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RESEARCH ARTICLE

Self-Solidifying and Self-Nanoemulsifying Drug Delivery System of Itraconazole

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Abstract

Objective: To improve solubility, dissolution, and permeability of BCS class II drug Itraconazole (ITZ) using a self solidifying self nano emulsifying drug delivery system (SNEDDS). **Method:** The solubility of ITZ was assessed in oils, surfactants, co-surfactants, and buffers. Surfactants, co-surfactants, and combination of surfactants (S-comb) were selected on the basis of emulsification efficacy test. The ability to solidify self emulsifying mixture was assessed and solid SNEDDSS was developed and optimised. **Finding:** Solubility of ITZ was found maximum in 0.1N HCl (0.120 ± 0.07mg/mL) followed by SGF (0.089 ± 0.01mg/mL). Out of 20 oils screened, Peceol showed highest solubility. Among all surfactants and co-surfactants studied, Labrafil M 1944 CS (LM 1944) showed highest potential to solubilize ITZ (14.91 mg/g). The globule size of the optimised formulation was found to be 40.11 nm with PDI of 0.144. Zeta potential study revealed the stability of the SNEDDS. Drug content of solid SNEDDS of ITZ was found within the range of 98.11% to 102.51%, which was found in the acceptable limit. It was observed that ITZ solid SNEDDS (S-SNEDDS) showed a promising improvement in the in vitro dissolution profile compared to the plain ITZ and marketed product in all three-dissolution media. Nearly 3-fold enhancement of permeability of ITZ was attributed by uniformly dispersed globules with nano size. Analytical characterization demonstrated that the drug and excipients are compatible with amorphous characteristics of the ITZ. **Novelty:** The developed self solidifying SNEDDS of ITZ showed enhanced solubility, dissolution, and permeability in comparison to the pure drugs (ITZ). Self solidifying SNEDDS would be a novel approach to overcome the limitations associated with liquid dosage forms.

Keywords: Itraconazole; Solubility; Bioavailability; Self Nano Emulsifying Drug Delivery System (SNEDDS); Permeability

1 Introduction

For a variety of drugs, oral administration is thought to be the best mode of administration, hence oral administration accounts for 80% of dosage forms now on the market. Furthermore, oral drug administration is preferred for a number of dosage forms (controlled release, sustained release, fast release, etc.) with quite distinct preparation techniques. To select the best route of administration, it is necessary to investigate and evaluate the drug's physicochemical properties. For instance, the majority of drugs have very low water solubilities (68% of oral drugs have poor solubility, <100 μg/mL), which results in insufficient absorption in the stomach milieu following oral administration, limited bioavailability, and consequently poor efficacy[1].

In order to increase the solubility of drugs that are lipophilic (Log P > 3), lipid-based formulations known as SNEDDS are utilised. SNEDDS is essentially a blend of oils from natural or synthetic origin and surfactants, or they can be a mixture of hydrophilic solvents and co-solvents/surfactants[2]. When taken orally, the preconcentrate of SNEDDS transforms into an oil-in-water type emulsion with small globules (microemulsion or nanoemulsion). A key phenomenon for self-emulsification is the transformation of SNEDDS preconcentrate into fine globules, which occurs as a result of modest agitation (*in vitro*) or digestive motility (*in vivo*) in the presence of simulated fluid or gastric fluid[2]. BCS class II and IV drug absorption and *in-vitro* drug dissolution rates are improved by formulating SNEDDS[3]. This dosage form offers benefits such drug protection against the unfavorable gut environment and targeted drug delivery to the GIT. A stable formulation that can be filled into capsules, high drug entrapment efficiency, spontaneous emulsion formation that helps skip the dissolution process, and prevention of drug degradation in gastric media are some common benefits associated with SNEDDS[4].

Itraconazole (ITZ) is triazole derivative and antifungal agent. ITZ is classified as BSC class II drug (low solubility, high permeability). ITZ is insoluble in water (0.00964 mg/mL) and absolute bioavailability of ITZ is 55% and it reaches to maximum when taken with food[5]. A number of attempts have been made by researchers to enhance solubility and bioavailability of ITZ by PLGA [poly (lactic-co-glycolic acid)] nanoparticles, L-SEDDS (Liquid self emulsifying drug delivery system), mucoadhesive tablet, bioadhesive film, nanoparticles, co-crystals, tablet, crystalline agglomerates, and solid dispersion[6–9]. All these developed technologies have their own drawbacks e.g., liquid formulations have stability issues and may promote microbial growth. Bioadhesive drug delivery systems have severe patient compliance issue. In such scenario, it is highly recommended to develop a dosage form which may overcome all these problems. It is reported that SNEDDS improves the oral bioavailability of poorly water-soluble drug compound by presenting and maintaining the drug at a molecular level in the solution form throughout its stay in the G.I tract[10].In present research self-solidifying self-nanoemulsifying drug delivery system of ITZ was developed which overcome disadvantages of liquid SNEDDS formulation and stability of ITZ. SNEDDS of ITZ is found to be stable only in acidic environment i.e., in presence of concentrated HCl[8]. Hence, in this research ITZ was stabilized without using concentrated HCl. Extensive literature search revealed no research attempt on self-solidifying SNEDDS of ITZ.

So far, very limited research work has been done with ITZ as self solidifying SNEDDS in which blend of oil, surfactant and co-surfactant was used at appropriate concentrations in such a way that the developed formulation gets solidified at room temperature. In addition to this there is no any external solidifying agent required which in turn would reduce the bulk of the formulation. It is believed that having a self-solidifying SNEDDS will lead to decreased production costs, improved formulation

stability, ease of handling, accurate dosage, and increased patient compliance. They could benefit from L-SNEDDS and solid dose form in combination (i.e., improved solubility and bioavailability). The objectives of present study was to design and formulate SNEDDS of poorly water soluble drugs Itraconazole (ITZ) with the intention of improving the solubility and dissolution profile of these drugs and to characterize the obtained SNEDDS in respect of the physicochemical properties, performance and stability.

