DEVELOPMENT OF SEMI-SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS) USING LIQUID MODEL DRUG FOR ORAL DRUG DELIVERY APPLICATION

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by

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LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

ACN	Acetonitrile
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Total area under the plasma concentration-time curve
AUC_{0-t}	Total area under the plasma concentration-time curve from the time zero to the last sampling time, t
$AUC_{t-\infty}$	Total area under the plasma concentration-time curve from the last sampling time to infinity
BCS	Biopharmaceutics classification system
C_{max}	Peak plasma concentration
CV	Coefficient of variation
DLS	Dynamic light scattering
D (v, 0.5)	Median volume diameter
G44/14	Gelucire® 44/14
GIT	Gastrointestinal tract
HCl	Hydrochloric acid
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
K_e	Elimination rate constant
Log P	Logarithm of octanol/water partition coefficient
o/w	Oil-in-water
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
R.S.D	Relative standard deviation
SD	Standard deviation

SEDDS	Self-emulsifying drug delivery systems
S.E.M	Standard error of mean
Tmax	Time to reach peak plasma concentration
USM	Universiti Sains Malaysia
UV	Ultraviolet
v/v	volume over volume
w/w	weight over weight

LIST OF APPENDICES

PEMBANGUNAN SISTEM PENGHANTARAN DRUG MENGEMULSI KENDIRI (SEDDS) SEMI PEPEJAL MENGGUNAKAN DRUG MODEL CECAIR UNTUK APLIKASI PENGHANTARAN DRUG SECARA ORAL

ABSTRAK

Satu kajian berkenaan pembangunan formulasi semi pepejal SEDDS dengan penambahbaikan biokeperolehan menggunakan Gelucire® 44/14 sebagai agen pengemulsi kendiri dan campuran tokotrienol (EVNol™ 50%) sebagai model drug cecair telah dijalankan. Satu siri formulasi dengan nisbah ekstrak tokotrienol dan Gelucire® 44/14 yang berbeza telah disediakan dan dinilai. Akhirnya, satu formulasi bernisbah 1: 1 bagi tokotrienol kepada Gelucire® 44/14 dengan penambahan 2.5% propilena glikol telah menghasilkan formulasi paling sesuai untuk mendapatkan SEDDS semi pepejal dengan sifat pengemulsi kendiri yang memuaskan. Daripada keseluruhan siri pengujian *in vitro*, formulasi tersebut yang mampu mengemulsi kendiri di bawah gegaran perlahan dan membentuk emulsi minyak di dalam air dalam jangkamasa 3 minit selepas disebarkan dalam media pengujian. Pembentukan emulsi tersebut adalah stabil di mana penyebaran saiz partikel emulsi mencapai skala nano.

Seterusnya, satu kajian telah dijalankan untuk menilai biokeperolehan bagi tokotrienol daripada penyediaan SEDDS semi pepejal dengan perbandingan antara formulasi minyak biasa (EVNol™ 50% oily formulation) dan produk komersial teremulsi kendiri (Tocovid Suprabio™ 50mg). Kajian tersebut dilakukan mengikut 3 tempoh, 3 turutan silang bagi tikus Sprague Dawley. Daripada keputusan yang diperolehi, penyediaan semi pepejal SEDDS telah mencapai kadar biokeperolehan yang lebih baik berbanding formulasi minyak biasa yang mana tidak mampu mengemulsi kendiri. Manakala, kadar

bioperolehan pepejal SEDDS adalah setanding dengan nilai biokeperolehan bagi produk komersial, Tocovid Suprabio™ (50mg) untuk ketiga-tiga tokotrienol.

DEVELOPMENT OF SEMI-SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS) USING LIQUID MODEL DRUG FOR ORAL DRUG DELIVERY APPLICATION

