that all formulations, except for those stabilized with PVP, were in sub-micron ranges of 314-968 nm and polydispersity index of less than 0.4. Thermal analysis, X-ray diffraction studies and scanning electron microscopy imaging showed reduced crystallinity of ITZ, which resulted in enhanced dissolution rate of 10-110 fold increases in the dissolution efficiency (DE) for the prepared nanosuspension compared to untreated ITZ and comparable to the commercially available product. MC proved to be successful in achieving a stable nanosuspension.

Keywords: Itraconazole; Nanosuspension; Stabilizers; Methylcellulose; Dissolution efficiency

Introduction

Itraconazole (ITZ) 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]. ITZ has been classified as a biopharmaceutics classification scheme (BCS) Class II drug. It is also very lipophilic with an octanol/water log partition coefficient of 5.66 at a pH of 8.1 [2-4]. It is practically insoluble in water (~4 ng/ml) [5]. Therefore, the bioavailability of unformulated ITZ is extremely low.

Since 1986, ITZ has become available in market (Sporanox®) in the form of pellets in which the drug has been layered onto sugar beads. Sporanox® is produced by a fluid bed bead layering process by dissolving the drug and hydroxypropylmethylcellulose (HPMC) in an organic solvent of dichloromethane and ethanol. This solution is used to coat the sugar particles with controlled drying producing a dispersion of drug in HPMC on the pellets [6]. The resultant product provides a significant enhancement of ITZ bioavailability with approximately 55% of the administered dose absorbed [7].

Many attempts have been made to increase the dissolution rate of ITZ adopting relatively simple techniques. These included dissolution enhancement by complexation with cyclodextrin derivatives, by adsorption on ordered mesoporous silica [8] and the use of self-emulsifying drug delivery systems (SEDDS) [9]. Furthermore, many reports have demonstrated that the dissolution and oral absorption properties of ITZ were particle size dependent and both were enhanced significantly when the particle size was reduced to the nanometer range. This was proved with the work of Sun et al [10], Cerdeira et al [11] and Mou et al [12] who investigated the ability of dried ITZ nanosuspensions to enhance the drug dissolution rate and oral bioavailability.

Nanosuspensions are sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants and/or polymers [12]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [13]. These can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level can attain faster. Furthermore, because of the little use of excipients with respect to drugs, the risk of toxicity from these components is reduced [14,15]. There are many administration routes for nanosuspensions, such as oral, parenteral, pulmonary, dermal and ocular. They can be formulated as solid dosage forms for the general population, or as suspensions for paediatric or geriatric patients.

The aim of this study is to prepare ITZ oral nanosuspension formulations with enhanced dissolution rate, using a simple and scalable method. Specific objectives will look at studying the effect of different stabilizers at different concentrations, with the introduction of a new candidate stabilizer; methyl cellulose, to achieve the most stable ITZ nanosuspension. This study also aimed at converting ITZ nanosuspension into a more stable dry form by freeze drying with optimum particle size and dissolution characteristics.

Materials and Methods

**Materials**

ITZ was kindly donated by Mylan Laboratories Limited, Hyderabad, India. Sodium lauryl sulphate (SLS), mannitol and sucore were purchased from El Nasr pharmaceutical Co. Egypt. Polyvinylpyrrolidone (PVP) K25 was purchased from BASF, USA. Hydroxypropylmethylcellulose (HPMC) E15, polyethylene–polypropylene glycol, Poloxamer188 (P188), Poloxamer 407 (P407) and methylcellulose (MC) were kindly gifted by Medizen pharmaceutical Co. Egypt. All other reagents were of analytical grade.

**Preparation of ITZ nanosuspension formulations**

Nanosuspensions of ITZ were prepared using the acid-base neutralization technique [16]. ITZ (100 mg) was dissolved in 1.1 ml mixture of hydrochloric acid solution (3N) and ethanol (1:10, v/v). Various stabilizers (HPMC E15, MC, P188, P407, SLS and PVP) at different concentrations were used to prepare 12 different nanosuspension formulations (Table 1). Each stabilizer was dissolved in 10 ml sodium hydroxide solution (0.0375N). The
acid solution of ITZ was added drop wise to the sodium hydroxide solution under moderate stirring (400 rpm) for 15 minutes to produce the nanosuspension. Effects of dilution and stirring time on particle size of ITZ were investigated using the two nanosuspension formulations F7 and F11 respectively. For F7, the stabilizer HPMC was dissolved in 50 ml sodium hydroxide solution (0.0375N) instead of 10 ml, while for F11, the nanosuspension was prepared under moderate stirring for 30 minutes instead of 15 minutes.

