Chapter 4

Preparation and characterization of itraconazole self-emulsifying formulation containing Soluphor® P

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Abstract

The objective of this study was to develop and evaluate self-emulsifying drug delivery system (SEDDS) for improving the delivery of a poorly water soluble antifungal agent, itraconazole (ITZ). Solubilisation efficiency of Soluphor® P (2-pyrrolidone) for itraconazole was exploited for developing a self-emulsifying formulation. Phase diagrams were constructed at different ratios of surfactant/co-surfactant (K_m) to determine microemulsion existence region. The system was characterized by measuring droplet size, polydispersity index and zeta potential of formed emulsion when diluted in three different media viz. Distilled water, 0.1N HCl and phosphate buffer saline pH 6.8. The optimized formulation was evaluated for dissolution, in vitro diffusion and ex vivo stomach and intestinal permeability in comparison to marketed capsule. A comparative pharmacokinetic study of a developed formulation and marketed capsule was performed in rats. The absorption of itraconazole from SEDDS resulted in about 2.9-fold increase in bioavailability compared with the marketed capsule. Our studies illustrated the potential use of Soluphor® P as one of the component in SEDDS for oral delivery of highly hydrophobic drug like itraconazole.
4.1. Introduction

The aqueous solubility of a drug is one of its most important physicochemical properties. A low aqueous solubility and slow dissolution can potentially limit a drug’s absorption from the gastrointestinal tract. The aqueous solubility of drug is of prime significance when a direct administration to the blood stream is required. From the drug development standpoint, often a solution of drug is required to conduct pharmacological, toxicological and pharmacokinetic studies. Thus, poor aqueous solubility not only limits a drug’s pharmacological applications but also challenges its pharmaceutical development. As a result, investigation into new solubilizers and techniques for solubility enhancement is important.

The excipients used to solubilize hydrophobic drugs for oral and parenteral delivery include pH modifiers, water-soluble organic solvents, surfactants, water-insoluble organic solvents, medium-chain triglycerides, long-chain triglycerides, cyclodextrins, and phospholipids. Two key aspects of any successful solution formulation are solubility and stability. The solvent system chosen must also be able to solubilise the drug at the desired concentration and must provide an environment where the drug has sufficient chemical stability. Sufficient stability is normally defined as < 5-10% degradation over 2 years under the specified storage conditions (Strickley, 2004).

N-methyl pyrrolidone (NMP) is a water miscible, aprotic solvent with a log $K_{ow}$ of −0.54. It has been reported to increase the solubility and permeability of several drugs (Aguiar, et al., 1987; Lee et al., 2005; Tarantino et al., 1994; Uch et al., 1999).

The NMP molecules (Figure 4.1) contain a polar disubstituted cyclic amide group, which can interact with water molecules ensuring their complete miscibility. The presence of the non-polar carbons of NMP can weaken the hydrogen-bonded structure of water, thus enabling it to act as a co-solvent. In addition, the presence of a substantially large and nearly planar non-polar region may result in hydrophobic interactions between NMP and drug molecule to form a complex. Such an association stabilizes the drug in dissolved form and further increase its solubility in NMP-water mixture. (Sanghvi et al., 2008)

\[ \text{H}_3\text{C} - \text{N} - \text{O} \]

**Figure 4.1:** Structure of N-methyl-2-pyrrolidone
NMP and 2-pyrrolidone (2P) have been widely used in preparing biodegradable in situ forming microparticle systems (ISM) (Elkharraz et al., 2011; Hatefi and Amsden, 2002; Kranz et al., 2007; Rungseevijitprapa and Bodmeier, 2009). A positively charged polymeric nanoparticle of meloxicam was prepared using Soluphor® P as a solubilizer (Khachane et al., 2011). The highly water-soluble oligonucleotide was encapsulated very efficiently into biodegradable polymeric microparticles by the o/o/o solvent extraction method based on less toxic, biocompatible and water miscible solvents like DMSO and 2-pyrrolidone (Elkharraz et al., 2011). WIPO WO 2011/048493 disclosed examples of pyrrolidone derivatives co-surfactants such as N-methyl pyrrolidone (e.g. Pharmasolve®) and 2-pyrrolidone (e.g. Soluphor® P) suitable for coated capsules and tablets of fatty acid oil mixture (Klaveness et al., 2011). Self-emulsifiable liquid florfenicol composition using Soluphor® P and NMP, intended to be incorporated into the drinking water of livestock was reported by Derrieu and Raynier (2011). N-methyl-2-pyrrolidone was used in the formulation of PLA (poly lactic acid) and PLGA (poly lactic glycolic acid) in situ implants containing secnidazole and/or doxycycline for treatment of periodontitis (Gad, et al., 2008). The systemic absorption of fenofibrate is dissolution rate limited and therefore an oral self-emulsifying formulation was dissolved in N-alkyl derivatives of 2-pyrrolidone, is mentioned in U.S. Patent (Liang et al., 2006).

A range of pyrrolidones and structurally related compounds have been investigated as potential penetration enhancers in human skin and has been well described in literature (Williams and Barry, 2004). Few research groups have reported the use of 2-pyrrolidone (Soluphor® P) for improvement of solubility and bioavailability of poorly water-soluble drugs as described in Chapter 2. Soluphor® P proved to be the best excipient for solubilising 8-methoxy psoralen (8-MOP) in formulating sublingual delivery for PUVA (Psoralen and Ultraviolet A) therapy and was checked for its pharmacokinetics in healthy volunteers (Shephard et al., 2001). U.S. Patent 0153585 relates pharmaceutical preparations comprising 2-pyrrolidone as solubilizer for poorly soluble medicinal active ingredients for oral administration (Schreder et al., 2003).

