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Evaluation of 2-pyrrolidone As a Potential Adjuvant in Self-Emulsifying Formulation for Oral Delivery of Poorly Water-Soluble Drugs

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Abstract

Solubilisation efficiency of Soluphor® P (2-pyrrolidone) for itraconazole was exploited for developing a self-emulsifying formulation. 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). 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 behaviour, *in vitro* diffusion and *ex vivo* stomach and intestinal permeability capabilities 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 components in SEDDS for oral delivery of highly hydrophobic drug like itraconazole.

Keywords: 2-pyrrolidone; solubility; Self-emulsifying; Phase diagram; Bioavailability

Introduction

The aqueous solubility of a drug is one of its most important physicochemical properties. A low aqueous solubility and slow dissolution can restrict a drug's absorption from the gastrointestinal tract. The aqueous solubility of drug is of utmost importance when a direct administration to the blood stream is required. For any drug development 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. Hence, exploration 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-miscible organic solvents, surfactants, water-immiscible 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 [1].

N-methyl pyrrolidone (NMP) is a water miscible, aprotic solvent with a log $K_{o/w}$ of −0.54. It has been reported to increase the solubility and permeability of several drugs [2-5]. The NMP molecules (Figure **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. Moreover, the presence of a large and nearly planar non-polar region may result in hydrophobic interactions between NMP and drug molecule to form a complex. Such bond stabilizes the drug in dissolved form and further increase its solubility in NMP-water mixtures [6].

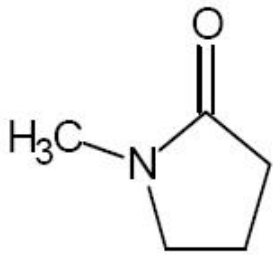

Figure 1: Structure of N-methyl-2-pyrrolidone

NMP and 2-pyrrolidone (2P) have been widely used in preparing biodegradable in situ forming microparticle systems (ISM) [7-9] and transdermal delivery system [10,11]. A positively charged polymeric nanoparticle of meloxicam was prepared using Soluphor® P as a solubilizer [12]. 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 [7]. WIPO WO 2011/048493 disclosed examples of pyrrolidone derivative 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 [13]. A 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 [14]. 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 [15]. 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, as mentioned in U.S. Patent 7022337 [16].

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 [17]. Few research groups have reported the use of 2-pyrrolidone (Soluphor®

P) for improvement of solubility and bioavailability of poorly water-soluble drugs. 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 [18]. U.S. Patent 0153585 relates to pharmaceutical preparations comprising 2-pyrrolidone as solubilizer for poorly soluble medicinal active ingredients for oral administration [19].

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 [20]. It has been put forwarded that 2-pyrrolidone which is structurally similar to NMP can simultaneously act as co-solvent and complexing agent [21].

2-pyrrolidone (CAS 616-45-5) is the lactam of gamma-aminobutyric acid (Figure **2**). It is used as a vehicle to increase and improve the absorption of dermally administered antimycotics in human medicines. For this purpose, it is often mixed (2:3) with N-methyl-2-pyrrolidone (NMP).

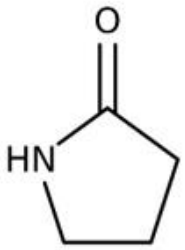

Figure 2: Structure of 2-pyrrolidone (Soluphor® P)

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. It has been shown that 2-pyrrolidone is able to cross blood-brain barrier and enzymatically hydrolyzed to gamma-amino butyric acid *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 [$^{2}H_{6}$]-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 [22]. 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 [23, 24]. A low aqueous solubility does not necessarily lead to a high solubility in excipients used in lipid-based formulations [24]. Therefore, new solvents or lipid excipients are constantly sought to solubilise the drugs before administration.

