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Application of Spherical Agglomeration Technique in Bioavailability Enhancement of Poorly Water Soluble Drug Itraconazole

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ABSTRACT

This study investigated the spherical agglomeration of itraconazole for enhanced drug dissolution rate and bioavailability at various polymers percentage like 0.2%, 0.4%and 0.6% with Soluplus®, HPMC and PEG-4000 by simple stirring at 900 r.p.m. (The spherical agglomerates(SA) were dried powdered and with method followed by characterized by differential scanning calorimetry and X-ray powder diffraction. The SAs of itraconazole were also evaluated by drug content study, solubility study and *in-vitro* dissolution study. The pharmacokinetic studies of the formulations and pure itraconazole were evaluated i.e. Cmax, Tmax and AUC *in vivo* study by pharmacokinetic model on wistar rats.

Keywords: Itraconazole, Spherical Agglomeration, Dissolution, Bioavailability.

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INTRODUCTION

Bioavailability is a fundamental property of a pharmaceutical product for a given route of administration. More specially, a low bioavailability can be a major source of therapeutic variability, whenever bioavailability is low drug companies may attempt to increase the dose to achieve an appropriate drug exposure[1]. Poor aqueous solubility of drugs is a major limiting factor with many new drugs in their successful launch in market in spite of their potential pharmacokinetic activity. Poor solubility (less than 10 %) of a drug leads to poor dissolution in the gastro intestinal tract (GIT) hence, incomplete and erratic absorption ultimately limits its clinical utility. Further, poorly soluble drugs are generally administered at much higher doses than the actual dose in order to achieve necessary drug plasma levels leading to increased adverse reaction & cost of therapy as well as erratic pharmacological response and hence poor patient compliance[2]. About 40 % of drugs being in the pipeline of pharmaceutical companies are poorly soluble, emphasizing the need of a technique to overcome such problems. Poorly water soluble drugs are becoming a problem in terms of obtaining satisfactory dissolution within the gastro intestinal tract, which is necessary for good bioavailability. Poorly water-soluble drugs are associated with slow drug dissolution followed by slow absorptioneventually leading to inadequate and variable bioavailability[3]. Itraconazole is a potent synthetic triazole antifungal drug with activities against broad spectrum of fungal species. According to the biopharmaceutics classification system[4], itraconazole is an extreme example of a class II compound meaning that its oral bioavailability is determined by dissolution rate in the GI tract[5]. In the spherical agglomeration technique, the itraconazole was choosen for development as its microcrystalline forms exhibited poor aqueous solubility. Novel spherical agglomeration procedure was developed by modifying the kawashima[5] techniques by incorporating the polymers (HPMC, PEG-4000 and SOLPLUS®) in the concentration of 0.2%, 0.4% and 0.6% during the agglomeration processes. The agglomerates were evaluated by x-ray diffraction (XRD), differential scanning calorimetry (DSC), solubility, *In-Vitro* dissolution study and pharmacokinetic studies.

MATERIALS AND METHODS

Materials

Itraconazole (ITRA) was obtained as gift sample from De NovoBiotech.Pvt. Ltd., India. , Hydroxypropylmethylcellulose (HPMC) and Poly Ethylene Glycol (PEG-4000) were purchased from Qualigens Fine Chemicals (Mumbai, India). Soluplus was obtained as gift sample from BASF, Mumbai, India. All other chemicals were of extra-pure reagent grade and used as and when received.

Methods: Preparation of spherical agglomerates of PEG-4000, HPMC and Soluplus of itraconazole in the concentration of(0.2%, 0.4% and 0.6% w/v of polymer)

Drug (2.5 g) was dissolved in 40 mL DMF by gentle warming upto 50ºCand then cooled to room temperature. A solution of 0.2%, 0.4% and 0.6% w/v of surfactant/polymer (Hydroxypropylmethyl cellulose (HPMC), PEG-4000, Soluplus) in distilled water (30 mL) was then added to drug solution in DMF with stirring. The precipitated solid was dissolved by further addition of 30 mL DMF and gentle warming. This solution was added with stirring to 400 mL distilled water contained in the agglomerating vessel. Chloroform (18 mL) was added drop wise with stirring at 900 rpm for 30 minutes. The precipitate obtained was collected and dried in vacuum and stored in desiccator for further studies[5,6]. A total of nine samples ITRA-01 to ITRA-09 were prepared using HPMC, PEG-4000 and Soluplus as shown in Table 1.