2 Methodology

2.1 Materials

Itraconazole was obtained as gift sample form Cadila Pharmaceuticals. Peceol, Gelucire 50/13 were procured from Gattefose, Mumbai. Cremophore RH 40 and Polaxamer were obtained as gift sample from BASF chemicals India Pvt. Ltd. Ethanol was obtained from S.D.fine Chemicals, Mumbai. Hard gelatine capsules were procured from Associate Capsules, Mumbai.

2.2 Solubility study of ITZ

An excess amount of ITZ was added to the distilled water, 0.1N HCL, SGF, and SIF and properly mixed by vortexing. The resulting mixtures were then kept at room temperature for 2 hr. to achieve equilibrium. The undissolved ITZ was separated by centrifugation at 1000 rpm for 10 minutes. The concentration of ITZ was measured using a UV-Visible spectrophotometer at 260 nm. For each vehicle, three replicate samples were used in the analysis.

2.3 Emulsification efficacy test

2.3.1 Selection of surfactant(s)

In brief, 300 mg of each surfactant and Peceol was taken in glass vial and gently heated up to 40-50°C using a water bath followed by vortexing on cyclomixer for 3-4min to homogenize the components and again heated gently at 40-50°C on a water bath. 50 mg mixture then diluted to 50.00 mL inside volumetric flask using double distilled water. The formulation was monitored for the total inversion required to produce clear emulsion. After 2 hours of storage of these flasks at room temperature, the resultant formulations were visually observed for the comparative turbid nature and phase separation if any. Then these nano-emulsions were evaluated for % transmittance (%T) at 638.2 nm by UV spectrophotometer. Also, emulsions formed must checked to see size of globule, polydispersity index (PDI) and zeta potential [11].

2.3.2 Selection of co-surfactant(s /co-solvent(s

The co-surfactants and co-solvents were screened to improve the emulsification ability of selected surfactants namely Labrasol, Cremophore RH 40 (Cr-RH40), Gelucire 50/13 (Gel 50/13) and Poloxamer L-188 (PoL-188), by following the same process as explained in session 2.3.1.

2.3.3 Preparation of surfactants combination (S-comb

Four different surfactant combinations (S-comb), at 1:1(w/w) ratio was prepared. Each of this combination contains one liquid state surfactant either Labrasol or Cr-RH40 and one solid state surfactant either Gel 50/13 or PoL-188.

S-comb 1: Labrasol + Gel 50/13
S-comb 2: Labrasol + PoL-188
S-comb 3: Cr- RH40 + Gel 50/13
S-comb 4: Cr-RH40 + PoL-188

2.3.4 Selection of co-surfactant/co-solvent for S-comb, by evaluating spontaneity of emulsification

Four co-surfactants/co-solvents viz. Ethanol, Plurol Oleique 497 (PO- 497), Labrafil M 2125 CS (LM-2125) and Labrafil M 1944 CS (LM-1944) were evaluated and compared for their efficiency (spontaneity) to emulsify oily phase i.e., Peceol.

Individual S-combinations as mentioned in section 2.3.3 were evaluated against co-surfactant and oily phase.

2.4 Optimization of Gelucire 50/13 (Gel 50/13 concentration)

The optimization of Gel 50/13 concentration was performed based on the ability to solidify Self-emulsifying (SE) mixture and spontaneity in dispersion and emulsification of solid SE mixture.

2.5 Ability to solidify Self-emulsifying (SE mixture)

Gel 50/13 was weighed according to its concentration and added to the glass vial, which was then heated on a hot plate above 50°C until a clear Gel 50/13 solution was formed. Equivalent concentrations (1:1:1 w/w) of oily phase; Peceol, surfactant; Cr-RH40, and co-surfactant/co-solvent; Ethanol were used to make the Self-emulsifying (SE) mixture. This SE mixture was mixed with molten Gel 50/13 in five different ratios: 30:70, 35:65, 40:60, 50:50, and 60:40. To ensure homogeneity, all samples were vortex mixed (5-10 minutes) in the molten state. The molten mixtures in the vials were then allowed to solidify at room temperature and then in the refrigerator, with the time necessary to solidify each mixture at both storage conditions recorded. SE combinations that solidify quickly at room temperature or in the refrigerator were tested further for their capacity to keep a solid state for 15 days at room temperature (25°C) and at 40°C, on both storage conditions independently. The optimal concentration of Gel 50/13 was found by observing the physical consistency of the mixture[12].

2.6 Spontaneity in dispersion and emulsification of solid SE mixture

A 50 mg SE mixture containing GEL 50/13 was added to a glass beaker containing 250 mL distilled water using a spatula. The solutions were gently stirred magnetically at 100 rpm at room temperature for this test. The time it took for the mixture to completely disperse and emulsify was recorded[11]. Visual observations and percent transmittance at 638.2 nm against distilled water were used to assess the clarity (look) of the microemulsions produced.

2.7 Formulation of ITZ Solid-SNEDDS (ISS and optimization

ITZ was dissolved in vial containing oily phase and ethanol. This oily mixture was heated in a water bath at 40 to 50°C, followed by sonication in a bath sonicator (5-10 minutes) to ensure complete solubilization of the drug. To ensure homogeneity, molten Cr-RH40 and Gelucire 50/13 (prewarmed in separate vial at 50-60°C) were combined and homogenized on cyclomixer for 10-15 minutes. With the use of a micropipette, the molten liquid (520 mg equivalent to 65 mg of ITZ) was then packed into hard gelatin capsules of size "0." Capsules filled with Liquid State SE mixture were allowed to solidify at room temperature or in the refrigerator, following which they were stored at room temperature for at least 24 hours to ensure complete solidification. The optimization of the formulation was done after storing at room temperature, the formulations were assessed by visual observations for their physical state. The formulations which do not solidify or does not maintain solid state at room temperature were not considered for further evaluation.

