Self-Micro Emulsifying Drug Delivery System: An Approach to Improve the Solubility of Itraconazole

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
September 2022

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "Self-Micro Emulsifying Drug Delivery System: An Approach to Improve the Solubility of Itraconazole." submitted by Alifa Ahmed (ID 18346002) of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 28th September, 2022.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

The study is designed to improve the bioavailability of Itraconazole by developing a self-micro emulsifying drug delivery system (SMEDDS). A series of SMEDDS were prepared with different ratios of oil, surfactant and co-surfactant and examined for emulsification. Pseudo-ternary phase diagram was used to find out the optimized concentration of the components. The dissolution profile of the optimized SMEDDS-ITZ was performed in aqueous media. The physicochemical characteristic of SMEDDS-ITZ were also examined by dynamic light scattering (DLS). The possible interaction between the components were checked by Fourier Transform Infrared spectroscopy (FT-IR). The results from the dissolution study of the prepared formulation suggests that the SMEDDS can be promising approach for Itraconazole and other Biopharmaceutics Classification System (BCS) Class II drugs to reduce dosing frequency and to improve the bioavailability.

Keywords: Self-Micro Emulsifying Drug Delivery System (SMEDDS); bioavailability; dissolution; BCS Class II drug; Itraconazole.

Dedication

To my mother and late father

Acknowledgement

I would like to begin by thanking the Almighty Allah to bless me with the strength, patience and the dedication to complete this project. However, it would have been impossible to complete this project without the support and guidance of several individuals and I would like to pay respect to them.

First of all, I would like to express my gratitude towards my supervisor Dr. Eva Rahman Kabir, (Professor and Dean, School of Pharmacy, Brac University) for giving a wonderful opportunity to work with her. I am immensely thankful to her for sparing her valuable time to share her expertise and also for her constant motivation, encouragement to complete this project. She has motivated me to be more engaged in research work. It would have been very difficult to complete this project without her immense expertise in this field.

Secondly, I would like to express my deepest appreciation to Dr. Shimul Halder (Associate Professor, Department of Pharmaceutical Technology, University of Dhaka) for constantly guiding me with his expert opinion and ease my project with his vast knowledge in the arena. He has been a constant support in every phase of this journey and inspired me to explore more in the research field.

I would also like to show my gratitude towards Dr. Hasina Yasmin (Professor, Assistant Dean and Program Director, School of Pharmacy), Namara Mariam Chowdhury (Lecturer, School of Pharmacy) and Tanisha Tabassum Sayka Khan (Lecturer, School of Pharmacy) to direct their guidance and help to solve the problems during my project along with supervisor.

Then I would also like to thank the lab officer, Tania Akter Tamanna to help me with all the necessary materials to continue my research work at the lab and to guide me in operating the laboratory equipment.

I would like to also acknowledge all the faculty members, lab officers, teaching assistants and staffs of the School of Pharmacy for their support during my B. Pharm Program.

Lastly, I would like to appreciate my mother and my family for their unconditional support, love and good wishes to give me patience and dedication to pursue my goals. I am eternally grateful to my late father for inspiring me in every aspect of my life.

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List of Acronyms

IFI	Invasive Fungal Infection
ITZ	Itraconazole
SMEDDS	Self-Micro Emulsifying Drug Delivery System
BCS	Biopharmaceutics Classification System
GI	Gastrointestinal
S-mix	Surfactant and Co-surfactant mixture
DLS	Dynamic Light Scattering
PDI	Polydispersity Index
FT-IR	Fourier Transform Infrared Spectroscopy

Chapter 1

Introduction

1.1 Background

The incidence and severity of fungal infections have recently increased and contributed to the substantial morbidity and mortality especially in patients with weak immune systems. Individuals taking carcinostatic drugs, steroids or antibiotics for a prolonged period, as well as patients with AIDS, terminal illness, undergoing chemotherapy or transplantation are more prone to acquiring infections caused by fungal pathogens (Chudasama et al., 2015; Firacative, 2020). 7 billion people suffer from fungal infections globally with the death of 1.5 million annually (Fuentefria, 2017). Fungi of *Candida, Cryptococcus, Pneumocystis and Aspergillus* genus are mainly responsible for fungal related deaths, as well as cutaneous, superficial and invasive fungal infections (IFI) (Van Daele et al., 2019). The mortality and morbidity from IFI have surpassed the rate of deaths from tuberculosis and malaria (Gonzalez-Lara et al., 2017). The occurrence of cutaneous, superficial skin, nail and hair fungal infections including vaginal candidiasis, mycotoxicosis dermatophytosis is even higher than that of IFI. Despite the huge magnitude of fungal infections and morbidity, few antifungal therapies are offered for the treatment (Table 1) (Seyedmousavi et al., 2017).

The prevailing five categories of antifungal therapies show limited efficacy with associated drawbacks including low aqueous solubility, relatively narrow therapeutic index, low bioavailability and higher inter-individual variability. There is, thus, an interest to investigate the drug delivery systems and drug formulations to tackle the limitations and contribute to the antifungal armamentarium (Mota Fernandes et al., 2021).

