TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

DEVELOPMENT AND CHARACTERIZATION STUDIES ON A SOLID SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM OF DEFERASIROX

Ph.D THESIS

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PHARMACEUTICAL TECHNOLOGY DEPARTMENT

MENTOR

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2020 NICOSIA

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ALAA ALGHANANIM

Acknowledgment

I am deeply indebted and would like to express my sincere thanks to my supervisor **Assoc. Prof. Dr. Yıldız ÖZALP,** It was an owner for me to be a Ph-D student under her supervision throughout my time as her student, I have been extremely lucky to have a supervisor who supported, guided and motivated me in each step through this journey. It would never have been possible for me to take this work to completion without her incredible support and encouragement and words fail to express my deepest regards towards her.

I would like to thank my co-supervisor **Prof. Dr. Sevgi GÜNGÖR**, she has provided constructive comments, valuable conversation and suggestions during my thesis as well as on the preliminary formalities towards the completion of this thesis,

Also I would like to thank **Prof. Dr. Yıldız Özsoy ERGİNER** and pharmaceutical technology department in Istanbul University, who was kindly agreed to host me for following up part of my thesis.

My sincere thanks to **Dr. Burcu MESUT** for following my progress in each step during my work in the laboratory. She provided me a motivating, passionate and relaxed atmosphere during discussions. I really appreciate her immense support and encouragement during my stay in Istanbul University, In spite of her busy schedule she was always available for discussing my progress in the research work.

I am truly grateful to **my parents, brothers (Mohammad and Ahmad), sister (Ayat)** and their families for their love and care. They have always encouraged me to explore my potential and chase my dreams. I dedicate this thesis to my family.

I am highly thankful to **Yağmur, Rafael, Günay, Mazen and Şeyma** for their support as my friends and made a wonderful working atmosphere in the lab and the great time we spend out of the lab.

My thanks also go to my colleagues and friends in pharmaceutical technology department **Nailla, Joseph, Mayowa, Asmaa and abdulkadir** for their help and support during Ph-D study.

To all my friends in Cyprus; **Sarah, Fawwaz, Alaa, Hala, Musaab, Omar, Majd, Samar, Sameer and Anas,** without all of you, all these hard years would not have been the same.

And I would like to thank my friends from Jordan, **Ruba** for helping in proofreading and standing by me since my first days in Cyprus, also my friends; **Shatha, Rawan, Rakhaa, Nidal, Yasmin, Loubna and Rawya** for their support even we have oceans and continents between us still we are connected by hearts.

Finally I would like to thank **Assis. Prof Abdelhadi Aljafari** and **Assoc. Prof. Shadi Gharaibeh** in Jerash Private University for supporting me to start my Ph-D journey.

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LIST OF ABBREVIATIONS

(LBDDS)	Lipid Based Drug Delivery System
°C:	Degree Celsius
µm	Micrometer
ABC:	ATP-binding cassette
API:	Active pharmaceutical ingredient
AUC	area under the curve
BCS:	Biopharmaceutics classification system
Cmax	Maximum concentration
conc.	Concentration
CT:	Clotrimazole
d.f.	Dilution factor
DDS	Drug delivery systems
DEF-SNEDDS:	Deferasirox loaded SNEDDS
DFX:	Deferasirox
DFX-S-SNEDDS:	Solid Deferasirox loaded SNEDDS
EMEA	European Medicines Agency
ES	Extrusion Spheronization
FDA:	Food and drug administration
FTIR:	Fourier transformed infrared spectroscopy

G	Free energy
g:	Gram
GIT:	Gastrointestinal tract
GRAS:	Generally regarded as safe
h.:	Hour
HLB:	Hydrophilic lipophilic balance
HPMC:	Hydroxypropyl methylcellulose
ICH:	International Conference on Harmonisation
IR:	Immediate released
IVIVC:	In vitro in vivo correlations
LBFs:	Lipid based formulations
LCT:	Long-chain triglycerides
LD:	Laser diffraction
LEMS:	Liquid encapsulation micro-spray sealing
LFCS:	Lipid formulation classification system
LNCs	Lipid nanocapsules
LOD:	Limit of detection
LOQ:	Limit of quantification
LPs	Liposomes
L-SNEDDS	Liquid self nano-emulsifying drug delivery system
m.p	Melting point

MCC:	Microcrystalline cellulose
MCT:	Short chain triglycerides
MEC	Minimum Effective Concentration
MG	Melt Granulation
mg:	Milligram
min. :	Minute
mL:	Milliliter
MRPs:	Drug resistance-associated proteins
n:	Release exponent
nm:	Nanometer
O/W	Oil in water
OAC	Oil adsorption capacity
P5-40-Syloid:	Solid Deferasirox loaded SNEDDS solidified by using Syloid XDP 3150
P5-40-UFL2:	Solid Deferasirox loaded SNEDDS solidified by using Neusilin UFL2
P5-40-US2:	Solid Deferasirox loaded SNEDDS solidified by using Neusilin US2
PCS:	Photon correlation spectroscopy
PDI	Polydispersity index
PEG:	Polyoxyethyleneglycols
PEG:	Polyethylene glycols

Pgp:	P-glycoprotein
pH:	Potential Hydrogen
pKa:	Acid dissociation constant
PPB	Porous polystyrene beads
PTPD	Pseudo terntary phase diagram
PWSD	Poorly water soluble drugs
Q5%:	Drug release within 5 min
QC:	Quality control
r.t	Room temperature
R^2:	Coefficient
rpm:	Revolutions per minute
SA	Surface area
SD:	Standard deviation
SEDDS:	Self-emulsifying drug delivery system
SEM:	Scanning electron microscopy
SGF:	Simulated gastric fluid
SIF:	Simulated intestinal fluid
SLN	Solid lipid nanoparticles
SMEDDS:	Self micro-emulsifying drug delivery system
SNEDDS:	Self nano-emulsifying drug delivery system
SUPAC:	Scale-Up and Post-Approval Changes

T, %:	Percentage transmittance determination
TEM:	Transmission electron microscopy
USFDA:	United States Food and Drug Administration
v/v%	Volume by volume concentration
W/W% :	Weight concentration
WHO	World Health Organization

ÖZET

Amaç: Araştırmamız deferasirox etken maddesinin çözünürlüğünü artırmak için deferasirox`un (DFX) kendinden emulsifiye olan ilaç taşıyıcı sistemler (SNEDDS) formülasyonu hazırlamaktır. Bu uygulama güvenli olup ve biyoyararlanımı geliştirme potansiyeline sahip olacaktır.

Gereç ve yöntem: DFX'in farklı bileşenlerdeki çözünürlük çalışmalarına göre SNEDDS bileşenleri seçilmiş ve Pseudo-terner faz diyagramları oluşturulmuştur. DFX yüklü SNEDDS hazırlanmış ve karakterize edilmiştir. Optimum DFX SNEDDS formülasyonları geliştirilmiştir. Optimize edilmiş SNEDDS formülasyonunun güvenliği, MTT hücre canlılık testi ve in vitro ilaç salım çalışmaları kullanılarak bir insanın ölümsüzleştirilmiş miyelojenöz lösemi hücre hattında, K562 hücrelerinde incelenmiştir.

Bulgular ve sonuçlar: SNEDDS formülasyonunun bileşenleri olarak Peceol, Kolliphor EL ve Transcutol seçildi ve karakterizasyon iyi stabil formülasyonun hazırlandığını gösterdi. Sitotoksisite çalışmaları, 40 μM'de saf DFX'e (% 3,99) kıyasla DFX yüklü SNEDDS'nin daha fazla hücre canlılığını (% 71,44) ortaya çıkardığı görülmüştür. Seçilen DFX-SNEDDS formülasyonu, gözenekli taşıyıcılara adsorbe edilerek S-SNEDDS'e dönüştürüldü ve in vitro ilaç salım çalışmaları, Neusilin UFL2 ile katılaşan S SNEDDS'den DFX salımının (% Q5)pazarlanan ürünle karşılaştırıldığında önemli ölçüde daha yüksek olduğunu (5 dakika içinde% 93.6 ± 0.7) (% 81,65 ± 2,10) gösterdi. Genel sonuçlar, DFX'in S-SNEDDS formülasyonunun DFX'in çözünürlüğünü artırma potansiyeline sahip olabileceğini gösterdi.

Anahtar kelimeler: deferasirox; SNEDDS; katı SNEDDS; katıtaşıyıcılar;çözünürlüğügeliştirme; oral dağıtım.

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SUMMARY

Aim: The research work was designed to develop a solid self-nanoemulsifying drug delivery system (S-SNEDDS) of deferasirox (DFX) in order to enhance the solubility of DFX which would in turn have the potential to improve its oral bioavailability as a safe novel delivery system.

Material and Method: According to the solubility studies of DFX in different components, the SNEDDSs components were selected and PTPD were constructed. DFX loaded SNEDDS were prepared and characterized. The optimum DFX-SNEDDS formulations were developed. The relative safety of the optimized SNEDDS formulation was examined in a human immortalized myelogenous leukemia cell line, K562 cells, using the MTT cell viability test and in vitro drug release studies.

Findings and Results: optimum DFX-SNEDDS formulation was prepared by Peceol, Kolliphor EL, and Transcutol showed good stable formulation and has droplet size of14.72±1.50 nm. Cytotoxicity studies revealed more cell viability (71.44%) of DFX loaded SNEDDS compared to pure DFX (3.99%) at 40 µM , DFX-SNEDDS formulation was successfully converted into S-SNEDDS by adsorbing into Neusilin UFL2 DFX release (Q5%) from S-SNEDDS solidified with Neusilin UFL2 was significantly higher (93.6±0.7% within 5 min) compared with the marketed product (81.65 ± 2.10%).

The overall results indicated that the S-SNEDDS formulation of DFX could have the potential to enhance the solubility of DFX.

Keywords: deferasirox; SNEDDS; solid SNEDDS; solid carriers; enhancement solubility; oral delivery

CHAPTER ONE

INTRODUCTION AND AIM

1.1 Self Nano-Emulsifying Drug Delivery Systems

Recently, drug discovery programs are finding new chemical entities where 40% are either insoluble or PWSD (Rohrer, 2018). Many strategies and formulation technologies were came out to increase and elevate the bioavailability of drugs which are PWSD; one efficient method known as formulation into lipid based formulations (LBFs) like liposomes, microemulsion, nanoemulsion, and SEDDSs (Shrestha, 2014).

LBFs approach is a big umbrella contains a broad group of formulations that defined as a lipophilic drug dissolved in a mixture of excipients up to 5 classes; these excipients vary by their physicochemical characteristics fluctuate from triglyceride oils as pure, mono- and diglycerides, and extensive percentage of hydrophilic or lipophilic surfactants and cosolvents/cosurfactants. Pouton introduced a model which classifies the LBFs according to the type and amount of excipients used called LFCS. (Pouton C. W., 2000). LFCS classification established to select the most proper formulation constituents according to the specific physiochemical properties for each molecule (Pouton C. W., 2008).

Summarily, Type I lipid formulations compromise drug dissolved in digestable oils that considered by agencies of regulatory as GRAS which means as Generally Regarded as Safe. Type I LBFs have poor drug capacity but can be efficient compounds of logp>4 and highly potent drugs. Type II LBFs are water insoluble SEDDSs which consist of oils and water insoluble surfactants (HLB<12). Type III LBFs consist of oils and water soluble surfactants (HLB>12) and hydrophilic cosurfactants, Type III formulations spitted into IIIB and IIIA based on percentage of surfactants and/or co-solvents/cosurfactnts which are soluble in water; where type IIIB includes extra percentage of the soluble surfactants and/or co-solvents than type IIIA. LBFs of type III involve SMEDDSs SNEDDSs where both differ by the size of the oil in water emulsion created upon dilution. type IV LBFs regarded the most hydrophilic formulations and contain only hydrophilic surfactants (HLB>12) and cosufractants. (Pouton C. W., 2000) (Pouton C. W., 2006).

Among the LBFs, SNEDDSs have gained great attention, as an approach to improve oral bioavailability of drug substances which have low aqueous solubility, SNEDDS are isotropic mixtures of drug, oil and hydrophilic surfactants and co-surfactant/co-solvent. Instantaneously, under dilution and mild agitation provided by the peristaltic motility in gastrointestinal tract, SNEDDs can form fine oil in water emulsion which has globule size less than 50 nm (Shakeel, 2014

In addition, SNEDDS are unique DDS that are characterized by thermodynamic stability of the nanoemulsion formed, rapid onset of action, ease of preparation process, and scale-up, in comparison with other LBDDS (Khan A. W.,2012) which make them attractive for industrial manufacturing.

In the last few decades SNEDDs gained attention in enhancing and increasing the solubility of PWSD thus improving their bioavailability through increasing solubility and keeping up these drugs dissolved as droplets of nano size within the gastrointestinal fluid (Gupta, 2013) therefore skip the dissolution step for the oral dosage form (Mobarak, 2019), and also by promoting lymphatic transportation through gastrointestinal walls for highly lipophilic drugs that results into by passing first pass metabolism. (Rehman, 2017)

Conventional L-SNEDDS are incorporated into a soft gelatin capsule; however, on long term storage, they could face some limitations, like precipitation at lower temperatures, drug leakages, excipient-capsule incompatibility, and handling and stability issues (Tang, 2008)In order to overcome these limitations, combining the advantages of traditional SNEDDS formulations and the solid dosage form by incorporating liquid SNEDDSs formulations into solid carrier and converting to solid SNEDDS(S-SNEDDS) formulations by using different techniques, like spray drying or by adsorbing into porous carriers, result in free-flowing powder which can be formulated as powders, granules, pellets, and tablets, or filled into capsules

1.2 Deferasirox Overview

Deferasirox is an orally a tridentate iron chelator agent that is approved by the United States FDA in 2005 and EMA in 2006 for chronic iron overload treatment as a result of blood transfusion in patients 2 years of age and older (Tanaka, 2014). (Cappellini, 2007). DFX is moderately lipophilic molecule (log P value of 3.52) and categorized as class II according to BCS which means that it has low water solubility

and high permeability through intestine (Al Durdunji, 2016). DFX bioavailability after oral administration compared to intravenous administration is 70% (90% confidence interval, 62%-80%) (Stumpf, 2007) mainly due to first pass effect (Waldmeier, 2010).

The importance need for DFX comes from the fact that excess iron which enters the body during blood transfusion has no physiological mechanism to be excreted therefore it forms insoluble complex with ferritin. Iron-Ferritin complex can deposit in the spleen, liver, and myocardium and ending with organ damage (Lindsey, 2007).

The dosage reduction to diminish its side effects and improvement of patient compliance particularly for pediatrics is important. Therefore, to enhance its oral bioavailability, increasing its aqueous solubility is crucial. So far, few studies were performed to improve the solubility of DFX, such as encapsulated imidazole-modified DFX into polymeric micelles as a nano carrier (Theerasilp, 2017)or to increase the solubility of DFX by decreasing its particle size and using sodium lauryl sulfate or Pluronic F127 as surfactants (Gulsun, 2019).

The dissolution of active drug substances is the rate limiting step for the absorption of BCS Class II compounds such as DFX. Therefore, increasing the solubility of DFX has a great importance, to improve its oral bioavailability. In the case of Exjade®, the commercial preparation of deferasirox, this step is overcome by the formulation of a tablet for oral suspension in which sodium lauryl sulphate is used as a solubilizing agent to improve the dissolution of DFX.

1.3 Aim and Scope

1.3.1 Research objective

The aim of this research study is to develop and characterize a novel SNEDDS loaded with DFX in order to increase its solubility and to improve its oral bioavailability and evaluating in vitro cytotoxicity effects of the optimized DFX-SNEDDS formulation. Furthermore, the optimum DFX-SNEDDS would be incorporated into a solid carrier, by adsorbing into different porous carriers to compare their dissolution behavior with marketed tablet of DFX.

To the extent of our knowledge, there has been no research study conducted to formulate DFX intoa SNEDDS formulation for increasing the solubility or bioavailability.

1.3.2 Work plan

1. Preliminary studies to select the components of SNEDDS formulation which comprise
   1.1. Investigation solubility of deferasirox in different excipients
   1.2. Pseudo-ternary diagram construction
   1.3. Nanoemulsion formation assessment
2. Measuring deferasirox equilibrium solubility in SNEDDS formulation
3. Formulation and optimization DFX-L-SNEDDS
4. Characterization of L-SNEDDS of deferasirox regarding droplet size, polydispersity index (PDI), thermodynamic stability, self-emulsification efficiency, robustness of dilution, effect of pH on droplet size and polydispersity index (PDI) and Transmission electron microscopy
5. *In vitro* cytotoxicity study of L-SNEDDS by MTT assay
6. Development of S-SNEDDS of deferasirox by adsorption into different porous carriers
7. Characterization of DFX-S-SNEDDS regarding Fourier transformed infrared spectroscopy and scanning electron microscopy.
8. Studies of *In vitro* dissolution release and compares with the market product

CHAPTER TWO

GENERAL INFORMATION

2.1 Oral Dosage Forms

Oral route believed as the main, most preferable routes for administration of drugs, where it comprises 80% of the commercially available dosage forms (Morishita, 2012).It is well known that administration of drugs orally is the most conventional and desirable route both patients and pharmaceutical companies compared to other alternative administration routes. Regarding pharmaceutical industry view, oral dosage forms are the most cost effective, need the least sterile manufacturing conditions and offer wide range of dosage forms designs while for the patients especially for chronic condition diseases and elderly patients, oral route administration improves patient adherence and provides better patient compliance. Moreover, oral route administration is comfortable for patients as it could help them in avoid hospitalization (Krishnaiah, 2010).

The major challenge for the pharmaceutical manufactures is the low bioavailability especially for the drug molecules that have been synthesized by secreening and drug discovery tools, where 70% exhibit low aqueous solubility (Ku, 2012).

2.1.1 Biopharmaceutics classification system (BCS)

BCS is a tool for development of drugs that permits evaluation of the effects of the three major factors solubility, dissolution, and intestinal permeability on the drug absorption from immediate release solid dosage forms as orally. BCS presents a categorization of drug substances based on solubility of the maximum dose and permeability. As stated by USFDA guidelines, API is regarded as highly soluble if the highest dose strength is soluble in aqueous medium that has pH range of 1-7.5 at temperature of 37 °C in 250 ml or less volume. where High dissolution stands for that 85 % of the administered dose as a minimum value is released as maximum of 30 minutes while highly permeable drug substance means that 90 % of the given dose as a minimum is absorbed through GIT walls based on a mass balance

determination or as a alternative way comparing to an intravenous reference dosage (FDA, 2000)

BCS classifies drug substances into four categories, as represented in table 2.1(Amidon, 1995)

Table 2.1 BCS characteristics

BCS class	Solubility	Permeability	Example
I	highly	highly	Metroplol
II	low	highly	Ibuprofen
III	highly	low	Metformin
IV	low	low	Hydrochlorothiazide

Drugs that belong to BCS class I are absorbed highly drugs and the rate limiting step or absorption is dissolution, in case of rapid dissolution then the rate limiting step regarded as gastric emptying, normally are formulated as immediate release dosage forms. The rate limiting step for Class II BCS drugs is absorption is considered to be the *In vivo* dissolution except the case of very high dose number. The low solubility of class II directly affects the bioavailability and formulation into LBFs and micro-sized formulations etc. would be an option for this type of drugs. Formulation designs in general have a little effect on Class III drugs which characterized by poor permeability especially through GIT membrane while in the case of class IV drugs which characterize by poor solubility and poor membrane permeability, the recommendation to increase the bioavailability is go back to lead optimization phase of ‘chemical discovery’ and find competitor with better physiochemical properties as displayed in Figure 2.1. (Pouton, 2006)

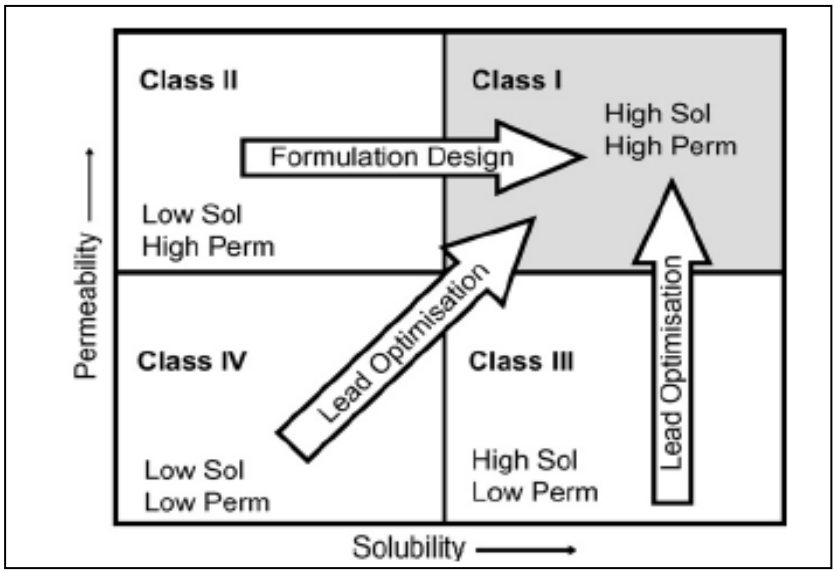

Figure 2.1 BCS classification (Pouton C. W.,2006)

United States FDA, WHO, and EMEA enclose using the BCS classification for authorizing using in vitro release data for establishing the in vivo bioequivalence studies. Also, the agencies permit a" BCS-based" biowaiver for drug products including BCS class I due to rapid dissolution (Amidon, 1995)

This classification provides guidance for waivers of in vivo clinical trials related to BA and BE clinical studies for immediate released (IR) solid dosage forms as orally by replacing clinical studies with an precise , exact *in vitro* dissolution release studies (Yu, 2002)

2.1.2 Physicochemical properties of drug molecules

2.1.2.1 Solubility

The solubility of API is the concentration of the drug particles in dissolved form, where the dissolved particles are in thermodynamic equilibrium and balance with the solid drug particles at a given specified temperature. solubilizing of the drug particles is an essential step for absorption step of any orally drug and for achieving the required concentration of the drug in systemic circulation which exerts pharmacological effect. The solubility of API should be performed accurately, where the solubility plays a key function in understanding quality control of the final formulation and choosing the appropriate drug delivery system.

The solubility depends on numerous determinants consisting of physico-chemical properties of the API (for instance effective SA, particle size of drug particles and the crystal form), solvent properties (for instance pH, polarity, surface tension, added surfactants, co-solvents, salts), and controlling solubility measurement parameters (such as temperature, time, agitation method).

According to USA Pharmacopeia solubility of API is defined as the parts of solvent required for solubilizing one part solute of drug, therefore, solubility of drugs can be categorized as illustrated in table2.2.(The United States Pharmacopeia, 2007). Solubility may be stated in any other analytical unit and also concentration units such as molality(m), weight/volume(w/v,%) …etc.

Table 2.2 Descriptive terms of solubility according to USA Pharmacopeia

Descriptive term	Parts of solvent required for one part of solute	Solubility range (mg/mL)	Solubility assigned (mg/mL)
Very soluble	< 1	> 1000	100
Freely soluble	1 to 10	100 – 1000	100
Soluble	10 to 30	33 – 100	33
Sparingly soluble	30 to 100	10 – 33	10
Slightly soluble	100 to 1000	1 – 10	1
Very slightly soluble	1000 to 10000	0.1 – 1	0.1
Practically insoluble	> 10000	< 0.1	0.01

PWSD exhibit low solubility and low dissolution rate in the gastrointestinal fluids that cause deficient bioavailability in particular for BCS classes especially class II, method for enhancing solubility will be discussed in section 2.6

2.1.2.2 Permeability

Intestinal permeability termed as the flow of API across the organ and how can a drug substance penetrate into the intestinal wall per time unit.For understanding the permeability concept first let's sees the mechanism and how molecules transportation across GI wall is happening

Drug transport mechanisms via gastrointestinal epithelium are divided into the following as shown in Figure 2.2: (Löbenberg, 2013)

1. Transcellular transportation where drug molecules pass across the cells and it has to pass the brush border membrane to enter the cell and crossing the basolateral membrane to leave the cell. The penetration mechanism through both membranes can be either by :
   a. Simple passive diffusion where API particles pass across membrane via passive diffusion where particlesare moving toward blood where it has low concentration of drug from high concentration in the GIT lumen
   b. Transportation via Carrier-mediated entails the passage of a molecule through the enterocyte of the gut using transporters. It comprises active transport and facilitated diffusion
2. Paracellular transportation which is passing through the spaces between the cells.

Physiochemical properties of API molecule specifically the lyphophilicity and hydrophilicity influence the transportation way for each molecule. The molecules transported *via* transcellular route have the ability to diffuse through the membrane are low molecular weight hydrophobic molecules where the hydrophilic small molecules are transported paracellularily. (Homayun, 2019).

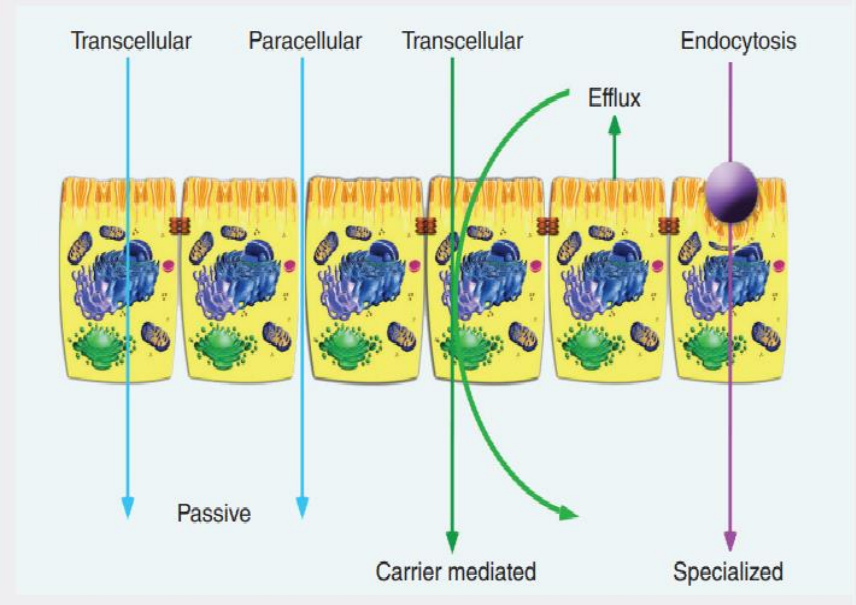

Figure 2.2 Mechanisms of API absorption across intestinal epithelium (Löbenberg, 2013)

Several methods are available for permeability measurement

1. *In situ methods*

In situ experiments involve studies on whole animals. It directly give an idea about absorption *in situ*, therefore, they are universally employed to learn drug kinetics regarding absorption and penetration

It includes intestinal perfusion, intestinal vascular and intestinal loops

2. *In vitro* methods

In vitro methods compromise dialysis bag, Using chamber and cell culture model

In vitro methods characterized by being simple, easy to perform and simple to control conditions of the experimental. on the other hand, In vitro methods facing some problems in estimating actual absorption of particle in nanosize in vivo.

Models based on cell culture are for studying the drug absorption at the cellular and molecular levels. One of the well-known models used is Caco-2cell model and used for intestinal epidermal cellular drug transport and metabolism where Caco-2 cell line is derived from human colon adenocarcinoma and provides a good model for simulation purposes and

distinguishing different absorption pathways in the intestinal cavity and to determine the drug absorptions' mechanisms and kinetics. One of the drawbacks of using Caco-2 cell is that it's a model of only epithelial cells in the intestinal epithelium while many other cell types like mucosal cells and M cells are present in the intestine but not in Caco-2 cell. And, also the lack of the mucous layer found in the intestinal wall. (Liu W. P., 2016)

Co-cultures of Caco-2 cells and mucus-producing goblet cells can provide a drug absorption model that incorporates the drug absorption to the mucus barrier. Incorporating goblet cells yield a mucus gel that covers the whole cell surface Therefore, co-cultures of Caco-2 cells with goblet cell lines such as HT29-MTX cell line have been proposed as an alternative of using Caco-2 cell monolayer alone. (Béduneau, 2014)

3. *In vivo* methods

In vivo evaluation always be required to confirm the true performance of an oral drug delivery system however the in vitro models were sophisticated. The most significant information is the drug release kinetics information either in blood or in urine. (Liu W. P., 2016)

2.1.2.3 Dissolution

It is described as process where solid particles transformed into solubilized particle and to a solution, in other words it's the mass transfer to liquid phase as solubilized from solid state (Viswanathan, 2017). Solid oral dosage form's dissolution is the process where drug particles are likely to dissolve in gastrointestinal fluids. While dissolution rate is the quantity of drug going in the solution in defined time unit in specific circumstances like temperature and solvent composition. Figure 2.3 represents the dissolution process either in vitro media or in gastrointestinal tract fluids.

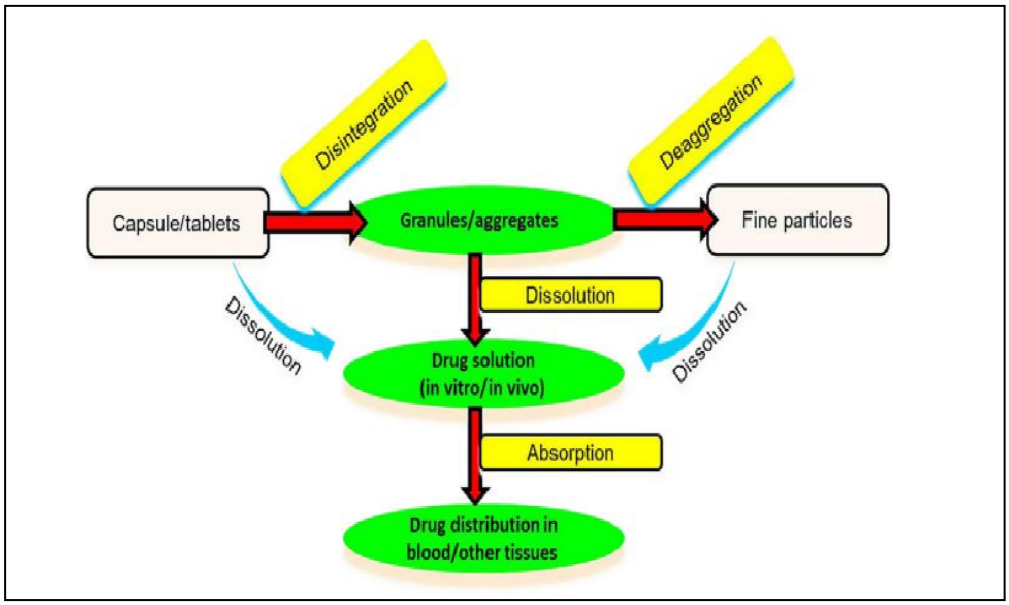

Figure 2.3 Dissolution processes of solid oral dosage forms either in GIT fluids or in vitro media (Kapoor, 2020)

2.1.2.3.1 *In vitro* dissolution testing

This test is used for determining the amount of API released into dissolution medium from a solid dosage form under managed circumstances of temperature and agitation speed using precise dissolution medium volume within a pre-verified duration of time. In vitro dissolution test is one of the important quality control (QC) tests which have a big role in different steps in drug formulation such as selecting a candidate for formulation, identifying critical manufacturing process parameters, and simulating the effect of food on bioavailability by using SGF or SIF as a dissolution media. also this test plays a major role in the "*in vivo"* prediction and evaluation of in vivo performance of the dosage form into the body and it specifically gives important idea on how active pharmaceutical ingredient (API) will be release into the GIT fluids from the orally intended dosage form for oral dosage forms so it finds the way for successful IVIVCs of a final drug dosage form. It also supports waivers for bioequivalence requirement and in addition, moreover this test is used as a requirement in case of changes in formulations components or formulating process as illustrated in SUPAC guidance. (Kapoor, 2020)

2.1.3 Physicochemical properties of lipophilic drugs

Upon oral administration of lipophilic drugs, only a portion of the dose is presented in systemic circulation, this is due to physiochemical properties of lipohpilic drugs. “Lipophilic drugs” term in general describes a diverse set of molecules that exhibits poor/low solubility in water and, these molecules are frequently soluble in a range of organic solvents according to the solubility categorization by United State Pharmacopeia illustrated in section 2.3.1. The descriptive terms: practically insoluble, very slightly soluble and slightly soluble are utilized to classify lipophilic API (Commission BP, 2001).

Lipophilic drugs are also characterized by their partition coefficient value, P, which is the ratio of the concentrations of a compound in a mixture of two immiscible phases of water and 1-octanol at equilibrium (Sangster, 1997). The partition coefficient is expressed as logP, normally.If logP is more than 3 it is considered as lipophilic compound (Mannhold, 2009).

The PWSD candidates exist in two forms of molecule arrangement, “grease ball” and “brick dust”. “Grease ball” molecules are molecules which are characterize by low melting point and high logP value by reason of no interactions with water. “Brick dust” molecules have melting point of 200 and more, low to moderate logP value. Their poor water-solubility is a reason of tough intermolecular-bonding and elevated crystal lattice energy in solid-state which is a mark of high melting point (Ditzinger, 2019).

2.1.4 Challenges facing oral drug delivery

In spite of countless benefits of oral delivery route, the development of orally administered dosage forms stills a big challenge due to the physicochemical characteristics of lipophilic API candidates, physiological barriers and pharmacological barriers that face the dug molecule in GIT.

These challenges which counter orally drug molecules result in low bioavailability and subsequently can cause an ineffective concentration of API molecule in the blood.

Bioavailability is termed as “the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of

action." It illustrates the process of API release from dosage form until reaching the site of action

Lipinski et al. have set up the "role of 5" for identifying the possible poorly bioavailable orally API candidates by identifying the incomplete absorption or permeation properties. The following properties of drugs which are in the discovery stage anticipated in poor bioavailability:

1. High molecular weight of more than 500 D

2. High lipophilicity; calculated Log P > 5 or MLogP1 > 4.15

3. 5 H-bond donors and more (like NH or OH functional groups)

4. 10 H-bond acceptors and more (like functional groups contain N or O atoms)).

"Role of 5" is applicable only for drug candidates that are not classified as substrates for active transporters and/or efflux mechanisms (Lipinski, 1997).

Incomplete bioavailability is the biggest challenge which faces the procedure for formulating oral dosage forms; Figure 2.4 shows the absorption steps for an oral dosage form and possibilities of incomplete absorption that lead to incomplete bioavailability.

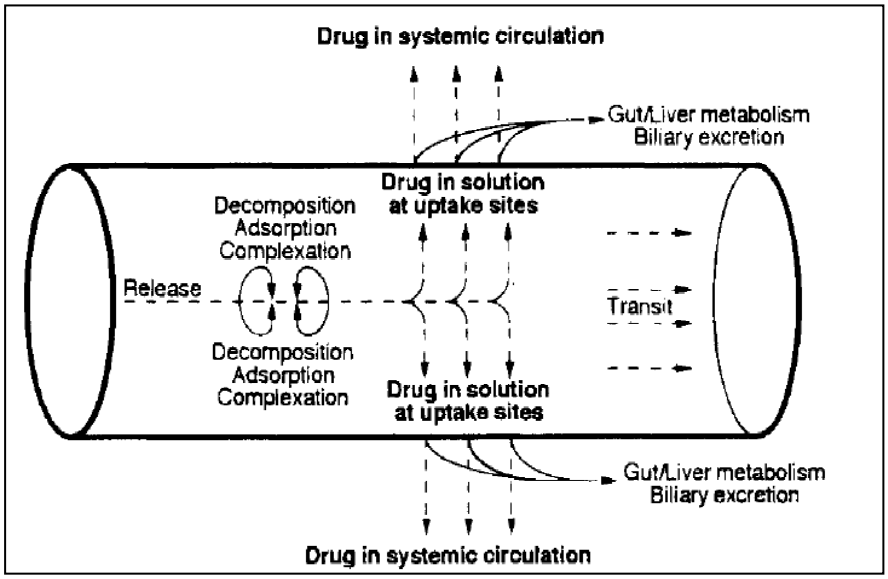

Figure 2.4 Source of incomplete bioavailability in drug absorbance steps

2.1.4.1 Challenges associated with physicochemical properties of drugs

2.1.4.1.1 Solubility

Solubility of the API is considered as one of the essential parameters for accomplishing the goal of therapeutic concentration of API in systemic circulation after absorption from GIT for reaching therapeutic plasma concentrations that leads to achieve the required pharmacological response. For absorption, the drug be required to be existing in solubilized form of in aqueous solution at absorption site, regarding poorly water soluble drugs; high doses are required in order to achieve MEC in systemic circulation. (Savjani, 2012)

The limiting step for absorption rate for API of BCS class II is the release of API from the dosage form and solubilization in the GIT fluid not the permeability, accordingly, the poor solubility and low dissolution rate of class II in the aqueous GIT fluids often leads to inadequate bioavailability. (Sharma, 2009)

Enhancing solubility and subsequently dissolution rate of this drug in GIT fluids is the main key for bioavailability enhancement of class II.

