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Research Article

Ditosylate Salt of Itraconazole and Dissolution Enhancement Using Cyclodextrins

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Abstract. Salt formation has been a promising approach for improving the solubility of poorly soluble acidic and basic drugs. The aim of the present study was to prepare the salt form of itraconazole (ITZ), a hydrophobic drug to improve the solubility and hence dissolution performance. Itraconazolium ditolenesulfonate salt (ITZDITOS) was synthesized from ITZ using acid addition reaction with *p*-toluenesulfonic acid. Salt characterization was performed using ^{1}H NMR, mass spectrometry, Fourier transform infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction. The particle size and morphology was studied using dynamic light scattering technique and scanning electron microscopy, respectively. The solubility of the salt in water and various pharmaceutical solvents was found multifold than ITZ. The dissolution study exhibited 5.5-fold greater percentage release value in 3 h of ITZDITOS (44.53%) as compared with ITZ (8.54%). Results of *in vitro* antifungal studies using broth microdilution technique indicate that ITZDITOS possessed similar antifungal profile as that of ITZ when tested against four fungal pathogens. Furthermore, the physical mixtures of ITZDITOS with two cyclodextrins, β-cyclodextrin (β-CD), and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared in different molar ratios and were evaluated for *in vitro* release. It was observed that in only 30 min of dissolution study, about 74 and 81% of drug was released from 1:3 molar ratios of ITZDITOS with β-CD and ITZDITOS with HP-β-CD, respectively, which was distinctly higher than the drug released from ITZ commercial capsules (70%). The findings warrant further preclinical and clinical studies on ITZDITOS so that it can be established as an alternative to ITZ for developing oral formulations.

KEY WORDS: antifungal; BCS class II; dissolution rate; insoluble; itraconazole.

INTRODUCTION

Salt formation (or salification) is an approach in which the non-optimal physicochemical and/or biopharmaceutical properties of an ionizable molecule can be modified by creating a complimentary form, wherein the ionizable moiety of the drug is paired with an oppositely charged "counter ion". It being convenient and inexpensive, is the widely preferred technique for improving bioavailability as well as for drug product development using conventional as well as novel delivery systems of insoluble drugs (1–3).

Frequently, the decisive goal of salt formation is to deliver the drug orally. As stated by Henderiksen *et al.*, adequate water solubility of a drug is essential property for its absorption through oral route (4). In addition to this, Lipinsky *et al.*, also established that the drugs with poor aqueous solubility usually have variable absorption and as a result erratic bioavailability, and, therefore, salt synthesis may be helpful in resolving this problem (5). Itraconazole was the model hydrophobic drug chosen for our study. The plan of the present study involved 2-fold objectives of firstly preparing, characterizing, and evaluating (in terms of physicochemical properties as well as antifungal efficacy) an acid addition salt of a poorly soluble compound itraconazole (ITZ) and secondly preparing and optimizing physical mixtures of the salt and cyclodextrins to further modulate the dissolution characteristics.

ITZ, an orally active antifungal agent is used to treat various fungal infections including histoplasmosis, blastomycosis, and oncomycosis. On tenets of the Biopharmaceutics Classification System (BCS), it is categorized as a class II drug. It is practically insoluble in water at physiological pH conditions and soluble only under extremely acidic media, leading to a poor oral bioavailability with large individual variability. ITZ is a weak base and pK_a of the piperazine ring is 3.7 (Fig. 1), while the other nitrogen atoms in ITZ molecule are not protonated at extremely acidic pH environments (6–8).

Successful attempts of preparing oral solid dispersions of ITZ using a variety of polymers and surfactants have been reported in recent past (9–14). Although the solid dispersions proved to be valuable formulations for improving the solubility and bioavailability of ITZ, they are associated with shortcomings like expensive pharmaceutical excipients, use of microwaves and constraints of using sophisticated machinery (10–13). In addition, micro- and nanoemulsified systems of

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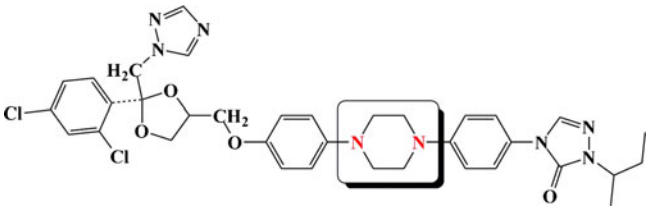

Fig. 1. Basic piperazine moiety of ITZ molecule

ITZ have been reported (15,16). These systems generally suffer from the problem of poorer physical stability than most other delivery systems. Also, castor oil derivatives, common emulsion excipients, have been found to cause adverse effects like anaphylactic shock (17). On the other hand, the micro- and nanoparticulate carriers explored by some workers are associated with drawbacks of increased final cost of formulations due to use of costly polymers and dedicated plant facility requirements (18–20).

The manufacturing process of marketed ITZ capsules involves use of halogenated alkanes and some additional steps including, coating inert sugar beads with the ITZ in polymer solution, drying them, and thereafter again coating them with polymer film to avoid agglomeration of prepared beads (21).

Although salt formation has been a classical approach for improving solubility of hydrophobic drugs, it is also sometimes associated with certain disadvantages including, propensity of disproportionation of certain drug salt(s) to low solubility forms, e.g., miconazole salt forms (22); increased possibility of formation of hydrates and polymorphs in salt forms, e.g., ganciclovir and diclofenac salts (23,24); increased corrosiveness of certain salt forms as compared with their free acid/base drug, e.g., salts of CGP 21495 A, a penicillin derivative (25); and inclusion of an additional step in the manufacturing process for synthesis of the salt.

Owing to the basic nature of ITZ and various drawbacks of marketed capsules and recently explored oral delivery systems, we performed the salification of ITZ in order to favorably enhance its physicochemical properties. Recently, improvement of solubility and dissolution characteristics of ITZ were reported by preparing dihydrochloride salt (26). Although the dihydrochloride salt enhanced aqueous solubility and dissolution behavior, under *in vivo* conditions due to the presence of chloride ions in the gastric juices, common ion effect may reduce its performance (27,28).

The synthesis of the ITZDITOS salt was performed using simple acid addition reaction, and characterized using different spectral and thermal analytical techniques. Broth microdilution method was used to determine the antifungal susceptibility and minimum inhibitory concentration of the salt against four different fungal strains. The *in vitro* dissolution profile of ITZDITOS was studied and compared with ITZ. The physical mixtures of ITZDITOS with β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared simply by sieving them together. Furthermore, *in vitro* dissolution profile of these ITZDITOS CD physical mixtures was performed and the results were compared with ITZ CD mixtures and commercial ITZ capsules (Candistat®).

MATERIALS AND METHODS

Chemicals and Reagent

Itraconazole bulk drug was received as a gift sample from Nosch Labs Pvt Ltd, Hyderabad, India. Commercial ITZ capsules (Candistat®, 100 mg/capsule, Merck India Ltd.) were purchased from local market. The cyclodextrins, peptone, yeast extract, dextrose, sucrose, and agar were procured from Himedia Laboratories, India. All other chemicals used were of analytical grade.

