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Formulation and Characterization of Itraconazole as Nanosuspension Dosage Form for Enhancement of Solubility

Asmaa M. Rashid*,1 and Shaimaa N. Abd-Alhammid*

*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Itraconazole (ITZ) is a triazole antifungal agent given orally for the treatment of oropharyngeal and vulvovaginal candidiasis, for systemic infections including aspergillosis, candidiasis, and for the prophylaxis of fungal infections in immunocompromised patients.

The objective of the study was to formulate a nanosuspension for a practically water-insoluble ITZ to increase aqueous solubility and improve its dissolution and oral bioavailability.

ITZ nanosuspension was produced by a solvent-antisolvent nanoprecipitation method in the presence of different stabilizers (Poloxamer-188 and HPMCE5) at different ratios with the drug alone or combination with surfactants (tween 80 and SLS).

The results exhibit that the particle size values of all prepared itraconazole formulations were in the nano size range. The best formula (F6) has a particle size of 42 nm and zeta potential of - 21.86 mV. *In vitro* cumulative release percent from the nanosuspension was 88 % at 30 min and from lyophilized nanoparticles was 98.2% when compared to the pure drug 13.5%. Additionally, the effects of different parameters on the prepared formulas were investigated.

Several characterization methods were done for the optimized nanoparticles prepared by lyophilization technique, such as FTIR, DSC, XRD and SEM, which showed smooth uniform particles within the nano size. FTIR shows no change in the position of the ITZ nanosuspension functional group; XRD and DSC show no significant change in the crystallinity of lyophilized nanoparticles.

Thus, nanosuspension appears to be a promising approach to increase the water solubility and the dissolution rate of ITZ.

Keywords: Itraconazole, Nanoprecipitation method, Nanosuspension

تصييغ وتوصيف وتقييم عقار الاتروكانازول كمعلق نانوي لزيادة الذوبانية

اسماء محمد رشيد*،١ و شيماء نزار عبد الحميد*

*فرع الصيدلانيات ،كلية الصيدلة، جامعة بغداد، بغداد،العراق.

الخلاصة

عقار الاتراكونازول من الادوية المضادة للالتهابات الفطرية كداء المبيضات الفموي-البلعومي وفطريات المبيضات المهبلية وداء الرشاشيات وللوقاية من الفطريات المرافقة لالتهابات نقص المناعة.

الهدف من هذه الدراسة هو تحضيير وتقييم معلق نانوي للاتراكونازول كشكل دوائي فموي لتحسين قابلية ذوبانه من اجل الحصول على شكل صيدلاني فموي مستقر.

الاتراكونازول كمعلق نانوي تم تحضيره باستخدام طريقة الترسيب بالمذيب ومضاد المذيب باستعمال مثبتات مختلفة مثل (بوليكسمر ١٨٨, هايدروكسي بروبيل مثيل سيليلوز E ٥ وبتراكيز مختلفة لوحدها او مع مثبت مساعد مثل (tween 80,SLS) مع دراسة تأثير عوامل مختلفة على التحضير.

اظهرت النتائج ان الحجم الحبيبي للجسيمات لعقار الاتراكونازول كانت ضمن حجم النانو لجميع التركيبات وكانت افضل صيغة رقم(٦) لها حجم حبيبي (٤٢) نانومتروجهد سطحي (-٢١,٨٦) ملفولت باستخدام مثبت مستقر(بوليكسمر-١٨٨) ومثبت مساعد (تووين ٨٠) وبنسب١:٢:٢.

وتراوحت النسبة المئوية لانحباس الدواء (٨٨%)في الدقيقة ٣٠ للصيغة السائلة و٩٨% للمعلق النانوي الجاف مقارنة بالعقار الاصلي والذي يبلغ ١٣,٥% لنفس المدة .خضعت الصيغة المختارة لتقييمات مختلفة لغرض فحص الحالة البلورية للمسحوق النانوي المجفف FTIR , DSC XRD,SEM , وكانت النتائج تشير الى كونها جزيئات ذات حجم نانوي وذات سطح ناعم مما يشير الى ان تقنية تصنيع المعلق النانوي لعقار الاتراكونازول بالترسيب بالمذيب من الطرق الواعدة والفعالة لصياغة اتراكونازول ذو قابلية ذوبان عالية وانتشار سريع.

الكلمات المفتاحية : اتراكونازول ، معلق نانوي،،الترسيب بالمذيب .

