

T.C  
YEDITEPE UNIVERSITY  
INSTITUTE OF HEALTH SCIENCES  
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

**Bioavailability Enhancement of Poorly  
Soluble Drugs with The Approach  
of Self-Emulsifying Drug Delivery Systems**

MASTER THESIS

RAWAN TAREK RANNA, B Pharm

ISTANBUL, 2021

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SUPERVISOR  
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## THESIS APPROVAL FORM

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### APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ  
Director of Institute of Health Sciences

## **DECLARATION**

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

30/06/2021

Rawan Ranna



I dedicate this thesis to my beloved father (Tarek Mohammad Mamoon Ranneh), my adorable mother (Saja Aslan Alhariri), my brothers (Mamoon, Mayar, and Sameh, my fiancé and life partner to be (Mohammad Harun Noori), and finally, my friends Doaa and Diana for their continuous support to me in all aspects of life. Moreover, encouraging me to be the best version of myself. I will always be grateful for everything they have done for me.

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## LIST OF SYMBOLS/ABBREVIATIONS

|           |                                                   |
|-----------|---------------------------------------------------|
| °C        | Degree centigrade                                 |
| ml/min    | Milliliter per minute                             |
| μm        | Micrometer                                        |
| N         | The number of droplets                            |
| nm        | Nanometer                                         |
| GI        | Gastrointestinal                                  |
| P-gp      | P-glycoprotein                                    |
| B-S-SEDDS | Blank solid self-emulsifying drug delivery system |
| GIT       | Gastrointestinal tract                            |
| BCS       | Biopharmaceutics classification system            |
| U.S.A.    | United states of America                          |
| LBDDS     | Lipid-based drug delivery systems                 |
| o/w       | Oil in water                                      |
| SMEDDS    | Self-micro-emulsifying drug delivery system       |
| MCM       | Medium-chain monoglycerides                       |
| r         | Radius                                            |
| MCT       | Medium-chain triglycerides                        |
| w/w       | Weight to weight                                  |
| B-SEDDS   | Blank self-emulsifying drug delivery system       |
| HLB       | Hydrophile-lipophile balance                      |
| w/o       | Water in oil                                      |
| PG        | Propylene glycol                                  |
| FDA       | Food and drug administration                      |
| PEG       | Polyethylene glycol                               |
| pH        | The potential of hydrogen                         |
| GIF       | Gastro-intestinal fluids                          |
| LCT       | Long-chain triglycerides                          |
| log p     | Partition coefficient                             |
| s         | The interfacial energy                            |
| π         | pi symbol                                         |

|                  |                                                    |
|------------------|----------------------------------------------------|
| %                | Percent sign                                       |
| DC               | Drug content                                       |
| Ca               | Actual concentration                               |
| Ct               | Theoretical concentration.                         |
| HPLC             | High-performance liquid chromatography             |
| N                | Normality (0.1 N)                                  |
| HCl              | Hydrochloric acid                                  |
| SEDDS            | Self-emulsifying drug delivery system              |
| Rpm              | Rounds per minute                                  |
| P <sub>o/w</sub> | Oil/water partition coefficient                    |
| hrs              | Hours                                              |
| vivo             | Within the living                                  |
| in vitro         | Within the laboratory                              |
| USP              | United states pharmacopeia                         |
| g                | Gram                                               |
| kg               | Kilograms                                          |
| B-L-SEDDS        | Blank liquid self-emulsifying drug delivery system |
| mL               | Milliliter                                         |
| SLS              | Sodium lauryl sulphate                             |
| min              | Minute                                             |
| R.I.             | Refractive index                                   |
| UV               | Ultraviolet radiation                              |
| w/v              | Weight to volume                                   |
| v/v              | Volume to volume                                   |
| C18              | The molecules contain 18 carbon atoms              |
| S-SEDDs          | Solid-self-emulsifying drug delivery system        |
| HPMC             | Hydroxypropylmethylcellulose                       |
| HPC              | Hydroxypropylcellulose                             |
| MCC              | Microcrystalline cellulose                         |
| SE               | Self-emulsify                                      |
| SMES             | Self-micro-emulsifying suppositories               |
| β                | Beta                                               |

|                       |                                                                                             |
|-----------------------|---------------------------------------------------------------------------------------------|
| CMC                   | Carboxymethyl cellulose                                                                     |
| BCNU                  | A brand name of carmustine                                                                  |
| LC-MS/MS              | Liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. |
| PLGA                  | Poly(lactic-co-glycolic acid)                                                               |
| SES                   | Self-emulsifying system                                                                     |
| PPB                   | Porous polystyrene beads                                                                    |
| GMO                   | Glyceryl monooleate                                                                         |
| PTX                   | Paclitaxel                                                                                  |
| o/w/o                 | Oil in water in Oil                                                                         |
| CoQ10                 | Coenzymum Q10                                                                               |
| L-SEDDs               | Liquid-self-emulsifying drug delivery system                                                |
| ZTO                   | Zedoary turmeric oil                                                                        |
| SEPS                  | Self-emulsifying phospholipid suspension                                                    |
| EPL                   | Eplerenone                                                                                  |
| log Ko/wOctanol/water | partition coefficient                                                                       |
| CYP3A4                | Cytochrome P450 3A4                                                                         |
| NIOS                  | No indication of segregation                                                                |
| HP-TLC                | High-performance thin-layer chromatography                                                  |
| µg/mL                 | Micrograms per milliliter                                                                   |
| r <sup>2</sup>        | Coefficient of determination                                                                |
| DG                    | Free energy                                                                                 |
| DLS                   | Dynamic light scattering                                                                    |
| DAD                   | Diode array detector                                                                        |
| CAN                   | Acetonitrile                                                                                |
| S\Cos                 | Surfactants\co-surfactants                                                                  |
| S-mix                 | Surfactants mixture                                                                         |
| oil:s-mix             | Oil-surfactant mixture                                                                      |
| SNEDDS                | Self-nano-emulsifying drug delivery system                                                  |
| PDI                   | Polydispersity index                                                                        |
| ZP                    | Zeta potential                                                                              |
| DS                    | Droplet size                                                                                |

|                  |                                                                                     |
|------------------|-------------------------------------------------------------------------------------|
| DSC              | Differential scanning calorimetry                                                   |
| EPL-S-SEDDs      | EPL loaded solid self-emulsifying drug delivery system                              |
| RV               | Rotational viscometer                                                               |
| FT-IR            | Fourier transform infrared spectroscopy                                             |
| cm <sup>-1</sup> | Inverse centimeter                                                                  |
| ng\mL            | Nanograms per milliliter                                                            |
| DMSO             | Dimethylsulfoxide                                                                   |
| F1               | Formulation 1                                                                       |
| F2               | Formulation 2                                                                       |
| F3               | Formulation 3                                                                       |
| SET              | Self emulsifying tablets                                                            |
| RP-HPLC          | Reversed-phase HPLC                                                                 |
| SD               | Standard deviation                                                                  |
| mV               | Megavolt                                                                            |
| NUS2             | Neusilin US2                                                                        |
| RH               | Relative humidity                                                                   |
| HPLC-ESI-MS      | High-performance liquid chromatography<br>electrospray ionization mass spectrometry |
| SEDDs-F1         | Self-emulsifying drug delivery system formulation 1                                 |
| kV               | Kilovolt                                                                            |
| A200             | Aerosil 200                                                                         |
| SIP              | Segregation is present                                                              |

## ABSTRACT

**Ranna, R (2021). Bioavailability Enhancement of Poorly Soluble Drugs with The Approach of Self-Emulsifying Drug Delivery Systems. Yeditepe University Institute of Health Sciences, Drug and Cosmetics Production Technology, İstanbul.**

Eplerenone (EPL) is the second aldosterone antagonist registered for usage in the USA. It is utilized for the cure of heart failure, hypertension, and ventricular-systolic dysfunction. EPL is considered a class II medication, as it has poor solubility - high permeability. Our purpose in this study is to increase the solubility of EPL by using a self-emulsifying drug delivery system (SED DS) and then solidifying it to form nanoparticles to be compared with the marketed and pure powder through in vitro dissolution studies. Solubility studies were done with different oils, surfactants, and co-surfactants to determine which one has the highest solubility for Eplerenone.

After that, the ternary phase diagram was formed to conduct the perfect percentage for the primary three components (oil, surfactant, and co-surfactant) to determine the optimum SED DS formulation composition. The solidification technique followed this procedure to increase the stability of liquid SED DS and many more advantages. The solidification process was carried out with adsorption to solid carriers method by using FLO (calcium silicate), HPC (Low-substituted hydroxypropyl cellulose), Aerosil 200, and Neusilin US2. The prepared liquid and powdered SED DS formulations were characterized and compared with the marketed product and pure drug of eplerenone.

The solubility screening results showed high solubility of EPL in triacetin as oil, Kolliphor EL and tween 80 as surfactant and finally PEG 200, DMSO, transcuto l p as co-surfactant and with ternary phase diagram optimum concentration of oil, surfactant and co-surfactant ratio have been determined. During the dissolution study, the results showed a remarkable increase in EPL dissolution rate (the entire dose in 30 minutes) when combined with S-SED DS-F3 X compared to the marketed and pure drug powder ( $p < 0.05$ ). Overall, this study approved that EPL's dissolution and oral bioavailability can be improved by SED DS technology.

**Key Words:** SED DS, S-SED DS, Eplerenone, Solidification, Adsorption to solid carrier

## ÖZET

**Ranna, R (2021). Kendiliğinden Emülsifiye Olabilen İlaç Taşıyıcı Sistemler ile Düşük Çözünürlük Gösteren İlaçların Biyoyararlanımının Arttırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü İlaç ve Kozmetik Üretim Teknolojisi, İstanbul.**

Eplerenon (EPL), ABD'de kullanım için kayıtlı ikinci aldosteron antagonistidir. Kalp yetmezliği, hipertansiyon ve ventriküler-sistolik disfonksiyon tedavisinde kullanılır. EPL, zayıf çözünürlüğü - yüksek geçirgenliği olduğu için sınıf II ilaç olarak kabul edilir. Bu çalışmadaki amacımız, kendi kendine emülsifiye edici ilaç taşıma sistemleri (SEDDS) ve bunları katı taşıyıcılar üzerine adsorbe ettirerek EPL'nin çözünürlüğünü arttırmak ve in vitro çözünme hızı çalışmaları ile ticari ilaç ve saf haldeki etken maddenin çözünürlük profillerini kıyaslamaktır. Çözünürlük çalışması yapılarak EP'un en fazla çözündüğü yağ, yüzey aktif madde ve yardımcı yüzey aktif madde bulunmuştur. Optimum SEDDS kompozisyonu üçgen faz diyagramı kullanılarak tespit edilmiştir. Sıvı SEDDS daha sonra stabilitesini artırmak ve daha birçok avantaj kazandırılabilmesi için inert katı bir taşıyıcı yüzeyine adsorbe ettirilmiştir. İnert katı taşıyıcı olarak FLO (calcium silicate), HPC (Low-substituted hydroxypropyl cellulose), Aerosil 200 and Neusilin US2 kullanılmıştır. Hazırlanan sıvı ve katı haldeki SEDDS lerin karakterizasyon çalışmaları yapılmış ve EP çözünme hızı piyasa ilacı ve saf ilaç etken maddesi ile kıyaslanmıştır. Çözünürlük çalışmaları sonucunda yağ olarak triasetin, yüzey etken madde olarak Kolliphor EL ve tween 80, yardımcı çözücü olarak PEG 200, DMSO ve transcutol p EPL un en fazla çözündüğü ortam olarak bulunmuş ve üçgen faz diyagramı kullanılarak yağ, yüzey etken madde ve yardımcı çözücünün optimum oranları belirlenmiştir. Katı hale getirilmiş kendiliğinden emülsifiye olabilen ilaç taşıyıcı sistemlerin çözünürlük profilinin (tüm doz 30 dk içerisinde çözünmüştür) ticari ürün ve saf haldeki ilaç etken maddesi çözünürlük profiline göre yüksek olduğu bulunmuştur ( $p < 0.05$ ). Bu çalışma ile elde edilen bulgular EPL'nin çözünme hızının ve oral biyoyararlanımının SEDDS teknolojisi ile artırılabilirliğini göstermiştir.

**Anahtar Kelimeler:** SEDDS, Katı SEDDS, Eplerenon, Katılaşma, Katı taşıyıcı yüzey üzerine adsorbsiyon

## **1. INTRODUCTION and PURPOSE**

### **1.1. AIM OF THE STUDY**

This thesis aims to boost solubility and, therefore, the bioavailability of low soluble drugs through the application of self-emulsifying drug delivery systems. To achieve that goal, we used Eplerenone as a low soluble antihypertensive agent, then combined it with the liquid SEDDS formulation and finally solidify it using adsorption to solid carrier technique. Applying this drug delivery system accommodates the occupying of the entire drug dosage within the formulation and raises the dissolution rate compared with the marketed and unformulated drug.

### **1.2. Oral drug delivery**

Oral administration is considered to be the most often utilized route of administration for medications used to treat chronic illnesses. The vast majority of medications are usually taken orally. [1] Such medicines' oral bioavailability is essentially determined by their solubility and dissolution, and with a low magnitude and a high inter and intra subject variation. Furthermore, oral bioavailability is influenced by a variety of other drug aspects, including GI fluid consistency, intestinal permeability, ability to resist metabolic processes by both the cytochrome P450 class of enzymes found throughout gastrointestinal enterocytes and hepatic hepatocytes, as well as ability to interact with efflux transporter systems including P-glycoprotein (P-gp). [2]

Approximately 40% of new chemical entities exhibit poor aqueous solubility and present a significant challenge to the modern drug delivery system. The rate-limiting step for absorbing these drugs is often their solubilization in the gastrointestinal tract (GIT). [3] A considerable formulation strategy is required to improve the bioavailability of several poorly water-soluble substances. [4] These drugs are classified as class II drugs by the Biopharmaceutics Classification System (BCS), with poor aqueous solubility and high permeability. [3]

### 1.3. Biopharmaceutics classification system (BCS)

The Biopharmaceutics classification system (BCS) is a reference supplied by the US Food and Drug Administration (FDA) for estimating gastrointestinal medicine absorption. From a biopharmaceutical viewpoint, BCS is a helpful resource for formulation development evaluation. [5] BCS classifies medications under four groups based on drug solubility as well as gut permeability, as illustrated in (Table 1.2).

**Table 1.2** The Biopharmaceutics classification system (BCS) [5]

|                   | High solubility | Low solubility |
|-------------------|-----------------|----------------|
| High permeability | Class 1         | Class 2        |
| Low permeability  | Class 3         | Class 4        |

When the greatest dose strength of a drug component is dissolved within 250 mL or less of aqueous solutions with a pH range of 1-6.8, it is classified as a highly soluble drug. On the other side, a drug substance is classified as high permeable when the amount of uptake in humans equals 85 percent or higher of an administered dose depending either on mass balance measurement or compared to an intravenously reference dosage. [6] Due to their inadequate permeability, Class III and Class IV drugs are poorly bioavailable, lowering their potential therapeutic impact or demanding large doses to accomplish it. [7]

### 1.4. Lipid-based formulations to improve the solubility of low soluble medicines

Various formulation strategies are exploited to overcome drug solubility problems, such as the use of surfactants, permeation enhancers, solid dispersions, salt formation, cyclodextrins, nanoparticles, and micronization. Each technique for boosting bioavailability has benefits and drawbacks. [8] Lipid-based drug delivery systems (LBDDS) have by far the most advanced capabilities and potent considerations among the existing formulation approaches to boost solubility. [9] Lipid-based formulations increase the solubilization of drugs during GI transit and fabricate the lipophilic ambiance at a micro level to oblige drug transportation towards intestinal absorptive sites. [10] Various factors can affect medication absorption through lipid-based

formulations, involving the size of particles, intensity of emulsification, rate of dispersing, as well as drug precipitating throughout dispersion. [11, 12]

#### **1.4.1. The benefits of LBDDS [13]**

- (1) Controlled and targeted release of drugs.
- (2) Increased bioavailability
- (3) Increased drug content.
- (4) The capability to transport both hydrophobic and hydrophilic medicines.
- (5) Biocompatible as well as biodegradable.
- (6) Excipients' adaptability
- (7) Product adaptability.
- (8) The level of risk is minimal.
- (9) The passive and noninvasive generation of a vesicular system has become widely viable.