Organisms

Asparagillus fumigates (MTCC-2551), *Microsporum canis* (MTCC-2820), *Microsporum gypsum* (MTCC-2830), and *Trichophyton rubrum* (MTCC-3272) were procured form Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. Czapek media was used for *A. fumigates* and Sabouraud media was used for *M. canis*, *M. gypsum*, and *Trychophyton rubrum.* The compositions of specific media for the fungal strains have been described in Table I.

Synthesis of Itraconazolium Ditoluenesulfonate Salt

ITZDITOS was synthesized using our previously reported method (29). Briefly, a solution of ITZ (5 g, 7.09 mmol) in chloroform (20 ml) was refluxed with 50% (*w*/*v*) methanolic solution of toulenesulfonic acid (14.88 mmol). The reaction was completed in around 25 min, and the reaction mixture was washed with water using separating funnel and dried over anhydrous sodium sulfate. Evaporation of chloroform under reduced pressure gave pale white residue, which was reprecipitated from methanolic solution by addition of water to give ITZDITOS salt, which was filtered and dried under vacuum.

Characterization of ITZDITOS

^1H NMR Spectroscopy

NMR spectra were recorded in deuterated chloroform using 400 MHz Bruker FT-NMR (Bruker India Scientific Pvt.

Table I. Media Compositions for Different Fungal Strains

Media	Composition	
	Ingredient	Quantity
Czapek media	Czapek concentrate[a]	10 ml
	Dipotassium hydrogen phosphate	1.0 g
	Yeast extract	5.0 g
	Sucrose	30.0 g
	Agar	15.0 g
	Water	q.s. 100 ml
Sabouraud media	Special peptone	10.0 g
	Dextrose	20.0 g
	Agar	15.0 g
	Water	q.s. 100 ml

[a] $NaNO_3$, 30.0 g; KCl, 5.0 g; $MgSO_4 \cdot 7H_20$, 5.0 g; and $FeSO_4 \cdot 7H_2O$, 0.1 g in up to 100 ml with water

Ltd., New Delhi, India) spectrometer using tetramethylsilane as internal standard, and the chemical shifts are reported in δ units.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) absorption spectra for both ITZ and prepared ITZDITOS salt were obtained on FTIR-spectrometer (PerkinElmer, England) over a range of 400 to 4,000 cm^{-1}. Dry KBr (50 mg) was finely ground in an agate mortar and the sample of drug or salt was subsequently added and mixed gently. A manual press was used to prepare the sample in KBr disc.

Mass Spectrometry

Mass spectrograph of ITZDITOS salt was obtained by LCQ Mass Spectrometer (Finnigan MAT, UK) in atmospheric pressure chemical ionization (APCI) mode with an inner temperature of 200°C. Sample was dissolved in methanol, filtered (0.45 μm), and analyzed in the range of 0–1,000 m/z. Data interpretation was performed using X'Calibur software.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis was performed using Mettler Toledo 821^e DSC (Mettler Toledo, Switzerland) operating with STAR^e software version Solaris 2.5.1. Temperature axis and cell constant were calibrated using Indium. Samples were heated at 10°C min^{-1} over the temperature range of 25–400°C under dry nitrogen with nitrogen flow rate set at 80 ml min^{-1} in pin-holed aluminum pans.

Hot Stage Microscopy

The hot stage microscopy (HSM) was performed using Zeiss Axioplan-2 microscope (Microptic, Netherlands) fitted with Linkam44 hot stage THMS600 (Linkam Scientific Instruments, UK). Images were captured using attached Nikon, Eclipse 80*i* camera. The samples were mounted in air/silicone oil and heated from 25–400°C.

Powder X-ray Diffraction

The powder X-ray diffraction (PXRD) patterns of ITZ and ITZDITOS were recorded on X-ray diffractometer (X.Pert Pro, Panalytical, the Netherlands) with Cu as tube anode. The diffractograms were recorded under following conditions: voltage, 40 kV; current, 45 mA; scan range, 3–50; scan rate, 4°/min; and measurement temperature, 25°C.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was performed using a Jeol Scanning microscope (Jeol Inc., Japan) with a 15-kV accelerating voltage. The surfaces of samples for SEM were previously made electrically conductive in a sputtering apparatus (Jeol Fine Coat, ion sputter, JFC-1100, Japan) by evaporation of gold. A magnification of ×5,000 was used.

Dynamic Light Scattering

The mean diameter and size distribution of ITZ and ITZDITOS powders were determined by dynamic light scattering (DLS) in Mastersizer 2000S (Malvern Instruments Ltd., UK). Dry samples (3 g) were analyzed using single narrow analysis model with enhanced sensitivity for a size range of 0.1–2,000 μm range.

Determination of Porosity and Bulk Density

The two parameters, bulk density and tapped density that govern the flow properties of a powder were calculated using methods provided in USP 2007 (30). Briefly, ITZDITOS powder was passed through 1-mm screen and was filled in a 100-ml graduated measuring cylinder up to the 50 ml. The bulk volume of the powder without disturbing the powder bed was measured and bulk density was calculated (Eq. 1).

$$\text{Bulk density}(B_d) = M/V_b \tag{1}$$

Where,

V_b bulk volume of the powder
M mass of powder with a bulk volume of 50 ml

Then the cylinder was tapped for around 500 times and final volume indicated tapped volume of the powder which provided tapped density (Eq. 2).

$$\text{Tapped density}(T_d) = M/V_f \tag{2}$$

V_f final tapped volume of the powder

The values of bulk volume and tapped volume were used for calculating Hausner's ratio (Eq. 3) and compressibility index (Eq. 4).

$$\text{Hausner's ratio} = V_b/V_f \tag{3}$$

$$\text{Compressibility index} = 100(V_b - V_f)/V_b \tag{4}$$

Quantitative Determination Using Ultraviolet Spectroscopy

A stock solution of a 100 μg/ml was prepared in simulated gastric fluid (SGF) without pepsin, pH 1.2. Stock solution was diluted to obtain standard solutions of different concentrations (2, 4, 8, 10, 15, 20, and 25 μg/ml) and then calibration curves of the drug and the salt were obtained by plotting the absorbance values at (λ_{max}, 254 nm) against different concentrations using ultraviolet (UV) spectrometer (Pharmaspec 1700, Shimadzu Inc. Japan). The method was validated with respect to linearity, accuracy, and precision.