Introduction

Low bioavailability is one of the serious problems correlated with poorly soluble drugs. Foremost struggles have been ended for the development of customized drug carriers to overcome the disappointing *in- vivo* fates of the drug. Hence, there is a growing necessity for a unique strategy that can tackle the formulation associated problems related to the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy concerning pharmacoeconomics. Various solubilization techniques have been used for the improvement of solubility and drug dissolution rates including a reduction of the particle size , by micronisation or

[1]Corresponding author: E-mail: asmaamohammad68@gmail.com
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nanonisation to increase the surface area, use of surfactants, cyclodextrin complexation, pro-drug formation, conversion of crystalline to amorphous forms, polymeric conjugates and solid dispersion[1]

Nanosuspension has shown to be a more cost-effective and technically simpler method. It can be defined as the dispersion of particles in the nanometer size range, where stabilization is accomplished by the addition of surfactants, stabilizer or a combination of both [2,3].

Nanosuspension consists of a poorly water-soluble drug without any matrix material suspended in dispersion resulting in the formulation having high dissolution velocity and increased saturation solubility [4]. It can be applied to several administration routes such as oral, parenteral, pulmonary, ophthalmic, and nasal routes [5].

ITZ is an active triazole, antifungal agent($C_{35}H_{38}Cl_2N_8O_4$), which is active against a broad spectrum of fungal species including Cryptococcus, Candida, Aspergillus, Blastomyces and Histoplasma capsulatum. It inhibits lanosterol 1,4- demethylase, the enzyme that converts lanosterol to ergosterol [6,7].

ITZ is belonging to class II as classified by the biopharmaceutical classification system (BCS), soluble in lipids and has a pKa of 3.7 [8]. ITZ has the characteristic of pH-dependent solubility having the highest solubility at the acidic media (4µg/ml) compared to basic pH (1µg/ml). It is slightly soluble in alcohols and freely soluble in dichloromethane and practically insoluble in water[9], indicating that poor aqueous solubility is the main reason for lower plasma concentrations. ITZ at a low pH, in gastric juice, will be ionized and therefore the gastric acidity is essential for adequate dissolution and its oral bioavailability was increased when taken with food [7,10].

In nanoprecipitation method, the drug is dissolved in a suitable solvent. This solution is mixed with a miscible antisolvent system in the presence of surfactants. Rapid addition of drug solution into the antisolvent leads to the sudden supersaturation of drug in the mixed solution forming ultrafine drug solids. Precipitation method involves two phases - nuclei formation and crystal growth [10].

The objective of this study is to optimize and characterize the formulations prepared by the nanoprecipitation method for the preparation of ITZ nanosuspension.

Materials and Methods

Materials

ITZ powder was purchased from Baji Gaokang Bio-Technology Co., Ltd., China. Tween 80 was purchased from Scharlau S. L Spain. Poloxamer 188 and HPMC E5 were provided by Hangzhou hyper chemicals limited, Zhejiang, China. Dialysis membrane70 provided by HIMEDIA (Mumbai, India). All other chemicals and solvents were of analytical reagent grade.

Methods

Saturation solubility determination

Solubility studies for the pure drug were achieved in distilled water, 0.1N HCl(pH 1.2) and phosphate buffer pH 6.8. In each case, the excess amount of sample added to 10 ml of solvent and agitated at 25°C in a rotary test tube shaker for 72 hrs. After equilibration, samples were filtered using 0.45 µm millipore filters, suitably diluted with the respective solution, and analyzed by measuring the absorbance at the determined wavelength of max. absorbance (256) nm, when this solution was scanned in the UV range from 200- 800nm using UV-Visible spectrophotometer, in 0.1 N HCl and (263)nm in methanol, D.W, and phosphate buffer pH 6.8 to determine the amount of the drug dissolved [11,32].

Preparation of Itraconazole nanosuspension by precipitation method

Nanosuspension precipitation method is used to prepare nanosuspension of ITZ using different concentrations of polymer and surfactant. In brief, 50 mg of ITZ was dissolved in an organic solvent (° ml of methanol). The aqueous solution containing the selected stabilizers p-188 and HPMC-E5 at different ratios (1:1), (1:2) and (1:4) drug to stabilizers, or in combination with co-surfactant (tween 80 and SLS), which acts as the antisolvent system. This was followed by the addition of the organic solution into stabilizer/surfactant aqueous solution at a rate (1ml /min) by the help of syringe, under high-speed mechanical agitation of 3000 rpm using, homo disperser for 30 min at 25±1°C to allow the organic solvent to evaporate and get the desired nanosuspension [13] **.** The batches were prepared according to the formulation design (Table 1)**.**

Table 1. Composition of itraconazole nanosuspension formulation.

Formula no.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
ITZ(mg)	50	50	50	50	50	50	50	50	50	50	50
P-188(mg)	50	100	200	400	100	100	200	200			
HPMCE5(mg)									50	100	200
Tween80(ml)						0.1					
SLS (mg)					50						
Methanol(ml)	5	5	5	5	5	5	5	5	5	5	5
DW(ml)	20	20	20	20	20	20	20	20	20	20	20
Speed(rpm)	3000	3000	3000	3000	3000	3000	1500	500	3000	3000	3000

F: Formula, ITZ: Itraconazole, P-188: Poloxamer 188, SLS: Sodium lauryl sulphate ,HPMCE5 :Hydroxypropyl -methylcellulose, rpm: Revolution per min.