Table 1. Formula composition of ITZ solid SNEDDS formulation

Batch No.	**ISS** $_1$	**ISS** $_2$	**ISS** $_3$	**ISS** $_4$	**ISS** $_5$	**ISS** $_6$	**ISS** $_7$	**ISS** $_8$	**ISS** $_9$
Ratio of Peceol: Cr-RH40	1:3	1:2	1:1.5	1:1	1.5:1	2:1	3:1	5:1	10:1
Ingredients	mg/unit								
ITZ	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00
Peceol containing Ethanol (4:1)	65.00	87.00	104.00	130.00	156.00	173.00	195.00	217.00	236.00
Cr-RH40	195.00	173.00	156.00	130.00	104.00	87.00	65.00	43.00	24.00
Gel 50/13	195.00	195.00	195.00	195.00	195.00	195.00	195.00	195.00	195.00
Total weight (mg)	520.00	520.00	520.00	520.00	520.00	520.00	520.00	520.00	520.00

2.8 Evaluation of ITZ S-SNEEDS Formulations

2.8.1 Spontaneity of emulsification and drug precipitation study

Visual observations were used to evaluate the spontaneity for self-emulsifying properties of ITZ S-SNEDDS (ISS3 and ISS4) formulations based on emulsification speed, clarity, and apparent stability of the resulting emulsion, as described in the previous section. Similarly, when solid aggregates disintegrated and dispersed readily in water, the tendency to scatter the solid mass to form fine microemulsion was graded "bad" while the tendency to disperse the solid mass to produce fine microemulsion was judged qualitatively as "good". The formulations were further classified as clear (transparent or transparent with a bluish tint), turbid (turbid), stable (no precipitation after 24 hours), or unstable (no precipitation after 24 hours) (showing precipitation within 24 hr.)[11].

2.8.2 Robustness to dilution

Each S-SNEDDS was diluted 50, 100, and 1000 times in distilled water, 0.1N HCl, SGF, and SIF, respectively. Magnetic stirring at 100 rpm for 5 minutes was used to disperse the solid mass. The percent transmittance of the resulting microemulsion was measured using a UV-Visible spectrophotometer after 2 hours of dispersion at 638.2 nm against distilled water as a blank. After storing the diluted microemulsion for 12 hours, any indications of phase separation or drug precipitation were examined [13].

2.8.3 Globule size, polydispersity index and zeta potential

Photon correlation microscopy with a Malvern zeta sizer was used to assess the mean globule size, Polydispersity index (PI), and zeta potential of the selected formulations. The S-SNEDDS (ISS3 and ISS4) were placed in a 100 mL beaker with 50 mL (1000-fold dilution) of distilled water, SGF, or SIF followed by magnetic stirring at 100 rpm for 5 minutes to disperse the solid mass. It was then placed in an electrophoretic cell to measure the globule size, PDI, and Zeta potential of globules.

2.8.4 Morphology of globules by Transmission Electron Microscopy (TEM)

The morphology of the resulting microemulsion droplets was studied using transmission electron microscopy. The S-SNEDDS (ISS4) samples were dispersed in distilled water (1000-fold dilution) to produce microemulsion, stained for 30 seconds with 2 percent (w/v) phospho-tungstic acid, and observed on 400-mesh copper grids with films.

2.8.5 Differential Scanning Calorimetric (DSC study)

METTLER TOLLEDO, DSC-822e was used to evaluate ITZ and an optimized S-SNEDDS formulation including ITZ. In the sample and control compartments of the furnace, around 1mg of sample and alumina are filled in an aluminium pan. Under an inert atmosphere with a nitrogen gas flow rate of 50mL/min, heat runs were set from 20°C to 250°C with a 5°C/min increment. The resulting thermograms were then compared to a standard ITZ powder thermogram.

2.8.6 Powder X-Ray Diffraction (PXRD study)

The physical state of ITZ was characterized by X-ray powder diffraction (PXRD) measurements. PXRD was performed on samples of plain ITZ powder and Solid SNEDDS formulation of ITZ. Operational conditions maintained were same as mentioned in session

2.8.7 Scanning Electron Microscopy (SEM)

The outer macroscopic structure of Plain ITZ, Carrier: Gelucire 50/13 and S-SNEDDS (ISS4) were investigated by scanning electron microscope (JSM-6510LV; Jeol, Japan), operating at 15KV accelerating voltage, a 1 μm-10μmworking distance, and a probe current of 3 $\times$ 10-11 A°. The sample was fixed on a SEM stub using double-sided adhesive tape and then coated with thin layer of gold ion.

2.8.8 Drug content

Accurately weight, 520 mg of S-SNEDDS (ISS4) (equivalent to 65 mg of ITZ) was transfer to 100 mL volumetric flask and the volume was made up to the mark with methanol, flask inversions was done to facilitate the dispersion of solid mass, to facilitate the extraction of API. 2.5 ppm solution was prepared and analyzed with UV spectroscopy at 260 nm using suitable blank solutions. The ITZ concentration in the resulting solution was calculated using standard calibration curve in methanol.

2.8.9 In vitro dissolution study

In vitro dissolution of plain ITZ drug, S-SNEDDS of ITZ (equivalent to 65 mg of ITZ) filled in hard gelatin capsules, and marketed formulation of ITZ capsule (100 mg) was investigated using USP apparatus I at 37.50°C at 100 rpm in 0.01N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer to investigate the effect of pH on drug release. To maintain the sink condition, 5 mL of aliquots were withdrawn from the dissolution media at predefined time intervals and replaced with fresh buffer. The aliquots were filtered using Whattman filter paper, and 1mL of the filtrate was diluted to 10mL using 0.1N HCl and buffer. UV-visible spectroscopy at 260 nm was used to assess the amount of ITZ released in the dissolution medium.