#### **1.4.2. Lipid formulation classification system**

The self-convening property of lipid has turned to account for a wide variety of colloidal drug carriers with diverse structure, for instance, micelles, microemulsion, emulsion, liquid crystalline nanoparticles, vesicular carriers such as liposomes and solid lipid nanoparticles, polymer-lipid hybrid nanoparticles, and self-emulsifying/self-micro-emulsifying drug delivery system (SEDDS). [14]

**Table 1.3.2.** The classification system for lipid formulations defines the 4 main categories of lipid formulations. [15]

| Formulation type | Substance                                                                 | Features                                                  | Advantages                                                                                     | Disadvantages                                                                                             |
|------------------|---------------------------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| Type I           | Lipid free of surfactant such as monoglycerides and diglycerides.         | Non-dispersing, involves digesting.                       | The product considered as safe; it is uncomplicated to use; and it has good capsule stability. | The formulation exhibits inadequate solvent capability except if the medicine is particularly lipophilic. |
| Type II          | Lipid and hydrophobic surfactant                                          | SEDDS generated without hydrophilic ingredients           | It is improbable that solvent capability will be lost during dispersion.                       | Dispersion of unclear oil in water droplets droplet diameter range of 0.25 to 2 $\mu\text{m}$ .           |
| Type III         | Lipid, surfactant, and co-surfactant [hydrophobic/hydrophilic excipients] | SEDDS generated with hydrophilic ingredients              | Transparent or nearly transparent dispersion, non-digestible medication absorption             | Dispersion may result in a decrease of solvent capability, making it hardly absorbed or digested.         |
| Type IV          | Hydrophilic surfactant and co-surfactant                                  | The formulation is usually distributed into the micelles. | Formulations offer a high solvent capability for a variety of medicines.                       | The probable decrease in solvent capability during dispersion could be hard to digest.                    |

#### 1.4.3. The role of excipients used in lipid-based formulation with P-glycoprotein (P-gp)

Plasma glycoprotein (P-gp) is an active, efflux, membrane-bound transport protein pump discovered in 1976. [16] P-gp is mainly spread and synthesized in the intestinal epithelium, in which it transports medicines straight into the lumen. [17] Interestingly,

various excipients (i.e., oils, surfactants, and cosurfactants) that have been used in the lipid-based formulation have the potential to inhibit P-gp activity. Also, the entrapment of P-gp substrate drugs in the ultrafine oil droplets could further shield against recognition by intestinal P-gp transporters, thus enhancing drug absorption. [18]

**Table 1.4.3.** Classification of lipid-based delivery systems depending on the excipients percentage. [12]

| –                                   | Type I                | Type II                                          | Type IIIA                              | Type IIIB                                                                   |
|-------------------------------------|-----------------------|--------------------------------------------------|----------------------------------------|-----------------------------------------------------------------------------|
| Typical composition (%)             | 100%                  | 40 to 80%                                        | 40 to 80%                              | 20%                                                                         |
| Triglycerides                       |                       |                                                  |                                        |                                                                             |
| Mixed glycerides                    | none                  | 20 to 60 % (HLB:12)                              | 20 to 40 % (HLB:11)                    | 20 to 50% (HLB:11)                                                          |
| Surfactant                          |                       |                                                  |                                        |                                                                             |
| Hydrophilic co-surfactant           | none                  | none                                             | 0 to 40%                               | 20 to 50%                                                                   |
| droplet diameter of dispersion (nm) | Larger than 250       | 100 to 250                                       | 100 to 250                             | 50 to 100                                                                   |
| Aqueous dilution's importance       | Less Significant      | Capability of the solvent remains unaltered      | Slight reduction in solvent capability | Substantial phase transformations and possible solvent capability reduction |
| Digestibility's Importance          | Unavoidable necessity | Not essential, but there is possibility to occur | Not essential but could be prevented   | Not essential and there is no possibility to occur                          |

The main mechanism through which a lipid formulation optimizes bioavailability is generally the prevention of the slow dissolution processes and reduces the bioavailability of lipophilic medicines within solid dosage forms. The formulation will ideally allow the medicine to remain dissolved during its passage through the GIT. The pharmaceutical formulation can improve absorption, mostly as its solubilize inside a colloidal dispersion. This purpose can be reached by utilizing the medicine in a self-emulsifying system. [19]

### **1.5. Self-emulsifying drug delivery system**

Self-Emulsifying Drug Delivery System (SEDDS) is described as an isotropic combination of synthesized or non-synthesized oils, liquid or solid surfactants, and one or more hydrophilic solvents and co-solvent. Those systems form fine o/w emulsions (or nanoemulsions) within GIT by moderate agitations produced by stomach movement after oral route intake. [8]

#### **1.5.1. Properties of SEDDS [20]**

1. They can self-emulsify quickly throughout GI secretions and produce a fine oil in water emulsion with the effect of mild agitation caused by peristaltic as well as other GIT motions.
2. They have the capacity to efficiently combine drugs (hydrophilic or lipophilic) into the oil and surfactant combination.
3. The ability to be utilized for both solid as well as liquid dosage forms.
4. They required a reduced dose of the medication compared to typical dosage formulations.

#### **1.5.2. Advantages of SEDDS**

Most of the drugs exhibit high intra and inter-subject diversity in absorption, resulting in a variation in plasma profile. Food is an important variable that influences a drug's therapeutic efficacy in the body. SEDDS generate a consistent plasma profile. [21] A medicine that is poorly soluble in water results in poor dissolution and bioavailability. SEDDS is a novel strategy for increasing both the solubility in water and, ultimately, the overall bioavailability of hydrophobic medicines. The capability of SEDDS to deliver the active ingredient into GIT in globules ranging in diameter from 1 to 250 nm, as well as the subsequent increase in particular surface area, allows for more efficient drug delivery via the intestinal aqueous boundary layer, resulting in enhanced bioavailability. [22] One of the most essential features that distinguish SEDDS from other drug delivery systems such as liposomes, solid dispersion, nanoparticles is the simplicity of production. For large mass production, SEDDS demands an affordable and simple production facility, such as a basic mixer with an agitator and volumetric liquid filling devices; this enables it an economically beneficial approach for the pharmaceutical

industry. [22] Smaller droplet size eliminated quickly from the stomach and enhances the widespread reach of the drug across the GIT, decreasing irritating that is often associated with prolonged contact between the drugs and gut membrane. [23] SEDDS also has the capacity to deliver peptides which are sensitive to the GIT enzymatic hydrolysis. [22]

### **1.5.3. Composition of SEDDS**

#### **1.5.3.1. Oil**

SEDDS were produced using long and medium chain triglycerides (such as Soyabean oil and Capmul MCM) with various degrees of saturation. Due to their biocompatibility, altered or hydrolyzed vegetable oils were significantly contributed to the accomplishment of SEDDS. [24] Medium-chain triglycerides (MCT) were recently displaced with new semi-synthetic medium-chain triglyceride comprising compounds, including Gelucire, Olive oil, animal fat, and soyabean oil also were excellent oils and fats choices. [25] Newer semi-synthetic MCT, described as hydrophilic groups with surfactant characteristics, gradually and efficiently replaces standard MCT oils within SEDDS. MCT is also more soluble and movable at lipid in water interactions over long-chain triglycerides (LCT), which leads to faster hydrolysis of MCT. In comparison to MCT, LCT requires a large concentration of cremophor RH40 to produce microemulsions. [26] Consumable oils are rarely chosen due to their inability to dissolve substantial quantities of lipophilic medicines. Vegetable oils that have been altered or degraded were frequently utilized as excipients. They produce an excellent emulsification system with different surfactants accepted for the oral route and have higher drug solubility features. [27]

#### **1.5.3.2. Surfactant**

SEDDS are composed of non-surfactants with high hydrophile lipophile balance (HLB) values, such as Tween 60, Span 80, ..etc. The standard surfactant quantity in SEDDS formulations ranges from 30 - 40 % of the entire formulation. [25] The great HLB and consequent hydrophilicity of surfactant are essential for the instant generation of oil\ water droplets and the quick distribution of the formulation in the aqueous phase, resulting in a positive dispersing / self-emulsifying function. [28] In consequence, crude

surfactants are favored over the synthetic ones, although they have a low potential for self emulsifying. There is a link between the diameter of the particles and the quantity of the surfactants. In certain situations, raising the surfactant quantity could reduce the average size of SEDDS particles; this may be explained by the stability of the particles owing to the surfactant particles' localisation at the w/o interface. Contrastingly, as surfactant amounts grow, the average particle size could increase. Surfactants can boost the bioavailability through several techniques, including enhancing the solubility, improve the permeability of the intestinal epithelium, and decreased p-glycoprotein-mediated drug permeability. [26]

#### **1.5.3.3. Co-surfactant**

The purpose of co-surfactant, in association also with surfactants, is to reduce interfacial or surface tension to a low value. Around this value, the interface would extend to produce excellently distributed particles and then absorb additional surfactant and co-surfactant up to their mass state is reduced enough for it to return interfacial tension to positive. The microemulsions are formed through a process called 'spontaneous emulsification'. [29] In most cases, the co-surfactant with is often used HLB value of 10 to 14. [25]

#### **1.5.3.4. Consistency builder**

Additional ingredients including cetyl alcohol, beeswax as well as stearic acid can also be used to change the structure of the emulsion. [30]

#### **1.5.3.5. Polymers**

The stationary polymer matrix, that is also not ionized in physiological pH and able to create the matrix, contributes to 5-40% of the content by weight. Examples of polymers used for this point: ethylcellulose. [31]

#### **1.5.3.6. Antioxidant**

Hydrophobic antioxidants (for example, ascorbic palmitate propyl gallate) help to maintain the oil content in the SEDDS formulations. [26]

#### 1.5.4. Classifications of SEDDS

According to the previous Tables that discussed the classification of lipid-based delivery systems depending on the excipients percentage (Table 1.3.2 and 1.3.3), SEDDS is organized as self-emulsifying, self-micro emulsifying, and self-nano emulsifying drug delivery systems [SEDDS-SMEDDS-SNEDDS]. They are categorized vary according to the diameter of the particles and the emulsion properties created following self-dispersing. SEDDS often generate dispersions with lipid particle sizes varying from 100 to 300 nm. [32] A brief of the differences between SEDDS types is presented in (Table 1.5.4).

**Table 1.5.4.** The differences between SEDDS and SMEDDS [33, 28]

| S.No. | SEDDS                                                                                                                                                                                                                                                    | SMEDDS                                                                                                                     |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| 1     | It can be a binary formulation consisting of the active ingredient and oily excipients that aid in self-emulsification when combined with gastrointestinal fluids (GIF), or it can be a system consisting of the active ingredient, surfactant, and oil. | They are mainly consisted of the active ingredient, a surfactant, a co solvent, and the oil.                               |
| 2     | The diameter of the oil particles in the dispersion varies between 200nm to 5 $\mu$ m, giving a high surface area and, therefore, high absorption.                                                                                                       | The diameter of the oil particles within the dispersion is 200 nm, resulting in a high surface area for better absorption. |
| 3     | The dispersion appears unclear.                                                                                                                                                                                                                          | The distribution has an apparent clarity ranging from clear to transparent.                                                |
| 4     | In the aqueous or in-vivo environment, SEDDS is considered physically/chemically unstable.                                                                                                                                                               | In the aqueous or in-vivo environment, SMEDDS is considered physically/chemically stable.                                  |
| 5     | SEDDS includes from 40 to 80% oil.                                                                                                                                                                                                                       | In comparison to SEDDS, the oil percentage on the SMEDDS is below 20%.                                                     |

### **1.5.5. Factors affecting SEDDS**

#### **1.5.5.1. The drug's origin and dosage**

A larger dose of medicines is not ideal for SEDDS unless they have a high solubility in minimum one of the SEDDS constituents, such as with the lipophilic phase ideally. SEDDS would be extremely challenging to deliver medications with poor solubility lipids and water. [29]

#### **1.5.5.2. The active ingredient degree of solubility in the oily phase**

The SEDDS capacity to retain the active ingredient solubilization is usually triggered by the medication's solubility within the lipophilic phase. Assume the surfactant / co-surfactant plays a more significant role in drug solubility. Therefore, the sediment may occur because the thinning of SEDDS consistency reduces the solvent ability of the surfactant and/or the co-surfactant. [22]

#### **1.5.5.3. Polarity of oil droplets**

The polarity of the lipid phase is one of the factors that govern the release from the SEDDS. HLB chain length and degree of unsaturation of the fatty acid, molecular weight of the hydrophilic portion, and concentration of the emulsifier govern the polarity of the droplets. The polarity reflects the drug's affinity for oil and/or water and the type of process involved. The high polarity will promote the rapid rate of release of the drug into the aqueous phase. [34]

#### **1.5.5.4. Equilibrium solubility**

Equilibrium-solubility studies could be used to predict potential causes of intestinal sedimentations. Crystallization, contrarily, may be delayed in the colloidal stabilising environment. According to Pouton's research, these formulations may need five days to achieve equilibrium, and the medicine can stay supersaturated for 24 hours post intestine emulsifying event. [22, 35]

#### 1.5.5.5. Concentration of surfactant or cosurfactant

Assume a surfactant/cosurfactant plays a significant role in drug solubility. There is a possibility of sediment in this situation since the SEDDS dilution may reduce the capability of surfactant/cosurfactant. [26]

#### 1.5.6. Mechanism of self-emulsification

Self-emulsifying happens once the entropy switching the dispersion exceeds the energy necessary to enhance the dispersion's surface area. [36] The free energy necessary to build a typical emulsion is related to the energy needed to build a new surface among the two phases. The following equation can be used to define it:

$$DG = SN\pi r^2$$

DG: The process's free energy

N: droplets number

S: indicates the energy present at the interface.