Table 1: Formulations Batches Depending on Different Polymers

Sr. No.	Formulations	Solvent	Polymers	Polymer conc. (%)
1	ITRA-01	DMF	HPMC	(0.2)
2	ITRA -02	DMF	HPMC	(0.4)
3	ITRA -03	DMF	HPMC	(0.6)
4	ITRA -04	DMF	SOLUPLUS®	(0.2)
5	ITRA -05	DMF	SOLUPLUS®	(0.4)
6	ITRA -06	DMF	SOLUPLUS®	(0.6)
7	ITRA -07	DMF	PEG-4000	(0.2)
8	ITRA -08	DMF	PEG-4000	(0.4)
9	ITRA -09	DMF	PEG-4000	(0.6)
10	ITRA -10	----------	------	

Evaluation and Characterization

Drug Content Studies

The individual formulations equivalent to 10mg of drug were weighed accurately and mixed with 100 ml of methanol. The solution was filtered through 22µm nylon disc filter and after further dilution (10 times with methanol) the drug content was determined at 263nm using UV spectrophotometer (Perkin Elmer EZ301, USA)[6].

Solubility Studies

Solubility study of the drug and its formulations was carried by shaking 10 mg of Std. drug and its formulations (equivalent to 10 mg drug) with 40ml of distilled water in 100ml volumetric flask for 72 hours on BOD shaker and then make up the volume up to 100ml. After 10 times dilution then filtering and determining the amount of drug dissolved spectrophotometrically at 275nm against suitable blank[6].

***In Vitro* Study**

The *in-vitro* dissolution study was carried out with 10 mg of Std. drug and its formulations (equivalent to 10 mg drug). Formulations (10mg) of itraconazole were placed inhard gelatine(00 size) capsule with 200 mg sprayed dried lactose in a rotating basket dissolution apparatus (USP XXII) and then placed in 900 ml 0.1% SLS solution and stirred at a speed of 50 rpm with temperature maintained at 37 ± 1ºc. Aliquots of 10ml were withdrawn at appropriate time intervals and an equal volume was replaced in the vessel. 0.1%SLS was used to explore the dissolution of drug replaced in the vessel[5]. Itraconazole in the aliquot was assayed spectrophotometically by measuring absorbance at275 nm against suitable blank.

Differential Scanning Calorimetry

DSC studies of the prepared samples were conducted immediately after preparation as well as after storage for 6 months. A (Q-10, TA) Instrument equipped with an intraocular 2p cooling accessory was used. Samples of 10mg to 5mg were placed in saturated aluminium pans and sealed with a lid. Heating scans by 10ºC /min applied with a purge of 50ml/min. Fast heating rates are preferred to prevent changes during scanning[6].

X-Ray Powder Diffractometry

X-ray powder diffraction patterns were recorded on a XPERTO-PRO x-ray diffractometer using Ni filtered, using a voltage of 45kV, and a 40mA current. The scanning employed was1 min- 1 over the 6 to 90 diffraction angle (2θ) range. The relationship used for the calculation of crystallinity was presented by relative degree of crystallinity[17]**.**

$$\text{Relative degree of crystallinity (RDC)} = \frac{I_{sam}}{I_{ref}}$$

Where,

I_{sam} = Peak height of the sample under investigation

I_{ref} = Peak height at the same angle for the reference with the highest intensity[5,6].