2-pyrrolidone is a useful solvent for the preparation of the graft copolymer solutions, which have the ability to undergo in situ gelation in an aqueous environment such as that on the mucosal surfaces in the body. Such solution is a convenient method of dispensing the controlled release mucoadhesive composition to the tissue. 2-pyrrolidone is highly advantageous for this purpose due to its relatively benign toxicological characteristics and
excellent solubility properties (Shah, 1999). It has been proposed that 2-pyrrolidone which is structurally similar to NMP can simultaneously act as co-solvent and complexing agent (Jain and Yalkowsky, 2007).

1-methyl-2-pyrrolidone (CAS No. 872-50-4) is the lactam of 4-methylamino-butyric acid. Reported LD$_{50}$ values for rats, mice and rabbits are as follows; rat: 3914 mg/kg bw (orally), 2266 mg/kg bw (intravenously), and 2472 mg/kg bw (intraperitoneally), mouse: 5130 mg/kg bw (orally), 1980 mg/kg (intravenously), 3564 mg/kg bw (intraperitoneally), rabbit (dermal route): 8000 mg/kg bw (intact skin), 4000 mg/kg bw (abraded skin) (EMEA/MRL/457/98: http://www.eudra.org/emea.html). A human stillbirth after an occupational exposure, including skin contact, to an unknown level of NMP has been described in a case report (Solomon et al., 1996). Furthermore, in rats, NMP have been reported to be a developmentally toxic compound after dermal and oral administration (Hass et al., 1995; Saillenfait et al., 2002). Retarded growth has been reported after inhalation exposure (Saillenfait et al., 2003).

2-pyrrolidone (CAS 616-45-5) is the lactam of gamma-aminobutyric acid (Figure 4.2). In human medicine 2-pyrrolidone is used mainly as vehicle for dermally administered drugs as for instance antimycotics, in order to enhance and accelerate absorption. For this purpose it is often mixed (2:3) with N-methyl-2-pyrrolidone (NMP).

![Figure 4.2: Structure of 2-pyrrolidone (Soluphor® P)](image)

2-pyrrolidone is a normal component of certain food items. The natural content of 2-pyrrolidone in plant foodstuffs was reported to be in the range of 0.1-2.2 mg/kg (e.g. prunes, orange juice, tomatoes) and up to 20 mg/kg in certain processed food (e.g. tomato pastes and sauces). In mammals 2-pyrrolidone is associated with the metabolism of glutamic acid, putrescine and gamma aminobutyric acid. There is evidence that 2-pyrrolidone, as a cyclic form of gamma aminobutyric acid, is a precursor of gamma aminobutyric acid in the central nervous system. 2-pyrrolidone is able to cross the blood-brain barrier and has been shown to be hydrolysed enzymatically to gamma amino butyric acid \textit{in vivo}. This conversion however appears to be effectively regulated by homeostatic
mechanisms preventing uncontrolled gamma aminobutyric acid formation. A relatively large intravenous dose of 200 mg/kg of \[^{3}H\_6\]-pyrrolidone given to mice was shown not to alter brain steady state levels of gamma aminobutyric acid at 30 minutes post dosing. Endogenous concentrations of 2-pyrrolidone in human, dog, rat and mouse plasma, cerebrospinal fluid or brain was reported to be in the range of 5-30 µg/L, but considerably higher amounts in brain and cerebrospinal fluid were also described in some investigations (up to 3500 µg/kg). 2-pyrrolidone is reported to be of low acute toxicity in mammals with oral LD\(_{50}\) values of above 6500 mg/kg bw in rats and guinea pigs, 800 mg/kg bw after intravenous injection in rabbits, 3000 and 3700 mg/kg bw after subcutaneous injection in rats and mice, respectively (EMEA/MRL/493/98 http://www.eudra.org/emea.html).

2-pyrrolidone is a normal component of the diet in humans, is of endogenous origin, rapidly metabolised after oral and parenteral administration in mammalian species.

Lipid based formulations are particularly effective for some of the most difficult compounds (Gursoy and Benita, 2004; Hauss, 2007). A low aqueous solubility does not necessarily lead to a high solubility in excipients used in lipid based formulations (Hauss, 2007). Therefore, new solvents or lipid excipients are constantly sought to solubilise the drugs before administration.

This makes it of interest to investigate if Soluphor\(^\text{®}\) P (2-pyrrolidone) could be utilized in oral delivery of itraconazole by means of self-emulsifying formulation. The overall aim of the present work was, therefore, to examine if Soluphor\(^\text{®}\) P is useful as an excipient in oral drug formulation for poorly water-soluble drugs, using both \textit{in vitro} and \textit{in vivo} methods.

Poorly water soluble model investigational antifungal drug, itraconazole offer challenges in developing a drug product with adequate bioavailability. The objective of the investigation was to improve the solubility and bioavailability of itraconazole using Soluphor\(^\text{®}\) P as a potential solvent in a self-emulsifying drug delivery system.