2.1.4.1.2 Permeability

Low permeability is a character of Class III and Class IV of BCS where the oral bioavailability is influenced barely by the solubility of the API in gastrointestinal lumen but as well with percentage of drug that can permit the gastrointestinal mucosa and reach circulation system. For drug to be highly permeable; more than 90% of an administered dose stand on either a mass-balance determination or in association to an intravenous dose (Amidon, 1995). Percentage reached the systemic circulation depending on the physiochemical characteristics of the drug molecule regarding lipophilicity and hydrophilicity of API molecule.

Many techniques are inverted to increase the permeability hence increase the bioavailability like :Cyclodextrin inclusion complex, Spray freeze dying, Chitosan derivatives and “SEDDS”. (MS, 2012)

2.1.4.1.3 Drug stability

For drug to have optimum bioavailability it should be chemically stable and resist the pH changes and enzymatic degradation in the gastrointestinal tract (Kumari, 2019)

Tests for measuring drug stability must include the sensitivity of drug in dissolved form to alkalis, oxidation, acids, photo and thermal-degradation which are very beneficial properties while designing drug delivery system.

2.1.4.2 Challenges resulted from physiological and pharmacological barriers

Many chemical and enzymatic barriers are present in GIT which have an effect on drugs delivery. Through the drug journey to reach final absorption site in the intestine, GI tract's pH changes from pH around 1.0 in stomach to the pH around 7.0 in the small intestine. In this case, drug candidates have to pass the different pH variation without any degradation.

In addition, the GIT transit time is an important feature that extensively influences bioavailability of many drugs. a lot of hacks were done to improve the absorption window by increasing the time that formulations spend in the gastrointestinal tract, like mucoadhesive dosage that can amplify the local drug concentrations available for oral absorption and advance the efficiency for extending drug effect. (Bravo-Osuna, 2007)

Also, a variety of enzymes such as lipases and proteases which are functioning in food digestion could have harm effect on drug molecules.

2.1.4.2.1 Intestinal efflux transporters

The carrier-mediated transports employ membrane-associated transporters which aid in the transfer of solutes. Subfamily ATP-binding cassette (ABC) transporters include drug resistance-associated proteins (MRPs which contain 9 members) and P -glycoprotein (P-gp, MDR1, and ABCB1), "ABC" transporters' role suppresses the accumulation of their substrates intracellularlly by facilitating efflux out of cells and preventing the influx. (Murakami, 2008)

P-glycoprotein is one of counter transport efflux proteins that is widely distributed and expressed in intestinal epithelia particularly on brush-border membrane of the distal intestine, drug-eliminating organs, and capillary

endothelial cells like blood-brain and pumps specific drugs back into the lumen of the gastrointestinal tract after absorption process.

Drugs under the umbrella of class I BCS which are substrates of P-gp can run off P-gp-mediated efflux where these compounds are highly soluble and can be absorbed rapidly prior to reach distal intestine while class II and IV P-gp substrates would be transferred into the distal intestine because of the low solubility in the proximal intestine as a result the distal intestinal absorption of class II and IV which are P-gp substrates is believed to be restricted by P-gp. In case of class II P-gp drug substrates water solubility can be improved and proximalintestine absorption would be increased hence the intestinal oral bioavailability would increase as a result of escaping the P-gpeffect(Varma, 2006)

The anticancer drugs like Vinblastine, Paclitaxel, Docetaxel, Etoposide, and Doxorubicin are substrates for P-gp which can clarify the low bioavailability of these drugs. (Murakami, 2008).

2.1.4.2.2 Drug metabolism

Until recent years drug metabolism processes were associated mainly with the activity of metabolic isoenzymes in the liver. Lately, a new hypothesis was raised by many research groups that for many drugs, poor oral bioavailability could be a reason of the action of intestinal enzymes.

Drug metabolism is known as first pass effect where biochemical transformation of pharmaceutical substances or xenobiotics through specialized enzymatic systems either in the gastrointestinal mucosa or in the liver. (Robertson, 2017). The difference between intestinal and hepatic is that the intestinal metabolism happens directly during absorption process in the Intestinal membrane and before reaching systemic circulation while the hepatic metabolism happens in a different way where the drug is absorbed by the small intestine into the “hepatic portal vein” to the liver where the process of biotransformationof fraction of absorbed dose begins by metabolism enzymes.(Robertson, 2017)

The systemic availability of API is largely diminished after being metabolized which affects the percentage of the API reaching site of action; the rate of metabolism determines the drug's pharmacological action intensity and duration.

Cytochrome P450 enzymes are the enzymes responsible for metabolizing a lot of medicines and endogenous molecules. The “CYP3A” family is the most plentiful subfamily of the CYP isoforms in the liver and intestine which has four isoforms: 3A7, 3A5, 3A4, and 3A43. CYP 3A4 is mainly the significant drug-metabolizing enzyme and extremely expressed in liver and small intestine. (Thummel, 1997)

Extensive intestinal metabolism was reported for many drug molecules which share the property that they are absorbed transcellularily like nisoldipine, tacrolimus and cyclosporine. Remarkably, a great number of Class 2 compounds are substrates for CYP3A. (Custodio, 2008)

2.1.5 Technologies to improve the solubility of PWSD

Increasing solubility of PWSD especially talking about class II BCS which had very good permeability will increase the bioavailability in a direct effect. Many technologies and approaches were used which can be divided as the followings:

2.1.5.1 Crystal modification

2.1.5.1.2 Metastable polymorphs

When the solids are in crystalline state, Polymorphism is important phenomena characterized as structures which have similar chemical composition, but dissimilar lattice structures and/or molecular conformations, polymorphs have unlike physicochemical characteristics, such as stability, density, m.p and solubility.

The majority of API can crystallize into numerous polymorphs. Every polymorph possesses dissimilar energy; in general, metastable polymorphic solubility is kinetically elevated than a thermodynamically more stable polymorph, where the variation of the solubility was accounted to be on average less than 2.0-fold.

This method is regarded as a very efficient method to increase API solubility hence the dissolution rate of a drug but one of the disadvantages is that the

metastable forms in time convert to the thermodynamically stable form. To maintain bioavailability after oral administration, polymorphic's transformation should be controlled throughout both the manufacture and final dosage form storage. (Kawabata, 2011)

2.1.5.1.2 Salt formation

In pharmaceutically industry field, an approach of salt formation is widely used as a tool for developing solubility and dissolution rate of an ionizable drug. Salts fomtaionare developed via proton transfer.

"salt-containing" counter ion modifies pH of dissolving surface of the salt particule in the diffusion layer, resultant in superior dissolving rate of the salts comparable to the corresponding free forms, and the change in pH has a considerable effect on the aqueous solubility of the ionizable drug. (Serajuddin A. 2., 2007)

Celecoxib, which is categorized as poorly "water-soluble" weak acid drug, once combined with Na and using of "precipitation inhibitor" an improvement of dissolution and bioavailability was noticed. (Guzmán, 2007).

2.1.5.1.3 Cocrystal formation

A lot of concentration was paid to co-crystal formation in recent years for increasing the rate of dissolution of PWSD. Cocrystal is generally termed as materials which are crystalline and involve minimum two separate components. "Pharmaceutical cocrystal" is usually involves stoichiometric ratio of an API and a "cocrystal former" which is non-toxic. usually, the API and "cocrystal former" need hydrogen bond for a stable complex. (Schultheiss, 2009)

The cocrystal approach may be an alternative good choice instead of other techniques for advancing rate of dissolution of PWSD, in particular for API candidates who are physiologically not ionised.

2.1.5.2 Particle size reduction

This concept is broadly employed to enlarge and enhance dissolution rate. As the SA of particles increase in a consequence of particle size reduction, rate of dissolution of a substance proportionally increases.

In line with the "Prandtl boundary layer" equation, by minimizing particle size, particularly down to <5 μm, a decline in diffusion layer's thickness will result, which consequently speeding up dissolution (Mosharraf, 1995)

2.1.5.2.1 Micronization

"Dry milling" is normally obtained by using different techniques mainly ball milling and high pressure homogenizatin for acquire drug particles that micronized. In case of solid powders, the minimum particle size that can be attained by is around 2μm.

Milling processes doesn't constantly increase drug's dissolution rate greatly, also it could increase drug particle agglomeration which may decrease "effective SA" for dissolution. In these situations, raising the "effective SA" by using wetting agents will play beneficial.

Micornization by these techniques results in evading conventional micronization shortcomings like poor flowability, agglomeration, insignificant or no dissolution improvement, and small bulk density

2.1.5.2.2 Jet milling

"Fluid jet mill" employs energy of air of high pressure to obtain ultra fine crush of powders. Jet milling has many profits of being a dry operation, micron particle size reduction with narrow PDI, lack of impurities and is ideal for heat-sensitive drugs (Midoux, 1999)

Example of a "class II BCS" is ibuprofen which was processed to coincident micronization by "fluid energy milling", resulting in micronized ibuprofen powders of particle size around 5 μm and enhancement of dissolution rate. (Han, 2011)

2.1.5.2.3 Ball milling

A pharmaceutical "ball mill" is typically crushing device of cylinder shape which is utilized by rotation about an axis to grind pharmaceutical powders. The tool is partially loaded with ground material plus medium normally ceramic balls, or stainless steel-balls (Khadka, 2014)

2.1.5.2.4 High pressure homogenization(HPH)

This method is known as top to down technology which is broadly used technique for developing nanosuspensions for PWSD. Using HPH was stated as a method for advancing dissolution rate and bioavailability of PWSD like budesonide by reducing size to nano-size (Savjani K. T., 2012.)

Also HPH was reported to overcome the weakness of conventional size reducing methods like; polymorph transformation and amorphization which are associated

with high mechanical energy. Accordingly, high pressure homogenization is valuable for milling of drug particles. In HPH, API particles are firstly distributed in an appropriate fluid, then pressurized by a nanosized aperture valve of a HPH, which is basically a bottleneck where the suspension travels at high speed, then instantly practice a sudden (Khadka, 2014).

2.2 Lipid Based Formulations (LBFs)

Recently, LBFs has gained more attention in “pharmaceutical research” area for enhance gastrointestinal absorption of PWSD.

“LBFs” consist of a homogenous mixture of PWSD dissolved in a mix of different excipients that characterize of an extensive diversity of physicochemical features mainly known as oil, surfactants and cosolvents/cosurfactant where different mixtures resulted with different resulted properties in (Pouton, 2000).

Drug formulation in a “LBFs” would be in many final dosage forms including: a simple solution, suspension, emulsion, nanoemulsion, SEDDS or dry emulsion. Effectiveness of a “LBF” is centered on the selection of appropriate excipients and a proper design of the delivery system.

Concerning the predicting of which classes of drugs is fitting and appropriate for “LBFs”; grease ball molecules are considered advantageous for LBFs that could be rationalized to their lipophilic naature and quite their crystal lattice energy which is weak, but it’s not the case for “brick dust” type drugs which they have strong solid-state forces that is the most limiting to absorption (Williams, 2019).

2.2.1 Absorption enhancement mechanisms

Enhanced absorption of lipophilic API released from “LBFs” can be attributed to several different factors:

1. Lipid presence in the GI tract encourages and increase biliary secretions, including phospholipids, cholesterol and bile salts that can form emulsions along with gastric movement subsequently enhance PWSD solubilization, also surfactants inclusion into these delivery systems may contribute to lipophilic drug solubilization.it was evidenced lately that gallbladder secretions can be triggered by small lipid amount. (TSO, 1985)
2. The exogenous lipid part of “LBFs” is subject to enzymatic digestion. Esters are rapidly hydrolyzed in the presence of pancreatic lipase, and after contact

with bile salts and phospholipids, the lipolytic products form various micellar species that prevent precipitation of the incorporated poorly water soluble drug. (Dahan, 2008)

3. Delay and extension of gastric residence time: ingestion of Lipids cause postpone in gastric emptying, in other words gastric transit time is increased. As a result, the residence time of the incorporated poorly water soluble drug in the small intestine increases. Thereby improves absorption.
4. Stimulation of "lymphatic transport pathway": Bioavailability of lipophilic drugs could be enhanced also by the stimulation of the intestinal lymphatic transport pathway

   Intestinal lymphatic transport is the way for highly lipophilic compounds to reach the systemic blood circulation and known as an intra-enterocyte process where intracellular association of the drug with the lipidic core of the chylomicron is developed, chylomicron is a lipoprotein that is synthesized in*situ* inside the enterocyte cells. Following this association, the chylomicron travels with the lipophilic molecule along the lymphatics until it drains into the systemic blood circulation as represented in Figure 2.5. (Porter, 2001)

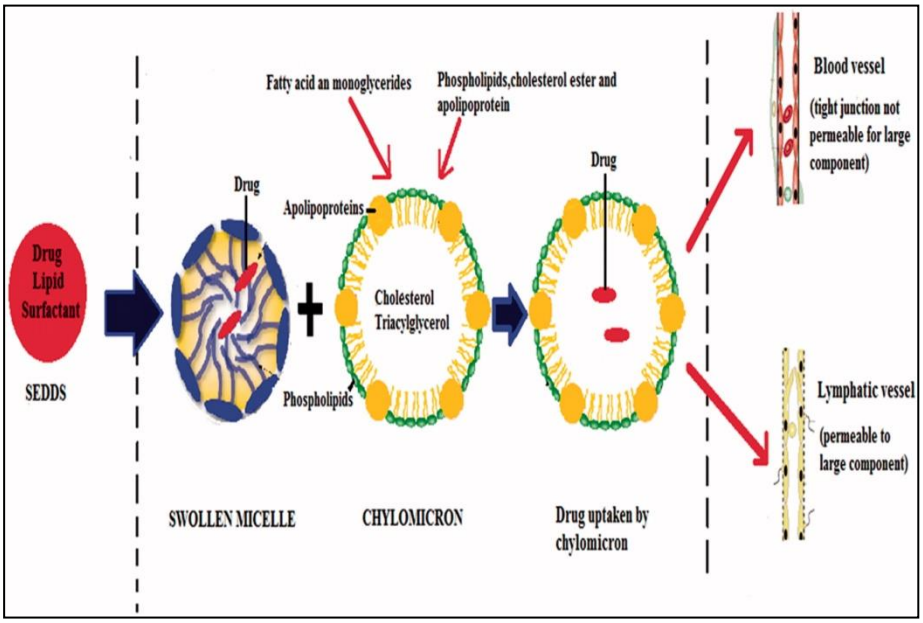

Figure 2.5 Lymphatic transport mechanisms from intestine (Mohammad Mahmoudian, 2020)

Drug that absorbed by intestinal lymphatic transport will avoid the first-pass metabolism indeed increase bioavailability.

5. Intestinal permeability enhancement: Different lipids were evidenced that it has effect in altering the physical barrier structure of gastrointestinal wall. This mechanism is not beneficial for enhancement of oral absorption of "Class II BCS" drugs which are highly permeable but have an effect for "class IV BCS" drugs (Dahan, 2008)
6. Reduce efflux activity and metabolism: specific lipid excipients like CremophorEL and Polysorbate80 were issued of reducing the efflux transporters activity in GI wall so increasing the percentage of drug reached systemic circulation (Nerurkar, 1996) and it could have effect on CYP3A4 enzyme activity as a reason of the interaction between "P-gp" and "CYP3A4" enzyme.

2.2.2 Lipid formulation classification system

Due to the big diversity in the excipients used, their physiochemical properties and the need for predicting the most proper formulation type for specific API in accordance with their physiochemical features, Pouton introduced LFCS in 2000 basing on:

1. type of lipid excipients
2. quantity of lipid excipients
3. morphology of lipid aggregates formulated while dilution in aqueous medium during dilution (Pouton, 2000)

And further updated in 2006 by dividing "type III LFCS" into "IIIA LFCS" and "IIIB LFCS", basis on ratio of lipophilic and hydrophilic constituents and type of dispersion formulated once diluted.(Pouton, 2006).

"LFCS "consists of four classes, as illustrated in table 2.3

Type I characterized as non dispensing and contain only oil as the excipient which is classified as Generally Regarded as Safe (GRAS) according to FDA. low capacity for API loading is the main drawback of type I. API that is fitting for merging into "Type I LFCS" and forming stable formulation should be highly lipophilic, and has more than 4 value for logP, and API should have an adequate solubility in specified lipid components.

Table 2.3 Characteristics of lipid formulation classification system

	Type I	Type II	Type IIIA	Type IIIB	Type IV
Typical composition (%)Triglyceride	100	40–80	40–80	20	0
Surfactants	-	20–60 (HLB<11)	20–40 (HLB>11)	20–50 (HLB>11)	30-100
Hydrophilic cosolvents	0	0	0–40	20–50	0-50
Particle size of dispersion (nm)	Coarse	100–250	100–250	50–100	<50
Significance of aqueous	Limited importance	solvent capacity unaffected	Some loss of Solvent capacity	Significant phase changes and potential loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	not likely to occur	Not important

"Type II LFCS" self-dispersing carriers which is known as "SEDDS" are comprise of mixture of active substance, lipids, and surfactants which under mild agitation and also upon dilution in GIT will form fine O/W emulsion spontaneously. Presence of a surfactant which have HLB of less than 11 that known to be "lipophilic", dramatically increases solubility rate of PWSD which they have logP value of 2-4. "SEDDS" of this type are opalescent, and have droplet size in range of 100-250 nm.

"Type III LFCS" self-dispersing carriers which is known as SMEDDS or SNEDDS are characterized by inclusion of hydrophilic surfactants which have HLB>12 and cosolvents in addition of oil.

Adding hydrophilic excipients other than oil into "LBF" have a positive effect on API dissolution and formulation dispersibility upon aqueous medium dilution either in GIT or in vitro. Throughout dilution, API dissolves in oil component where as the surfactant forms a film between active substances dissolved in oil component.

resulting in forming a film between oil and water phase where this film has large SA, the rate of dissolution of PWSD is increased, in that way supporting the active substance and oily component to stay dissolved, the disadvantage of this system is the loss of solvent capacity due to that hydrophilic components have the tendency to disunite from the oil component throughout dispersion, and dissolved in the aqueous phase as a result precipitation could happen.

Alot of 'LBFs" marketed products belongs to "Type III LFCS", this class is widely used as an effect of broad diversity in the fractions of hydrophobic and hydrophilic excipients.

"Type IV LFCS" is made of mixture of active substance dissolved or solubilized in surfactants and cosolvents or just surfactants. The interesting in this type doesn't contain any lipid component and it's the most hydrophilic type of LFCS. Micellar solution is formed during dissolution with water that permits rapid release of API and sequentially a higher absorption. The disadvantages including probability of API precipitation while travelling in GIT, poor GIT tolerance, and possible irritation of GIT mucosa by reason of high conc. of surfactants.

2.2.3 Formulations approach of lipid based drug delivery system(LBDDS)

Various "LBDDS" were developed and investigated; "LBFs" possibly will be developed in various ways to accomplish the objectives of the favoured formulation. The development process should start from the selection of the most suitable lipid excipients taking into account their stability, fatty acid content, compatibility, HLB value and digestibility (Kalepu, 2013).

Lipid based formulations can be classified to different types:

2.2.3.1 Lipid solutions

The best way to increase the bioavailability is to dissolve a poorly soluble product in lipids. Due to the broad variability in physicochemical properties and the digestibility of lipids which may affect the solubilization of the product, careful selection of the correct lipid excipient is essential for this formulation (Mu, 2013).

Additionally, oral administration of both seocalcitol lipid solutions in rats outcome in increase in the drug's bioavailability of 2 folds, in comparasopn to just 10 percent bioavailability obtained from a reference propylene glycol solution. This enhancement in solubility was for the reason that ability of lipid formulations to hold

the drug solubilized in the GIT until full absorption. No major variations between the medium and long chain triglycerides were observed. (Grove, 2005)

2.2.3.2 Lipid suspensions

Lipid-based suspensions can be helpful when lipid solubility is very low. A research showed that the oral bioavailability of danazol from various lipid-based suspensions did not differ from that achieved when a lipid solution was given orally in rats.(Larsen, 2008). on the other hand, suspension physical stability and the critical need for sedimentation control can limit the formulation of lipid suspensions

2.2.3.3 Liposomes

"LPs" are vesicles which are enclosed and made up of phospholipid as two bilayers which surround a central aqueous cavity. liposomes have the capability to incorporate both lipophilic and hydrophilic drugs due to their biphasic property (Krishnaiah, 2010).

Such liposomal formulations based on lipids were evidenced as a method for oral absorption enhancement of various types of API such as insulin (Hu, 2013).

Improved oral absorption by liposomal formulations was due to the potential increase in drug solubility, protection against digestive degradation and enhanced intestinal permeation; liposomal phospholipids can also combine with bile salts in the gastrointestinal tract to produce mixed micelles which increase oral PWSD absorption (Hu, 2013)

Various liposomal modifications were investigated to evaluate stability of liposomes such as interaction of bile salts with phospholipid and polymer coating of the vesicles, Accurate prediction of the stability of liposomes in human GIT from in vitro stability assays depends, in addition to the type of animal model selected for the assays, on the degree of simulation of the physiological conditions(Parmentier, 2012)

2.2.3.4 Solid lipid nanoparticles (SLNs)

"SLNs" are nano-sized colloidal carriers consisted of melted solid lipids like triglycerides highly purified, monoglycerides, and complex glyceride mixtures in suitable ratios and they are highly stable matrix systems that utilize non-toxic solid lipids for drug delivery. "SLNs" can be produced by different technologies such as HPH and microemulsion techniques, HPH methods may produce particle size of an average of 500 nm. (Mehnert, 2012)

Enhanced oral bioavailability of several drugs such as cyclosporine has been reported when these drugs were loaded into solid lipid nanoparticles. Improved oral absorption from SLN was attributed to API solubilization into micelles that form upon degradation for lipids in the gut wall (Müller, 2008)

While solid lipid nanoparticles are safe and tolerable, they have a relatively low capacity for drug loading and possible displacement of the embedded drug from the shaped SLN during storage may occur. Expulsion of the drug from the SLN may be due to the potential crystallization of the lipids during storage and subsequent production of a more tightly populated crystalline structure with a low drug content affinity(Müller, 2008)

2.2.3.5 Lipid nanocapsules (LNCs)

"LNCs" present a new oral nano-technology. "LNCs" are an additional type of LBFs, consisting of core that consist of either semi-liquid or liquid oil and also a external core shell that made of solid lipid layer , "LNCs" represent a promising biocompatible drug delivery system, which provides ananometer range with narrow size distribution and provides unique properties such as high bioavailability and controlled release profiles. (Battaglia L, 2012)

Lately, many lipophilic drugs were introduced as "LNCs" dosage form for example, "LNCs" loaded with ibuprofen as pain killer and various hydrophobic anticancer agents (Thakkar, 2015). As a result, "LNCs" is an excellent method for oral delivery fir highly lipophillic API.

2.2.3.6 Self-emulsifying drug delivery systems (SEDDSs)

Defined as a type of "LBFs", the next sections will discuss SEDDS and SNEDDS in details

2.2.4 Lipid based formulations in the market

LBFs have proven enhancing the bioavailability for a variety of PWSD. Even though, conforming to a study carried out in Japan, UK and USA, commercial "LBFs" drug products account for just 4 per cent of pharmaceutical market. (Hauss, 2007) According to Savla R. et. al, the number of oral lipid based formulations approved by the FDA is 36 formulations for different drugs molecules (Savla, 2017)
The reason of few drug products which are "LBFs" in market, regardless of the potentially great benefits as oral systems, owing to a number of aspects:

1. Manufacturing processes for LBFs can utilize costly and complex instruments
2. Not all active ingredients are suitable for LBF formulation because of stability issues or low solubility in the formulation excipients (Gupta, 2013)
3. "LBFs" can cause API precipitation when the formulation diluted in GI fluids, thereby failing in the purpose of retaining the API solubilized in solution (Mohsin, 2009)
4. No full understanding of the ability to expect "LBFs" function upon entering in GI tract. (Gupta, 2013)
5. Effect of lipids on the total absorption of drugs is still not full predictable attributable to many simultaneous complex processes effects by lipids orally.

Commercial products in pharmaceutical market are represented in table 2.4, and first lipid based formulation on the market will be discussed in section 5.1

Table 2.4 The commercial products present in pharmaceutical market which were manufactured as SEDDS formulation (Rajesh, 2010)

Drug Name	Compound	Dosage form	Company	Indication
Neoral®	Cyclosporine A/I	Soft gelatin capsule	Novartis	Immune suppressant
Novir®	Ritonavir	Soft gelatin capsule	Abbot Laboratories	HIV antiviral
Fortovase ®	Saquinavir	Soft gelatin capsule	Hoffmann-La Roche inc.	HIV antiviral
Agenerase®	Amprenavir	Soft gelatin capsule	Glaxo Smithkline	HIV antiviral
Convulex®	Valporic acid	Soft gelatin capsule	Pharnacia	Antiepileptic
Lipirex®	Fenofibrate	Hard gelatin capsule	Genus	Antihyperlipoproteinemic
Sandimmune ®	Cyclosporine A/II	Soft gelatin capsule	Novartis	Immune suppressant
Targetin®	Bexarotene	Soft gelatin capsule	Ligand	Antineoplastic
Rocaltrol®	Calcitirol	Soft gelatin capsule	Roche	Calcium Regulator
Gengraf®	Cyclosporine A/III	Hard gelatin capsule	Abbot Laboratories	Immune suppressant

2.2.5 Selection of Suitable LBFs

Goal of utilizing any drug into "LBFs" is to increase the absorption of API from GI mucosa which directly improves the bioavailability but of course there are criteria on choosing the suitable type of lipid formulation for specific drug to achieve this goal:

1. Physicochemical considerations

   The choice of the most appropriate lipid-based formulation for specific drugs is primarily determined by their physicochemical properties. The followings are the conditions which were set by Pouton and Porter of how to choose the right formulation according to physiochemical properties and keeping in mind the stability as physical and chemical concepts of API in the formulation components must be essentially considered before selection of a suitable excipients:

   - PWSD that characterized by poor solubility in glycerides, bile salts mixed micelles and lecithin are not suitable of being formulated in "Type I LFCs", "Type II LFCs", or "Type IIIA LFCs",
   - Drugs with log *P* value approximately 2; limited solubility in both lipid and water are not likely to have improved absorption by formulating into lipid formulations
   - If the value of log *P* is higher than 5, the bioavailability may be greater from lipid formulations due to incorporation into mixed micelles and enhancement of their dissolution in the gut lumen for more efficient absorption

2. Dose of the drug

   The optimum formulation should be able to solubilize unit dose of the preparation and maintain in a solubilized state until GI absorption and also keeping the solvent capacity. Solvent capacity could happen by different ways:

   - Inclusion of surfactants and co-solvents into lipid formulations should be assessed carefully to prevent drug precipitation on dilution. The loss of the solvent potential of lipid formulations after dilution has been reported to be more prevalent if a co-solvent is incorporated rather than those containing non-ionic surfactant

- Solvent capacity for Type III and Type IV formulations could be vanished upon dilution as a reason of having surfactant which are water-soluble and partitioning into continuous part. for that reason, for predicting the precipitation that could happen in the intestinal lumen these types of formulations must be tested first for in vitro dispersion (O'Dwyer, 2019)

3. susceptibility of lipid formulations to degradation in intestine

   Lipid components undergo digestion in GIT by lipolysis and consequently, their digestion products may interact with biliary secretion like bile salts present in the intestinal lumen to form micellar structures to solubilize drugs. This activity was reported to result in loss of solvent ability and subsequent reduction of the API solubility in GIT, which would result in precipitation of the active substance and reduction of the absorption rate. For inhibition and minimizing this, incorporating of surfactants in Types "Type II LFCs", "Type III LFCs", or "Type IV LFCs",may stabilize oil component and inhibit the digestion of the oil within these formulations(Pouton, 2006)

2.2.6 Self-Emulsifying Drug Delivery Systems(SEDDS)

The following sections of this thesis will shine the spotlight on "SEDDS" as part of the proposed thesis.

2.2.6.1 Definition and general properties

"SEDDS" are emulsion or emulsion pre-concentrates in anhydrous forms, compose of mixtures of oils with hydrophilic or lipophilic surfactant, co-solvents, and the solubilized lipophilic drug substance (Kohli, 2010). SEDDS classified under the "Type II LFCs" and "Type III LFCs", and can form fine O/W emulsion very fast; microemulsions or nanoemulsions once dispersed in the gastrointestinal fluids under mild disturbance mvemnts in digestive motility in GI system either produced by the stomach or the intestines.

Advantages of SEDDS over other LBDDS: (Khedekar, 2013)

1. SEDDS have the potential to rapidly self-emulsify when a fine O/W emulsion is produced once contacted with GIF, by the effect of gentle churning induced by peristaltic and other gastrointestinal movements.

2. Drugs formulated by using SEDDS approach selectively target site by heading towards specific GIT absorption sites.
3. SEDDS result in increased solubility by making lipophilic drugs more soluble in aqueous media.
4. Drugs formulated in SEDDS are protected from the intestinal hostile environment. Sensitive drugs may be formulated into SEDDS formulation for protection reasons
5. Food has the effect of increasing variation on drug action. SEDDS has the ability to reduce the variability of the drug action
6. Hydrophobic or Hydrophilic drugs can be effectively integrated into an oil-surfactant mixture.
7. SEDDS yield interfacial SA for partitioning API between water and oil which is big compared with oily formulations.
8. SEDDS can be formulated in liquid dosage forms and also in solid dosage forms.
9. Drugs developed as SEDDS are administered in lower dosages relative to "conventional dosage forms"
10. The oil droplets of SEDDS are very fine and quickly facilitate the wide distribution of API all over the stomach and support the broad distribution of the drug incorporated into SEDDS formulation throughout the GI tract, reducing the irritation often experienced during the prolonged contact between API and intestine wall.
11. For manufacturing either in small or large scale, SEDDS have many advantages that make it special when compared with other DDS such as liposomes, nanoparticles, and others, like easiness of manufacture because they need easy manufacturing equiments such as simple agitator mixer.

General limitations and disadvantages of SEDDS: (Shukla, 2010) (Kumar, 2012)

1. One of important barriers for formulation of “LBFs” and particularly SEDDS is the lack in vitro models for adequate predicative for formulation’s estimation.
2. Dissolution studies in traditional methods will not work beneficially, since “SEDDS” can rely on digestion before releasing the drug.
3. Supplementary research based on IVIVC is needed and therefore in an appropriate animal model so diverse prototype “LBFs” should be building up and checked for in vivo.
4. One of the limitations of SEDDS includes chemical instability and degradation of drugs as a result of high amounts of surfactants and cosurfactants.
5. High concentrations of surfactants in formulations (30-60 %) that could irritate the GIT. Using the minimum amount of surfactants while formulating stable “SNEDDS” would solve the problem.
6. For traditional self-emulsifying formulations, volatile co-solvents are reported to migrate to capsule shell, so precipitation of lipophilic drug out from formulation would happen.
7. Because of using hydrophilic solvent and dilution effect in the fluids, the precipitation tendency of the drug may be higher.
8. SEDDSs formulations which contain several components are becoming more challenging to validate.
9. For controlled release dosage forms, SEDDSs formulations are not satisfactory.
10. Expensive production cost

2.2.6.2 Self emulsification mechanism

Reiss has stated that “self-emulsification” occurs when entropy toward dispersion is superior on energy required to raise the dispersion SA. (Reiss, 1975)

Free energy”G” needed for formation of normal emulsion is directly related to interfacial energy needed to build a new interface among oil and water phases. Free energy “G” represented by following mathematical equation:

$$\Delta G = \sum \mathrm{N}\pi \mathrm{r}2\sigma$$

Where

G: free energy related to “emulsification process”

N : number of droplets with radius and ‘σ’ as the interfacial energy.

r: is radius of resulted droplets

σ: interfacial energy of resulted droplets

It is noticeable that spontaneous creation of interface between oil/water phases is not privileged regarding energy value and normally an emulsion is an unstable system since the two phases be inclined for splitting to minimize the system’s high free energy at the interface, normally the conventional emulsifying agents provide stability for the emulsions droplets by settling a monolayer on the surface so interfacial energy is reduced and a barrier would be developed for preventing coalescence.

Interestingly, “G” needed for emulsion formation resulted from self-emulsifying formulations is small and emulsification process occurs spontaneously.

It was suggested that the ease of emulsification is linked to how water penetrate easily into the different phases of LC or gel shaped on droplet surface (Rang, 1999)

Upon water addition to binary mixture (oil/non-ionic surfactant), the interface between two phases will form then as the first step water penetration throughout the interface then solubilization of water within the oil phase to reach the limit of solubilization. Then water dispersion could result in construction of dispersed liquid crystal phase. In the end, LC will be formed. As a consequence, as gentle agitation of “SEDDS” water quickly penetrate the core and cause interruption and formation of droplet. As a result of creation of LC interface around droplets, SEDDS would be very stable and no very less chances for coalescence (Wakerly, 1987) (Gursoy, 2004).

2.2.7 SEDDS, SMEDDS and SNEDDS terms

Upon taking a look to the literature data base, uncountable SEDDS, SMEDDS and SNEDDS formulation were formulated, some researches were clearly defined as either “SMEDDS” or “SNEDDS”, though some studies used both “SEDDS” and “SMEDDS” as a wide-ranging term.

SNEDDS and SMEDDS are modified types of SEDDS formulation that they are self emulsified to dispersion of less than 100 nm in case of SNEDDS and 100 to 250 nm in case of SMEDDS (Chatterjee, 2016).

The following points illustrate how the researches differentiate between SEDDS, SMEDDS and SNEDDS:

- The composition of “SMEDDS” and “SNEDDS” include co-solvent(s) that is not present in SEDDS.
- Droplet size of emulsion resulted from dilution of SMEDDS is lower than that resulting from dilution of SEDDS which is the reason for transparent or translucent emulsion (Oh, 2011)
- SMEDDS and SNEDDS classified as type “IIIA LFCS” or “IIIB LFCS”, that contain extra amount of hydrophilic excipients while “SEDDS” could classify under type II or IIIA so it maybe it will not have any hydrophilic components. (Čerpnjak, 2013)
- Dispersion systems developed from SEDDS and SMEDDS formulations are thermodynamically stable while dispersion resulted from SNEDDS is kinetically stable. (Čerpnjak, 2013)
- Kohliet. al. differentiated SNEDDS and SMEDDS formulation c according to the droplet size of the resulted emulsion upon dispersion in aqueous media, SMEDDS form droplet size of minimum 100 nm but oil droplet resulted from “SNEDDS” is maximum of 100 nm. (Kohli, 2010)
- Dispersion resulted from SNEDDS is optically clear appearance while from SMEDDS is optically clear to translucent appearance.

2.2.8 General Components of SNEDDS

SNEDDS comprises three major components other than API; oil or lipids, hydrophilic surfactants and co-surfactants are represented in Figure 2.6.

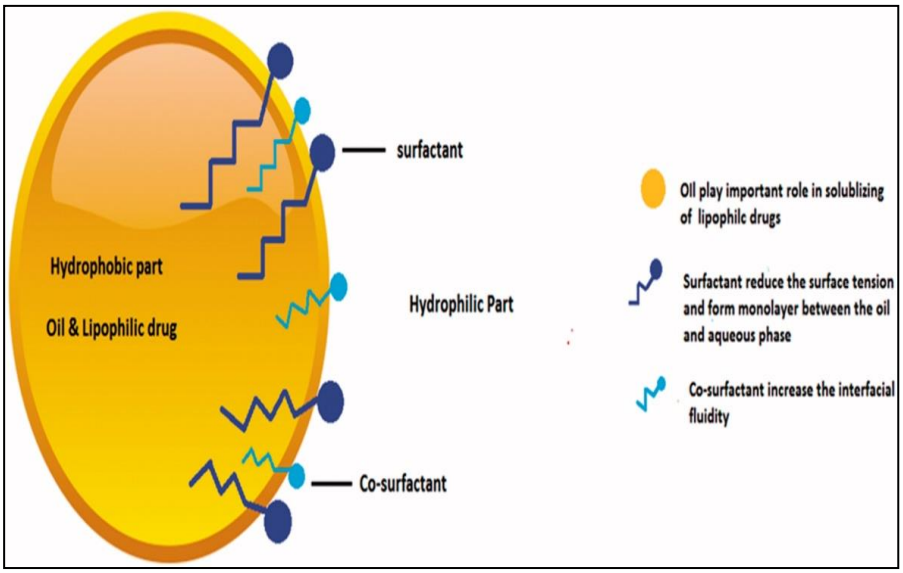

Figure 2.6 Components of SNEDDS (Mohammad Mahmoudian, 2020)

2.2.8.1 Lipid/Oil

Choosing the oil process is always balanced between the oil's strength to solubilize API and its capacity to promote nanoemulsion along desirable properties.