### Preparation of dried ITZ nanosuspensions by lyophilisation

Two lyophilized ITZ nanosuspension formulations were prepared from F10 employing mannitol (F.D.1) and sucrose (F.D.2) as cryoprotectants. Each cryoprotectant (50% of the weight of ITZ) was added, with moderate stirring, to a volume of the ITZ nanosuspension, equivalent to 300 mg ITZ and containing MC as the stabilizer (MC:ITZ, 0.4:1 w/w). The resulting nanosuspensions were then transferred into 30ml Falcon tubes. Thereafter, the nanosuspensions were frozen by immersing the Falcon tubes in liquid nitrogen. The freeze drying was performed at a temperature of -50°C with a pressure below 1 mbar and the vials were removed after 48 hours of drying. Each formulation was produced in triplicate. A control lyophilized formulation, F.D.3, was prepared without any cryoprotectant for comparison.

### Particle size analysis

The average particle sizes of ITZ nanosuspension formulations were measured using photon correlation spectroscopy (Zetasizer, Nano ZS, Malvern, UK) at 25°C. Prior to measurements, the samples were diluted (1:1000) with previously filtered distilled water. All measurements were performed in triplicate and the average value was used. Lyophilized ITZ nanosuspensions (F.D.) were also subjected to this test after reconstitution of 5 mg formulations in 10 ml distilled water and further diluted prior size measurements.

### Morphology studies

**Transmission electron microscopy for nanosuspensions:** ITZ nanosuspension formulations (F7 and F11) were imaged using a transmission electron microscope, JEOL, JEM-100S, Tokyo, Japan. The samples were placed on a carbon-coated copper grids and air dried prior to imaging.

**Scanning electron microscopy for lyophilized nanosuspensions:** Morphological examination of ITZ coarse particles and lyophilized ITZ nanosuspensions were performed using a scanning electron microscope (SEM, JSM-5300, JEOL, Japan) with an accelerating voltage of 10 kV. Prior to imaging, the mounted samples were coated with gold.

### Differential scanning calorimetry (DSC)

Thermal analysis of ITZ, sucrose, mannitol and the three lyophilized nanosuspension formulations were studied using DSC (DSC-60, Shimadzu, Japan). An ITZ sample of 2-3 mg or its equivalent weight was loaded into an aluminum pan and the lid was crimped using a Shimadzu crimper. The thermal behaviour of each sample was recorded under nitrogen at a heating rate of 10°C/min, covering temperature ranges of 30–300°C. The extrapolated onset temperature was used to define the melting point (T_m).

**X-Ray powder diffraction (XRPD):** XRPD patterns were determined for dried ITZ nanosuspension without cryoprotectant (F.D.3), ITZ using an X-ray diffractometer (PW 1830/40, Philips, Netherlands). The XRPD was performed in a symmetrical reflection mode using Cu-Kα radiation with λ= 1.54 Å (40 kV and 30 mA). The sample was placed on a flat aluminum sample holder. Data were collected by scanning from 10° to 80° with 0.01° steps, and the measuring time per step was 0.5 s.

### In-vitro release study

**In-vitro drug release was determined for all the prepared ITZ formulations under study following the FDA dissolution method for Sporanox® [17]**. Dissolution experiments were performed in a dissolution USP apparatus 2 (Hanson, 107081172, Hanson research cooperation, USA). The dissolution medium consisted of 900 ml of 0.1N hydrochloric acid solution (pH 1.2). The amount of sample for each experiment was equivalent to 100 mg of ITZ. The dissolution experiments were performed at 37 ± 0.5°C with a paddle speed of 100 rpm. Aliquots of 5 ml were withdrawn at 10, 20, 30, 45, 60 and 90 minutes, and then replaced by an equal volume of fresh dissolution media. The solution was then filtered through a 0.22 µm syringe filter. The first 2 ml were discarded and the remainder was diluted with the dissolution medium and analyzed spectrophotometrically at λmax 254 nm (UV-1800 Shimadzu Spectrophotometer, Shimadzu, Kyoto, Japan). All dissolution data represent the mean of triplicates. The dissolution efficiency (DE) of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It is calculated according to the following equation [18]:

\[
DE = \frac{\int y \times dr}{y_{100} \times t} \times 100%
\]