Pharmacokinetic Study in rats

The pharmacokinetics of drug and test (ITRA-01 and ITRA-06) was evaluated following oral administration. The study was conducted in accordance with the regulation specified by the Institutional Animal Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSEA). In total 04 groups (6 per group) Wistar rats (6-7weeks old) weighing between 180-240 g were used for the study. The rats were housed in a cage and maintained on a 12h light/dark at room temperature (21ºC to 24ºC) and relative humidity of 50 to 70% and acclimatized to study area conditions for atleast 5 days before dosing. General and environmental conditions were strictly monitored. Each group was orally administered 1ml of

0.2%w/v methylcellulose aqueous suspension containing the drug and formulations of itraconazole (equvivalent to itaconazole 10mg/kg body weight) respectively. Blood samples were collected from the retro-orbitall vein from inner canthus of eyes using micro heamatocrit capillaries. Blood samples were collected at 0, 1, 2, 3, 4, 8, 12, 24 and 48 h post-dose in EDTA vials then centrifuged at 4000 rpm for 10 minutes to obtain plasma and stored at- 80ºC until bioanalysis[8].

Bioanalysis

The samples were analyzed using High performance liquid chromatography (HPLC Model Water) equipped with reverse phase column (Hypersil BDS C18 150×4.6mm) and UV detector at 255nm using 40:40:20 methanol: acetonitrile: water. The calibration curve was also drawn for analysis which was found to be linear from 62.5ng/ml to 1000ng/ml[9].

Pharmacokinetic data analysis

The area under the drug concentration-time curve from zero to 48h (AUC) was utilized for pharmacokinetic drug analysis. The maximal plasma concentration of drug (Cmax) and the time to reach maximal plasma concentration (Tmax) were directly obtained from data and area under the curve (AUC)[9].

RESULTS AND DISCUSSION

Drug content study

The drug content of the prepared formulations of Itraconazole was observed to be varying from 43.29% to 13.66% and it was maximum with formulation (ITRA-06) and minimum in formulation(ITRA-07) as shown in Table 2. This studies shows that formulations with PEG-4000 show the maximum drug content.

Table 2: Content, solubility and % release of Itraconazole from different formulations in comparison with original drug

Sr. No.	Formulations	Drug Content (Equivalent to 10mg)	Solubility Studies (mg/100ml)	*In-Vitro* release in 4 hrs. (%)
1	ITRA-01	17.27	3.77±0.18	38.8%
2	ITRA -02	20.87	2.41±0.13	26.1%
3	ITRA -03	36.12	2.37±0.06	24.1%
4	ITRA -04	17.85	2.55±0.03	26.1%
5	ITRA -05	38.91	2.09±0.06	22.8%
6	ITRA -06	43.29	3.31±0.12	34.1%
7	ITRA -07	13.66	2.04±0.11	21.3%
8	ITRA -08	19.15	2.95±0.06	29.2%
9	ITRA -09	36.89	3.55±0.04	31.1%
10	ITRA -10	10	1.52±0.10	14%

Solubility studies

Solubility studies were performed with several hydrophilic carriers (HPMC, PEG-4000 and Soluplus®) maximum augmentation of solubility was observed with HPMC (0.2%). The minimum with formulation ITRA- 07(2.04±0.11 ug/ml), ITRA-05(2.09±0.06 ug/ml) and ITRA-10**(**1.52±0.10 ug/ml) as shown in (Table 2 and Figure 1). Studies show that spherical agglomeration technique has the maximum solubility with PEG-4000(0.6%), Soluplus® (0.6%) and with HPMC (0.2%) as compared to the pure drug of itraconazole **ITRA-10 (1.52±0.10 ug/ml)** this technique show maximum drug solubility. The spherical agglomeration technique is the best method for the increases in the solubility of the itraconazole with Soluplus® and PEG-4000 in higher and HPMC in lower concentration among all solubility studies conducted in our research.