Table 1: List of drugs currently used in the treatment of fungal infections (Seyedmousavi et al., 2017)

	Antifungal Class	Example
1	Polyenes	Amphotericin B, Nystatin
2	Azoles	Itraconazole, Posaconazole, Ketoconazole, Voriconazole, Fluconazole
3	Echinocandins	Caspofungin, Micafungin, Anidulafungin
4	Antimetabolite	Flucytosine
5	Allyl amine	Terbinafine

1.2 Drugs for the Treatment and Prophylaxis of Systemic Mycoses

Systemic mycoses occur by both opportunistic, endemic fungi and may have a high rate of mortality if not diagnosed or treated for a long period of time (Souza & Amaral, 2017). Azole antifungals are recognized as the first-line therapy for the prophylaxis of systemic mycoses due to its wide spectrum and potent fungicidal activity (Gintjee et al., 2020). They have a five membered azole ring with two (imidazoles) or three atoms of nitrogen (triazoles) that is attached to a side chain (Souza & Amaral, 2017).

Despite the wide spectrum activity, poor response rate, relapse of the infections and drug toxicities have triggered the need for improving the efficacy of the existing drugs or the search for new drugs. One such approach may involve the derivatives of azole group known as triazoles (Seyedmousavi et al., 2017). Triazoles showed better tolerance and were more effective than the imidazole drugs. These drugs also exhibited broader antifungal spectrum, more potency and resistance to metabolic degradation (Souza & Amaral, 2017). However,

though triazole drugs have made tremendous advancement in the field of the fungal treatment, there were reports of complications including non-linear pharmacokinetics, low aqueous solubility and bioavailability, fatal drug-drug interactions, inter-individual variability (Gintjee et al., 2020; Mota Fernandes et al., 2021). Itraconazole (ITZ) has thus been chosen as the main drug of interest in this study to improve its aqueous solubility with an attempt to address the current treatment concerns.

1.2.1 Itraconazole

Itraconazole (ITZ) is the first orally active triazole class of antifungal agent which is found to have wide spectrum activity against major opportunistic and true fungi including *Cryptococcus, Candida, Aspergillus, Blastomyces and Hispolasma capsulam.* ITZ is one of the most used drugs in the prophylaxis of penicilliosis, blastomycosis, superficial candidiasis, aspergillosis, histoplasmosis, coccidioidomycosis (Hong et al., 2006; Lestner & Hope, 2013).

The main target of ITZ is the ergosterol synthesis pathway by inhibiting lanosterol located in the cell membrane of fungi. The free nitrogen atom of the azole ring binds to the heme group of fungal cytochrome P450 14-α-demethylase (CYP51). CYP51 enzyme is responsible for the ergosterol synthesis (Seyedmousavi et al., 2017). By inhibiting the enzyme, azoles ultimately cause depletion of ergosterol and accumulation of toxic methylated sterols occurs in the cell membrane. As a consequence, the integrity of the cell membrane is impaired which ultimately alters the permeability and fluidity of the membrane. Thus, ITZ exert its fungicidal and fungistatic activity by mainly interfering with the fungal cell growth, proliferation, and subsequent accumulation of toxic substances in the cell (Souza & Amaral, 2017).

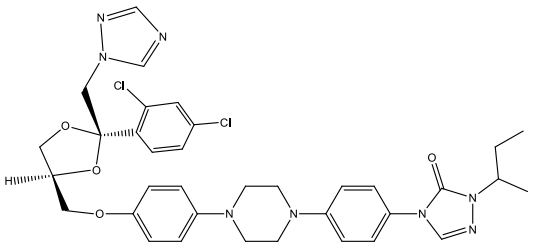


Figure 1: Chemical Structure of ITZ

1.2.2 Physiochemical Characteristics of Itraconazole

ITZ is a water-insoluble, strongly lipophilic drug with high molecular weight. (Seyedmousavi et al., 2017). It exhibits non-linear or saturable pharmacokinetics. It is highly bound to plasma protein which is up to 99.8% and only 0.2% drug is distributed in the system. As a result, it exhibits minimal penetration into eye fluid and cerebrospinal fluid. Although it is extremely plasma bound, it shows relatively good concentration in the tissues with a volume of distribution of 11L/kg. The terminal clearance of ITZ is prolonged and accumulation occurs slowly. The half-life of ITZ is estimated to be 24h and it can take as long as 14 days to reach steady state. The elimination of ITZ primarily occurs in the liver and produces more than 30 metabolites which is subsequently eliminated through bile. 3 to 18% of non-absorbed drugs are found in the faeces while none in the urine. (De Beule & Van Gestel, 2012; Lestner & Hope, 2013).

The solubility of ITZ is pH dependent with a comparatively better solubility in acidic pH than in basic pH (Chivate et al., 2021). The aqueous solubility of ITZ is as low as 1 ng/mL at neutral pH, 4µg/mL at pH 1 and the pka is 3.7 with log P of 5.66 (Chudasama et al., 2015). ITZ is a BCS Class II drug (Biopharmaceutics Classification System) with low solubility and high permeability (Deshpande et al., 2018).

Currently ITZ drug is marketed as a capsule or in the form of oral solution which shows extremely low bioavailability after oral administration (Chudasama et al., 2015). According to De Beule & Van Gesten, 2012, ITZ capsules show variability in absorption and in plasma

concentration which lead to inter-individual variability in terms of bioavailability. For the prophylaxis of systemic mycoses, it is necessary to obtain a constant plasma concentration. However, the variable absorption of ITZ capsules limits the application of the drug (De Beule & Van Gestel, 2012). The bioavailability of ITZ is greatly affected by food as gastric juice is increased after a meal in comparison to fasting state (Hong et al., 2006). Individuals with low gastric acidity or in the fasting state, the absorption of the drug decreases noticeably which ultimately contributes to the inter-individual variability and unpredictable drug response (Chudasama et al., 2015). Thus, the study focuses on designing a ITZ formulation with improved aqueous solubility to achieve desirable oral absorption and bioavailability.