Where y is the drug percent dissolved at time t. Dissolution efficiency (DE) % after 30 minutes was calculated for some selected formulas. Areas under the curves were estimated using the trapezoidal method.

### Stability study

To assess the physical stability of ITZ formulations, selected ITZ nanosuspensions (F1, F2, F3, F4, F7, F8, F9 and F10) were stored in clean well closed dark glass bottles and kept at room temperature (25°C). These formulations were assessed by monitoring dissolution over one week period of time (fresh, day 2, day 5 and day 7) to select the most appropriate nanosuspension for further processing (lyophilisation).

Another long term stability study was conducted on the optimized ITZ nanosuspension (F10) stored in a clean well closed dark glass bottle and kept in the refrigerator (4°C) for 9 months. This formulation was assessed for any change in the average particle size

### Table 1: Composition of ITZ nanosuspension formulations and % dissolution efficiency after 30 minutes (% DE-30)

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Stabilizer</th>
<th>Stabilizer:ITZ ratio</th>
<th>% DE-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Poloxamer 188</td>
<td>0.5:1</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>F2</td>
<td>Poloxamer 188</td>
<td>1:1</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>Poloxamer 407</td>
<td>0.5:1</td>
<td>49 ± 2.5</td>
</tr>
<tr>
<td>F4</td>
<td>Poloxamer 407</td>
<td>1:1</td>
<td>-</td>
</tr>
<tr>
<td>F5</td>
<td>SLS</td>
<td>0.5:1</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>F6</td>
<td>PVP K25</td>
<td>0.5:1</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>F7</td>
<td>HPMC E15</td>
<td>0.5:1</td>
<td>38 ± 2.5</td>
</tr>
<tr>
<td>F8</td>
<td>HPMC E15</td>
<td>1:1</td>
<td>-</td>
</tr>
<tr>
<td>F9</td>
<td>MC</td>
<td>0.5:1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>F10</td>
<td>MC</td>
<td>0.4:1</td>
<td>-</td>
</tr>
<tr>
<td>F11</td>
<td>MC</td>
<td>0.3:1</td>
<td>-</td>
</tr>
<tr>
<td>F12</td>
<td>MC</td>
<td>0.2:1</td>
<td>-</td>
</tr>
</tbody>
</table>
and polydispersity index by photon correlation spectroscopy using Zetasizer Nano ZS (Malvern) at 0, 3, 6, and 9 months of storage. It was also assessed for any change in the in vitro drug release profile by monitoring dissolution at different time intervals over a period of 4 months.

**Statistical analysis**

Paired two tailed t-test was carried out with a 5% significance level using Microsoft Excel 2007 software to investigate some formulation variables on the particle size and release of prepared formulations under study. Difference factor (f1) was also calculated to compare dissolution profiles, using DD Solver program. The difference factor (f1) measures the percent error between two curves over all time points.

**Results and Discussion**

**Effect of dilution and stirring time on particle size**

To optimize the different parameters of the preparation technique; such as dilution and stirring time, different ITZ nanosuspensions were investigated for particle size and polydispersity index employing photon correlation spectroscopy.

The results revealed that particle size and polydispersity index of ITZ nanosuspension F7 formulated using HPMC as a stabilizer (stabilizer:ITZ 0.5:1) changed from 682.3 nm ± 55 nm (PDI 0.38 ± 0.05) to 828 nm ± 86.3 µm (PDI 0.48 ± 0.104) after dilution. This indicates that dilution significantly increased ITZ particle size (p < 0.01) which could be attributed to lower HPMC (stabilizer) concentration and accordingly the dispersed nanoparticles were prone to aggregation and size enlargement. The use of an inadequate amount of stabilizer did not provide a complete coverage of the drug molecule surface needed to provide steric stabilization of the suspension [19].