This makes it of interest to investigate if Soluphor® P 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® P is useful as an excipient in oral drug formulation for poorly water-soluble drugs, using both *in vitro* and *in vivo* methods. The poorly water-soluble model in-

vestigational antifungal drug, itraconazole, offers challenges in developing a drug product with adequate bioavailability. The excipient will improve the solubility and dissolution of the poorly soluble antifungal active ingredient when incorporated in self-emulsifying drug delivery system. The objective of this investigation was to improve the solubility and bioavailability of itraconazole using Soluphor® P as a potential adjuvant in a self-emulsifying drug delivery system.

Materials and Methods

Materials

Itraconazole (ITZ) was a generous gift from Matrix Laboratories Ltd. (Hyderabad, India); Propylene glycol monocaprylate (Capryol™ 90), Isopropyl myristate (IPM), Propylene Glycol Dicaprylate/Dicaprate (Captex® 200) and Glycerol Caprylate Caprate (Captex® 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.

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 [25]. The supernatant portion of the supersaturated solution was pipetted out and suitably diluted with methanol, and solubility of itraconazole was determined using HPLC (Jasco, Japan) at 263 nm [26-28]. 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

Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams were constructed using water dilution method [29]. 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.

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 of 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.

Characterization of Self-Emulsifying Drug Delivery System

Drug Content

A SEDDS formulation equivalent to 25 mg of itraconazole was taken and diluted in methanol. The 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 determined at 258 nm using a UV visible spectrophotometer (Shimadzu UV-2450, Japan) with an equal

mixture of methanol and 0.1N HCl (1:1) as reference. Placebo was also treated in the same way to check for interference, if any.

Spectroscopic Characterization of Optical Clarity

The optical clarity of aqueous dispersions of the SEDDS formulation was measured spectrophotometrically. The composition was prepared according to the design and diluted to 100 times with distilled water and 0.1N HCl. The % transmittance of the solution was measured at 650 nm, using distilled water as a reference [30-32].

Morphological Characterization

The morphology of the self-emulsifying formulation was characterized 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).

The 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 film grids and observed after drying.

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.

Dissolution Study

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

In Vitro Diffusion Study

An *in vitro* diffusion study was performed for the developed itraconazole SEDDS (S1) formulation, using a dialysis technique [33-35]. A 0.1N HCl was used as a dialyzing medium. One end of dialysis tubing (Dialysis membrane 70, HIMEDIA; MWCO 12,000-14,000 Da; 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 using a magnetic stirrer at 37°C. Aliquots of 5 mL were removed at different time intervals. An aliquots of fresh dialyzing medium was replaced each time. These samples were analyzed quantitatively for itraconazole dialyzed across the membrane at the corresponding time using UV-visible spectrophotometer at 255 nm.

For the 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.

Ex vivo Stomach Permeability

Male Sprague-Dawley rats (250-300 g) were euthanized in a 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. The tissue was washed with physiological acid solution containing 100 mM HCl and 54 mM NaCl to remove the mucous and gastric contents. The tissue was rinsed with 100 mM HCl. The method employed was modified from experimental procedures well described in the literature [36-38].

The 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 sutures. Similarly, 2 mL extemporaneous suspension of market capsule (Itaspor®-INTAS) equivalent to 10 mg of drug was filled into the stomach tissue and was placed in a beaker with constant stirring at a 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, against the appropriate blank. The percent permeation of drug was calculated and plotted against time.

Ex vivo intestinal permeability

This was carried out by the method described in the literature [39, 40]. A carbon-dioxide vacuum chamber was used for euthanizing Male Sprague-Dawley rats (250-300 g). 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. A 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 buffer (USP 35) [41] to remove any mucous and lumen contents.

One millilitre of SEDDS formulation was 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 sutures. A suspension was extemporaneously prepared from the marketed capsule (Itaspor®-INTAS), and 1 mL equivalent to 5 mg of drug was filled in intestinal 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 buffer containing 20% PEG 400. The absorbance was measured at 255 nm, against the appropriate blank. The percent diffusion of drug was calculated against time and plotted on a graph.