2.2.8.1.1 Triglycerides

Vegetable oils Triglycerides of are glyceride esters of mixed LCT. Triglyceride vegetable oils have many advantages where they are considered as the most natural lipid vehicles and recognized as “GRAS” status by FDA and they are normally found in food, and being fully digested and therefore absorbed easily, which means no concern about safety concerns (Gibson, 2007) Although their use in SEDDDS formulations is less so because of insufficient capability of LCT to dissolve big quantities of API. Examples of different oils originated from vegetable sources are represented in table 2.5 with their fatty acids composition.

Favorable outcome of “SNEDDS” comes from using MCT that have fluctuating saturation degrees and hydrolysis Good examples is the oil resulted from distillation of Coconut oil for generating MCT known as glyceryl tricaprylate/caprate commonly consist of glyceryl esters with mostly saturated C8 and C10 fatty acids.

MCTs show a good solubilizing capacity even higher than LCT for less lipophilic drugs with good self-dispersing ability. Also MCT Are not susceptible to oxidation (Stuchlík, 2001)

2.2.8.1.2 Semi-synthetic and synthetic lipid

Chemical interaction of either medium chain triglycerides or the glycerides derivative from natural oils, which posses hydrophilic entities has developed large variety of liquids or semi-solids (thermo softening) excipients that found broad use in lipid formulations due to its solubilizing, surfactant, and wetting properties.

Excipients of mixed glyceride may be produced by partial hydrolysis of triglycerides resulted of variety content of mono- , di- and triglycerides. Mixed glycerides resulted in different properties including the chemical composition, melting behavior, physical appearance, and HLB values, which are affected by the original source of triglycerides and the degree of hydrolysis generated during the production process are affected (Gibson, 2007)

Common excipients of this type include:Capmul® MCM (glyceryl monocaprylocaprate), Geleol®, Imwitor® (191glyceryl monostearate), Peceol™ (glyceryl monooleate) and Maisine™ 35-1(glyceryl monolinoleate) (Kalepu, 2013).

Another example of oil pharmaceutical excipients which is used efficiently in lipid based formulations are , polyoxylglycerides or macrogolglycerides, which are produced by polyglycolysis of vegetable oils with polyoxyethyleneglycols (PEG) of molecular weight as a limit, PEG aid vegetable oils for dispersion in water.

Depending on their composition of different glycerides and different esters, the physical appearance of these excipients may range from liquids of high viscosity to solids structure at r.t.

Examples of this type of excipients that have unsaturated “LCT” which include Labrafil®M1944CS (oleylpolyoxylglycerides) labrafil®M2125CS (linoleylpolyoxylglycerides), saturated medium chain fatty acid esters such as Gelucire®44/14 (lauroylpolyoxylglycerides) or saturated long chain fatty acid esters such as Gelucire®50/13 (steroylpolyoxylglycerides) ((Kalepu, 2013)

Mixed monoglycerides and diglycerides that have long-chain fatty acids are appropriate for formulating liquids when technical difficulties occur with waxy content. Mixed long-chain glycerides are more beneficial for solubilizing drugs

compared to triglycerides, but highly lipophilic drugs are an exception to this rule, especially in “Type II LFCS” and “Type III LFCS” (Pouton,2000)

Mixed glycerides of medium-chain fatty acids have gained considerable attention compared to conventional MCTs due to their higher solvent capacity, lower oxidation susceptibility and additional surfactant properties which improve their emulsification power However, digestion of medium chain glycerides should be tested for lipid-based formulations prior to their selection (Porter C. J., 2008)

Oily excipients which considered more polar such as sorbitan fatty acid esters (Spans) are similar to mixed glycerides in their physical features and their ability to improve solvent capacity and formulation dispersion.. Examples of polar oils include the more lipophilic Span 85 (sorbitantrioleate), Span 80 (sorbitanmonooleate) and free fatty acids such as oleic acid (Strickley, 2007, Gibson, 2007).

Using the oil phase as a mixture may was used to assemble the optimal characteristics of oily phase. Example when Kassem et al. prepared SNEDDS loaded formulation wit clotrimazole (CT) and used a mixture of ratio of 7.5:2.5 and 6.7:3.3 of oleic acid and coconut oil respectively. (Kassem, 2010)

Also Basalious et al. prepared SNEDDSs formulation of lacidipinee for improving dissolution and oral absorption by using oil mixture of ratio of 2:1 w/w Labrafil/Capmul (Basalious, 2010)

Oils which are semi-synthetically could form systems of good emulsification properties once used with solubility enhancers known as surfactant that are acclaimed for oral use.

Table 2.5 Oils originated as vegetable sources and their fatty acid composition (Rahman, 2013).

Oil	C8	C10	C12	C14	C16	C18	C18:1	C18:1-OH	C18:2	C18:3
Apricot Kernel	-	-	-	-	-	1.0	64.2	-	28.3	0.2
Canola	-	-	-	-	4.7	2.0	60.0	-	21.7	9.1
Castor	-	-	-	-	2.0	1.0	7.0	87	3.0	-
Coconut	2.4	2.7	4.4.4	16.6	9.6	2.8	17.8	-	3.1	0
Corn	-	-	-	-	10.7	1.6	24.5	-	10.7	1.1
Olive	-	-	-	-	12.9	71.2	71.2	-	10.0	0.9
Palm	-	-	-	1.0	45.0	40.0	40.0	-	2.0	-
Palm Kernel	-	4.0	48.0	16.0	8.0	15.0	15.0	-	36.0	-
Peanut	-	-	-	-	12.0	48.0	48.0	-	36.0	0
Safflower	-	-	-	-	5.5	11.1	11.1	-	81.4	0.4
Sesame	-	-	-	-	9.0	41.0	41.0	-	45.0	-
Soybean	-	-	-	-	10.4	21.5	21.5	-	51.5	7.8
Sunflower	-	-	-	-	6.2	20.7	20.7	-	67.9	0.2

2.2.8.2 Surfactants

Surfactants are essential component in SEDDS and critical for the self-emulsifying properties, choosing of surfactant with the appropriate properties is also critical for the formulation of SEDDS, They have important outcome on emulsification process and the droplet size obtained of diluted nanoemulsion. (Date, 2010)

Due to their nature surfactants characterized to be amphiphilic, surfactants can enclose elevated proportions of hydrophobic drug in formulation also; they facilitate the dispersion process through diminishing the interfacial tension between water/oil phases with concomitant creation of a flexible film around droplets of oil and hydrophobic drug (Nielsen, 2008).

The mainly suggested surfactants are non-ionic surfactants which have elevated HLB of more than12 in case of SNEDDS optimization for promoting rapid dispersion in the aqueous GI fluids and instant configuration of fine o/w droplets also other properties should be considered while choosing the surfactant like oily phase affinity, viscosity and cloud point and in case of oral administration the GRAS status should be taken into consideration. (Pandey, 2018)

Surfactants as medium chain monoglycerides (MCM) and lecithin which are of natural origin are considered safer than synthetic surfactants, but they showed limited capacity for self-emulsification (Constantinides, 1995).

Non-ionic surfactants are less toxic than ionic surfactants and is the preferred for type for self emulsifiedsystems (Pouton, 2000)

Water-soluble surfactants of HLB more than 12 are used for optimization class IIIA and IIIB LFCS. According to the method of production, the lipophilic part of water-soluble surfactants may contain fatty acids either in saturated or unsaturated form such as the polyalcohol esters fatty acids which are generated by esterification of vegetable oils, based on the process of synthesis the HLB can be ranged from medium to high (Rajebahadur, 2006). Examples of this group include Plurol™Oleique CC497, Tween®80, Lauroglycol™90, Mirj®52, Mirj®45, Solutol®HS15 and Capryol™90,.

Most of the orally accepted surfactants interact with efflux transporters like Pgp transporter and inhibitory effects were often experienced with such surfactants, table 2.6 shows surfactants which were proved for their Pgp and MRPs inhibitions (Kuentz, 2011)

Table 2.6 Inhibition of efflux transporters effect by surfactants

Excipient	Transporter	Reference
Polyoxyl 40 hydrogenated castor oil (Cremophor RH40)	Pgp	(Tayrouz, 2003)
Polyoxyl 35 castor oil (Cremophor EL)	Pgp	(Hugger, 2002)
Caprylocapryolmacrogol glycerides (Labrasol)	Pgp	(Cornaire, 2004)
Lauryl macrogol glycerides (Gelucire 44/14)	Pgp	(Sachs-Barrable, 2007)
Mixture of diacylglycerols (Peceol)	Pgp	(Sachs-Barrable, 2007)
Polyoxyethylenesorbitan monooleate (Polysorbate 80)	Pgp, MRPs	(Hugger, 2002)
Polyoxyethylenesorbitan monooleate (Polysorbate 20)	Pgp, MRPs	(Yamagata, 2007)

2.2.8.3 Co-surfactants

A significant decrease of the interfacial tension for getting the goal of ensuring a spontaneous dispersion which is thermodynamically stable can be achieved with a high surfactant concentration as 30%-50%. However, surfactants usually results in irritation in GI mucosa along with toxicity at such elevated concentrations. This difficulty could be solved by addition of another excipient called co-surfactant that can help sustain an optimal degree of surfactant quantity while not causing safety issues and at the same time decrease the interfacial tension to optimum values. Many co-surfactants which are alcohols of medium chain length, as PEG 400, carbitol, isopropyl alcohol and n-butanol, give a hand in further reducing interfacial tesnsion and improve interface fluidity, thereby enhance system entropy (Djekic, 2008)On the other side, volatile cosolvents like alcohols have limitation of fade away to soft gelatin or hard capsule shells in traditional L-SNEDDS associated with further drug precipitation (Rahman, 2013)

2.2.8.4 Excipients selection

The method of self-emulsification was proven to be specific to nature of excipients used and their concentration also ration of oil phase to surfactant phase and also temperature when emulsification process occurred. Accordingly, it was observed that few particular blends of excipients would generate effective self-emulsification systems (Charman, 1992). Once an index of appropriate components has been recognized, a binary drug-excipient test for compatibility, stability and solubility must be followed to spot the most fitting excipients for specific API in progress.

The components are selected for the following objectives: (Rahman, 2013)

- Achieving maximal drug loading
- Achieving minimal time for emulsification which has the least droplet size in gastric lumen
- Reducing dissimilarity in size of the resulted droplets once changing pH of media used
- Prohibit or diminish API degradation in the physiological environment.

2.2.9 Approaches for oral delivery of SEDDS

Development of "SEDDS" in an appropriate final oral dosage form must be considered. In general, SEDDSs formulations final dosage form is the liquid form. But, some drawbacks associated with these liquid formulations have resulted in the development of "S-SEDDSs" as an substitute method for "SEDDS" formulations.

2.2.9.1 Capsule filling of L-SEDDS

Capsule filling considered to be the easiest and simplest method for encapsulating liquid SEDDSs formulations for oral drug delivery. It involves two steps, first filling the SEDDS formulation into the capsule body which will then be sealed with the capsule cap either by using liquid encapsulation micro-spray sealing (LEMS) or banding with a gelatin band technology.

Many reasons, consists of restricted stability and transportability of L-SEDDS , the solubility of different ingredients including the drug in the final product may be affected by the storage temperature which was reported that API and excipients could precipitate at low storage temperatures, low drug loading, tendency for API

crystallisation and, poor IVIVC, the need for large dosage volumes for administration, costly manufacturing, difficult handling and transportation, in addition to limited choices of dosage forms were attributed to the incapability for the commercial success of SEDDS to completely give full idea about their therapeutic potential (Tang, 2008).

2.2.9.2 Solid self-emulsifying drug delivery systems (S-SEDDSs)

As a substitute solution to "L-SEDDS" formulations, "S-SEDDS" were used. These systems are developed by solidifying liquid SEDDSs into powders that could be formulated as a diverse solid dosage forms types for oral route of administration such as tablets, beads, pellets and of course capsules. Conversion of "L-SEDDS" to "S-SEDDS" should not change release characteristics of API or the self-emulsification process that will take place in the GI tract. Therefore, S-SEDDSs possess the rewards of both "L-SEDDS" formulations and solid dosage forms (Feeney, 2016).

"S-SEDDS" systems are distinguished by high formulation stability and reproducibility, better drug solubility and bioavailability, ease of handling and transport, as well as better patient compliance (Gupta, 2013)

a huge amount of benefits were achieved by utilizing S-SEDDS compared to the normal "L-SEDDS" (Joyce, 2019)

- Extending absorption time by increasing gastric residence which means extending the total transit time, this could be achieved by many ways like solidifying with polymers that reveal favorable interaction with epithelial cells in stomach such as hydroxypropyl methylcellulose (HPMC) and microcrystalline cellulose (MCC) and including excipients that are floating which enable it to stay in floating state within the gastric media
- Enhance solubility of API in intestine: S-SEDDS can afford increasing of intestinal solubility by different mechanisms; modulating lipolysis of digestible lipids and stabilizing supersaturated drug states. While the function of digestive enzymes is also altered by changing in chemistry's surface of solid carrier, extent and rate of release of lipid products is regulated for controlling accordingly inhibitory effect of precipitation and lipolysis products solubilizing process increase the intestinal solubility of API.

- Enhanced API permeability: using intestinal permeation enhancers in SNEDDS as mucoadhesive polymers and chitosan for encouraging API permeability crosswise intestinal epithelium which is promising way for using SEDDS for class IV.

2.2.10 Techniques for SEDDS Solidification

2.2.10.1 Adsorption to solid carriers

Simple adsorption engages adding "L-SEDDS: to a solid carrier by combining in a mixer to obtain a free-flowing particles as powder that can be directly packed in capsules or pressed as tablets after mixing with suitable excipients.

In this technique, good content uniformity can be obtained and up to 70% of lipid formulation may be adsorbed onto a selected carrier (high lipid exposure). However, as a result of subsequent dilutions of LBFs throughout mixing with solid carriers and then during mixing with other excipients required for compression into tablets a decreased of drug loading capacity may be encountered during adsorption process (Jannin, 2008)

Solid carriers that are capable to adsorb large quantities of L-SEDDS hould be selected to ensure increased drug loading capacity as well as lipid exposure.

Solid carriers types can be divided into many types as the followings :

1. Inorganic microporous substances
2. Inorganic colloidal adsorbent substances of high SA
3. Nanoparticle adsorbents or polymers which are cross-linked.

Polymers which are cross-linked produce favorable environment for sustaining drug dissolution consist of porous silicon dioxide and carbon nanotubes. (Gupta, 2013)

For example silicates, silica, different grades of Neusilin®, magnesium trisilicate, different grades of Florite® TM RE, crospovidone, talcum, and microcrystalline cellulose (MCC) as Avicel® were used successfully as adsorbents to transform liquid SEDDS into solid SEDDS.

Examples of SEDDSs formulation used Neusilin®US2 as a solid carrier when Beg et al. formulated solid SNEDDS of olmesartanmedoxomil from an

optimized liquid SNEDDS and this produce an improvement in release rate of drugs compared to other adsorbents tested, such as Aerosil ® 200. (Beg, 2016)

Different grades of Florite® (calcium silicate) were used to formulate solid SEDDS of gentamicin where a positive impact on dissolution and consequently on bioavailability of API was achieved (Ito, 2005).

2.2.10.2 Spray drying

The L-SEDDS is inserted into a solution which contain solid carrier and keep stirring unti emulsion of oil in water is formed. After that in a hot dry chamber the prepared mixture atomized into a spray of droplets, and the volatile parts evaporates and dry particles formed under controlled airflow conditions and temperature. Such solid powder would be more filled into capsules or pressed into tablets.

Parameters like drying chamber design, atomizer, most fitting airflow prototype and temperature are chosen according to specifications of powder and drying nature of final product.

Balakrishnan et al. utilized spray drying to formulating loaded "S-SEDDS" of dexibuprofen using Aerosil® 200 as a solid carrier, two fold higher bioavailability of dexibuprofen following oral administration to rats when compared to dexibuprofen powder. These findings suggested that even after solidification, the self-emulsification properties of the liquid SEDDS were conserved. (Balakrishnan, 2009)

2.2.10.3 Lyophilization

Freeze drying or lyophilization or involves transmit of mass and heat from the product under preparation. Lyophilization was known as mixing technique in a molecular stage; both API and carrier are codissolved in solvent. The possible utilization of lyophilization in SEDDS manufacturing have successfully been studied, Jain et al. developed S-SNEDDS combination of tamoxifen and quercetin formulation with the lyophilization technique, the formulations showed improved therapeutic efficacy and reduced toxicity of tamoxifen citrate in comparison to free tamoxifen citrate (Jain, 2014)

During lyophilization, strong stabilising effects was related to cooling rate slow and adding up cryoprotectants. In case of formulating tablets, maltodextrins are considered to be an important matrix-forming agent (Gupta, 2013)

2.2.10.4 Melt granulation (MG)

MG is a technique where the agglomeration of powder is attained through adding binder which is lipid that softens or melts at low temperature degrees. It has many profits above traditional granulation, where liquid addition and following drying process is not there.

Main parameters which control granulation process are mixing time impeller speed, and the viscosity and particle size of binder.

Broad variety lipids which are solids or semi-solid can be used as meltable binders. For example, Gelucires are capable to amplify the dissolution rate once compared with polyethylene glycol, probably because of its self emulsified property (Verreck, 2004). An important stat for lipids to be used that it must be semisolid at r.t; examples of other lipid based excipients used for solidification of formulations by met granulatin are lecithin, partial glycerides, or polysorbates.

2.2.10.5 Extrusion Spheronization(ES)

ES is a method of transforming a plastic-property raw material into a uniformed shape product through pushing it into die in specific conditions conditions; pressure, temperature and liquid flow.

ES is a process that characterized by being free of solvent and allows drug loading of 60%, and frequently employed in pharmaceutical industry to create pellets of uniform sized. Size of the resulting spheroids depends on extruder aperture size.

ES process involves the subsequent steps: dry mixing of the formulation ingredient to attain a powder which is homogenous; first wetting the mixture with binder; then extrusion into rope-like extrudate; cut extrudate to uniform-sized spheroids then drying process.(Gupta, 2013).

2.2.11Solid Self-Emulsifying dosage forms

2.2.11.1 Self-Emulsifying capsules

In addition to liquid filling, liquid self emulsified ingredients in solid or semi-solid state may also be filled into capsules; self emulsified capsules were orally administered to improve patient compliance

After administration of capsules including either traditional L-SEDDS or filled with the "S-SEDDS", droplets subsequently spread in GIT to enter absorption sites.

2.2.11.2 Self-Emulsifying Sustained/Controlled-Release Tablets.

For significantly reducing solidifying excipients amounts needed to turn L-SEDDS into S-SEDDS, a gelled "SEDDS" had build up, Where Aerosil 200 as gelling agent was used, which has give out double function of dropping excipients amount required and helping to slow the release of drugs. (Yetukuri, 2012)

The resultant self emulsified tablets every time preserve an elevated API conc. in plasma compared to the same profile of conventional tablet.

2.2.11.3 Self-Emulsifying Beads

One of the solid dosage forms of self-emulsifying system that utilizing solidifying excipients of minimum amounts is formulating into self-emulsifying beads. Patil and Paradkar explored uploading the solid self emulsified into the microchannels of PPB by using a method called solvent evaporation. (Patil, 2006)

PPB is an inert structure that has stability in a broad pH range, humidity and temperature.

PPB has been identified as potential carriers for solid emulsified systems, with low PPB amount required to solidify high amount of L-SEDDS. The pore structure of PPB and bead size has been shown to influence loading effectiveness and in vitro release of the drug from of solid emulsified systems -loaded PPB drugs. (Patil, 2006)

2.2.11.4 Self-Emulsifying solid dispersions

Solid dispersion is a formulation involving the dispersion of one or more drugs into self-emulsifying solid excipients. The dispersed drug may be found

as dissolved molecules or as amorphous or crystalline particles where as carriers are present in amorphous or crystalline states. solid dispersion formulation has been identified as a concept for developing solubility and dissolution process of PWSD by different mechanisms including reducing the particle size to molecular level, enhancing porosity, wettability and changing drug state from crystalline to an amorphous state. (Vo, 2013)

As a concept targeting enhancing bioavailability of PWSD, solid dispersion technology has many advantages over other techniques that can be used for the same purposes, such as salt forming, nanosizing, micronisation, solubilization by co-solvents and others, these conventional techniques for size reduction produce particles minimum of 2 μm that can easily produce agglomerate in the formulation either during dissolution studies or storage while solid dispersions technique result in particle size decreasing to molecular level. (Vo, 2013), the interesting that solid despersions will not form agglomerates as a result of their interaction with the carrier. So upon contact with GI fluids the drug is released or dissolved rapidly to form a state of supersaturation that facilitates rapid drug absorption. And even though if the drug particles precipitated from solid dispersions, the precipitated particles still will show higher in vitro dissolution due to their preserved submicron size (< 1 μm) And their higher energy state can also contribute to faster in vivo dissolution (Serajuddin, 1999)

Self emulsified excipients were used intensively as carriers for solid dispersants like Labrasol, Transcutol1, Gelucire150/02 and Gelucire44/14. (Rahman, 2013)

2.2.12 *in Vitro* Characterizations of SNEDDS

2.2.12.1 Pseudo-ternary phase diagram

Construction of PTPD is typically applied in evolution of SNEDDS. PTPD allows assessment of various surfactants and the combining result of incorporating the co-surfactant. PTPD facilitate define the optimal conc. levels of diverse components of SNEDDS formulation and regions for self-emulsification.

The limits of the phase regions is assessed by visually.

2.2.12.2 Dispersibility Test

SNEDDS dispersibility test performed for evaluate SNEDDS ability to be dispersed into the emulsion, define the size of the resulted droplets. This test performed by employing paddle dissolution apparatus.

Once titrated in purified water, SNEDDS formula develop a mixture of diverse types depending on formulation output in vitro could be evaluated using the grading system illustrated in table 2.7 (Rajeshwar, 2018)

Table 2.7 Grading system

Formulation grade	Characteristics
Grade A	Nanoemulsion rapidly forming (within 1 min), having a clear or bluish appearance.
Grade B	Rapidly forming, slightly less clear emulsion, having a bluish white appearance.
Grade C	Fine milky emulsion that formed within 2 min.
Grade D	Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).
Grade E	Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Grade A and B formulations are dispersed as nanoemulsion. Where as Grade C formulation may be recommended for formulating SEDDS.

In according to the visual appearance, formulation types can be categorized as illustrated in table 2.8

Table 2.8 formulation category according to visual observation

Type of formulation	Mixture/Gel
Micro emulsion	Transparent Mixture
Micro emulsion gel	Transparent Gel
Emulsion	Milky or Cloudy Mixtrure
Emulgel	Milky Gel

2.2.12.3 Thermodynamic stability studies

A formulation's physical stability is significant for its efficiency, because stability has an effect on drug precipitation out from the excipients. Poor formulation and physical instability causes phase separation of API from excipients (Rajinikanth PS, 2012). The following cycles were performed to address the thermodynamic stability:

1. Formulations are subjected to 6 cycles of heating and cooling among elevated temperature (45°C±2) and refrigerator temperature (4°C±2) and at individual temperature exposure formulations are kept for 48 h. minimum.
2. Formulations which are succeed previous phase are subjected to centrifugation for 30 min at 3000 rpm. To pass this phase formulations shouldn't show any precipitation or separation.
3. Three freezing cycles between -21°C and 25°C with minimum of 48 hours of storage at indiviual temperature, To pass this phase formulations shouldnt show phase separation or creaming and are called to be thermodynamically stable.

2.2.12.4 Robustness to dilution

Emulsions resulted from dilution with various dissolution media should not reveal any phase separations or precipitation of drug even after 12 hours of storage. Such formulation is considered to be robust for dilution

2.2.12.5 Droplet size and particle size distribution

The precise estimation of the droplet size is extremely significant as it can affect the *in vivo* performance of "LBFs". Droplet size can be measured by using a number of methods: photon correlation spectroscopy (PCS) also known as dynamic light scattering, laser diffraction (LD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). PCS and LD are the most widely used for droplet size assessment of dispersion systems. By this technique droplet size is measured on the basis of the fluctuation of the intensity of the scattered light caused by the movement of the droplets. LD measures the droplet size by the diffraction angle of the droplet radius when the more intense scattering is caused by smaller particles. (Khan, 2012)

2.2.12.6 Drug release studies

Released mechanism of drugs incorporated into SNEDDS is usually by erosion of surface's particle and biodegradation or diffusion through the matrix.

Release studies could be conducted for release kinetics. But, it must be put emphasis on that release kinetics rely on the parameters of release, like Sink conditions, release media and others.

Using of simple aqueous media to test lipophilic drug dissolution profiles is restricted by the drug's low intrinsic aqueous solubility, which leads to difficulties in maintaining sink conditions, this can result in irreproductive and unreliable dissolution data and dissolution profile assessment.

Thus, a modified dissolution media was developed to develop IVIVC, which more precisely express solubilizing capacity of GI fluids. In general, dissolution media which has bile salts and phospholipids, imitating fasted and fed GI conditions.

2.2.12.7 Zeta potential

Zeta capacity is used to characterize "SNEDDS" oil droplet charges. Oil droplets charge is negative in conventional SNEDDS as a reason of free fatty acids presence.

When zeta potential is high, means that the system is stabile and extended shelf life ofthe droplets in SNEDDS emulsion. Once zeta potential is low, attractive forces would surpass this repulsion and can split and accumulate the emulsion.

Many investigators consider zeta potential as an auxiliary parameter for "SNEDDS", since "SNEDDS" is just a preconcentrate drug mixed in oil and surfactant and only emulsified in vivo. (Gupta, 2013)

The zeta potential of SNEDDSs resulted emulsion is usually explored by ZetaSizer

2.2.12.8 Morphology

Morphology of droplets resulted from dispersion into nanoemulsion would be assessed by TEM. In addition, PDI can be additionally validated by using TEM. Scattering by small-angle X-ray is utilized to find out if the structure of the droplet is in micro or nano scale in terms of such parameters as shapes, average droplet size, and PDI (Kohli, 2010)

2.3 Deferasirox Overview

Deferasirox was chosen to be the model dug for performing, optimizing and characterization of "S-SNEDDS" formulation. This chapter will discuss Deferasirox as a physiochemical properties, pharmacological and pharmacokinetics of the molecule.

Deferasirox (ICL670, Exjade; Novartis Pharma AG, Basel, Switzerlandis) is iron chelator agent characterized by being tridentate that is approved by the United States FDA in 2005 and EMA in 2006 for chronic iron overload treatment because of blood transfusion in patients 2 years of age and older, specifically indicated for β-Thalassemia, Sickle Cell disease, Myelodysplastic syndrome and any hereditary diseases whose patients receiving blood transfusion. (Tanaka, 2014)

Specifically DFX is used for getting rid of excess chronic iron in patients who are of minimum age of 6 years and they have anemia depending on blood transfusion, also DFX could be prescribe for patients aged less than 6 years old who are treated by deferoxamine and not sufficiently treated, and also DFX is effective in case of patients who are more than 10 years old and have thalassemia syndromes and treated with non-transfusion blood.

2.3.1 Physiochemical Properties

Deferasirox chemical name is "4-[3,5-bis-(2-hydroxyphenyl)-[1,2,4]-triazol 1-yl] benzoic acid" (Figure 2.7), DFX molecular weight is 373.4 , DFX have pH of 4.1 in water at around 22 ℃ Deferasirox is powder of a white to slightly yellowish color and has m.p around 264 °C (Tanaka, 2014).

Deferasirox shows pH dependent solubility; insoluble at low pH and DFX solubility equal to 0.4 mg/mL at a physiological pH of 7.4 at 25℃ (Nick, 2003)deferasirox'spka value is 4.57 (Nick H. W., 2002)

Deferasirox is categorized as "class II BCS" according to BCS system which has low solubility and high permeability(Al Durdunji, 2016)Deferasirox has high logP value of 3.52.

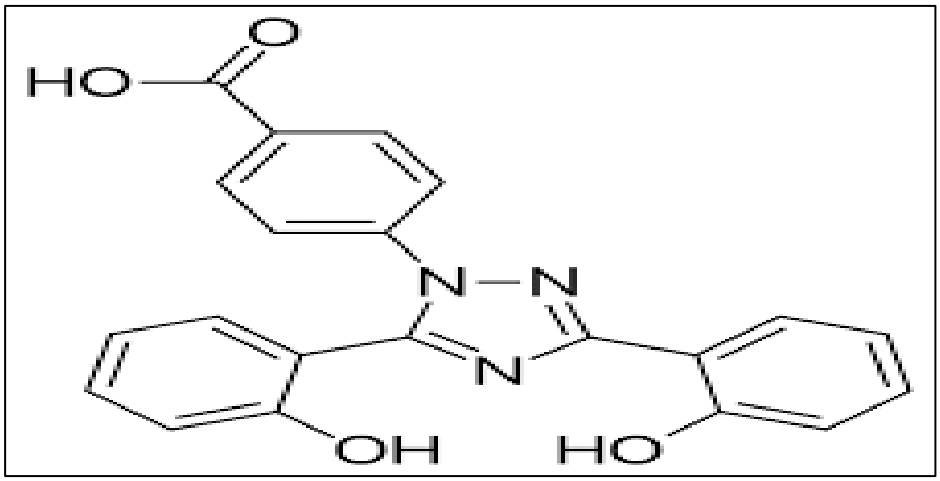

Figure 2.7 Chemical structure of deferasirox (Nick H. W., 2002)

2.3.2 Mechanism of action

DFX is an orally tridentate iron chelator greatly selective for iron and form a stable complex, each stable complex compose of 1 molecule Fe+3 and 2 molecules Deferasirox (Cappellini, 2007), excretion of this complex will be into feces. (Lindsey, 2007).

Iron is a required element in many body's physiological processes including energy production etc. (Lindsey, 2007)Normally iron is maintained in a homeostasis situation, where the iron input into the circulation system from both the absorption of iron from gastrointestinal and the release of iron from hepatocytes and macrophages is equilibrium with the physiological processes' requirements (Lindsey, 2007), in this circumstances the circulating iron is bound to transferrin, which is a protein with a high affinity for iron as ferric form (Fe3+) (Cappellini, 2007). However, in case of blood transfusion where each unit of blood transfused encloses 180 to 200 mg iron (Poggiali, 2012),the transferrin's capacity exceeded and no physiological mechanism to excrete the excess iron , excess iron form complex which are insoluble with Fe^{+3} that can deposit in liver, and myocardium and ending with organ damage and here comes the importance of deferasirox to bind to excess iron and excrete out of the body so will not make a complex with transferrin and deposit in the organs.

2.3.3 Pharmacokinetics

Deferasirox bioavailability of Exjade® after oral administration compared to intravenous administration is 70% (90% confidence interval, 62%-80%) The results

of an open label study performed by using DFX tablet of 375 mg strength and DFX intravenous infusion of 130 mg DFX are represented by Figure (2.8). (Sechaud, 2008)

Deferasirox is 99% plasma protein bounded mainly to serum albumin, V_{dss} is almost 14 Liter a sign of low tissue distribution and also it has a low clearance(Cl) of about 4 Liter per hour (Sechaud, 2008).

DFX and its metabolites are mainly excreted by feces and minimal by kidneys, its elimination half life is between 8-16 hours, and the long half life makes Deferasirox superior on the other chelator agents that allowing once daily dosing regimen which can sustain the plasma levels within the therapeutic range over a 24-hour period (Nisbet-Brown, 2003).

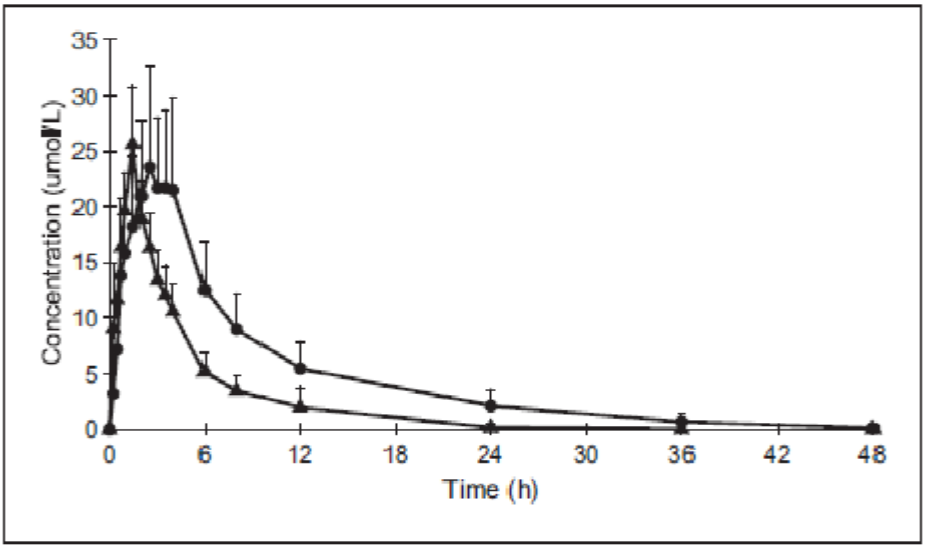

Figure 2.8 Mean plasma conc. of DFX after a single oral dose DFX tablets and IV infusion DFX; •: DFX tablet, ▲: DFX IV infusion(Sechaud, 2008)

The plasma concentration reached its maximum upon oral administration of 20mg/kg/day value after 1-2 hour and Tmax was independent on the dose administered. Regarding the Cmax and AUC over the dose concentration range of 2.5–80 mg/kg, it showed dose dependent pattern (Shirley, 2014).

2.3.4 Deferasirox products in market

Deferasirox was first produced by Novartis as Exjade® as a tablet for oral suspension with three dosage strengths of 125, 250, and 500 mg

Then as a tablet dosage form with the name Jadenu® again in three different dosage strength of 90, 180, and 360 mg, and Jadenu® Sprinkle Granules with the

same dosage strength like Jadenu® tablet of 90, 180, and 360 mg. In the latter two commercial products of DFX, its dosage strength has been reduced by approximately 30% with a Pluronic-containing formulation.

Different attempts were performed to enhance the solubility of deferasirox had been discussed in section 5.2

2.4 Literature Review

2.4.1 Literature review of SNEDDS

Neoral® was introduced in the market in 1995 is the first IIIA LFCS and many more formulations were also approved as LBFs before Neoral® as "Class I LFCS" and some as "Class II LFCS" formulations.

