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A novel technique to enhancing the bioavailability of Itraconazole using freeze drying

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ABSTRACT

The aim of the present work was to develop a Itraconazole freeze dried tablet (FDT). Which dissolve instantaneously in the mouth. To enhancing the bioavailability of poorly water-soluble Itraconazole was improved by preparing a FDT of Itraconazole using freeze-drying technique. The FDT was prepared by dispersing the drug in an aqueous solution of highly water-soluble carrier materials consisting of gelatin, glycine, and sorbitol. The mixture was poured in to the pockets of blister packs and then was subjected to freezing and lyophilization. The FDT was characterized by DSC, XRD and SEM and was evaluated for saturation solubility and dissolution and compared with physical mixture (PM) and pure drug. The samples were stored in stability chamber to investigate their physical stability for 90 days. Result obtained by DSC and X-ray studied showed that Crystalline state of Itraconazole in FDT transformation to amorphous state during the formation of FDT. SEM result suggests reduction in Itraconazole particle size. The solubility of Itraconazole from the FDT showed seven times greater than pure drug was due to super-saturation generated by amorphous form of the drug. Dissolution studies showed that dissolution rate of FDT of Itraconazole significantly improved compared with the PM and the pure drug. More than 90% of Itraconazole in FDT dissolved within 5 min compared to only 27.38% of Itraconazole pure drug dissolved during 60 min. In stability test, the dissolution release profile of the Itraconazole in FDT was almost unchanged as compared with the freshly prepared FDT.

Introduction

Itraconazole (ITZ) is a potent broad-spectrum triazole antifungal drug with activity against Cryptococcus, aspergillum, Candida, blast-mycosis, onychomycosis, and histoplasmosis organisms (1-4). The compound is insoluble in water (S ≈ 0.050 µg/ml at neutral pH and S = 1.2 µg/ml in 0.1mol/l HCl), and has an ionization constant of 3.7 and a very high n-octane/water partition coefficient (log P >5) (5). According to the biopharmaceutical classification system (BCS), itraconazole is an extreme example of a class II compound, dissolution dependent drug absorption (6-9).

Currently, several approaches in use widely used to fabricate Rapid Dissolving tablet (RDT) including lyophilisation, solid dispersion, mucoadhesive micro-particulate, direct compression and moulding. However, in terms of sales value, sales volume and number of products available in the market, freeze drying (lyophilisation) method has been the most successful (10, 11). The fabrication of , freeze drying RDTs is based on creating a porous matrix by subliming the water from pre-frozen aqueous formulation of the drug containing matrix forming agents and other excipients such as preservatives, flavors and lyoprotectants (12). The matrix of the freeze drying RDT consists of two components that work together to ensure the development of a successful formulation. The first component is water soluble polymers such as gelatin, dextrin, alginate (13) and maltodextrin (14). This component maintains the shape and provides mechanical strength to the tablets (binder).The second constituent is matrix supporting/disintegration enhancing agents such as sucrose and mannitol, which acts by cementing the porous frame work, provided by the water soluble polymer and accelerates the disintegration of the RDT (15, 16, 17). Although there is wide availability of literature describing the preparation of RDTs by freeze drying, the number of matrix supporting/disintegration enhancing agents used has been limited to saccharides and polyols with majority of the work dedicated to the inclusion of mannitol (14, 16, 17). This is primarily because the incorporation of these matrix forming agents requires fulfillment of stringent characteristics such as reasonable drying time, stability during freeze-drying process, as well as formation of elegant tablets with short disintegration time and adequate mechanical properties. However, high concentration of saccharides and polyols is required to achieve these quality features (14,16,17), thus restrains their application in delivering drugs for the treatment of long-term chronic conditions especially for children, diabetic and obese patients, due to limited intake requirement. Therefore, this present study aim to develop novel excipients by investigating the feasibility of using amino acids as matrix supporting agents (second component) in the fabrication of rapid dissolving tablets prepared by freeze drying in order to produce tablets with enhanced properties and wider application to pediatric and geriatric patient population.

In this present study, Itraconazole FDT were prepared and evaluated by DSC, XRD, and SEM analysis for the

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physicochemical properties. Formulated FDT was compared with its physical mixture and pure drug. Solubility and dissolution characteristics of the Itraconazole were also compared with FDT.