HPLC Method Development and Validation

The concentration of itraconazole in the plasma samples was analyzed by a slight modification of the reported HPLC methods [27, 42, 43]. The HPLC apparatus consisted of a 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 limit of detection and quantitation for ITZ was 15 ng/mL and 50 ng/mL, respectively. The assay was linear (r^2=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 levels (150, 590 and 2300 ng/mL) as per ICH guidelines [44].

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 included in the study, and four rats were 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 the marketed capsule (Itaspor®) were given orally using feeding snode. Dose for the rats was selected as reported [45, 46] and calculated based on the weight of the rats (30mg/kg body weight) according to the surface area ratio [47, 48]. 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.

Plasma Analysis

The frozen plasma samples were thawed just prior to extraction. 100 µL of plasma sample was transferred to a 2 mL centrifuge tube (Tarson, Kolkata, India). To that 300 µL of acetonitrile containing ketoconazole as an internal standard (200 ng/mL in acetonitrile) was added and vortex-mixed for 1 min [49]. The tube was centrifuged at 15,000 rpm for 20 min at 4° C. The organic layer was carefully decanted and was transferred to a clean tube and dried under a 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 ($AUC_{0\to t}$) from zero to 24 hour was estimated by the linear trapezoidal method [50, 51]. The relative bioavailability (F) of SMEDDS to the suspensions was calculated using the following equation:

$$F = (AUC_{test}/AUC_{reference}) \times 100\%$$

Statistical Analysis

The differences in diffusion, permeability and *in vivo* absorption profiles of itraconazole SEDDS and the marketed preparation were assessed by the use of Student's *t*-test. The pharmacokinetic data for the different formulations were compared using Student's *t*-test. Statistical probability (P) values less than 0.05 were considered significantly different.

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 [52]. 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 the different aforementioned storage conditions for up to 3 months. Samples were removed at 0, 1, 2 and 3 month intervals and analysed for itracona-

zole content (by HPLC), droplet size, polydispersity index and zeta potential after diluting the formulation 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.

Results

Screening of Oils and Surfactants

The solubility of itraconazole in various vehicles is shown in Table **1**. A pseudo-ternary phase diagrams were constructed with constant drug level fixed at 2.4% (w/w) to obtain the components and their concentration ranges. While screening the oil phase, drug loading capability is the main factor for hydrophobic drugs in developing such lipid-based delivery systems.

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

Table 1: Solubility of itraconazole in various oils and surfactants at 25°C (n =3).

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 **3**. The area of microemulsion isotropic region changed slightly in size with the increasing ratio of surfactant to co-surfactant.

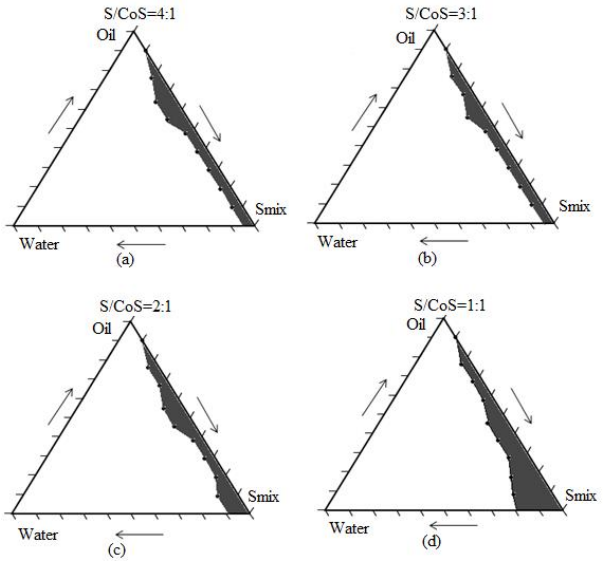

Figure 3: Pseudoternary phase diagrams showing the o/w microemulsion (shaded area) regions of Capryol 90 (Oil), Soluphor® P (Surfactant), Transcutol P (Co-surfactant) at different S_{mix} ratios a) S_{mix} 4:1 b) S_{mix} 3:1 c) S_{mix} 2:1 and d) S_{mix} 1:1

Characterization of Self-Emulsifying Drug Delivery System

Drug Content

Assay of the itraconazole SEDDS was carried out by UV-visible spectrophotometry. 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. The assay was found to be 102.56 % with a standard deviation of ± 1.54%.