\textbf{4.2. Materials and methods}

\textbf{4.2.1. Materials}

Itraconazole (ITZ) was a generous gift from Matrix Laboratories Ltd. (Hyderabad, India); Propylene glycol monocaprylate (Capryol\(^\text{TM}\) 90), Isopropyl myristate (IPM), Propylene Glycol Dicaprylate/Dicaprate (Captex\(^\text{®}\) 200) and Glycerol Caprylate Caprate (Captex\(^\text{®}\) 355) were obtained from Subhash Chemical Industries (Pune, India); PEG-8 glycol
caprylate (Labrasol®) and diethylene glycol monoethyl ether (Transcutol P®) were provided by Gattefosse, France. 2-Pyrrolidone (Soluphor® P®) was obtained from BASF Corp., Germany. PEG-20 sorbitan monolaurate (Tween 20) and Tocopherol acetate were purchased from Merck (Mumbai, India). Capsule shells were provided by Capsugel, Mumbai. Ketoconazole was purchased from HIMEDIA. All other chemicals and solvents used were of analytical grade.

4.2.2. Solubility studies

The solubility of itraconazole in various oils, surfactants and co-surfactants was determined. An excess amount of itraconazole was added to 5 ml of each selected oils and surfactants and was shaken reciprocally at 25°C for 24 hrs. The supernatant portion of the supersaturated solution was carefully withdrawn and suitably diluted with methanol, and solubility of itraconazole was determined using HPLC (Jasco, Japan) at 263 nm (Shim et al., 2006; Yang et al., 2008; Yoo et al., 2000). The HPLC system consisted of RP column (LCGC Qualisil BDS C18; 5 µm 250 mm x 4.6 mm i.d) and acetonitrile: water (90:10) as a mobile phase. A 40µL volume was injected into the column at a flow rate of 0.7mL/min.

4.2.3. Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were constructed using water dilution method (Djordjevic et al., 2004). Capryol 90 was used as the oil phase, Soluphor® P as the surfactant and Transcutol P as the co-surfactant. Phase diagrams were constructed with 9:1 to 1:9 v/v ratio of oil to surfactant and various ratios of surfactant/co-surfactant (4:1, 3:1, 2:1, and 1:1 v/v). The data obtained was subjected to Tri plot v1.4 software (David Graham and Nicholas Midgley, Loughborough, Leicestershire, UK) for fabrication of ternary plot.

4.2.4. Preparation of itraconazole SEDDS:

The developed formulation consisted of itraconazole: capryol 90: Soluphor® P: Transcutol P (2.4: 9.0: 60.06: 28.0) (% w/w). Itraconazole was weighed accurately and to it Capryol 90 was added. This was vortexed followed by addition in a mixture of surfactants and vortexed again for 2 min. The mixture was then heated on water bath for 5-10 min at 60°C until a transparent solution was obtained.
4.2.5. Characterization of self emulsifying drug delivery system

4.2.5.1. Drug content

SEDDS formulation equivalent to 25 mg of itraconazole was taken and diluted in methanol. Volume was made up to 25 mL with methanol (1mg/mL). 0.2 ml was withdrawn from the above solution and to it 4.8 ml of methanol was added and mixed well. 5 mL of 0.1 N HCl was added to above mixture and mixed well (20 µg/mL). Samples were prepared in triplicate and absorbances were taken at 258 nm on UV visible spectrophotometer (Shimadzu UV-2450, Japan) keeping equal mixture of methanol and 0.1N HCl (1:1) as a reference. Placebo was also treated in the same way to check interference, if any.

4.2.5.2. Spectroscopic characterization of optical clarity

The optical clarity of aqueous dispersions of SEDDS formulation was measured spectrophotometrically. Composition was prepared according to the design and diluted to 100 times with distilled water and 0.1N HCl (USP 30) (USP 30/NF25). The % transmittance of solution was measured at 650 nm, using distilled water as a reference (Cirri et al., 2007; Subramanian et al., 2004; Zhang et al., 2008).

4.2.5.3. Morphological characterization

The morphology of self emulsifying formulation was observed by using a transmission electron microscope (TEM) (Phillips Tecnai 20, Holland) at an acceleration voltage of 200 kV and typically viewed at a magnification of 43,000×. The size of the colloidal structures was determined using AnalySIS® software (Soft Imaging Systems, Reutlingen, Germany).

Formulation was diluted with distilled water 1:25 and shaken. Carbon-coated copper grids were glow-discharged (Edwards E306A Vacuum Coater, England) and 10 µL of sample adsorbed on to these holey film grid and observed after drying.

4.2.5.4. Determination of droplet size and zeta potential

Droplet size and zeta potential of the formed emulsion were determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle.
4.2.5.5. Dissolution Study

Dissolution studies of itraconazole capsule and itraconazole SEDDS filled in hard gelatine capsule (size # “000”) was carried out using USP XXIII Dissolution apparatus I (basket type) at speed of 50 rpm in 900ml for 100 mg marketed capsule and 225 mL for 25 mg SEDDS 0.1 N HCl at 37 ± 0.5 °C. Aliquots of 5 ml were removed at 2, 5,10,15,30,45,60,75 and 120 min. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for amount of itraconazole released at corresponding time by using UV-visible spectrophotometer (Shimadzu UV-2450, Japan) at 255 nm.

4.2.5.6. In vitro diffusion study

In vitro diffusion study was performed for the developed itraconazole SEDDS (S1) formulation, using a dialysis technique (Dixit et al., 2010; Kang et al., 2004; Patil et al., 2004). The dialyzing medium was 0.1N HCl. One end of dialysis tubing (Dialysis membrane 70, HIMEDIA; MWCO 12,000-14,000 daltons; pore size: 2.4 nm) (7 cm in length) was clamped and then 1.1 mL (=25 mg of drug) of self-emulsifying formulation was placed in it. The other end of the tubing was also secured with dialysis closure clip (HIMEDIA, Mumbai) and was allowed to rotate freely in 225 mL of dialyzing medium and stirred continuously with magnetic bead on magnetic plate at 37°C. Aliquots of 5 mL were removed at different time intervals. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for itraconazole dialyzed across the membrane at corresponding time by using UV-visible spectrophotometer at 255 nm.