Materials and method:

Materials

Itraconazole, micronized gelatin, glycine, and sorbitol were gifted by IPCA pharmaceutical Ltd. Mumbai, India. All other materials used were of analytical grade.

Preparation of Itraconazole Freeze dried Tablets

A 2% w/v solution of gelatin in water was prepared by soaking the gelatin in water until complete hydration. The hydrated gelatin was stirred using a magnetic stirrer until clear solution was obtained. Equal weights of glycine (0.886% w/v) and sorbitol (0.886% w/v) were added to the gelatin solution while stirring until completely dissolved. An accurately weighed amount of Itraconazole powder (2.5% w/v) was dispersed. 1 ml of the resulting suspension was poured into each of the pockets of a tablet blister to contain Itraconazole dose of 25 mg.. The tablet blister packs, each packs containing 8 tablets, were transferred to a ultra low freezer kept at -40 ^{0}C for 24 hr. the frozen tablets were placed in a lyophilizer for 24 h using a Freeze Dryer (IISHIN Lab. Co. Ltd. Korea) with a condenser temperature of -40^{0}C and a pressure of 40 mbar followed by a secondary drying at 25 ^{0}C for 12h.The FDTs were kept in a desiccator at room temperature until further experiment. Five blister packs containing a total of 40 tablets were produced in each batch. Eight tablets randomly selected for drug content uniformity. The mean persentage drug content of Itraconazole was estimated by standard method and found to be 98.75% ± 0.017%.

Preparation of the Physical Mixtures

Itraconazole was uniformly mixed with gelatin, glycine and sorbitol in the same percentage used in the FDT using a mortar and pestle. The prepared mixtures were kept in desiccators until used (11).

Differential scanning calorimetry (DSC)

DSC study was carried out to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC DuPont 9900, differential scanning calorimeter with a thermal analyzer.

X-ray Diffraction analysis

X-Ray powder diffraction patterns were obtained at room temperature using a Philips X' Pert MPD diffractometer, with Cu as anode material and graphite monochromator, operated at a voltage of 40 mA, 45 kV. The process parameters used were set at scan step size of 0.0170 (2θ).

Scanning electron microscopy (SEM)

Scanning electron microscopic (Joel- LV-5600, USA, with magnification of 250x) photographs were obtained to identify and confirm morphological nature and Surface topography of the crystals.

Solubility studies

Itraconazole (100mg), its FDTs and PMs Equivalent to 100 mg Itraconazole were placed in glass stopper flasks were shaken in a water bath at 37^{0}C for 24 hr. The solutions were filtered through a membrane filter (0.45µg) and the dissolved drug was measured spectrophotometrically at 285 nm. Analyses were carried out (14, 15).

Dissolution studies

The dissolution of pure Itraconazole was compared with the PM and FDT. Dissolution studies were carried out using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). All studies were conducted in 900 ml of distilled water maintained at 37 ± 0.2^{0}C with a paddle rotation speed at 100 rpm. After specified time intervals, samples of dissolution medium were withdrawn and replaced by equal amount of fresh medium and then filtered, the amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 285 nm. Each sample was done in triplicate (16, 17).

Determination the physical stability

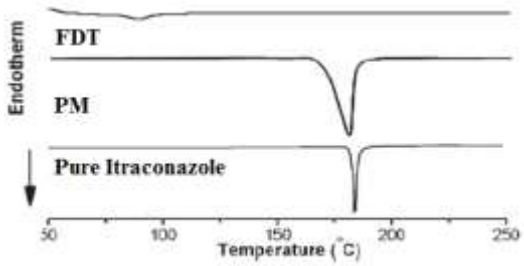
The physical stability of FDT was assessed by keeping the formulation at 20^{0}C and 45% relative humidity (RH), For 90 days, The % drug release of Itraconazole in the FDTs sample was determined by dissolution study and compared with freshly prepared FDT (16).

Results and discussions:

Itraconazole Freeze dried Tablets (FDT) were formulated using different fast dissolving carrier materials. Glycine was used to prevent shrinkage of the tablet during manufacturing and sorbitol was used to impart crystallinity, hardness, and elegance to the tablet. There exciepients are well-known and acceptable materials used in preparing of freeze-dried tablets. The percentage excipients used was optimized during the formulation process, to obtain a strong and elegant tablet that could be handled with ease (16).