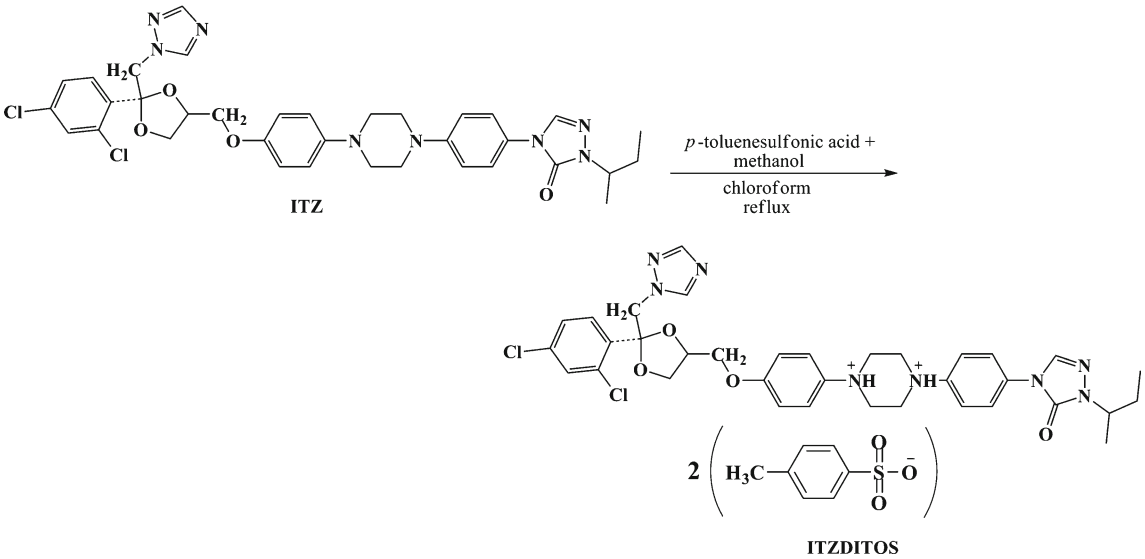

Fig. 2. Synthesis of ITZDITOS

Evaluation of ITZDITOS

Antifungal Susceptibility Test

Preparation of Inoculum. Filamentous moulds were allowed to grow media plates for different organisms at 25°C, until sufficient numbers of conidia were formed. The surface of thallus was scraped with a sterile loop, suspended in phosphate buffer (5.0 mM, pH 7), and was allowed settle for 2 h. The supernatant was taken out and adjusted to make the 1.0 OD at 600 nm. The suspension of the spores recovered by the above procedure was diluted 1,000 times in nutrient media and utilized as inoculum in microdilution plate method for determination of MIC (31,32).

MIC Determination. Broth micordilution method has been suggested for the determination of MIC of antifungal agents (33,34). The filter sterilized stock solutions of ITZ and ITZDITOS were prepared in dimethyl sulfoxide. The wells of sterilized microplates were filled with 280 μL of sterile media and thereafter 10 μL of inocula were added in each well and mixed thoroughly. Various strengths of filter sterilized solutions (10 μL) of ITZ and ITZDITOS were poured in the wells to make their final concentration of drug in 0.03125–16 μg/ml range. Microplates were incubated at 25°C for 5 days. The growth was observed in the form of turbidity with naked eyes under the light background by comparing the clarity of the blank media. The MIC was defined as "the lowest concentration that produced inhibition of visible growth".

Preparation of Cyclodextrin Physical Mixtures

The mixing of 500 mg of ITZ (or 743.8 mg of ITZDITOS) with 804.3, 1,608.5, and 2,412.8 mg of β-CD and

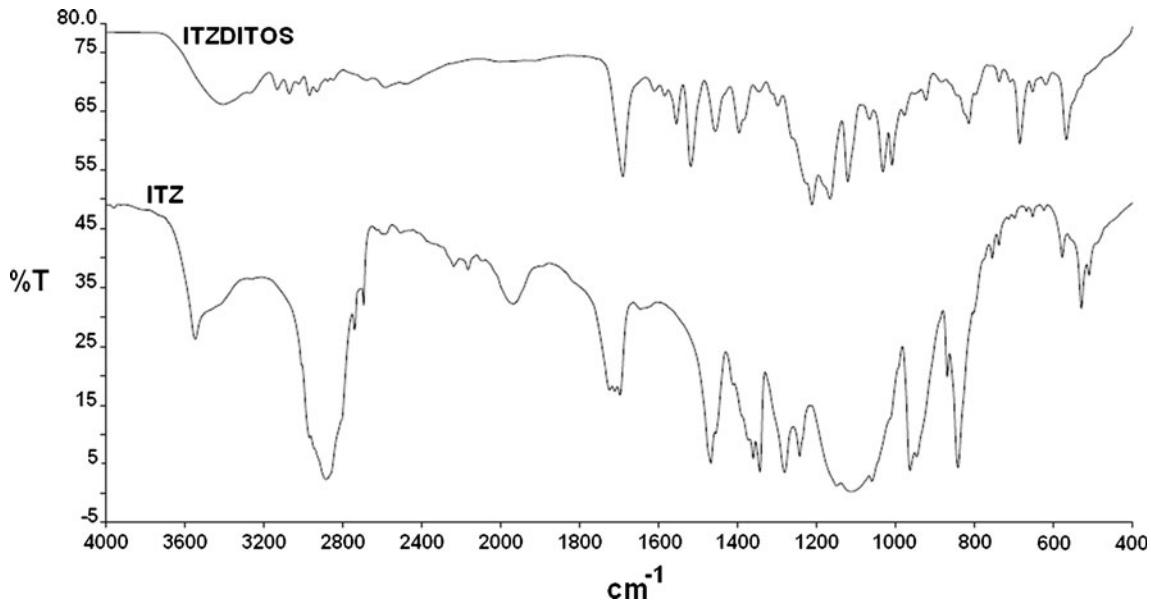

Fig. 3. FTIR spectra of ITZ and ITZDITOS

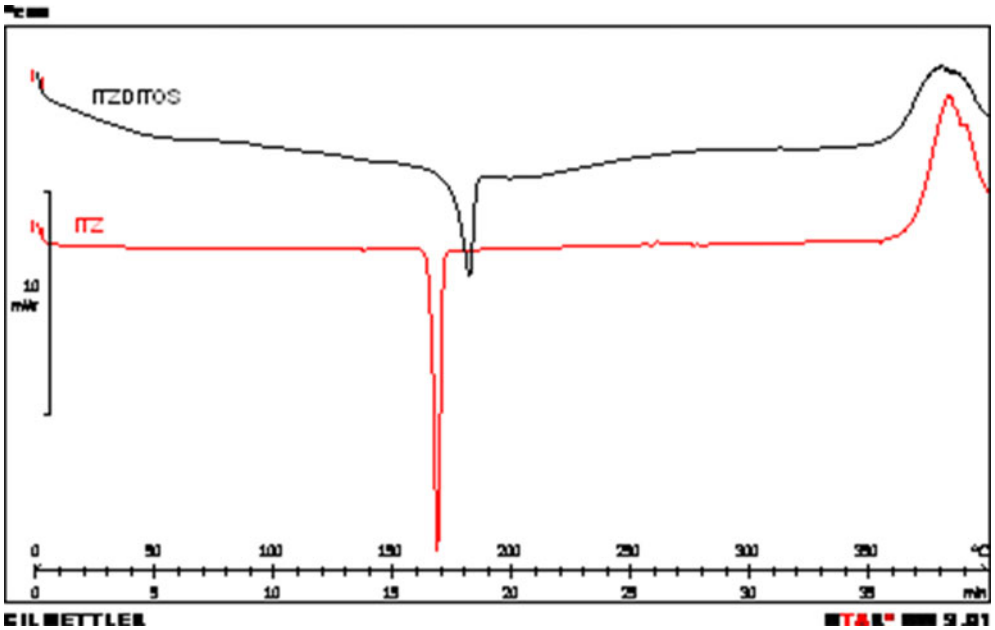

Fig. 4. DSC overlay plot of ITZ and ITZDOTOS (the heat of fusion values were −83.94 and −60.83 $J g^{-1}$ for ITZ and ITZDOTOS, respectively)