For marketed formulation (Itaspor®-INTAS, pellets in capsule), capsules were crushed in mortar with pestle. From the crushed pellets, blend equivalent to 25 mg (113.975 mg blend) was weighed and made suspension by adding 3 mL of DW and was filled in dialysis membrane. The membrane was sealed from both the end with the help of dialysis closure clips and was placed in 225 mL of 0.1N HCl

4.2.5.7. Ex vivo stomach permeability

Male Sprague-Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and
Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the stomach permeability, the stomach part was isolated carefully and taken for the *ex vivo* permeation study. Then this tissue was washed with physiological acid solution containing 100 mM HCl and 54 mM NaCl to remove the mucous and gastric contents. Then the tissue was rinsed with 100 mM HCl. The method employed was modified from experimental procedures well described in the literature (Gharzouli et al., 1995; Hung and Neu, 1997; Shah and Khan, 2004).

SEDDS formulation, 0.4 mL (equivalent to 10 mg of drug) was injected into the stomach and tissues were tightly closed with the help of threads. Similarly 2 mL extemporaneous suspension of market capsule (Itaspor®-INTAS) equivalent to 10 mg of drug was filled in stomach tissue and was placed in a beaker with constant stirring and temperature of 37°C on a magnetic stirrer. The receiver compartment was filled with 30 mL of 100 mM HCl. The absorbance was measured at 255 nm, keeping the respective blank. The percent permeation of drug was calculated against time and plotted on a graph.

**4.2.5.8. *Ex vivo* intestinal permeability**

This was carried out by the method well described in the literature (Araya et al., 2006; Ghosh et al., 2006). Male Sprague-Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the intestinal permeability, small portion of small intestine was isolated and used for the *ex vivo* permeability study. The tissue was thoroughly washed with pH 6.8 phosphate-buffered saline (USP 30) (USP 30/NF25) to remove any mucous and lumen contents.

One millilitre of SEDDS formulations were diluted to 5 mL with distilled water (outside mixing for 1 minute by vortex mixer), the resultant sample (5 mg/mL) was injected into the lumen of the duodenum using a syringe, and both the sides of the intestine were tightly closed with the help of threads. Extemporaneously suspension was prepared from market capsule (Itaspor®-INTAS), and 1 mL equivalent to 5 mg of drug was filled in stomach tissue similar to that of SEDDS formulations. Then the tissue was placed in a beaker with constant stirring and temperature of 37°C on magnetic stirrer. The receiver compartment
was filled with 30 mL of phosphate-buffered saline containing 20% PEG 400. The absorbance was measured at 258 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

4.2.6. HPLC method development and validation

The concentration of itraconazole in the plasma samples were analyzed by slight modification of the reported HPLC methods (Kapsi & Ayres, 2001; Rabinow et al., 2007; Yang et al., 2008). The HPLC apparatus consisted of Jasco PU-980 Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco FP-920 Intelligent Fluorescence detector (Jasco, Japan), an autosampler Jasco AS-950-10 Intelligent sample (Jasco, Japan), a Jasco Borwin Chromatography Software (version 1.50) integrator software and a LCGC Qualisil BDS C18 (4.6 mm × 250 mm and 5 μm particle size) column. The mobile phase consisted of a mixture of 5 mM sodium phosphate buffer pH 5.7 and acetonitrile (38:62 v/v) at a flow rate of 1.0 mL/min that led to retention time of 13.72 min when detection was carried out at excitation 263 nm and emission 380 nm. The assay was linear (r²=0.999) in the concentration range of 50-3000 ng/mL. The method was validated with respect to accuracy and inter- and intra-day precision at three quality control samples (150, 590 and 2300 ng/mL) as per ICH guidelines (ICH Q2(B)).

4.2.7. Bioavailability studies

Bioavailability studies were performed in male Wistar rats weighing 280 to 350 g. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of Sri Dhanvantary Pharmaceutical Analysis and Research Centre, Surat, India and were in accordance with guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Two groups were made for the study, and four rats were kept in each group. The animals were kept under standard laboratory conditions, temperature at 25 ± 2°C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water ad libitum. The animals were fasted overnight prior to the experiment but had free access to water.

The formulations (SEDDS and extemporaneous suspension prepared from marketed capsule (Itaspor®, B.No. DL3229 MFD Oct 2010 EXP SEP 2012)) were given orally using feeding snode. Dose for the rats was selected as reported (Shin et al., 2004; Van Cauteren
et al., 1987) and calculated based on the weight of the rats (30mg/kg body weight) according to the surface area ratio (Akhila et al., 2007; Paget and Barnes, 1964). The animals were anesthetized using ether and blood samples (approximately 500 μL) were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0 (pre-dose), 1, 2, 4, 8, 12, 16 and 24 hours after oral administration of formulations. The blood samples were collected into a vacutainer tube (13 x 75mm, 2mL, Accuvac, BD, NJ), mixed and centrifuged on a laboratory centrifuge at 5000 rpm for 20 min at ambient temperature. The supernatant plasma was carefully separated, filled in 1.5 mL screw-capped polypropylene vials and kept at –20°C (Thermo Scientific, USA) until analysis was carried out using validated HPLC.