2.8.10 Ex vivo intestinal permeability study by everted sac technique

Permeability study of ITZ from plain ITZ solution and microemulsion produce by ITZ S- SNEDDS was evaluated. Non-everted chicken intestinal sacs were used to preform Ex-vivo permeability study. Intestinal sac was placed in cold pH 7.2, KRBS. Solution was aerated with electrical aerator. Long sac was prepared (approximately 5-6 cm) using cotton thread and tying of the two ends of the sac. 5 mL of S-SNEDDS of ITZ and plain ITZ solution were put in the two different sacs for comparison. The sacs were

kept into two different beakers having 100 mL KRBS with supply of atmospheric air, at 37±0.5°C and 50 rpm. Aliquots were taken out at particular time point with a calibrated syringe and same volume of fresh KRBS solution was added to maintain the sink condition. The permeability study was performed for 60 minutes. The quantity of ITZ from S-SNEDDS permeated through the intestinal sac was analyzed by measuring the absorbance at 260 nm.

2.8.11 Stability study of Optimized ITZ S-SNEDDS

According to ICH guidelines, the stability of ITZ S-SNEDDS (ISS4) was studied at 40±2°C/75±5% RH. The molten formulation was filled into glass vials and hard gelatin capsules and allowed to solidify at room temperature. After ensuring that the formulations had solidified, vials were sealed with rubber corks and crimped with aluminium caps. Individual capsules were wrapped in aluminium foil and stored in an airtight container. All samples were kept in a stability chamber for 6 months and at 0, 30, 60, 90, and 180 days, the samples were withdrawn. Stability samples removed from vials were evaluated for physical appearance, drug content, dispersion and self-emulsification behaviour, globule size, and compatibility with capsule shells, while stability samples of formulations filled in capsules were evaluated for compatibility with capsule shell and dissolution efficiency at 20min (Q20 min) in SGF by comparing with percent cumulative drug release shown by 0th day stability formulation at 20min in SGF considering the release at 0th day as 100% at 2 days [14].

3 Result and Discussion

3.1 Solubility studies

The results suggested that the ITZ having pH dependent solubility, amongst the buffer tried solubility of ITZ was found maximum in 0.1N HCl (0.120 ± 0.07mg/mL) followed by SGF (0.089 ± 0.01mg/mL). Out of 20 oils screened, Peceol showed highest solubility (250 mg/g). Peceol is glycerol monoolete (type 40), comprised primarily of a mixture of mono and diglycerides of oleic acid, which closely resembles the end-products of intestinal lipid digestion. It is reported that Peceol increases the bioavailability of the poorly water-soluble drug compounds like Ontazolast (10-fold increase in bioavailability) by enhancing the lymphatic drug transport. Peceol has been reported to increase the oral bioavailability of model drug Varapamil by inhibiting the pre-systemic drug metabolism by regulating and inhibiting the P-gp protein expression [15]. Peceol was selected as oily phase to produce SNEDDS of ITZ because of its higher potential to solublise ITZ and its bioactive role, which could enhance the oral bioavailability of ITZ. Among all surfactants and co-surfactants studied, Labrafil M 1944 CS (LM 1944) showed potential to solubilize ITZ (14.91 mg/g). Amongst the all vehicles tried, the higher solubility of ITZ was found in Ethanol (23.12 ± 3.56 mg/g). Solubility profile of ITZ in oils, surfactants, and co-surfactants is presented in Figure 1.

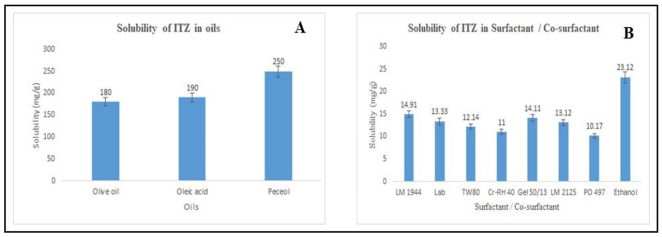

Fig 1. Solubilityprofile of ITZ in oils, surfactants, and co-surfactants

3.2 Surfactant and co-surfactant selection

The results of the emulsification ability of different surfactants are summarized in Table 2. Study reveals that, Cr-RH40 have the maximum ability to emulsify Peceol which produce emulsion with 90.12%T and required 10 flask inversions (10FI), followed by Gel 50/13 (88.11% T, 15FI). Labrasol and PoL-188 produces microemulsion which were similar in appearance with about 86%T but to accomplish it they differed from each other by required number of flask inversion (10 and 25FI respectively). In our study, it was observed that single surfactant with co-surfactant will not be efficient to emulsify Peceol and an additional surfactant would be required to do so. Hence, combinations of surfactants were tried along with the individual co-surfactant to emulsify Peceol efficiently.

Study reveals that among the four-combination tried Labrasol shows poor emulsification ability along with both solid-state surfactants i.e., Gel 50/13 and PoL-188. Surfactant in Combinations; Labrasol + Gel 50/13 and Labrasol + Pol-188 found

Table 2. Emulsification ability of different surfactants

Sr. No.	Surfactant	No. of flask inversion (FI)	% Transmittance	Appearance
1	Cremophore RH 40	10	90.12	Bluish white
2	Gelucire 50/13	15	88.11	Whitish
3	Poloxamers L-188	25	86.11	Whitish
4	Labrasol	10	85.89	Whitish

incapable to emulsify Peceol and produced emulsions were lower in %T than the %T observed in case the where Labrasol used alone as a surfactant without solid surfactant (Table 3).

On the other hand, Cr-RH40 produce fine microemulsion in combination of both Gel 50/13 as well as PoL-188 with nearly all co-surfactants tried, amongst the co-surfactants PO 497, LM 2125 and Ethanol produce fine microemulsion with more than 90 %T. So, as far as the emulsification (in combination with solid surfactant) is concern, Cr-RH40 was found to be better choice as compared to Labrasol to formulate ITZ SNEDDS. Based on the obtained results ethanol was selected as co-surfactant, and co-solvent to be blended with oily phase Peceol for formulating S-SNEDDS of ITZ. Thus, Peceol, Cr-RH 40, Gel 50/13 and ethanol were selected as the optimized excipients for the development of ITZ loaded Solid SNEDDS.