Spectroscopic Characterization of Optical Clarity

The SEDDS were diluted with water to confirm the formation of a microemulsion with the external phase of the system without phase separation. In order to assess the optical clarity quantitatively; a UV-visible spectrophotometer was used to measure the light at 650 nm wavelength transmitted by the solution. Cloudier solutions scatter more incident light and results in lower transmittance. However, an optically clear solutions should have a higher transmittance. The absorbance of optically clear solutions are small and such aqueous dispersions are thought to be in the finer state. 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.

Morphological characterization

The TEM photomicrograph is shown in Figure **4**.

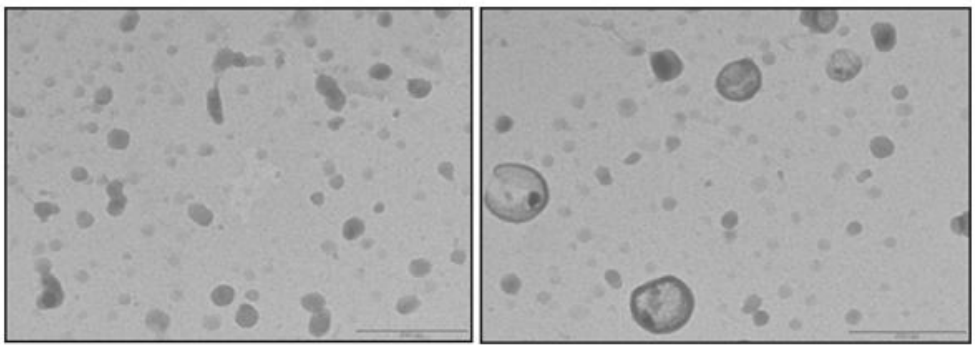

Figure 4: Transmission electron microscopic image of itraconazole self emulsifying system after dilution showing size of some oil globules. (Scale: 500 nm)

Droplet Size and Zeta Potential Analysis

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 **2**.

Table 2: Initial characterization of itraconazole SEDDS S1

	Droplet sizeD (nm)	Polydispersity Index (PdI)	Zeta potential (mV)	Drug content(%)
Distilled water	183.9	0.134	-3.52	102.56 ± 1.54
0.1N HCl	266.9	0.512	2.70	
PBS	246.92	0.010	-2.24	

PBS Phosphate Buffer Saline

In vitro evaluations

There was a significant difference between itraconazole SEDDS and the marketed capsule with respect to dissolution (Figure **5**), *in vitro* diffusion (Figure **6**), *ex vivo* stomach permeability (Figure **7**) and *ex vivo* intestinal permeability (Figure **8**) at $p = 0.05$.

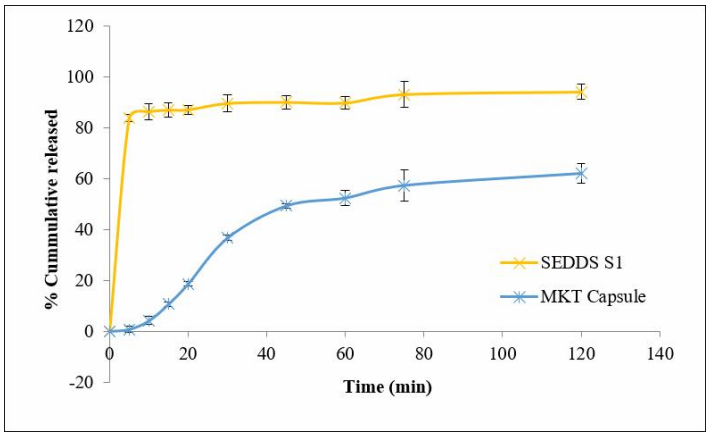

Figure 5: Dissolution profiles of itraconazole SEDDS S1 and marketed capsule in 0.1N HCl (*n*=6)