Neoral® as a commercial product represented how a self-emulsifying system can deal with clinical worries and establish a final product that administer a better results for patients, the formulations consists of Corn oil mono-ditriglycerides as the oil component, "Polyoxyl 40 hydrogenated castor oil" as surfactant and Ethanol 11.9%, glycerol, propylene glycol as the cosolvents.(Savla, 2017)

Kang et al., 2012 formulated flurbiprofen loaded Liquid SNEDDS and prepared S-SNEDDS by using spray dryer mechanism with different carrier and examined the effects of using different solid carriers many properties like crystalline properties, dissolution profile and bioavailability. The optimum liquid SNEDDS formulation contained LabrafilM 1944 CS, Labrasol, TranscutolHP and flurbiprofen. S-SNEDDS formulated with hydrophobic carriers of silicon dioxide produced droplets which their size is around 10 nm while magnesium stearate showed larger droplet size and with improved oral bioavailability and dissolution rate. Hydrophilic carriers; such as sodium carboxy methyl cellulose (NA-CMC), polyvinyl alcohol (PVA) have almost improved the dissolution rate, but have been found to be comparatively lower than S-SNEDDS produced by the use of silicon dioxide. (Kang, 2012)

Nasr et al., 2016 prepared solid SNEDDS of olmesartan for enhancing the solubility as well as dissolution rate. Optimized formulation was optimized by Capryol90, CremophorRH40 and TranscutolHPas the SNEDDS components. Where Aerosil® was used as carrier. Optimization and evaluation results revealed that the size of droplets was within the range of nanometer size and that the polydispersity

value was also within the acceptable range. Prepared batches displayed high stability, good optical clarity, fast emulsification time and high drug content. In vitro release be evidence for 90 percent of the olmesartan was released in less than 90 minutes and, based on the results obtained, an optimized batch was chosen for solidification by spray drying technique. The prepared "S-SNEDDS" was examined and the obtained results showed a powder had good-flow properties and high drug content. (Nasr, 2016)

Beg and others, 2012 prepared solid SNEDDS of valsartan, by using CapmulMCM, Tween20 and Labrasol. Where Box Behnken principle used for optimizing SNEDDS using surface response methodology. Different carriers like Neusilin® US2 and Sylysia® (350, 550 and 730) were used to form free flowing granules for the optimum L-SNEDDS. *In vitro* dissolution studies showed 3.5 folds increased in dissolution rate of valsartan. also*in-vivo* test performed for S-SNEDDS resulted in systolic blood pressure decrease in Wistar rats. (Beg, 2012)

Ameeduzzafar et al., 2019 developed "SNEDDS" formulation as an oral dosage form of dapagliflozin by using eucalyptus oil, tween80 and PEG400 as the components and then using adsorbent avicel PH-101 for converting to S-SNEDDS. The droplet size of "SNEDDS" and reconstituted "S-SNEDDS" was found to be around 65.2 nm and 74.3 nm respectively. The *in vitro* dissolution studies disclosed that release percentage of API from "S-SNEDDS" in one hour was three times more than the pure API with zero order release kinetics (El-Bagory, 2019)

Kumar et al. 2019 Prepared Fisetin loaded SNEDDS which was composed of castor oil, LauroglycolFCC, tween80 and TranscutolP and the formulation was formulated by using Box Behnken Design, the release of Fisetin from "SNEDDS" formulation was increased about 7.9 folds HCL buffer of pH 1.2 in the first 5 min as compared to pure Fisetin .Also toxicity studies results exhibited more cell viability of "fisetin- SNEDDS" (89.05%) as compared to pure Fisetin (10.8%) (Kumar, 2019).

Al-Nimry and others 2019, Formulated “SNEDDS” formulation of omeprazol by using Capryol 90, Cremophor RH40 and ethanol as the components and solidified by using Neusilin US2 as a solid carrieras a result the dissolution rate enhanced if compared with unprocessed omeprazol and products in market. (Al-Nimry, 2020)

2.4.2 Literature review of deferasirox

Deferasirox classified as “class II BCS” which it has low solubility and high permeability so most of the studies were performed to boost DFX solubility but neither of these studies was formulating deferasirox as self emulsification formulation as this will be the first study.

Gulsun et al 2019, used Ball milling method to produce small particle size of deferasirox and stabilized by adding surfactants like Pluronic F127 or sodium lauril sulfate (SLS) were selected at different concentrations. The dissolution studies showed that the time needed for 85% released of deferasirox was significantly deceased. (Gulsun, 2019)

Theerasilp et al. 2017, encapsulated deferasirox in polymeric micelles and showed good chelating efficiency and cytotoxicity against cancer cells also the outcomes showed that DFX release from polymeric micelles was lower at pH 4.5 compared to pH 7.4 (Theerasilp, 2017)

Khatamifar et al. 2015, successfully prepared deferasirox particles in the nano size by using bath ultrasound radiation (Khatamifar, 2015)

Patel et al. 2017, improved the solubility of deferasirox by pressing tablets in direct compression method, deferasirox was in micro size and was complexed to resin by drug dispersion technique. (Patel, 2017)

Akdag et al. 2020, prepared fast disintegration tablets formulations of deferasirox by direct compression and lyophilization methods which showed fast disintegration time and the very rapid dissolution (Akdag, 2020)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

DFX was kindly gifted from Sanovel Drug Company, Turkey. Labrafac,Lipophile WL1349, Labrafac PG, Peceol, Transcutol HP, Labrasol ALF, Labrafil M2125, Maisine, Gelucire 44/14 and Gelucire 48/16 wereakindgift from Gatteffosse (France). Kolliphor PS20, Kolliphor PS60, Kolliphor PS80, Kolliphor CS12 and Kolliphor HS15, Kolliphor EL, Kolliphor ELP, Kollisov PEG 400 and were kindlygifted from BASF (Germany).Syloid XDP 3150 was gifted fromGrace GmbH & Co.KG(Germany).Neusilin UFL2 and Neusilin US2 were gifted from Fuji chemical (Japan) The acetonitrile and methanol of analytical reagent grade were purchased from Merck (Germany).Purified water was used during the whole study

3.2 Chemical and Physical Properties of Excipients Used in SNEDDSs Formulations

Oils, surfactants and cosurfacant used in this research with their properties are listed in table 3.1, 3.2 and 3.3 respectively

Table 3.1 Trade name, chemical name, chemical description, Source, Physical properties and HLB of the Oils used. (Data supported by manufacturers).

Trade name	Chemical name	Chemical description	Physical properties	HLB
Peceol	Glyceryl monooleate	Mono-, di- and triglycerides of oleic (C18:1) acid	Liquid viscosity = 220 mPa.s at 20°C	1
Oleic acid	Octadec-9-enoic acid	Monounsaturated omega-9 fatty acid with lipid number of 18:1	Colorless to pale yellow liquid with a mild odor.	1
Labrafac PG	Propylene glycol dicaprolate	propylene glycol esters of caprylic (C8) and capric (C10) acids	Liquid viscosity = 9 – 12 mPa.s at 20°C	1

Lipophile WL 1349	Medium chain Triglycerides	Mixture of caprylic (C8) and capric (C10)	Liquid viscosity =25 – 33 mPa.s at 20°C	1
Maisine CC	Glyceryl monolinoleate	Mono-, di- and triglycerides of mainly linoleic (C18:2) and oleic (C18:1) acids	Liquid viscosity = 120 mPa.s at 20°C	1

Table 3.2 Trade name, chemical name, composition Physical properties and HLB of the surfactants used. (Data supported by manufacturers).

Trade name	Chemical name	Composition	Physical properties	HLB
Kolliphor EL	Polyoxyl castor oil	Glycerol polyethylene glycol ricinoleate. Together with fatty acid esters of polyethylene glycol	Pale yellow oily liquid that is clear at temperatures above 26 °C	12 -14
Kolliphor ELP	Polyoxyl castor oil. Different from Kolliphor EL that Kolliphor ELP followed by purification step after preparation	Glycerol polyethylene glycol ricinoleate. Together with fatty acid esters of polyethylene glycol	White to yellowish paste or cloudy liquid	12 -14
Kolliphor PS 20	Polysorbate 20	Laurate ester of sorbitol and its anhydrides, copolymerized with ethylene oxide	Oily, light yellow to brownish-yellowish, clear or slightly opalescent liquid with a faint odour.	16.7

Kolliphor PS 60	Polysorbate 60	Stearate and palmitate partial esters of sorbitol and sorbitol anhydrides condensed with ethylene oxide (C2H4O	Yellowish brown gelatinous mass which becomes a clear liquid at temperatures above 25 °C.	15
Kolliphor PS 80	Polysorbate 80	Sorbitan oleic acid ester copolymerized with ethylene oxide	Oily, colorless or brownish yellow, clear or slightly opalescent liquid.	15
Kolliphor CS 20	Polyoxyl 20 cetostearyl ether	Mixture of mono-cetostearyl (mixed hexadecyl and octadecyl) ethers of mixed polyoxyethylene diols	White or yellowish white waxy powder	15
Kolliphor HS 15	Polyoxyl 15 Hydroxystearate	Polyglycol mono and diesters of 12-hydroxystearic acid and about 30% of free polyethylene glycol	Yellowish white paste at room temperature that becomes liquid at approx. 30 °C.	15
Gelucire 44/14	Lauroylpolyoxyl/macrogol 32 glycerides	Small fraction of mono, di- and triglycerides and mainly PEG-32 (MW 1500) mono- and diesters of lauric acid (C12)	Semi-solid excipient with a melting. point of 44°C	11
Gelucire 48/16	Polyethylene glycol monostearate	PEG-32 esters of palmitic (C16) and stearic (C18) acids.	solid at ambient temperature with melting	12

			point of 48°C	
Labrasol ALF	Caprylocaproyl macrogol-8 glycerides EP	mono-, di- and triglycerides and mono- and di- fatty acid esters of polyethylene glycol (PEG)-8 and free PEG-8,	Liquid	12
Labrafil M2125 CS	Linoleoyl Polyoxyl-6 glycerides	mono-, di- and triglycerides and PEG-6 (MW 300) mono- and diesters of linoleic (C18:2) acid	Liquid	9

Table 3.3 Trade name, chemical name and Physical properties and HLB of the solvents used as cosurfactants (Data supported by manufacturers).

Trade name	Chemical name	Physical properties
Transcutol HP	Diethylene glycol monoethyl ether	Colourless liquid
Kollisolv PEG 400	Polyethylene glycol	Colourless liquids at room temperature

3.3 Construction Of Standard Calibration Curve Of Deferasirox In Acetonitril And Methanol (50:50, v/v)

A stock solution of deferasirox (100 mg / 100 ml) was prepared in acetonitril and methanol (50:50, v/v). Then, serial dilutions were prepared from deferasirox stock solution to obtain different concentrations varying from 2.5 to 45 μg/ml. The absorbance of the different diluted concentrations was determined by using UV-VIS spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan) at λmax 245 nm, using acetonitril and methanol (50:50, v/v) as a reference. the standard calibration curve was prepared by ploting eachmeasured absorbance against the corresponding concentrations.

3.4 Optimization of DFX-L-SNEDDS

3.4.1 Equilibrium solubility of DFX in the L-SNEDDS components

Equilibrium solubility of DFX in different types of oils (Peceol,Oleic acid, Labrafac PG, Labarafac, Lipophile WL 1349 and Maisine CC), surfactants (Kolliphor EL, Kolliphor ELP, Kolliphor PS 20, Kolliphor PS 60, Kolliphor PS 80, Kolliphor CS 20, Kolliphor HS 15, LabrasolALF,Labrafil M2125 CS, Gelucire 44/14, Gelucire48/16) and co-surfactants (Transcutol HP and Kollisov PEG 400) were determined by using a shaking flask method. The samples were analysed spectrophotometrically at 245 nm to quantify DFX amount in the samples(Mohammed, 2014).The supernatant was appropriately diluted to obtain samples which are in the linearity range at spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). The mixture of acetonitril and methanol (50:50, v/v) was used as a diluent to provide sink conditions. All measurements were done in triplicate.

3.4.2. Construction of pseudo-ternary phase diagram

To recognize the phase behaviour and to observe the SNEDDS formation ratios of the SNEDDS excipients, a pseudo-ternary diagram needed to be created by using aqueous titration technique (Chaudhary, 2019). From the solubility studies stated above, Peceol, Kolliphor EL, and Transcutol were selected

3.4.3 SNEDDS formation assessment

For checking nanoemulsion formation, each oil and Smix ratio previously prepared for pseudo-ternaryphase diagram was assessed for nano emulsion formation by diluting 50 mg of each of the mixtures to 50 ml with double distilled water and, checked visually for the formation of nanoemulsion and subjected for droplet size measurement and PDI by using ZetaSizer Nano ZS (Malvern, UK)(Kheawfu, 2018). The transparent emulsions formed were visually assessed for clarity and stability for 48 hours at room conditions.

3.5 Equilibrium Solubility of DFX in Selected SNEDDS

The goal of a SNEDDS formulation is to develop formulation that is capable to upload maximum amount of DFX into 1 ml of the SNEDDS mixture. Thus, equilibrium solubility of DFX was measured in the selected SNEDDSs formulations by using shaking flask method. All measurements were done in triplicate.

3.6 Preparation of DFX-SNEDDSS Formulations

Based on the solubility data of DFX in the selected formulations; DFX-SNEDDS which differ by the amount loaded were prepared by adding accurately weighted DFX to 1mL of each of selected formulations and, mixing using a magnetic stirrer.

3.7 Characterization of DFX-L-SNEDDSs Formulations

DFX-SNEDDS were characterized in terms of their droplet size, polydispersity index (PDI), thermodynamic stability, transmittance percentage, dispersibility test, robustness to dilution and Effect of pH of the dispersion media on droplet size and PDI values as explained below.

3.7.1Droplet size and PDI determination

For measuring droplet size and PDI of SNEDDSs formulations, SNEDDS 100-fold diluted were prepared and analysed through a ZetaSizer (Malvern, UK). The formulations which have droplet size less than 50 nm and optimum PDI values closed to zero were subjected forfurther characterization tests(Shakeel, 2014).

3.7.2 Thermodynamic stability studies

These studies comprising of centrifugation, heating–cooling cycle and freeze–thaw cycle (Kassem, 2016).

The selected DFX- SNEDDSs formulations which passed the requirements for droplet size and PDI were subjected to centrifugation, heating cooling cycle and freeze thaw cycle.

3.7.3 %T determination

Nanoemulsions resulted from 100-fold dilution of DFX-SNEDDS in purfied water were checked for their turbidity by measuring percent transmittance (T, %). (Abd-Elhakeem, 2019).

3.7.4 Dispersibility test

The efficiency of self-emulsification of DFX-SNEDDSs formulations were assessed by using a standard USP-dissolution apparatus-II. (Nasr, 2016).

The time and efficacy for self- emulsifying were evaluated according to the grading system illustrated in table 2.7 Section 2.2.12.2.

3.7.5 Robustness to dilution

In this test, 50 and 100 times dilution of DFX-SNEDDS into 0.1N HCl and phosphate buffer of different pH values; 4.5, 6.8 and 7.4 were carried out and checked visually for any phase separation. (Selvam, 2013).

3.7.6 Effect of pH of the dispersion media on droplet size and PDI

Stability of DFX-SNEDDSs in different pH buffer solutions was verified by 100 fold dilution in each of the buffer solutions, then subjected to droplet size and PDI measurements by ZetaSizer and compared the values with droplet size and PDI resulted from dispersions in double distilled water (Kumar Mantri, 2012).

3.8 In Vitro Cytotoxicity Studies

To evaluate the relative safety of the selected DFX-SNEDDS, P5-40 coded SNEDDS formulation was selected for in vitro cytotoxicity study which had less concentration of surfactant to avoid unexpected irritation of gastrointestinal tract [47]. The cytotoxic effects of DFX-SNEDDS (P5-40) and the drug-free same SNEDDS formulation (P5°) were compared to that of pure DFX itself (Pure DFX).

3.8.1 MTT assay

To quantify cell viability and proliferation of K562 cells after treatment with selected DFX-SNEDDS (P5-40), MTT assay was applied by using Cell proliferation Kit I (MTT) (Roche, Germany). Untreated cells were considered as experimental control in line with the literature and the MTT protocol and similar protocol was applied. The cytotoxic activity was stated as cell viability (%)

$$\text{Cell viability (\%)} = \frac{(\text{Absorbance of treated cells} - \text{Absorbance of blank})}{(\text{Absorbance of control} - \text{Absorbance of blank})} \times 100 \quad (1)$$

3.8.2 Investigating cell morphology and cell proliferation using light microscope

Morphology and proliferation of K562 cells were investigated under a light microscope (Leica Microsystems, Germany).

3.9 Development of DFX-S-SNEDDS

The solid DFX-SNEDDS were achieved by adsorbing DFX-SNEDDS formulation (P5-40) on solid carriers. Three different solid carriers were used; Neusilin® US_2, Neusilin® UFL_2 and Syloid® XDP 3150 for preparing different batches of solid DFX-SNEDDS for P5-40 formulation and, weremeasured OAC for each solid carrier (Beg S. S., 2012). Specific amount of each carrier was placed separately and the P5-40 was added drop wisely with good mixing until a free flowing powder obtained, the weight of P5-40 formulation used was documented for the calculation of its OAC by applying gravimetric method. (Rajesh, 2018).

3.10 Characterization of DFX-S-SNEDDS

3.10.1 Fourier transformed infrared spectroscopy (FTIR)

FTIR analysis of DFX, prepared DFX-S-SNEDDS and pure carriers were carried out. The spectra were recorded using Fourier transform infrared spectrophotometer (Perkin Elmer Spectrum One, USA) in the range of 4000-650 cm-1. (Parmar K. P., 2015)

3.10.2 SEM Imaging

Scanning electron micrographs for DFX, DFX-S-SNEDDSs formulation and Images with Zeiss Evo LS 10 scanning electron microscope (Germany) has been implemented. The images were obtained under a 7 kV acceleration voltage using the secondary electron detector . (Parmar, 2011)

3.11 In Vitro Dissolution Studies of DFX-S-SNEDDS

In vitro dissolution studies were performed for optimum DFX-S-SNEDDS formulation (P5-40) solidified with different adsorbents mentioned above and a market product of DFX (Exjade®, Novartis, Switzerland) using USP dissolution apparatus II (Sotax Smart AT7, Switzerland). As specified by FDA. The blank used for S-SNEDDS formulation was S-SNEDDS formulation without DFX to avoid interferences from the excipients used in the formulation (Kanuganti, 2012).

Dissolution studies were performed in phosphate buffer 6.8 without surfactants addition. Besides, the release of DFX from P5-40-UFL2 and market product was performed in pH 1.2±0.05 dissolution medium and the same time keeping the other dissolution parameters constants

3.12 Kinetic Analysis of DFX Release Data

For analyzing the *in vitro* release data, the *in vitro* release profile for market product and DFX-S-SNEDDS of different carriers were fitted in various kinetic models. Models Zero order, First order, Higuchi model, Hixson-Crowell and Korsemeyer-Peppaswere applied, analyzed and determination coefficients (r^2) were calculated for each model.

3.13 Statistical Analysis

The results were evaluated by using two-way ANOVA test, GraphPad Prism 8.0.1software was used. *P* value less than 0.05 is considered to be significant.

CHAPTER FOUR

FINDINGS

4.1 Analytical Method for DFX Analysis

As illustrated in Figure 4.1, calibration curve for DFX in acetonitril:methanol (50:50v/v%)showed a good linear relationship over the concentration range of 2.5–12.5 µg/mL of DFX,with a high correlation coefficient (R^2 = 0.9987).

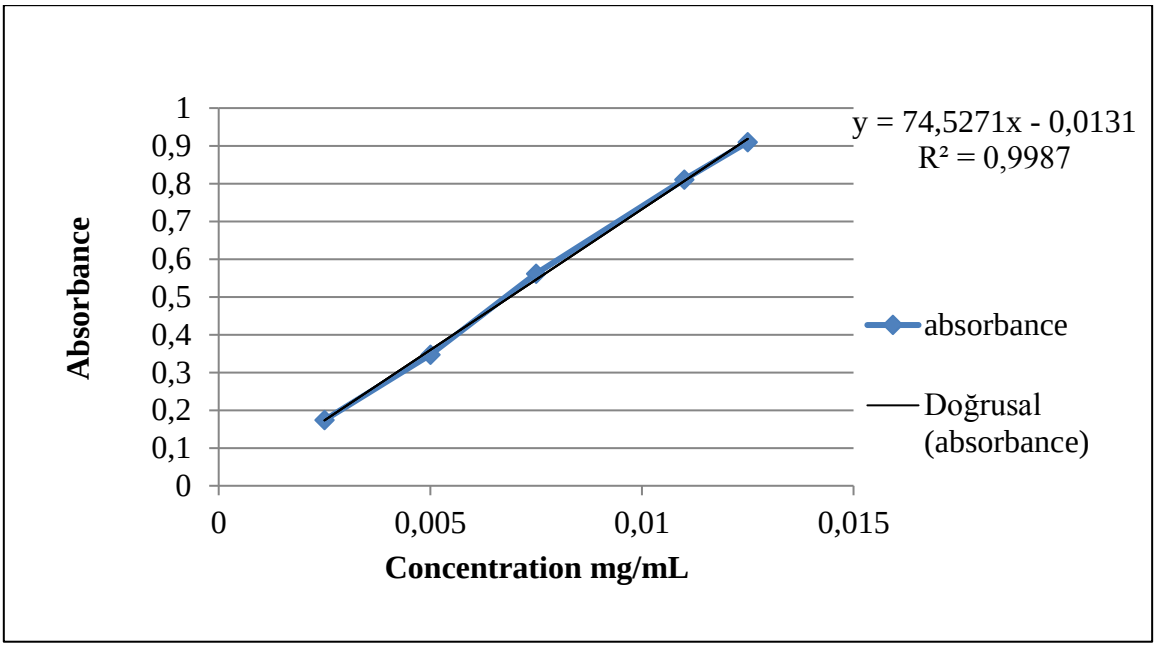

Figure 4.1 Calibration curve of deferasirox in acetonitrile/methanol (50:50v/v%)

4.2 Optimization of DFX-L-SNEDDS

4.2.1. Equilibrium solubility of DFX in the SNEDDSS components

The mean concentration of DFX saturated solubility is shown in Figure 4.2 A–C,

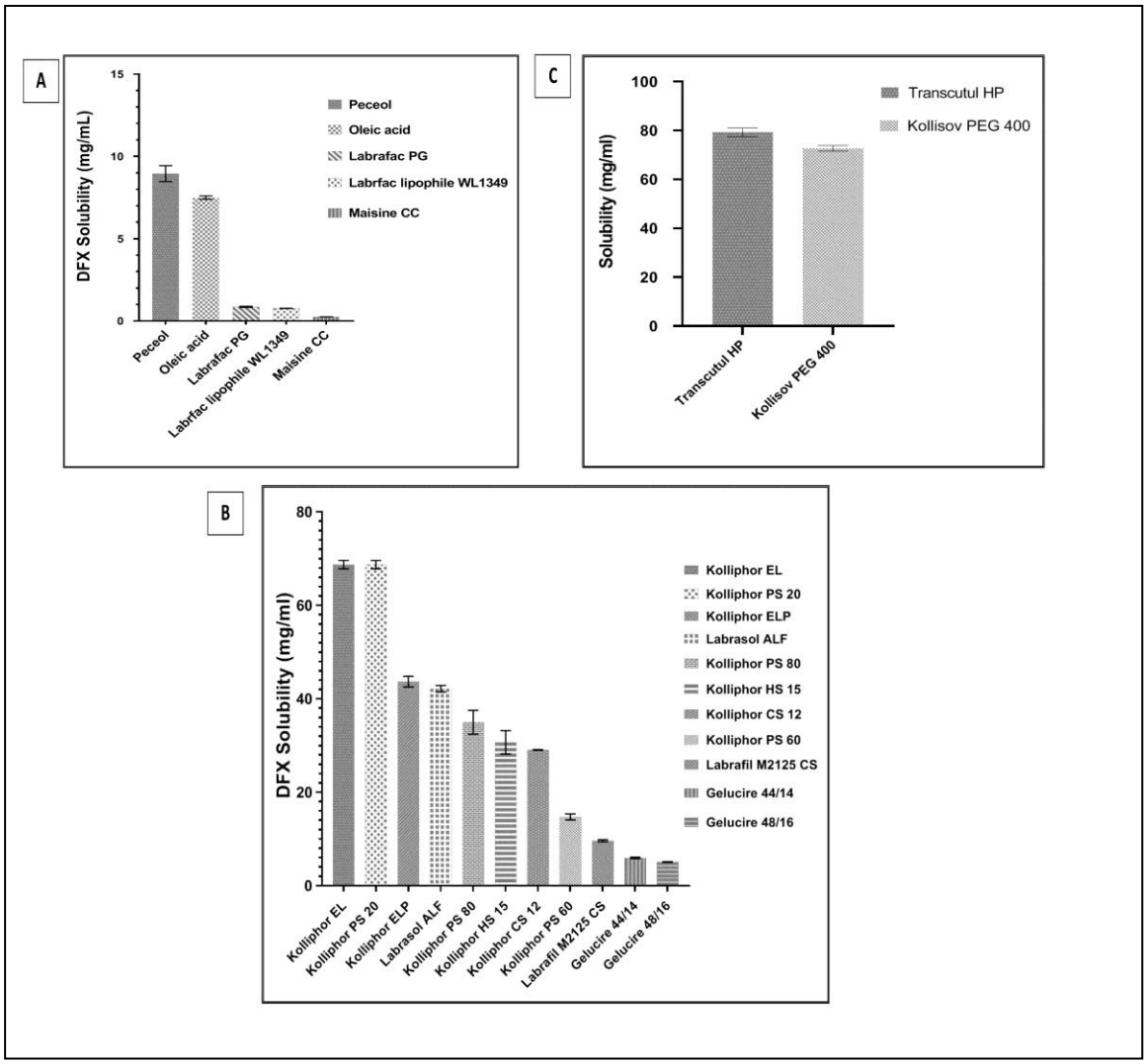

Figure 4.2 The solubility of deferasirox (DFX)in different excipients

Accordingly Peceol, Kolliphor EL, and Transcutol HP are the promising DFX-SNEDDSs components.

4.2.2. Construction of pseudo-ternary phase diagrams

The PTPD at diverse Smix (KolliphorEL :Transcutol HP) ratios (1:1, 1:2, 2:1, 2:3, 3:1, 3:2 and 4:1) are shown in figure 4.3, the area of nanoemulsion is symbolized by colored-region.

Transparent systems formed when 10% peceol was used, as the bi-phasic system formed from high peceol ratio (Gupta, 2013).

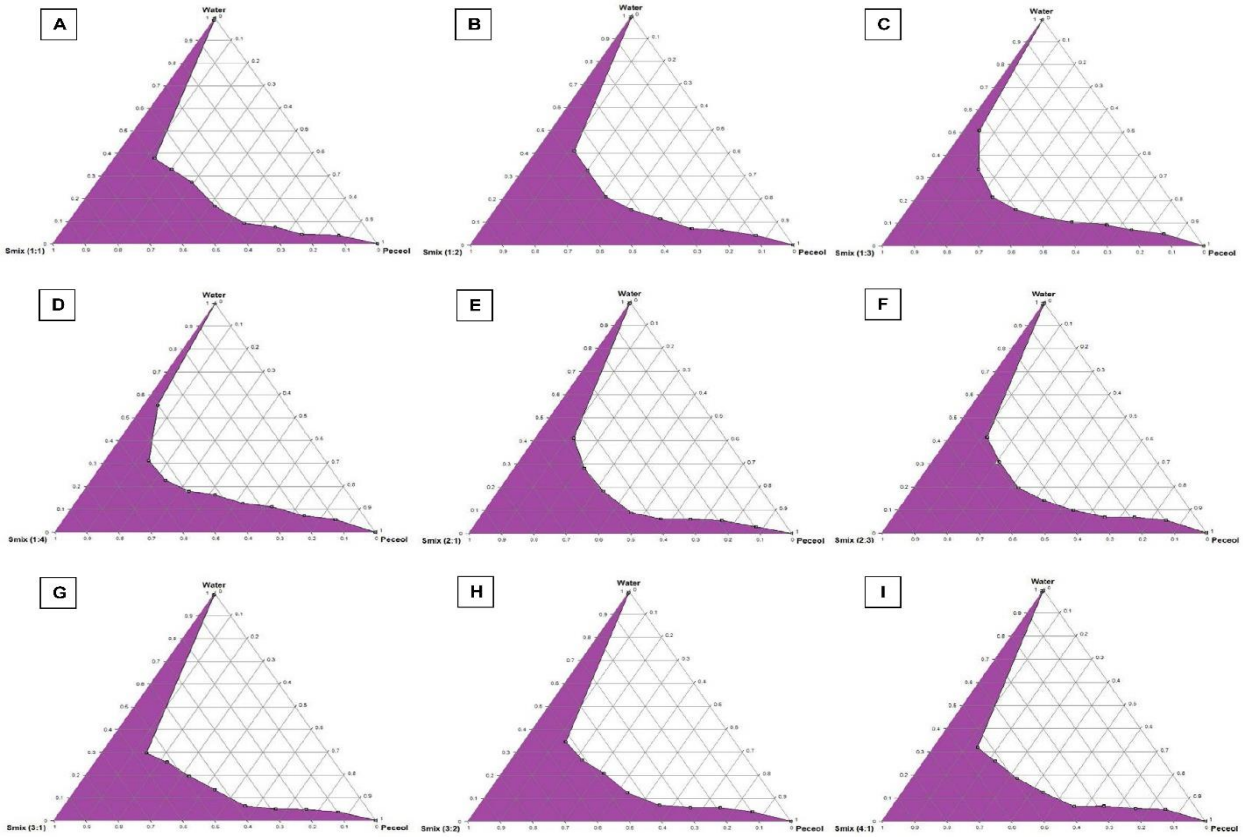

Figure 4.3 PTPD of Peceol, Kolliphor EL, and Transcutol at Smix ratios (**A**)1:1, (**B**)1:2, (**C**)1:3, (**D**)1:4, (**E**)2:1, (**F**) 2:3, (**G**)3:1, (**H**)3:2 and (**I**)4:1.

4.2.3 SNEDDS formation assessment

The seven mixtures dispersed into nanoemulsion dispersions have a droplet size of less than 50 nm and PDI of less than 0.3 as shown in Table 4.1.

Table 4.1 Droplet size and PDI values of SNEDDS combinations

SNEDDS Formulation Code	Mean Droplet Size (nm) (±SD)	Mean PDI (±SD)
P1	20.46 ± 0.20	0.17 ± 0.04
P2	24.57 ± 0.14	0.19 ± 0.01
P3	16.04 ± 0.55	0.04 ± 0.01
P4	21.01 ± 0.09	0.17 ± 0.02
P5	15.30 ± 0.29	0.14 ± 0.01
P6	16.71 ± 0.89	0.19 ± 0.01
P7	15.13 ± 0.12	0.1 ± 0.01

4.3 Equilibrium Solubility of DFX in Selected SNEDDS

DFX solubility results in seven SNEDDS are shown in Figure 4.4, where all of them have DFX capability more than 50 mg/mL.

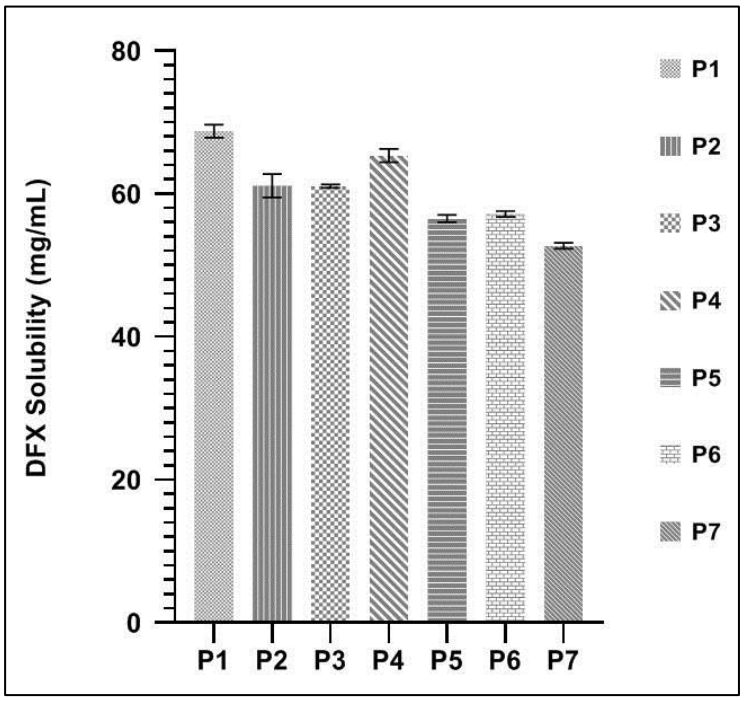

Figure 4.4 Solubility of DFX in selected SNEDDS

4.4. Preparation of DFX-SNEDDS Formulations

In line with results of highest amount of DFX could be solubilized in SNEDDS, each SNEDDS formulation was loaded with 50, 45, or 40 mg of DFX. And further characterized.

4.5 Characterization of DFX-L-SNEDDS Formulations

4.5.1 Droplet size and PDI determination

Amongst the prepared formulations, P5-40 and P7-40 formulations have small droplet sizes of 14.72 and 15.77 nm, and narrow PDI of 0.214 and 0.174, respectively.

Table 4.2 droplet size and PDI of SNEDDS loaded with DFX (*n*=3).

SNEDDS Formulation Code	Mean Droplet Size (nm) (±SD)	Mean PDI (±SD)
P1-50	128±26.20	0.379±0.08
P1-45	109.1±1.70	0.498±0.09
P1-40	73.71±4.59	0.496±0.01
P2-50	670±147.95	0.885±0.16
P2-45	239.4±47.87	0.706±0.27
P2-40	189.5±2.20	0.496±0.01
P3-50	95.12±14.70	1.000±0.001
P3-45	120.5±0.32	0.444±0.01
P3-40	75.53±63.4	0.546±0.20
P4-50	165.6±2.20	0.352±0.12
P4-45	140.62±74.18	0.686±0.11
P4-40	122.3±1.82	0.348±0.04
P5-50	39.20±12.34	0.745±0.28
P5-45	27.82±0.83	0.803±0.03
P5-40	14.72±1.50	0.214±0.036
P6-50	81.56±2.12	0.544± 0.004
P6-45	41.28±0.90	1.000±0.001
P6-40	19.57±0.30	0.578±0.02
P7-50	33.79±26.68	0.486±0.12
P7-45	29.02±12.44	0.478±0.19
P7-40	15.77±3.56	0.174±0.03

4.5.2 Thermodynamic stability studies

DFX-SNEDDS formulations P7-40 and P5-40, demonstrate no signs of instability like turbidity, precipitation, creaming or cracking at the end of the three cycles.

4.5.3 %T determination

DFX-SNEDDS formulations P7-40 and P5-40 produced a dispersion which is clear and have %T values of 99.7% and 99.6%, correspondingly.

4.5.4 Dispersibility test results

Observations done visually confirmed both DFX-SNEDDS P7-40 and P5-40 produced a nano-emulsion which is clear in very short time and accordingly assign that these formulations belong to A grade.

4.5.5 Robustness to dilution

As presented in Table 4.3, P7-40 and P5-40 DFX-SNEDDS formulations were stable at 100 and 50 times dilution at pH 7.4, 6.8, 4.5, and 1.2 and no precipitation, cloudiness or observation of phase separation for 24 hour

Table 4.3 dilution and pH effect on stability of DFX-SNEDDS

SNEDD Formulation Code	**0.1 N HCl pH 1.2**		**Phosphate Buffer pH 4.5**		**Phosphate Buffer pH 6.8**		**Phosphate Buffer pH 7.4**	
	50 d.f	**100 d.f**	**50 d.f**	**100 d.f**	**50 d.f**	**100 d.f**	**50 d.f**	**100 d.f**
P5-40	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
P7-40	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

4.5.6 Effect of pH of the dispersion media on droplet size and PDI

As illustrated in Table 4.4, droplet size and PDI of the resulted nanoemulsions did not much change once using dispersion media of different pH.

Table 4.4 pH Effect on droplet size and PDI (*n*=3).

Formulation	Droplet Size (nm) and PDI				
	Purified Water	pH 1.2 (1 N HCl)	pH 4.5 (Phosphate Buffer)	pH 6.8 (Phosphate Buffer)	pH 7.4 (Phosphate Buffer)
P5-40	14.72-0.21	27.93-0.25	27.39-0.17	12.37-0.17	15.11-0.07
P7-40	15.77-0.17	28.91-0.24	33.98-0.35	12.56-0.16	14.97-0.06

4.6 In vitro Cytotoxicity Studies

4.6.1 MTT assay

Cell viability% data shown in Figure 4.5, these data showed that DFX as pure had low Cell viability% compared with the negative control group and DFX-SNEDDS. The lowest Cell viability% is detected at 40µM of pure DFX of only 3.99%, meanwhile, Cell viability% of DFX-SNEDDS/ P5-40 was 71.44%.