The stirring effect was also investigated on ITZ nanosuspension (F11) formulated using MC as a stabilizer (stabilizer:ITZ 0.3:1). Doubling the stirring time increased particle size and polydispersity index significantly from 844.3 nm ± 41 µm (PDI 0.49 ± 0.057) to 945.8 nm ± 0.86 µm (PDI 0.496 ± 0.137) with p = 0.0305 (p < 0.05). This shows that increasing the stirring time does not result in smaller ITZ particle size.

**Effect of type and concentration of stabilizers on particle size**

To gain more insights into the manufacture of nanosuspensions, six different stabilizers were investigated at different concentrations. The stabilizers used included polymers such as HPMC E15, PVP K25 and MC as well as the anionic surfactant (SLS) and the non-ionic surfactants P188 and P407. Four different MC:ITZ ratios; 0.2:1, 0.3:1, 0.4:1 and 0.5:1 were also tested.

**Effect of stabilizer type**

Figure 1 shows both average particle size diameters and polydispersity index (PDI) values for 6 formulations having a stabilizer:ITZ ratio of 0.5:1 w/w. The formulations tested were F1 (P188), F2 (P407), F5 (SLS), F6 (PVP K25), F7 (HPMC E15) and F9 (MC). Poloxamers-stabilized nanosuspensions demonstrated the lowest particle sizes, PVP-stabilized nanosuspension showed the largest ones, whereas the average sizes of SLS and cellulose-stabilized nanosuspensions were intermediate (Figure 1). P188 (F1) and P407 (F2) stabilized nanosuspensions showed average particle sizes (z-average sizes) of 499 and 314 nm respectively. These results are supported by the results reported by Pandey [20], where the optimized ITZ formulation, prepared by pearl milling technique, showed an average size of 283 nm, when 3.0% w/v of poloxamer 407 was used as a stabilizer. Sun, et al. [10] also reported that P407 stabilized suspension produced the most efficient particle size reduction of ITZ nanosuspension prepared by high pressure homogenization.

Poloxamers have the same overall molecular structure: linear ABA triblock polymer chain (A stands for hydrophilic polyethylene oxide (PEO) segment and B stands for hydrophobic polypropylene oxide (POO) segment). The hydrophobic POO chains can drive the polymer to be adsorbed on the surface of drug particles, while the hydrophilic PEO chains surround the drug particles providing a sterically hindrance against aggregation. The P188 has a lower molecular weight than the P407 (8400 g/mol vs. 12,600 g/mol), which may exert less kinetic restriction in the adsorption process and faster diffusion [21]. Furthermore, the non-ionic surfactants poloxamers were significantly more efficient than the anionic surfactant SLS in producing nanosuspensions. These findings warrant further investigation of the effect of hydrophilic-lipophilic balance (HLB) on stabilization of nanosuspensions.

It can be concluded from Figure 1 that polymeric stabilizers were less effective (z-average > 680 nm) than surfactants (< 650 nm) in terms of particle size reduction. This is in agreement with Sun, et al. [10], who reported that polymeric stabilizers were also less effective (z-average >1500 nm) than surfactants (z-average <550 nm) when used as stabilizers for the preparation of ITZ nanosuspension by high pressure homogenization.

In general, surfactants can provide more effective wetting of the drug particles than macromolecular hydrocolloids (PVP, HPMC and MC). This would result in a better dispersion of the drug particles and less aggregation. Furthermore, the higher molecular weight of P407 (vs. P188) and its relatively longer polymer chains can lead to a higher degree of steric stabilization for the drug particles, which can enhance particle dispersion and result in a greater extent of size reduction.

Growth of ITZ particles was obviously enhanced with PVP. This can be explained as follows: (1) there was low affinity between PVP and the newly formed ITZ crystal during the process of acid–base neutralization; (2) PVP was not effective enough to disperse ITZ nanoparticles and inhibit aggregation of nanoparticles at the high supersaturated concentration created by acid–base neutralization due to relatively lower viscosity compared with HPMC and MC; (3) PVP adsorbed onto ITZ nanoparticles acted as an impurity that enhanced the growth rate of crystals [16].