$\Pi$ : pi

r: droplets radius

The interfacial area, and the resultant emulsion, are stabilised over time through typical emulsifiers that build a monolayer surrounding the emulsion particles, lowering the interfacial-energy and forming a barrier to avoid coalescence. [37] Emulsification, on the contrary, happens instantaneously in SEDDS due to the low free energy necessary to produce the emulsion, regardless if its negative or positive. [38] During emulsification, the interfacial structure should provide little or no aversion to surface breaking. [39] It's been claimed that the simplicity of emulsion formation is connected with water entry into the different gel phases or liquid crystals generated upon the surface of the droplet. [40] When a binary combination, i.e., oil and nonionic surfactant, is added to water, the interface that happens between both the oil and the aqueous phases is produced. [41] Following that, as a consequence of water entry throughout the interface, solubilization happens within the oil medium. This process continues up until the solubility limit at the interphase is reached. Additionally, water entry results in the creation of the dispersion of the liquid crystal phase. Eventually, anything near the interface will turn into liquid

crystals, the quantity that will rely on the binary mixture's emulsifying agents [42]. As a result, when the self-emulsifying system is gently agitated, water immediately enters the water cores, disrupting the interface and forming droplets. The SEDDS are generally stable towards coalescence as a consequence of the creation of the liquid crystal interface around the oil droplets. Additionally, the drug compound's existence may modify the emulsion's properties, potentially by interaction with the liquid crystal phases. Nonetheless, a link between the generation of liquid crystals and self-emulsification has yet to be established. [43, 44]

#### **1.5.7. Construction of ternary phase diagrams**

Before beginning the formulation, it is necessary to design Ternary Phase Diagrams. It is beneficial to determine the optimal emulsification zone for oils, surfactants, and cosurfactants. The ternary phase diagrams of the three main components will be plotted, and each of the apexes in the triangle indicates one of the phases. [45] These diagrams demonstrate the self-dispersing potential of SEDDS as a thermodynamically stable nano-microcarrier to deliver in the GI lumen, providing information on phase behavior concerning different formulation aspects. Low free energy forms nano-sized oil droplets for the thermodynamically spontaneous formulations developed. The higher the water content penetrates the oil droplet, the more quickly it undergoes the self-emulsification process. This process helps prevent coalescence through the mechanical barrier. This diagram is designed to adjust the amounts of various ingredients used in the creation of SEDDS, which exhibits a high ability for self-emulsifying and is visually homogenous and physically/chemically stable. It illustrates robustness to dilution in the GI lumen apart from self-emulsification performance and ratio of co-surfactant used. [46] Ternary Phase Diagrams can be observed in two ways: by water titration or dilution techniques. Firstly, the optimized surfactant can be dissolved in the oil phase in the ratio 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, and 1:1 in a glass test tube. Each surfactant and oil phase ratio should then be titrated against the aqueous phase. Turbidity is referred to as an endpoint. It is necessary to quantify the element's proportions within the ternary system, followed by plotting the obtained data on triangle coordinates for the phase diagram production. The areas enclosed inside the plotted triangular coordinates are referred to as a biphasic system (heterogeneous), and the unenclosed areas are referred to as a

monophasic system (homogeneous). Different surfactants, co-surfactant, and oil ratios as per requirement are incorporated in preparing a ternary mixture in the dilution method. The formation of nano-emulsion is evaluated by diluting the ternary mixture with a suitable volume of double-distilled water. The size of the globule formed by resulted dispersion is determined using spectroscopy. The ternary phase diagram is plotted for the nano-emulsion formed. The phase diagram determines the area of nano-emulsion formed with the desired size of globule size were obtained. [26]

#### **1.5.8. SEDDS assessment**

##### **1.5.8.1. Drug content**

The active ingredient is separated from SEDDS simply by dissolving the formulation using a sufficient solvent. The solvent extractor then tested in triplicate to measure the drug content. The following formula estimates the drug content (%):

$$DC = Ca/Ct \times 100$$

DC: Drug quantity percentages

Ct: The theoretical concentration

Ca: The concentration obtained through high-performance liquid chromatography. [47, 48]

##### **1.5.8.2. Turbidimetric evaluation**

Turbidity is a factor used to determine the diameter of particles and the time required for self-emulsifying. [49] A constant amount of SEDDS is applied to a constant amount of Phosphate Buffer/0.1 N HCl mediums while mixing continuously at 50 rpm using magnetic bars set to the optimal temperature. Subsequently, utilizing a turbidimeter, the turbidity is assessed. Due to the short time needed for total emulsification, monitoring the speed of turbidity, including emulsification, is difficult. Turbidimetric analysis is used to determine the formation of particles following emulsification. [26]

##### **1.5.8.3. Droplets polarity**

The polarity of oil particles is determined via the value of the HLB in the oil, fatty acid degree of unsaturation, the molecular weight of the hydrophilic component, the emulsifying agent concentration, and the chain length of the fatty acids. A mixture of

small particles and their suitable polarity (low Po/w of the active ingredient) allows for an adequate release rate. The Po/w of lipophilic medicines is also used to determine the polarity of the particles. [28]

#### **1.5.8.4. Globule size**

The emulsion's droplet diameter is critical for self-emulsification performance since it controls the rate and degree of drug release as well as absorption. [50] The most often used technique for measuring the droplet diameter of SEDDS formulations is the Malvern Zeta Sizer, which is focused on dynamic light scattering (DLS) concept. [19]

#### **1.5.8.5. Morphologic evaluation**

The nano/micro-emulsion morphology of SEDDS formulations can be characterized by Scanning electron microscopy/Transmission electron microscopy devices. [19]

#### **1.5.8.6. Robustness to dilution**

Emulsions must not exhibit precipitation of the medication or any phase separation after 12 hours of preservation, and those formulations are classified as robust to dilution. This test is critical for SEDDS formulations to certify that the emulsion produced has related features at various dilutions in order to obtain a consistent medication release profile and also to prevent the active ingredient from precipitating at high dilutions in vivo, which would substantially reduce the drug's absorption of the formula. [51, 31, 52]

#### **1.5.8.7. Zeta-potential measurements**

The charge of the particles is determined via Zeta-Potential Assessment. The charge in SEDDS oil particles is an important feature to evaluate. [53] The zeta potential of SEDDS is frequently determined utilizing the Malvern Zeta nano sizer, which depends upon the electrical conductivity as well as the electrophoresis of the generated nano/micro-emulsion. [19]

#### **1.5.8.8. Self-emulsification time**

The time of self emulsification is measured by the USP dissolution device. The test is usually done by adding 0.5 g of SEDDS liquid formulation into 250 mL of SLS

(0.5%)/HCl (0.1N) solutions at a speed of 50 rpm. The time required for self-emulsifying at room temperature is referred to as the formulation's self-emulsification time. [20]

#### **1.5.8.9. Liquefaction time**

Liquefaction Time is an additional approach for measuring the duration needed for solid-SEDDS formulations to dissolve in vivo in the lack of any movement with artificial GI fluid. [54] The formula then gets enclosed in a translucent polyethylene film and attached to the thermometer's bulb. The thermometer is subsequently inserted into the round bottom flask that has been loaded with artificial stomach fluid that doesn't contain pepsin. A heating mantle is used to control the temperature at 37°C [26]

#### **1.5.8.10. Thermodynamic stability studies**

SEDDS must be sustainable at a range of temperatures and retain their capability to self-emulsifying after dilution. [55] Thus, phase separation, as well as thermodynamic stability tests, have to be conducted to determine the ability of developed SEDDS to tolerate stress environments. These tests are conducted in the subsequent cycles.

##### **1.5.8.10.1. Heating cooling cycle**

Six cooling and heating cycles across (4°C) and (45°C) temperatures are performed, involving an incubation time of at least 48 hours for each temperature. Stable formulations are then centrifuged using a centrifugation test. [56]

##### **1.5.8.10.2. Centrifugation test**

A centrifugation test is usually performed to examine the SEDDS's sustainability upon the emulsion formation. To do this, diluted samples are centrifuged at a speed of 4000 rpm for 30 minutes and afterward evaluated for any sign of phase separation. The freeze-thaw stress test is then performed on formulations which do not exhibit a phase separation. [47, 19]

#### **1.5.8.10.3. Freeze-thaw stress cycle**

Three to four freeze-thaw cycles may be performed on all the formulations, chilling at -4°C/24 hrs time duration then thawing it at 40°C/24 hrs. This test must verify that the formulations are stable, without any phase separation, creaming, or cracking. [25]

#### **1.5.8.11. In vitro diffusion study**

In-vitro diffusion study can be used to evaluate the performance of final product release by the dialysis method, which typically uses 6.8 pH phosphate buffer as a dialyzing medium by USP dissolution test device. A 900 mL dialyzing medium was preserved at 37°C and agitated at a speed of 100 rpm. Blank SEDDS (B-SEDDS), a placebo formulation was evaluated concurrently in similar conditions to examine whether there was any interference. 5 mL of samples will be withdrawn at various times (5, 10, 15, 30, 45, 60, 120 minutes) and refilled by a new dissolution medium. The collected samples will then be filtered using a membrane filter before being tested through an HPLC device. [57]

#### **1.5.8.12. In-vitro dissolution study**

In-vitro dissolution test is usually used to evaluate the drug release of the oil phase to the hydrophilic phase in the generated SEDDS formulations as well as in the unformulated/pure drug using a USP dissolution apparatus. [26, 58]

#### **1.5.9. Solidification of SEDDS**

SEDDS has benefits in terms of drug solubility and enhancing absorption. Oral dosage forms are considered to be the most favored route of administration, especially as lipid formulations, due to their ability to be made as solutions/semisolids/solids. Typical SEDDS are formed regularly in liquid form, which has various problems, including irrevocable drug or excipient sedimentation, a large dose quantity, low stability, limited selections of dosage forms, and difficulties during handling/transportation. To counter these issues, solid-SEDDS was considered as a possible substitute. [19]

#### **1.5.9.1. Advantages of solidification approach of SEDDS**

Including low manufacturing costs, manufacturing simplicity, easy process control, extended presence in the GI, stability, and repeatability are considered to be high, increased permeability of medicines, improve absorption rate and extent, resulting in more reliable blood time patterns, patient acceptance, increased solubility within the intestine, and increased permeability of medicines. [33, 59, 45]

#### **1.5.9.2. Techniques for solidifying liquid SEDDS**

##### **1.5.9.2.1. Adsorption to solid carriers**

Adsorption to solid carriers featuring a highly porous structure and particular area and has been the most widely used method to acquire a lipid formulation in solid form/dry emulsion/solid SEDDS. [60] The adsorption technique is basic, involving the addition of the liquid formulation into the adsorbents through the blender. The resultant powder can be directly filled in the hard gelatin capsule or combined with appropriate excipients prior to compressing it as tablets. One of the advantages of the adsorption method is the high degree of uniformity in the contents. Examples of solid adsorbents including Dextran 40, Neusilin US, magnesium trisilicate, and talcum. [33]

##### **1.5.9.2.2. Spray drying**

Formulation preparation in this method includes combining the oil, surfactant/co-surfactant, the active ingredient, as well as the solid adsorbent prior to spray drying. The atomized liquid composition is afterward sprayed in the form of droplets. As the droplets/particles are placed in the drying chamber, the volatile phase, such as the water included in the formulated emulsion, evaporates, producing dry droplets in sustained temperature and airflow settings. [61] These droplets can then be converted to dosage forms like capsules/tablets. The atomizer, appropriate airflow flow, temperature as well as drying chamber design are all chosen based on the product's drying properties and powder specifications. [19]

##### **1.5.9.2.2.1. Spray drying advantages**

Including; capability to work in a variety of applications, containing not many moving parts, and proper component selection can yield in a system without any moving parts in

close interaction to the product hence eliminating corrosion risks. The spray drying technique is considered to be fast. The final powder consistency remains stable throughout the dryer's operation, Offered in different designs to accommodate the product requirements, and it is compatible with any components that are both heat sensitive/resistant. [62]

#### **1.5.9.2.3. Melt granulation**

Melt granulation takes place when a binder that dissolves or loosens at low temperatures is added to the agglomerated powder. Melt granulation consists of a single step process and has significant benefits compared to typical wet granulation, as the addition of liquid and drying it afterward is not required. [61]

#### **1.5.9.2.4. Extrusion spheronization**

This solidification method is widely used in the pharmaceutical industry and is the most thoroughly researched process for producing self-emulsifying pellets. Extrusion is the process of transforming a plastic raw substance into a cylinder configuration with an agglomeration of equal densities and is used to distribute the drug within a thermal carrier substance. Spheronization occurs afterward, during which the extrudate is shattered and converted into spherical pellets having a narrow droplet diameter distribution, and high flowability characteristics. [60, 63, 64]

#### **1.5.9.2.5. Spray congealing**

In this method, a cooling container is used to spray the melted material inside. When the melted particles interface with the cooling air, they become crystallized as round solid droplets that settle towards the bottom of the container as a dusty powder. Following that, this powder can be utilized to produce formulations, including capsules or tablets. [65]

#### **1.5.10. Dosage forms of SEDDS**

Considering the fact that most of the excipients utilized in SEDDS are liquid at room temperature, liquid dosage forms usually limit SEDDS. Alternative SEDDS dosage forms that can overcome this problem are mentioned as follow:

#### **1.5.10.1. Dry emulsions**

This dosage form is consisting of o/w emulsion and produced through many techniques, including; rotary evaporation, spray congealing, etc. involving a solid adsorbent within the aqueous medium. Dry emulsions usually disperse naturally when applied to an aqueous medium. It can be utilized in tablet and capsule production. [61] This approach can minimize the usage of hazardous or toxic solvents and successfully eliminates the compatibility issues involved in regular emulsion formulas, i.e., cracking, creaming, or sedimentation. [26]

#### **1.5.10.2. Self-emulsifying suppositories**

Certain researchers have demonstrated that Solid-SEDDS could enhance GI and rectal absorption. [34] Self-emulsifying suppositories SES containing  $\beta$  artemether showed rapid absorption with sustained efficacy when delivered through the rectal route. [66] Glycyrrhizin was another drug with low bioavailability administrated orally and also acquired better bioavailability results for chronic liver disorders when administered through rectal-SES. [61]

#### **1.5.10.3. Self-emulsifying capsules**

Capsule dosage form is one of the convenient and widely used methods for Self-Emulsifying formulas for oral administration. Filling into the capsule has a number of advantages, including significant pharmaceutical loading capability, eligibility for efficient, low-dose medications, and ease of manufacture. [67, 19, 26]

#### **1.5.10.4. Self-emulsifying implants**

Self-Emulsifying implant has significantly increased the usage and applicability of solid SEDDS. [19] One of the drugs used as SE-Implantation was Carmustine, an anticancer medication intended for malignant brain tumors. Carmustine has short  $t_{1/2}$ ; therefore, it is ineffective as a drug. A self emulsification approach was established to improve drug bioavailability. According to The observations, SE-implants prolonged the drug  $t_{1/2}$  in contrast to pure Carmustine. [68]

#### **1.5.10.5. Self-emulsifying tablets**

Self-Emulsifying Tablets (SET) are usually prepared by adsorbing the nano/micro-emulsion on granulated substances and compressing them to become tablets. SET can release nearly 80 to 90 % of the medication in under 45 min. [26]

#### **1.5.10.6. Self-emulsifying nanoparticles**

The manufacturing of Self-Emulsifying nanoparticles has improved from nano-particle approaches. One of these strategies includes solvent insertion. The oil, surfactant/cosurfactant, and active ingredient were combined together and then delivered drop-wise over an agitated nonsolvent using this approach. Self-Emulsifying nanoparticles were then purified and dehydrated. This strategy resulted in nanoparticles of approximately 100 nm with 74% of the active ingredient - loading efficiency. [69]

#### **1.5.10.7. Self-emulsifying controlled release tablets**

Oli and surfactant configurations have demonstrated significant ability to develop Self-Emulsifying tablets that have been commonly studied. [61] Patil created a gelled SEDDS to considerably minimize the quantity of solid carriers necessary to convert SEDDS to solid oral dosage forms. Aerosil 200 was chosen as the solid carrier for the lipophilic systems in their research because it provided the double function of decreasing the quantity of solid adsorbant needed and assisting with delaying the release profile of the active ingredient. [70]

#### **1.5.10.8. Self-emulsifying solid dispersions**

Solid dispersions have been commonly applied to boost the bioavailability of insoluble medicines as sustainability is a major challenge in their production. [26] Serajuddin pointed out that these difficulties could be surmounted by the use of SE excipients such as Gelucire 44/14, Gelucire 50/02, Labrasol, and Transcutol [71, 72] Those excipients have the capability to improve the absorption of inadequately hydrophilic medicines and can potentially be added quickly into molten gelatin capsules, avoiding the need for grinding and mixing prior to filling. [73, 74]

#### **1.5.10.9. Self-emulsifying phospho-lipid suspension (SEPS)**

A self-emulsifying phospho-lipid suspension (SEPS) containing a high concentration of phospho-lipids may help in retaining the active ingredient solubilized in vivo, which is critical for improving the bioavailability. Phospho-lipids are considered endogenous lipids with a high capacity for emulsifying in vivo. This dosage form needs a small quantity of surfactant and co-surfactant, therefore, causing fewer health risks. [75]



## 1.6. Eplerenone

### 1.6.1. General information of Eplerenone

**Table 1.6.1.** General information regarding Eplerenone. [76, 77, 78, 79, 80, 81, 82]

|                                    |                                                                                                     |
|------------------------------------|-----------------------------------------------------------------------------------------------------|
| Name of the active ingredient      | Eplerenone (EPL)                                                                                    |
| Name of the commercial product     | inspra <sup>®</sup>                                                                                 |
| BCS classification                 | Class II [low solubility and high permeability]                                                     |
| The drug approval date in the FDA  | 27/9/2002                                                                                           |
| Drug usage                         | For heart failure and hypertension                                                                  |
| The nature of the drug             | Second aldosterone antagonist                                                                       |
| Aldosterone definition             | Hormone in the renin angiotensin aldosterone system                                                 |
| Mechanism of action of Aldosterone | Controlling the amount of electrolytes in the body, thereby affecting blood pressure                |
| EPL molecular weight               | 414.49 g/mol                                                                                        |
| EPL powder characteristic          | White crystalline powder                                                                            |
| Solubility of EPL                  | Low soluble in water, moderate solubility in methanol                                               |
| EPL Partition coefficient          | log K <sub>o/w</sub> is 7.1                                                                         |
| Pharmacokinetic of EPL             | Highest plasma concentration in 1.5 to 2 hours upon oral administration                             |
| Metabolism of EPL                  | High hepatic first-pass metabolism is nearly 12.6% to 27.1%, and only 5% excreted through the urine |
| Bioavailability of EPL             | 69%                                                                                                 |
| EPL half life                      | 3-6 hrs                                                                                             |

FDA; Food and Drug Administration.