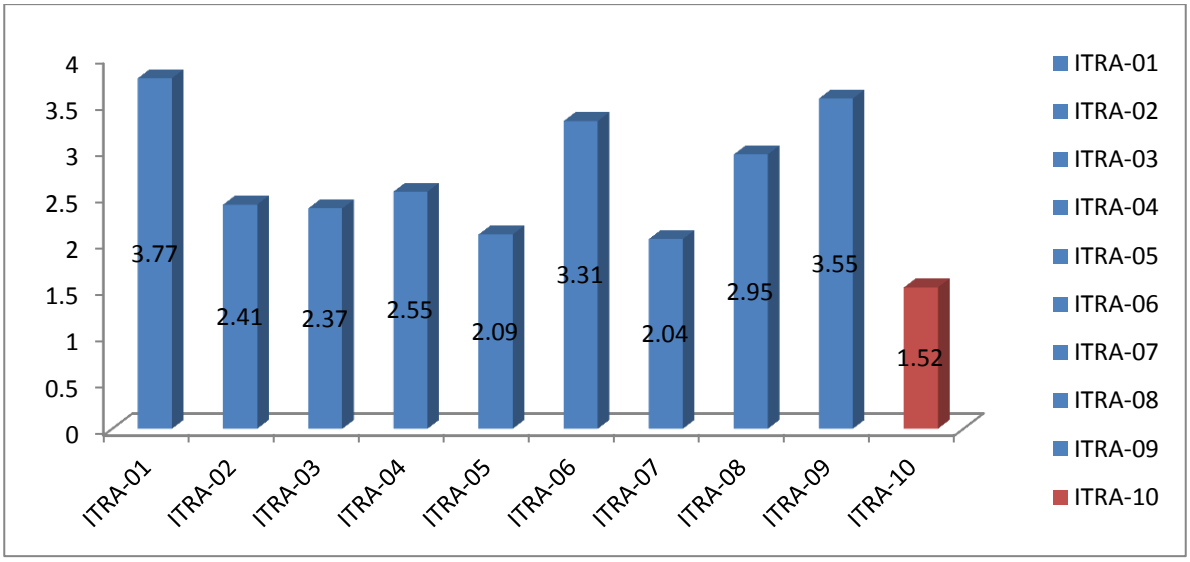

Figure 1: Solubility studies of Itraconzole (ITRA-10) and its formulations.

***In-Vitro* Dissolution studies**

In all formulations, the drug content was taken as 10 mg per capsule in 900 ml dissolution medium, 37˚C containing SLS (0.1 % w/v) and at different time intervals, 10ml of the solution was withdrawn until 4 hr. In Vitro dissolution study of the different formulations of Itraconazole prepared by spherical agglomeration technique, it was observed that the formulation ITRA-01, ITRA-06 and ITRA-09 show the maximum increase in the %release of the drug from the formulations. The formulation ITRA-02, ITRA-03 Show the lesser % release of the drug in the dissolution medium with the HPMC polymer. It was observed that the formulation ITRA-06 show the maximum increase in the %release of the drug with the Soluplus and ITRA-04 show the lesser % release from the formulation. The formulation ITRA-09 shows the maximum % release with the PEG-4000 polymer and ITRA-07 shows the lesser % release of the drug from the formulations.