1.3 Proposed Formulation and Drug Delivery System of ITZ

Poor aqueous solubility and limited dissolution in the Gastrointestinal (GI) fluid after oral administration is considered to be the rate-limiting factor for drug absorption of BCS class II drugs (Hong et al., 2006). As a result, the study aims to facilitate the development and optimization of formulation of itraconazole through the development of self-micro emulsifying drug delivery system (SMEDDS), that will improve oral bioavailability and dissolution. SMEDDS is a dynamic approach which aids in enhancing the aqueous solubility of lipophilic drugs by delivering the drug as an oil-in-water emulsion form to the gastrointestinal tract. This drug delivery system employs an isotropic mixture of oil, surfactants of high Hydrophilic-Lipophilic balance (HLB) value and co-surfactants which will be easily emulsified in the GI fluid upon mild agitation (Kim et al., 2019).

Microemulsions are thermodynamically stable formulations with mixtures of oil, water and two emulsifiers – surfactant and cosurfactant. Surfactant is mainly soluble in water while co-surfactant is oil soluble. Surfactant reduces the interfacial tension to mainly facilitate the drug dispersion. The HLB value of the surfactants should be higher than 12 to obtain high emulsifying performance. It also assists to develop o/w droplets and to achieve rapid spreading

of the formulation in the aqueous media. Co-surfactant is associated with reducing the interfacial tension to a negative value which is crucial for the formation of microemulsion and is considered to be the main driving force (Dokania & Joshi, 2015). Moreover, the surface area of interaction is increased between the GI fluid and formulation by SMEDDS ITZ (Kim et al., 2019). The resultant droplet size is less than 250nm which ultimately increases the penetration of the drug across the GI membrane. This particular property makes SMEDDS a convenient technique to deliver orally active lipophilic drug which show adequate solubility in the mixture of oil and surfactant (Woo et al., 2008). According to a study by Woo et al., in 2008 on a rat model, an improvement in the rate and extent of absorption of itraconazole was observed which was unaffected by the presence of food. The onset of action of the drug was also shown to be increased. Therefore, SMEDDS was chosen in the study as the preferred drug delivery system for ITZ to improve its aqueous solubility to achieve a formulation with better bioavailability.

1.4 Solubility Optimization

Solubility is considered to be one of the most important physico-chemical factors that affect drug absorption and efficacy. It is important for a drug to have good aqueous solubility to ensure that the drug is sufficiently absorbed from the absorption site. Low aqueous solubility and limited dissolution rate ultimately results in poor bioavailability. Moreover, the clinical efficacy of poor soluble drugs is hindered as they are eliminated from the body before dissolution in GI fluids (Aboutaleb et al., 2016; Savjani et al., 2012). Large doses and frequent dose regimens are required for these drugs to achieve the minimum therapeutic concentration which reduces patient compliance (Sarfraz et al., 2017). Therefore, drug solubility is considered as one of the important determinants of drug efficiency (Dizaj et al., 2015). In case of the drug of interest in the study, ITZ, is practically insoluble according to USP criteria with an aqueous solubility of 1ng/mL in neutral pH. Therefore, solubility enhancing approaches were considered for ITZ to improve its clinical efficacy and bioavailability.

Table 2: United States pharmacopoeia solubility criteria

Descriptive term	**Parts of solvent required for one part of solute**	**Solubility range (mg/mL)**	**Solubility assigned (mg/mL)**
Very soluble	< 1	≥ 1000	1000
Freely soluble	From 1 to 10	100-1000	100
Soluble	From 10 to 30	33-100	33
Sparingly soluble	From 30 to 100	10-33	10
Slightly soluble	From 100 to 1000	1-10	1
Very slightly soluble	From 1000 to 10,000	0.1-1	0.1
Practically insoluble	≥ 10,000	< 0.1	0.01

1.4.1 Biopharmaceutics Classification System (BCS)

According to the characteristics of solubility and permeability, drugs are categorized into four classes by the Biopharmaceutics Classification System (BCS) (Table 3).

Table 3: Biopharmaceutics classification system (BCS),(adapted from, Nikolakakis & Partheniadis, 2017)

BCS Class	Solubility	Permeability	Marketed & Candidates
Class I	High	High	Marketed 35% - Candidates 5-10%
Class II	Low	High	Marketed 30% - Candidates 60-70%
Class III	High	Low	Marketed 25% - Candidates 5-10%
Class IV	Low	Low	Marketed 10% - Candidates 10-20%

Oral route is the most convenient route of drug administration due to its flexibility in design, improved patient compliance and decreased sterility requirements. As a result, pharmaceutical industry focuses its resource towards developing bioequivalent orally active drugs (Chivate et

al., 2021; Savjani et al., 2012). However, 40% of the new drug candidates are found to have poor aqueous solubility, low dissolution rate, resulting in poor bioavailability of drug after oral administration, a major concern in developing orally active formulations (Dokania & Joshi, 2015; Khadka et al., 2014). The rate limiting step for the bioavailability of BCS class II drug is the low rate of dissolution. Despite of low solubility, these drugs have high permeability and as a result, some approaches for formulation development are still emphasized on the BCS class II drugs. In this study, ITZ, a BCS class II drug, was considered to attempt to improve its aqueous solubility and bioavailability.