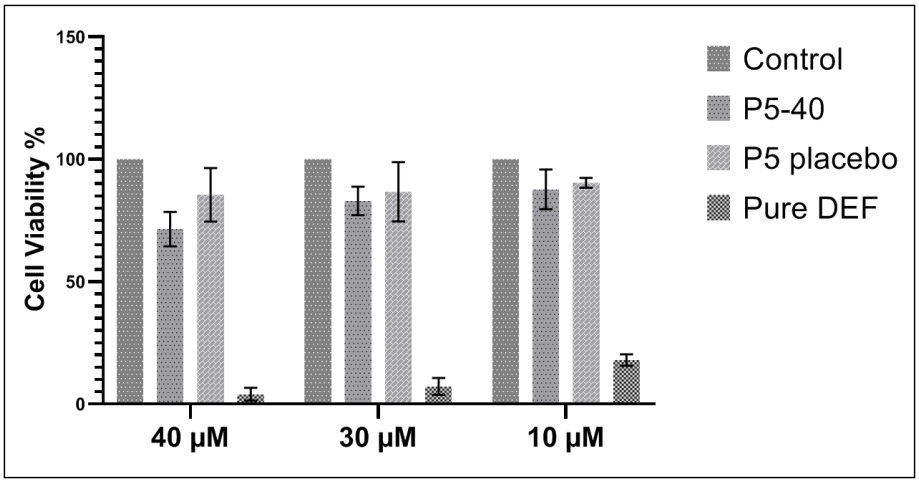

Figure 4.5 K562 cell viability results.

4.6.2 Investigating cell morphology and cell proliferation using a light microscope

The images shown in Figure 4.6 reveal changes in the morphology of K562 cells at both 24 and 48 h.

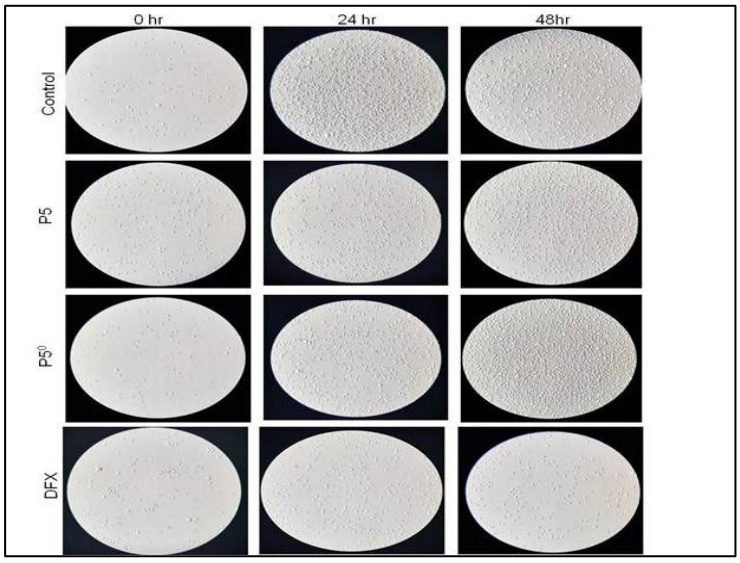

Figure 4.6 Light microscope images of K562 cells

4.7. Development of DFX-S-SNEDDS

The OAC for different carriers was attained to be the same for Neusilin UFL2 and NeusilinUS2, where 300 mg DFX-SNEDDS needed 150 mg carrier and less when Syloid XDP 130 was used, where 300 mg DFX-SNEDDS needed 175 mg Syloid XDP 130. The characteristics of different carriers used are shown in Table 4.5; among different carriers, the carrier which has biggest particle size and least porosity is Syloid XDP 130 which is the reason of low OAC. P5-40 formulation was solidified withNeusilinUFL2 and Neusilin US2 and three different DFX-S-SNEDDS batches were prepared for further testing. The components DFX-S-SNEDDS batches are showed in Table 4.6

Table 4.5 OAC of carriers studied for the preparation of DFX-S-SNEDDS.

Type of Carrier	Chemical Name	Appearance	Average Particle Size(µm)[a]	Oil Adsorbing Capacity (mg)	SNEDDS to Adsorbent Ratio
Neusilin US2	Magnesium aluminometasilicate	White granules	106	150	2:1
Neusilin UFL2	Magnesium aluminometasilicate	Amorphous white powder	3.1	150	2:1
Syloid XDP 3150	Mesoporous silica	White free flowing powder	150	175	1.75:1

[a]Data published by Fuji Chemical and Grace Company.

Table4.6 Components of DFX-S-SNEDDSs formulations

DFX-S-SNEDDSs Formulations Code	Adsorbent Type
P5-40-US2	Neusilin U2
P5-40-UFL2	Neusilin UFL2
P5-40-SYLOID	Syloid XDP 3150

.

4.8 Characterization of DFX-S-SNEDDS

4.8.1. Fourier transformed infrared spectroscopy (FT-IR)

The FT-IR spectra for pure DFX is shown in Figure 4.7A DFX-S-SNEDDS formulations; P5-40-US2, P5-40-SYLOID and P5-40-UFL2 are shown in Figures 4.7 B-D and carriers used are shown in Figures 4.7 E-G .

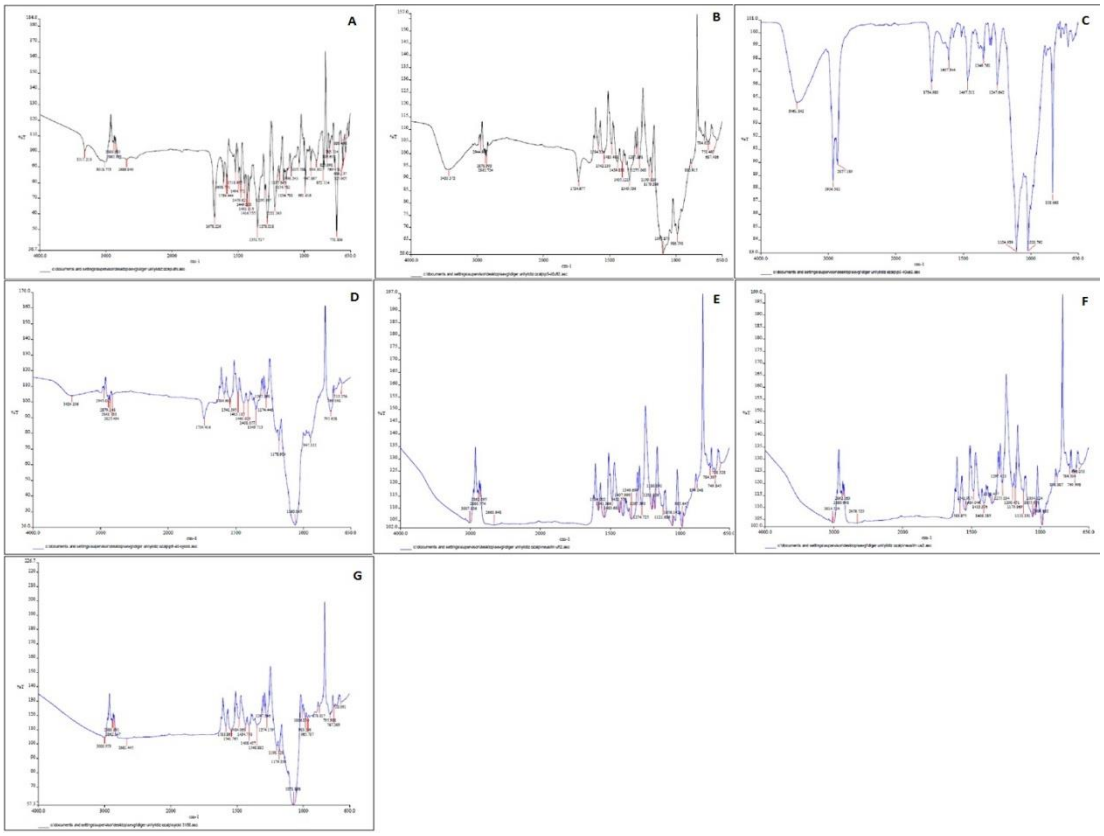

Figure 4.7 FT-IR spectra of (A) pure DFX and (B) P5-40-UFL2 (C) P5-40-US2 (D) P5-40-SYLOID (E) Neusilin UFL2 (F) Neusilin US2 (G) Syloid XDP 3150.

4.8.2. SEM imaging

The SEM images DFX is shown in Figure 4.8, P5-40-SYLOID formulation is shown in Figure 4.9 B and Syloid XDP 3150 shown in Figure 4.9 A.

SEM image of formulation P5-40-UFL2 is represented in Figure 4.10 B while SEM image of Neusilin® UFL2 is in Figure 4.10 A also SEM image of P5-40-US2 is shown in Figure 4.11 B and Neusilin® US2 in Figure 4.11 A

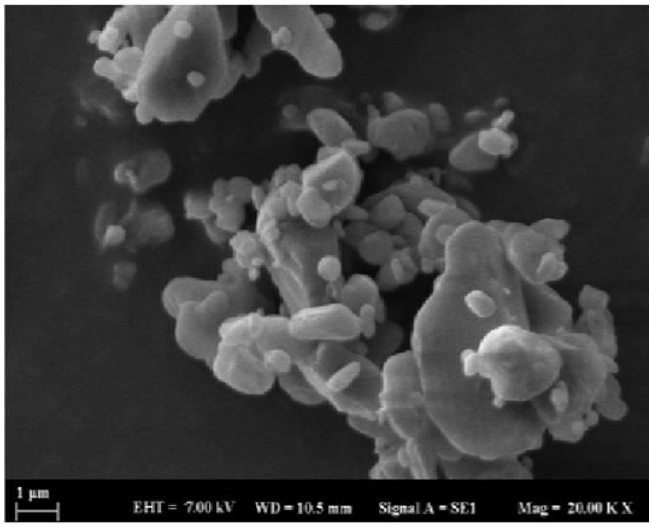

Figure 4.8 SEM image of Pure DFX

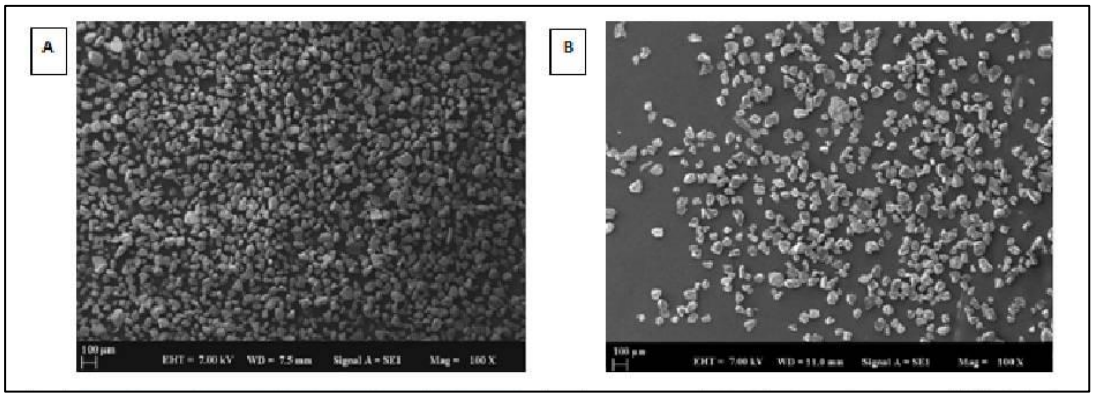

Figure 4.9 SEM image of (A) Syloid XDP 3150, (B) P5-40-SYLOID

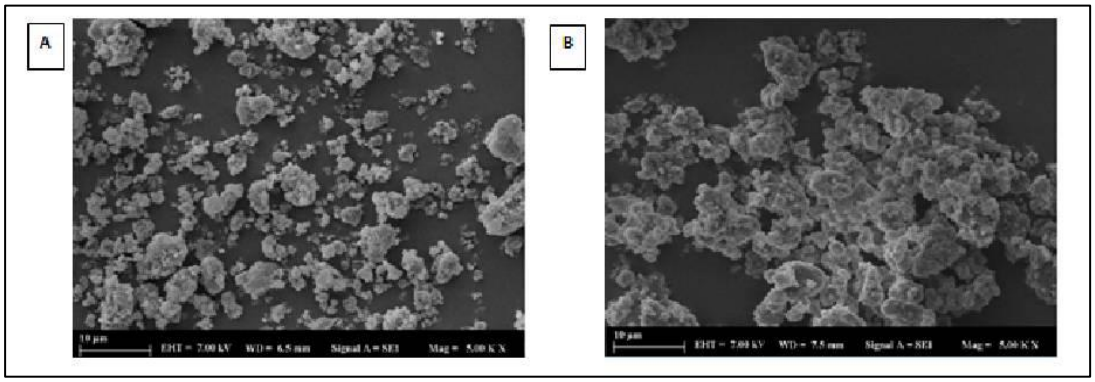

Figure 4.10 SEM image of (A) Neusilin UFL2, (B) P5-40-UFL2

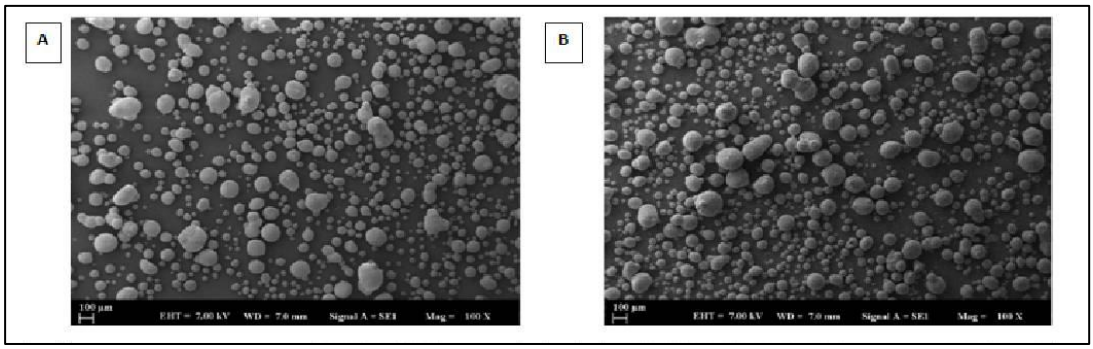

Figure 4.11 SEM image of (A) and (C) Neusilin US2, (B) and (D) P5-40-US2

4.9 In Vitro Dissolution Studies of DFX-S-SNEDDS

The disslution profiles of DFX-S-SNEDDS formulations and Exjade® are shown in Figure 4.12

The dissolution data signified that the release performance of DFX from S-SNEDDS formulations was considerably enhanced.

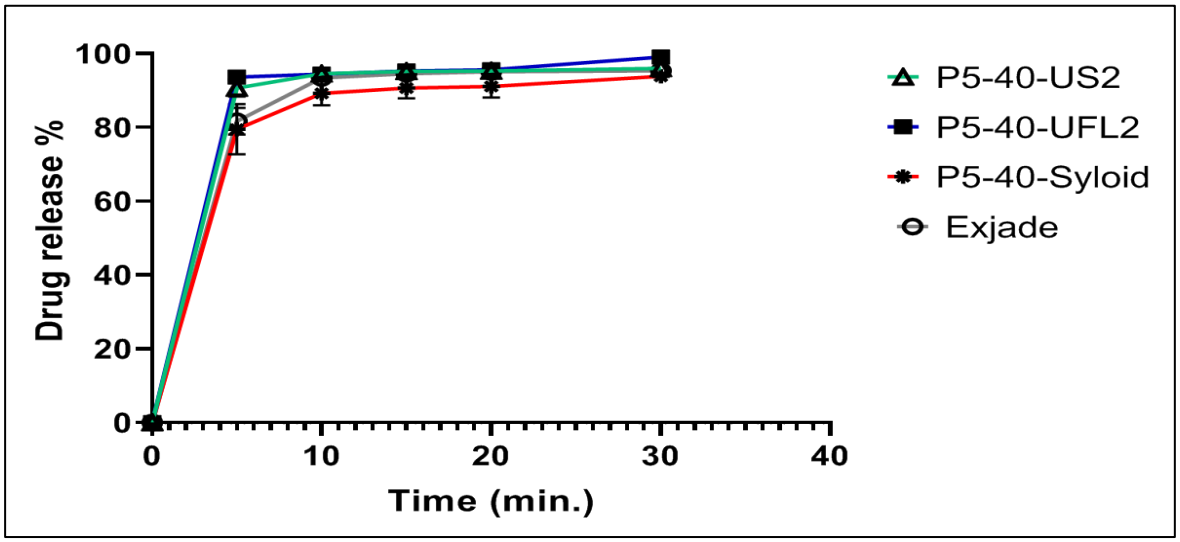

Figure 4.12 Drug release % of DFX from optimized S-SNEDDS and its commercial tablet in phosphate buffer of pH 6.8 containing 0.5% Tween 20

As shown in Figure 4. the percentage released of DFX from P5-40-UFL2 is almost 3 times higher than that of market product Exjade.

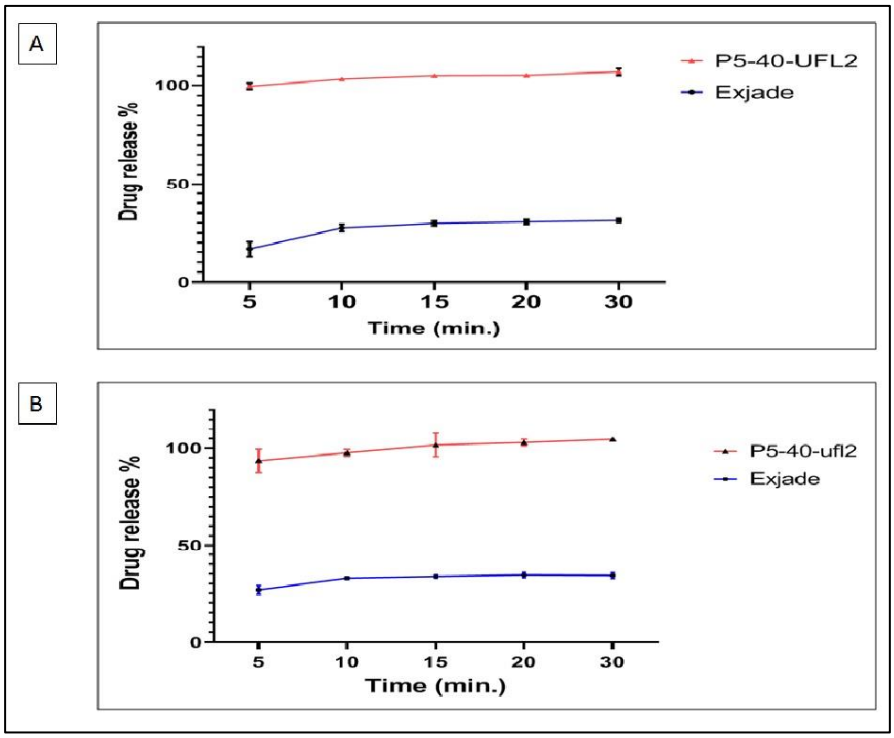

Figure 4.13 Drug release % of DFX from P5-40-UFL2 and its commercial tablet in (**A**) phosphate buffer of pH 6.8 and (**B**) pH 1.2.

4.10Kinetic Analysis of DFX Release Data

Table 4.7 showed that the determination coefficients r^2 values for DFX release was the highest for the Korsemeyer–Peppas model.

Table 4.7 the determination of coefficient (R^2) and release exponent (n) values

Formulation	**Zero Order** R^{2*}	**First Order** R^{2*}	**Higuchi Model** R^{2*}	**Hixon Crowell Model** R^{2*}	**Korsmeyer–PeppasModel** R^{2*}	***n*Value**
Market product (Exjade®)	0.459	0.657	0.762	0.531	**0.842****	1.323
P5-40-US2	0.403	0.562	0.707	0.672	**0.788****	1.318
P5-40-UFL2	0.65	0.881	0.859	0.642	**0.914****	1.385
P5-40-Syloid	0.479	0.725	0.775	0.847	**0.848****	1.31

*R^2 (R squared) value is a statistical measure of how close the data to the fitted regression line for each model.

**values in bold represent the highest r2 value for each formulation in comparison with different models

CHAPTER FIVE

RESULTS AND DISCUSSIONS

5.1 Analytical Method for DFX Analysis

The calibration curves used to analyze the concentration of DFX in different excipients.

5.2 Optimization of DFX-L-SNEDDS

5.2.1. Equilibrium solubility of DFX in the SNEDDSS components

Taking into consideration the therapeutic dose strength of DFX, an elevated drug loading capacity of SNEDDS is essential. The primary choice of oil type, surfactant, and co-surfactant used were determined depending on maximum drug solubilizing capacity. As it is recognized, higher drug solubility in SNEDDS components facilitates higher DFX loading (Patil Prashant, 2016) .

The oil phase takes a part of being the major in solubilizing the obligatory doses of API and transporting it by the intestinal lymphatic system. Peceol was selected as the oil phase which exhibited the maximum DFX solubilization. it was stated in literature that Peceol was used successfully in a lot of SNEDDS due to its solubilizing capacity and capability to inhibit Pgp mediated efflux (Park, 2018) (Sachs-Barrable, 2007). Kolliphor EL as the surfactant is a hydrophilic non-ionic surfactant, which its HLB value is 12–14, has the capacity to diminish P-glycoprotein activity, therefore has a appositive effect in increasing absorption (Hugger, 2002) and a role in suppress many types of Cytochrome enzymes, as a result a appositive effect in bioavailability enhancement (Jakab, 2018). The co-surfactant as Transcutol HP has high DFX solubility ability, and it also has a ability to assemble a stable interfacial film with Kolliphor EL because of its HLB value of 4.2. It was before stated that Transcutol HP has a part in the bioavailability improvement of PWSD (Yan, 2011)

5.2.2. Construction of pseudo-ternary phase diagram

The importance of construction PTPD is to mark the self-nanoemulsifying regions and recognize proper concentrations of excipients for the formulation of a firm formulation, that will not allow DFX precipitation and lose solvent capacity once diluted in body (Pouton, 2000) Increasing Kolliphor EL ratio in Smix led to a

broad nanoemulsion region, but Transcutol HP high percentage in Smix ratios decreased nanoemulsion region.

This could be as a result of that Kolliphor EL as a surfactant effectively diminish surface tension by forming a stable layer all over Peceol droplet and, while Transcutol HP have slight effect on interfacial tension in a straight way; therefore, Transcutol HP concentration increasing will not be sufficient to diminish the surface tension and preserve stability of formulated droplets once SNEDDS diluted (Inugala, 2015).

5.2.3 SNEDDS formation assessment

Visual observations showed seven formulations which have 10% Peceol as oil component formed transparent nanoemulsions once diluted 100times in purified water.

5.3. Equilibrium Solubility of DFX in Selected SNEDDS

Changing in the percentage of Kolliphor EL and Transcutol HP didn't significantly influence DFX solubility.

5.4 Characterization of DFX-L-SNEDDS Formulations

5.4.1 Droplet size and PDI determination

The droplet size of SNEDDS has a huge effect on amount and rate of DFX that is absorbed GIT after oral administration. Decrease in droplet size cause an amplify in interfacial SA, that cause dramatic enhancement in absorption (Mohd, 2015). How droplet size distribution is homogenized can be understood by PDI value, where highly homogenized distribution can concluded from low value of PDI.

Loading high amount of DFX into SNEDDS would result in larger droplets and high droplet size distribution, where DFX precipitated out in short time, that means instable nanoemulsion it was noted that droplet size and droplet size distribution would decrease in case of higher surfactant amount and lower DFX amount uploaded, as a reason of reducing interfacial energy by surfactant which have the ability to make layer around droplet and so resulted in stable system in opposition to coalescence (Jaiswal, 2014)

P5-40 and P7-40 rewarded SNEDDS requirements as regards their droplet size and PDI values; where droplet size is less than 50 nm and low PDI values point toward a narrow size distribution of droplets formed.

5.4.2 Thermodynamic stability studies

Stability of SNEDDS regarding kinetic way is examine by exposing formulations to conditions of stress like centrifugation different cooling and heating temperatures and freeze-thaw of temperature less than zero. The goal is to discriminate normal emulsion nanoemulsion, therefore exclude meta-stable formulations (Syukri, 2018).

5.4.3 %T determination

P5-40 and P7-40 formed a clear dispersion with high %T value

5.4.4 Dispersibility test results

the grading system explained before, Grade A formulation signify P5-40 and P7-40 formulations are self-emulsified robustly in less than sixty seconds once diluted and they formed nanoemulsion which is clear(Usmani, 2019)

5.4.5 Robustness to dilution

Once DFX-SNEDDS formulation facing dilutions in different dilution factor in the pH values in GIT, possibility of Precipitation of DFX could happen and accordingly retarding drug absorption will happen (Balakumar, 2013) as a result, the robustness of dilutions were verified through dilution P7-40 and P5-40 for 100 and 50 d.f in different media that mimic in vivo GIT at (pH 7.4, 6.8, 4.5 and 1.2).

The results are a good sign of stability of resulted emulsion. Findings give an proof that P7-40 and P5-40 were robust to and DFX was stabile in emulsion fromed and did not influenced with dilution.

5.4.6 Effect of pH of the dispersion media on droplet size and PDI

significant factor related to nanoemulsion stability and precipitation of drug is the droplet size, once SNEDDS formulation subjected to dilution in different pH ranges of GIT that have pH range of acidic to basic that will cause an amplify in size of droplet; as a result, droplet size should not vary in a critical value once facing pH changes in GIT (Kang, 2004)

Droplet size and PDI values of the resulted nanoemulsions give a signal that P7-40 and P5-40 would form a stable nanoemulsion once diluted in GIT.

5.5 In vitro Cytotoxicity Studies

5.5.1 MTT assay

P5-40 formulation which has less surfactant f 67.5% compared to P7-40 formulation of 72% was chosen for additional in vitro cytotoxicity test. The importance of cytotoxicity test to understand the probablity of DFX and DFX-SNEDDS in prohibit test cell growth.

The MTT data represented in figure 4.7 in section 4.5.1 suggesting a minimum toxic effect of P5-40 compared to DFX pure that is a reason of nanoemulsion formation once P5-40 was diluted that support DFX stayed in oil globule as O/W emulsion means minimum interaction of DFX with cells (Kumar, 2019)

5.5.2 Investigating cell morphology and cell proliferation using a light microscope

The light microscope images shown in figure 4.8 in section 4.6.2 reveal that an antiproliferative effect on K562 cells was noticed.

5.6 Development of DFX-S-SNEDDS

In recent times, adsorption to solid carriers has become the most intensively studied approach to get S-SNEDDS formulations. Solid carrier Syloid XDP 3150, Neusilin US2 and Neusilin UFL which were used are documented as GRAS status and was reported as effective carriers in producing successful S-SNEDD. (Mandić, 2017). Maximal OAC was attained founded on the least carrier amount needed to whole adsorption for obtaining flow free powder.

5.7 Characterization of DFX-S-SNEDDS

5.7.1 Fourier transformed infrared spectroscopy (FT-IR)

The FT-IR spectrum for DFX as pure in figure 4.9 secion 4.8.1 declared specific absorption bonds (Thomas, 2012). it was noticed that all absorption bonds due to DFX functional group were presented in the three SNEDDS formulations.

5.7.2 SEM imaging

SEM image of pure DFX consists of mixture of large and small crystals. While Syloid XDP 3150 had irregular crystalline shape (Figure 4.11 A,C) SEM image of P5-40-SYLOID, NeusilinUFL2 and NeusilinUS2 shows full adsorption of DFX-L-SNEDDS into the three types that can be understood by the absence of L-SNEDDS globules and no appearance of DFX crystalline shape.

5.8 In Vitro Dissolution Studies of DFX-S-SNEDDS

The increase in the percentage of DFX released from S-SNEDDS formulations is likely due to the spontaneous formation of nanoemulsion with small globules in the nano size (Rahman, 2018) that could induce a higher absorption and higher oral bioavailability of DFX. (Inugala, 2015)

5.9 Kinetic Analysis of DFX Release Data

The n value associated with Korsemeyer–Peppas model indicates the characteristic of release mechanisms (Costa, 2001) An n value higher than 1.0 implies that DFX release from S-SNEDDS formulations follows the Super case-II transport release mechanism (Eltobshi, 2018)

CHAPTER SIX

CONCLUSION

In this study, a novel DFX-SNEDDS was formulating consisting of Peceol (10%), Kolliphor EL (67.5%), and Transcutol HP (22.5%) as the excipients. The optimum SNEDDS formulation was further characterized. DFX-SNEDDS dispersed into stable clear nanoemulsion with a droplet size of 14.72±1.50 nm upon dispersion in purfied water, and even stable against dilution and pH changes. The cell viability effect of optimum DFX-SNEDDs formulation was discovered to be relatively safe in comparison with the pure DFX. in addition, the selected DFX-SNEDDS was converted to DFX-S-SNEDDSs formulations through adsorbing into different carriers. S-SNEDDSs formulations of DFX conserved the self-emulsification performance of the SNEDDS solidified with Neusilin UFL2 and exhibited the fastest in vitro DFX dissolution rate than other adsorbents, and even with its commercial product in different dissolution media. These findings signify enhanced dissolution of DFX by S-SNEED formulations

Overall, our data support the solubility enhancement capability of DFX by optimized S-SNEDDS formulation, and also, indicated that the optimized S-SNEDDS formulation of DFX has a potential for improving its oral bioavailability.

REFERENCES

Abd-Elhakeem, E. T. (2019). Bioavailability enhanced clopidogrel-loaded solid SNEDDS: Development and in-vitro/in-vivo characterization. *Journal of Drug Delivery Science and Technology ,49,* 603-614.

Akdag, Y. G. (2020). Characterization and comparison of deferasirox fast disintegrating tablets prepared by direct compression and lyophilization methods. *Journal of Drug Delivery Science and Technology,* 101760.

Al Durdunji, A. A.-G. (2016). Development of a biphasic dissolution test for Deferasirox dispersible tablets and its application in establishing an in vitro–in vivo correlation. *European Journal of Pharmaceutics and Biopharmaceutics,102,* 9-18.

Al-Nimry, S. S. (2020). Solid self-nanoemulsifying drug delivery system filled in enteric coated hard gelatin capsules for enhancing solubility and stability of omeprazole hydrochloride. *Pharmaceutical Development and Technology* , *25*(5), 588-600.

Amidon, G. L. (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical research, 12* (3), 413-420.

Amin, M. M.-G.-H.-A. (2016). Effect of formulation variables on design, in vitro evaluation of valsartan SNEDDS and estimation of its antioxidant effect in adrenaline-induced acute myocardial infarction in rats. *Pharmaceutical development and technology* ,*21*(8), 909-920.

Balakrishnan, P. L. (2009). Enhanced oral bioavailability of dexibuprofen by a novel solid self-emulsifying drug delivery system (SEDDS). *European Journal of Pharmaceutics and Biopharmaceutics, 72*(3), 539-545.

Balakumar, K. R. (2013). Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids and Surfaces B: Biointerfaces,112,* 337-343.

Baloch, J. S. (2019). Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation. *Medicina*, *55*(5), 210.

Basalious, E. B.-E. (2010). SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: development and optimization. *International journal of pharmaceutics*, *391*(1-2), 203-211.

Battaglia L, G. M. (2012). Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery. *Expert Opinion on Drug Delivery* , *9*(5), 497-508.

Béduneau, A. T. (2014). A tunable Caco-2/HT29-MTX co-culture model mimicking variable permeabilities of the human intestine obtained by an original seeding procedure. *European Journal of Pharmaceutics and Biopharmaceutics* , *87*(2), 290-298.

Beg, S. K. (2016). Solid self-nanoemulsifying systems of olmesartan medoxomil: Formulation development, micromeritic characterization, in vitro and in vivo evaluation. *Powder Technology* , *294*, 93-104.

Beg, S. S. (2012). Development, optimization, and characterization of solid self-nanoemulsifying drug delivery systems of valsartan using porous carriers. *Aaps Pharmscitech* , *13*(4), 1416-1427.

Bravo-Osuna, I. V. (2007). Mucoadhesion mechanism of chitosan and thiolated chitosan-poly (isobutyl cyanoacrylate) core-shell nanoparticles. *Biomaterials* , *28*(13), 2233-2243.

Cappellini, M. D. (2007). Exjade®(deferasirox, ICL670) in the treatment of chronic iron overload associated with blood transfusion. *Therapeutics and clinical risk management*, *3*(2), 291.

Čerpnjak, K. Z. (2013). Lipid-based systems as a promising approach for enhancing the bioavailability of PWSD. *Acta pharmaceutica* , *63*(4), 427-445.

Charman, S. A. (1992). Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. *Pharmaceutical research* , *9*(1), 87-93.

Chatterjee, B. H. (2016). Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. *Drug delivery* , *23*(9), 3639-3652.

Chaudhary, S. A. (2019). Self-nanoemulsifying drug delivery system of nabumetone improved its oral bioavailability and anti-inflammatory effects in rat model. *Journal of Drug Delivery Science and Technology* , *51*, 736-745.

Constantinides, P. P. (1995). Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharmaceutical research* , *12*(11), 1561-1572.

Cornaire, G. W. (2004). Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. *International journal of pharmaceutics* , *278*(1), 119-131.

Costa, P. &. (2001). Modeling and comparison of dissolution profiles. *European journal of pharmaceutical sciences* , *13*(2), 123-133.

Custodio, J. M. (2008). Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Advanced drug delivery reviews* , *60*(6), 717-733.

Dahan, A. &. (2008). Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. *Journal of controlled release* , *129*(1), 1-10.

Date, A. A. (2010). Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. *Nanomedicine* , *5*(10), 1595-1616.

Ditzinger, F. P. (2019). Lipophilicity and hydrophobicity considerations in bio-enabling oral formulations approaches–a PEARRL review. *Journal of Pharmacy and Pharmacology* , *71*(4), 464-482.

Djekic, L. &. (2008). The influence of cosurfactants and oils on the formation of pharmaceutical microemulsions based on PEG-8 caprylic/capric glycerides. *International Journal of Pharmaceutics* , *352*(1-2), 231-239.

El-Bagory, I. A. (2019). Development of novel dapagliflozin loaded solid self-nanoemulsifying oral delivery system: Physiochemical characterization and in vivo antidiabetic activity . *Journal of Drug Delivery Science and Technology* , *54*, 101279.

Eltobshi, A. A. (2018). Self-nanoemulsifying drug-delivery systems for potentiated anti-inflammatory activity of diacerein. *International journal of nanomedicine*, *13*, 6585.

FDA, U. (2000). Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification systemGuidance for industry.

Feeney, O. M. (2016). 50 years of oral lipid-based formulations: provenance, progress and future perspectives. *Advanced drug delivery reviews* , *101*, 167-194.

Gibson, L. (2007). Lipid-based excipients for oral drug delivery. *Drugs and the Pharmaceutical sciences* , *170*, 33.

Grove, M. P. (2005). Bioavailability of seocalcitol I: relating solubility in biorelevant media with oral bioavailability in rats—effect of medium and long chain triglycerides. *Journal of pharmaceutical sciences* , *94*(8), 1830-1838.

Gulsun, T. A. (2019). Effect of particle size and surfactant on the solubility, permeability and dissolution characteristics of deferasirox. *Marmara Pharmaceutical Journal*, *23*(5).

Gupta, S. K. (2013). Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. *ISRN pharmaceutics*, *2013* .

Gursoy, R. N. (2004). Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine & pharmacotherapy* , *58*(3), 173-182.

Guzmán, H. R. (2007). Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. *Journal of pharmaceutical sciences*, *96*(10), 2686-2702.

Han, X. G. (2011). Simultaneous micronization and surface modification for improvement of flow and dissolution of drug particles. *International journal of pharmaceutics*, *415*(1-2) , 185-195.

Hauss, D. J. (Ed.). (2007). *Oral lipid-based formulations: enhancing the bioavailability of PWSD* (Vol. 170). CRC Press.

Homayun, B. L. (2019). Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics* , *11*(3), 129.

Hu, S. N. (2013). Integrity and stability of oral liposomes containing bile salts studied in simulated and ex vivo gastrointestinal media. *International journal of pharmaceutics*, *441*(1-2), 693-700.

Hugger, E. D. (2002). A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity in vitro. *Journal of pharmaceutical science 91*(9), 1991-2002.

Inugala, S. E. (2015). Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: in vitro and in vivo evaluation. *European Journal of Pharmaceutical Sciences* , *74*, 1-10.

Ito, Y. K. (2005). Oral solid gentamicin preparation using emulsifier and adsorbent. *Journal of Controlled Release* , *105*(1-2), 23-31.

Jain, A. K. (2014). Solidified self-nanoemulsifying formulation for oral delivery of combinatorial therapeutic regimen: part II in vivo pharmacokinetics, antitumor efficacy and hepatotoxicit. *Pharmaceutical research* , *31*(4), 946-95.

Jaiswal, P. A. (2014). Development of self-microemulsifying drug delivery system and solid-self-microemulsifying drug delivery system of telmisartan. *International journal of pharmaceutical investigation* , *4*(4), 195.