Characterization of the prepared nanosuspension: Particle Size and Polydispersity Index (PDI)

Particle size analysis and PDI of the prepared ITZ nanosuspension were measured by dynamic light scattering (DLS) using Nano Brook 90Plus particle size analyzer (Brookhaven instruments. USA). Measurements were performed in triplicate by measuring the intensity of light scattered by the molecules in the sample as a function of time at 90° scattering angle and constant temperature 25 °C. The PDI was determined which measured the width of the size distribution of each formula, it is an index of spread or variation within the particle size [5,14].

Determination of entrapment efficiency (%DEE)

Ten ml of freshly prepared nanosuspension was centrifuged at 6000 rpm for 20 minutes using ultracentrifuge. The supernatant solution was filtered and separated. One ml of this filtrate was diluted with 10ml water, and the absorbance at maximum λ max was measured by UV spectrophotometer using water as blank. The amount of unincorporated drug in the formulations was measured, and the entrapment efficiency was calculated by subtracting the quantity of free drug in the supernatant from the initial amount of drug taken (eq.1). The outcomes were analyzed in triplicate[15,20].

$$DEE\% = \frac{(total\ drug\ in\ the\ formula - free\ drug)}{total\ drug\ in\ formula} \times 100 \quad (1)$$

Determination of Zeta Potential

The Zeta potential of the selected formula of nanosuspension was measured by using 'Nano Brook 90Plus-zeta seizer (Brookhaven Instruments USA). Sample of the selected formula was placed in the electrophoretic cell and measured three times at 25 ±1° C, and the average values were calculated. Zeta potential gives information about the surface charge properties and furthers the long-term physical stability of the nanosuspension. To obtain an electrostatically stabilized nanosuspension, a minimum zeta potential of ± 30mV is required. In the case of a combination of electrostatic and steric stabilization, a minimum zeta potential of ± 20mV is desirable [16,25].

Scanning electron microscopy (SEM)

The morphology of pure drug and the selected ITZ nanosuspension (F6) were examined by scanning electron microscope (VEGA3Tuscan Czech republic) operated with a secondary detector at different acceleration voltage and at different magnification .The morphology of pure drug was done by direct deposition of powder on double-sided carbon tape and coated with gold at 1K, 2K, 5K and 500x magnification. while for liquid sample F6, it was prepared by the droplet evaporation technique. A droplet of liquid was deposited on double-sided carbon tape and dried at room temperature for the evaporation of water and then coated with gold [17,18].

Lyophilization of selected ITZ- nanosuspension

Lyophilization of the selected formula was accomplished using christ (ALPHA 1-4 LD plus) to recollect nanoparticles in a dried-powder state from the nanosuspension and to complete characterization of nanosuspension and show the effect of lyophilization on nanoparticles size and solubility. The selected formula was used containing 2% w/w mannitol ,as the cryoprotectant, was frozen in a refrigerator at -70 °C for 24 h. Then the sample was lyophilized using vacuum freeze dryer at a controlled temperature of -58 °C and the pump operating at the pressure of 150 militorr over the range of 48–72 hr. The obtained powder was used for further studies [5,13].

Characterization of ITZ lyophilized nanosuspension

Differential scanning calorimetry (DSC)

The DSC of pure ITZ powder and lyophilized ITZ nanoparticles formulation were taken on (Shimadzu DSC-60 plus, Japan). Ten milligrams were sealed in the flat-bottomed aluminium pan of the differential scanning calorimeter. Data collection was achieved at a temperature range of 0–200°C and the heating rate

was 5°C/min under nitrogen gas at a flow rate of 25 ml/min [19].

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of bulk ITZ, and lyophilized ITZ nanoparticles were recorded using FTIR -7600, Australia spectrophotometer. Powders were mixed with potassium bromide and compressed into disks using hydraulic press before scanning from 4000 to 400 cm^{-1} [19].

X-ray powder diffractometry studies

The XRD patterns for bulk ITZ and Lyophilized ITZ nanoparticles were analyzed using an XRD-6000, Shimadzu-Japan. The freeze-dried samples scanning was conducted over powder X-ray diffractometer at the axis of 2 thetas ,with the continuous scan range of 5-80 degree. The operating voltage was 40 kV, and current 30mA and scan step size of 0.050° (2θ) and scan step time of 60 seconds [19].