ABSTRACT

A study was conducted to formulate a semi-solid SEDDS with enhanced bioavailability using Gelucire® 44/14 as the self-emulsifying agent and a tocotrienols mixture (EVNol™ 50%) as the liquid model drug. A series of formulations with different proportions of tocotrienol extract and Gelucire® 44/14 were prepared and evaluated. Finally, a formulation with 1:1 ratio of tocotrienols to Gelucire® 44/14 with the addition of 2.5% of propylene glycol was found most suitable to obtain a semi-solid SEDDS with satisfactory self-emulsifying properties. From a series of *in vitro* studies, the formulation was able to self-emulsify under gentle agitation and formed an oil in water emulsion within 3 minutes upon contact with the dissolution media. The emulsion formed was stable, with the emulsion particle size distribution achieving nano-range. Subsequently, a study was conducted to evaluate the bioavailability of the tocotrienols from the semi-solid SEDDS preparation in comparison to a normal oily formulation (EVNol™ 50% oily formulation) and a commercial tocotrienols self-emulsifying product (Tocovid Suprabio™ 50mg). The study was performed according to a three-period, three-sequence cross-over design in Sprague Dawley rats. From the results obtained, the semi-solid SEDDS preparation was found to achieve better bioavailability than the normal oily formulation which does not have self-emulsifying properties, and slightly lower but nearly comparable bioavailability when compared to the self-emulsifying commercial product, Tocovid Suprabio™ (50mg) for all 3 tocotrienols.

CHAPTER 1

INTRODUCTION

1.1 ORAL DELIVERY OF LIPOPHILIC DRUGS

Oral dosage forms dominate more than half of the products in the market and the oral route is the most preferred way of drug administration (Zhong *et al.*, 2018; Brayden and O'Mahony, 1998). The oral route offers many advantages to the patients resulting in better patient compliance. However, the orally delivered drug may or may not be absorbed during its passage in gastro-intestinal tract (GIT). This is especially true for drugs that are lipophilic where the absorption process is hindered due to poor dissolution. Under the Biopharmaceutics Classification System (BCS) such drugs are classified under class II category. BCS II drugs are those with low water solubility and high intestinal permeability. Most BCS II drugs suffer from poor absorption and highly variable bioavailability (Holm, 2019; Zhang *et al.*, 2014). The major factors affecting oral bioavailability of a drug are solubility and permeability. In general, dissolution is usually the rate limiting step in drug absorption when the drug solubility in water is less than 10 mg / ml (Habib, 2000).

Successful development and commercialization of new drugs in the pharmaceutical industry is a major challenge, with more than 40% of new drugs have little or no water solubility (Connors & Elder, 2004). A drug whether in a liquid, semi-solid or solid form must be dissolved in the GI-fluid before it can be absorbed. Therefore, its absorption rate and extent depend largely on the rate of dissolution. The dissolution rate limits the absorption of poorly water-soluble drugs resulting in low oral bioavailability. The use of lipid materials in self-dispersing formulations has become a common way of improving oral bioavailability for lipophilic drugs in

particular (Pouton, 2000). In the case of poorly water-soluble drugs, lipid-based formulations are usually developed as liquids, as most lipids and surfactants suitable for dissolving drugs exist at room temperature as liquids. Oral lipid drug delivery is effective because the slow dissolution step that limits availability from the dosage form is avoided. Bioavailability of poorly water-soluble drugs can be enhanced by formulation in digestible oils. The drug remains in the solution during its passage along GIT prior to absorption. Lipid vehicles can enhance the absorption of poorly water-soluble drugs because the presence of lipids slows the gastric emptying time, promotes uptake from the intestinal lumen into the lymphatic system, improve solubilization with the formation of mixed micelles and increase the permeability of the epithelial membrane (Porter, Trevaskis and Charman, 2007). Co-administration with a suitable lipid vehicle and surfactant, has been shown to promote the uptake of highly lipophilic compounds into the lymphatic system (Kollipara and Gandhi, 2014).

The highly lipophilic compound namely tocotrienols was used in this study, is present as an oily liquid form by its natural occurrence. Its adsorption is thought to be identical to lipids and other lipophilic substances with a log P values over 5, making the tocotrienols a good candidate for lipid-based formulation (Agrawal *et al*., 2012). As reported by O'Driscoll, (2002) the absorption process of lipid-based formulations had occurred through lympatic transport for a drug molecule with a partition coefficient (log P) > 5 and triglyceride solubility>50mg/mL that had been used in lipid-based formulations. Studies with rats found that the absorption of tocotrienols by oral route was poor and incomplete (Yap and Yuen, 2004). Biodiscrimination occurred among the tocotrienols isomers in oral absorption and disposition. The absorption process may be altered by changes in the lipophilicity of the molecules between those three isomers. Alpha tocotrienol has three methyl groups, compared to two in gamma-