1.4.2 Methods of Solubility Enhancement

Many formulation strategies have been reported in the literature to enhance the solubility of drugs. The main methods of enhancing aqueous solubility include modifying drug structure, enlargement of size, reduction of particle size, modifying surface area, solid dispersion, prodrug approach, supercritical fluid, complexation, micronization, using salts of weak acids and bases, holt melt extrusion, microemulsion etc (Chivate et al., 2021; Kumar et al., 2013)

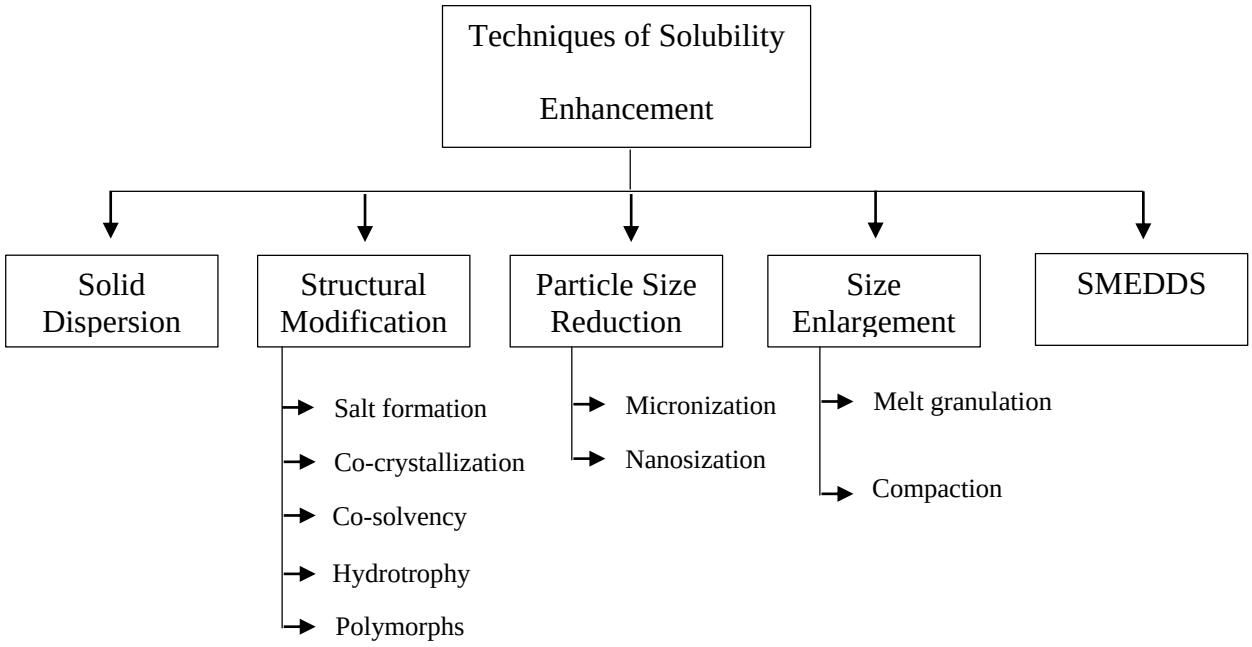


Figure 2: Techniques of Solubility Enhancements (adapted from, Kumar et al., 2013)

Among the methods of solubility enhancements, lipid-based drug delivery systems have become one of the popular approaches to subdue the challenges with poor solubility and bioavailability of drugs due to the accessibility of lipid excipients. Self-emulsifying drug delivery system (SEDDS), SMEDDS and self-nanoemulsifying drug delivery system (SNEDDS) are focused in numerous literatures as appropriate method to enhance the aqueous solubility, rate of dissolution and bioavailability (Čerpnjak et al., 2013). In this study, SMEDDS was chosen as the preferred method of drug delivery. SMEDDS is thermodynamically stable, can be stored very easily and creaming does not occur with prolonged period. There is no water in SMEDDS which contributes to better physical and chemical stability during its shelf-life. They are also easy to administer with a very small volume, and thus, patient compliance is achieved with SMEDDS. More importantly, the effect of food on the drug absorption is also tackled with SMEDDS formulation and rapid oral absorption is achieved with a quicker onset on action.

Table 4: List of SMEDDS designed for the oral delivery of lipophilic drugs (Agrawal et al., 2012)

Types	**Drug**	**Lipid**	**Surfactant:Co-surfactant**	**Dose**	**Route**
SMEDDS	Peuraria lobata	Ethyl oleate	Tween 80: Transcutol P	20% w/w	Oral
SMEDDS	Clotrimazole	Oleic acid	Tween 20: PEG 200 n-butanol	40 mg	Oral
SMEDDS	Ibuprofen	Campul MCM C8	Cremophore EL: Carbitol	5mg	Oral
SMEDDS	Furosemide	Miglyol	Labrasol: Plurol oleique	-	Oral
SMEDDS	Acyclovir	Sunflower oil	Tween 60: Glycerol	50mg	Oral

1.5 Objective of the Study

Despite having wide spectrum activity against fungi, ITZ shows limited therapeutic efficacy due to its poor solubility attribute. Therefore, the objective of this study was to develop a lipid-based drug delivery system, SMEDDS, for ITZ to improve the solubility and oral bioavailability of the drug. The formulation would ensure better biopharmaceutical performance, resulting in decreased dosing frequency and enhanced patient compliance.

1.6 Literature Review

A thorough and extensive literature review was performed for the proper selection of drug, polymers, materials, methods and experimental conditions for the study. Following is the list of journal and databases that were used for this study:

1. American Association of Pharmaceutical Scientists
2. Pharmaceutical Development and Technology
3. Drug Design, Development and Therapy
4. International Journal of Pharmaceutics
5. Frontiers in Cellular and Infection Microbiology
6. PubMed
7. Elsevier

Chapter 2

Methodology

2.1 Materials

ITZ was provided by the Square Pharmaceuticals Ltd., Dhaka, Bangladesh. Kollisolv® PG, Kolliphor® P 407 and Propylene glycol were from the BASF Bangladesh. The chemicals and solvents were of analytical grade.