In -vitro dissolution studies

The *in-vitro* dissolution test was estimated using (paddle assembly) type II dissolution test apparatus. Accurately weighed bulk drug and the selected nanosuspension formula (F6) and the lyophilized formula (equivalent to 50mg nanoparticles)were determined under sink conditions using Himedia dialysis membrane 70 (MWCO 12 KD). Briefly, 20ml of optimized formulations of ITZ nanosuspension was placed in the pretreated dialysis bag soaked in dissolution media overnight before use; and fitted with a paddle then dispersed in 900ml of 0.1 N HCl (pH 1.2) kept at 37± 0.5 °C at a rotation speed of 100 rpm. Sink condition was maintained throughout the study. An aliquot of 5ml samples was withdrawn from the receiver compartment at predetermined time intervals (5, 10 , 15 ,20 ,30 , 45, 60 ,90 ,and 120 min) respectively and refilled with the equivalent volume of fresh dissolution medium to maintain the constant volume. Then, samples were filtered and the amount of drug determined spectrophotometrically on UV spectrophotometer at the determined wave length of max. absorbance in 0.1N HCl at 257 nm wavelength. The experiment repeated in triplicate for each formulation [5,16].

Results and Discussion

Saturation solubility of pure ITZ and freeze-dried nanoparticles

The solubility of pure ITZ and ITZ-nanosuspension (F6) (ITZ: P-188: tween80 1:2:2) that was selected for lyophilization; was carried out in purified water, pH 1.2 HCl buffer and phosphate buffer pH 6.8. The saturation solubility of the lyophilized ITZ - nanosuspension was increased significantly($P< 0.05$) over the pure drug in all vehicles used. This increment due to the decrease in particle size and increasing the surface area as illustrated in figure 1 [20].

Particle size analysis and polydispersity index measurement

The mean particle size of various batches of nanosuspension prepared is depicted in table 2. All the prepared formulations were in the nano size. The mean particle size varied from 42nm – 685.2 nm. The stabilizer p-188 and tween 80 resulted in smaller particle size indicating effective stabilization of prepared nanosuspension which agrees with that obtained by Kalvakuntla who formulate aprepitant nano-suspension[27]. The PDI of each formula was determined which is varied from 0.005-0.336.

The entrapment efficiency of ITZ-nanosuspension

The results of entrapment efficiency have been shown in table 2. The values of drug entrapment efficiency of F3, F6, F7 was high compared to other formulations in figure 2 which may be attributed to the presence of optimum concentrations of stabilizers (P-188) and the presence of surfactant (tween 80). It is clear that increasing the stabilizer concentration, and the addition of surfactant increased the drug entrapment efficiency. The low values of drug entrapment efficiency point out the relatively low affinity of the drug with the polymer matrix. The concentration of stabilizer used is the most effective factor in entrapment efficiency, and this agrees with that obtained by Preeti Singh. et al. who formulates satranidazole nanosuspension and by Patil et al. who formulates spry dried chitosan nanoparticles containing doxorubicin [21,22].

Effect of stabilizer concentration on the particle size and polydispersity index

Figure 3 shows the effect of stabilizer concentration on particle size and PDI by using four different ratios of polymers 1:1,1:2, 1:4 and 1:8 (F1, F2, F3 and F4 for P-188) and(F9,F10,F11 for HPMC E5). The formulation showed PDI in the range of (0.005-0.336), and this low value will indicate good stability of the nanosuspension. The results showed that the particle size decrease with the increasing of concentration of polymer. Mean particle size of formula F1 was 531.2 nm compared with F4 that had mean particles size 53 nm for P-188 and for HPMC E5 ,F9 was 241.6 nm compare to F11 that had mean particles size 90 nm. The choice of suitable stabilizers and its concentration are the most important factors to control the size and stability of the nanosuspension during nanoprecipitation methods [23].

Table 2.Physicochemical characterization of itraconazole nanosuspension

Formula no.	Stabilizer	Drug:stabilizer Co-stabilizer	Stirring speed (rpm)	Particle size(nm)	Polydispersity index	%EE
F1	P-188	1:1	3000	532 ±0.0	0.269 ±0.0	96.4±0.2
F2	P-188	1:2	3000	196.2±0.0	0.336 ±0.0	98.1±0.2
F3	P-188	1:4	3000	131.8±0.0	0.005 ±0.0	99.98±0.3
F4	P-188	1:8	3000	53±0.0	0.041 ±0.0	99.2±0.32
F5	P-188:SLS	1:2:2	3000	540.1±0.0	0.321 ±0.0	93.41±0.5
F6	P-188: Tween80	1:2:2	3000	42±0.0	0.086 ±0.0	99.99±0.11
F7	p-188	1:4	1500	424±0.0	0.005 ±0.0	99. 4±0.05
F8	p-188	1:4	500	685.2±0.0	0.005 ±0.0	97.82±0.53
F9	HPMCE5	1:1	3000	241.6±0.0	0.260 ±0.0	81.94±0.38

Effect of stirring speed on the particle size and polydispersity index

Three different speeds 3000, 1500 and 500 rpm were used to prepare three formulas(F3 ,F7 and F8) to show the effect of stirring speed on particle size as shown in figure 4. In this study the optimum speed for formulas have a drug to stabilizer ratio 1:4 was found to be 3000 rpm that produces mean particle size 131.8nm. PDI of these formulations was 0.005 An increasing stirring speed would result in lower particle size. As a result, high shear stress was necessary to break down particles to the submicron range [(24).]