tocotrienol and one in delta-tocotrienol, therefore the lipophilicities of the three molecules differ, with alpha-tocotrienol having the highest lipophilicity, followed by gamma-tocotrienol, and delta-tocotrienol. Due to the poor and erratic bioavailability of tocotrienols, they are appropriate candidates to be studied in order to improve their oral bioavailability using systems such as self-emulsifying formulations. Tocotrienols are commonly prepared as liquid in SES and delivered using soft gelatin capsules. In recent years, there is increasing interest in semi-solid SEDDS such semi-solid SEDDS systems involve the solidification of liquid self-emulsifying (SE) ingredients into semi-solid / paste with incorporation of appropriate surfactants. The benefits of such SEDDS (enhanced the solubility and bioavailability) are combined with solid dosage forms (low cost of manufacturing, process control simplicity, good efficiency and reproducibility, greater compliance with patients) (Jannin, *et al.*, 2008; Tang *et al*., 2008). As reported in recent studies, semi-solid self-emulsifying drug delivery systems for drug progesterone had shown the enhanced in solubility of the poorly water-soluble in various media (Hassan and Mäder, 2015; Hassan *et al*., 2014).

1.2 SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS)

Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, and one or more hydrophilic solvents and co-solvents that spontaneously form oil-in-water (o/w) emulsions upon incorporation into the aqueous phase with mild agitation. Increased absorption of drugs with a low bioavailability can be achieved when the drugs are in the form of small droplets of oil and the formulation emulsifies rapidly in the stomach's aqueous contents (Nirosha *et al.*, 2016; Cerpnjak *et al.*, 2013; Singh *et al.*, 2009). SEDDS are normally formulated in the form of oily formulations encapsulated in soft gelatin capsules.

Traditionally, SEDDS are prepared by dissolution of drugs in oils by blending it with appropriate solubilizing agents. However, this conventional self-emulsifying method usually results in a liquid form, which has several drawbacks. High cost of production, low stability, portability, and drug loading as well as limited choice of dosage forms are some of the frequently reported disadvantages (Tang *et al.*, 2008).

Various studies have been conducted to investigate SEDDS as a useful candidate to overcome the poor oral bioavailability of newly discovered drugs with low in water solubility. Utilization of SEDDS had been reasonably effective in increasing the oral bioavailability of lipophilic and poorly water-soluble drugs (Kohli *et al.*, 2010). Pouton (1985) had conducted thorough discussions and reviews on the development of self-emulsifying systems, especially on various formulation aspects, followed by work by Gershanik and Benita (2000). These studies suggest that to enhance drug bioavailability, the emulsion formed by the self-emulsifying systems should have droplets of very fine sizes, preferably in colloidal dimensions. In contrast, for self-emulsifying systems that produce coarse emulsions, subsequent digestion or lipolysis of the oil droplets are required to improve drug bioavailability. SEDDS can also appear in the form of semi-solid when their formulation utilizes semi-solid oils or surfactants. As a result, semi-solid SEDDS could potentially incorporate the benefits of SEDDS (i.e., increased solubility and bioavailability) and solid dosage forms (e.g., low production costs, ease of process control, high stability and reproducibility), as well as improved patient compliance (Mandić *et al.*, 2017). Typically, hard gelatin capsules are used to encapsulate these semi-solid formulations. Compared to the oily self-emulsifying formulations, semi-solid SEDDS differ only in terms of their physical appearance. Lahr *et al.*, (1986) had developed a semi-solid formulation for nifedipine which was filled into the hard gelatin capsules; the results of the semi-solid dosage

form was shown to be bioequivalent to nifedipine oral liquid and soft gelatin capsules (Hermann *et al.*, 1986). In another study, Ito *et al.*, (2006) had discovered that the clinical treatment of venous thrombo-embolism, low molecular weight heparin (LMWH) was only available via parenteral route. Then, they had successfully formulated the LMWH as self -emulsifying formulations in solid dosage forms and filled into hard capsules which were suitable to be consumed through oral route.

In this study, a suitable candidate for developing the lipid-based formulation was chosen namely mixed tocotrienols, which can be formulated into semi-solid SEDDS. In summary, for semi-solid SEDDS of tocotrienols to be successful, some formulation aspects must be considered including of the type and amount of oil as well as emulsifying agents/surfactants used, the usage of co-surfactant/solvent, the size of emulsion product droplets and the final bioavailability enhancement of the system. One example of successful utilization of tocotrienols has already been mentioned earlier, that is Hovid Sdn. Bhd, a Malaysian pharmaceutical company which has patented a tocotrienols formulation that can increase the extent of bioavailability of the three isomers tocotrienol by approximately 300%.