2.2 Standardization of Itraconazole

The preparation of stock solution involved dissolving 10mg of standard ITZ (API) in 100mL methanol in a volumetric flask, to make a concentration of 0.1mg/mL. 2mL of the stock solution was diluted using 8mL of water to form the primary solution of 20µg/mL. This primary solution was further diluted to prepare the solution of ITZ of varying concentrations of 10µg/mL, 5µg/mL, 2.5µg/mL and 1µg/mL. UV-visible Spectrophotometer was used to record the absorbance of the solution and these absorbance values were plotted in a scatter diagram. It was used to determine the linear relationship between concentration and absorbance and to obtain the standard curve. The extent of linearity between these two variables was shown by the value of regression coefficient.

2.3 Optimization of Vehicle

Kollisolv® PG, Kolliphor® P 407 and Propylene glycol (PG) was used as oil, surfactant and co-surfactant respectively to formulate the lipid vehicle. Thirty different 100mg formulations of the lipid vehicle were prepared with varying concentration of oil, surfactant and co-surfactant mixture (S-mix) and were evaluated to find out the optimized concentration of the vehicle (Table 5).

Oil, surfactant and co-surfactant were weighed accurately as per the ratio to formulate thirty 100mg of oil phase. After precise weighing, it was properly mixed in the vortex machine for 2-3minutes. The mixture was heated at 50^{o}C for 15 minutes in the ultrasonic water bath and 10mL of water was added after cooling. The oil and water phase were properly mixed in the vortex machine for 1-2 minutes and visually evaluated against a black paper to assess for emulsification and precipitation. Ratio 6 and Ratio 29 showed good results. Finally, data from the pseudo-ternary diagram confirmed the fine emulsion region to develop the SMEDDS-ITZ formulation with optimized concentration of Oil and S-mix.

Table 5: Titration chart to find out microemulsion region

Formulation No.	**Ratio A:B:C**	**A (Kollisolv® PG) µL**	**B (Kolliphor ® P 407) mg**	**C (Proplylene glycol) µL**	**Water (mL)**
1	25:70:5	23.81	70	4.80	10
2	25:65:10	23.81	65	9.61	10
3	30:60:10	28.57	60	9.61	10
4	30:55:15	28.57	55	14.41	10
5	30:50:20	28.57	50	19.21	10
6	35:45:20	33.33	45	19.21	10
7	35:40:25	33.33	40	24.02	10
8	20:75:5	19.05	75	4.80	10
9	15:80:5	14.29	80	4.80	10
10	10:85:5	9.52	85	4.80	10
11	20:70:10	19.05	70	9.61	10
12	20:65:15	19.05	65	14.41	10
13	25:60:15	23.81	60	14.41	10
14	15:75:10	14.29	75	9.61	10
15	40:45:15	38.10	45	14.41	10
16	35:50:15	33.33	50	14.41	10

17	35:55:10	33.33	55	9.61	10
18	30:65:5	28.57	65	4.80	10
19	40:50:10	38.10	50	9.61	10
20	45:50:5	42.86	50	4.80	10
21	25:70:5	23.81	70	4.80	10
22	40:55:5	38.10	55	4.80	10
23	35:60:5	33.33	60	4.80	10
24	12:78:10	11.43	78	9.61	10
25	42:53:5	40.00	53	4.80	10
26	25:65:10	23.81	65	9.61	10
27	20:75:5	19.05	75	4.80	10
28	24:60:16	22.86	60	15.37	10
29	20:64:16	19.05	64	15.37	10
30	32:60:8	30.48	60	7.68	10

2.3.1 Pseudo-ternary Phase Diagram

Pseudo-ternary phase diagram of oil, surfactant and co-surfactant was constructed using the aqueous titration method. The boundaries of phase diagrams designated the system's three components; one axis representing the oil phase, the second for the surfactant, and the third representing the co-surfactant. The aqueous phase (distilled water) was added gradually to the mixtures of oils and S-mix. Each mixture was titrated with water and visual assessment was performed for emulsification. The amount of water and time at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was also taken in consideration to find out the region of fine emulsion. The area of micro emulsion formation was identified for the respective systems by using software Sigma plot V14 (Systat Software Inc, UK). After the fine emulsion region was confirmed, the suitable ratio of oil and S-mix was chosen for further physicochemical analysis.

2.4 Preparation and Visual Assessment of SMEDDS-ITZ

10% (w/w) ITZ was added to the optimized concentration of oil and S-mix. These mixtures were properly mixed in the vortex machine to achieve uniform phase and was sonicated for 30minutes at 50^{o}C. In SMEDDS, the main assessment of emulsification is the visual estimation. 100 microliters of itraconazole SMEDDS was diluted with purified water (100 ml) and gently stirred with magnetic stirrer at 90-100 rpm, at 37˚C temperature for visual assessment.

2.5 Dissolution Study

SMEDDS-ITZ containing approximately 10mg ITZ was added to the dissolution apparatus, USP Type II, equipped with a water bath. The dissolution media was 900mL water and the study was performed at 37 ± 0.5 ℃ using the USP paddle method. When the temperature rose to 37^{o}C, the machine was set to rotate at 50rpm. At 15, 30, 45, 60, 120 minutes respectively, 10mL of testing solution was carefully withdrawn from the middle point of the dissolution vessel ensuring no undissolved drug powders were taken. Simultaneously, 10mL of water was added in the vessel at each timepoint to maintain the original volume. The withdrawn solution from the vessel was then filtered and 2mL of the filtrate was taken to the test tube. It was diluted 5 times by adding 8mL of methanol and determined by UV-vis spectrophotometer at 262nm wavelength. For each analysis, three replicates were performed.