**Effect of stabilizer concentration**

To investigate the effect of stabilizer concentrations on particle size and polydispersity index of ITZ nanosuspension, four
nanosuspensions with different MC:ITZ ratios were prepared and measured using photon correlation spectroscopy. The choice of methylcellulose as the stabilizer for studying the effect of stabilizer concentration was based on its well-known properties as a suspending agent for orally administered liquids and its ability to delay the settling of suspensions and increase the contact time of drugs in the stomach [22].

Most of the experimental work reported on ITZ nanosuspensions in the literature was dedicated to other polymeric stabilizers such as HPMC and non-ionic surfactants such as poloxamers, neglecting MC despite its promising properties, even in low concentrations, to retard aggregation and inhibit Ostwald ripening of nanocrystals [16,20].

The influence of different concentrations of MC on the average size of ITZ nanosuspensions was studied (Table 2). The average size was significantly decreased from 490.2 ± 60.7 nm (PDI 0.54 ± 0.057) to 394.1 ± 47.4 nm (PDI 0.33 ± 0.003) when the concentration of MC was increased from 30% to 40% (MC:ITZ 0.3:1 to 0:4:1). No further decreases of particle sizes (967.7 nm) were recorded when MC concentration reached 50% (MC:ITZ 0.5:1). On the contrary, the Z-average size increased significantly (967.7 ± 53.1 nm). It was not preferable to use 0.2:1 MC:ITZ ratio, although yielding reasonable average particle size of 450.7 ± 4.24 nm (PDI 0.45 ± 0.041), as this low concentration might not provide complete surface coverage of ITZ nanocrystals needed to provide steric repulsion between nanoparticles in the suspension. Therefore, 40% MC was used as the optimum concentration for preparing ITZ nanosuspension for further processing (lyophilization).

**Transmission electron microscopy of nanosuspensions**

The size and morphology of ITZ nanosuspensions for F7 and F10 stabilized by HPMC and MC at 0:5:1 and 0:4:1 respectively were observed using the transmission electron microscope (Figure 2). HPMC-stabilized (F7) formulation shows spherical aggregated ITZ nanocrystals (130–170 nm), whereas MC-stabilized formulations (F10) revealed spherical ITZ nanocrystals (170–297 nm). Both HPMC and MC can coat the surface of nanocrystals and provide steric stabilization but clearly MC seems to be more promising in inhibiting the growth and aggregation of formed ITZ nanocrystals.

**Table 2: Particle size (nm) and dissolution efficiency % after 30 (DE% 30) minutes of different ITZ nanosuspensions with different MC : ITZ ratios.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>MC : ITZ Ratio</th>
<th>z-average ± SD(nm)</th>
<th>DE% 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>F12</td>
<td>0.2:1</td>
<td>450.7 ± 2.44</td>
<td>54.5</td>
</tr>
<tr>
<td>F11</td>
<td>0.3:1</td>
<td>490.2 ± 6.07</td>
<td>51.2</td>
</tr>
<tr>
<td>F10</td>
<td>0.4:1</td>
<td>394.1 ± 47.4</td>
<td>53.4</td>
</tr>
<tr>
<td>F9</td>
<td>0.5:1</td>
<td>967.7 ± 53.1</td>
<td>29.7</td>
</tr>
</tbody>
</table>

**Figure 2: TEM micrographs for ITZ Nanosuspension F7 with HPMC:ITZ ratio of 0.5:1 (left) and ITZ nanosuspension F10 with MC:ITZ ratio of 0.4:1 (right).**