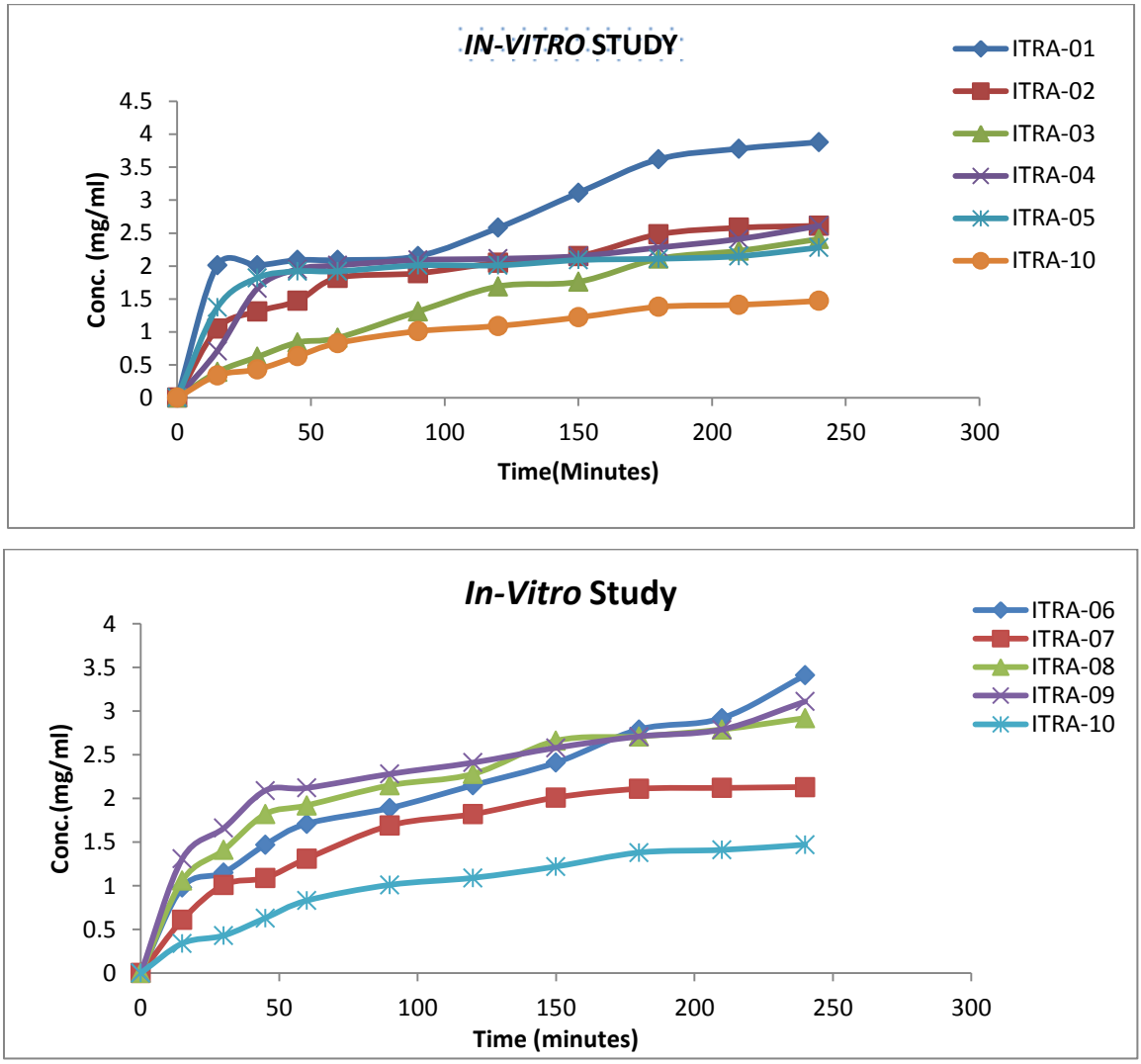

Figure 2: Dissolution profile of pure itraconazole and its formulations prepared by spherical agglomeration (ITRA-01, toITRA-10)

The formulations which are to be kept in the capsule as compared to the std. drug profile. The formulations show % release of the drug in the dissolution medium as compared to the std. drug release profile as shown in (Figure 2- Figure -3 and Table 2).

Differential scanning calorimetric (DSC) study

DSC thermograms of pure drug and corresponding drug carrier system are depicted in Figure 4. The DSC curve of Itraconazole(ITRA-10) shows a sharp endothermic peak (Tpeak=173.11ºC) corresponding to its melting, indicating its crystalline nature. However, the characteristic endothermic peak, corresponding to drug melting was broadened and shifted toward lower temperature, with reduced intensity, in all the formulations of ITRA-01(Tpeak=164.31ºC), ITRA-06(Tpeak=168.21 ºC) and ITRA-09 (Tpeak=169.21 ºC). This could be attributed to higher polymer concentration and uniform distribution of drug in the crust of polymer, resulting in complete

miscibility of molten drug in polymer. Moreover, the data also indicate that there may be no interaction between the components of binary system.