Table 3. Ability of co-surfactant to enhance the spontaneity S-comb to emulsify of oily phase

Co-Surfactant	S-comb:1 Lab+ Gel 50/13		S-comb:2 Lab+ PoL188		S-comb:3 Cr-RH 40+ Gel 50/13		S-comb:4 Cr-RH 40 + PoL188	
	FI	%T	FI	%T	FI	%T	FI	%T
Ethanol	7	69.45	15	67.88	3	98.89	15	85.67
PO 497	8	79.16	17	73.11	4	94.91	16	88.19
LM 2125	9	87.31	15	86.43	5	96.11	20	93.12
LM 1944	5	87.89	16	87.18	5	94.26	18	87.51

3.3 Optimization of Gelucire 50/13 (Gel 50/13 concentration)

The potential of solid carrier; Gel 50/13 was investigated to solidify and to maintain the solid state in 1:1:1 ratio of self-emulsifying (SE) mixture of Peceol: Cr-RH 40: Ethanol. It was observed that Gel 50/13 can solidify SE mixture as high as about 1.5 times at room temperature within 1h and maintained SE mixture in solid state for longer duration at room temperature and even at 40°C (Table 4). This system (40:60) disperses spontaneously (within 5 min) in water and produce microemulsion with more than 93%T. When ratio of Gel 50/13: SE was tried at 60:40 and 50:50, it was observed that these systems get solidified easily at room temperature and maintain the solid state even at 40°C, but these systems having a limitation of poor dispersion on aqueous dilutions. These systems produce fine microemulsion with more than 94%T but required more time to disperse in water (>15 min). Concentration of SE mixture above 1.5 times of the Gel 50/13 concentration (system 35:65) could not solidify easily at room temperature and required freezing to get solidified, but after removing from refrigerator and storing at room temperature, the consistency of this system changes from solid to semi solid[16]. In case, the preparation consists of 1 part of Gelucire 50/13 and more than 1.85 parts of SE mixture (system 30:70), could not completely solidify neither at room temperature nor on refrigeration. From the above findings it was concluded that, to make a spontaneously dispersing and stable solid SNEDDS formulations of ITZ, consisting equal proportion of Peceol, Cr-RH 40 and Ethanol, concentration of Gel 50/13 should be maintained at 40% w/w to the total mass of formulation i.e., ratio of Gel 50/13 to SE mixture 40:60 w/w.

3.4 Saturated solubility of ITZ in self-emulsifying mixture

The saturated solubility of ITZ in different SNEDDS formulations was found in the range of 75.11 to 125.11 mg/g. It was observed that the solubility of ITZ was increased as increase in the concentration of oily phase since the oily phase is made up of Peceol blended with Ethanol (4:1w/w) so which increases the concentration of Ethanol in total mass. All the anhydrous formulations (520 mg) were able to solubilised 115 % to 192 % of the target dose of ITZ (65 mg), in the other words all these formulation systems contain the ITZ concentration less than 85% of ITZ's saturated solubility in respective formulation systems.

Table 4. Determination of optimum concentration of Gelucire 50/13

	SE mixture= Peceol + Cr-RH 40 + Ethanol; 1:1:1				
Gel 50/14: SE mixture	30:70	35:65	40:60	50:50	60:40
Solidification ability					
At Room temperature	Not solidify even after 24 hr	Not solidify even after 24 hr	Solidify within 1 hr	Solidify within 1 hr	Solidify within 30 min
Cooling at refrigerator	Partially solidify	Solidify after 1 hr	Solidify within 10 min	Solidify within 10 min	Solidify within 5 min
Solid state maintenance after solidification					
At Room temperature	Completely liquid with increased viscosity	Liquidity on 1 hr standing	Remain as solid mass	Remain as solid mass	Remain as solid mass
At 40°C for 15 days	Nor performed	Nor performed	Remain as solid mass	Remain as solid mass	Remain as solid mass
Dispersibility test					
Dispersibility	Rapid	Rapid	Rapid	Poor	Poor
Time required to produce uniform emulsion	Less than 5 min	Less than 5 min	Less than 5 min	More than 15 min	More than 20 min
Appearance	Turbid	White	White bluish	White bluish	White bluish
%T	75.11	85.67	94.33	95.66	95.11

3.5 Optimization of formulations based on solidification ability

Study reveals that, since Cr-RH40 is viscous in nature which may get solidified easily in comparison to Peceol and Ethanol. The formulations containing high concentration of Cr-RH40 (ISS_1, ISS_2, ISS_3, and ISS_4) get solidified easily even at room temperature or in refrigerator and maintain its solid state in both the storage conditions i.e., at room temperature and at 40°C. On the other hand, formulations with lower concentration of Cr-RH40 and high concentrations of Peceol and Ethanol (ISS_9, ISS_8, and ISS_7) do not solidify at room temperature for even after 24h on standing, these formulations get solidified after placing inside the refrigerator but shows inability to maintain the solid state even for 1h after removing it from refrigerator and placing at room temperature or at 40°C. In case of formulations consist of moderate amount (20% to 15%) of Cr-RH40 formulation code ISS_5 and ISS_6 showed potential to get solidified at room temperature as well as refrigerator and they maintain their solid state in room temperature but gets converted in liquid state again at 40°C. Based on the above findings, only four formulations (ISS_1, ISS_2, ISS_3,and ISS_4) are evaluated further which gets solidified easily (5-10min) and maintain solid state for study period (15days) even at 40°C, were evaluated further, while the other formulations were omitted from the further study because of their failure in solidification and/or maintaining the solid consistency. Table 5 shows the solidification ability developed by ITZ SNEDDS.