Jakab, G. F. (2018). Optimization of quality attributes and atomic force microscopy imaging of reconstituted nanodroplets in baicalin loaded self-nanoemulsifying formulations. *Pharmaceutics 10*(4), 275.

Jannin, V. M. (2008). Approaches for the development of solid and semi-solid lipid-based formulations. *Advanced drug delivery reviews* , *60*(6), 734-746.

Joyce, P. D. (2019). Solidification to improve the biopharmaceutical performance of SEDDS: Opportunities and challenges. *Advanced Drug Delivery Reviews* , *142*, 102-117.

Kalepu, S. M. (2013). Oral lipid-based drug delivery systems–an overview. *Acta Pharmaceutica Sinica B* , 361-372.

Kang, B. K. (2004). Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *International journal of pharmaceutics* , *3*(6), 65-73.

Kang, J. H. (2012). Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS). *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(2), 289-297.

Kanuganti, S. J. (2012). Paliperidone-loaded self-emulsifying drug delivery systems (SEDDS) for improved oral delivery. *Journal of dispersion science and technology* , *33*(4), 506-515.

Kapoor, D., Maheshwari, R., Verma, K., Sharma, S., Pethe, A., & Tekade, R. K. (2020). Fundamentals of diffusion and dissolution: dissolution testing of pharmaceuticals. In *Drug Delivery Systems* (pp. 1-45). Academic Press.

Kassem, A. A. (2016). Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design,

optimization, in vitro and in vivo evaluation. *Journal of molecular liquids* , *218*, 219-232.

Kawabata, Y. W. (2011). Formulation design for PWSD based on biopharmaceutics classification system: basic approaches and practical applications. *International journal of pharmaceutics*, *420*(1), 1-10.

Khadka, P. R. (2014). Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian journal of pharmaceutical sciences* , *9*(6), 304-316.

Khan, A. W. (2012). Potentials and challenges in self-nanoemulsifying drug delivery systems. *Expert Opinion on Drug Delivery* , *9*(10),1305-1317.

Khatamifar, M. R. (2015). Preparation of Deferasirox in nano-scale by ultrasonic irradiation and optimization the amount and reaction time parameters. *International journal of nano dimension* , 363-369.

Kheawfu, K. P. (2018). Development and characterization of clove oil nanoemulsions and self-microemulsifying drug delivery systems. *Journal of Drug Delivery Science and Technology* , *46*, 330-338.

Khedekar, K. &. (2013). Self emulsifying drug delivery system: A review. *International journal of pharmaceutical sciences and research*. *4*(12), 4494.

Kohli, K. C. (2010). Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. *Drug discovery today* , *15*(21-22), 958-965.

Krishnaiah, Y. S. (2010). Pharmaceutical technologies for enhancing oral bioavailability of poorly soluble drugs. *J Bioequiv Availab* , *2*(2), 28-36.

Ku, M. S. (2012). A biopharmaceutical classification-based Right-First-Time formulation approach to reduce human pharmacokinetic variability and project cycle time from First-In-Human to clinical Proof-Of-Concept. *Pharmaceutical development and technology*, *17* (3), 285-302.

Kuentz, M. (2011). Oral self-emulsifying drug delivery systems, from biopharmaceutical to technical formulation aspects. *Journal of Drug Delivery Science and Technology* , *21*(1), 17-26.

Kumar Mantri, S. P. (2012). Development and characterization of self-nanoemulsifying drug delivery systems (SNEDDS) of atorvastatin calcium. *Current drug delivery* , *9*(2),182-196.

Kumar, R. K. (2019). Self-nanoemulsifying drug delivery system of fisetin: Formulation, optimization, characterization and cytotoxicity assessment. *Journal of Drug Delivery Science and Technology* , 101252.

Kumar, S. G. (2012). Self-emulsifying drug delivery systems (SEDDS) for oral delivery of lipid based formulations-a review. . *African Journal of Basic & Applied Science* , *4*, 7-11.

Kumari, N. (2019). Various Challenges and Opportunities in Oral Delivery of Anticancer Drugs. *Journal of Advances in Medical and Pharmaceutical Sciences* , 1-18.

Larsen, A. H. (2008). Lipid-based formulations for danazol containing a digestible surfactant, Labrafil M2125CS: in vivo bioavailability and dynamic in vitro lipolysis. *Pharmaceutical research* , *25*(12), 2769-2777.

Leo, A. H. (1971). Partition coefficients and their uses. *Chemical reviews* , *71*(6), 525-616.

Lindsey, W. T. (2007). Deferasirox for transfusion-related iron overload: a clinical review. *Clinical therapeutics* , 2154-2166.

Lipinski, C. A. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews* , *23*(1-3), 3-25.

Liu, W. P. (2016). Developments in methods for measuring the intestinal absorption of nanoparticle-bound drugs. *International journal of molecular sciences* , *17*(7), 1171.

Löbenberg, R. A.-C. (2013). Mechanism of gastrointestinal drug absorption and application in therapeutic drug delivery. In *Mechanism of gastrointestinal drug absorption & application.*

Mandić, J. P. (2017). Overview of solidification techniques for self-emulsifying drug delivery systems from industrial perspective. *International journal of pharmaceutics* , *533*(2), 335-345.

Mannhold, R., Poda, G. I., Ostermann, C., & Tetko, I. V. (2009). Calculation of molecular lipophilicity: State-of-the-art and comparison of log P methods on more than 96,000 compounds. *Journal of pharmaceutical sciences*, *98*(3), 861-893.

Mehnert, W. &. (2012). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, *64*, 83-101.

Midoux, N. H. (1999). Micronization of pharmaceutical substances in a spiral jet mill. *Powder Technology* , *104*(2), 113-120.

Mobarak, D. S. (2019). Improvement of dissolution of a class II poorly water-soluble drug, by developing a five-component self-nanoemulsifying drug delivery system. *Journal of Drug Delivery Science and Technology* , *50*, 99-106.

Mohammad Mahmoudian, H. V.-M. (2020). Enhancement of the intestinal absorption of bortezomib by self-nanoemulsifying drug delivery system. *Pharmaceutical Development and Technology* , *25*(3), 351-358.

Mohammed, S. S. (2014). Formulation of Deferasirox into Dispersible Tablet. *Asian Journal of Research in Chemistry and Pharmaceutical Sciences* , 118-130.

Mohd, A. B. (2015). Solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of glimepiride: development and antidiabetic activity in albino rabbits. *Drug delivery* , *22*(4), 499-508.

Mohsin, K. L. (2009). Design of lipid-based formulations for oral administration of PWSD: precipitation of drug after dispersion of formulations in aqueous solution. *Journal of pharmaceutical sciences*, *98*(10), 3582-3595.

Morishita, M. &. (2012). Advances in oral drug delivery: improved biovailability of poorly absorbed drugs by tissue and cellular optimization. *Advanced drug delivery reviews, 64* (6).

Mosharraf, M. N. (1995). Mosharraf, M., & Nyström, C. (1995). The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. . *International journal of pharmaceutics*, *122*(1-2), 35-47.

MS, S. D. (2012). Permeability enhancement techniques for poorly permeable drugs: A review. *Journal of Applied Pharmaceutical Science*, *2*(06), 34-39.

Mu, H. H. (2013). Lipid-based formulations for oral administration of PWSD. *International journal of pharmaceutics* , 215-224.

Müller, R. R. (2008). Cyclosporine-loaded solid lipid nanoparticles (SLN®): Drug–lipid physicochemical interactions and characterization of drug incorporation. *European journal of pharmaceutics and biopharmaceutics*, *68*(3), 535-544.

Murakami, T. &. (2008). Intestinal efflux transporters and drug absorption. *Expert opinion on drug metabolism & toxicology*, *4*(7), 923-939.

Nasr, A. G. (2016). Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. *Pharmaceutics*, *8*(3), 20.

Nerurkar, M. M. (1996). The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. *Pharmaceutical research* , *13*(4), 528-534.

Nick, H. A. (2003). Development of tridentate iron chelators: from desferrithiocin to ICL670. *Current medicinal chemistry*, *10*(12), 1065-1076.

Nick, H., Wong, A., Acklin, P., Faller, B., Jin, Y., Lattmann, R., ... & Schnebli, H. P. (2002). ICL670A: preclinical profile. In *Iron Chelation Therapy* (pp. 185-203). Springer, Boston, MA.

Nielsen, F. S. (2008). Bioavailability of probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*(2), 553-562.

Nisbet-Brown, E. O. (2003). Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. *The Lancet*, *361*(9369), 1597-1602.

O'Dwyer, P. J. (2019). In vitro methods to assess drug precipitation in the fasted small intestine–a PEARRL review. *Journal of Pharmacy and Pharmacology* , *71*(4), 536-556.

Oh, D. H. (2011). Comparison of solid self-microemulsifying drug delivery system (solid SMEDDS) prepared with hydrophilic and hydrophobic solid carrier. *International journal of pharmaceutics*, *420*(2), 412-418.

Pandey, V. &. (2018). Lipids and surfactants: the inside story of lipid-based drug delivery systems. *Critical Reviews™ in Therapeutic Drug Carrier Systems*, *35*(2).

Park, J. H. (2018). Comparison of a revaprazan-loaded solid dispersion, solid SNEDDS and inclusion compound: physicochemical characterisation and pharmacokinetics. *Colloids and Surfaces B: Biointerfaces*, *162*, 420-426.

Parmar, K. P. (2015). Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. *asian journal of pharmaceutical sciences*, *10* (5), 396-404.

Parmar, N. S. (2011). Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) selfnanoemulsifying drug delivery system. *Colloids and Surfaces B: Biointerfaces*, *86*(2), 327-338.

Parmentier, J. T. (2012). Exploring the fate of liposomes in the intestine by dynamic in vitro lipolysis. *International journal of pharmaceutics*, *437*(1-2), 253-263.

Patel, K. (2017). Formulation and evaluation of immediate release tablet of deferasirox. *World journal of Pharmacy and pharmaceutical sciences*, *6*(5), 1188-1203.

Patil Prashant, P. V. (2016). Potential investigation of peceol for formulation of ezetimibe self nano emulsifying drug delivery systems. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *6*(54), 20-47.

Patil, P. &. (2006). Porous polystyrene beads as carriers for self-emulsifying system containing loratadine. *Aaps Pharmscitech*, *7*(1), E199-E205.

Poggiali, E. C. (2012). An update on iron chelation therapy. *Blood transfusion*, *10*(4), 411–422.

Porter, C. J. (2008). Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Advanced drug delivery reviews* , *60*(6), 673-691.

Porter, C. J. (2001). Intestinal lymphatic drug transport: an update. *Advanced drug delivery reviews* , *50*(1-2), 61-80.

Pouton, C. W. (2008). Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Advanced drug delivery reviews* , *60*(6), 625-637.

Pouton, C. W. (2000). Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying'drug delivery systems. *European journal of pharmaceutical sciences* , *11*, S93-S98.

Pouton, C. W. (2006). Formulation of PWSD for oral administration: physicochemical and physiological issues and the lipid formulation classification system. *European journal of pharmaceutical sciences* , *29*(3-4), 278-287.

Rahman, M. A. (2018). Development of self-nanoemulsifying tablet (SNET) for bioavailability enhancement of sertraline. *Brazilian Journal of Pharmaceutical Sciences* . *54*(1).

Rahman, M. A. (2013). Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS). *Drug Development and Industrial Pharmacy* , *39*(1), 1-19.

Rajebahadur, M. Z. (2006). Mechanistic study of solubility enhancement of nifedipine using vitamin E TPGS or solutol HS-15. *Drug delivery* , *13*(3), 201-206.

Rajesh, B. V. (2010). Lipid based self-emulsifying drug delivery system (SEDDS) for PWSD: A review. *J. Glob. Pharma Techno* , *2*, 47-55.

Rajesh, S. Y. (2018). Impact of various solid carriers and spray drying on pre/post compression properties of solid SNEDDS loaded with glimepiride: in vitro-ex vivo evaluation and cytotoxicity assessment. *Drug development and industrial pharmacy* , *44*(7), 1056-1069.

Rajeshwar, V. &. (2018). Self emulsifying drug delivery system (SEDDS): A conventional and alternative approach to improve oral bioavilability. *International Journal of Pharmaceutical Sciences and Research*, *9*(8), 3114-3127.

Rajinikanth PS, S. Y. (2012). Development and in-vitro characterization of self-nanoemulsifying drug delivery systems of valsartan. *World Academy of Science Engineering and Technology*, *72*, 1418-1423.

Rang, M. J. (1999). Spontaneous emulsification of oils containing hydrocarbon, nonionic surfactant, and oleyl alcohol. *Journal of colloid and interface science*, *209*(1), 179-192.

Rehman, F. U. (2017). From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS). *Expert opinion on drug delivery*, *14*(11), 1325-1340.

Reiss, H. (1975). Entropy-induced dispersion of bulk liquids. *Journal of colloid and Interface Science* , *53*(1), 61-70.

Robertson, D. (2017). First pass metabolism. *Nurse Prescribing* , *15*(6), 303-305.

Rohrer, J. Z.-S. (2018). Design and evaluation of SEDDS exhibiting high emulsifying properties. *Journal of Drug Delivery Science and Technology*, *44*, 366-372.

Sachs-Barrable, K. T. (2007). Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. *J Pharm PharmSci* , *10*(3), 319-31.

Sangster, J. (1997). *Octanol-water partition coefficients: fundamentals and physical chemistry* (Vol. 1). John Wiley & Sons.

Savjani, K. T. (2012.). Drug solubility: importance and enhancement techniques. *ISRN pharmaceutics, 2012*.

Savla, R. B. (2017). Review and analysis of FDA approved drugs using lipid-based formulations. *Drug development and industrial pharmacy*, *43*(11), 1743-1758.

Schultheiss, N., & Newman, A. (2009). Pharmaceutical cocrystals and their physicochemical properties. *Crystal growth and design*, *9*(6), 2950-2967.

Sechaud, R. R. (2008). Absolute oral bioavailability and disposition of deferasirox in healthy human subjects. *The Journal of Clinical Pharmacology* , *48*(8), 919-925.

Selvam, P. R. (2013). Preparation and evaluation of self-nanoemulsifying formulation of efavirenz. *Indian Journal of Pharmaceutical Education and Research*, *47*(1), 47-54.

Serajuddin, A. 2. (2007). Salt formation to improve drug solubility. *Advanced Drugb delivery reviews* , *59*(7), 603-616.

Serajuddin, A. T. (1999). Solid dispersion of PWSD: Early promises, subsequent problems, and recent breakthroughs. *Journal of pharmaceutical sciences* , *88*(10), 1058-1066.

Shakeel, F. M. (2014). Thermodynamics and solubility prediction of talinolol in self-nanoemulsifying drug delivery system (SNEDDS) and its oil phase components using mathematical modeling. . *Journal of Drug Delivery Science and Technology* , *24*(5), 533-538.

Sharma, D. S. (2009). Solubility enhancement–eminent role in poorly soluble drugs. *Research Journal of Pharmacy and Technology* , *2*(2), 220-224.

Shirley, M. &. (2014). Deferasirox: a review of its use for chronic iron overload in patients with non-transfusion-dependent thalassaemia. *Drugs* , *74*(9), 1017-1027.

Shrestha, H. B. (2014). Lipid-based drug delivery systems. *Journal of pharmaceutics 2014* .

Shukla, J. B. (2010). Self micro emulsifying drug delivery system. *Pharm Sci Monit* , *1*, 19-33.

Stuchlík, M. &. (2001). Lipid-based vehicle for oral drug delivery. *Biomedical Papers-Palacky University In Olomouc* , *145*(2), 17-26.

Stumpf, J. L. (2007). Deferasirox. *American Journal of Health-System Pharmacy* , *64*(6), 606-616.

Syukri, Y. M. (2018). Novel Self-Nano Emulsifying Drug Delivery System (SNEDDS) of andrographolide isolated from AndrographispaniculataNees: characterization, in-vitro and in-vivo assessment. *Journal of Drug Delivery Science and Technology* , *47*, 514-520.

Tanaka, C. (2014). Clinical pharmacology of deferasirox. *Clinical pharmacokinetics, 53*(8) , 679-694.

Tang, B. C. (2008). Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug Discovery Today* , *13*(13-14), 606-12.

Tayrouz, Y. D.-T. (2003). Pharmacokinetic and pharmaceutic interaction between digoxin and Cremophor RH40. *Clinical Pharmacology & Therapeutics* , *73*(5), 397-405.

Thakkar, A., Chenreddy, S., Wang, J., & Prabhu, S. (2015). Evaluation of ibuprofen loaded solid lipid nanoparticles and its combination regimens for pancreatic cancer chemoprevention. *International journal of oncology*, *46*(4), 1827-1834.

The United States Pharmacopeia, U. 3.-N. (2007).

Theerasilp, M. C. (2017). Imidazole-modified deferasirox encapsulated polymeric micelles as pH-responsive iron-chelating nanocarrier for cancer chemotherapy. *RSC advances* , *7*(18), 11158-11169.

Thomas, S. J. (2012). Identification, characterization and quantification of a new impurity in deferasirox active pharmaceutical ingredient by LC–ESI–QT/MS/MS. *Journal of pharmaceutical and biomedical analysis* , *63*, 112-119.

Thummel, K. E. (1997). Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Advanced drug delivery reviews* , *27*(2-3), 99-127.

TTSO, P. (1985). Gastrointestinal digestion and absorption of lipid. In *Advances in lipid research* (Vol. 21, pp. 143-186). Elsevier.

Usmani, A. M. (2019). Development and evaluation of doxorubicin self nanoemulsifying drug delivery system with Nigella Sativa oil against human hepatocellular carcinoma. *Artificial cells, nanomedicine, and biotechnology* , *47*(1), 933-944.

Varma, M. V. (2006). Functional role of P-glycoprotein in limiting peroral drug absorption: optimizing drug delivery. *Current opinion in chemical biology* , *10*(4), 367-373.

Verreck, G. &. (2004). Melt extrusion-based dosage forms: excipients and processing conditions for pharmaceutical formulations. *Bulletin Technique Gattefossé* , *97*, 85-95.

Viswanathan, P., Muralidaran, Y., & Ragavan, G. (2017). Challenges in oral drug delivery: a nano-based strategy to overcome. In *Nanostructures for oral medicine* (pp. 173-201). Elsevier.

Vo, C. L. (2013). Current trends and future perspectives of solid dispersions containing PWSD. *European journal of pharmaceutics and biopharmaceutic* , *85*(3), 799-813.

Wakerly, M. G. (1987). Evaluation of the self-emulsifying performance of a non-ionic surfactant-vegetable oil mixture. *J Pharm Pharmacol*, *39*(6), b70.

Waldmeier, F. B. (2010). Pharmacokinetics, metabolism, and disposition of deferasirox in β-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. . *Drug metabolism and disposition* , *38*(5), 808-816.

Williams, H. D. (2019). Unlocking the full potential of lipid-based formulations using lipophilic salt/ionic liquid forms. *Advanced Drug Delivery Reviews* , *142*, 75-90.

Yamagata, T. K. (2007). Improvement of the oral drug absorption of topotecan through the inhibition of intestinal xenobiotic efflux transporter, breast cancer resistance protein, by excipients. *Drug metabolism and disposition* , *35*(7), 1142-1148.

Yan, Y. D. (2011). Enhanced oral bioavailability of curcumin via a solid lipid-based self-emulsifying drug delivery system using a spray-drying technique. *Biological and Pharmaceutical B*, *34*(8), 1179-1186.

Yetukuri, K. &. (2012). Approaches to development of Solid-Self Micron Emulsifying Drug Delivery System: Formulation Techniques and Dosage Forms: A Review. *International Journal of Pharmaceutical Sciences and Research* , *3*(10), 3550.

Yu, L. X. (2002). Biopharmaceutics classification system: the scientific basis for biowaiver extensions. . *Pharmaceutical research, 19* (7), 921-925.

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A Solid Ultra Fine Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) of Deferasirox for Improved Solubility: Optimization, Characterization, and In Vitro Cytotoxicity Studies

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Received: 18 June 2020; Accepted: 21 July 2020; Published: 24 July 2020

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Abstract: The research work was designed to develop a solid self-nanoemulsifying drug delivery system (S-SNEDDS) of deferasirox (DFX). According to the solubility studies of DFX in different components, Peceol, Kolliphor EL, and Transcutol were selected as excipients. Pseudo-ternary phase diagrams were constructed, and then SNEDDS formation assessment studies and solubility of DFX in selected SNEDDSs formulations were performed. DFX loaded SNEDDS were prepared and characterized. The optimum DFX-SNEDDS formulations were developed. The relative safety of the optimized SNEDDS formulation was examined in a human immortalized myelogenous leukemia cell line, K562 cells, using the MTT cell viability test. Cytotoxicity studies revealed more cell viability (71.44%) of DFX loaded SNEDDS compared to pure DFX (3.99%) at 40 μM. The selected DFX-SNEDDS formulation was converted into S-SNEDDS by adsorbing into porous carriers, in order to study its dissolution behavior. The in vitro drug release studies indicated that DFX release (Q5%) from S-SNEDDS solidified with Neusilin UFL2 was significantly higher (93.6 ± 0.7% within 5 min) compared with the marketed product (81.65 ± 2.10%). The overall results indicated that the S-SNEDDS formulation of DFX could have the potential to enhance the solubility of DFX, which would in turn have the potential to improve its oral bioavailability as a safe novel delivery system.

Keywords: deferasirox; SNEDDS; solid SNEDDS; solid carriers; enhancement solubility; oral delivery

1. Introduction

Deferasirox (DFX) is an orally tridentate iron chelator agent that was approved by the United States Food and Drug Administration (FDA) in 2005, and the European Medicines Agency (EMA) in 2006, for chronic iron overload treatment as a result of blood transfusion in patients who are 2 years and older [1].Commercially, DFX is available on the market in three different dosage forms; EXJADE®, as a tablet for oral suspension (125, 250, and 500 mg), JADENU®, as a tablet dosage form (90, 180, and 360 mg), and Jadenu® Sprinkle Granules (90, 180, and 360 mg). In the latter two commercial products

of DFX, its dosage strength has been reduced by approximately 30% with a Pluronic-containing formulation [2].

DFX is relatively a lipophilic molecule (log P = 3.52). It is classified as a Class II drug, according to the Biopharmaceutics Classification System (BCS), which is characterized by its low aqueous solubility of 0.038 mg/mL at 37 °C [3] and high intestinal permeability [4]. The oral bioavailability of DFX was studied on EXJADE®, as a tablet for oral suspension dosage form is believed to be about 70%, due to its low solubility and first pass effect [5]. The dissolution of active drug substances is the rate limiting step for the absorption of BCS Class II compounds such as deferasirox [6]. Therefore, increasing the solubility of deferasirox has a great importance, to improve its oral bioavailability. In the case of Exjade®, the commercial preparation of deferasirox, this step is overcome by the formulation of a tablet for oral suspension in which sodium lauryl sulphate is used as a solubilizing agent to improve the dissolution of DFX.

Due to the higher dose required for DFX to exert its therapeutic benefits, re-formulation of DFX into a new oral dosage form that has higher solubility and bioavailability is still desired. The dosage reduction to diminish its side effects and improvement of patient compliance particularly for pediatrics is important. Therefore, to enhance its oral bioavailability, increasing its aqueous solubility is crucial. So far, few studies were performed to improve the solubility of DFX, such as encapsulated imidazole-modified DFX into polymeric micelles as a nano carrier [7], or to increase the solubility of DFX by decreasing its particle size and using sodium lauryl sulfate or Pluronic F127 as surfactants [8].

Lipid-based formulations (LBFs) are one of the efficient technologies to improve aqueous solubility, and thus to improve the bioavailability of lipophilic drug molecules [9]. Among the LBFs, self-nanoemulsifying drug delivery systems (SNEDDSs) have gained great attention, as an approach to improve oral bioavailability of drug substances which have low aqueous solubility. SNEDDSs are isotropic mixtures of the drug, oil, and hydrophilic surfactants and co-surfactant/co-solvent. Instantaneously, under dilution and mild agitation provided by the peristaltic motility in the gastrointestinal tract, they can form fine oil in water emulsion which has a globule size of less than 50 nm [10]. The oil component of the SNEDDS can be short chain triglycerides, medium chain triglycerides, or long chain triglycerides with different degrees of saturation, while surfactants are mainly non-ionic surfactants with a high hydrophilic lipophilic balance (HLB)value of more than 12 [11].

In addition, SNEDDSs are unique drug delivery systems that are characterized by thermodynamic stability of the nanoemulsion formed, rapid onset of action, ease of preparation process, and scale-up, in comparison with other lipid-based drug delivery systems [12], which make them attractive for industrial manufacturing.

Conventional liquid SNEDDSs are incorporated into a soft gelatin capsule; however, on long term storage, they could face some limitations, like precipitation at lower temperatures, drug leakages, excipient-capsule incompatibility, and handling and stability issues [13]. In order to overcome these limitations, combining the advantages of traditional SNEDDSs formulations and the solid dosage form by incorporating liquid SNEDDSs formulations into solid carrier and converting to solid SNEDDS (S-SNEDDSs) formulations by using different techniques, like spray drying or by adsorbing into porous carriers, result in free-flowing powder which can be formulated as powders, granules, pellets, and tablets, or filled into capsules [14].

To the extent of our knowledge, there has been no research study conducted to improve the solubility or oral bioavailability of DFX by developing a SNEDDS formulation. Thus, the aim of this research study is, for the first time, to develop and characterize a novel DFX-SNEDDS in order to increase its solubility and to potentially improve its oral bioavailability in order to evaluate the in vitro cytotoxicity effects of the optimized DFX-loaded SNEDDS formulation. Furthermore, the optimized DFX loaded SNEDDS would be incorporated into S-SNEDDS formulation, by adsorbing into different porous carriers to allow to compare their dissolution behavior with the commercially available tablet formulation of DFX.

2. Results and Discussions

2.1. Analytical Method for DFX Analysis

The calibration curves used to analyze the concentration of DFX in different excipients showed a good linear relationship over the concentration range of 2.5–12.5 µg/mL of DFX, with a high correlation coefficient (R^2 = 0.9987) and precise intra- and inter-day variation (<2%) and accurate mean recovery(>98%). The limit of detection (LOD) and limit of quantification (LOQ) values were 0.596 and 1.806 µg/mL, respectively.

2.2. Optimization of DFX Loaded SNEDDS

2.2.1. Equilibrium Solubility of DFX in the SNEDDSs Components

The development of a successful SNEDDS formulation depends on choosing the right excipients which reveal the best solubilizing potential for the drug, to ensure maximum drug loading and to keep the emulsification performance. In addition, the solubility of the drug in the proper excipients has a major role in the stability of the final formulation; if the solubility of the drug is not enough, then precipitation could happen in the early stages of development [15].

Considering the therapeutic dose strength of DFX, a higher drug loading capacity of SNEDDS formulation is crucial. The initial choice of the type of oil, surfactant, and co-surfactant used in the compositions of SNEDDSs formulations were decided based on the maximum drug solubilizing capacity. As it is well-known, higher drug solubility in SNEDDS formulation components enables higher loading capacity in the SNEDDS [16]. The mean concentration of DFX saturated solubility in oil, surfactant, and co-surfactant screened are shown in Figure 1A–C, respectively.

The oil phase of SNEDDS formulation plays a major role in solubilizing the required doses of the drug and transporting it via the intestinal lymphatic system. Peceol (glycerol monooleate) was chosen as the oil phase which revealed the maximum DFX solubilization value of 8.95 ± 0.48 mg/mL (Figure 1). As reported in the literature, Peceol has been successfully used in many SNEDDS formulations because of its solubilizing capacity and ability to reduce P-glycoprotein (Pgp)-mediated efflux [17,18]. Kolliphor EL (glycerol polyethylene glycol ricinoleate), as the surfactant, displayed a solubility value of 68.71 ± 1.57 mg/mL. It is a hydrophilic non-ionic surfactant, which has a value of 12–14 HLB, has the ability to inhibit P-glycoprotein activity in the gut wall, thus increasing absorption after oral administration [19], and also inhibiting different Cytochrome enzymes, therefore, it increases oral bioavailability [20]. Transcutol HP (diethylene glycol monoethyl ether), as the co-surfactant has a quite high DFX solubility capability (79.26 ± 3.05 mg/mL), and it also has a capability to construct a stable interfacial film with surfactants due to its HLB value of 4.2. Transcutol HP has been previously reported to have a role in enhancing the bioavailability of poorly soluble drugs [21]; thus, it was concluded that Peceol, Kolliphor EL, and Transcutol HP are the promising components for developing SNEDDS of DFX, and they were checked for the formation of stable nano-size emulsion upon dispersion in water.

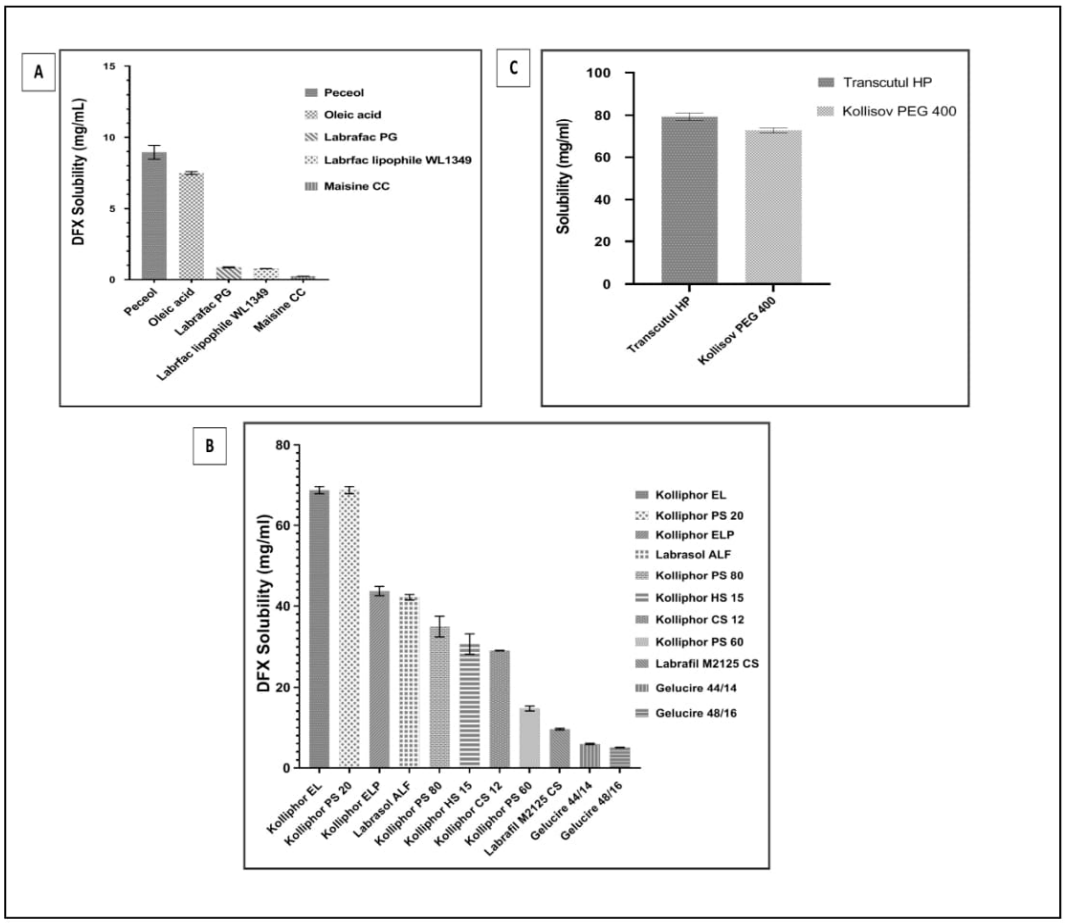

Figure 1. The solubility of deferasirox (DFX) in (**A**) different types of oils, (**B**) different types of surfactants, and (**C**) different types of co-surfactants. Each value represents the mean ± SD (n = 3).

2.2.2. Construction of Pseudo-Ternary Phase Diagrams

The importance of pseudo-ternary phase diagram construction is to label the self-nanoemulsifying regions and to identify the appropriate concentrations of oil (Peceol), surfactant (Kolliphor EL) and co-surfactant (Transcutol HP) for the formulation of a stable SNEDDS formulation, which will not lose its solvent capacity of DFX and precipitate after dilution with the body fluids [22]. Nanoemulsion formation is defined as clear and homogenous systems obtained upon dilution; the area of nanoemulsion is represented by the colored region in the pseudo-ternary phase diagrams in Figure 2A–I.

The pseudo-ternary phase diagrams at different Smix (KolliphorEL: Transcutol HP) ratios (1:1, 1:2, 2:1, 2:3, 3:1, 3:2 and 4:1) show clear-transparent systems formed when the oil content was 10%, while the bi-phasic system formed from higher oil percentage indicates a higher percentage of Smix form fine clear nanoemulsion [23]. However, increasing the surfactant ratio (Kolliphor EL) in Smix ratios(1:1, 2:1, 3:1 and 4:1) led to an increase in the region of nanoemulsion formation, while an increase in co-surfactant (Transcutol HP) ratio in Smix ratios (1:1, 1:2, 1:3, 1:4) decreased the region of nanoemulsion formation. This could be due to the fact that the surfactant forms a layer around the oil globule and efficiently reduces surface tension, though co-surfactants have little effect on reducing the interfacial tension directly; therefore, increases in co-surfactant concentration will not be enough to reduce the surface tension and maintain thermodynamic stability of the formulated systems upon dilution [24].

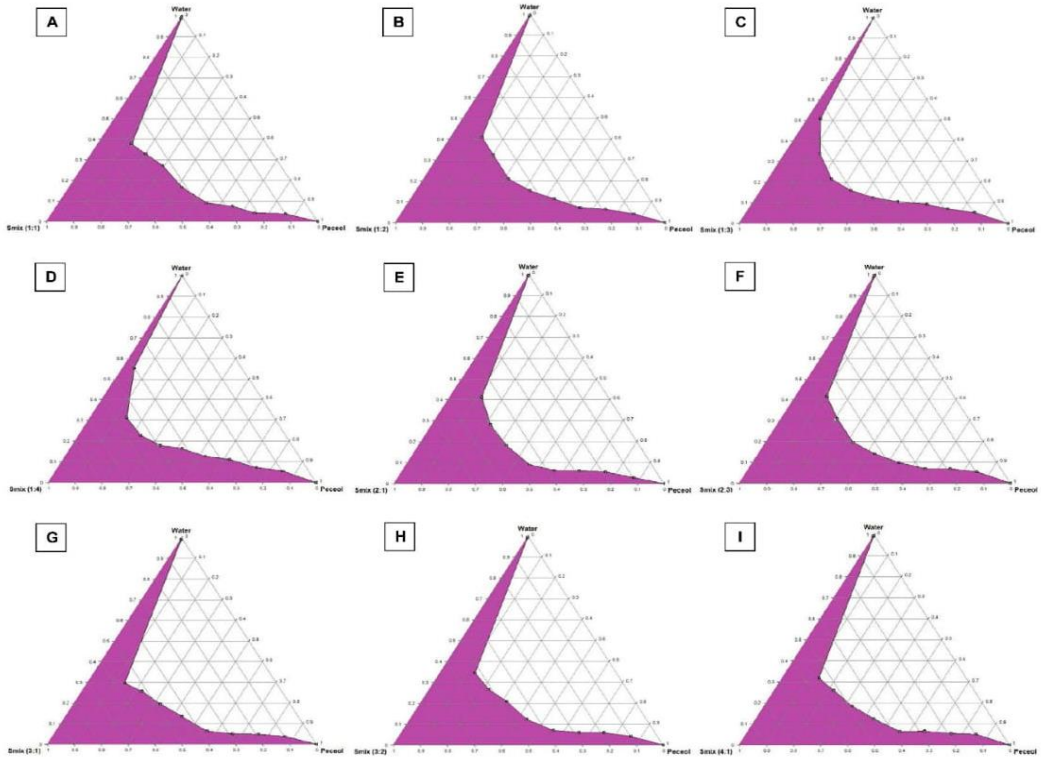

Figure 2. Pseudo-ternary phase diagrams of Peceol, Kolliphor EL, and Transcutol at Smix ratios (**A**) 1:1, (**B**) 1:2, (**C**) 1:3, (**D**) 1:4, (**E**) 2:1, (**F**) 2:3, (**G**) 3:1, (**H**) 3:2 and (**I**) 4:1. The colored region represents the nanoemulsion formation region.