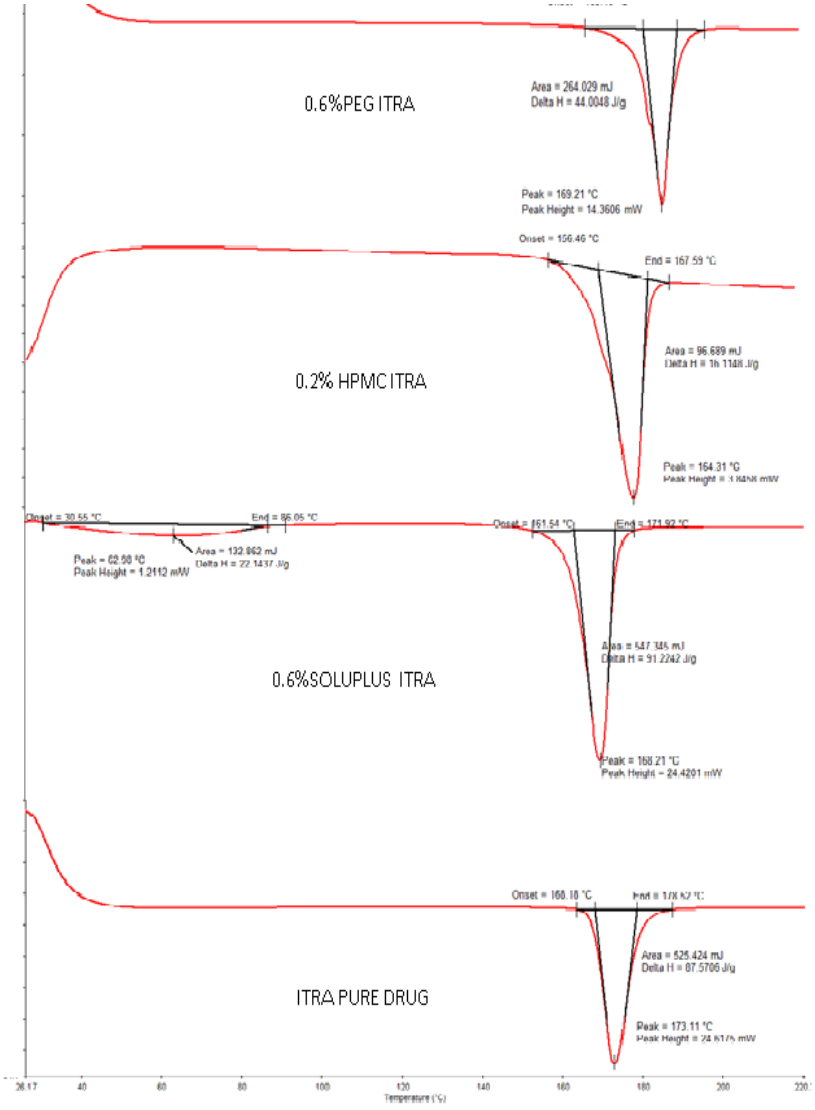

Figure 4: DSC thermogram of pure itraconazole and its formulations.

X-ray Diffraction Study

X-ray diffracometry (XRD) spectra of pure compound and binary systems with carriers are presented in figure 5. The x-ray diffractogram of ITRA-10 has sharp peaks at diffraction angles (2θ) 21.37°, 26.33°, 15.92°, and 20.14º. It is showing a typical crystalline pattern. However, all major characteristic crystalline peaks appear in the diffractogram of all the formulations (ITRA-01, ITRA-06, and ITRA-09). Pure drug peak at 21.37° (2θ) was used for calculating RDC of formulations of itraconazole. The RDC values of ITRA-01, ITRA-06 and (ITRA-09) were 0.9772, 0.6031 and0.5886 respectively. Moreover, the relative intensity and 2θ angle of these peaks remains practically unchanged.