Table 5. Solidification ability developed by ITZ SNEDDS

B. No.	**ISS** $_1$	**ISS** $_2$	**ISS** $_3$	**ISS** $_4$	**ISS** $_5$	**ISS** $_6$	**ISS** $_7$	**ISS** $_8$	**ISS** $_9$
Solidification ability (Time required for solidification)									
At Room temperature	Within 15 min	Within 15 min	Within 30 min	Within 30 min	Within 1 hr	Within 1 hr	Not solidify after 24 hr	Not solidify after 24 hr	Not solidify after 24 hr
In refrigerator (2-8°C)	Within 5 min	Within 5 min	Within 10 min	Within 10 min	Within 10 min	Within 15 min	After 1 hr	After 1 hr	Partially solidify after 1 hr
Solid state stability after solidification									
At Room temperature	Stable	Stable	Stable	Stable	Stable	Stable	Liquify after 1 hr	Liquify after 1 hr	Liquify after 1 hr
At 40°C for 15 days	Stable	Stable	Stable	Stable	Liquify after 7 days	Liquify in 1 day	Not done	Not done	Not done

3.6 Spontaneity of emulsification and drug precipitation

Spontaneity of dispersion of solid mass and its self-emulsification of selected formulation was evaluated, the ability of each selected formulation is presented in Table 6 . Results from this study suggested that there is significant effect of oil to surfactant ratio on dispersibility and the appearance of produced emulsion. It was clear from the results that the optimum concentration of oil and surfactant is required to produce fine microemulsion with potential to avoid precipitation of drug on aqueous dilution.

Table 6. Dispersion and self-emulsification of developed ITZ SNEDDS

Batch No.	Time required for dispersion and self-emulsification (min)	Appearance	Drug precipitation after 24 hr	Grade
ISS_1	6-9 min	White bluish	Unstable (precipitation occur)	II/III
ISS_2	5-6 min	White bluish	Stable	II
ISS_3	Less than 5 min	Clear and transparent	Stable	I/II
ISS_4	Less than 5 min	Slightly bluish	Stable	I

3.7 Robustness to dilution

The result of these study suggested that, both these formulation systems found robust to various dilution fold using different dilution medium differing in pH. ISS_3 produce fine microemulsion which was slightly bluish or bluish white in appearance with more than 92%T but less than 95%T. On the other hand, ISS_4 produce slightly bluish and even clear and transparent microemulsion on dilution with transmittance value in between 94.29 to 98.89%T. The observation of robustness to dilution study of both selected ITZ S-SNEDDS formulation; ISS_3 and ISS_4 are compared and tabulated in Table 7.

Table 7. Data of robustness to dilution study of selected ITZ S-SNEDDS formulations

Batch No.	Dilution media	Dilution	Evaluation parameters		
			% T	Appearance	Drug precipitation
ISS $_3$	Distilled water	50	92.01	Bluish white	Stable
		1000	93.11	Slightly bluish	Stable
	0.01 N HCl	50	92.31	Bluish white	Stable
		1000	95.09	Slightly bluish	Stable
	0.1 N HCl (SGF)	50	93.56	Bluish white	Stable
		1000	95.43	Slightly bluish	Stable
	pH 6.8 phosphate buffer (SIF)	50	92.00	Bluish white	Stable
		1000	93.98	Slightly bluish	Stable
ISS $_4$	Distilled water	50	95.09	Slightly bluish	Stable
		1000	96.72	Slightly bluish	Stable
	0.01 N HCl	50	95.13	Slightly bluish	Stable
		1000	96.22	Slightly bluish	Stable
	0.1 N HCl (SGF)	50	97,12	Slightly bluish	Stable
		1000	98.89	Slightly bluish	Stable
	pH 6.8 phosphate buffer (SIF)	50	94.29	Slightly bluish	Stable
		1000	96.11	Slightly bluish	Stable

*Values are expressed as mean (n=2)

3.8 Globule size, polydispersity index and zeta potential

The findings reflects that the mean globule size of oil droplet produce by ISS_4 (40.11 nm in water) are nearly 15 nm smaller in diameter than that of ISS_3 (55.16 nm in water). Zeta potential of droplets produce from both formulations is observed with negative charge irrespective of the dilution medium and it was in the range of -9.21 to – 11.50 mV. In summary, based on all

above findings formulation system; ISS_4 was selected as optimized Solid SNEDDS of ITZ and evaluated for further studies. The comparative difference between ISS_3 and ISS_4; ITZ S-SNEDDS on their globule size and zeta potential analysis is provided in Table 8 .

Table 8. Dataof Globule size, Polydispersity Index (P.I.) and Zeta potential of selected ITZS-SNEDDS Formulation

Batch No.	Dilution media	Globule size (nm)#	Zeta potential* (mV)	P.I*
ISS $_3$	Distilled water	55.16	-10.11	0.145
	0.1 N HCl (SGF)	55.13	-9.21	0.129
	pH 6.8 phosphate buffer (SIF)	56.17	-11.01	0.150
ISS $_4$	Distilled water	40.11	-11.13	0.144
	0.1 N HCl (SGF)	38.37	-11.50	0.125
	pH 6.8 phosphate buffer (SIF)	45.11	-10.88	0.132

Values are expressed as mean ± SD (n=2). * Values are expressed as mean (n=2)

3.9 Evaluation of optimized formulations of ITZ S-SNEDDS (ISS_4)

3.10 Morphology of globules by Transmission Electron Microscopy (TEM)

Morphology of microemulsion globules obtained after 1000 folds water dilution of optimized ITZ S-SNEDDS was assessed by Transmission electron microscopy. The obtained image is presented in Figure 2**,** The observations confirm the ability of optimized ITZ S-SNEDDS to produce uniformly distributed, spherical shaped oil globules of nano size. This observation of TEM image is in agreement with results obtained from globule size analysis.