DSC studied were carried out to evaluate the crystalline property of Itraconazole in FDT, PM and pure drug. In DSC curve (fig. 1) of pure Itraconazole had a sharp endothermic peak at 170°C that corresponds to the melting point of Itraconazole which is in accordance with a previous report (4, 6). The thermogram of the PM showed the endothermic peak of Itraconazole, although broader, spitted, and slightly shifted to the left, indicating that the crystalline state is maintained but decreased in the PM and shown sharp endothermic peak at 169°C. However, the melting endotherm was absent on the DSC thermogram of the FDT, suggesting absence of crystalline or amorphous form of the drug. This could be because of Itraconazole was molecularly dispersed in the freeze dried tablet (FDT).

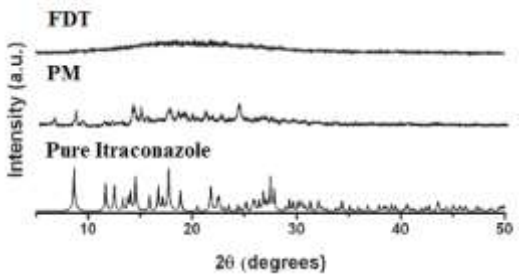
Figure 1 DSC spectrum of Itraconazole samples



X-Ray diffraction was used to analyze potential changes in the inner structure of Itraconazole during the formulation of FDT. The extent of such changes depends on the chemical nature and physical hardness of the active ingredient. The powder X-ray diffraction patterns of the pure drug, physical mixture and FDT are shown in Fig. 2. The results of DSC were further conformed by X-ray diffraction studies. The XRD spectra of the Itraconazole appeared in the 2θ range of 10–30^{0,} indicating that the unprocessed Itraconazole was a crystalline material (4, 6). The pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The X-ray diffraction study of the PM showed the peak corresponding to the crystalline drug molecules

present in the mixture, although their intensity was lower due to the high excipients-drug ratio employed. The diffraction pattern of the FDT of drug showed absence, broadening and reduction of major Itraconazole diffraction peaks indicating that mostly an amorphous form (disordered state) existed in the FDT or drug exists as molecular dispersion in FDT. These results could explain the observed enhancement of solubility and rapid dissolution of Itraconazole in FDT.

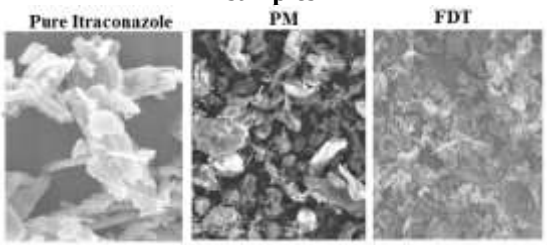
Figure 2 X-ray powder diffraction spectrums of Itraconazole samples



SEM micrographs of Pure Itraconazole, PM, and FDT are shown in Fig. 3. The result showed that Itraconazole crystals could be seen in the PM while the micrograph of FDT shows a matrix in which no crystals of Itraconazole could be seen. The SEM micrograph of FDT suggests that the particles of drug might have been reduced during dissolution in the gelatin-glycine -sorbitol solution. This could therefore indicate that Itraconazole particle size has been reduced which also accelerates solubility and dissolution.

The solubility of Itraconazole was increase from FDT (0.153 mg/ml), nearly five fold increases when compared to the solubility of the pure drug (0.27 mg/ml), suggesting the presence of high amount of amorphous form or molecular form of the Itraconazole drug in FDT, that indicates the super-saturation obtained from the FDT. Increase in solubility of Itraconazole from the PM (0.51 mg/ml), almost two times higher than the pure drug. Solubility result (Table .1) showed that increase in solubility of Itraconazole from FDT could be due to the solubilizing effect of highly water soluble carrier materials used in the formulation such as glycine and sorbitol. The higher solubility of Itraconazole from FDT may be due to the increased in surface area, wetability and solubilizing effect of highly water soluble carrier materials used in the formulations.