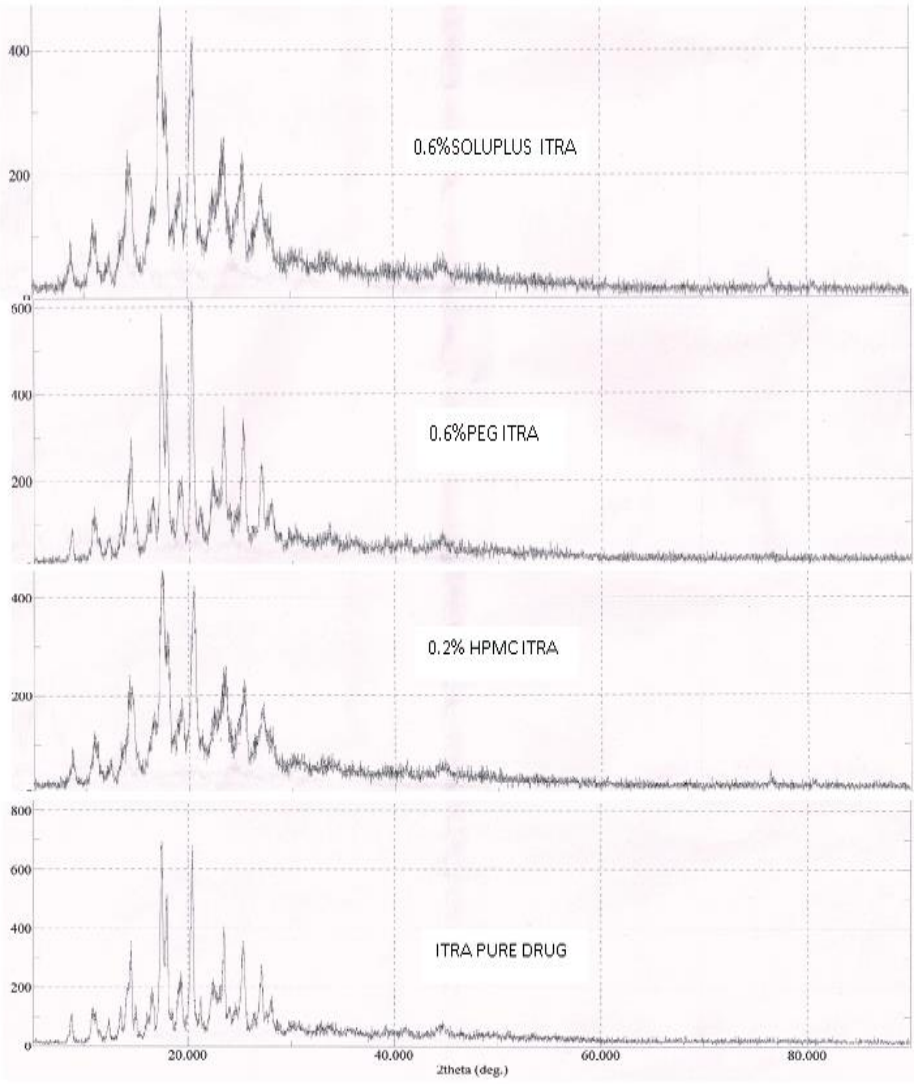

Figure 5: Powder X-ray diffraction spectra of pure Itraconazole and its formulations (ITRA-01, ITRA-06 and ITRA-09).

Effect of different carriers on the dissolution of Itaconazole

Spherical agglomeration techniques were developed for improving the solubility of microcrystalline itaconazole. The process involved agglomerating microcrystal using agglomerating solvents. Temperature and speed of agitation were optimized to obtain spherical agglomerates in a desired range, which was found to be essential for enhancing the solubility. Incorporation of polymer/surfactant (HPMC, SOLUPLUS) during agglomeration significantly enhanced the dissolution rate of itaconazole. In additional flow, and compressibility properties of the drug are improved. The enhancement of dissolution of drug from drug carrier systems can be ascribed to several factors. The mechanism of dissolution rate improvement from solid dispersion

is lack of crystallinity and particle size reduction considered to be important factors for dissolution rate enhancement. Mixing of drug with a hydrophilic carrier results in greater wetting and increase surface available for dissolution by reducing interfacial tension between the hydrophilic drug and dissolution media. It was noted that drug carrier system sink immediately, while pure drug keeps floating on the surface for a longer time interval**.** The dissolution parameters of itraconazole with various carriers (HPMC, PEG-4000, SOLUPLUS) in same concentration of each carrier. The dissolution rate of pure drug is low even in the surfactant based medium, as 14% of the drug gets dissolved in 240 min. Spherical agglomerates formulated with all the carriers exhibited significant improvement in the dissolution parameters of drug. The order of dissolution enhancement with various binary systems was found to be (ITRA-01>ITRA-06>ITRA-09>ITRA-10) for itraconazole. The increase in the dissolution rate of the solid mixtures might be due to size reduction and increase in the wettability of the drug molecules in presence of the surfactants. Surfactants also lower the effect of surface tension, hence increasing the solubilizing effect. This kind of technique can be extended for improving dissolution rate of drug showing poor dissolution profiles and causing erratic bioavailability[10, 11].