4.2.7.1. Plasma analysis

Frozen plasma samples were thawed just prior to extraction. A 100 μL of plasma sample was transferred in 2 mL centrifuge tube (Tarson, Kolkata, India). To that 300 μL of acetonitrile containing ketoconazole as an internal standard (Vaughn et al., 2006) (200 ng/mL in acetonitrile) was added and vortex-mixed for 1 min. The tube was centrifuged at 15,000 rpm for 20 min at 4°C. The organic layer was carefully decanted and was transferred to clean tube and dried under nitrogen stream at ambient temperature. The residue was reconstituted with a 100 μL aliquot of mobile phase, and 50 μL was injected directly onto the HPLC column at a flow rate of 1.0 mL/min.

Plasma concentration versus time data of itraconazole for rats was analyzed using standard non-compartment analysis. The area under the plasma concentration-time curve (\(AUC_{0→t}\)) from zero to 24 hour was estimated by the linear trapezoidal method (Han and Lee, 1999; Lee and Ku, 1999). The relative bioavailability (\(F\)) of SMEDDS to the suspensions was calculated using the following equation:

\[ F = \frac{(AUC_{\text{test}} / AUC_{\text{reference}})}{\times 100\%} \]

4.2.8. Statistical analysis

Statistical analysis for the determination of differences in diffusion, permeability and \textit{in vivo} absorption profiles of itraconazole SEDDS and the marketed preparation was assessed by the use of Student’s \(t\)-test. The pharmacokinetic data between different formulations were compared for statistical significance by Student’s \(t\)-test. Statistical probability (\(P\)) values less than 0.05 were considered significantly different.
4.2.9. Stability studies

Chemical and physical stability of itraconazole SEDDS (S1) were assessed under various storage conditions namely room temperature (RT), 30±2°C/65±5% RH and 40±2°C/75±5% RH in ICH certified stability chamber (Humidity Chamber, EIE Instruments Ltd., Ahmedabad, India) as per ICH guidelines (ICH Q1A(R2)). Itraconazole SEDDS (S1) equivalent to 25 mg was filled in a glass vial with a rubber closure and aluminium-crimped tops. Eight such glass vials were filled and stored at various aforementioned storage conditions up to 3 months. Samples were removed at 0, 1, 2 and 3 months of interval and checked for itraconazole content (by HPLC), droplet size, polydispersity index and zeta potential after diluting formulation to 100 times with three different media viz. Distilled water (DW), 0.1N HCl and phosphate buffer saline pH 6.8 (PBS) maintained at 37°C.

4.3. Results and Discussion

4.3.1. Screening of oils and surfactants

The solubility of itraconazole in various vehicles is shown in Table 4.1. The components and their concentration ranges can be obtained by the construction of a pseudo-ternary phase diagram with constant drug level fixed at 2.4% (w/w). While screening the oil phase, drug loading capability is the main factor for hydrophobic drugs in developing such lipid-based delivery systems.

Capryol 90 showed good solubility among oils. Among the surfactants, Transcutol P, Tween 20 and Labrasol were selected for the preliminary studies. In preliminary trials solubility of itraconazole was checked in different ratios of Transcutol P and Tween 20 (4:1, 3:1, 2:1 and 1:1). For this 50 mg of itraconazole was weighed and dissolved by gentle warming. Drug was precipitated in all those four formulations. Systems were unable to dissolve even 25 mg of itraconazole.

Several trials were taken using Capryol 90, Transcutol, Tween 20, PEG 400 and Soluphor® P in various ratios and combination, but no stable system was obtained.

Soluphor® P and Transcutol were found to be excellent solubilizer for itraconazole and hence they were used. Uch et al (1999) have reported that 1-methyl pyrrolidone as a potential solubilising agent for itraconazole. In the present study, the selected solubilizer is 2-pyrrolidone and our result are also in agreement with that of observed by Uch and his colleagues. Complexation effect of 2-prrolidone may be considered for solubilisation of
hydrophobic drug, itraconazole. It is believed that 2-pyrrolidone molecule can undergo stacking with the drug molecule in presence of polar environment. Such an arrangement will stabilize the dissolved drug resulting in an increase in its solubility (Sanghvi et al., 2008).

Different ratios of surfactant: co-surfactant and oil were used to construct ternary phase diagrams. Self emulsification region with highest drug solubility were determined and formulation was optimized. Capryol 90 (oil), Soluphor® P (surfactant) and Transcutol (co-surfactant) showed maximum solubility of drug were selected to plot the ternary phase diagrams and surfactant: co-surfactant 1:1 shows maximum microemulsion region was selected for incorporating the drug.

Table 4.1: Solubility of itraconazole in various oils and surfactants at 25°C ($n=3$)

<table>
<thead>
<tr>
<th>Oil /Surfactant</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl myristate</td>
<td>0.206 ± 2.07</td>
</tr>
<tr>
<td>Captex 200</td>
<td>1.008 ± 2.08</td>
</tr>
<tr>
<td>Captex 355</td>
<td>2.127 ± 1.97</td>
</tr>
<tr>
<td>Tocopherol acetate</td>
<td>5.22 ± 1.87</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>22.132 ± 1.58</td>
</tr>
<tr>
<td>Tween 20</td>
<td>3.709 ± 1.04</td>
</tr>
<tr>
<td>Labrasol</td>
<td>7.147 ± 1.65</td>
</tr>
<tr>
<td>Transcutol</td>
<td>4.6 ± 1.48</td>
</tr>
<tr>
<td>Soluphor</td>
<td>29.733 ± 1.57</td>
</tr>
</tbody>
</table>

4.3.2. Construction of pseudo ternary phase diagrams

The pseudo-ternary phase diagrams with various weight ratios of Soluphor® P to Transcutol P are depicted in Figure 4.3. The area of microemulsion isotropic region changed slightly in size with the increasing ratio of surfactant to co-surfactant.