2.6 Physicochemical Study

2.6.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The compatibility of the components of the SMEDDS-ITZ and the possibility of interaction was analysed with FT-IR. One-channel $LiTaO_3$ pyroelectric detector was used to record the IR spectra of the samples. Samples were put individually in the sample platform of the instrument

(Perkin Elmer, L160000A, USA) in the range of 4000-700 cm^{-1}. The samples were placed in a pre-assembled sealed rectangular liquid cell with a path length of 0.5mm. For proper analysis, the adjustment and correction of the baseline was performed with each sample. To achieve fine spectra, a 9-point smoothing function was employed

2.6.2 Dynamic Light Scattering (DLS)

DLS method was used to record mean droplet size and polydispersity index (PDI) of SMEDDS-ITZ with a Zetasizer Ultra (MAL-VERN, USA). 1ml of SMEDDS-ITZ was suspended in water and the distribution of droplet size, PDI of the formed emulsion was determined by DLS. The rate and extent of drug release as well as absorption is highly related to the size of the emulsion droplet. It is a major factor affecting the self-emulsification performance of the formulation and PDI determines the droplet size uniformity.

Chapter 3

Results

3.1 Standardization of Itraconazole

A linear calibration curve was obtained when the absorbance was plotted against the concentration, with a correlation coefficient (R^2) of 0.9902 (Figure 3).

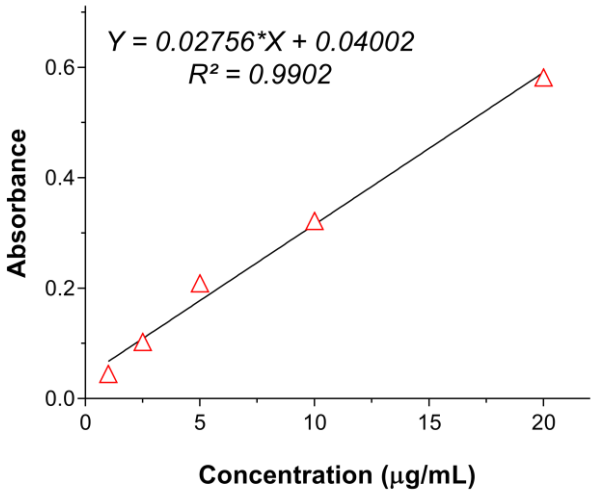


Figure 3: Standard Curve of ITZ in 50% methanol

3.2 Pseudo-ternary Phase Diagram

To formulate SMEDDS-ITZ, it was required to determine the optimized concentrations of the components. Pseudo-ternary diagram confirmed the fine emulsion region to obtain the optimized composition of SMEDDS-ITZ (Table 6).

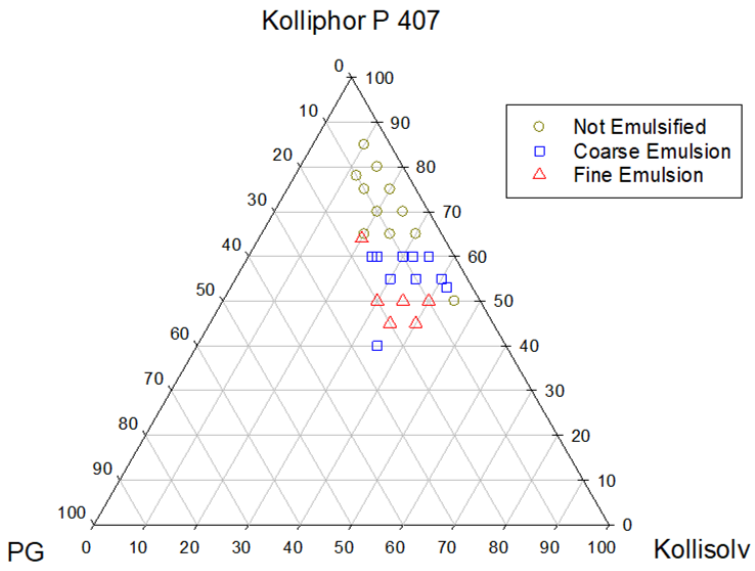


Figure 4: Pseudo-ternary Phase Diagram of SMEDDS-ITZ

3.3 Dissolution Study

Dissolution study was performed with the prepared optimized SMEDDS-ITZ and was compared with the standard ITZ. The study was focused to determine the rate of dissolution of the prepared formulation to find out if the proposed delivery system had significant improvement over the standard ITZ. The study was conducted three times and mean of these experiments were plotted in the diagram (Figure 5).