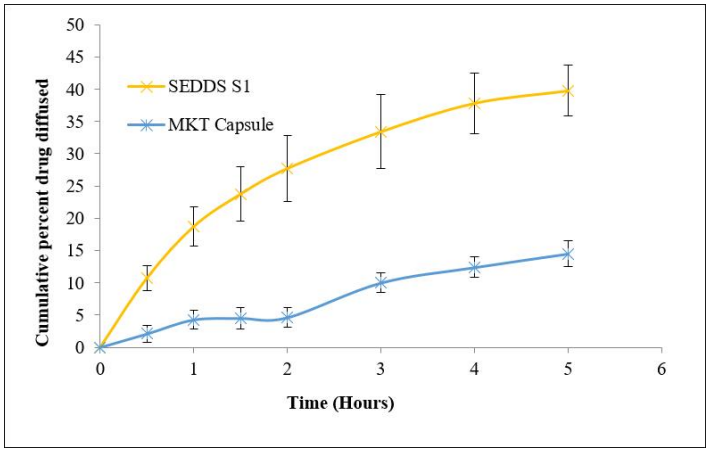

Figure 6: In vitro diffusion profile of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in 0.1N HCl (*n*=6; 25mg)

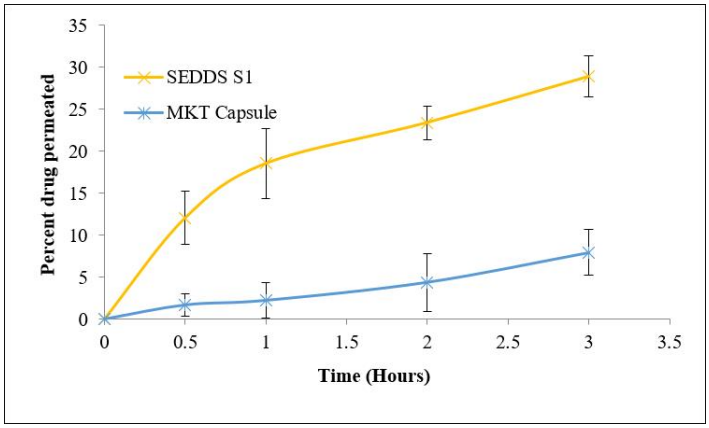

Figure 7: Ex vivo stomach permeability of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in 100 mM HCl (*n*=3; 10mg/0.4mL)

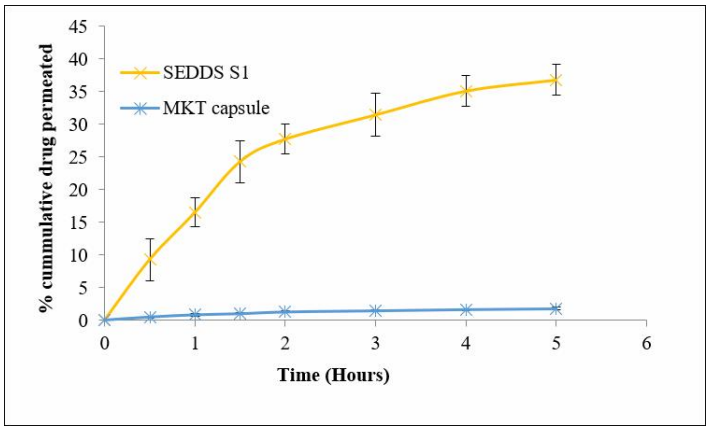

Figure 8: Ex vivo intestinal permeability of itraconazole SEDDS S1 and extemporaneous suspension of marketed capsule in pH 6.8 PBS (n=3; 5mg/mL)

Bioavailability Studies

Table 3 summarises mean pharmacokinetic parameters for the different formulations. The plasma concentration profile of Itraconazole after oral administration of the SEDDS and an extemporaneous suspension of the marketed capsule to rats has been shown in Figure 9.