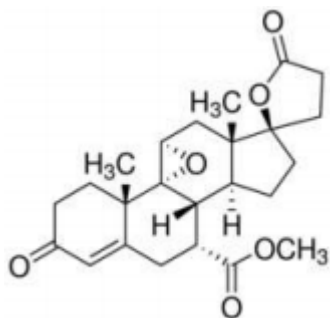


**Figure 1.6.1.** Chemical structure of Eplerenone. [83]

#### **1.6.1.1 Eplerenone validation and calibration analysis:**

Several analytical approaches have been published for evaluating Eplerenone, including the HP-TLC, RP-HPLC, and the UV method. Eplerenone is considered water-insoluble and has moderate solubility in methanol. [83]

#### 1.6.1.1.1. HPLC analysis of Eplerenone

**Table 1.6.1.1.1.** Summary of chromatography methods for Eplerenone

| Sr.no | Method       | Mobile phase                                                                                                                    | Stationary phase                                          | Wavelength | References |
|-------|--------------|---------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|------------|------------|
| 1     | RP-HPLC      | As a mobile phase, a 60:40 % v/v combination of buffer (pH 3.5) as well as methanol was utilized with a flow rate of 1.0ml/min. | C18, (150mm×4.6mm, 5µm) column.                           | 276 nm     | [83]       |
| 2     | RP-HPLC      | A 50:50% v/v combination of acetonitrile + phosphoric acid 0.1%.                                                                | C18, 250 x 4.6 mm, 5 microns.                             | 241 nm.    | [84]       |
| 3     | Aluminum TLC | Ethyl acetate: toluene 7:3 (v/v) as mobile phase                                                                                | Silica gel 60F254 was used as the stationary phase        | 238 nm     | [85]       |
| 4     | HPTLC        | Toluene–ethyl acetate–glacial acetic acid (7:3:0.1, V/V) as the mobile phase.                                                   | Aluminum-backed silica gel 60 F254 plates.                | 247 nm     | [86]       |
| 5     | LC-MS/MS     | Acetonitrile:water (40:60, v/v) containing 10 mM ammonium acetate (pH 7.4)                                                      | Reversed-phase Zorbax XDBC8 HPLC column (2.1/50 mm, 5 mm) | -          | [87]       |
| 6     | HPLC-ESI-MS  | Mobile phase of 10 mmol·L <sup>-1</sup> ammonium acetate water solution-methanol (30:70, v/v)                                   | Reversed-phase C18 column                                 | -          | [88]       |
| 7     | (RP) HPLC    | 55:45 (v/v) 50 mM ammonium acetate buffer (pH 7)–acetonitrile as isocratic mobile phase                                         | A 250 mm × 4.6 mm, 5-µm particle, C18 column              | 240 nm     | [89]       |

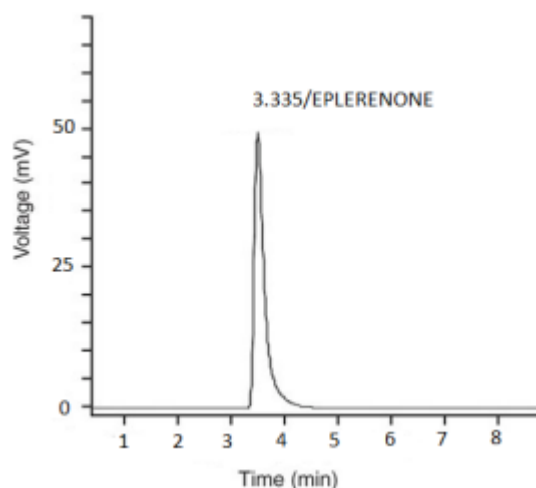


**Figure 1.6.1.1.1.** Isolated peak of eplerenone by RP-HPLC method [84]

#### 1.6.1.1.2. UV Spectroscopy analysis of Eplerenone

**Table 1.6.1.1.2.** Summary of spectroscopic methods for Eplerenone.

| Sr.no | Method | Wavelength | Linearity<br>(ug/ml) and<br>Range | Recovery                           | References |
|-------|--------|------------|-----------------------------------|------------------------------------|------------|
| 1.    | UV     | 241 nm     | 4 - 24 µg/mL<br>( $r^2= 0.9996$ ) | In the range of 99.84 -<br>100.39% | [90]       |
| 2.    | UV     | 430 nm     | 5- 35 µg /ml                      | In the range of 98.56-<br>99.12%   | [91]       |
| 3.    | UV     | 245 nm     | 2.5-20 µg/ml                      | In the range of 90-110 %           | [92]       |
| 4.    | UV     | 245 nm     | 5-15µg/ml                         | In the range of 98-102%            | [93]       |

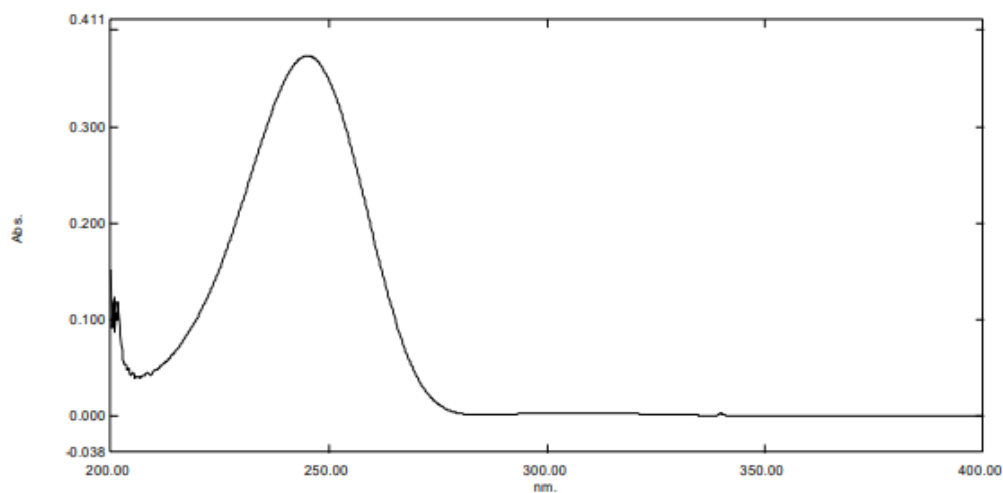


**Figure 1.6.1.1.2.** UV spectrum figure of Eplerenone standard [92]

#### 1.6.1.2. Formulation approaches to enhance the bioavailability of Eplerenone

**Table 1.6.1.2.** Summary of formulation approaches used to improve the bioavailability of Eplerenone

| Sr.no | Method                           | References |
|-------|----------------------------------|------------|
| 1.    | Nanoemulsion approach            | [94]       |
| 2.    | Chitosan microspheres approach   | [95]       |
| 3.    | Nanoemulsion Liquisolid approach | [82]       |
| 4.    | Liquisolid approach              | [96]       |
| 5.    | Solvent Melt Method              | [97]       |

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. The active ingredient

Eplerenone was a kind gift from Neutec Pharmaceuticals - Turkey.

Epleday® tablets containing 25 mg of EPL were also a kind gift from Neutec Pharmaceuticals (Istanbul, Turkey) and used as a reference product.

#### 2.1.2. The excipients

##### 2.1.2.1. The oils

**Table 2.1.2.1.** The oil phases that were considered in the study

| The Name of the oil | Booked\Gifted | Company name  | Origin country |
|---------------------|---------------|---------------|----------------|
| Triacetin 99%       | Booked        | Acros         | Belgium        |
| Ethyl oleate        | Booked        | Aldrich       | Germany        |
| Crodamol GTCC       | Gifted        | Croda         | Spain          |
| Cetiol J600         | Gifted        | Verser Vinmar | USA            |
| Kollicream 3C       | Gifted        | BASF          | Germany        |

##### 2.1.2.2. The surfactants

**Table 2.1.2.2.** The surfactants that were considered in the study

| The Name of the Surfactant | Booked\Gifted | Company name       | Origin country |
|----------------------------|---------------|--------------------|----------------|
| Birj 35                    | Booked        | Carl Roth Gmbh     | Germany        |
| Tween 80                   | Booked        | Neofroxx Gmbh      | Germany        |
| Kolliphor EL               | Booked        | Sigma              | USA            |
| Caprol PGE 860             | Gifted        | ABITEC corporation | USA            |
| Kolliphor P188             | Gifted        | BASF               | Germany        |

### 2.1.2.3.The Co-Surfactants

**Table 2.1.2.3.** The co-surfactants that were considered in the study

| The Name of the Co-surfactant     | Booked\Gifted | Company name  | Origin country |
|-----------------------------------|---------------|---------------|----------------|
| Tetraglycol                       | Booked        | Sigma         | USA            |
| Diethylene glycol monoethyl ether | Booked        | Sigma-Aldrich | Germany        |
| Sorbitol                          | Booked        | Sigma         | USA            |
| PEG 200                           | Booked        | Sigma         | USA            |
| Dimethylsulfoxide                 | Booked        | Merck         | Germany        |

### 2.1.2.4.Additives

**Table 2.1.2.4.** The additives that were considered in the study

| The Name of the Additive | Booked\Gifted | Company name            | Origin country |
|--------------------------|---------------|-------------------------|----------------|
| Calcium silicate         | Booked        | Aldrich                 | Germany        |
| Hydroxypropylcellulose   | Booked        | Tokyo Chemical Industry | Japan          |
| Aerosil 200              | Booked        | Evonik – GmbH           | Germany        |
| Neulisin US2             | Booked        | Fuji Chemical Industry  | Japan          |
| Carboxymethyl cellulose  | Booked        | Sigma                   | USA            |

### 2.1.3. Devices and Instruments

**Table 2.1.3.** The device\instruments used in the study.

| Device\Instrument name             | Company name                                       | Origin country |
|------------------------------------|----------------------------------------------------|----------------|
| HPLC                               | Agilent Technologies 1260 infinity                 | USA            |
| Centrifuge                         | 1) Sigma 3-16 PK                                   | USA            |
|                                    | 2) Spectrofuge 7M                                  | USA            |
| DLS                                | Malvern Nanozen 3600                               | UK             |
|                                    | Dynamic Light Scattering                           | UK             |
| Viscometer                         | Brookfield                                         | Canada         |
| Homogenizer                        | Heldol PH Silent Crusher M Homogenizer             | Germany        |
| Ultrasonic-Homogenizer             | Bandelin Sono Plus Ultrasonic Homogenizer          | Germany        |
| Ultrasonic Bath                    | Bandelin Sonorex Super RK 156 BN                   | Germany        |
| Water Shaking Bath                 | 1) Wise Bath                                       | 1) Japan       |
|                                    | 2) GFL                                             | 2) Germany     |
| Hot plate                          | Heidol ph (MR 3004 Safty)                          | Germany        |
| DSC                                | Diffrential Setaram DSC 131                        | USA            |
| Infrared                           | Nicolet is 50 FTIR Spectrometer                    | USA            |
| Vortex Mixer                       | Velp Scientifica ZX3 Advanced Vortex Mixer         | Italy          |
| Ultrapure Type I Water Machine     | Simplicity UV Merck Life Science                   | Germany        |
| Vacuum Pump                        | Pall Life Science Corporation                      | USA            |
| Weighting Device                   | Analytical Plus OHAUS                              | USA            |
| Dissolution Apparatus              | PT-DT70 Low-Head Tablet Dissolution Test Apparatus | Germany        |
| Scanning Electron Microscopy (SEM) | Zeiss Evo40                                        | Germany        |
| pH meter                           | Mettler Toledo Quattro pH meter                    | Switzerland    |

## **2.2. Methods**

### **2.2.1. HPLC analysis**

EPL was assayed by reverse-phase high-performance liquid chromatography (HPLC). This method consists of a HPLC instrument (Agilent Technologies 1260 Infinity) connected with a diode array detector (DAD) (Agilent Tech – Germany). Using an infinityLab poroshell 120 EC-C18 column ( $3.0 \times 150$  mm,  $2.7 \mu\text{m}$ ) (Agilent Tech – Germany). The isocratic mobile phase consisted of distilled water, pure methanol, and pure acetonitrile (ACN) (50:30:20, v/v). The mobile phase was first filtered by a vacuum pump through a  $0.45 \mu\text{m}$  membrane filter and ultrasonically degassed for 15 min. The DAD detector was operating at a wavelength of (240 – 245 nm). Samples were eluted using a flow rate of 0.6 mL/min. The column was preserved at a temperature of  $55^\circ\text{C}$ . The injection volume was set at  $10 \mu\text{L}$  of sample. EPL had a retention time of 2.6 minutes. The correlation coefficient was ( $R^2 = 0.9989$ ) indicated that the calibration curve was generally linear within 50 - 1000 ng/mL ranges.

### **2.2.2. Solubility screening**

The excipients were chosen primarily for their capacity to dissolve the maximum amount of the drug. [98] EPL was soluble in a variety of oils, surfactants, and cosurfactants. This test utilized 1.0 mL of every excipient within a sealed sample vial incorporated with a large quantity of EPL (20 mg). The combination was then sonicated for 10 minutes, vortexed for 5 minutes to ensure equal distribution, and then agitated for 3 days at 75 rpm,  $25^\circ\text{C}$ , using a water shaking bath. Following that, the specimens were centrifuged for 20 minutes at 15,000 rpm. The supernatant was then extracted and diluted in the mobile phase and filtered through a  $0.45 \mu\text{m}$  membrane filter. EPL concentration was measured using the HPLC device.