Influence of stabilizer type on particle size and PDI

Two types of stabilizer were used to prepare nanosuspension , HPMC E5 and p-188 at the (1:1,1:2,1:4) ratio. As shown in figure 5 , the mean particle size obtained is (241.6,95,90 nm) for HPMC E5 , while for p-188 stabilizer the mean particle size obtained is (531.2,196.2,131.8nm) for the same ratios. Comparing results, HPMC E5 stabilized nanosuspension , demonstrated the lowest particle sizes than p-188. HPMC E5 has a good affinity toward the drug particles, and thereby, it can provide an active steric barrier against particles growth [(26)].

Zeta potential

The zeta potential for the selected formulation of ITZ nanosuspension was - 21.86 mV, as shown in figure 6. The charge was negative due to adsorbed tween 80 and p-188 on the drug particles; The importance of zeta potential that it reflects the degree of repulsion between adjacent, similarly charged particles in the dispersion. The obtained value for selected formulation indicates that stable nanosuspension value can be related to the stability of colloidal dispersions. Zeta potential is an important parameter for the prediction of the stability of nanosuspension [(24)].

***In vitro* drug release**

The release of ITZ from the nanosuspension of selected formulation and the lyophilized nanoparticles was higher than the release profile of the pure drug in 120 min as shown in figure 7. The %CDR of the selected lyophilized nanoparticles was 98% in 30min and 88% for ITZ-nanosuspension formula in 0.1N HCl media as compared to 13.5% of the pure drug in the same media indicating the poor drug solubility and thereby dissolution [(25)]. So there is significant differences ($p<0.05$) in the release of the pure drug and selected formula ,which coordinated with Noyes–Whitney equation, in which the dissolution rat is enhanced as the saturation solubility increased and the particle size decreased[(23)] .

Other factor that may contributing to the fast release is the entrapment efficiency which has a direct effect on the drug release profile. As it increased, the release rate also increased [(26)].

Scanning electron microscope (SEM)

The SEM of pure ITZ is presented in figure 8 ,showed agglomerates of irregular, rough surface, large shape of ITZ particles while the images of the SEM of the selected formula of the nanosuspension (F6) is represented in figure 9 , showed smooth uniform particles within the nano size which could be assigned to the presence of stabilizer that coated particles, the surfactant which was stabilized the particles could be adsorbed to the surface of the crystals by hydrophobic interaction. The SEM images confirmed that an increase in particle size was observed after freeze-drying, but it was still in the submicron level and lower in size in comparison with the pure drug, but not below 100 nm [(16,27)].

Differential scanning calorimetry (DSC)

The DSC was used to estimate the effect of excipients and conditions on the physical properties of drug. The DSC of pure ITZ powder, (Figure10) depicted a sharp endothermic peak at 166.19 °C indicating no change in its melting temperature and the drug has a crystalline nature with high purity. For lyophilized powder, (Figure11), the slightly lower melting temperature, at 165.59ºC might be due to small size of ITZ crystals resulted from surrounding of the stabilizer to drug crystals and small particle size [(5.13)].

Powder X-ray diffraction analysis (PXRD):

To affirm the crystallinity of ITZ, X-ray diffraction was performed for pure ITZ and lyophilized nanoparticles. The X-ray diffraction pattern of ITZ exhibited characteristic sharp peaks (Figure12) at 14.48°, 17.51°, and 20.37° that indicate the crystalline state of ITZ [20].Some of characteristic peaks appeared related to p-188 at 19.1^0, broader one between 22°, and 23° and a characteristic peaks at 9.7° appeared for mannitol [30]. The diffractogram of lyophilized nanoparticles exhibited the diffraction peaks at 9.63^0, 13.55^0, 19.37^0, 20. 29^0 21.83^0, 23.11^0, 24.56^0, 36.58^0, 37.79^0 and 44.01^0 which arise due to the overlapping contribution of excipients and drug. [20]

However, slight decrease in intensity of peaks was observed with and the absence of two characteristic peaks in the XRD pattern of selected lyophilized nanoparticles compared to the pure ITZ as seen in figure 13 may explain a reduction in the size of ITZ crystal and nanoparticles was not affected significantly by the freeze-drying process [5,16].