with 1,092.3, 2,184.7, and 3,277 mg of HP-β-CD provided 1:1, 1:2, and 1:3 molar ratios, respectively. These mixtures were made homogenous by repeated screening through 80 mesh. The 1:1, 1:2, and 1:3 molar ratios of the physical mixtures of ITZ with β-CD will further be notated as ITZ β-CD (1:1), ITZ β-CD (1:2), and ITZ β-CD (1:3), respectively, whereas those with HP-β-CD will be referred to as ITZ HP-β-CD (1:1), ITZ HP-β-CD (1:2) and ITZ HP-β-CD (1:3), respectively. Similarly, the physical mixtures of ITZDITOS with β-CD and HP-β-CD will be named as ITZDITOS β-CD (1:1), ITZDITOS β-CD (1:2), ITZDITOS β-CD (1:3), ITZDITOS HP-β-CD (1:1), ITZDITOS HP-β-CD (1:2), and ITZDITOS HP-β-CD (1:3) in identical manner.

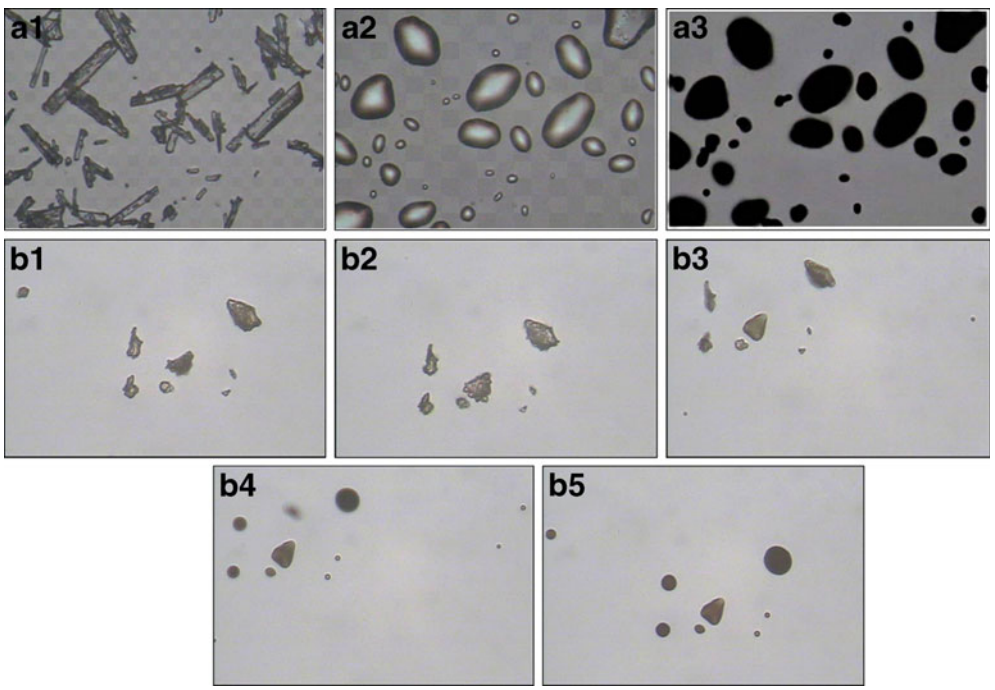

Fig. 5. The HSM images of ITZ and ITZDITOS. ITZ depicted acicular crystal habit prior to melting (*A1*); melting initiated at 172°C (*A2*) followed by decomposition over temperatures above 350°C (*A3*), whereas ITZDITOS revealed plated particles which showed no melting at 100°C (*B1*) and 150°C (*B2*). Melting initiated at a temperature of 178°C (*B3*) and completed at 185°C (*B4*), followed by decomposition above 348°C (*B5*)

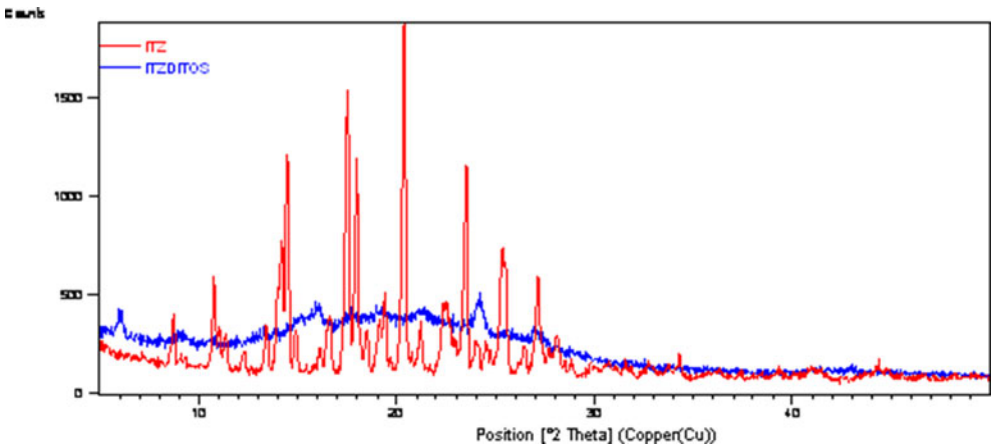

Fig. 6. X-ray diffractograms of ITZ and ITZDITOS

Dissolution Testing

Dissolution study of ITZ (100 mg) as well as ITZDITOS and the CD physical mixtures (equivalent to 100 mg ITZ) was performed using USP paddle method (LabIndia DS 8000, LabIndia Ltd.) in 900 ml of enzymeless SGF, pH 1.2±0.02, maintained at 37±1°C and stirred at 100 rpm. Five-milliliter aliquots of dissolution medium were withdrawn at predetermined time intervals and replaced by 5 ml of fresh dissolution medium. The filtrates of the samples were analyzed for the content of drug by UV spectrophotometer at 254 nm. The experiments were done in triplicate. The dissolution profiles of various ratios of CD physical mixtures of ITZ and ITZDITOS were compared by employing two-factor ANOVA test with replication and cumulative percent drug released (in 180 min) as response using statistical software (SigmaStat 3.5), for significant difference at 5% confidence limit.