The phase study revealed that the maximum proportions of oil were incorporated in microemulsion systems when the surfactant-to-co-surfactant ratio was 1:1 but the ratio 2:1 has shown maximum solubility of drug and there was slight difference in the dilution potential. Therefore, ratio 2:1 was selected for microemulsion formulation.
4.3.3. Characterization of self-emulsifying drug delivery system

4.3.3.1. Drug content

Assay of prepared itraconazole SEDDS was carried out by UV-visible spectrophotometer. A linear calibration curve was obtained at 255 nm in the range of (1-40 µg/mL) with a correlation ($r^2$) of 0.999. Assay was found to be in the range of 102.56% with a standard deviation of ±1.54%.

4.3.3.2. Spectroscopic characterization of optical clarity

SEDDS was diluted with water to confirm the formation of microemulsion with the external phase of the system without phase separation. In order to assess the optical clarity quantitatively, UV-visible spectrophotometer was used to measure the transmitted light at 650 nm wavelength transmitted by the solution. Higher transmittance should be obtained with optically clear solutions, since cloudier solutions will scatter more of the incident radiation, resulting in lower transmittance. Aqueous dispersions with small absorbance are
optically clear and oil droplets are thought to be in a state of finer dispersion. A clear o/w micro-emulsion was formed in both the dilution media. On 100 fold dilution percent transmittance of the studied aqueous dispersion of itraconazole SEDDS was found to be 17.731 with distilled water and 73.668 with 0.1N HCl.

4.3.3.3. Morphological characterization

The TEM picture is shown in Figure 4.4. The microemulsion droplets were observed to be spherical and homogenous with large population of the smaller droplet in the size range of less than 200 nm. The droplet size of formed emulsion determined by TEM is in correlation with that of measured by photon correlation spectroscopy (PCS).

![TEM image](image)

**Figure 4.4:** Transmission electron microscopic image of itraconazole self emulsifying system after dilution showing size of some oil globules. (Scale: 500 nm)

4.3.3.4. Droplet size and zeta potential analysis

Droplet size distribution following self-emulsification is a critical factor to evaluate a self-emulsifying system. Droplet size is thought to have an effect on drug absorption as has been illustrated in several research papers. In our study, we investigated the effect of distilled water, 0.1N HCl and phosphate buffer saline pH 6.8 as three different dilution media on droplet size. The average droplet size, polydispersity index and zeta potential of microemulsion dispersed from the itraconazole SEDDS after 100 times dilution in various media are shown in Table 4.2. Size distribution by intensity records of diluted SEDDS are shown in Figure 4.5 (a), (b) and (c).

**Table 4.2:** Initial characterization of itraconazole SEDDS S1

<table>
<thead>
<tr>
<th></th>
<th>Droplet size D (nm)</th>
<th>Polydispersity Index (PdI)</th>
<th>Zeta potential (mV)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>183.9</td>
<td>0.134</td>
<td>-3.52</td>
<td>102.56 ± 1.54</td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>266.9</td>
<td>0.512</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>246.92</td>
<td>0.010</td>
<td>-2.24</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.5(a): Droplet size distribution of itraconazole SEDDS S1 after dilution in distilled water.

Figure 4.5(b): Droplet size distribution of itraconazole SEDDS S1 after dilution in 0.1N HCl.
4.3.3.5. In vitro evaluations

There was significant difference between itraconazole SEDDS and market capsule with respect to dissolution study, *in vitro* diffusion, *ex vivo* stomach permeability and *ex vivo* intestinal permeability studies at statistical probability value less than 0.05.

---

**Figure 4.5(c):** Droplet size distribution of itraconazole SEDDS S1 after dilution in phosphate buffer saline pH 6.8

**Figure 4.6:** Dissolution profiles of itraconazole SEDDS S1 and marketed capsule in 0.1N HCl \((n=6)\)
**Figure 4.7:** *In vitro* diffusion profile of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in 0.1N HCl ($n=6$)

**Figure 4.8:** *Ex vivo* stomach permeability of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in 100 mM HCl ($n=3$)
4.3.4. HPLC method development and validation

The method was found to be specific, as IS and ITZ were well resolved and no interfering peaks from endogenous components of normal plasma were observed (Figure 4.10(a) and Figure 4.10(b)). The method was found to be linear over the range 50-3000 ng/mL (Figure 4.11).

Figure 4.9: *Ex vivo* intestinal permeability of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in pH 6.8 PBS (n=3)

**Figure 4.10(a):** A representative HPLC chromatogram obtained for blank plasma used for preparing standards and quality control samples of itraconazole
Figure 4.10(b): A representative chromatogram of plasma spiked with standard itraconazole and Ketoconazole (IS) for standards and quality control samples of itraconazole

The calibration ranges were selected based on the expected concentration in bioavailability study samples. The least square linear regression analysis with weighing factor 1/x of the peak areas ratios of drug and IS (y) versus concentration of itraconazole in plasma (x), obtained by assaying plasma sample spiked with itraconazole with correlation coefficient values above 0.99 is shown in Table 4.3.
The LLOQ was 50 ng/mL. Sensitivity of the method is the lowest concentration that can be measured with an acceptable limit of accuracy and precision. The accuracy and precision at LLOQ was determined by analyzing at least 5 replicates of the sample at the LLOQ concentration on at least one of the validation days. These samples were independent of those used for construction of the calibration curve. All the results of sensitivity samples were also used to calculate accuracy and precision. The intra-day accuracy at LLOQ (50 ng/mL) was found to be 101.9 % with precision of 4.31 % (%RSD) (Table 4.4). The values are within the limits of specified in the guidelines of bioanalytical method validation (ICH Q2(B)). Accuracy should be ± 20% and the RSD should be less than 20% at LLOQ.
Table 4.4. Intra-day accuracy and precision (%RSD) of 5 determination for estimation of itraconazole in rat plasma on the same day.