2.2.3. SNEDDS Formation Assessment

Visual observations revealed that seven mixtures containing 10% Peceol as the oil component were clear transparent dispersions upon dilution with purified water for 100 times. The seven mixtures dispersed into nanoemulsion dispersions have a droplet size of less than 50 nm and Polydispersity index (PDI) of less than 0.3 as shown in Table 1.

Table 1. Droplet size and Polydispersity index (PDI) values of self-nanoemulsifying drug delivery system (SNEDDS) combinations for emulsification efficient (n = 3).

SNEDDS Formulation Code	Oil:Smix	Peceol:KolliphorEL:Transcutol HP (*w/w*, %)	Mean Droplet Size (nm) (±SD)	Mean PDI (±SD)
P1	1:9	10:45:45	20.46 ± 0.20	0.17 ± 0.04
P2	1:9	10:30:60	24.57 ± 0.14	0.19 ± 0.01
P3	1:9	10:60:30	16.04 ± 0.55	0.04 ± 0.01
P4	1:9	10:36:54	21.01 ± 0.09	0.17 ± 0.02
P5	1:9	10:67.5:22.5	15.30 ± 0.29	0.14 ± 0.01
P6	1:9	10:54:36	16.71 ± 0.89	0.19 ± 0.01
P7	1:9	10:72:18	15.13 ± 0.12	0.1 ± 0.01

2.3. Equilibrium Solubility of DFX Solubility in Selected SNEDDS Formulations

Solubility results of DFX in the seven formulations are given in Figure 3, which shows that all SNEDDS formulations have DFX a solubility capability of more than 50 mg/mL. The surfactant and co-surfactant amount changes in SNEDDS formulations could not have a significant effect on the solubility of DFX.

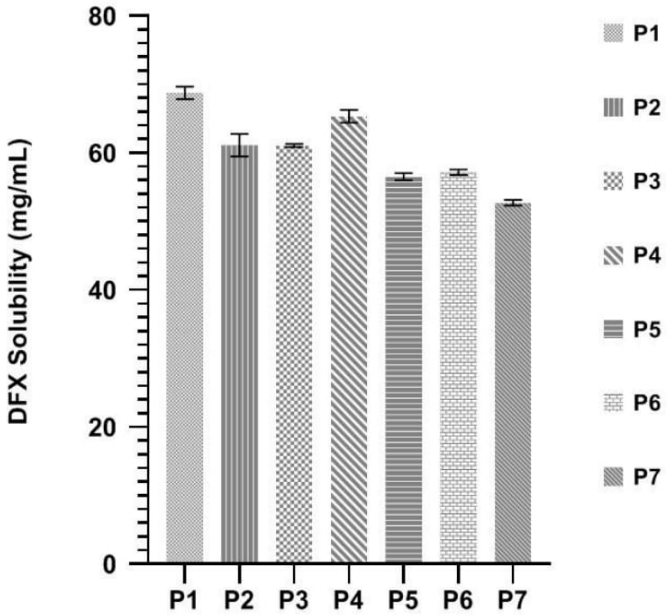

Figure 3. Solubility of DFX in selected SNEDDSs formulations. Each value represents the mean ± SD (n = 3).

2.4. Preparation of DFX Loaded SNEDDS Formulations

According to the equilibrium solubility results of SNEDDS formulations, each of the seven SNEDDS formulations loaded with 40, 45, or 50 mg of DFX were prepared for further investigation of the stability of DFX into the prepared SNEDDS formulations and droplet size measurement of the resulting nanoemulsions after dilution.

2.5. Characterization of DFX Loaded SNEDDS Formulations

2.5.1. Droplet Size and PDI Determination

The droplet size of SNEDDS has a great impact on the rate and amount of drug which is dissolved and absorbed in the gastrointestinal tract after oral administration. Reduction in droplet size results in an increase in interfacial surface area, which leads to an improvement in absorption [25]. The PDI value of SNEDDS indicates the homogeneity of the droplet size distribution and a lower value of PDI indicates that the droplet size range distribution is highly homogenized.

Uploading 50 mg of DFX into the formulation resulted in higher droplet size and high PDI values, and DFX precipitated after 6 h out of the dispersion, which is a sign of instability of the nanoemulsions. As shown in Table 2, decreasing the amount of DFX uploaded and increasing the amount of surfactant, droplet size, and PDI values of SNEDDS were decreased, due to the ability of surfactant to form a layer around the emulsion droplet and reduce the interfacial energy, hence stabilizing the system against coalescence [26].

Among the evaluated formulations, two formulations P5-40 and P7-40 showed small droplet sizes of 14.72 ± 1.50, 15.77 ± 3.56 nm, and narrow PDI of 0.214 ± 0.036 and 0.174 ± 0.03, respectively. This fulfilled the requirements for SNEDDS, regarding their droplet size and PDI values; droplet size of less than 50 nm and low PDI values indicate a narrow size distribution of the emulsion formed. Therefore, P5-40 and P7-40-coded SNEDDS formulations of DFX were selected for further characterization studies.

Table 2. Effect of amount of DFX loaded into the SNEDDS formulations on the droplet size and PDI of SNNEDS ($n = 3$).

SNEDDS Formulation Code	DFX Amount (mg/mL)	Peceol:KolliphorEL:Transcutol HP (*w*/*w*, %)	Mean Droplet Size (nm) (±SD)	Mean PDI (±SD)
P1-50	50	10:45:45	128 ± 26.20	0.379 ± 0.08
P1-45	45		109.1 ± 1.70	0.498 ± 0.09
P1-40	40		73.71 ± 4.59	0.496 ± 0.01
P2-50	50	10:30:60	670 ± 147.95	0.885 ± 0.16
P2-45	45		239.4 ± 47.87	0.706 ±0.27
P2-40	40		189.5 ± 2.20	0.496 ± 0.01
P3-50	50	10:60:30	95.12 ± 14.70	1.000 ± 0.001
P3-45	45		120.5 ± 0.32	0.444 ± 0.01
P3-40	40		75.53 ± 63.4	0.546 ± 0.20
P4-50	50	10:36:54	165.6±2.20	0.352 ± 0.12
P4-45	45		140.62±74.18	0.686 ± 0.11
P4-40	40		122.3±1.82	0.348 ± 0.04
P5-50	50	10:67.5:2.5	39.20 ± 12.34	0.745 ± 0.28
P5-45	45		27.82 ± 0.83	0.803 ± 0.03
P5-40	40		**14.72 ± 1.50** *	0.214 ± 0.036
P6-50	50	10:54:36	81.56 ± 2.12	0.544 ± 0.004
P6-45	45		41.28 ± 0.90	1.000 ± 0.001
P6-40	40		19.57 ± 0.30	0.578 ± 0.02
P7-50	50	10:72:18	33.79 ± 26.68	0.486 ± 0.12
P7-45	45		29.02 ± 12.44	0.478 ± 0.19
P7-40	40		**15.77 ± 3.56** *	0.174 ± 0.03

* Values in bold represent the optimum droplet size values for formulations P5-40 and P7-40.

2.5.2. Thermodynamic Stability Studies

Kinetic stability of colloidal nano-sized carriers is investigated by subjecting formulations to different stress conditions; centrifugation cycle, heating-cooling cycle, and freeze-thaw cycle to differentiate nanoemulsion from emulsion formation, thus removing metastable formulations [27]. Both selected SNEDDS formulations of DFX, P5-40 and P7-40, showed no signs of precipitation, cracking, turbidity, or creaming following the application of centrifugation, heating-cooling cycles, and freeze-thaw cycles.

2.5.3. Percentage Transmittance Determination (% T)

The nanoemulsion resulting from the dilution of SNEDDS formulations is an optically isotropic mixture of water, oil, and a mixture of surfactant and co-surfactant; hence, since the nanoemulsion formed is a single thermodynamically stable solution, the clarity of this solution should be determined. Both SNEDDS formulations P5-40 and P7-40 formed a clear dispersion with transmittance percentage values of 99.6% and 99.7%, respectively.

2.5.4. Dispersibility Test Results

Visual observations showed that both selected SNEDDS formulations of DFX (P5-40 and P7-40) formed a clear nanoemulsion in less than one minute and this is referred to be grade A, according to the grading system mentioned in method Section 3.6.4. Grade A formulation indicates that the formulation is robust enough to self-emulsified in less than one minute to form a clear nanoemulsion, when exposed to dilution [28].

2.5.5. Robustness to Dilution

Precipitation of a drug in vivo, when exposed to different dilutions in the pH ranges in the gastrointestinal tract, will affect the drug absorption and retard it [29]. Therefore, the robustness of dilution was checked by dilution P5-40 and P7-40 for 50 and 100 times with different dilutions which mimic the in vivo environment (pH 1.2, pH 4.5, pH 6.8, and pH 7.4).

As shown in Table 3, P5-40 and P7-40-coded SNEDDS formulations of DFX were stable at 50 and 100 times dilution at pH 1.2, 4.5, 6.8, and 7.4 and neither precipitation were formed, nor cloudiness or phase separation observed, even for 24 h, which indicates the stability of the reconstituted emulsion. The results confirmed that the formulations P5-40 and P7-40 were robust to dilution with different dilution volumes of various media, and the stability of DFX in the emulsion did not influence the dilution process.

Table 3. Effect of dilution and pH on the stability of optimized DFX loaded SNEDD formulations.

SNEDD Formulation Code	0.1 N HCl pH 1.2		Phosphate Buffer pH 4.5		Phosphate Buffer pH 6.8		Phosphate Buffer pH 7.4	
	50 d.f	100 d.f	50 d.f	100 d.f	50 d.f	100 d.f	50 d.f	100 d.f
P5-40	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
P7-40	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

2.5.6. Effect of pH of the Dispersion Media on Droplet Size and PDI

Droplet size is an important parameter for the stability of nanoemulsion, precipitated drug. SNEDDS formulation one diluted in different pH ranges of gastrointestinal tract, ranging from acidic to basic, will lead to an increase in droplet size; therefore, droplet size should not change significantly upon changes in the pH of the GIT [30].

As shown in Table 4, droplet size and PDI of the result and nanoemulsions did not influence changing the pH of the dispersion media used, an indication that these formulations have the ability to form a stable emulsion upon dilution in the gastrointestinal fluids. The droplet size distribution of formulation P5-40 in purified water is given in Figure 4, which shows one homogenized peak of 14.72–0.21 nm value and narrow size distribution.

Table 4. Effect of pH of the dispersion media on droplet size (nm) and PDI (n = 3).

Formulation	Droplet Size (nm) and PDI				
	Purified Water	pH 1.2 (1 N HCl)	pH 4.5 (Phosphate Buffer)	pH 6.8 (Phosphate Buffer)	pH 7.4 (Phosphate Buffer)
P5-40	14.72–0.21	27.93–0.25	27.39–0.17	12.37–0.17	15.11–0.07
P7-40	15.77–0.17	28.91–0.24	33.98–0.35	12.56–0.16	14.97–0.06

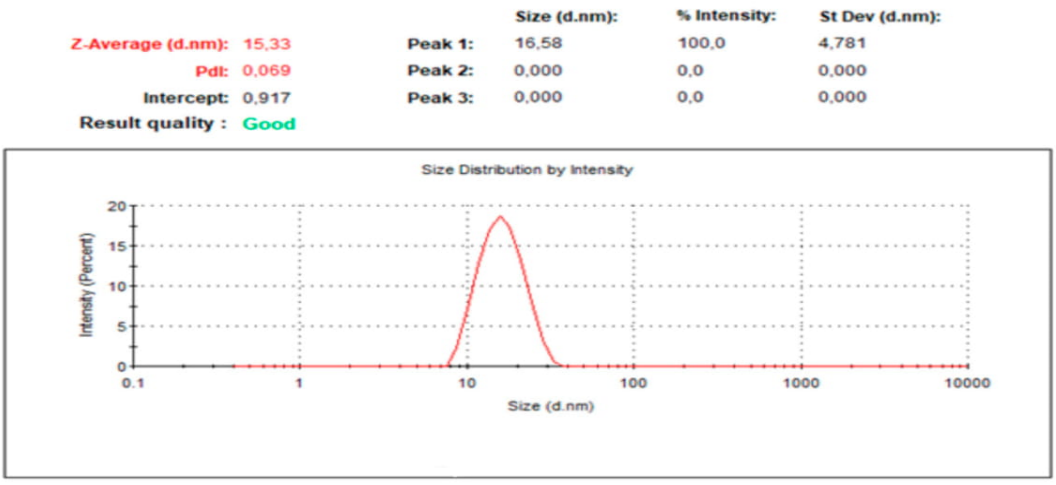

Figure 4. P5-40 formulation droplet size graphic reconstituted in distilled water.

2.5.7. Transmission Electron Microscopy (TEM)

The nanoemulsion globules of the formulation P5-40 which was selected for further investigations were spherical with no signs of droplet aggregation, as shown in Figure 5.

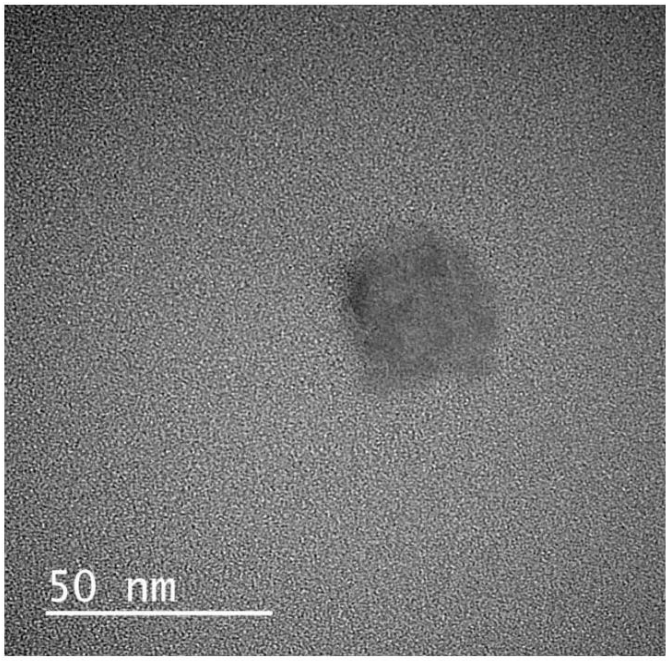

Figure 5. TEM image of the optimized SNEDDS formulation P5-40.

2.6. In Vitro Cytotoxicity Studies

2.6.1. MTT Assay

SNEDDS formulation P5-40, which has less surfactant concentration (67.5%) compared with SNEDDS formulation P7-40 (72%), was chosen for further in vitro cytotoxicity test. The cytotoxicity test revealed the potential of DFX and DFX loaded SNEDDS in inhibiting the growth of the test cell. The cytotoxic potential for SNEDDS formulation P5-40 was assessed in comparison with that of a negative control (cells without any treatment). In the research study, the human immortalized myelogenous leukemia cell line K562 was used and these cells were treated with 10, 30, and 40 µM of DFX consisting of the P5-40 formulation, placebo SNEDDS formulation (P5°), and pure DFX to examine if P5-40, P5°, and pure DFX have any effect on cell viability. The results indicated that cells that were exposed to P5-40, P5°, and pure DFX were comparable to cells without any treatment as a negative control.

The MTT data represented in Figure 6 illustrated that pure DFX showed low percent cell viability in a concentration-dependent manner compared with the negative control group and DFX loaded SNEDDS formulation (P5-40). The maximum cell death was produced at 40 µM of pure DFX where 3.99% cell viability was detected, while, at the same concentration, the cell viability percentage (%) of DFX loaded SNEDDS formulation (P5-40) was 71.44%, suggesting a least/non-toxic effect. This effect of the developed DFX loaded SNEDDS formulation is likely due to the formation of nanoemulsion upon dilution where DFX remained in the globule as an oil/water emulsion and resulted in less interaction with the cells [31].

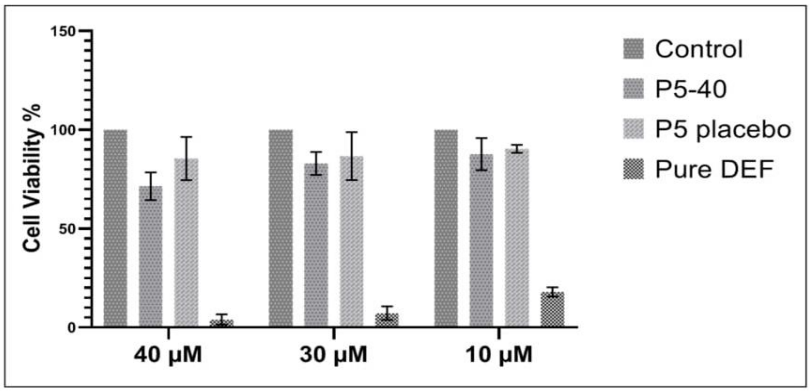

Figure 6. K562 cell viability of pure DFX, P5-40, and $P5^0$ formulations at different concentrations of 10, 30, and 40 µM of DFX. ($n = 3$). (p value < 0.05).

2.6.2. Investigating Cell Morphology and Cell Proliferation Using a Light Microscope

The images shown in Figure 7 reveal that both 30 and 40 µM of P5-40, $P5^0$, and pure DFX had an antiproliferative effect on K562 cells, both at 24 and 48 h. The antiproliferative effect observed for the 30- and 40-µM concentrations have not been observed for the 10 µM of P5-40, $P5^0$, and pure DFX at the same extend. Changes in the morphology of K562 cells were also observed at 30 and 40 µM of P5-40, $P5^0$, and pure DFX at both 24 and 48 h. The circular shape of the K562 cells was disrupted at both time points, whilst the 40-µM concentration was affecting the morphology greatly. Moreover, as the cells were exposed to P5-40, $P5^0$, and pure DFX more at 48 h, the morphological changes observed were considerable.

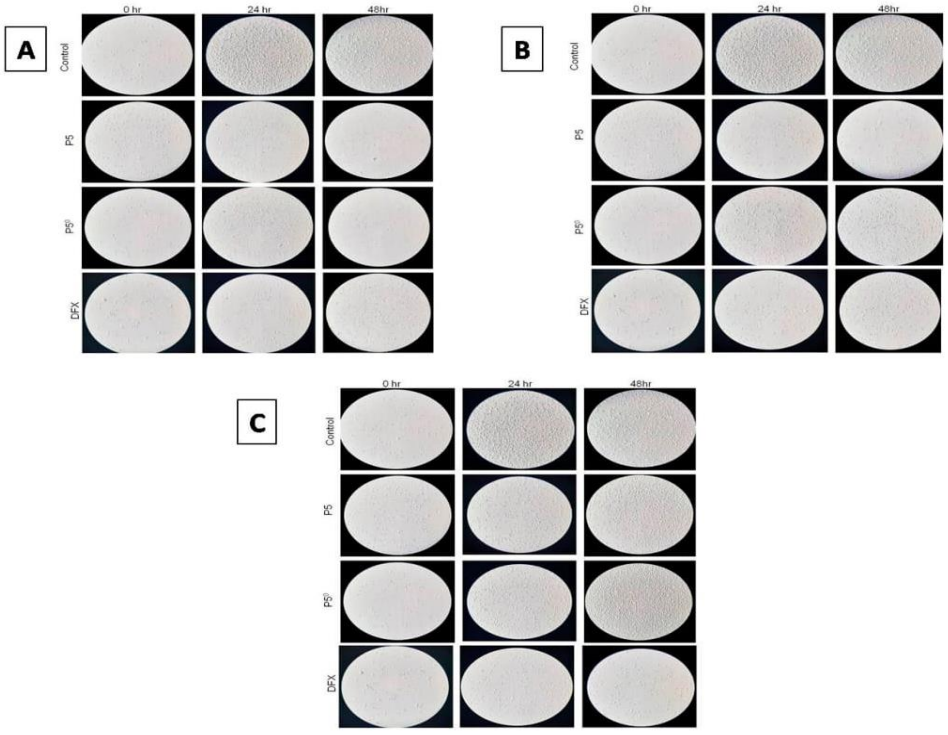

Figure 7. Light microscope images of K562 cells which were exposed to P5-40, $P5^0$, and pure DFX of concentrations (**A**) 40 µM, (**B**) 30 µM, and (**C**) 10 µM for 24 and 48 h and control for comparison.

2.7. Development of DFX Loaded Solid-SNEDDS (S-SNEDDS)

Recently, adsorption to solid carriers has become the most intensively investigated approach to obtain S-SNEDDS formulations. Solid carrier employed in this research work (Neusilin UFL2, Neusilin US2, and Syloid XDP 3150) are recognized as safe (GRAS status) and could be effectively used as carriers to produce solid SNEDDS formulations [32]. Maximal oil adsorption was achieved based on the minimum amount of carrier material required for complete adsorption to obtain powder flow freely.

The oil adsorption capacity for the carriers was found to be equal for both Neusilin UFL2 and NeusilinUS2 (150 mg for 300 mg of SNEDDS formulation) and less in the case of Syloid XDP 130 (175 mg for 300 mg of SNEDDS formulation). The properties of the carriers are presented in Table 5; Syloid XDP 130 has the highest particle size and lower porosity compared with the other carriers that could lead to lower oil absorption capacity. According to adsorbing capacity, the P5-40 formulation solidified with three different adsorbents (NeusilinUS2, Neusilin UFL2, and Syloid XDP 130) which were prepared for further characterization. The components of each formulation (DFX-S-SNEDDS) are illustrated in Table 6.

Table 5. Oil adsorption capacity of porous carriers studied for the preparation of DFX- Solid-SNEDDS (S-SNEDDS).

Type of Carrier	Chemical Name	Appearance	Average Particle Size(μm) [a]	Oil Adsorbing Capacity (mg)	SNEDDS to Adsorbent Ratio
Neusilin US2	Magnesium aluminometasilicate	White granules	106	150	2:1
Neusilin UFL2	Magnesium aluminometasilicate	Amorphous white powder	3.1	150	2:1
Syloid XDP 3150	Mesoporous silica	White free flowing powder	150	175	1.75:1

[a] Data published by Fuji Chemical and Grace Company.

Table 6. Components of DFX-S-SNEDD formulations prepared for further characterization.

DFX-S-SNEDD [a] Formulation Code	Peceol:KolliphorEL:Transcutol HP (%, *w/w*)	Adsorbent Type
P5-40-US2	10:67.5:22.5	Neusilin U2 [b]
P5-40-UFL2	10:67.5:22.5	Neusilin UFL2 [b]
P5-40-SYLOID	10:67.5:22.5	Syloid XDP 3150 [c]

[a] 40 mg/mL of DFX was loaded into formulations, [b] 200 mg of weight adsorbent is required for 1 *g* of SNEDDS, [c] 175 mg of weight adsorbent is required for 1 g of SNEDDS.

2.8. Characterization of DFX-S-SNEDDS

2.8.1. Fourier Transformed Infrared Spectroscopy (FT-IR)

The FT-IR spectrum for DFX as pure, DFX-S-SNEDDS formulations and carriers used are provided in the Supplementary Materials. FT-IR spectrum of pure DFX is shown in Figure S1. It revealed absorption bonds at 3317 cm^{-1} (OH, stretching), 1687 cm^{-1} (acid, conjugated C=O stretching), and 1584 cm^{-1} (aromatic, C=C stretching) [33]. The IR spectra for the DFX-S-SNEDDSs formulations (P5-40-UFL2, P5-40-US2, and P5-40-SYLOID) are presented in Figures S2–S4, respectively, while the IR spectra of Neusilin UFL2, Neusilin US2, and Syloid XDP 3150 are presented in Figures S5–S7, respectively. The data indicated that all the absorption bonds due to the functional group of DFX were presented in the three SNEDDS formulations (P5-40-UFL2, P5-40-US2, and P5-40-SYLOID), and there was no significant difference found in wave number (cm^{-1}) in the spectrum of the prepared S-SNEDDS formulations. This finding indicates that there was no unwanted interaction between DFX and other used excipients in the study.

2.8.2. *Scanning Electron Microscopy (SEM) Imaging*

The SEM images of DFX, DFX-S-SNEDDs formulation, and carriers used for solidification are shown in Figure 8A–G. Figure 8A shows that pure DFX consists of asymmetrically shaped crystals as a mixture of small and large crystals. When comparing SEM images of S-SNEDDS formulations with the three types of carriers, Syloid XDP 3150 (Figure 8B) had irregular crystalline shape and the P5-40-SYLOID formulation kept the irregular shape of the Syloid XDP 3150 particles and had no oil globules (Figure 8C). In the case when both Neusilin® UFL2 and Neusilin® US2 were used as carriers, both carriers have a sphere-like shape (Figure 8D,F, respectively). Following mixing of L-SNEDDS with the solid carrier, the L-SNEDDS formulation was adsorbed totally into the internal Neusilin pores and both P5-40-US2 (Figure 8E) and P5-40-UFL2 (Figure 8G) kept the sphere-like shape of both carriers. Overall, these findings indicate the complete adsorption of liquid DFX-SNEDDS into the three types of carriers which were noticed by the absence of oil globules and disappearance of the DFX crystalline shape.

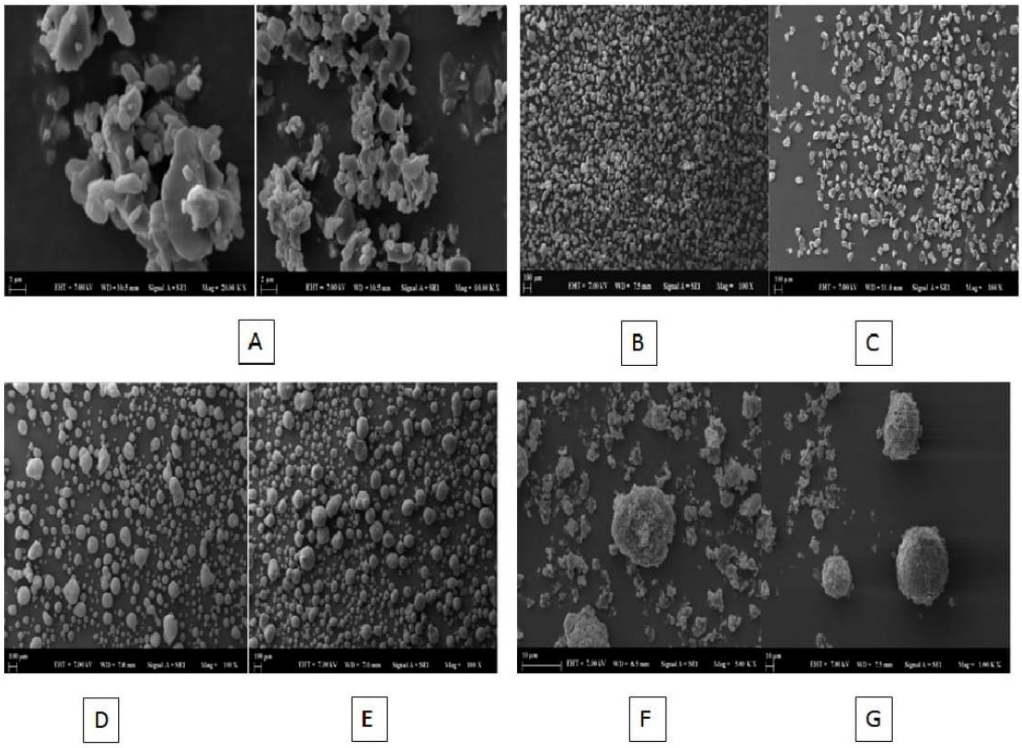

Figure 8. Scanning electron microscopy photographs of (**A**) Pure DFX, (**B**) Syloid XDP 3150, (**C**) P5-40-SYLOID, (**D**) Neusilin US2, (**E**) P5-40-US2, (**F**) Neusilin UFL2, and (**G**) P5-40-UFL2.

2.9. In Vitro Dissolution Studies of DFX-S-SNEDDS

In vitro dissolution studies were conducted to evaluate the release characteristics of DFX from developed DFX-S-SNEDDS formulations (P5-40-UFL2, P5-40-US2, and P5-40-SYLOID) and to compare their drug release characteristics with that of the market product of DFX (Exjade®, Novartis, Switzerland) in a dissolution medium of phosphate buffer of pH 6.8 containing 0.5% Tween 20. The release profiles of DFX from S-SNEDDS formulations and Exjade® are shown in Figure 9.

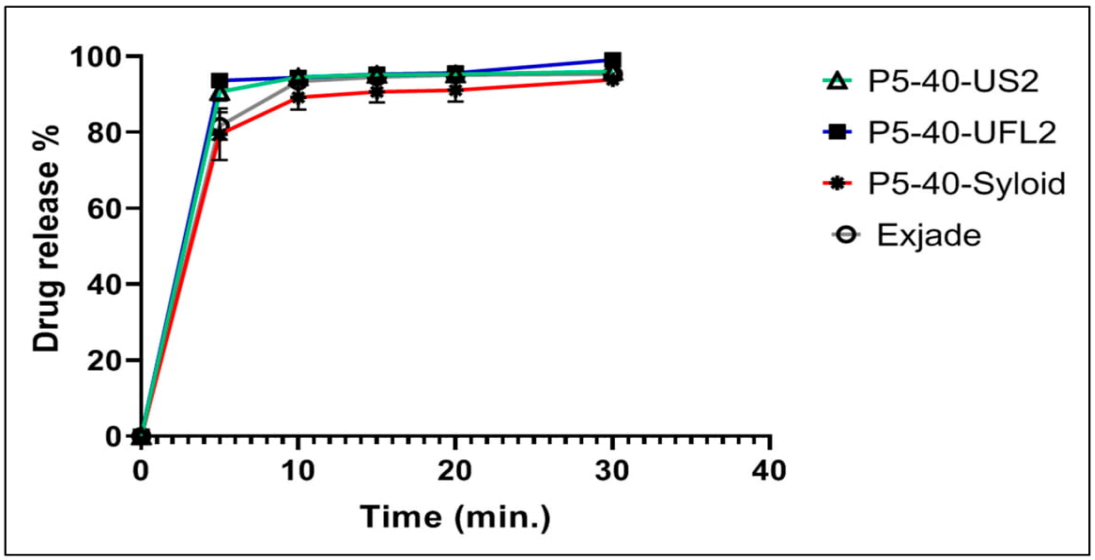

Figure 9. Drug release % of DFX from optimized S-SNEDDS formulated with different carriers and its commercial tablet (Exjade®) in phosphate buffer of pH 6.8 containing 0.5% Tween 20 (dissolution media recommended by the FDA). The data represent drug release (%) versus time (in min) in terms of mean ± SD ($n = 3$) ($p < 0.05$).

The dissolution data indicated that the release performance of DFX from S-SNEDDS formulations was significantly improved. Drug release occurred within 5 min. The Q5% observed by the P5-40-UFL2 formulation (93.6 ± 0.7%) and P5-40-US2 formulation (90.67 ± 2.35%) was significantly higher than that of the market tablet preparation of DFX (81.65 ± 2.10%) ($p < 0.05$). However, in the case of the P5-40-SYLOID formulation, the Q5% of DFX was found to be 82.95 ± 4.59%, which is quite closer to the concentrations of DFX released from the market product.

The increase in the percentage of DFX released from S-SNEDDS formulations is likely due to the spontaneous formation of nanoemulsion with small globules in the nano size once contacted with the dissolution media, which results in higher surface area, which permits a faster rate of drug release and extent [34]. That could induce a higher absorption and higher oral bioavailability of DFX. The enhancement of the dissolution profile of P5-40-UFL2 and P5-40-US2 could also be related to the lower particle size of both adsorbents compared with the particle size of the Syloid XDP 3150 used in the P5-40-SYLOID formulation, which offer a higher surface area for dissolution. In addition, Neusilin US2 and Neusilin UFL2 are highly porous solids that allow a quick entrance of dissolution medium into the pores and rapid emulsification [24].

When comparing the drug release within 5 min (Q5%) from P5-40-UFL2 and P5-40-US2, P5-40-UFL2 exhibited a higher Q5% value compared withP5-40-US2, which was aligned with Park's study [35] reporting that Neusilin US2 used in the formulation of P5-40-US2 had a larger particle size and a similar specific surface area compared with Neusilin UFL2 which was used in formulation P5-40-UFL2. Therefore, this result could be due to the presence of larger numbers of long and narrow intra-particular pores where DFX could entrap inside these pores and cause a slightly less percentage of released DFX.

P5-40-UFL2 exhibited the highest drug release within 5 min. P5-40-UFL2 was selected for performing a dissolution study to check DFX release in phosphate buffer of pH 6.8 without using Tween 20 and to compare with the DFX release from the market product (Exjade®). As illustrated in Figure 10A, 31.25% of DFX was released from the market product at 30 min, while for P5-40-UFL2, the percentage released at 5 min was 99%. DFX release from formulation P5-40-UFL2 and market product Exjade® in a medium of pH 1.2 was also performed to mimic the stomach pH (Figure 10B).

This profile shows that the percentage released of DFX from P5-40-UFL2 is almost 3 times higher than that of market product Exjade.

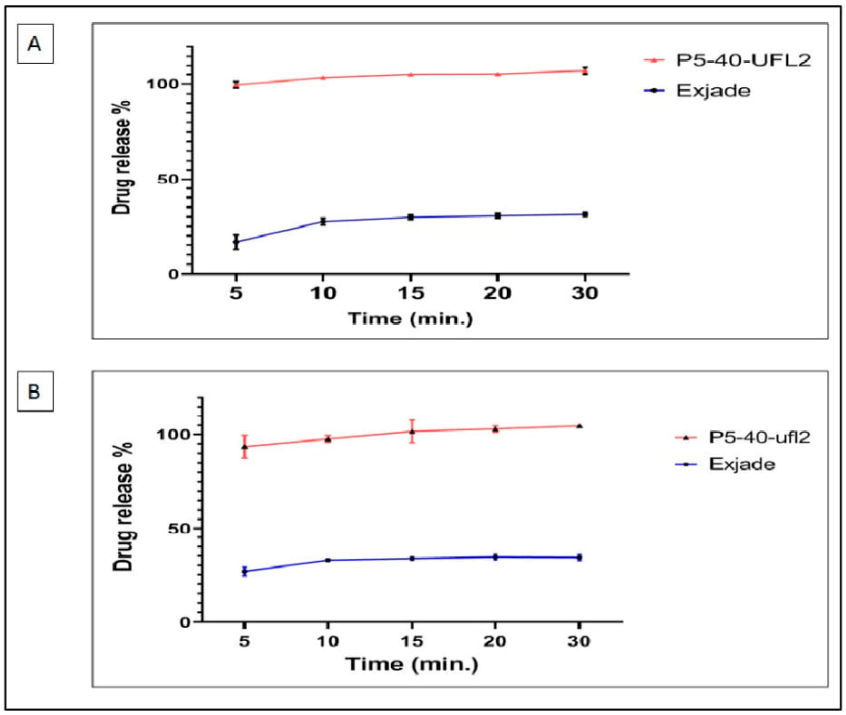

Figure 10. Drug release % of DFX from P5-40-UFL2 and its commercial tablet (Exjade®) in (**A**) phosphate buffer of pH 6.8 and (**B**) pH 1.2. The data represent drug release (%) versus time (in min) in terms of mean ± SD ($n = 3$) ($p < 0.05$).

These results of in vitro release tests emphasized that the aim of formulating DFX into SNEDDS formulations was successfully achieved for increasing its solubility. It has been previously explained by Ameeduzzafar et al. that surfactants and co-surfactants in SNEDDS reduce interfacial tension and that SNEEDS adsorbed to the carrier improves dissolution with high surface area, high porosity, small droplet size, rapid emulsification without the need for a special environment to obtain sink condition. [36]. Our dissolution data also supported that phenomenon.

2.10. Kinetic Analysis of DFX Release Data

The best fitting kinetic model for the in vitro release of DFX from S-SNEDDS formulations and for the market product (Exjade®) can be calculated from the highest values of the obtained determination coefficients (R^2). Table 7 showed that the r^2 values for DFX release from the market product (Exjade®), P6-40-UFL2, P6-40-US2, and P6-40-Syloid were the highest for the Korsemeyer–Peppas model with values of 0.842, 0.788, 0.914, and 0.848, respectively. The n value indicates the characteristic of release mechanisms [37]. An n value higher than 1.0 which was calculated for all formulations implies that DFX release from S-SNEDDS formulations follows the Super case-II transport release mechanism, which refers to the erosion of the polymer chain and explains the initial burst release [38].