FT-IR analysis

The FTIR spectra of pure ITZ is shown in figure14.The main peaks of pure ITZ were observed at wave numbers, 3466, 3130, 2965, 3067, 1698, 2822,1611, 1512 and 1453 cm^{-1}.The absorption bands between 2800 cm^{-1} and 3200 cm^{-1} were vibration stretching of C-H for both alkane and aromatic C-H. The band located at 3466 cm^{-1} was assigned to the stretching vibration of C-H of furan ring group and 733cm^{-1}due to C-Cl stretching in ITZ molecule and those at 3130 and 3067 cm^{-1} resulted from stretching vibration of the amino group. A sharp band at 1698 cm^{-1} is due to C= O, and the bands at 1611 and 1454 cm^{-1} are assigned to stretching vibration of (C= C) and (C-N)bonds, respectively. It should be noted that all the characteristic bands of ITZ were detected in the spectrum of the lyophilized nanoparticles as shown in figure 15, indicated that the drug and the stabilizer and other excipients used in the formulation are compatible with each other.[20,32].

Conclusion

Nanoprecipitation method was successfully used to produce stable ITZ nanosuspension by using the proper stabilizer (p-188) and surfactant (tween 80**).**

Different parameters, such as stabilizer type, concentration, stirring speed, were investigated and optimized to produce the smallest drug nanoparticles. The dissolution rate of the prepared ITZ nanosuspension and lyophilized nanoparticle were significantly enhanced as compared with the pure ITZ powder.