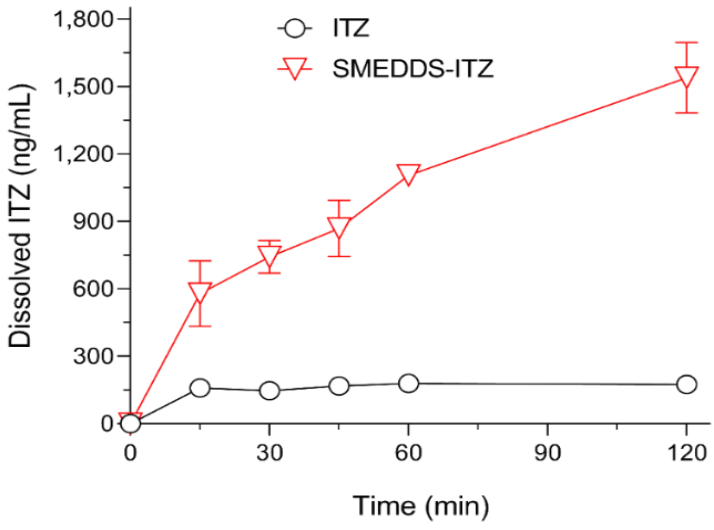
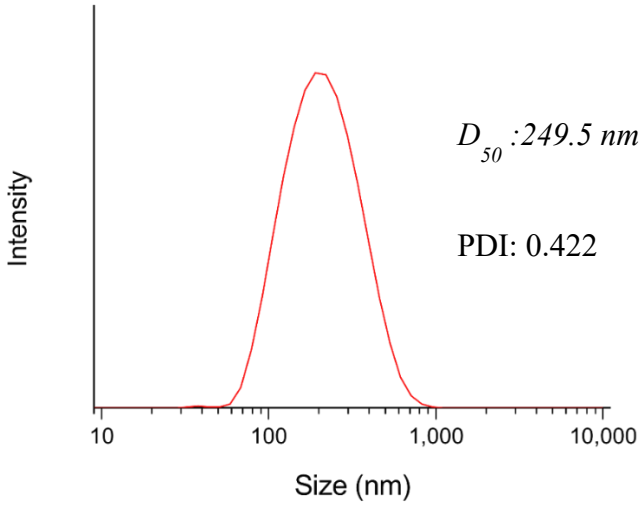


Figure 5: In-vitro Dissolution Study of ITZ and SMEDDS-ITZ

3.4 Physicochemical Characterization

3.4.1 DLS Study

DLS study was performed with the optimized SMEDDS-ITZ formulation to determine the particle size distribution and the uniformity of the particles. Mean particle size and droplet size distribution were analysed from the data of Figure 6. The potential interaction between the SMEEDS components and also how densely the surfactant molecules were packed around the droplet was analysed from the data obtained (Halder, Islam, et al., 2021).



D_{50}, mean particle size; PDI, polydispersity index

Figure 6: Particle Size Distribution of SMEDDS-ITZ Sample in Water

3.4.2 FT-IR analysis

FTIR analysis were performed to check for compatibility of the components of SMEDDS and ITZ. The study was analysed to determine if there was any band shift, as well as to confirm the absence of any additional peaks and bonds between the drug and the polymers (Kim et al., 2019).

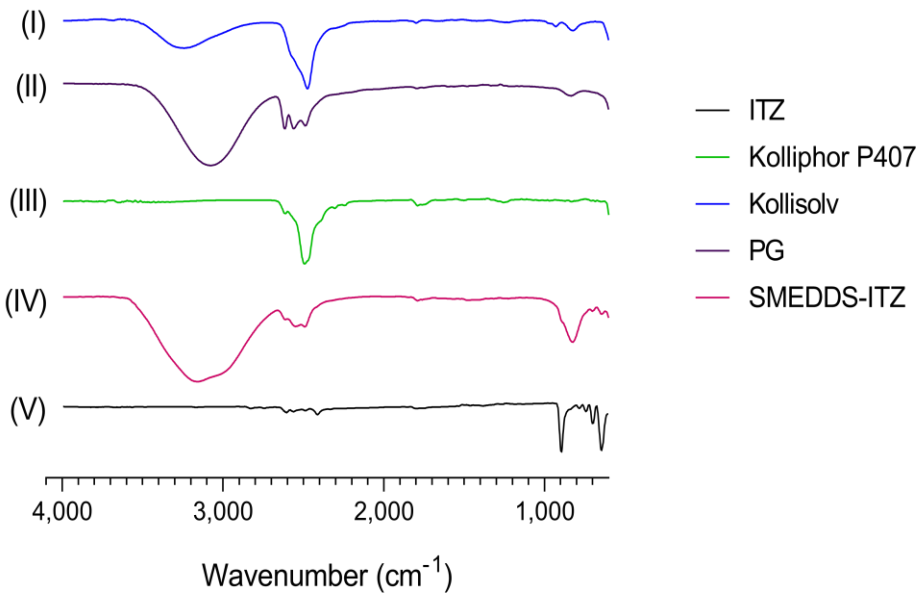


Figure 7: FT-IR Analysis to Determine ITZ-polymer Compatibility

Chapter 4

Discussion

4.1 Composition of SMEDDS by Pseudo-ternary Phase Diagram

To obtain spontaneous self-emulsification, it is essential to optimize the ratio of oil and S-mix of the formulation. The fine emulsion region and the optimized concentrations of oil, surfactant and co-surfactant were determined by the pseudo-ternary phase diagram.

From Figure 4, it was apparent that fine emulsion region starts with Kolliphor® P 407 (surfactant) at a concentration of 45% and the range should be between 45-50%. It was observed that with gradual increase in the surfactant concentration than this range, the ability of self-emulsification of the formulation declined and formed no emulsion to coarse emulsion. The data also confirmed the concentration of Kollisolv® PG (oil) which should be between 30-40% to obtain successful dispersion of lipid and fine emulsion. Then from figure 4, the concentration of the co-surfactant (PG) should be ≥ 10% in the formulation. Below 10% of PG, there was no fine emulsion region. Moreover, a decrease in oil and surfactant concentrations in the formulation than these optimized ranges, lead to coarse emulsion or no emulsification at all. Finally, combined S-mix concentration of ≥ 55% was found to be required for proper fine spontaneous emulsification.