<table>
<thead>
<tr>
<th>Itraconazole concentration in ng/mL</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>50.95</td>
<td>152.365</td>
<td>590.7</td>
<td>2371</td>
</tr>
<tr>
<td>SD</td>
<td>2.106</td>
<td>4.09</td>
<td>6.63</td>
<td>29.91</td>
</tr>
<tr>
<td>%RSD a</td>
<td>4.13</td>
<td>2.68</td>
<td>1.12</td>
<td>1.26</td>
</tr>
<tr>
<td>Accuracy b</td>
<td>101.9</td>
<td>101.57</td>
<td>100.11</td>
<td>103.10</td>
</tr>
</tbody>
</table>

* a %RSD = SD/Mean * 100

b Accuracy = Concentration found/actual concentration * 100

Accuracy and precision of the analytical method was determined for both intra- and inter-runs. They were determined by analyzing quality control (QC) samples at a minimum of 3 concentrations (low, mid and high), representing the entire range of the calibration curve (50-3000 ng/mL). As the concentration of low QC (LOQ) should be near the lower limit of quantification and should not be more than 3 times the LLOQ concentration and hence 150 ng/mL was selected as LQC. The mid QC concentration should be somewhere in the middle of the calibration range and hence 590 ng/mL was selected as MQC. The high QC concentration should be near the upper end of the calibration curve (within the upper quartile of the calibration range). HQC selected was 2300 ng/mL. At least five replicates at each concentration were analyzed. The results of the intra-day accuracy and precision are shown in Table 4.4 and inter-day accuracy and precision data is shown in Table 4.5.

Table 4.5: Inter-day accuracy and precision (%RSD) of 3 determination for estimation of itraconazole in rat plasma on the same day.

<table>
<thead>
<tr>
<th>Itraconazole concentration in ng/mL</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>50.12</td>
<td>151.83</td>
<td>592.38</td>
<td>2308</td>
</tr>
<tr>
<td>Day 2</td>
<td>52.9</td>
<td>146.78</td>
<td>586.32</td>
<td>2390</td>
</tr>
<tr>
<td>Day 3</td>
<td>52.41</td>
<td>151.94</td>
<td>588.10</td>
<td>2390</td>
</tr>
<tr>
<td>Mean</td>
<td>51.81</td>
<td>150.18</td>
<td>588.93</td>
<td>2387</td>
</tr>
<tr>
<td>SD</td>
<td>1.48</td>
<td>2.94</td>
<td>3.11</td>
<td>6.08</td>
</tr>
<tr>
<td>%RSD a</td>
<td>2.86</td>
<td>1.96</td>
<td>0.52</td>
<td>0.25</td>
</tr>
<tr>
<td>Accuracy b</td>
<td>103.62</td>
<td>100.12</td>
<td>99.81</td>
<td>103.78</td>
</tr>
</tbody>
</table>

* a %RSD = SD/Mean * 100

b Accuracy = Concentration found/actual concentration * 100

The method precision (%RSD) at LLOQ were 4.13 % (intraday) and 2.86 % (interday) and accuracy of 101.9 % and 103.62 % for drug, intra- and inter-day respectively (Table 4.4 and 4.5). As per the bioanalytical method validation guidelines (ICH Q2(B)), the mean of the observed concentration should be within ± 15% of the nominal at all
concentrations of the QC samples. Coefficient of variation (indication of precision) around the mean observed concentration should not exceed 15% at all concentration, and the CV should be less than 15%, at all concentrations. If the QC concentration is at the lower limit of quantification, the RE% at the CV can be up to 20%.

Stability of the itraconazole in plasma samples was observed for three freeze-thaw cycles is reported in Table 4.6.

**Table 4.6:** Stability of itraconazole in rat plasma

<table>
<thead>
<tr>
<th></th>
<th>Sample concentration (ng/mL)</th>
<th>Stability sample concentration (ng/mL)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bench top</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (150)</td>
<td></td>
<td>149.33 ± 2.15</td>
<td>99.55</td>
</tr>
<tr>
<td>HQC (2300)</td>
<td></td>
<td>2586.66 ± 83.266</td>
<td>112.46</td>
</tr>
<tr>
<td><strong>Freeze-thaw</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (150)</td>
<td></td>
<td>150.83 ± 2.12</td>
<td>100.55</td>
</tr>
<tr>
<td>HQC (2300)</td>
<td></td>
<td>2366.66 ± 45.52</td>
<td>102.89</td>
</tr>
<tr>
<td><strong>Post preparative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (150)</td>
<td></td>
<td>149.87 ± 0.96</td>
<td>99.91</td>
</tr>
<tr>
<td>HQC (2300)</td>
<td></td>
<td>2660 ± 26.45</td>
<td>115.65</td>
</tr>
</tbody>
</table>

\* After 6 h at room temperature  
\^ After 3 freeze-thaw cycles  
\^c After 24 h at 25°C in Autosampler  
\^d Mean ± SD (n=3)

Reconstituted organic extracts were stable for 24 h in the autosampler at 25 °C as indicated by post preparative stability in Table 4.6. The spiked plasma samples as well as the drug stock solutions were stable for a minimum of 6 h at room temp (Table 4.6). The dried extracts of samples showed stability when left overnight at -80 °C.