Table 3: Relative bioavailability and pharmacokinetic parameters of itraconazole after oral administration of itraconazole SEDDS and suspension to the rats (n=4).

	MKT	SEDDS (S1)
$AUC_{0\to t}$ (ng.h/mL)	3037.268 ± 198.86	9299.188 ± 3152.50
$AUC_{0\to\infty}$ (ng.h/mL)	5264.334 ± 1705.79	15324.2 ± 8667.05
C_{max} (ng/mL)	171.956 ± 5.715	821.75 ± 188.952
T_{max} (h)	1.0 ± 0.0	1.25 ± 0.5
Relative bioavailability (%)	---	291.09

MKT = marketed capsule

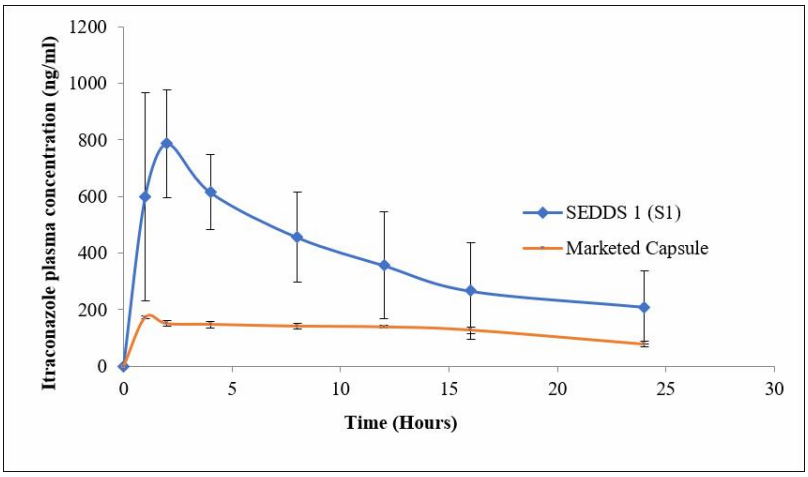

Figure 9: Plasma concentration profile of itraconazole after oral administration of SEDDS and marketed suspension in rats (n=4; 30mg/kg)

Stability Studies

During stability studies, the physical parameters such as homogeneity and clarity were not changed. There was no change in droplet size, polydispersity index and zeta potential even after storing the formulations under the different environmental storage conditions for 3 months, indicating that the developed formulations were physically stable (Table 4).

Table 4: Stability studies of itraconazole SEDDS S1 at various storage conditions

Storage conditions →		**25 ± 2°C (RT)**			**30 ± 2°C/65 ± 5% RH**			**40 ± 2°C/75 ±5%RH**		
Parameters ↓	**Medium↓**	**1 M**	**2 M**	**3 M**	**1 M**	**2 M**	**3 M**	**1 M**	**2 M**	**3 M**
Droplet Size D (nm)	DW	190.43	186.28	192.72	182.88	178.34	177.3	287.67	188.2	192.34
	0.1N HCl	254.39	271.39	263.71	260.53	258.99	267.27	257.5	268.3	251.46
	PBS	238.47	243.39	242.94	236.33	244.3	241.35	235.5	224.87	231.33
Polydisper-sity Index (PdI)	DW	0.136	0.125	0.2	0.125	0.134	0.128	0.231	0.221	0.219
	0.1N HCl	0.415	0.442	0.442	0.432	0.231	0.301	0.478	0.268	0.363
	PBS	0.12	0.236	0.236	0.231	0.253	0.223	0.361	0.331	0.298
Zeta Potential (mV)	DW	-4.75	-6.31	-5.72	-3.22	-3.2	-4.01	-4.53	-4.7	-5.04
	0.1N HCl	4.58	3.5	3.86	3.88	3.8	3.92	4.46	5.3	5.41
	PBS	-3.42	-3.54	-3.28	-4.62	-4.32	-5.21	-5.46	-6.38	-6.62
Drug Content (%)		104.242 ± 1.89	101.515 ± 1.81	101.81 ± 1.04	100.505 ± 0.46	102.02 ± 1.66	100.60 ± 0.80	100.50 ± 0.92	101.41 ± 0.63	100.30 ± 0.30