### **2.2.3. Construction of pseudo ternary phase diagram**

A pseudo ternary phase diagram consisting of oil, surfactant, and cosurfactant has been developed to determine the areas that self-emulsify as diluted with gently agitated. [99, 100] The ternary phase diagram of oil, water, and surfactant\co-surfactants (S\Cos) combination was developed in a non-drug environment utilizing the water titration technique. [101] The chosen surfactant, co-surfactant were integrated into a surfactant mixture (S-mix) in various weight proportions (1:1 to 1:9). Each (S-mix) has been added to the chosen oil in various fixed proportions (oil: S-mix proportions of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40). At a temperature of 25°C, water was poured drop-wise into the oily combination while magnetic stirring. The combination was then visually analyzed for clarity after adding the water. The weight readings were used to determine the volume of water at which the turbidity to clarity or clarity to turbidity transitions happened. These results were then utilized to identify the micro/nano emulsion region correlated to both the oil as well as S\Cos values. [102]

### **2.2.4. Preparation of SEDDS formulations**

#### **2.2.4.1. Preparation of liquid-SEDDS formulation**

Optimum ratios of oils, surfactants, and co-surfactant were selected from the phase diagrams. [102] Each formulation was stirred at room temperature and homogenized on a magnetic stirrer for 10 min, then sonicated for 5 min. 0.0025g of Carboxymethyl cellulose (CMC) was then added to each of the blank formulations as stabilizing agent while mixing it using the magnetic bar. EPL was added lastly to the mixture in a concentration of 25 mg. These mixtures were mixed at room temperature until EPL was dissolved entirely, which resulted in a transparent liquid. Both Blank Liquid-SEDDs (B-L-SEDDs) and EPL based formulations (EPL-L-SEDDS) were prepared for further characterization of DS, PDI, and ZP utilizing a DLS device (Malvern Instrument, UK). The emulsion particle diameter is critical for self-emulsifying efficiency since it is directly linked to the quantity and the drug release rate and absorption. [103]

#### **2.2.4.2. Preparation of solid-SEDDS formulation**

To solidify L-SEDDS in this study, the adsorption to solid carriers technique was utilized. This process is basic, including the addition of L-SEDDS to the specified adsorbant and blending them thoroughly. The S-SEDDS generated using this technique will have stability and easily flowable [60, 65] hydroxypropyl cellulose (HPC) as water soluble solid adsorbant and calcium silicate, Aerosil 200, and Neusilin US2 (CS, A200, NUS2) as water insoluble solid adsorbents were all assessed to determine which one was most appropriate for converting the B-L-SEDDS and EPL-L-SEDDS into B-S-SEDDS and EPL-S-SEDDS. To start, the adsorption capability within each of the solid carriers was determined by adding it using a dropwise technique and blending the specific ratio of approximately 535.6 mg or 0.5 mL of the B-L-SEDDS with a similar ratio of the solid adsorbent using a mortar and pestle to form a nonadhesive powder. The resulting S-SEDDS are then allowed to settle and dry at room temperature for 24 hours. 10 mg of the produced S-SEDDS was mixed with 10 mL of purified water and was mixed for 10 minutes using a magnetic bar before being filled in a cuvette in the DLS device for DS, PDI, and ZP quantification. Meanwhile, the produced S-SEDDS with water insoluble solid adsorbents were centrifuged at 10000 rpm for 10 minutes to isolate any water insoluble particles and filled into the DLS cuvette for further characteristic analysis. [104, 105]

#### **2.2.5. SEDDS features**

##### **2.2.5.1. L-SEDDS features**

##### **2.2.5.1.1. Thermodynamic stability**

Physical stability is considered to be critical for the formulation's efficiency since it could be impacted by sedimentation of the active ingredient in the excipient mixture. Due to the formulation's low physical stability, excipients may phase separate, negatively affecting the bioavailability as well as therapeutic effectiveness [58] The selected EPL-L-SEDDS and EPL-S-SEDDS formulations were subjected to three weeks of stability testing to determine the most efficient SEDDS formulation. Six heating and cooling cycles have been performed upon these formulations, involving 48 hours of cooling at 4°C accompanied by 48 hours of heating at 40°C. After centrifugation at 4000 rpm for 30 minutes, the samples were then subjected to two freeze-thaw stress

cycles, which also involved 48 hours of freezing at -4°C followed by 48 hours of thawing at 40°C. After each cycle completion, the formulations were analysed for phase separation visually and DS, PDI, and ZP values.

#### **2.2.5.1.1.1. Chemical stability**

The selected optimum SEDDS formulations were examined for their chemical stability in the long term (3 months) using a stability chamber ( $25 \pm 2$  °C /  $60 \pm 5$  % RH). The DS, PDI, and ZP measurements were collected for comparison purposes.

#### **2.2.5.1.1.2. Visual evaluation of self-emulsification effectiveness**

Self-emulsification capacity was determined visually by mixing the SMEDDS formulation in 20 mL of water. When water droplets were lightly stirred using a magnetic stirrer; the potential to generate emulsion was evaluated as "acceptable" if they quickly spread in water and remain clear without turbidity or any form of isolation. If turbidity or a whitish look developed in the formulation, then the inclination will be considered "unacceptable."

#### **2.2.5.1.1.3. Determination of rheological characteristics**

Viscosity measurements are required to define and control the system's physical stability in SEDDS. The rheological properties (viscosity, flow) of the microemulsion are evaluated by use of Brookfield viscometer (Japan) use of spindle RV-7 at (10,20,30,40,50,100, and 200 rpm) at  $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . This viscosity determination confirms whether the formulation has non-newtonian pseudoplastic flow results or not and if it's o/w or w/o. If the formula had a minimal viscosity, then it is an o/w system, while if it had a higher viscosity, then it is a w/o system. [57] This test has been done on B-L-SEDDS-F3 as well as EPL-L-SEDDS-F3 for comparison.

#### **2.2.5.1.1.4. Drug content (L-SEDDS)**

To measure the drug loading ability, large quantity of EPL was incorporated in 2 ml L-SEDDS formulations and stirred for 30 minutes utilizing a magnetic bars. Centrifugation at 10,000 rpm for 15 min is being used to eliminate the precipitated drug. The supernatant was separated and reduced by adding mobile phase and filtered 0.45

µm membrane filter, and the drug content was measured utilizing an HPLC device. [84] The analyses were conducted as three replicates.

#### **2.2.5.1.5.Determination of droplet size for L-SEDDS**

The droplet size of the emulsion affects the drug's absorption and bioavailability. Small droplets provide a wider surface area, resulting in a rapid release of the medication into the aqueous phase [106] Gershanik et al. studied the influence of emulsion droplet size on intestinal mucosa permeability. [23] The Researchers discovered that the ideal droplet size ranged between (100 to 500 nm). [107] In DS, PDI, and ZP, both B-L-SEDDs and their equivalent EPL-L-SEDDs formulas were more featured (after dilution 100 times with water). The EPL-L-SEDDs formulations with the greatest EPL concentration, DS 100 nm, PDI 0.5, and the greatest ZP value were chosen to be included in the solidifying method.

#### **2.2.5.2. S-SEDDS features**

##### **2.2.5.2.1. Drug content (S-SEDDS)**

The EPL content in EPL-S-SEDDS-F3 formulation was measured through mixing the formulation powder in 10 mL of mobile phase and stirring for 30 minutes using a magnetic bar. The mixture was then sonicated for 2 minutes in an ultrasonic bath. Sufficient aliquots were collected and diluted with the mobile phase, then filtered through a 0.45µm membrane filter before injection to the HPLC.

##### **2.2.5.2.2. Determination of droplet size for S-SEDDS**

As described in Subsubsection 2.2.4.2, the S-SEDDS-F3 were solidified with a water-insoluble solidifying agent. As a result, S-SEDDS (10 mg) was distributed in 10 mL of sterile distilled water and vortexed firmly to combine. Following combining, an aliquot of the solution was centrifuged at 10000 rpm for 10 minutes to eliminate hydrophobic components, and the droplet particle was quantified using a DLS apparatus.. The results were compared with previous L-SEDDS-F3 droplet size analysis findings.

#### **2.2.5.2.3.Differential scanning calorimetry (DSC)**

DSC was used to perform thermal studies on all solid powder samples, including Pure EPL, EPL-S-SEDDS-F3-NUS2, and EPL-S-SEDDS-F3-A200 (Differential Setaram DSC 131, USA). The samples (6-8 mg) were fully sealed in aluminum pans with a hole and warmed at a 10°C/min scanned rate in dry nitrogen flow (10 ml/min) within a temperature range between (25 to 300°C). Calibration of the DSC temp and enthalpic measurements was performed by an indium standard. Thermal analysis results were visualized using the (SETSOFT 2000, version 3.0) software package.

#### **2.2.5.2.4.FT-IR scanning**

Fourier transform infrared spectroscopy (FT-IR) was used to determine the complex formation and chemical characteristics of powdered materials (Nicolet, 50 FTIR Spectrometer, USA) by compression, the powder of Pure EPL, EPL-S-SEDDS-F3-NUS2, and EPL-S-SEDDS-F3-A200 in the IR device. The spectrum was scanned within the wavenumber range between (400 to 4400 cm<sup>-1</sup>). The FT-IR spectrum data were presented using (OMNIC, version 9.2) software program.

#### **2.2.5.2.5.Morphological analysis of S-SEDDS**

The surface morphology of the plain solid carriers (Aerosil 200 and Neusilin US2) and Drug loaded-SEDDs with each solid carrier were examined by scanning electron microscopy (SEM) (Zeiss Evo40, Germany). The samples were fixed on a SEM stub using double-sided adhesive carbon tape (Pelco tabs, 12mm, Ted Pella, USA). The samples were then sputter-coated with using a palladium layer (Bal-Tek sputter coater, SCDOOS, USA) at 15 mA for 20 seconds under a high vacuum evaporator prior to scanning. The technique was carried out at a voltage of (10) kilovolts. The machine then acquired the scanned images at the specified magnifications.

#### **2.2.6. In-vitro dissolution studies**

The drug release characteristics of the developed SEDDs formulations were determined by using USP Tablet Dissolution Test Apparatus (PT-DT70 Low-Head, Germany) in 500 mL of 0.1 N HCl (pH 1.2) at 50 rpm and 37 ± 0.5 C. At fixed time intervals, a 5 mL sample was collected and replaced with a fresh medium solution. HPLC was used to

determine the overall percentage of release profile. [84, 82] Similarly, the dissolution rate of the marketed and pure EPL was measured. Six replicates were taken as an average.

### **2.3. Statistical analysis**

The overall average and standard deviation are used to express the results. The data were analyzed statistically using the DesignExpert program (Version 13, USA). A Single-factor ANOVA test was done for the selected tests. In addition to that, samples were used as triplicated during each experiment. Means of the triplicated were used during statistical analysis. The significance level was accepted [ $p < 0.05$ ] for all formulations used in the dissolution test and was not significant for the unformulated pure drug used in the dissolution test nor SEDDS formulations in the chemical stability study. [ $p > 0.05$ ]

### 3. RESULTS

#### 3.1. Solubility screening

The levels of EPL solubility in diverse types of oils, surfactants, and cosurfactants are organized in (Figure 3.1, Table 3.1).

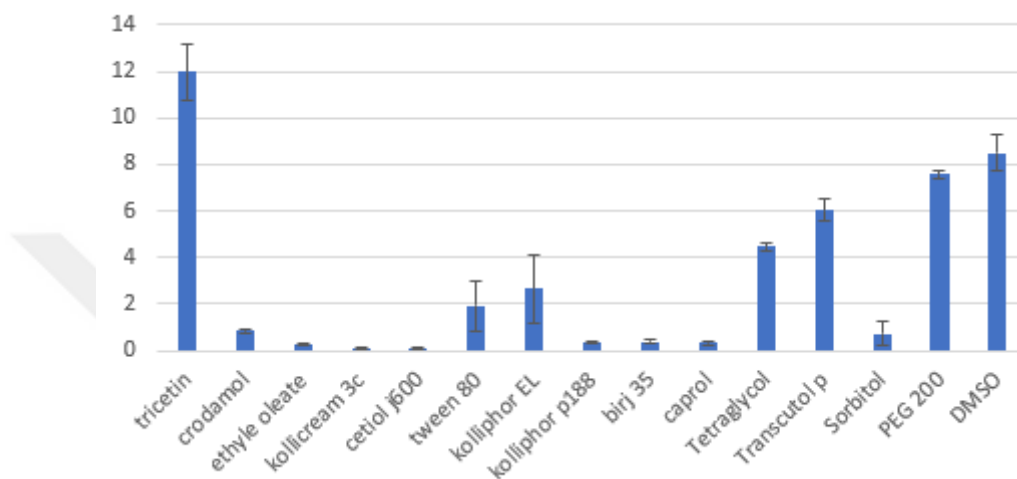


**Figure 3.1.** EPL's solubility in a variety of components

**Table 3.1.** EPL's solubility (mg / mL) in a variety of components.

| Oil\surfactant\cosurfactant | Mean $\pm$ SD    |
|-----------------------------|------------------|
| Tricetin                    | 11.99 $\pm$ 1.20 |
| Crodamol                    | 0.835 $\pm$ 0.10 |
| ethyle oleate               | 0.305 $\pm$ 0.04 |
| kollicream 3c               | 0.125 $\pm$ 0.02 |
| cetiol j600                 | 0.083 $\pm$ 0.01 |
| tween 80                    | 1.918 $\pm$ 1.06 |
| kolliphor EL                | 2.650 $\pm$ 1.45 |
| kolliphor p188              | 0.364 $\pm$ 0.01 |
| birj 35                     | 0.375 $\pm$ 0.1  |
| Caprol                      | 0.316 $\pm$ 0.11 |
| Tetraglycol                 | 4.476 $\pm$ 0.18 |
| Transcutol P                | 6.035 $\pm$ 0.47 |
| Sorbitol                    | 0.708 $\pm$ 0.50 |
| PEG 200                     | 7.572 $\pm$ 0.15 |
| DMSO                        | 8.501 $\pm$ 0.79 |

DMSO: Dimethylsulfoxide; PEG: Poly ethylene glycol;SD: Standard deviation

### 3.2. Construction of pseudo-ternary phase diagram

The investigated systems are presented according to their compositions in (Table 3.2). The region of the stable formulations in the pseudo ternary phase diagrams was highlighted in pink (Figures 3.2.A, 3.2.B, and 3.2.C).

**Table 3.2.** Composition of SEDDS formulations.

| Component    | SEDDS-F1     | SEDDS-F2  | SEDDS-F3     |
|--------------|--------------|-----------|--------------|
| Oil Phase    | Triacetin    | Triacetin | Triacetin    |
| Surfactant   | Kolliphor EL | Tween 80  | Tween 80     |
| Cosurfactant | PEG 200      | DMSO      | Transcutol p |

(DMSO: Dimethylsulfoxide SEDDS: Self-emulsifying drug delivery systems; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3.)