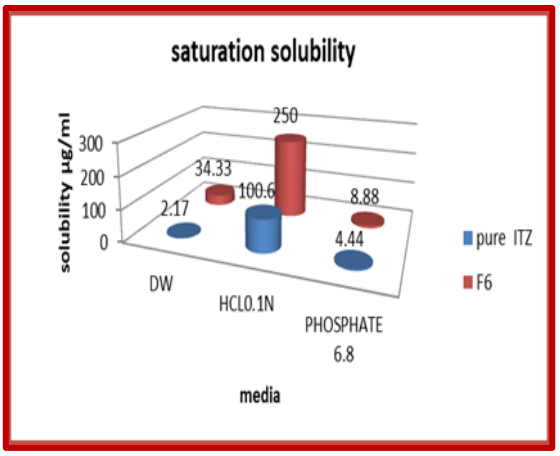

Figure 1. Saturated solubility of pure ITZ and selected lyophilized formula

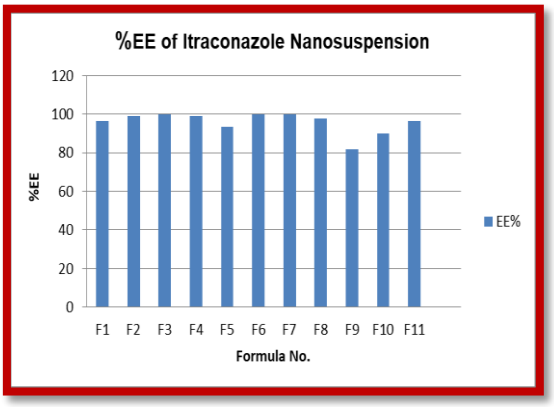

Figure 2. The entrapment efficiency of ITZ nanosuspension

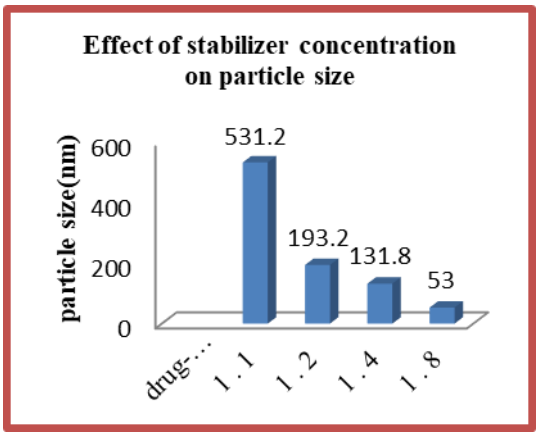

Figure 3. Effect of stabilizer concentration on the particle size

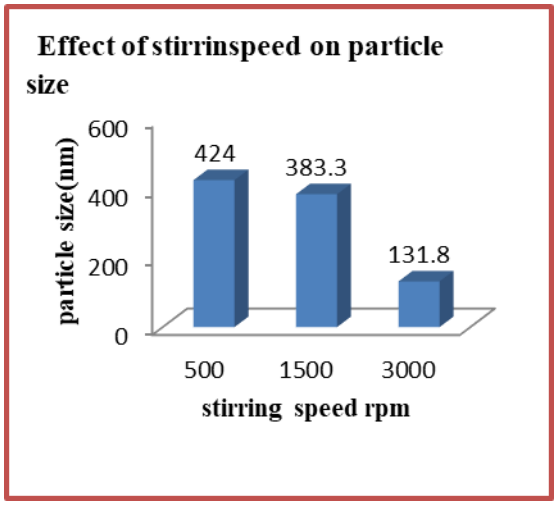

Figure 4. Effect of stirring speed on particle size

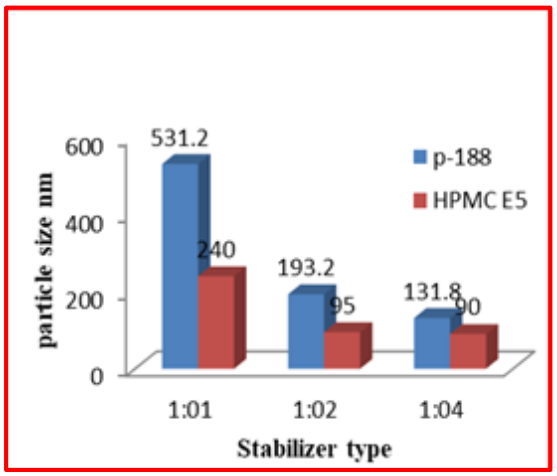

Figure 5.Effect of stabilizer type on particle size

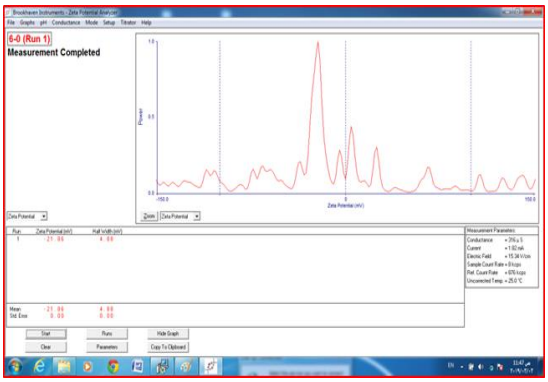

Figure 6. Zeta potential of the selected formula (F6) ITZ-NS

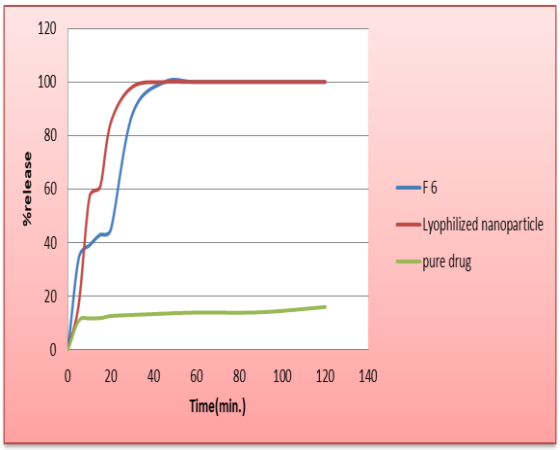

Figure 7. Release profile for pure drug, nanosuspension formulation(F6) and ITZ lyophilized nanoparticles (LN)

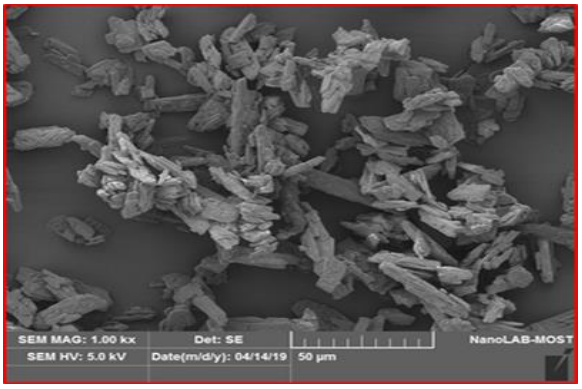

Figure 8. SEM of pure ITZ

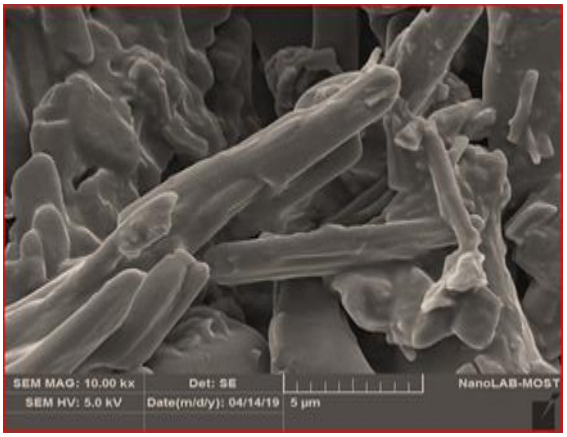

Figure 9. SEM of ITZ- selected formula (F6)