**In vitro release studies**

Figure 3 shows different dissolution profiles of untreated ITZ, commercial nanosuspension and ITZ from the prepared nanosuspension formulations (F1, F3, F5, F6, F7 and F9). Due to the extremely low solubility of ITZ (2µg/ml), it was not possible to achieve sink conditions throughout the dissolution experiments. Thus, all experiments were performed under non-sink conditions. Untreated ITZ showed extremely poor dissolution rate where less than 2% was dissolved over 90 minutes. This can be attributed to poor wettability and extremely poor solubility of the coarse ITZ particles. On the other hand, all ITZ nanosuspension formulations demonstrated comparable release rate to the commercial product but showed highly significant (P < 0.001) faster dissolution rates compared with untreated ITZ. Percentage dissolution efficiency at 30 minutes (% DE 30) for the prepared nanosuspensions demonstrated 10- to 110-fold higher than that for untreated ITZ (Table 2). The linear portion of each curve was quite brief as saturation solubility was quickly reached. All formulations, with the exception of SLS-stabilized nanosuspension, exceeded 60% release after one hour followed by a plateau. It is worth noting that whilst ITZ-suspension stabilized by PVP was in the micro-scale, the dissolution rate was comparable to other nanosuspensions. This indicates that at least part of the primary particles was nanosized, although they were aggregated to larger clusters. The aggregated particles were likely to disintegrate into submicron sizes without time lag, resulting in a fast drug release rate like in the case of the nanosuspensions [23]. Furthermore, the hydrophilic PVP surrounding the ITZ particles may improve the dissolution rate to some extent also by a wetting effect [24].

Table 1 shows also DE% at 30 minutes for the tested formulations. Superior dissolution efficiency was estimated for F1 and F3, poloxamers-stabilized nanosuspensions, of the lowest average nano-sizes. On the contrary, SLS-stabilized nanosuspension showed significantly lower dissolution rate compared with PVP-stabilized formulation, although SLS-stabilized formulation is in sub-micron sizes. PVP is a hydrophilic macromolecular polymer which diffuses out slowly compared with small detergent molecules of SLS. This is likely due to better drug wettability in the micro-diffusion layer of PVP-stabilized formulation compared with SLS-stabilized one.

The effects of different stabilizer concentrations on the release profile of ITZ nanosuspension were also assessed by comparing % DE 30 of four different ITZ nanosuspensions (F9, F10, F11 and F12).
with different MC:ITZ ratios. The results showed that the respective values were 30, 53, 51 and 55. These results demonstrated that the relation between the average particle size (nm) of ITZ nanocrystals and the dissolution behaviour; as particle size decreases, surface area increases, thereby leading to improved dissolution rates.

**Stability studies**

The biggest challenge facing the pharmaceutical nanosuspensions is the stability issue. Good physical stability is related to how long the stabilizer maintains the original and homogeneous sizes of the nanocrystals. In this study, preliminary short-term physical stability of different ITZ nanosuspensions at 25°C was investigated over one week period of time to evaluate whether the nanosuspension was sufficiently stable for further processing such as freeze drying.

Figure 4 shows that ITZ nanosuspension formulations (F1, F2, F3 and F4) formulated using P188 and P407 as stabilizers (non-ionic surfactants) are unstable at room temperature due to decrease in % drug released from 60% to 20% after one day. However, there was no remarkable change in the dissolution profiles of the other ITZ nanosuspension formulations (F7, F8, F9 and F10) using HPMC and MC as polymeric stabilizers the following day showing better stability than poloxamers at room temperature. Long swinging hydrophilic chains on the particle surface provide an optimal steric hindrance, which prevents the particles from aggregating. Moreover, the poorly soluble drugs and homogeneous particles hinder the dissolution of smaller particles and growth of larger particles, i.e. Ostwald ripening [24]. Similarly, it was reported that the good physical stability of ascorbyl palmitate nanosuspensions was attributed to the inhibition of Ostwald ripening by the steric stabilization effect of stabilizers [25].

Comparing the dissolution profiles of all ITZ nanosuspension formulations after 5 days, ITZ nanosuspension (F10) formulated using MC:ITZ ratio 0.4:1 showed better stability compared to other nanosuspensions making it a suitable candidate for further processing into the lyophilized form. To investigate the physical stability of ITZ nanosuspension formulation (F10) stored at 4°C, average particle sizes and polydispersity index were monitored for a period of 9 months and dissolution profiles were monitored for a period of 4.5 months.