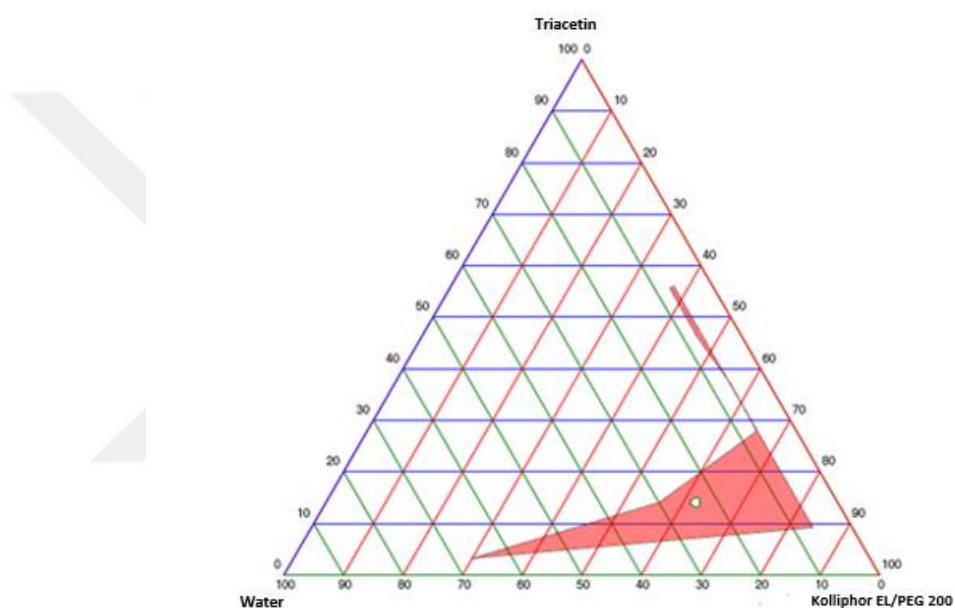


**Figure 3.2.A.** Pseudo ternary phase diagram of Triacetin, Kolliphor EL/PEG 200, and water (SEDDS-F1). The ratio 1:1 showed the best self-emulsifying properties of all ratios.

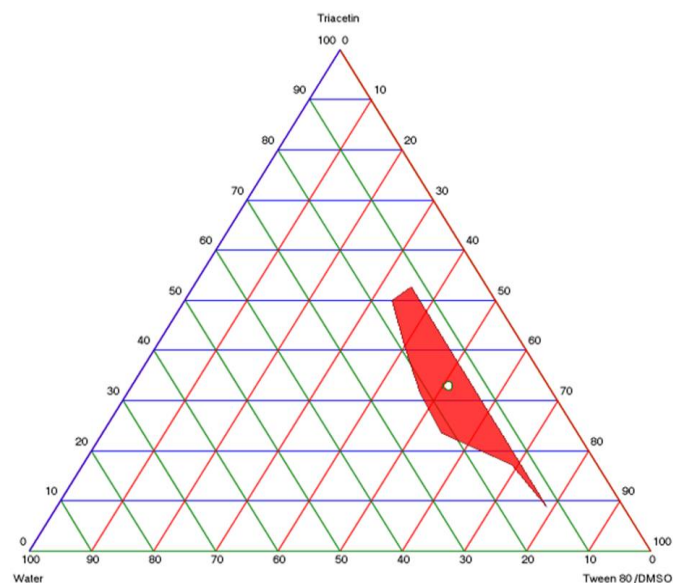


**Figure 3.2.B.** Pseudo ternary phase diagram of Triacetin, Tween 80/DMSO, and water (SEDDES-F2). The ratio 1:6 showed the best self-emulsifying properties of all ratios.

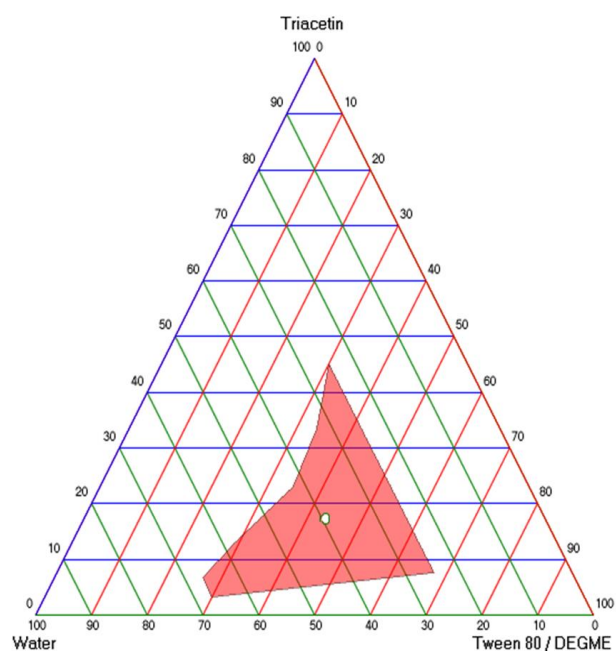


**Figure 3.2.C.** Pseudo ternary phase diagram of Triacetin, Tween 80/Transcutol p, and water (SEDDES-F3). The ratio 1:5 showed the best self-emulsifying properties of all ratios.

### 3.3. Preparation of SEDDS formulations

#### 3.3.1. Preparation of L-SEDDS formulation

Different formulations were prepared to select the optimum SEDD formulation for EPL and study the effects of the formulation variables. Oils and surfactants and co-surfactant mixtures were weighed into a glass beaker at a ratio of (18.44:40.77:40.77 w/w/w), (39.3:8.6:52 w/w/w), and (28.4:11.9:59.6 w/w/w) for B-L-SEDDS and EPL-L-SEDDS-Formulation 1 (F1), F2, and F3, respectively. The DS, PDI, and ZP determinations results are shown in (Table 3.3.1.A, 3.3.1.B, and 3.3.1.C).

**Table 3.3.1.A.** DLS results of SEDDs-F1. Mean  $\pm$  SD, n = 3.

| Parameters\Formulation code | B-L-SEDDs-F1     | EPL-L-SEDDs-F1    |
|-----------------------------|------------------|-------------------|
| DS (nm)                     | 13.46 $\pm$ 0.03 | 13.96 $\pm$ 0.391 |
| PDI                         | 0.09             | 0.067             |
| ZP (mV)                     | -5.63 $\pm$ 4.68 | -1.58 $\pm$ 1.23  |

(DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; EPL: Eplerenone ; F1: Formulation 1;B: Blank ; L: Liquid).

**Table 3.3.1.B.** DLS results of SEDDs-F2. Mean  $\pm$  SD, n = 3.

| Parameters\Formulation code | B-L-SEDDs-F2      | EPL-L-SEDDs-F2    |
|-----------------------------|-------------------|-------------------|
| DS (nm)                     | 189.4 $\pm$ 8.839 | 250.2 $\pm$ 62.31 |
| PDI                         | 0.414             | 0.422             |
| ZP (mV)                     | -34.7 $\pm$ 2.91  | -38.7 $\pm$ 2.03  |

(DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; B: Blank ; EPL: Eplerenone)

**Table 3.3.1.C.** DLS results of SEDDs-F3. Mean  $\pm$  SD, n = 3.

| Parameters/Formulation code | B-L-SEDDs-F3     | EPL-L-SEDDs-F3   |
|-----------------------------|------------------|------------------|
| DS (nm)                     | 35.05 $\pm$ 5.85 | 48.38 $\pm$ 0.36 |
| PDI                         | 0.317            | 0.233            |
| ZP (mV)                     | -28.3 $\pm$ 1.88 | -25.8 $\pm$ 5.75 |

(DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; EPL: Eplerenone ; F3: Formulation 3;B: Blank ;L: Liquid).)

### 3.3.2. Preparation of S-SEDDS formulation

Accordingly, Neusilin US2 and Aerosil 200 were selected as the optimized solidifiers for producing the S-SEDDs-F3. The characterization results of the solidified B-S-SEDDS and EPL-S-SEDDs are presented in (Table 3.3.2.C).

**Table 3.3.2.A.** Adsorption capacity of B-S-SEDDs-F1. Mean  $\pm$  SD, n = 3.

| Solid adsorbant  | Adsorption capability (SEDDs-F1): Solid adsorbant | DS (nm)           | PDI   | ZP (mV)            |
|------------------|---------------------------------------------------|-------------------|-------|--------------------|
| Calcium silicate | 1:2                                               | 527.56 $\pm$ 4.31 | 0.641 | -10.948 $\pm$ 4.31 |
| HPC              | 1:3                                               | 605.7 $\pm$ 1.37  | 0.691 | -6.24 $\pm$ 1.37   |
| Aerosil 200      | 1:0.6                                             | 398.1 $\pm$ 2.23  | 0.39  | -8.07 $\pm$ 2.23   |

(HPL, hydroxypropyl cellulose; DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems ; F1: Formulation 1).

**Table 3.3.2.B.** Adsorption capacity of B-S-SEDDs-F3. Mean  $\pm$  SD, n = 3

| Solid adsorbant  | Adsorption capability (SEDDs-F3): Solid adsorbant | DS (nm)           | PDI   | ZP (mV)          |
|------------------|---------------------------------------------------|-------------------|-------|------------------|
| Calcium silicate | 1:2                                               | 971 $\pm$ 1.14    | 0.756 | -3.31 $\pm$ 1.14 |
| HPC              | 0.18:1                                            | 832.8 $\pm$ 4.29  | 0.606 | -10.1 $\pm$ 4.29 |
| Aerosil 200      | 1:0.6                                             | 351.5 $\pm$ 1.75  | 0.411 | -8.28 $\pm$ 1.75 |
| Neulisin US2     | 1:0.6                                             | 343.0 $\pm$ 64.88 | 0.412 | -29.0 $\pm$ 4.24 |

(HPL, hydroxypropyl cellulose; DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; F3: Formulation 3)

**Table 3.3.2.C.** DLS results of S-SEDDS-F3 formulations. Mean  $\pm$  SD, n = 3

| Parameters\Formulation code | B-S-SEDDS-NUS2    | EPL-S-SEDDS-NUS2  | B-S-SEDDS-A200   | EPL-S-SEDDS-A200 |
|-----------------------------|-------------------|-------------------|------------------|------------------|
| DS (nm)                     | 343.0 $\pm$ 64.88 | 432.6 $\pm$ 65.03 | 351.5 $\pm$ 1.75 | 385 $\pm$ 0.68   |
| PDI                         | 0.412             | 0.446             | 0.411            | 0.388            |
| ZP (mV)                     | -29.0 $\pm$ 4.24  | -28.13 $\pm$ 1.20 | -8.28 $\pm$ 1.75 | -6.58 $\pm$ 0.45 |

(DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; NUS2: Neusilin; SEDDS: Self emulsifying drug delivery systems; EPL: Eplerenone; NUS2: Neusilin US2; A200: Aerosil 200; F3: Formulation 3; S: Solid; B: Blank.)

### 3.4. SEDDS features

#### 3.4.1. L-SEDDS features

##### 3.4.1.1. Thermodynamic stability

SEDDS must remain stable in a variety of conditions and maintain their ability to self-emulsify after dilution. [55] As a result, phase separation and thermodynamic stability experiments were conducted to determine the ability of developed SEDDS to tolerate stress environments [107]. These studies were performed in the following cycles, and the results were presented in (Table 3.4.1.1). The thermodynamic stability experiments confirmed that B-L-SEDDS-F1, B-L-SEDDS-F3, and EPL-L-SEDDS-F3 all pass with no indication of phase separation.

### 3.4.1.1.1. Chemical stability

Chemical stability DLS outcomes are presented in (Table 3.4.1.2). Previous DLS results at the beginning of the study are shown in (Table 3.3.2.C, Table 3.4.2.2) for comparison purposes.

**Table 3.4.1.1.** Thermodynamic stability studies (Visual examination) of all formulations.

| Formulation code       | Heating-cooling cycle | Centrifugation | Freeze-thaw stress cycle | Pass/not pass |
|------------------------|-----------------------|----------------|--------------------------|---------------|
| B-L-SEDDs-F1 2W        | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-L-SEDDs-F2 2W        | SIP                   | SIP            | SIP                      | Not pass      |
| B-L-SEDDs-F3 2W        | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-L-SEDDs-F3 2W      | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-L-SEDDs-F3 M1        | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-L-SEDDs-F3 M1      | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-A200 M1   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-A200 M1 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-NUS2 M1   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-NUS2 M1 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-A200 M2   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-A200 M2 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-L-SEDDs-F3 M2        | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-L-SEDDs-F3 M2      | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-NUS2 M2   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-NUS2 M2 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-A200 M3   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-A200 M3 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-S-SEDDs-F3-NUS2 M3   | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-S-SEDDs-F3-NUS2 M3 | NIOS                  | NIOS           | NIOS                     | Pass          |
| B-L-SEDDs-F3 M3        | NIOS                  | NIOS           | NIOS                     | Pass          |
| EPL-L-SEDDs-F3 M3      | NIOS                  | NIOS           | NIOS                     | Pass          |

(SEDDs: Self-emulsifying drug delivery systems; EPL: Eplerenone; W= Week; M= Month; NUS2: Neusilin US2; A200: Aerosil 200; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; S: Solid; NIOS: No indication of segregation ; SIP: Segregation is present; L: Liquid; B: Blank.)

**Table 3.4.1.2.** Droplet size results after thermodynamic stability studies. Mean  $\pm$  SD, n = 3.

| Formulation code              | DS (nm)          | PDI   | ZP (mV)          |
|-------------------------------|------------------|-------|------------------|
| B-L-SEDDs-F1 (week 1)         | 36.68 $\pm$ 3.73 | 0.187 | -1.77 $\pm$ 1.14 |
| B-L-SEDDs-F1 (week 2)         | 13.47 $\pm$ 5.50 | 0.192 | -3.31 $\pm$ 7.50 |
| B-L-SEDDs-F3 (week 1)         | 43.97 $\pm$ 1.75 | 0.174 | -10.4 $\pm$ 1.45 |
| B-L-SEDDs-F3 (week 2)         | 59.16 $\pm$ 2.84 | 0.192 | -6.61 $\pm$ 1.01 |
| EPL-L-SEDDs-F3 (week 1)       | 64.53 $\pm$ 6.23 | 0.43  | -16.7 $\pm$ 0.57 |
| EPL-L-SEDDs-F3 (week 2)       | 86.39 $\pm$ 1.05 | 0.357 | -6.18 $\pm$ 0.24 |
| B-L-SEDDs-F3 (Month 1)        | 29.52 $\pm$ 3.84 | 0.35  | -9.43 $\pm$ 1.26 |
| EPL-L-SEDDs-F3 (Month 1)      | 48.07 $\pm$ 5.24 | 0.282 | -6.27 $\pm$ 0.42 |
| B-L-SEDDs-F3 (Month 2)        | 22.10 $\pm$ 0.65 | 0.222 | -14.3 $\pm$ 0.49 |
| EPL-L-SEDDs-F3 (Month 2)      | 14.70 $\pm$ 0.40 | 0.324 | -5.53 $\pm$ 1.64 |
| B-L-SEDDs-F3 (Month 3)        | 15.39 $\pm$ 1.26 | 0.45  | -5.04 $\pm$ 0.89 |
| EPL-L-SEDDs-F3 (Month 3)      | 15.10 $\pm$ 0.44 | 0.404 | -7.25 $\pm$ 1.22 |
| B-S-SEDDS-F3-A200 (Month 1)   | 229.3 $\pm$ 7.77 | 0.327 | -18.6 $\pm$ 1.43 |
| EPL-S-SEDDS-F3-A200 (Month 1) | 341.7 $\pm$ 5.45 | 0.398 | -5.90 $\pm$ 2.76 |
| B-S-SEDDS-F3-A200 (Month 2)   | 265.2 $\pm$ 0.59 | 0.462 | -13.1 $\pm$ 1.66 |
| EPL-S-SEDDS-F3-A200 (Month 2) | 279.5 $\pm$ 0.77 | 0.396 | -8.35 $\pm$ 3.77 |
| B-S-SEDDS-F3-A200 (Month 3)   | 375.1 $\pm$ 1.47 | 0.578 | -6.00 $\pm$ 3.86 |
| EPL-S-SEDDS-F3-A200 (Month 3) | 398.4 $\pm$ 0.54 | 0.47  | -12.7 $\pm$ 2.95 |
| B-S-SEDDS-F3-NUS2 (Month 1)   | 349.6 $\pm$ 11.5 | 0.424 | -2.50 $\pm$ 0.42 |
| EPL-S-SEDDS-F3-NUS2(Month 1)  | 410.7 $\pm$ 17.1 | 0.475 | -23.8 $\pm$ 2.75 |
| B-S-SEDDS-F3-NUS2 (Month 2)   | 689 $\pm$ 8.24   | 0.453 | -12.9 $\pm$ 1.17 |
| EPL-S-SEDDS-F3-NUS2(Month 2)  | 733.5 $\pm$ 5.52 | 0.399 | -6.39 $\pm$ 0.03 |
| B-S-SEDDS-F3-NUS2 (Month 3)   | 766.6 $\pm$ 50   | 0.591 | -6.05 $\pm$ 2.09 |
| EPL-S-SEDDS-F3-NUS2(Month 3)  | 758.03 $\pm$ 31  | 0.346 | -5.90 $\pm$ 0.70 |

(Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; EPL: Eplerenone; NUS2: Neusilin US2; A200: Aerosil 200; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; S: Solid; L: Liquid; B: Blank)

#### 3.4.1.2. Visual evaluation of self-emulsification effectiveness

When SEDDS are added in aqueous media, they develop oil/water emulsions with agitation. Due to the relatively small amount of free energy necessary to produce an emulsion, its development is thermodynamically spontaneous. [108] The visual examination is used to determine the apparent spontaneously with which an emulsion forms. Three formulations of SEDDS were developed, and their self-emulsifying capabilities were visually assessed in (Table 3.4.1.3).