Table 7. The determination of coefficient (R^2) and release exponent (n) values for in vitro release profiles of the market product (Exjade®) and DFX S-SNEDD formulations prepared by different carriers.

Formulation	Zero Order	First Order	Higuchi Model	Hixon Crowell Model	Korsemeyer–Peppas Model	
	R^2 *	R^2 *	R^2 *	R^2 *	R^2 *	nValue
Market product (Exjade®)	0.459	0.657	0.762	0.531	**0.842** **	1.323
P6-40-US2	0.403	0.562	0.707	0.672	**0.788** **	1.318
P6-40-UFL2	0.65	0.881	0.859	0.642	**0.914** **	1.385
P6-40-Syloid	0.479	0.725	0.775	0.847	**0.848** **	1.31

* R^2 (R squared) value is a statistical measure of how close the data to the fitted regression line for each model.
** Values in bold represent the highest r2 value for each formulation in comparison with different models.

3. Materials and Methods

3.1. Materials

DFX was kindly gifted from Sanovel Drug Company, Turkey. Labrafac, Lipophile WL1349, Labrafac PG, Peceol, Transcutol HP, Labrasol ALF, Labrafil M2125, Maisine, Gelucire 44/14 and Gelucire 48/16 were kindly gifted from Gatteffosse (Lyon, France). Kolliphor PS20, Kolliphor PS60, Kolliphor PS80, Kolliphor CS12 and Kolliphor HS15, Kolliphor EL, Kolliphor ELP, and Kollisov PEG 400 were kindly gifted from BASF (Ludwigshafen, Germany). Syloid XDP 3150 was gifted from Grace (Columbia, Maryland, USA). Neusilin UFL2 and Neusilin US2 were gifted from Fuji chemical (Tokyo, Japan). All other chemicals were of analytical grade.

3.2. Analytical Method for DFX Analysis

The analysis of DFX was performed using UV-VIS spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan) at 245 nm. The analysis method was validated according to International Conference on Harmonization (ICH) Q2. The diluent consisted of acetonitrile and methanol (50:50, *v*/*v*) [39]. It was also used for the analysis of the DFX solubility in different excipients and SNEDDSs formulations.

3.3. Optimization of Loaded L-SNEDDS

3.3.1. Equilibrium Solubility of DFX in the L-SNEDDS Components

Equilibrium solubility of DFX in different types of oils, surfactants, and co-surfactants were determined using the shaking flask method. An excess amount of DFX was added to 1 mL of each component in an Eppendorf tube, solid excipients were heated by using a water bath at 45 °C whenever necessary to facilitate melting, stirred by using a vortex mixer (Nuve NM 110, Biobase®, Shandong, China). The sealed vials were kept on an orbital shaker (Model 420, Thermo Electron Corporation®, Waltham, MA, USA) at 37 ± 0.5 °C for 48 h to attain an equilibrium, and then centrifuged (Model D-7200, Hettich®, Tuttlingen, Germany) at 5000 rpm for 15 min. [36]. The supernatant was filtered through PTFE filter membrane (0.45 µm, Alwsci®, Hangzhou, China) and analyzed at UV Spectrophotometer, as described in Section 3.2.

3.3.2. Construction of Pseudo-Ternary Phase Diagram

To understand the phase behavior and to observe the SNEDDS formation ratios of the SNEDDS excipients which are oil, surfactant, and co-surfactants, a pseudo-ternary diagram was constructed by using an aqueous titration technique [40], with oil, Smix (the mixture of surfactant to co-surfactant), and water, each representing an apex of the triangle. Based on solubility studies stated above, Peceol, Kolliphor EL, and Transcutol were selected as the oil phase, the surfactant, and the co-surfactant phase of SNEDDS, respectively.

Surfactant (Kolliphor EL) and co-surfactant (Transcutol) were mixed (Smix) with different weight ratios (1:1, 1:2, 1:3, 4:1, 2:1, 2:3, 3:1, 3:2, and 4:1) for each phase diagram. Specific Smix ratio was mixed with oil (Peceol) with different ratios (from 1:9 to 9:1) in separated glass vials stirred at 50 rpm. Then, they were titrated with purified water drop by drop and, vortexed after each addition at room temperature was observed by the naked eye for any turbidity or phase changes were reported and the weight of the water was recorded for use in the coming concentration measurements for constructing the phase diagram. The nine phase diagrams, one for each Smix, were constructed by using a ProSim Ternary Phase Diagram Software (Strategie, Cedex®, Toulouse, France).

3.3.3. SNEDDS Formation Assessment

In order to check nanoemulsion formation, each oil and Smix ratio previously prepared for pseudo-ternary phase diagram preparation was evaluated for the formation of nanoemulsion by diluting 50 mg of each of the mixtures to 50 mL with purified water (Merck Milli Q, Darmstadt, Germany) in a volumetric flask, and checked visually for the formation of nanoemulsion. The mixtures which formed stable and transparent dispersions were subjected to droplet size measurement and PDI by using ZetaSizer Nano ZS (Malvern 1000 HS®, Worcestershire, UK) [41]. These measurements were done at 37 °C after an equilibration time of 120 s. Each sample was measured 3 times with 12–17 runs for each measurement.

3.4. Equilibrium Solubility of DFX in Selected SNEDDSs Formulations

The goal of a SNEDDS formulation is to develop a formulation that is capable to upload maximum amount of DFX into 1 mL of the SNEDDS mixture. Therefore, equilibrium solubility of DFX was measured in the selected SNEDDSs formulations, by applying the same procedure for measuring DFX solubility in the excipients. An excess amount of DFX was added to 1 mL of each of the combinations in an Eppendorf tube, stirred by using a vortex mixer (Nuve NM 110, Biobase®, Shandong, China). The sealed vials were kept on the orbital shaker (Model 420, Thermo Electron Corporation®, Waltham, MA, USA) at 37 ± 0.5 °C for 48 h to attain an equilibrium, then centrifuged (Model D-7200, Hettich®, Tuttlingen, Germany) at 5000 rpm for 15 min. The supernatant was filtered through a PTFE filter membrane (0.45 µm, Alwsci®, Hangzhou, China) and analyzed at a UV Spectrophotometer, as described in Section 3.2.

3.5. Preparation of DFX Loaded SNEDDS Formulations

Based on solubility data for DFX in the selected formulations, DFX loaded SNEDDS (DEF-SNEDDS), which differ by amount loaded, were prepared by adding accurately weighted DFX to 1 mL of each of selected formulations and, mixing using a magnetic stirrer at 50 rpm for 30 min to allow solubilization. The mixtures were kept at room temperature in tightly closed glass bottles for further use. Table 8 below illustrates SNEDDS formulation codes and the composition of each formulation.

Table 8. Composition of the formulations prepared for further investigation.

SNEDDS Formulation Code	DFX Amount (mg/mL)	Peceol (*w*/*w*,%)	Kolliphor EL (*w*/*w*,%)	Transcutol HP (*w*/*w*,%)
P1-50	50	10	45	45
P2-50			30	60
P3-50			60	30
P4-50			36	54
P5-50			67.5	22.5
P6-50			54	36
P7-50			72	18

Table 8. *Cont.*

SNEDDS Formulation Code	DFX Amount (mg/mL)	Peceol (*w*/*w*,%)	Kolliphor EL (*w*/*w*,%)	Transcutol HP (*w*/*w*,%)
P1-45	45	10	45	45
P2-45			30	60
P3-45			60	30
P4-45			36	54
P5-45			67.5	225
P6-45			54	36
P7-45			72	18
P1-40	40	10	45	45
P2-40			30	60
P3-40			60	30
P4-40			36	54
P5-40			67.5	22.5
P6-40			54	36
P7-40			72	18

3.6. Characterization of DFX Loaded SNEDDS Formulations

3.6.1. Droplet Size and PDI Determination

In order to measure the droplet size and PDI of SNEDDS formulations, SNEDDS formulations 100-fold diluted in purified water were prepared and analyzed through a ZetaSizer (Malvern 1000 HS, Worcestershire, UK). These measurements were done at 37 °C after an equilibration time of 120 s, and each sample was measured 3 times with 12–17 runs for each measurement. The formulations which had a droplet size of less than 50 nm and optimum PDI values closer to zero were subjected to further characterization tests [41].

3.6.2. Thermodynamic Stability Studies

Thermodynamic stability studies were performed to evaluate visually the phase separation and effect of temperature variations on DFX loaded SNEDDS stability comprising centrifugation, heating–cooling cycle and freeze–thaw cycle [15].

The selected DFX loaded SNEDDS formulations which passed the requirements for droplet size and PDI were diluted with purified water (1:20) and centrifuged at 3500 rpm for 30 min to find out their stability as an isotropic single-phase system. Formulations that showed no signs of phase separation, creaming, or cracking were subjected to three heating cycles and three cooling cycles in which samples were incubated at 4 and 45 °C for 48 h. The formulations which passed heating and cooling cycles were more subjected to three freeze–thaw cycles at temperatures between −20 °C and 25 °C in a deep freezer for 48 h minimum [42]. Formulations which passed the stability test were subjected to further characterization.

3.6.3. Percentage Transmittance Determination (% T)

Nanoemulsions obtained from 100-fold dilution of DFX loaded SNEDDS in purified water were checked for their turbidity by measuring the percent transmittance (T, %) using UV–visible spectrophotometer at 638 nm; a blank used was purified water [43].

3.6.4. Dispersibility Test

The efficiency of self-emulsification of DFX loaded SNEDDSs formulations were assessed by using a standard USP-dissolution apparatus-II. To be specific, 1mL of each formulation was added separately to 500 mL distilled water pre-heated at 37 ± 0.5 °C with continuous gentle agitation at 50 rpm [44].

The time and efficacy for self-emulsifying were evaluated according to the following grading system: Grade A: refers to rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance; Grade B: indicated that emulsion rapidly forming (within 2 min), slightly less clear nanoemulsion, having a bluish white appearance; Grade C: being fine milky emulsion that was formed within 2 min; Grade D: was a dull-greyish white emulsion having slightly oil appearance that was slow to emulsify (longer than 2 min); Grade E: represented a formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface (longer than 3 min) [42].

3.6.5. Robustness to Dilution

In order to simulate in vivo environmental conditions and to examine the effects of dilution in solutions which mimic pH of physiological fluids of the gastrointestinal system, 50 and 100 times dilution of DFX loaded SNEDDS formulations into 0.1N HCl and phosphate buffer of different pH values of 4.5, 6.8, and 7.4 were performed. The prepared nanoemulsions were stored at ambient conditions for 12 h and checked visually for any phase separation or precipitation formation [45].

3.6.6. Effect of pH of the Dispersion Media on Droplet Size and PDI

The stability of DFX loaded SNEDDS formulations in different pH buffer solutions (pH 1.2, Ph4.5, pH 6.8 and, pH 7.4) was checked by 100-fold dilution in each of the buffer solutions, then subjected to droplet size and PDI measurements by ZetaSizer (Malvern 1000 HS, Worcestershire®, UK), and the values were compared with droplet size and PDI that resulted from dispersions in purified water [46].

3.6.7. Transmission Electron Microscopy (TEM)

For observing the surface morphology, SNEDD formulations were diluted 1:100 with purified water and the prepared samples were dropped onto the TEM grid (carbon coated, 300 mesh copper grid). The excess volume of the sample was removed with a filter paper. Samples were loaded into the microscope after 5 s of plasma cleaning. The images were captured and analyzed by a Digital Micrograph with JEM-ARM200, 200 kV, JEOL (Tokyo, Japan) [36].

3.7. In Vitro Cytotoxicity Studies

To evaluate the relative safety of the selected DFX loaded SNED formulation, P5-40 coded SNEDDS formulation was selected for in vitro cytotoxicity study which had less concentration of surfactant to avoid unexpected irritation of gastrointestinal tract [47]. The cytotoxic effects of DFX loaded SNEDDS formulation (P5-40) and the same drug-free SNEDDS formulation (P5°) were compared to that of pure DFX itself (Pure DFX). For this purpose, the human immortalized myelogenous leukemia cell line, K562, was used. K562 is a previously established and well-characterized leukemia cell line from American Type Culture Collection (Rockville, MD, USA). In recent studies, K562 cells are highlighted and used promising venues for various diseases as well as cancer research. These cells were maintained in RPMI-1640 medium (Biological Industries, Bait HaEme, Israel) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), penicillin (64 µg/mL), streptomycin (0.1 mg/mL) and L-glutamine at 5% CO_2 and at 37 °C. Cell survival was assessed using an MTT test.

3.7.1. MTT Assay

To quantify cell viability and proliferation of K562 cells after treatment with selected DFX loaded SNEDDS (P5-40), MTT assay was applied by using Cell proliferation Kit I (MTT) (Roche, Penzberg, Germany); 100 µL of K562 cells at a concentration of 4×10^4 cells/mL in culture medium were seeded into each well of a flat-bottomed 96- well plate and incubated 24 h in 5% CO_2 and at 37 °C. Thereafter, these cells were treated with 10, 30, and 40 µM of DFX loaded SNEDDS formulation (P5-40), placebo SNEDDS (P5°), and pure DFX and incubated for 24 h in 5% CO_2 and at 37 °C. Following this, 10 µL of MTT solution was added into each well, mixed gently, and incubated at 37 °C for 4 h. Then, formazan

crystals were dissolved by adding 100 µL DMSO into each well [31]. The absorbance was read at 570 nm using a microplate reader (VersaMax™, Molecular Devices LLC, San Jose, California, USA). Untreated cells were considered as experimental control in line with the literature and the MTT protocol and a similar protocol was applied in triplicate. The absorbance measurement for the research study was performed at 630 nm, where neither MTT nor formazan absorbs, to eliminate possible errors. The cytotoxic activity was expressed as cell viability (%), the concentration of viable cells, and all experiments were performed in triplicate.

$$\text{Cell viability } (\%) = \frac{(Absorbance\ of\ treated\ cells - Absorbance\ of\ blank)}{(Absorbance\ of\ control - Absorbance\ of\ blank)} \times 100 \quad (1)$$

3.7.2. Investigating Cell Morphology and Cell Proliferation Using Light Microscope

The morphology and proliferation of K562 cells were investigated under a light microscope (Leica Microsystems, Wetzlar, Germany). Cells were incubated for 24 and 48 hat 10, 30 and 40 µM of DFX loaded SNEDDS formulation (P5-40), placebo SNEDDS (P5°), and pure DFX, in order to see the cytotoxic effect to them.

3.8. Development of DFX Loaded Solid-SNEDDS (S-SNEDDS)

The solid DFX loaded SNEDDS formulations were attained by adsorbing DFX-SNEDDS formulation (P5-40) on solid carriers. Three different solid carriers were used: Neusilin®, US2, Neusilin® UFL2 (Fuji Chemical, Tokyo, Japan), and Syloid® XDP 3150 (Grace, Columbia, Maryland, USA) for preparing different batches of DFX-S-SNEDDS formulations. The oil adsorption capacity was measured for each solid carrier, which is defined as the amount of porous carrier required for transforming the unit dose of liquid oily formulation into the solid free flowing powder [48]. The 200 mg of each carrier was placed separately in a mortar and the formulation P5-40 was added drop wisely with good mixing after each addition until a non-sticky free flowing powder was obtained and the weight of P5-40 formulation used was recorded [49].

3.9. Characterization of DFX-S-SNEDDS

3.9.1. Fourier Transformed Infrared Spectroscopy (FT-IR)

FT-IR analysis of DFX, prepared DFX-S-SNEDDS and pure carriers (Neusilin® UFL2, Neusilin® US2 and Syloid®XDB 3150) were carried out. The spectra were recorded using Fourier transform infrared spectrophotometer (Perkin Elmer Spectrum One, MA, USA) in the range of 4000–650 cm^{-1}. The background was taken with air before shooting. By applying pressure to the sample, the peaks are provided more clearly [50].

3.9.2. SEM Imaging

Scanning electron micrographs for DFX, DFX-S-SNEDDSs formulation, and pure carriers (Neusilin® UFL2, Neusilin® US2, and Syloid® XDB 3150) were taken. Powder samples were woven on double-sided carbon bands and excess powders were swept with nitrogen gases. Carbon bands containing sample powders placed on the sample holders were subjected to the Gold–Palladium alloy coating process for 75 s using the Quorum SC7620 sputter coated (UK). Images with Zeiss Evo LS 10 scanning electron microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) have been implemented. The images were obtained under a 7 kV acceleration voltage using the secondary electron detector [43].

3.10. In Vitro Dissolution Studies of DFX-S-SNEDDS

In vitro dissolution studies were performed for optimum DFX loaded S-SNEDDS formulation (P5-40) solidified with the different adsorbents mentioned above and a market product of DFX (Exjade®, Novartis, Switzerland), using a USP dissolution apparatus II (Sotax Smart AT7, Thun, Switzerland).

As specified by the FDA [51], the paddle rotation speed was 75 rpm maintained at 37 ± 0.5 °C in 900 mL phosphate buffer with a pH value of 6.8 containing 0.5% Tween 20 as dissolution medium. A 3-mL volume of the aliquots was withdrawn at predetermined time points (5, 10, 15, 20, and 30 min), and filtered through a PTFE filter membrane (0.45 µm, Alwsci®, Hangzhou, China). The S-SNEDDS formulation (weight differ according to different carriers) which corresponded to 40 mg of DFX was added to each of the vessels. The blank used for S-SNEDDS formulation was S-SNEDDS formulation without DFX to avoid interferences from the excipients used in the formulation [52], while for the market product, the blank used was the same dissolution medium. These experiments were carried out in triplicate for each formulation.

Dissolution studies were performed in phosphate buffer at pH 6.8 without surfactants addition to see the effect of formulating DFX into SNEDDS formulation where the optimum formulation was compared with the market product. The other dissolution parameters were kept constant, except the dissolution medium of phosphate buffer with a pH value of 6.8 ± 0.05. Besides, the release of DFX from P5-40-UFL2 and market product was performed in a dissolution medium of pH 1.2 ± 0.05 while at the same time keeping the other dissolution parameter constants to mimic gastrointestinal media.

The amount of DFX dissolved in each medium was determined by separated validated spectrophotometric analysis method (UV-1601; Shimadzu, Japan) at 245 nm after a proper dilution with the medium used.

The amount of drug released into the medium was calculated (Equation (2)) and accordingly, the percentage of drug released was calculated according to Equation (3). The drug released (%) was plotted against time points.

$$\text{Amount of drug released} = \frac{\text{Concentration} \times \text{Dissolution path volume} \times \text{Dilution factor}}{1000} \quad (2)$$

$$\text{Drug release } (\%) = \frac{(\text{Amount of drug released into medium})}{\text{Total amount of drug}} \times 100 \quad (3)$$

3.11. Kinetic Analysis of DFX Release Data

For analyzing the in vitro release data, the in vitro release profile for market product and DFX S-SNEDDS of different carriers were fitted in various kinetic models. Models Zero order, First order, Higuchi model, Hixson–Crowell and Korsemeyer–Peppaswerewere applied, analyzed, and determination coefficients (R^2) were calculated for each model.

The drug release rate in the Zero order model is independent of its concentration in the systems (Equation (4)), while for the First order model, the release rate is concentration dependent (Equation (5)). Higuchi model describes the release of drugs from the insoluble matrix as a square root of time-dependent process based on Fickian diffusion (Equation (6)). The Hixson–Crowell model describes the drug release from systems where surface area and diameter of particles or tablets changing with time (Equation (7)). The Korsemeyer–Peppas model describes drug release from a polymeric system (Equation (8)) [37].

$$Qt = Q0 + k0t \quad (4)$$

K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$Log\ Ct = LogC0 - kt/2.303 \quad (5)$$

C_0 is the initial concentration of drug and K is first order constant.

$$Qt = Kt_{1/2} \quad (6)$$

K is the constant reflecting the design variables of the system.

$$Q0^{1/3} - Qt^{\frac{1}{3}} = K_{HC}t \quad (7)$$

Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in tablet and K_{HC} t is the rate constant for the Hixson–Crowell rate equation.

$$\frac{M_t}{M_\infty} = Ktn \quad (8)$$

M_t/M_∞ is fraction of drug released at time t, k is the rate constant and n is the release exponent.

3.12. Statistical Analysis

The results were compared by using two-way ANOVA test using GraphPad Prism 8.0.1 software. A p value of less than 0.05 was considered to be significant.

4. Conclusions

In the research study, a novel SNEDDS of DFX was developed consisting of Peceol (10%), Kolliphor EL (67.5%), and Transcutol HP (22.5%) as an oil phase, a surfactant, and a co-surfactant, respectively. The optimum SNEDDS formulation was further characterized, and it provided good thermodynamic stability with good self-emulsification efficiency. The developed clear nanoemulsion with a droplet size of 14.72 ± 1.50 nm upon dispersion with water was stable, and even stable against dilution and pH changes. The cell viability effect of SNEDDs formulation of DFX optimized in the research study was found to be relatively safe compared with the pure drug itself. Furthermore, the selected SNEDDS formulation of DFX was converted to DFX-S-SNEDDSs formulations by adsorbing it on different carriers (Neusilin UFL2, Neusilin US2, and Syloid XPD 3150). S-SNEDDSs formulations of DFX preserved the self-emulsification performance of the SNEDDS solidified with Neusilin UFL2 and exhibited the fastest in vitro DFX dissolution rate than those of the other adsorbents used, and even with its commercial product (Exjade®) in different dissolution media. These findings indicate enhanced dissolution of DFX by S-SNEED formulations.

Overall, our data support the solubility enhancement capability of DFX by optimized S-SNEDDS formulation, and furthermore, indicated that the optimized S-SNEDDS formulation of DFX has a potential for improving its oral bioavailability. Therefore, further in vivo studies would be investigated to confirm the bioavailability enhancement of the new solid SNEDDS formulation of DFX.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-8247/13/8/162/s1, Figure S1. FT-IR Spectrum of Pure Deferasirox, Figure S2. FT-IR Spectrum of P5-40-Ufl2 Formulation, Figure S3. FT-IR Spectrum of P5-50-Us2 Formulation, Figure S4. FT-IR Spectrum of P5-40-Syloid, Figure S5. FT-IR Spectrum of Neusilin Ufl2 Carrier, Figure S6. FT-IR Spectrum of Neusilin US2 Carrier, Figure S7. FT-IR Spectrum of Syloid XDP 3150 Carrier.

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Funding: This research received no external funding.

Acknowledgments: This work is a part of a PhD Thesis. Near East University authors would like to acknowledge Istanbul University for making use of their laboratories and for the support in developing this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tanaka, C. Clinical pharmacology of Deferasirox. *Clin. Pharm.* **2014**, *53*, 679–694. [CrossRef]
2. Hcp.novartis.com. JADENU®(Deferasirox) Safety & Side Effects|HCP. 2020. Available online: https://www.hcp.novartis.com/products/jadenu/chronic-iron-overload/safety-profile/ (accessed on 10 April 2020).
3. Dannenfelser, R.M.; Yalkowsky, S.H. Data base of aqueous solubility for organic non-electrolytes. *Sci. Total Environ.* **1991**, *109*, 625–628. [CrossRef]
4. Al Durdunji, A.; AlKhatib, H.S.; Al-Ghazawi, M. Development of a biphasic dissolution test for Deferasirox dispersible tablets and its application in establishing an in vitro–in vivo correlation. *Eur. J. Pharm. Biopharm.* **2016**, *102*, 9–18. [CrossRef]
5. Waldmeier, F.; Bruin, G.J.; Glaenzel, U.; Hazell, K.; Sechaud, R.; Warrington, S.; Porter, J.B. Pharmacokinetics, metabolism, and disposition of deferasirox in β-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. *Drug Metab.* **2010**, *38*, 808–816. [CrossRef] [PubMed]
6. Reddy, B.B.K.; Karunakar, A. Biopharmaceutics classification system: A regulatory approach. *Dissolut Technol.* **2011**, *18*, 31–37. [CrossRef]
7. Theerasilp, M.; Chalermpanapun, P.; Ponlamuangdee, K.; Sukvanitvichai, D.; Nasongkla, N. Imidazole-modified deferasirox encapsulated polymeric micelles as pH-responsive iron-chelating nanocarrier for cancer chemotherapy. *RSC Adv.* **2017**, *7*, 11158–11169. [CrossRef]
8. Gülsün, T.; Akdağ, Y.; Izat, N.; Öner, L.; Şahin, S. Effect of particle size and surfactant on the solubility, permeability and dissolution characteristics of deferasirox. *J. Res. Pharm.* **2019**, *23*, 851–859. [CrossRef]
9. Shrestha, H.; Bala, R.; Arora, S. Lipid-based drug delivery systems. *J. Pharm.* **2014**, *2014*, 801820. [CrossRef]
10. Shakeel, F.; Mohsin, K.; Alanazi, F.K.; Alsarra, I.A.; Haq, N. Thermodynamics and solubility prediction of talinolol in self-nanoemulsifying drug delivery system (SNEDDS) and its oil phase components using mathematical modelling. *J. Drug Deliv. Sci. Technol.* **2014**, *24*, 533–538. [CrossRef]
11. Pouton, C.W.; Porter, C.J. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies. *Adv. Drug Deliv. Rev.* **2008**, *60*, 625–637. [CrossRef]
12. Khan, A.W.; Kotta, S.; Ansari, S.H.; Sharma, R.K.; Ali, J. Potentials and challenges in self-nanoemulsifying drug delivery systems. *Expert Opin. Drug Deliv.* **2012**, *9*, 1305–1317. [CrossRef] [PubMed]
13. Tang, B.; Cheng, G.; Gu, J.C.; Xu, C.H. Development of solid self-emulsifying drug delivery systems: Preparation techniques and dosage forms. *Drug Discov. Today* **2008**, *13*, 606–612. [CrossRef] [PubMed]
14. Chatterjee, B.; Almurisi, S.H.; Dukhan, A.A.M.; Mandal, U.K.; Sengupta, P. Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. *Drug Deliv.* **2016**, *23*, 3639–3652. [CrossRef] [PubMed]
15. Kassem, A.A.; Mohsen, A.M.; Ahmed, R.S.; Essam, T.M. Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design, optimization, in vitro and in vivo evaluation. *J. Mol.* **2016**, *218*, 219–232. [CrossRef]
16. Patil Prashant, P.; Vaishali, K.; Santosh, P. Potential investigation of peceol for formulation of ezetimibe self nanoemulsifying drug delivery systems. *Asian J. Biomed. Pharm. Sci.* **2016**, *6*, 20–47.
17. Park, J.H.; Kim, D.S.; Mustapha, O.; Yousaf, A.M.; Kim, J.S.; Kim, D.W.; Yong, C.S.; Youn, Y.S.; Oh, K.T.; Lim, S.J.; et al. Comparison of a revaprazan-loaded solid dispersion, solid SNEDDS and inclusion compound: Physicochemical characterisation and pharmacokinetics. *Colloid Surf. B* **2018**, *162*, 420–426. [CrossRef]
18. Sachs-Barrable, K.; Thamboo, A.; Lee, S.D.; Wasan, K.M. Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. *J. Pharm. Sci.* **2007**, *10*, 319–331.
19. Hugger, E.D.; Novak, B.L.; Burton, P.S.; Audus, K.L.; Borchardt, R.T. A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity in vitro. *J. Pharm. Sci.* **2002**, *91*, 1991–2002. [CrossRef]
20. Jakab, G.; Fülöp, V.; Bozó, T.; Balogh, E.; Kellermayer, M.; Antal, I. Optimization of quality attributes and atomic force microscopy imaging of reconstituted nanodroplets in baicalin loaded self-nanoemulsifying formulations. *Pharmaceutics* **2018**, *10*, 275. [CrossRef]
21. Yan, Y.D.; Kim, J.A.; Kwak, M.K.; Yoo, B.K.; Yong, C.S.; Choi, H.G. Enhanced oral bioavailability of curcumin via a solid lipid-based self-emulsifying drug delivery system using a spray-drying technique. *Biol. Pharm. Bull.* **2011**, *34*, 1179–1186. [CrossRef]

22. Pouton, C.W. Lipid formulations for oral administration of drugs: Non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.* **2000**, *11*, S93–S98. [CrossRef]
23. Gupta, S.; Chavhan, S.; Sawant, K.K. Self-nanoemulsifying drug delivery system for adefovirdipivoxil: Design, characterization, in vitro and ex vivo evaluation. *Colloids Surf. A Physicochem. Eng. Asp.* **2011**, *392*, 145–155. [CrossRef]
24. Inugala, S.; Eedara, B.B.; Sunkavalli, S.; Dhurke, R.; Kandadi, P.; Jukanti, R.; Bandari, S. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) ofdarunavir for improved dissolution and oral bioavailability: In vitro and in vivo evaluation. *Eur. J. Pharm. Sci.* **2015**, *74*, 1–10. [CrossRef]
25. Mohd, A.B.; Sanka, K.; Bandi, S.; Diwan, P.V.; Shastri, N. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of glimepiride: Development and antidiabetic activity in albino rabbits. *Drug Deliv.* **2015**, *22*, 499–508. [CrossRef]
26. Jaiswal, P.; Aggarwal, G.; Harikumar, S.L.; Singh, K. Development of self-microemulsifying drug delivery system and solid-self-microemulsifying drug delivery system of telmisartan. *Int. J. Pharm. Investig.* **2014**, *4*, 195. [PubMed]
27. Syukri, Y.; Martien, R.; Lukitaningsih, E.; Nugroho, A.E. Novel Self-Nano Emulsifying Drug Delivery System (SNEDDS) of andrographolide isolated from AndrographispaniculataNees: Characterization, in-vitro and in-vivo assessment. *J. Drug Deliv. Sci. Technol.* **2018**, *47*, 514–520. [CrossRef]
28. Usmani, A.; Mishra, A.; Arshad, M.; Jafri, A. Development and evaluation of doxorubicin self nanoemulsifying drug delivery system with Nigella Sativa oil against human hepatocellular carcinoma. Artif. *Cells Nanomed. Biotechnol.* **2019**, *47*, 933–944.
29. Balakumar, K.; Raghavan, C.V.; Abdu, S. Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids Surf. B* **2013**, *112*, 337–343. [CrossRef]
30. Kang, B.K.; Lee, J.S.; Chon, S.K.; Jeong, S.Y.; Yuk, S.H.; Khang, G.; Lee, H.B.; Cho, S.H. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *Int. J. Pharm.* **2004**, *274*, 65–73. [CrossRef]
31. Kumar, R.; Khursheed, R.; Kumar, R.; Awasthi, A.; Sharma, N.; Khurana, S.; Kapoor, B.; Khurana, N.; Singh, S.K.; Gowthamarajan, K.; et al. Self-nanoemulsifying drug delivery system of fisetin: Formulation, optimization, characterization and cytotoxicity assessment. *J. Drug Deliv. Sci. Technol.* **2019**, *54*, 101252. [CrossRef]
32. Mandić, J.; Pobirk, A.Z.; Vrečer, F.; Gašperlin, M. Overview of solidification techniques for self-emulsifying drug delivery systems from industrial perspective. *Int. J. Pharm.* **2017**, *533*, 335–345. [CrossRef] [PubMed]
33. Thomas, S.; Joshi, S.C.; Vir, D.; Agarwal, A.; Rao, R.D.; Sridhar, I.; Mathela, C.S. Identification, characterization and quantification of a new impurity in deferasirox active pharmaceutical ingredient by LC–ESI–QT/MS/MS. *J. Pharm. Biomed.* **2012**, *63*, 112–119. [CrossRef]
34. Rahman, M.A.; Mujahid, M. Development of self-nanoemulsifying tablet (SNET) for bioavailability enhancement of sertraline. *Braz. J. Pharm. Sci.* **2018**, *54*, e17232. [CrossRef]
35. Park, J.B.; Choi, B.K.; Kang, C.Y. Effects of absorbent materials on a self-emulsifying drug delivery system for a poorly water soluble drug. *Int. J. Pharm. Investig.* **2018**, *45*, 529–539. [CrossRef]
36. El-Bagory, I.; Alruwaili, N.K.; Elkomy, M.H.; Ahmad, J.; Afzal, M.; Ahmad, N.; Elmowafy, M.; Alharbi, K.S.; Alam, M.S. Development of novel dapagliflozin loaded solid self-nanoemulsifying oral delivery system: Physiochemical characterization and in vivo antidiabetic activity. *J. Drug Deliv. Sci. Technol.* **2019**, *54*, 101279.
37. Costa, P.; Lobo, J.M.S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **2001**, *13*, 123–133. [CrossRef]
38. Eltobshi, A.A.; Mohamed, E.A.; Abdelghani, G.M.; Nouh, A.T. Self-nanoemulsifying drug-delivery systems for potentiated anti-inflammatory activity of diacerein. *Int. J. Nanomed.* **2018**, *13*, 6585. [CrossRef]
39. Mohammed, S.S. Formulation of deferasirox into dispersible tablet. *AJRBPS* **2014**, *2*, 118–130.
40. Czajkowska-Kośnik, A.; Szekalska, M.; Amelian, A.; Szymańska, E.; Winnicka, K. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules* **2015**, *20*, 21010–21022. [CrossRef]
41. Shakeel, F.; Haq, N.; Alanazi, F.K.; Alsarra, I.A. Thermodynamic modeling for solubility prediction of indomethacin in self-nanoemulsifying drug delivery system (SNEDDS) and its individual components. *Drug Dev. Ind. Pharm.* **2014**, *40*, 1240–1245. [CrossRef]

42. Khan, A.W.; Kotta, S.; Ansari, S.H.; Sharma, R.K.; Ali, J. Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: Design, characterization, in vitro and in vivo evaluation. *Drug Deliv.* **2015**, *22*, 552–561. [CrossRef] [PubMed]
43. Parmar, N.; Singla, N.; Amin, S.; Kohli, K. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) selfnanoemulsifying drug delivery system. *Colloid Surf. B* **2011**, *86*, 327–338. [CrossRef] [PubMed]
44. Nasr, A.; Gardouh, A.; Ghorab, M. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartanmedoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. *Pharmaceutics* **2016**, *8*, 20. [CrossRef] [PubMed]
45. Selvam, P.R.; Kulkarni, P.K.; Dixit, M. Preparation and evaluation of self-nanoemulsifying formulation of efavirenz. *Indian J. Pharm. Educ.* **2013**, *47*, 47–54.
46. Kumar Mantri, S.; Pashikanti, S.; Ramana Murthy, K.V. Development and characterization of self-nanoemulsifying drug delivery systems (SNEDDS) of atorvastatin calcium. *Curr. Drug Deliv.* **2012**, *9*, 182–196. [CrossRef] [PubMed]
47. Date, A.A.; Desai, N.; Dixit, R.; Nagarsenker, M. Self-nanoemulsifying drug delivery systems: Formulation insights, applications and advances. *Nanomed. J.* **2010**, *5*, 1595–1616. [CrossRef]
48. Ghosh, D.; Singh, S.K.; Khursheed, R.; Pandey, N.K.; Kumar, B.; Kumar, R.; Gulati, M. Impact of solidification on micromeritic properties and dissolution rate of self-nanoemulsifying delivery system loaded with docosahexaenoic acid. *Drug Dev. Ind. Pharm.* **2020**, *46*, 597–605. [CrossRef]
49. Beg, S.; Swain, S.; Singh, H.P.; Patra, C.N.; Rao, M.B. Development, optimization, and characterization of solid self-nanoemulsifying drug delivery systems of valsartan using porous carriers. *AAPS Pharmscitech* **2012**, *13*, 1416–1427. [CrossRef]
50. Parmar, K.; Patel, J.; Sheth, N. Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. *Asian J. Pharm. Sci.* **2015**, *10*, 396–404. [CrossRef]
51. Dissolution Methods. Available online: https://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm (accessed on 10 April 2020).
52. Kanuganti, S.; Jukanti, R.; Veerareddy, P.R.; Bandari, S. Paliperidone-loaded self-emulsifying drug delivery systems (SEDDS) for improved oral delivery. *J. Dispers. Sci. Technol.* **2012**, *33*, 506–515. [CrossRef]

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High school	Subaihi high shcool	2007

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Part time lecturer		Jordan University	Jun 2015-Aug 2015
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English	Very good	Very good	Very good
-	-	-	-

Foreign Language Examination Grade								
YDS	ÜDS	IELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
-	-	-	-	-	-	-	-	-

	Math	Equally weighted	Non-math
ALES Grade	-	-	-
(Other) Grade	-	-	-

Computer Knowledge

Program	Use proficiency
GraphPad Prism	Very good

*****Evaluate as very good, good, moderate, poor.