In addition, the pharmacokinetic parameters like percentage drug dissolution in 10 min (DP_{10}) and half-life of release ($T_{50\%}$) were used to evaluate improvement of dissolution rate of ITZDITOS and ITZDITOS with CD physical mixtures as compared with ITZ as well as marketed ITZ capsules.

Another parameter, dissolution efficiency (DE%) at 180 min was determined to assess the enhancement in extent of dissolution (35). It is defined as the area under the dissolution curve up to a certain time t and is expressed as a percentage of the curve at maximum dissolution (Y_{100}), over the same time period (Eq. 5).

$$DE\% = \frac{\int_0^t Y \cdot dt}{Y_{100} \cdot t} \cdot 100 \tag{5}$$

Where,

Y % age of dissolved drug
t given time interval

The dissolution profiles of ITZDITOS as well as physical mixtures with CDs were compared against dissolution profile of commercial ITZ capsule using the FDA approved model independent approach of similarity factor (36). The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of similarity in the percent dissolution between the two curves and is expressed as the Eq. 6 (37).

$$f_2 = 50 \cdot \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \cdot 100\right\} \tag{6}$$

Where,

n number of time points
R_t dissolution value of the reference (prechange) batch at time t
T_t dissolution value of the test (postchange) batch at time t

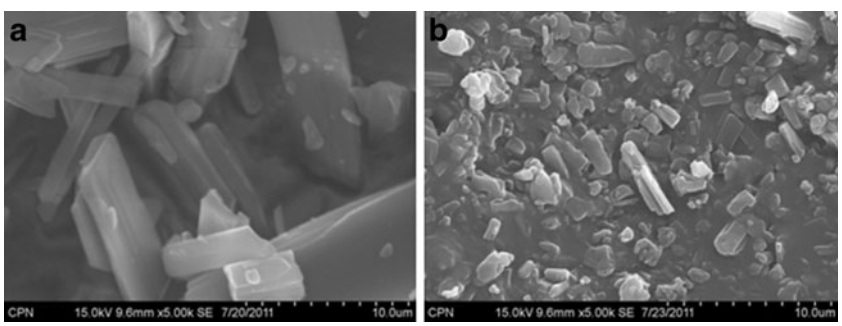

Fig. 7. SEM micrographs (×5,000) of ITZ (**a**) and ITZDITOS (**b**)

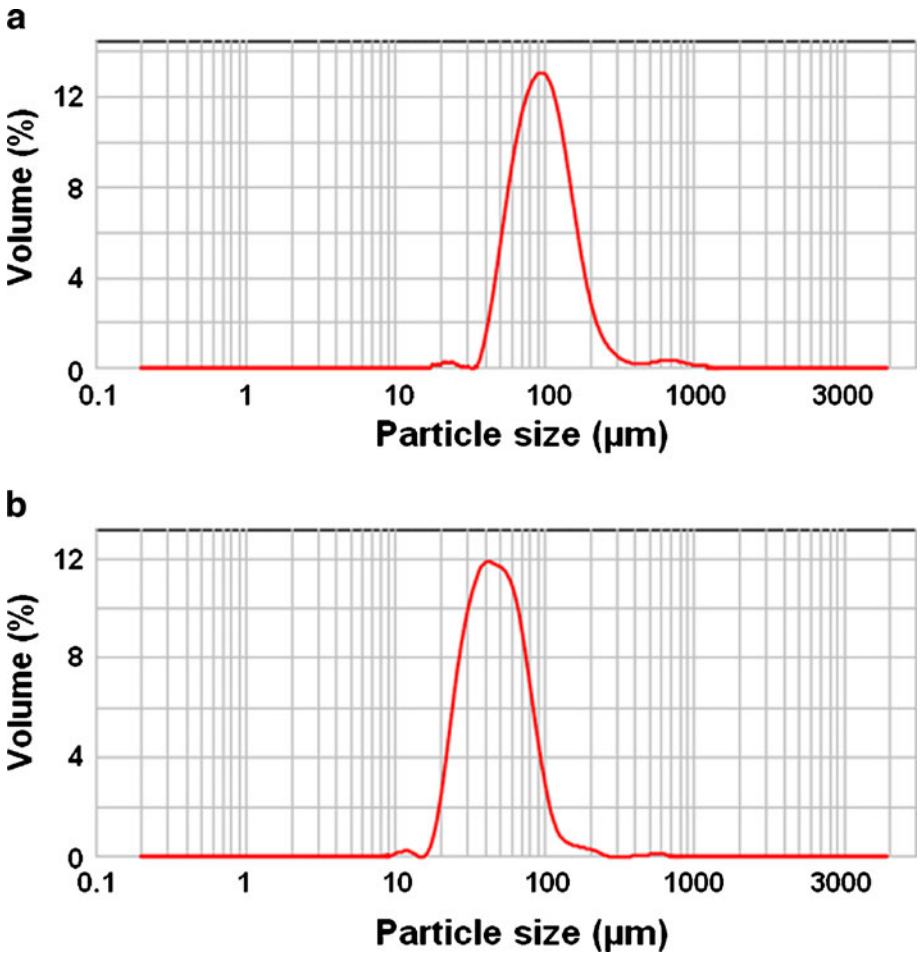

Fig. 8. Particle size distributions of ITZ (**a**) and ITZDITOS (**b**)

Similarity factor (f_2) above 50 indicates the sameness of reference and test curves. The similarity factor (f_2) values for ITZ, ITZDITOS, and their CD physical mixtures were calculated using commercial ITZ capsule taken as reference.

RESULTS AND DISCUSSION

ITZ is a potent antifungal agent effective against a variety of fungi. Being a BCS class II drug, it is slowly absorbed from the solid dosage forms (commercial capsules), consequently making dissolution the rate-limiting step in the absorption of the drug (38). The dissolution rate directly affects the onset, intensity, and duration of action. Therefore, enhancing the dissolution rate of ITZ becomes an important task for its oral formulation development.

Through salt formation, the physicochemical properties like aqueous solubility (and hence, bioavailability) of an ionizable drug could be improved due to polar character imparted by the counter anions.

Table II. List of Powder Flow and Compaction Parameters (mean± SD, n=3)

Parameter	ITZ	ITZDITOS
Bulk density (B_d (g/ml))	0.126	0.189
Tapped density (T_d (g/ml))	0.150±0.002	0.329±0.008
Hausner's ratio	1.191±0.014	1.745±0.0 46
Compressibility index	16±1.000	42.667±1.528

ITZDITOS was obtained as a pale white powder with the reaction yield of 88% (Fig. 2). As mentioned in our previous report, the solubility of the salts in water, SGF, and other pharmaceutical solvents like ethanol and propylene glycol was also improved to a great extent (20). The characterization of ITZDITOS was performed using various spectral, microscopic, and thermal techniques.