**Table 3.4.1.3.** Visual evaluation of self-emulsification effectiveness of all the SEDDS formulations

| Formulation code | Amount of water added in (mL) | Clear\Turbid | Pass\not pass |
|------------------|-------------------------------|--------------|---------------|
| SEDDs-F1         | More than 20 ml               | Clear        | Pass          |
| SEDDs-F2         | Less than 5 ml                | Turbid       | Not pass      |
| SEDDs-F3         | More than 20 ml               | Clear        | Pass          |

(SEDDs: Self emulsifying drug delivery systems; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3)

#### 3.4.1.3. Rheological properties determination

All of the Physico-chemical parameters have been examined to test all of the variables that could affect the drug release of the S-SEDDS and the interactions between the adsorbed liquid and the solid carriers. [109] The viscosity values measured for SEDDs-F1, SEDDs-F2, and SEDDs-F3 are depicted in (Table 3.4.1.4). All of the investigated formulations revealed low viscosity and Nonnewtonian pseudoplastic flow, which indicates an easy pourability characteristic.

**Table 3.4.1.4.** Rheological properties of all formulation

| rpm\Formulation code | B-SEDDS-F1 | B-SEDDS-F2 | B-SEDDS-F3 | EPL-SEDDS-F3 |
|----------------------|------------|------------|------------|--------------|
| 10 rpm               | 400 cp     | 400 cp     | 800 cp     | 400 cp       |
| 20 rpm               | 200 cp     | 200 cp     | 400 cp     | 200 cp       |
| 30 rpm               | 133.3 cp   | 133.3 cp   | 266 cp     | 133.3 cp     |
| 40 rpm               | 100 cp     | 100 cp     | 200 cp     | 100 cp       |
| 50 rpm               | 160 cp     | 80 cp      | 80 cp      | 80 cp        |
| 100 rpm              | 120 cp     | 40 cp      | 40 cp      | 40 cp        |
| 200 rpm              | 100 cp     | 20 cp      | 0 cp       | 0 cp         |

(SEDDS: Self emulsifying drug delivery systems; rpm: rounds per minute; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; EPL: Eplerenone; B: Blank)

#### 3.4.1.4. Drug content (L-SEDDS)

The percentage of drug loading capacity was ( $34.73 \pm 1.14$ ) for EPL-L-SEDDs-F1 and ( $73.59 \pm 1.06$ ) for EPL-L-SEDDs-F3 which was practically equals to 2.85 ml of F1-SEDDS and 2 ml of F3-SEDDS that acquired to dissolve the drug at room temperature entirely without the need for heating to prevent the chance of crystallization caused by heating the formula.

**Table 3.4.1.5.** Eplerenone (EPL) quantity in L-SEDDS, S-SEDDS formulations. Mean  $\pm$  SD, n = 3.

| ● Formulation code          | Percentage of drug content % |
|-----------------------------|------------------------------|
| EPL-L-SEDDs-F1 (randomly)   | $34.73 \pm 1.14$             |
| EPL-L-SEDDs-F3 (randomly)   | $73.59 \pm 1.06$             |
| EPL-S-SEDDs-F3-A200 (~25mg) | $106.8 \pm 0.18$             |
| EPL-S-SEDDs-F3-NUS2 (~25mg) | $103.3 \pm 0.92$             |

(SEDDS: Self emulsifying drug delivery systems; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3 EPL: Eplerenone; L: Liquid; S: Solid)

#### 3.4.1.5. Determination of droplet size for L-SEDDS

As described in Subsubsection 2.2.5.1.5, B-L-SEDDS, as well as EPL-L-SEDDS formulations features, were evaluated utilizing DS, PDI, ZP. EPL-L-SEDDS-F3 was selected for the solidifying process due to its highest EPL content (37.164 mg/mL), (DS =  $48.38 \pm 0.36$ ), (PDI = 0.233), and the highest (ZP =  $-25.8 \pm 5.75$ ) value as shown previously in (Table 3.3.1.C)

#### 3.4.2. S-SEDDS features

##### 3.4.2.1. Drug content (S-SEDDS)

EPL content was also examined in S-SEDDS with different solid carriers in (Table 3.4.1.5) The percentage of drug loading capacity was ( $106.8 \pm 0.18$ ) for EPL-S-SEDDS-F3-A200 and ( $103.3 \pm 0.92$ ) for EPL-S-SEDDS-F3-NUS2.

##### 3.4.2.2. Determination of droplet size for S-SEDDS

The results of the S-SEDDS analysis were evaluated by comparing it to those of the previous L-SEDDS particle size analysis in (Table 3.4.2.2).

**Table 3.4.2.2.** DLS results of liquid and solid EPL-SEDDS formulations. Mean  $\pm$  SD, n = 3.

| Parameters/Formulation code | EPL-L-SEDDS-F3   | EPL-S-SEDDS-F3-NUS2 | EPL-S-SEDDS-F3-A200 |
|-----------------------------|------------------|---------------------|---------------------|
| DS (nm)                     | $48.38 \pm 0.3$  | $432.6 \pm 65.03$   | $385 \pm 0.68$      |
| PDI                         | 0.233            | 0.446               | 0.388               |
| ZP (mV)                     | $-25.8 \pm 5.75$ | $-28.13 \pm 1.20$   | $-6.58 \pm 0.45$    |

(DS, Droplet size; PDI, The polydispersity index; ZP, Zeta potential; SEDDS: Self emulsifying drug delivery systems; F3: Formulation; EPL: Eplerenone)

### 3.4.2.3. Differential scanning calorimetry (DSC)

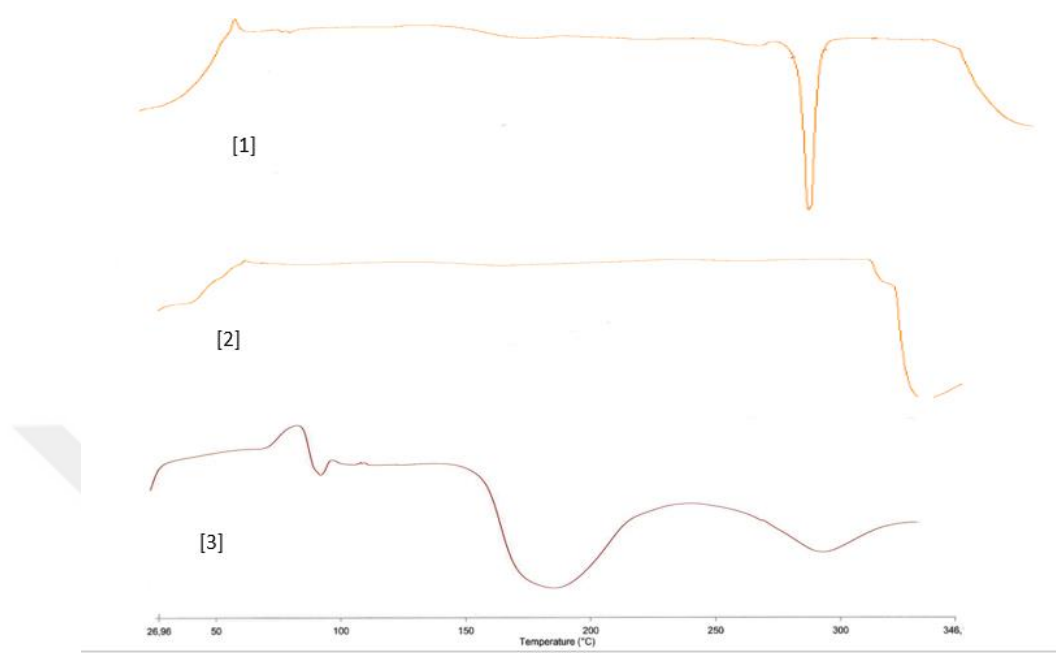


**Figure 3.4.2.3.** Solid-state characterization of [1]: Pure-EPL, [2]: EPL-S-SEDDS-F3-NUS2, and [3]: EPL-S-SEDDS-F3-A200 using DSC device.

#### 3.4.2.4. FT-IR scanning

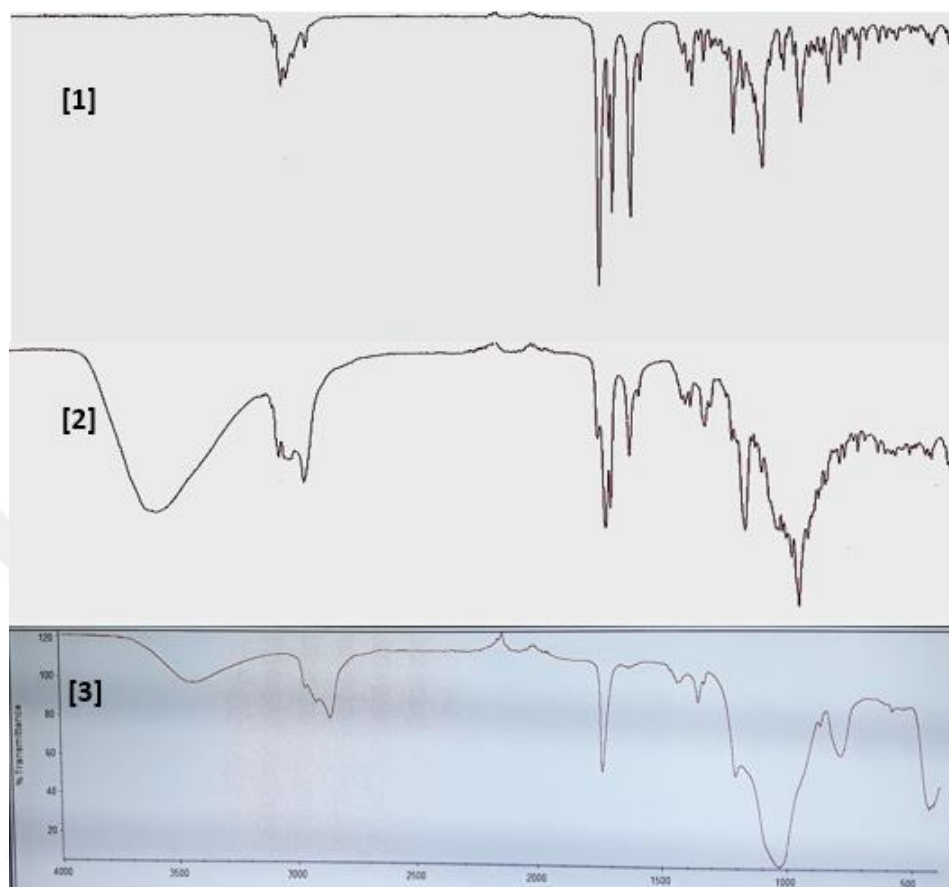


**Figure 3.4.2.4.** Solid-state characterization of [1]: Pure-EPL, [2]: EPL-S-SED DS-F3-NUS2, and [3]: EPL-S-SED DS-F3-A200 using FTIR.

### 3.5. Morphological analysis of S-SEDDS

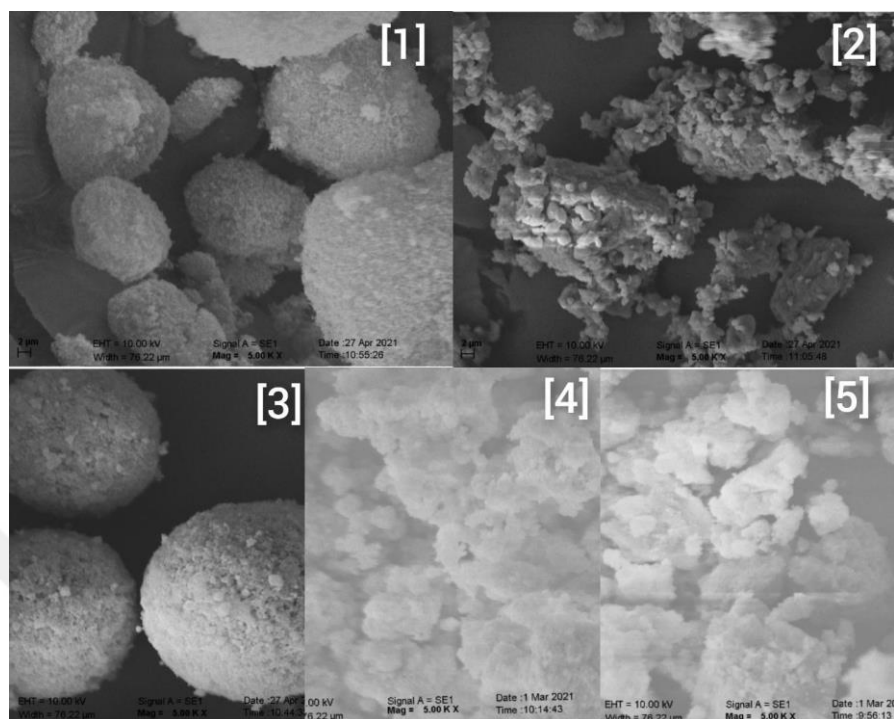


**Figure 3.5.** SEM images of [1]: Pure Aerosil 200, [2]: Pure EPL, [3]: Pure Neusilin US2, [4]: EPL-S-SEDDS-F3-NUS2, [5]: EPL-S-SEDDS-F3-A200. The magnification bar is referred in all pictures.