The above findings confirmed the concentration of the components of SMEDDS to ensure spontaneous emulsification and the ratio of oil, surfactant and co-surfactant of 35:45:20 (ratio 6) was chosen for preparation of SMEDDS-ITZ.

Table 6: Optimized composition of SMEDDS-ITZ

Component material	Component ratio (% w/w)
Kollisolv® PG	35
Kolliphor® P 407	45
PG	20
ITZ	10

4.2 In-Vitro Dissolution Test of SMEDDS-ITZ

The optimized SMEDDS-ITZ was evaluated for dissolution behaviour. Dissolved ITZ (ng/mL) versus time in comparison to ITZ was plotted to observe the dissolution rate of the optimized formulation. From Figure 5, SMEDDS-ITZ showed quicker onset of release and better dissolution profile than ITZ over a 2h period.

At the 15min, approximately 600ng/mL ITZ was dissolved from the SMEDDS-ITZ whereas the standard ITZ showed only a release of 145ng/mL. It was indicative of an initial quick release from the prepared formulation. Thus, initially SMEDDS-ITZ showed 4 times higher release rate. ITZ showed highest release of 155ng/mL. On the contrary, SMEDDS-ITZ showed a release of 1500ng/mL at 120min point which is around 9.68 folds higher than ITZ. Comparing the release profile of ITZ and SMEDDS-ITZ, the prepared formulation showed better dissolution behaviour with a final 9.67-fold higher dissolution at 2h than that of ITZ. Thus, SMEDDS could be a promising approach to improve the dissolution profile of ITZ.

4.3 Physicochemical Characterization

4.3.1 Particle Size Distribution and PDI by DLS

Droplet size distribution greatly affects the stability of SMEDDS and the efficacy of self-emulsification also depends on the droplet size upon exposed into the aqueous media (Halder, Islam, et al., 2021). In this study, DLS analysis was performed to characterize self-emulsification of SMEDDS-ITZ. DLS data of SMEDDS-ITZ showed fine emulsion with a mean droplet size of 249.5nm with a PDI of 0.422. Thus, the formulation showed nanosized distribution of particle which would provide a large surface of area and increase the interfacial solubility of the formulation. Reduced particle size would also facilitate penetrating across the GI membrane and improve drug absorption (Dokania & Joshi, 2015). PDI value greater than 0.7 indicates broad size distribution of particles. The PDI was found to be less than 0.7 which supports promising particle size reduction. The decreased particle size may have a positive impact on the dissolution and absorption of the drug (Halder, Azad, et al., 2021). Therefore, SMEDDS-ITZ should be focused and developed to improve the dissolution profile of ITZ as it produces nanosized particles when introduced to aqueous media.

4.3.2 ITZ-polymer Interaction by FT-IR Analysis

The potential interaction between the SMEDDS components and the drug was identified by FT-IR analysis. The corresponding FT-IR spectra are shown in Figure 7. In this study, there were negligible differences observed in the patterns of IR spectrum of ITZ, Kolliphor® P 407, Propylene glycol, Kollisolv® PG and SMEDDS-ITZ.

FT-IR spectrum of ITZ showed an intense sharp peak at 900 cm^{-1} of C-H and medium peak at 790 cm^{-1} of C=C. Again, a strong sharp peak at 680 cm^{-1} of C-Cl and weak peaks at 2695cm^{-1} of C-H. Then Kolliphor® P 407 showed a strong sharp peak at around 2695 cm^{-1} of C-H. A strong peak at 3200-2700 cm^{-1} of OH was observed for Kollisolv® PG and FTIR spectrum of

PG was exhibited at 3550-3200 cm^{-1} of OH and a strong sharp peak was achieved at 2830-2695 cm^{-1} for C-H. In contrast, in the IR spectrum of SMEDDS-ITZ, a strong sharp peak was achieved at 800 cm^{-1} of C-H which was slightly shifted from 900 cm^{-1} of the ITZ. Again, a medium peak was achieved at 2695 cm^{-1} of C-H and a broad strong peak was achieved at 2700-3200 cm^{-1} of OH. All the characteristic peaks of the components were observed in the FT-IR of SMEDDS-ITZ without any additional new peaks. Therefore, it indicated that, the polymers were compatible with the drug.

Chapter 5

Conclusion

A large number of drugs in the drug delivery pipeline are highly hydrophobic and it poses a serious obstacle for formulation scientists. A lipid-based drug delivery system or SMEDDS is mainly employed to enhanced the solubility and bioavailability of hydrophobic drugs (Dokania & Joshi, 2015). In this study, SMEDDS-ITZ was prepared with a blend of oil and S-mix. This new approach showed better dissolution profile with a quicker onset of release. The prepared SMEDDS-ITZ exhibited 9.67 times better dissolution in comparison to ITZ. The particle size of the formulation was also found to be micronized range which ultimately would improve the absorption and solubility of the drug. The components of SMEDDS and ITZ were also compatible. Thus, preparation of SMEDDS-ITZ can be a potential approach to improve the solubility of the drug. To conclude, the optimized SMEDDS-ITZ may result in a delivery system that ensures desirable dissolution and could be a promising technique for drugs solubilization.

Chapter 6

Future Work

Future work can be focused on preparing SMEDDS with various polymer ratios and comparing that with the commercial products of ITZ. In-vivo pharmacokinetic studies could be performed to support the in-vitro dissolution study of Itraconazole in improving solubility and bioavailability. The technique can also be applied to other members of BCS class II to enhance their solubility and bioavailability properties.

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