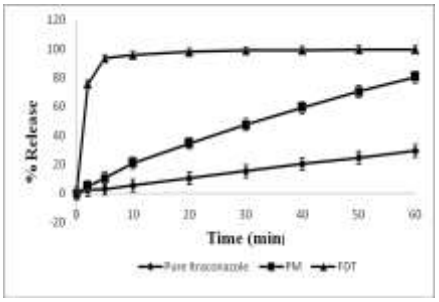
Figure 3 Scanning electron micrographs of Itraconazole samples



The dissolution curves of Itraconazole in distilled water shown in Fig. 4. The rate of dissolution of pure Itraconazole was slow compared with physical mixtures and FDT. Itraconazole in the FDT was immediately dispersed and almost completely dissolved (92.22%) in 5 min. Initial dissolution rate of Itraconazole in the FDT increased markedly about thirty two fold compared to pure Itraconazole in 5 min. The dissolution rate was also higher and faster in FDT than in PM. The percentage of Itraconazole dissolved from its PM for 60 min (78.39%) increased approximately two and half fold compared to Itraconazole pure alone (27.38%).

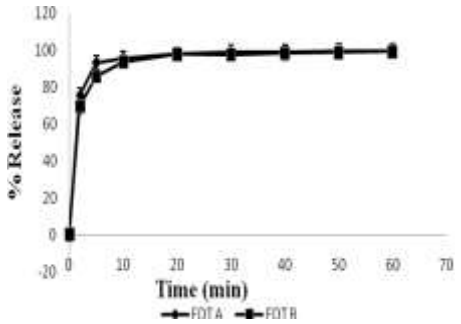
The increased dissolution rate of Itraconazole from its FDT suggesting that Itraconazole FDT might have a rapid oral absorption following disintegration in the mouth and dissolution in the saliva since solubilized Itraconazole is absorbed rapidly and completely from the gastrointestinal tract after oral administration. The enhancement in solubility and dissolution rate of Itraconazole in its FDT may be attributed to the formation of amorphous state in the FDT of the fast dissolving carrier materials.

Figure 4 Dissolution Profiles of Itraconazole pure drug, PM and FDT in Distilled Water at 37^{0}C



The dissolution behavior of Itraconazole FDT must remain unchanged during storage. The best way to guarantee this is by maintaining their physical state and molecular structure. For optimal stability of amorphous FDT, the molecular mobility should be as low as possible. However, FDT, partially or fully amorphous, are thermodynamically unstable and will have a natural tendency to crystallize, because the crystalline state has a lower energy compared to amorphous material. However, amorphous material can be kinetically stable, which implies that the equilibrium state, i.e. crystalline, is not reached within the timeframe of the experiment or shelf life of the product. Therefore, the physical state should be monitored because changes therein are likely to alter the drug release. The results of the stability study of FDT stored at 20 ^{0}C and 45% relative humidity for 90 days were shown in Fig. 5.

Figure 5 Stability Dissolution Profiles of Itraconazole samples FDT A) Freshly FDT, FDT B) FDT after 90 days of storing.



The influence of FDT on the physical stability of Itraconazole was investigated. The percentage of drug release from FDT almost same i.e. (99.67%) after 90 days of storing when compare with the freshly prepared FDT i.e. (99.73 %) after 60 min. Above result showed that FDT of Itraconazole was stable after 90 days storing at 20 ^{0}C and 45% relative humidity.

Conclusion:

From the present study it could be suggested that successful formulation of Freeze dried tablet of can be developed, that is water-soluble excipient and is feasible for enhancing the solubility and dissolution rate of Itraconazole. Since, the results

obtains were attributed to the formation of an amorphous state of the Itraconazole freeze dried tablet and probability to reduction of Itraconazole particle size. The physical stability of FDT result showed that FDT was stable and release from FDT almost unchanged after 90 days. Based on these results, it can be concluded that the freeze dried Itraconazole tablet could be a suitable in terms of solubility and dissolution in water.

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Table 1 Solubility of Itraconazole Pure Drug, PM and FDT in Distilled water at 37^0C

Itraconazole samples (mg/ml)	Solubility mg/ml (± SD, n=3)
Pure Itraconazole	0.27 ±0.015
PM	0.51 ±0.011
FDT	0.153 ±0.014