### 3.6. In vitro dissolution studies

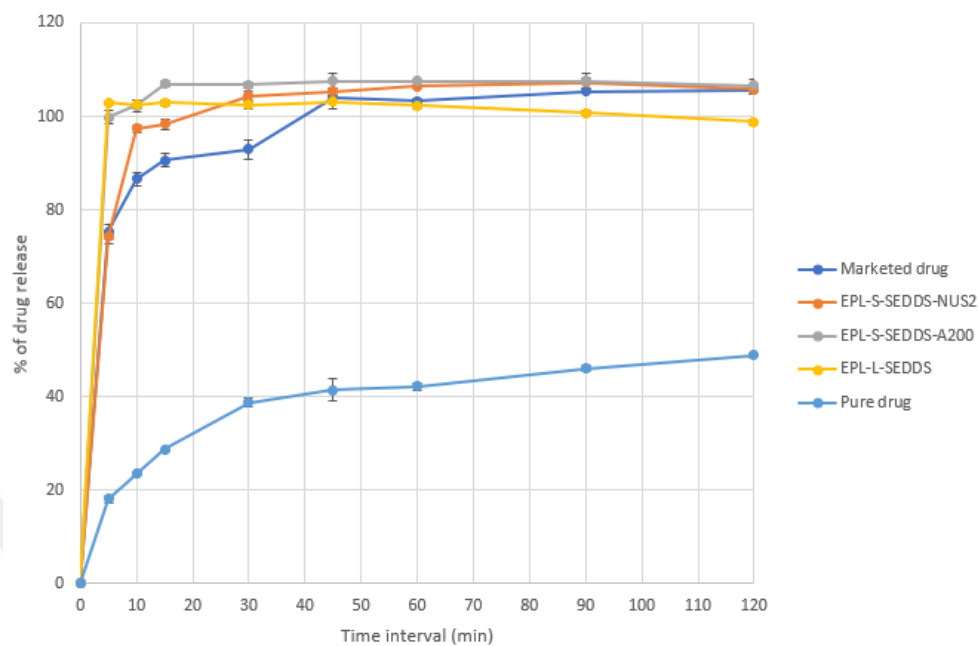


**Figure 3.6.** EPL release profiles obtained for the formulations as unformulated drug, marketed, EPL-L-SEDDS-F3, EPL-S-SEDDS-F3-A200, EPL-S-SEDDS-F3-NUS2 in 0.1N HCl.

## 4. DISCUSSION and CONCLUSION

### 4.1. Discussion

Eplerenone is a class II antihypertensive medication with low aqueous solubility and a high permeability, as stated by the Biopharmaceutics classification system (BCS). [3] with only 69% bioavailability. Eplerenone was taken as a model drug to enhance its solubility by using many formulation approaches in (Table 1.5.1.2).

SEDDS was selected in this study due to its significant advantages. [22, 21, 23] Many articles proved the ability of SEDDS to improve the solubility of active pharmaceutical ingredients with increased bioavailability on animal studies. [8, 104, 68]

Medicines solubility in oil is a critical factor in developing stable SEDDS formulations. The medicine should have a high solubility in the solvent to prevent sedimentation during the formulation's shelf life and upon dilution in the aqueous phase. [99, 110] EPL solubility in a variety of components is discussed in (Figure 3.1). The highest solubility of EPL was demonstrated in oils such as Triacetin (11.99 mg/mL), surfactants such as Kolliphor EL (2.65 mg/mL), and Tween 80 (1.91 mg/mL), as well as co-surfactants including Dimethylsulfoxide (DMSO) (8.50 mg/mL) and PEG 200 (7.57 mg/mL) and diethylene glycol monoethyl ether (Transcutol p) (6.03 mg/mL). Triacetin was an excellent oil selection for EPL compared with different oils reported in previous studies. [82, 96] Triacetin's high solubility is mainly due to the medium-chain fatty acids as well as the smaller molar volume in the Triacetin triglycerides, which facilitate EPL solubility. [111]

Additionally, the components that produced the highest solubility of EPL were applied to generate the pseudo ternary phase diagram. The choosing of a surfactant in SEDDS formulation is primarily determined by its compatibility, safety, and capacity to reduce the surface free energy within the w/o interface, resulting in stable systems physically. [112] Nonionic surfactant has a lower physiological toxic effect than the ionic equivalent. Additionally, nonionic surfactants show emulsification features at lower doses, as shown by a reduced critical micelle concentration, which is ideal for oral delivery. Surfactants at elevated doses cause GIT irritating. Additionally, in o/w SEDDS, a water sustainable nonionic surfactant [HLB value more than 10] exhibits an increased positive surface excess, leading to an accretion across the o/w interface efficient surface tension reduction and better stable system. [42] During this study, two

nonionic surfactants with such an elevated HLB were assessed for appropriateness in the formulation of EPL-SEDDS, depending on their sustainability. Kolliphor EL and Tween 80 exhibited the highest EPL solubility beside a high HLB (HLB = 14) and (HLB = 15), respectively, making them great surfactant choices to produce a stable EPL-SEDDS. All of the excipients chosen had a long history of usage in cosmetics, foods, as well as in medicinal formulations administered orally, topically, or parenterally. They are commonly considered to be generally harmless and nonirritating compounds, specifically biologically suitable and safe excipients.

SEDDS produce o/w emulsions once added in an aqueous solution with only mild stirring. On the interface, surfactant, co-surfactant adheres and lower the interfacial energy. The reduction in the free energy needed to produce the emulsion increases the formulation's thermodynamic stability. [99, 100] Pseudo ternary phase diagram was developed to detect the self-emulsifying zones and calculate the optimal surfactant, oil, and co-surfactant quantities of three distinct SEDDS formulations. Any increases within the co-surfactant quantity resulted in an increase as well in the self-emulsifying zone as shown in formulation 3 diagram (Figure 3.2.C). [107] During the preparation of L-SEDDS, it was found that B-L-SEDDS-F1, B-L-SEDDS-F3, EPL-L-SEDDS-F1, and EPL-L-SEDDS-F3 median particle size was less than 100 nm. The detected increase in the DS ( $p > 0.05$ ) in B-L-SEDDS-F2 and EPL-L-SEDDS-F2 was most likely associated with an elevation in the oil phase concentration and a decline in the surfactant quantity. [107] The ZP was utilized to determine the droplet's voltage charge. The ZP value represents the strength of electrostatic attraction among droplets in a dispersion. The strong ZP ensures stable dispersion and inhibits agglomeration. Stable SEDDS formulations would have a ZP greater than  $\pm 25$  mV. The charges of the oil particles in typical SEDDS usually negative due to the availability of free fatty-acids. [113, 114, 107] B-L-SEDDS-F2 and EPL-L-SEDDS-F2, and F3 have shown ZP readings higher than 25 mV. Additionally, it was noticed that surfactant addition helped to increase the ZP value. [107] The solidification technique of the liquid SEDDS is a new approach to upgrade the SEDDS, and it has many advantages. [33, 59, 45] Adsorption to solid carriers is a basic technique and provides a good content uniformity. [33,] The procedure begins with the addition of the L-SEDDS to solid carriers and combines them using a mortar and pestle.

As described in Subsubsection 2.2.4.2, the most often utilized solidifiers for the preparation of S-SEDDs formulations include non-water soluble solidifiers like Neulisin US2, Aerosil 200, calcium silicate, and water-soluble solidifiers, including HPC. [115, 116 ] The adsorption capability of these solidifiers (reported as a weight ratio of L-SEDDs: Solidifier). Each solidifier had a varied capability for adsorption, resulting in a free-flowing powder of S-SEDDs formulations as indicated in (Table 3.3.2.A and 3.3.2.B). [104] Both Neusilin US2 (NUS2), as well as Aerosil 200 (A200), had the highest capability for adsorption of (1:0.6). Additionally, they developed B-S-SEDDS and EPL-S-SEDDS formulations with DS values within the acceptable range (100-500 nm) and PDI values less than 0.5. Similarly, the same pattern of feature changes was observed upon solidification of the L-SEDDs formulation in previously published articles. [117, 104]

SEDDs-F1 and SEDDs-F3 exhibited excellent emulsification efficacy in the visual evaluation of the self-emulsification effectiveness test. It was discovered that increasing the amount of co-surfactant in the self-emulsifying formulation increased the spontaneous nature of the self-emulsification process. When a co-surfactant is applied into the formulation, the interfacial tension between both the water and oil interface is further reduced. Additionally, it affects the curvature of the interfacial film, which rapidly reshapes the surrounding oil particles. [118, 119]

In the drug content test, the L-SEDDS formulations that contained a higher oil concentration carried more quantities of the drug, which was associated with the drug's high solubility in oil such as triacetin and supports the necessity of drug solubility being the significant factor for oil selection. [82]

The high drug content in S-SEDDs indicates an excellent drug occupation characteristic due to the high surface area in both solid carriers (Aerosil 200 and Neusilin US2) used in the formulations. [120]

DS measurements showed an increased pattern from EPL-L-SEDDs-F3 to EPL-S-SEDDs-F3. EPL-S-SEDDs-F3 had a larger PDI value than EPL-L-SEDDs-F3. The high DS and PDI values could be due to solid carriers' influence (A200, NUS2). The average DS of S-SEDDS formulations was observed to be based on the solid carriers in previous studies as well. [121, 117]

The physical condition of EPL within the formulation is essential for the release of drug in vivo and in vitro. [122] Differential scanning calorimetry (DSC) is an important device for determining the physical properties of the active ingredient. [123] The DSC thermograms of Pure EPL, EPL-S-SEDDs-NUS2, and EPL-S-SEDDS-A200 are given in (Figure 3.4.2.3). Pure EPL in (Figure 3.4.2.3, section [1]) was identified as a broad endothermic peak in the range of temperature between 25 to 300 °C related to the evaporation of water, accompanied with a sharp peak having a starting temperature of approximately 254.39°C caused by water evaporation and melting. [124] However, there were no drug peaks obtained in the thermogram for EPL-S-SEDDS-F3-NUS2 (Figure 3.4.2.3, section [2]) suggests changes in the melting behavior of EPL and inhibition of crystallization and change it to amorphous states during the preparation of S-SEDDs using lipid surfactants and solidifying materials (NUS2). [125] It might also be that the excipients have absorbed the energy (enthalpy) raised from the heat before the active substance. Contrastingly, the DSC thermogram of EPL-S-SEDDS-F3-A200 in (Figure 3.4.2.3, section [3]) showed two endothermic peaks at 194.03°C (enthalpy of fusion, 361.58 J/g) and 305.98 °C (enthalpy of fusion, 105.849 J/g) while only one exothermic peak at 89.61°C (enthalpy of fusion 255.040 J/g). The first exothermic peak corresponds to desolvation of formulation crystals depending on the excipients followed by recrystallization process indicated by the endothermic peak at 194.03°C, which belongs to the EPL. It was observed that the decrease in the melting point depends on the lipid and surfactant/cosurfactant within the content. However, the melting point corresponds to the second endothermic peak at 305.98 °C, which is associated with the adsorbant (A200) recrystallization process.

The results of the FT-IR spectrum (Figure 3.4.2.4, section [1]) of Pure EPL in the current studies showed that the primary feature functional groups are located at 2968.96  $\text{cm}^{-1}$  (C–H), 1773.59  $\text{cm}^{-1}$  (O–C=O), 1736.61  $\text{cm}^{-1}$  (C=O), and 1654.80  $\text{cm}^{-1}$  (C=O) based on previously released data. [78, 82] The representative EPL-S-SEDDS-F3-NUS2 spectra (Figure 3.4.2.4, section [2]) exhibited an additional spectrum, including the absorbance bonds of EPL and NUS2, the adsorbent utilized. It is also exhibited a distinct absorption bond frequency than Pure EPL. i.e., the formulation's (O–H) bond formed at a frequency of (3431.89  $\text{cm}^{-1}$ ), therefore was inside alcohol stretching absorption range (3200–3650  $\text{cm}^{-1}$ ) due to the presence of the co-surfactant (Transcutol

p). On the other hand, EPL-S-SEDDS-F3-A200 (Figure 3.4.2.4, section [3]) showed similarity with the EPL-S-SEDDS-F3-NUS2 IR spectrum with smaller alcohol stretching absorption band. Thus, the total FT-IR data revealed that neither structural alteration nor chemical interactions happened between all the oil, solid carrier NUS2, A200, and EPL. And all EPL functional groups were maintained during the transformation of EPL's physical characteristics in typical S-SEDDs. which confirmed the compatibility between EPL and formulation additives. [126, 82]

Scanning electron micrographs of the pure solid carriers (Aerosil 200, Neusilin US2), Pure EPL, and EPL-S-SEDDS-F3-A200, EPL-SEDDS-F3-NUS2 are shown in (Figure 3.5). Pure Aerosil 200 carrier and Pure EPL particles produced aggregates of irregularly shaped droplets. However, Pure Neusilin US2 particles were regular spherical shaped. Both EPL-S-SEDDS-F3-A200 and EPL-S-SEDDS-F3-NUS2 exhibit relatively smooth agglomerated droplets, implying that L-SEDDS is coated or absorbed within the pores of the two solid carriers utilized.

The chemical stability studies of B-S-SEDDS-F3 and EPL-S-SEDDS-F3 with two different solid carriers formulations showed increases in DS were observed due to its preservation in a humidity chamber ( $25 \pm 2$  °C/ $60 \pm 5\%$  RH) for three months ( $p > 0.05$ ). However, only B-L-SEDDS-F3 and EPL-L-SEDDS-F3 showed decreases in the droplet size simultaneously ( $p < 0.05$ ). To illustrate, over three months inside the humidity chamber, all formulations' ZP values varied and altered. A similar increase in results was found in the PDI values, including all formulations. Throughout the stability studies, no modification in physical parameters, including clarity or homogeneity, was noticed. Depending on the degree of the modification, special labeling, packaging, refrigerator storage may be required to overcome any complications.

The drug release profiles obtained for the five examined formulations in 0.1 N HCl (pH 1.2) are depicted in (Figure.3.6). The percent drug release showed an enhancing of drug solubility from all SEDD formulations, in which drug release was completed within the 5 minutes for both L-SEDDS and S-SEDDS-A200 and within 30 minutes for S-SEDDS-NUS2 in comparison to 95.3% and 39.4% for the Marketed and Unformulated drug releases, respectively. All SEDDS formulations had a rapid release pattern at 15, 30 min ( $p < 0.05$ ) compared to marketed and unformulated drug. Contrastingly, unformulated drug exhibited slower drug release ( $p > 0.05$ ) in all the time intervals than

SEDDS formulations and Marketed drug. It could be indicated that the formulations including SEDDS-A200 and L-SEDDS spontaneously produced a nanoemulsion having a small DS (~Below 400 nm), this allowed for a more rapid drug release rate in the aqueous medium as compared to S-SEEDS-NUS2 with a larger droplet size even though NUS2 revealed higher surface tension capacity. Consequently, this greater availability of dissolved EPL from the S-SEED-A200 formulation could lead to higher absorption and higher oral bioavailability. Ritger and Peppas confirmed the influence of PDI on the release of the drugs through nano/microemulsion systems. [127] These results verify that both Liquid and S-SEDDS s significantly ( $p < 0.05$ ) enhanced the dissolution of EPL.



## 4.2. CONCLUSION

EPL is a BCS class II medication that commonly undergoes excessive hepatic degradation. Triacetin, Tween 80, and Transcutol p are all suitable oils, surfactants, and co-surfactants. To generate the EPL-S-SEDDS formulations in this study, L-SEDDS was adsorbed with solid adsorbents, including Aerosil 200 and Neusilin US2. The S-SEDDS formulations showed an excellently combined droplets morphology and even surface. The capability of the S-SEDDS-F3 to self-emulsify was identical to that of the L-SEDDS-F3. Both DSC and FTIR tests revealed that the EPL-S-SEDDS-F3 retained the molecular dispersion condition, and no interactions seen between the active ingredient and excipients occurred. In vitro dissolution test demonstrated that the S-SEDDS released EPL more rapidly and efficiently than the marketed and pure drug ( $p < 0.05$ ). Therefore, these findings confirm the hypothesis that EPL-S-SEDDS is a potential oral dosage form that enhances the dissolution rate, thereby improving the bioavailability of EPL.

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