Figure 4: Dissolution profiles of different ITZ nanosuspensions (A) F1, (B) F2, (C) F3, (D) F4, (E) F7, (F) F8, (G) F9 and (H) F10 when freshly prepared and after storage for a duration of 1-7 days at room temperature.
As shown in Figure 5 average particle sizes of ITZ started to increase by time indicating agglomeration and growth of ITZ nanocrystals (Ostwald ripening). However, no significant changes were recorded in the PDI of the nanosuspension during the first 6 months indicating homogeneity of the nanocrystals. After 9 months of storage, the nanosuspensions turned to become a microsuspension with an average particle size exceeding 1µm and a very high polydispersity index of one. Therefore, steric stabilization effect of hydrophilic polymer methylcellulose was able to stabilize ITZ nanosuspension stored at 4°C within a period of 6 months only in terms of maintaining the nanometer size range.

There was no significant change in the overall dissolution profile of ITZ nanosuspension after 1 week reaching a plateau value around 65% \((p = 0.404, >0.05)\). However, after 2 weeks, a significant decline in the dissolution pattern to 60% was observed \((p = 0.001138, <0.05)\). The lowest drug release was recorded after 4.5 months reaching a plateau of 50% after one hour. This is in agreement with the findings that ITZ crystallizes upon storage and tends to agglomerate. This in turn increases the particle size which would be reflected on lower dissolution profiles and drug release as described by Noyes-Whitney equation. These findings reinforce the necessity to convert the nanosuspension into a dry form by lyophilization.

**Characterization of ITZ lyophilized nanosuspensions**

Based on the above results, MC-stabilized nanosuspension was chosen for preparing lyophilized ITZ nanosuspensions (MC:ITZ ratio, 0.4:1). Prior to lyophilization, cryoprotectants (sucrose and mannitol) were dissolved into the nanosuspensions as matrix-formers. The traditional matrix-formers sucrose and mannitol were chosen as being commonly used and readily available excipients. To evaluate the effect of matrix formers on lyophilized ITZ nanosuspensions, a third nanosuspension, as a control, was lyophilized in absence of any of the two matrix formers.

**Characterization of ITZ crystalline state**

The crystalline state of ITZ after lyophilization was evaluated using DSC and XRD investigative studies (XRPD). Untreated ITZ showed a single sharp endothermic peak due to melting at 167°C; mannitol showed a single sharp endothermic peak due to melting at 166°C. Similar patterns were reported with pure ITZ (167°C) and sucrose (189.5°C).

**Figure 5:** Particle size (nm) and polydispersity index of ITZ nanosuspension using MC as a stabilizer (F10) at 0, 3, 6 and 9 months of storage at 4°C.

FD.1 showed partially resolved two peaks of much lower intensity at 142.2°C and 151.5°C (Figure 6); FD.2 showed a broad endothermic peak at 144.2°C; FD.3 showed a broad endothermic peak with an onset of 131°C and end-set at 195°C. In case of FD.2 and FD.3, the broad endothermic peak with an onset of 34.4°C and end-set of 81.6°C was attributed to the release of the adsorbed moisture from the sample containing the amorphous polymer MC as a stabilizer. The shift of ITZ peaks to lower melting temperatures were observed simultaneously in all lyophilized formulations. This could also be explained by the presence of the stabilizers in the samples which could be captured on the surfaces of the nanocrystals and also due to the smaller particle size [24]. Peak broadening observed with FD.2 and FD.3 could be attributed to the transformation of ITZ from crystalline into crystallites/or a partially amorphous form. Similar results were reported elsewhere; Bernard, et al. [26] reported that ITZ peaks showed some peak broadening in the nano-sized product, lyophiliized without matrix former; which can originate from smaller particle sizes (crystallites) and/or from stress and strain due to the milling process. As well as ITZ melting peak tended to shift to a lower temperature in the nanosized products (158.2 ± 0.4°C).

Supporting to DSC results, the crystalline state of ITZ before and after lyophilization without matrix former (FD.3) was examined using XRPD (Figure 7). Characteristic peaks of crystalline ITZ completely disappeared, thereby indicating that ITZ existed as an amorphous state in the dried nanosuspension without matrix former: The diffraction pattern of FD.3 exhibited characteristic peaks at 2θ 30 32 which represents sodium chloride derived from

**Figure 6:** DSC thermograms of untreated ITZ, different cryoprotectants used and lyophilized nanosuspensions.
the acid–base neutralization reaction [27]. Therefore, it could be concluded that ITZ was amorphous in dried ITZ nanosuspension. A similar observation was also reported by Mou, et al. [16] with the dried ITZ nanosuspension (HPMC:ITZ 0.5:1) without matrix former.