The validation results of bioanalytical method for estimation of itraconazole in rat plasma indicate that the method is simple, specific, precise, accurate and suitable for estimation of itraconazole from plasma samples of bioavailability studies in rats.

**4.3.5. Bioavailability studies**

Typical chromatograms obtained showing peaks for ITZ at 1 h after administration of marketed suspension and SEDDS are shown in Figure 4.12(a) and 4.12(b), respectively.
Figure 4.12(a): Typical chromatogram obtained while analyzing plasma for ITZ level after administration of the extemporaneous suspension of marketed capsules at 1 h.

Figure 4.12(b): Typical chromatograms obtained while analyzing plasma for ITZ level after administration of itraconazole SEDDS S1 at 1 h.
Table 4.7 summarises mean pharmacokinetic parameters for various formulations. Figure 4.13 depicts the plasma concentration profile of itraconazole after oral administration of SEDDS and extemporaneous suspension of marketed capsule to rats. Significant differences were found between the two formulations with regard to $AUC_{0→t}$, $AUC_{0→∞}$ and $C_{max}$ parameters at $P < 0.05$.

Table 4.7: Relative bioavailability and pharmacokinetic parameters of itraconazole after oral administration of itraconazole SEDDS and suspension to the rats ($n=4$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MKT</th>
<th>SEDDS (S1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0→t}$ (ng.h/mL)</td>
<td>3037.268 ± 198.86</td>
<td>9299.188 ± 3152.50</td>
</tr>
<tr>
<td>$AUC_{0→∞}$ (ng.h/mL)</td>
<td>5264.334 ± 1705.79</td>
<td>15324.2 ± 8667.05</td>
</tr>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>171.956 ± 5.715</td>
<td>821.75 ± 188.952</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.0 ± 0.0</td>
<td>1.25 ± 0.5</td>
</tr>
<tr>
<td>Relative bioavailability (%)</td>
<td>---</td>
<td>291.09</td>
</tr>
</tbody>
</table>

As a weakly basic drug with poor aqueous solubility and high lipophilicity, itraconazole should be rapidly transported into the intestinal mucosa, provided it can be dissolved. 2-pyrrolidone has improved itraconazole solubility in aqueous media to keep itraconazole in solution. SEDDS formulation containing 2-pyrrolidone was also readily permeated across
rat stomach and intestinal tissues, indicating that drug in solubilised state could have been taken up by passive diffusion as that of observation made with N-methyl-2-pyrrolidone.

The total amount of drug absorbed marketed capsule is not even the half the amount of that absorbed from SEDDS.

### 4.3.6. Stability Studies

No change in the physical parameters such as homogeneity and clarity was observed during the stability studies. Droplet size, polydispersity index and zeta potential remained unchanged, as compared to initial characterization at 0 month (Table 4.2), even after keeping the formulations at different environmental storage conditions for 3 months, indicating the physical stability of developed formulations (Table 4.8). No decline in the drug content was observed at the end of 3 months indicating that drug remained chemically stable in SEDDS.

#### Table 4.8: Stability studies of itraconazole SEDDS S1 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>25 ± 2°C (RT)</th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th>40 ± 2°C/75 ± 5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>1 M</td>
<td>2 M</td>
<td>3 M</td>
</tr>
<tr>
<td>Droplet Size D (nm)</td>
<td>DW</td>
<td>190.43</td>
<td>186.28</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>254.39</td>
<td>271.39</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>238.47</td>
<td>243.39</td>
</tr>
<tr>
<td>Polydispersity index (PdI)</td>
<td>DW</td>
<td>0.136</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>0.415</td>
<td>0.442</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>0.12</td>
<td>0.236</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>DW</td>
<td>-4.75</td>
<td>-6.31</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>4.58</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>-3.42</td>
<td>-3.54</td>
</tr>
<tr>
<td>Drug Content (%)</td>
<td>104.242</td>
<td>± 1.89</td>
<td>101.515</td>
</tr>
</tbody>
</table>

D (nm) = diameter in nanometer; DW = distilled water; PBS = phosphate buffer saline pH 6.8; RT = room temperature; M = month; RH = relative humidity

### 4.4. Conclusion

A SEDDS containing poorly water-soluble drug, itraconazole, was formulated for oral administration. The components and their ratio ranges for the formulation of SEDDS were obtained by solubility study, pseudo-ternary phase diagram construction, and droplet size analysis. The optimum formulation of the SEDDS consisted of 9.76 % of Capryol 90 as oil, 58.53% of Soluphor® P as surfactant and 29.27% of Transcutol P as co-surfactant., which had sufficient drug loading, rapid self-emulsification in aqueous media, and forming droplet size in the range of microemulsion. Our study indicated that the developed itraconazole SEDDS formulation showed greater dissolution, diffusion, stomach and intestinal permeability than the marketed suspension. The in vivo study data of a
developed SEDDS using Soluphor® P as one of its component showed improved bioavailability compared to marketed capsule. The results obtained suggest that the developed itraconazole SEDDS could be able to keep the itraconazole in a dissolved state in the gut and help in better absorption from the entire gastrointestinal tract of a rat. To conclude, Soluphor® P (2-pyrrolidone) appears to be a promising solubilising agent and a preferable excipient for developing self emulsifying drug delivery systems for lipophilic drugs like itraconazole to improve its bioavailability.
REFERENCES


Chapter 4

Itraconazole


ICH harmonized tripartite guideline, Stability testing of new drug substances and products Q1A (R2).

ICH Q2(B) Guidelines: Validation of Analytical Procedure: Methodology Q2(B), 2003.