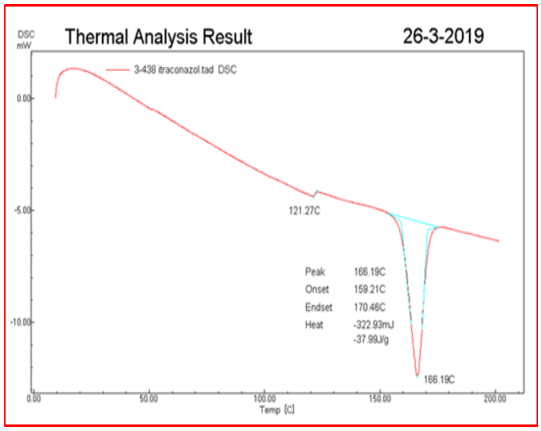

Figure 10. DSC thermograms of pure ITZ

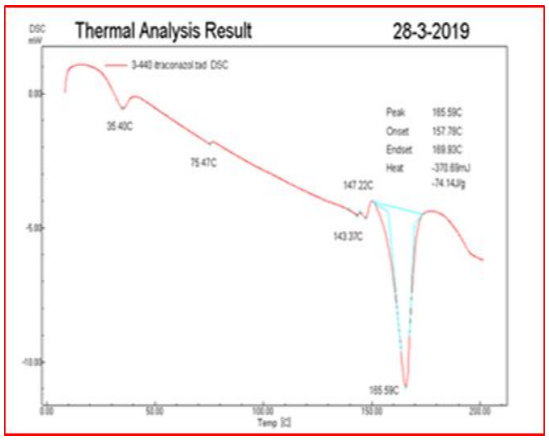

Figure 11. DSC thermograms of ITZ lyophilized powder of selected formula (F6).

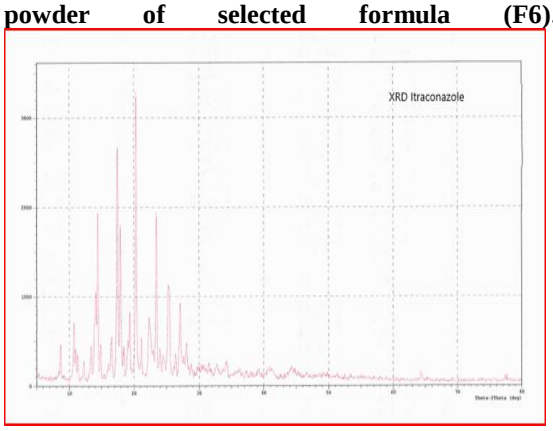

Figure 12. XRD of pure ITZ

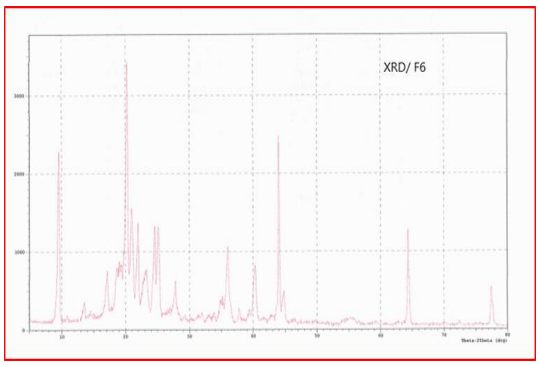

Figure 13. XRD of ITZ lyophilized powder of selected formula (F6)

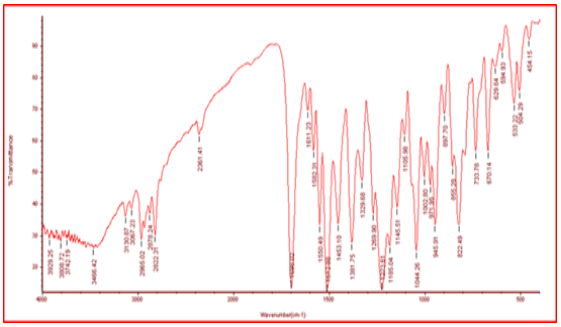

Figure 14. FTIR of itraconazole

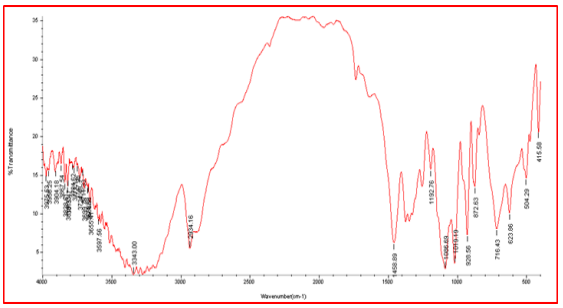

Figure15. FTIR of ITZ lyophilized powder of selected formula (F6)

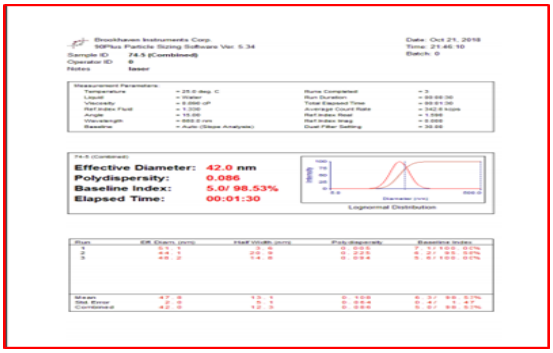

Figure 16. Particle size distribution of the selected formula (F6)

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