^{1}H NMR Spectrum

The ^{1}H NMR spectrum for ITZDITOS as exhibited peaks attributable to ITZDITOS structure, for instance singlet peak at δ 2.35 for six protons due to presence of two *p*-methyl groups of the benzene rings of toluenesulfonate ions. In addition to this, the eight aryl protons of the two toluenesulfonate ions exhibited two different signals, doublet peaks at δ 7.17–7.19 and δ 7.54–7.60.

Mass Spectrum

In APCI mode of mass spectrometry (MS), ITZDITOS salt gave a molecular (M^++1) ion peak at 705.53m/z. The molecular ion peak corresponding to molecular weight of ITZ confirmed the integrity of the parent molecule after salt formation.

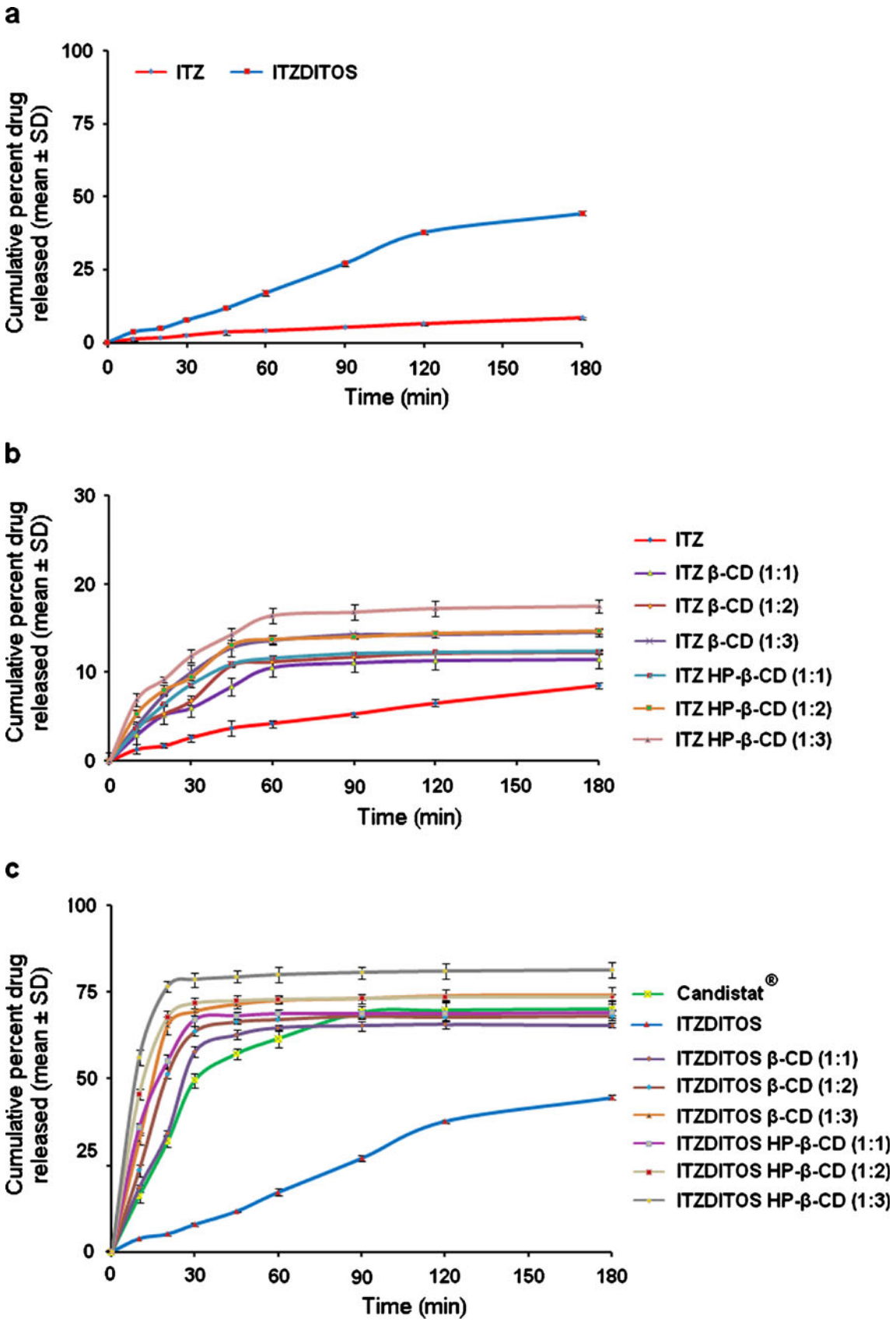

Fig. 9. Dissolution profiles of ITZ and ITZDITOS (**a**), ITZ CD physical mixtures (**b**), and ITZDITOS CD physical mixtures and Candistat® (**c**)

Table III. The MIC of ITZ and ITZDITOS Against Four Fungal Strains

Fungal strain	MIC (μmol)	
	ITZ	ITZDITOS
Asparagillus fumigates	0.5	0.5
Trychophyton rubrum	0.25	0.25
Microsporum canis	0.25	0.25
Microsporum gypsum	0.25	0.25

FTIR Spectrum

FTIR spectrum for ITZDITOS showed the characteristic sulfate asymmetric SO_3stretch band at 1,121 cm^{-1}, the peak which was not present in spectra of ITZ base, affirming the incorporation of *p*-toluenesulfonate ions in ITZ molecule (Fig. 3). The broad band at 3,406 cm^{-1} may be associated to O–H stretch due to presence of traces of water molecules in the ITZDITOS.

DSC Thermogram

In accordance to earlier reports (13,15,27), DSC thermogram of ITZ showed its characteristic melting endotherm at 170°C, whereas that of ITZDITOS exhibited a relatively broader endotherm starting at around 175°C to 185°C (Fig. 4), which infers the loss of crystalline nature of ITZ free base form after salt formation.

Hot Stage Microsopy

The HSM results were in concordance with thermal events evident from DSC profile (Fig. 5). The ITZ crystals started melting at a temperature of 172°C and the melting was completed at 176°C and decomposition was observed above 350°C. ITZDITOS on the other hand started melting at a temperature of 178°C and melting was complete at 185°C.

X-ray Diffractogram

As reported in some previous works (19,39), the PXRD patterns for ITZ, exhibited sharp characteristic peaks, which can be used as a fingerprint. In contrast to this, broad peaks comparatively smaller in number, having different positions and low intensities were observed for ITZDITOS (Fig. 6). The peak corresponding to 100% relative intensity was observed at 2θ value of 20.384° in diffractogram of ITZ, whereas the same for ITZDITOS was encountered at 2θ value of 18.844°. Also, the area under the peak (expressed as cts x 2θ) corresponding to 100% intensity of ITZ was found to be 365.40, which reduced to 53.00 after salt formation thus signifying the loss of crystalline nature of ITZ. Additionally, the other important peaks present in ITZ diffractogram corresponding to 61.04%, 57.22%, and 58.22% relative intensity occurring at 2θ values of 14. 471°, 17.971°, and 23.518° respectively were replaced by new peaks corresponding to 60.26%, 49.57%, and 54.15% at 2θ values of 17.631°, 25.037°, and 26.033°, respectively.