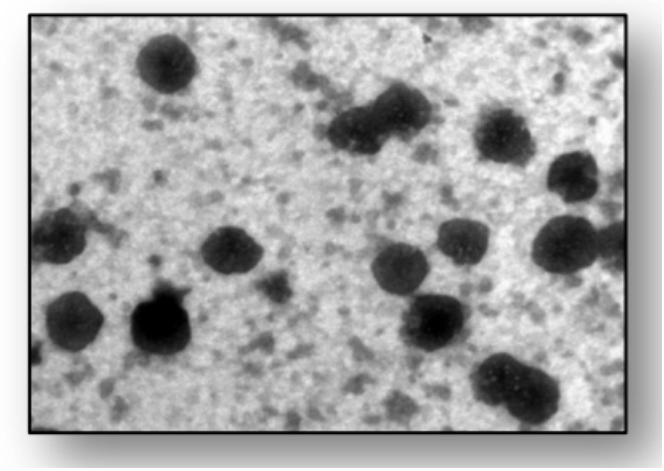

Fig 2. TEM image of microemulsion from ITZ S-SNEDDS (ISS_4)

3.11 Differential Scanning Calorimetric (DSC study)

The DSC thermograms of plain ITZ and S-SNEDDS formulation are shown in Figure 3 A and B respectively. Plain ITZ showed sharp endothermic peaks at 168.2°C indicating that the drug is highly crystalline. The absence of obvious peak in the S-SNEDDS formulation indicates change in the melting behaviour of Obvious ITR and inhibition of crystallization following solubilization using lipid surfactants and oil. Apart from this, no polymorphic changes were observed in the optimized S-SNEDDS of ITZ.

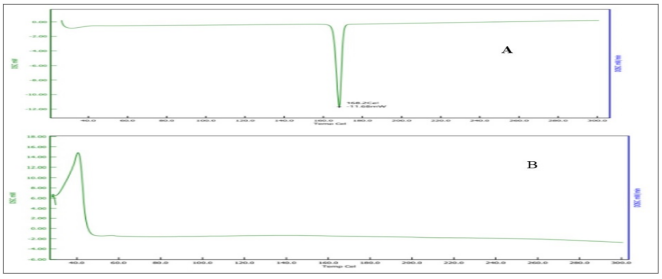

Fig 3. DSC thermogram of A: ITZ and B: ITZS SNEDDS (ISS_4)

3.12 Powder X-Ray Diffraction (PXRD study)

The X-ray diifractograms of pure ITZ drug and P-SNEDDS are represented in Figure 4 A and B. It is evident from the obtained results that, pure ITZ drug has sharp peaks at the diffraction angles of 2Θ shows a typical crystalline pattern. Whereas, P-SNEDDS has lesser sharp peaks at the diffraction angles of 2Θ thus indicating that crystalline nature of ITZ has completely changed to amorphous form. However, any different new peak was not appeared in the S-SNEDDS formulation, suggesting compatibility of ITZ with other excipients of SNEDDS.

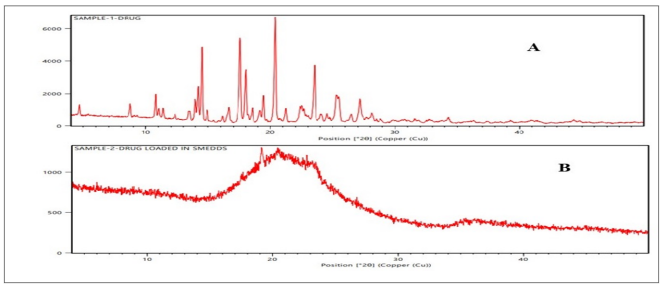

Fig 4. XRD peak for A: ITZ and B: ITZ S-SNEDDS

3.13 Scanning Electron Microscopy (SEM)

Scanning electron micrograph of plain ITZ shows needle shaped crystals indicating the crystalline nature of the drug (Figure 5 A). Neat Gel 50/13 pellets shows smooth surface (Figure 5 B). The SEM images of mixture of NBL dissolved in GEL 50/13 (Figure 5 C) showed few small crystalline particles of drug, indicate the change in surface morphology of drug particle due to entrapment in the Ge 50/13 and image of S-SNEDDS are shown in Figure 5 D ensures the complete solubility of ITZ since no crystals (ITZ) was seen and it's an irregular mass produce of S-SNEDDS. This surface modification ensures the decrease in crystallinity and complete solubility of ITZ particle in ITZ S-SNEDDS.

3.14 Drug content and *in vitro* dissolution studies

Drug content of solid SNEDDS of ITZ was found within the range of 98.11% to 102.51%, which was found in the acceptable limit. It is evident from the observation that ITZ S-SNEDDS showed a promising improvement in the in vitro dissolution profile compared to the plain ITZ powder filed in hard gelatin capsule and marketed product in all three-dissolution media studied (Figure 6 A, B and C). ITZ S-SNEDDS showed more than 85% ITZ released in 15 min and nearly complete (more than 95%) ITZ was released after 30 min of dissolution irrespective of the dissolution media, indicating that the release of ITZ from S-SNEDDS was independent of the pH of the dissolution medium. On the other hand, plain ITZ showed poor rate and extent of dissolution (less than 70% after 45min). 55.78%, 59.11% and 59.78% of drug release was observed in 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer. Marketed reference product showed more than 85% release in 30 min in all media. Test product was faster as compared to marketed product.