**ITZ particle size measurements and morphology of lyophilized formulations**

The average particle sizes of lyophilized ITZ nanosuspension formulations were measured after reconstitution. The average sizes and PDI values of reconstituted ITZ nanosuspensions for F.D.1 (mannitol) showed the smallest particle size (308.9 ± 33.8 nm) and PDI of 0.487 ± 0.104, whereas F.D.2 (sucrose) demonstrated average size of 591.3 ± 14.78 nm and PDI of 0.639 ± 0.059. F.D.3 (no cryoprotectant added) had an average particle size of 410.3 ± 17.39 nm and PDI of 0.464 ± 0.136. The average size of lyophilized ITZ nanoparticles was highly dependent on the type of cryoprotectant added. For example, the average size for sucrose-lyophilized formulation was markedly larger (1.5-fold greater) than that for the original nanosuspension (394.1 ± 47.4 nm), whereas mannitol-lyophilized nanosuspension showed a slightly lower size.

SEM micrographs of the lyophilized formulations are shown in Figure 8. Obvious differences in morphology were recorded for untreated ITZ powder and lyophilized nanosuspension formulations. The particles of untreated ITZ were found to be large and orthorhombic crystals with a particle size of approximately 15 µm, relatively more uniform spheres were recorded for lyophilized formulations F.D.1, F.D.2 and F.D.3 with an average size of 103-195 nm, 144-153 nm and 313-441 nm respectively. These results are in agreement with the results obtained by photon correlation spectroscopy using Zetasizer Nano ZS (Malvern) where ITZ nanosuspension formulation lyophilized using mannitol as the cryoprotectant showed the smallest particle size of ITZ nanocrystals. Similarly, Pandey [20] reported small and uniform particles after lyophilization indicated by SEM of lyophilized nanosuspension formulation using mannitol as a cryoprotectant (1:1 ratio). A similar observation was also reported by Sun et al [10] where the average size of lyophilized ITZ nanocrystals (without cryoprotectant) prepared by high pressure homogenization was approximately 300 nm with a relatively narrow size distribution.

**In vitro release studies of lyophilized formulations**

Since the main attribute of a nanosuspension is the increased dissolution rate resulting from the high specific surface area of the particles, dissolution of the powders was performed as a pharmaceutical evaluation. It is important to realize that nanoparticles agglomeration is likely to be inevitable during drying of a nanosuspension to which no additional matrix formers are added. Aggregations could decrease the dissolution rate of the drug. However, it was possible for dried drug nanosuspensions to maintain the high dissolution rate of nanosuspensions if the aggregation was easy to be redispersed in water in the presence of matrix formers (such as mannitol and sucrose) [16]. From the point of dissolution performance, the key question is therefore not whether nanoparticle agglomeration will occur or not, rather the central question is how easily the agglomerates break up during dissolution [28].

Results revealed that no significant differences between the dissolution profiles of the three lyophilized formulations after 60 minutes were observed (p values were 0.4836, 0.448 and 0.35, > 0.05) F.D.1 and F.D.2 showed release patterns similar to that of F.D.3 formulation with difference factors (f1= 3.76 and 3.26 respectively) and F.D.1 also showed similar release pattern to that of F.D.2 with difference factors (f1) of 0.97 indicating similar release patterns of all three lyophilized formulations which show that the type and presence of a cryoprotectant did not influence the release profile of the lyophilized ITZ formulations.

**Conclusion**

Itraconazole (ITZ) is a potent and broad spectrum antifungal drug, but its extremely poor aqueous solubility hinders consistent bioavailability. A simple and scalable technique was adopted to prepare ITZ nanosuspensions. These novel formulations demonstrated comparable in vitro dissolution profiles and acceptable physical stability to the commercially available product. This study also warrants methyl-cellulose (MC) as a potential stabiliser for nanocrystal formulations.

**References**