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

Discussion

Capryol 90 showed good solubility among the oils. Among the surfactants, Transcutol P, Tween 20 and Labrasol were selected for the preliminary studies. In preliminary trials the solubility of itraconazole was checked in different ratios of Transcutol P and Tween 20 (4:1, 3:1, 2:1 and 1:1). For these studies, 50 mg of itraconazole was weighed and dissolved by gentle warming. Drug was precipitated in all those four formulations, when allowed to cool. The 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 combinations, but no stable system was obtained.

The combination of 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 is a potential solubilising agent for itraconazole [5]. In the present study, the selected solubilizer was 2-pyrrolidone and our results are also in agreement with that observed by Uch and colleagues. The complexation effect of 2-prrolidone may be considered for solubilisation of hydrophobic drug, itraconazole. It is believed that the 2-pyrrolidone molecule can undergo stacking with the drug molecule in a polar environment. Such an arrangement will stabilize the dissolved drug resulting in an increase in its solubility [6].

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 and were selected to construct the pseudo- ternary phase diagrams. Surfactant:co-surfactant 1:1 showing the maximum microemulsion region was selected for incorporating the drug.

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 2:1was selected for microemulsion formulation.

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 [22, 33]. In our study, we investigated the effect of three different dilution media (distilled water, 0.1N HCl and phosphate buffer saline pH 6.8) on droplet size. The *in vitro* and *ex vivo* studies clearly showed a better diffusion and permeation of poorly soluble drug ITZ from the SEDDS than that from the extemporaneously prepared suspension of the marketed capsule.

Significant differences were found between the two formulations with regard to $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} parameters at $P < 0.05$. As a weakly basic drug with poor aqueous solubility and high lipophilicity, itraconazole should be rapidly transported in to the intestinal mucosa, provided it can be dissolved. 2-pyrrolidone has improved itraconazole solubility in aqueous media to keep itraconazole in solution. A SEDDS formulation containing 2-pyrrolidone also readily permeated across rat stomach and intestinal tissues, indicating that drug in solubilised state could have been diffused across cell membrane by passive diffusion as was observed with N-methyl-2-pyrrolidone [2-5]. The total amount of drug absorbed from the marketed capsule was less than half the amount absorbed from the SEDDS formulation. The stability studies showed that there was no decrease in the drug content at the end of 3 months indicating that the formulation remained chemically stable.

Although the use of any new excipient(s) that provides benefits, they bring challenges too. The lack of sufficient safety data for the proposed dose, period of use, such excipient(s) are considered 'new' under regulatory definitions. Hence safety/toxicity assessments should be the subject of further development in the future. Regulatory bodies demand the sufficient data of safety from animal to human studies to get any new excipients to be included in the inactive ingredients database of the regulatory bodies.

Conclusion

A SEDDS containing the poorly water-soluble drug, itraconazole, was formulated for oral administration. The components and their ratio ranges for the formulation of the 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 the oil, 58.53% of Soluphor® P as the surfactant and 29.27% of Transcutol P as the co-surfactant, and had sufficient drug loading, rapid self-emulsification in aqueous media, and forming droplets in the size range of a microemulsion. The present 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 the developed SEDDS using Soluphor® P as one of its components showed improved bioavailability compared to the 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 their bioavailability.

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Authors Declaration of Conflicting Interest

The authors declare no conflict of interest, financial or otherwise.

Author's Contributions

Conceptualization, AC, VP and CS; methodology, VP, AC and MN; software, VP; formal analysis and interpretation of results, KV, MN and CS; resources, CS and KV; writing-original draft preparation, AC; writing-review and editing, VP, CS and KV; supervision, KV and MN. All authors have read and agreed to the published version of the manuscript.

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