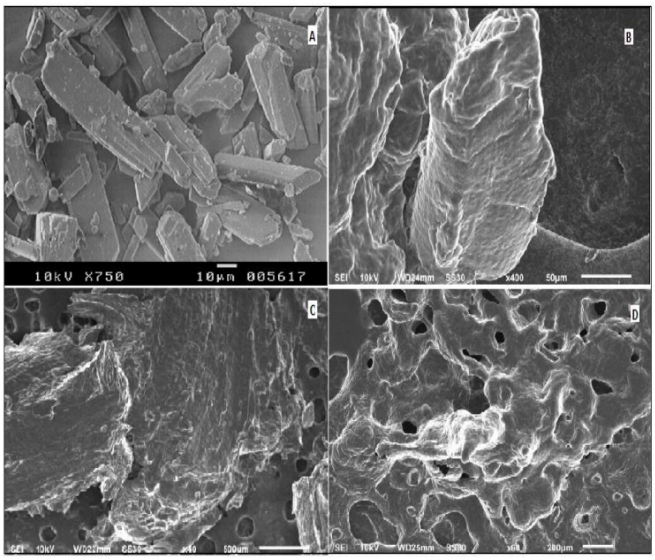

Fig 5. SEM of plain ITZ (A), Neat Gel 50/13 (B), physical mixture of ITZ and Gel 50/13(C)and ITZ S-SNEDDS (D)

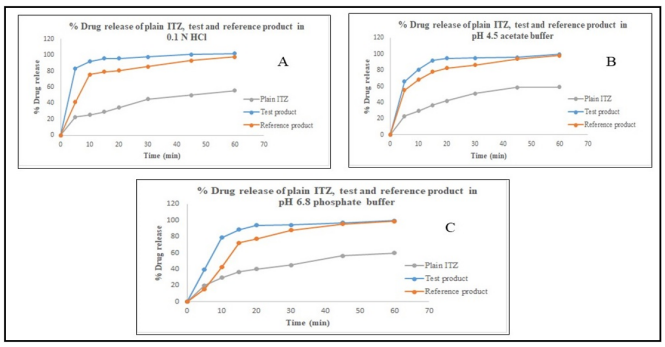

Fig 6. Dissolutionprofile of plain ITZ, S SNEDDS of ITZ and marketed reference product in 0.1 NHCl (A), pH 4.5 acetate buffer (B) and pH 6.8 phosphate buffer (C)

3.15 *Ex vivo* intestinal permeability study by everted sac technique

L-SNEDDS showed 90.11% release in 30 min while plain ITZ solution was showed incomplete release i.e., 42% at 60 min (Figure 7). It showed that, permeability of ITZ was improved when it gets converted into L-SNEDDS. This 3-fold enhancement of permeability of ITZ was attributed by many reasons mainly the uniformly dispersed globules with nano size. ITZ is present in the dissolved state, these fine globule size increases the surface area facilitates the permeability of drug [17].

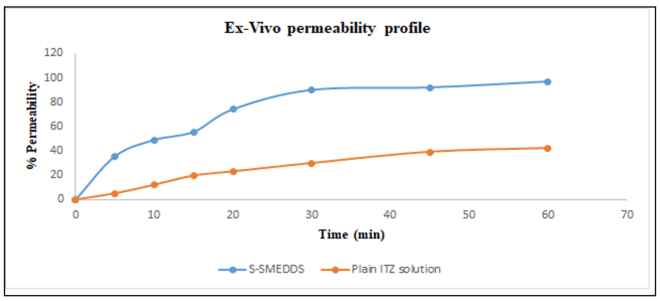

Fig 7. *Ex-vivo* permeability profile of ITZ by everted sac technique

3.16 Stability study of optimized ITZ S-SNEDDS

The results of the stability studies indicated that the Solid SNEDDS containing Itraconazole maintained the drug content, solid state, similar dissolution efficiency and very importantly the self-emulsification ability for over the period of 6 months (Table 9). This formulation had reasonable stability.

Table 9. tability data of ITZ S-SNEDDS (ISS_4)capsule at 40°C ± 2°C / 75 % ± 5 % RH for 6 months

Parameter	Condition				
	Initial	1 Month	2 Month	3 Month	6 Month
Physical appearance	No change in colour from initial.				
Compatibility with capsule shell	Compatible				
Disintegration time*	5-7 min	5-7 min	4-7 min	5-8 min	5-8 min
Assay (%) *	98.76 %	98.11 %	99.13 %	97.66 %	97.89 %
Dissolution-Q point 20 min (%) in 0.1 N HCl	95.83 %	95.19 %	96.17 %	94.11 %	95.43%
Assessment of SNEDDS in distilled water					
Visual observation of grade	I (Slight bluish)	I	I	I/II	I/II
Globule size (nm)	38.37	39.11	39.17	38.19	42.29
Polydispersity index	0.125	0.114	0.156	0.129	0.131

*Values are expressed as mean (n=3)

4 Conclusion

The present research work describes very novel approach for the development of ITZ loaded self-solidifying SNEDDS using Gel 50/13 as emulsifying, and solidifying agent with enhanced solubility and permeability. The formulations may be filled into hard gelatin capsules in their molten state as they solidify as hard masses inside the capsules. The optimized formulation's globule size was determined to be 40.11 nm with a PDI of 0.144. The stability of the SNEDDS was demonstrated by a zeta potential analysis. The drug concentration of solid SNEDDS of ITZ was found to be within the permissible range of 98.11% to 102.51%. When compared to plain ITZ and marketed products in all three-dissolution medium, it was found that ITZ S-SNEDDS displayed a potential improvement in the *in vitro* dissolution profile. Uniformly scattered globules with a nano size were responsible for a nearly 3-fold increase in the permeability of ITZ. Analytical characterization showed that the amorphous properties of the ITZ were compatible with any excipient. The developed formulations were found to be stable over the period of 6 months. ITZ loaded SNEDDS would be potential approach to overcome the limitations of incompatibility with hard gelatin capsules and to improve patient compliance. However, the feasibility of the formulation development at larger scale need to check. S-SNEDDS was found to be a potential approach to overcome the limitations of incompatibility of L-SNEDDS with hard gelatin capsules and to improve patient compliance. Itraconazole S-SNEDDS has many advantages over other reported formulation due to ability of self-solidification. It also reduces the cost and time required for the conversion of L-SNEDDS to S-SNEDDS.

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