Scanning Electron Micrographs

The scanning electron micrographs are presented in Fig. 7. The SEM images of ITZ illustrated its acicular needles, in agreement with some previous literature reports (40–42). On the other hand, ITZDITOS exhibited smaller particles with less crystalline nature.

Dynamic Light Scattering

The DLS technique showed prominent reduction in particle size of the drug after salt formation (Fig. 8). The mean diameter of ITZ powder was found to be around 95 μm, whereas that of ITZDITOS salt was found to be 42.7 μm. Thus, the salt powder exhibited less than half of the mean diameter than the free base form.

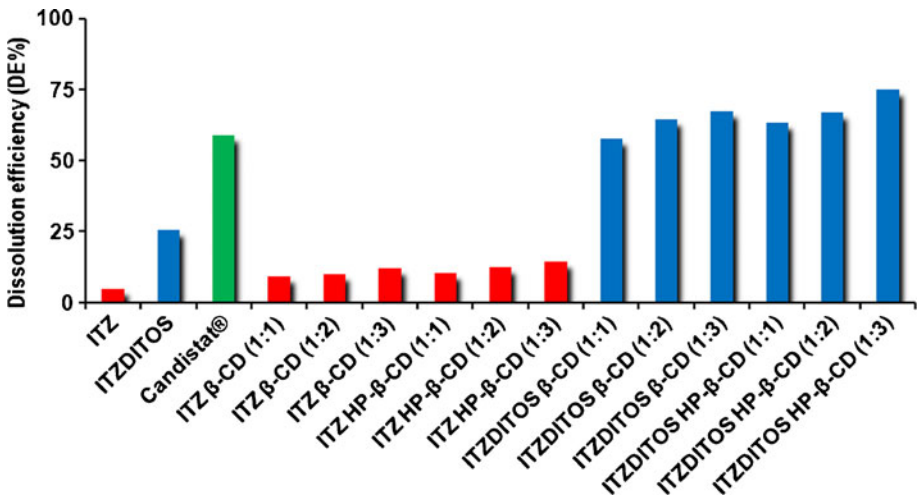

Fig. 10. Dissolution efficiency (*DE*%) of ITZ, ITZDITOS, their CD mixtures, and Candistat®

Table IV. The Cumulative Percent Drug Released in 10 min (DP_{10}, in percent) and $T_{50\%}$ (min) of ITZ, ITZDITOS, Candistat®, and CD Physical Mixtures in Various Molar Ratios

Entries	DP_{10} (%)	$T_{50\%}$ (min)
ITZ	1.37	–
ITZDITOS	3.92	–
Candistat®	16.25	30.7
ITZ β-CD (1:1)	2.93	–
ITZ β-CD (1:2)	3.82	–
ITZ β-CD (1:3)	4.01	–
ITZ HP-β-CD (1:1)	3.62	–
ITZ HP-β-CD (1:2)	5.38	–
ITZ HP-β-CD (1:3)	6.95	–
ITZDITOS β-CD (1:1)	18.46	26.5
ITZDITOS β-CD (1:2)	23.54	19.3
ITZDITOS β-CD (1:3)	32.30	15.4
ITZDITOS HP-β-CD (1:1)	35.77	16.9
ITZDITOS HP-β-CD (1:2)	45.58	11.7
ITZDITOS HP-β-CD (1:3)	56.01	8.0

Powder Flow and Compressibility Parameters

The powder flow and compressibility parameters are explained in Table II. The mean values of bulk density and tapped density were increased for ITZDITOS in comparison to ITZ. The relative greater difference in bulk density and tapped density of ITZDITOS, Hausner's ratio greater than 1.5 and the compressibility index value of above 40 for ITZDITOS reflects its poorer flow properties of the salt in comparison to ITZ.

Antifungal Susceptibility Study

Four most common fungal strains causing infections in humans were used to determine susceptibility against ITZDITOS. The MIC for ITZ and ITZDITOS exhibited no difference, and hence no loss of antifungal efficacy of the drug against all four fungal strains studied (Table III). In addition to this, the broth microdilution study also elucidated the order of antifungal activity of ITZDITOS, which was found to have similar hierarchy of antifungal activity as ITZ. The results of antifungal broth microdilution assay suggested that the antifungal activity of ITZ against the four different fungal strains was retained after salt formation.

Dissolution Study

The dissolution studies were carried out and the quantitative measurements were performed at λ_{max} of 254 nm. The dissolution profiles of ITZ and ITZDITOS in simulated gastric fluid are represented in Fig. 9a. It was observed that only about 8.40% of drug was released from free base form in 3 h, whereas from ITZDITOS salt form approximately 44.5% drug was released in the same time. Thus, more than 5-fold enhancement of dissolution from ITZDITOS in comparison to ITZ was achieved.

Figure 9b shows the dissolution profiles of the physical mixtures of ITZ with both cyclodextrins. It was found that physical mixtures of ITZ with different ratios of and β-CD and HP-β-CD resulted in enhancement of drug release with a maximum of about 2-fold (17.54%) using ITZ HP-β-CD (1:3).

The CD physical mixtures of ITZDITOS exhibited noticeable enhancements as is evident from Fig. 9c. In case of ITZDITOS β-CD physical mixtures, 1:1 and 1:2 molar ratios provided around 65% and 68% drug release, respectively, whereas 1:3 molar ratio gave around 74% drug release. The ITZDITOS HP-β-CD physical mixtures illustrated even better drug release profiles. The ITZDITOS HP-β-CD in 1:1, 1:2 and 1:3 ratios exhibited approximately 69%, 74%, and 81% drug release, respectively. Therefore, it was found that the percent drug releases from ITZDITOS β-CD (1:3) and ITZDITOS HP-β-CD (1:2 and 1:3) in 3 h were better than the commercial Candistat® capsule, which showed drug release value of about 70% in the same time.