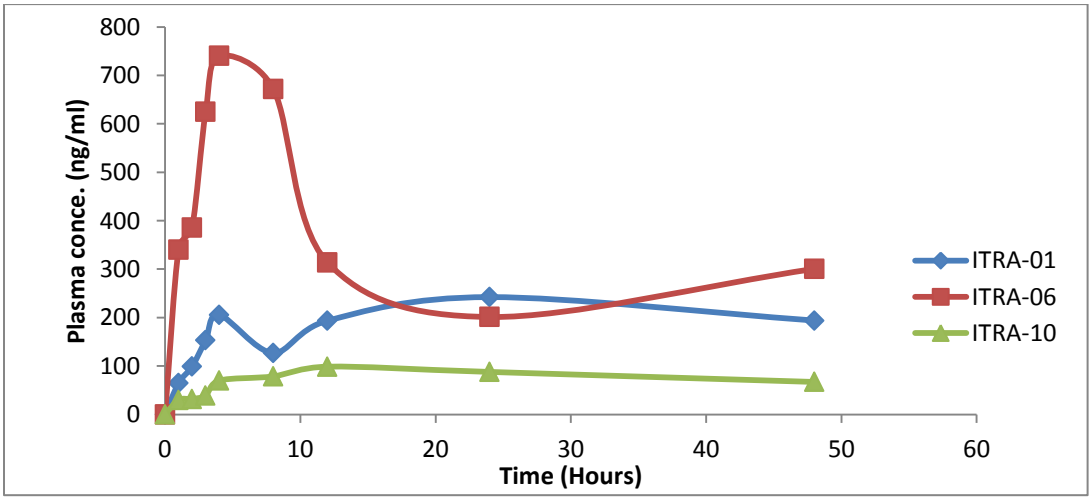

Figure 3: Mean plasma concentration–time profiles of itraconazole and its formulation after an oral administration(10 mg/kg) in different formulations to rats

Pharmacokinetic studies

As ITRA-01 and ITRA-06 formulations significantly improved the solubility and the dissolution rate of itraconazole, the effect of formulation on the oral exposure of itraconazole was examined in rats. Mean plasma concentration–time profiles of itraconazole and its formulations were evaluated in rats after an oral administration (10 mg/kg) of pure Itraconazole and its formulations summarized in Figure. 6. The pharmacokinetic parameters were also determined and summarized

in Table 3. As illustrated in Figure 6, the plasma concentration–time profiles of spherical agglomerates differ from that of pure itraconazole, the peak plasma concentration (Cmax) of ITRA-01 andITRA-06 were significantly higher than that of pure Itraconazole (approximately 2 and 7.4 folds higher for ITRA-01 SA and ITRA-06 SA, respectively). In addition, the area under the plasma concentration–time curve (AUC) of itraconazole tends to be increased via the SA formulation. Particularly, the AUC of ITRA-01 SA was enhanced significantly ($p < 0.05$, approximately 5 folds) compared to the pure itraconazole. The results indicated that the enhanced solubility and dissolution of itraconazole via the SA formulations could lead to improved oral bioavailability of itraconazole. Given that the disconnect between in vitro and in vivo results of itraconazole in inhibition could be due to the low bioavailability of itraconazole, the enhanced oral exposure of itraconazole via the SA preparation may lead to the improved in vivo performance of itraconazole[12].

Table No. 3. Pharmacokinetic Parameters

Parameters	**(ITRA-10)**	**(ITRA-01)**	**(ITRA-06)**
AUC	3763.54	9572.145	15632.49
Cmax(ng/ml)	98.62	205.38	740.53
Tmax (h)	12	4	4

CONCLUSION

Spherical agglomeration is a better technique for the solubility enhancement of Itraconazole because the drug loaded to the carriers in very significant amounts due to which the bulkiness of the dosage form is reduced and the solubility and oral bioavailability of the drug improved more than twice the pure drug. The techniques also gave better results in the in vitro studies but their limitation is that the drug content in the formulations is very less, so it enhances the bulkiness of the dosage form. Present investigation successfully enhanced the solubility and dissolution profile of the drug under investigation by enhancing the bioavailability of drugs and improving the patient compliance.

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