Table IV depicts the dissolution rate parameters for ITZ, ITZDITOS and their CD physical mixtures. Superior percent releases in 10 min were reported for both types of CD mixtures of ITZDITOS as compared with ITZ and its CD mixtures. From the commercial capsule, 16.25% drug was released in 10 min, whereas, higher DP_{10} values using ITZDITOS β-CD physical mixtures (18–32% approximately) and

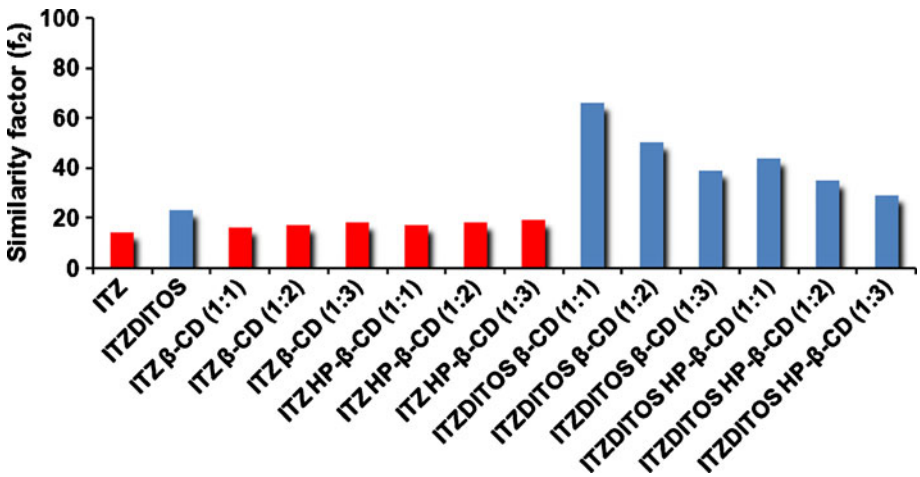

Fig. 11. Values of similarity factor (f_2) values of ITZ, ITZDITOS, and their respective CD physical mixtures in various molar ratios compared with marketed capsule Candistat®

ITZDITOS HP-β-CD physical mixtures (36–56% approximately) were achieved.

$T_{50\%}$ is considered as the factor, describing the dissolution rate of drug, and it was observed that all ratios of ITZDITOS HP-β-CD showed lesser $T_{50\%}$ values of all CD physical mixtures of ITZDITOS were noticeably lesser than Candistat® suggesting instant release of drug from these physical mixtures and slow release from Candistat®.

The DE% in 180 min was enhanced significantly for both ITZDITOS and ITZDITOS CD physical mixtures in comparison to ITZ and ITZ CD mixtures (Fig. 10). The DE% value of ITZ was mere 5.11%, which was improved after physical mixtures were prepared with β-CD and HP-β-CD, and the maximum DE% was nearly 15% for ITZ β-CD (1:3). The three molar ratios, 1:1, 1:2, and 1:3 of ITZDITOS with β-CD exhibited DE% of 57.85, 61.46, and 67.03, respectively. While, the values of DE% for ITZDITOS with HP-β-CD in 1:1, 1:2, and 1:3 molar ratios were 63.11%, 67.96%, and 75.07%, respectively. Thus, the DE% values of ITZDITOS β-CD and ITZDITOS HP-β-CD mixtures were found to be notably higher than that of plain ITZDITOS (25.47%). Also, it was found that DE% of ITZDITOS β-CD (1:1) was almost equal to Candistat® (58.70) and that of other molar ratios with both CDs were significantly higher than Candistat® indicating its more complete dissolution than marketed capsules.

Two factor ANOVA was performed to assess the influence of two factors explicitly salt formation and CD content in physical mixtures on dissolution profile. The CD mixtures with both CD types were prepared in three different molar ratios of CDs to drug. The results of two-way ANOVA test performed individually for β-CD and HP-β-CD physical mixtures of ITZ and ITZDITOS depicted significant dissolution rate enhancement, which was influenced greatly by the two factors studied, salt formation and increase in CD molar content in physical mixtures. The dissolution profile for 1:3 ratios of β-CD and HP-β-CD was found to be best among the three ratios studied for both CDs.

The similarity factor f_2 values of CD mixtures of ITZDITOS were found to be better than ITZ, ITZ CD mixtures and ITZDITOS. The values inferred considerable sameness of dissolution profile of ITZDITOS β-CD in 1:1 and 1:2 molar ratios to that of commercial capsules (Fig. 11).

The significant enhancements in dissolution rate of ITZDITOS as well as their CD mixtures could be explicated by several ways. Firstly, the reduction in particle size of ITZ after salt formation as depicted by DLS, optical microscopy (25), and SEM images, as the smaller-sized particles expose more surface area of the powder water resulting in better wettability and solubility. Secondly, lower crystalline nature of the salt was shown by thermal characterization techniques like DSC and HSM. This was further confirmed by X-ray diffraction patterns and SEM images. As non-crystalline states of a molecule are the higher energy states and therefore, have better solubility usually than corresponding crystalline forms (43,44). In addition to this, the inclusion effects of cyclodextrins on ITZDITOS in presence of an aqueous medium as explained in a previous study can be another major reason for this amplification in dissolution rate (15). This effect was more prominent in case of physical mixtures of ITZDITOS and HP-β-CD than that in ITZDITOS and β-CD, which may be explained on the basis of better aqueous solubility of HP-β-CD than β-CD. The dissolution profile exhibited faster dissolution of ITZDITOS with CD physical mixtures than ITZ with CD physical mixtures. Among the three molar ratios studied, ITZDITOS β-CD (1:3) and all ITZDITOS HP-β-CD ratios showed markedly higher dissolution profile of ITZDITOS.

CONCLUSIONS

Based on the present results, it could be concluded that ITZDITOS could be conveniently and cost effectively prepared. The augmented dissolution profiles of ITZDITOS as well as its CD physical mixtures than ITZ, its CD mixtures and even a commercial ITZ product and in addition its similar antifungal efficacy of the parent drug against tested fungal strains emphasize the extensive investigation of the salt as a candidate for clinical use. As itraconazole is a BCS class II drug, its bioavailability is dissolution rate limited. Therefore, ITZDITOS salt with enhanced dissolution may possess improved bioavailability and can be used as an alternative ITZ for development of various oral dosage forms. However, these are only the preliminary studies and they suggest further preclinical and clinical evaluation of ITZDITOS.

Itraconazolium ditoluenesulfonate. Yield, 88%; white solid; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89–0.92 (*t*, 3H, CH3, *J*= 7.40 Hz), 1.39–1.40 (*d*, 3H, CH3, *J*=6.68 Hz), 1.69–1.90 (*m*, 2H, CH2), 2.35 (*s*, 6H, ×2 CH3, Ar-CH3), 3.78–3.82 (*m*, 2H, dioxolane CH2), 3.90–3.93 (*m*, 11H, O-CH2, ×4 CH2, dioxolane CH), 4.25–4.38 (*m*, 3H, N-CH2), 4.88 (*s*, 2H, ×2 OH), 6.98–7.00 (*d*, 2H, Ar-H, *J*=8.84 Hz), 7.17–7.19 (*d*, 4H, ×4 Ar-H, *J*= 7.92 Hz), 7.26–7.28 (*d*, 2H, ×2 Ar-H, *J*=9.00 Hz), 7.48 (*s*, 1H, triazolone CH), 7.54–7.60 (*m*, 3H, ×3 Ar-H), 7.66 (*s*, 1H, Ar-H), 7.78–7.83 (*m*, 6H, ×6 Ar-H), 8.22 (*s*, 1H, triazole CH), 9.13 (*s*, 1H, triazole CH); MS (APCI)—705.6 (M+1). *Anal.* Calcd. for $C_{49}H_{52}Cl_2N_8O_{10}S_2$—C, 56.16; H, 5.00; N, 10.69; and S, 6.12. Found—C, 55.87; H, 5.20; N, 10.77; and S, 5.92.

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