1.2.1 EXCIPIENTS USED IN SEDDS

Oils, surfactants, and co-surfactants or co-solvents all contribute to the self-emulsifying properties of self-emulsifying drug delivery systems. SEDDS can be formulated using a wide variety of excipients, including natural or synthetic oils, solid and liquid surfactants with varying degrees of hydrophilicity, and co-solvents with varying degrees of solvation capacity. A satisfactory formulation of SEDDS requires the use of optimal excipient (Nardin and Köllner, 2019; Kollipara and Gandhi, 2014).

1.2.1 (a) OILS

Oils serve as an important component in the formulation of SEDDS. It is mainly utilized as a carrier to dissolve lipophilic drug. Due to their poor ability to dissolve a large amount of lipophilic drug, naturally derived oils are seldom used as the oil portion in SEDDS may be too large. For this reason, synthetic or chemically modified oils, such as hydrolysed vegetables oils are selected and widely used in SEDDS. Chemically modified oils possess unique surfactant properties and had been shown to have better ability to facilitate self-emulsification in addition to having better drug solubility properties (Cerpnjak *et al.*, 2013). Also, higher fluidity, better solubility properties and self-emulsification ability of medium chain triglycerides made this class of oil be utilized in earlier works to formulate SEDDS rather frequently (Kollipara and Gandhi, 2014). However, the emergence of novel semi synthetic oils with amphiphilic properties such as polyglycolized glycerides with varying fatty acids and polyethylene glycol chain lengths made the former class of oil to be less attractive.

1.2.1 (b) SURFACTANT

Surfactants, or surface-active agents, are amphiphilic molecules that contain both hydrophilic and lipophilic components. Surfactants are typically used to aid in the self-emulsification process of the SEDDS, thereby improving or increasing the bioavailability of poorly absorbable drugs. The hydrophile-lipophile balance (HLB) values of surfactants are used to classify them. This HLB value indicates a surfactant's hydrophilicity. Surfactants that are more hydrophilic have a higher HLB value. Surfactants with HLB values between approximately 8 and 18 are typically used to formulate SEDDS (Chen *et al.*, 2011; Hauss, 2007). Generally, the required non-ionic hydrophilic surfactants which have been used for SEDDS formulation (i.e; Gelucire®

44/14, Gelucire® 50/13, Labrasol®, Cremophor® EL, Cremophor® RH 40, etc.) are types of surfactant with HLB values above 12 (Cole *et al.*, 2008). These SEDDS formulations usually spontaneously form oil-in-water dispersions with droplet size in nano scale range upon dilution with digestive fluids in GIT (Pouton *et al.*, 2007). Previous works had been reported by Gursoy and Benita (2004) and indicate that incorporated high HLB values type surfactants gave rapid emulsification of the formulation. Thus, it led to the formation of very fine o/w droplets due to their excellent spreading properties and rapid formation for cloudy emulsion. C8/C10 polyglycolized glycerides (Labrasol) were chosen for self-emulsification properties studies because they had the highest HLB value of 14 and the most satisfactory self-emulsification properties.

When selecting the type of surfactant to use in the formulation of SEDDS, several factors are considered, including emulsification performance, safety, and the stability of the emulsion formed upon contact with aqueous medium. While naturally derived surfactants are safe to consume, they have a limited capacity for self-emulsification. Synthetic surfactants on the other hand can be divided into non-ionic and ionic types. Among them, non-ionic surfactants are widely recommended due to their less toxic nature. From studies conducted on concentration of surfactants, the range of 30% to 60% (w/w) was recommended to form stable SEDDS (Gursoy and Benita, 2004). The amount of surfactants used is critical. If the amount used is too high, it may cause irritation of the gastrointestinal tract whilst the self-emulsification ability of SEDDS might be compromised if insufficient amount of surfactants is used (Agrawal *et al.*, 2012). Surfactants that are frequently used in SEDDS include ethoxylated polyglycolized glycerides in both solid and liquid form, as well as polyoxyethylene 20 oleate (polysorbate 80) (Gursoy and Benita, 2004). When Pouton

et al. (2007) investigated a wide variety of industrial non-ionic surfactants, they discovered that the most efficient SEDDS could be formulated using surfactants with predominantly unsaturated acyl chains.

1.2.1 (c) CO-SURFACTANT /CO-SOLVENT

Increasing the solvent capacity of the formulations can be achieved by incorporating co-surfactant/ solvents into SEDDS especially in those which contain a large amount (30% - 60%) of hydrophilic surfactants. High solvent capacity in SEDDS is necessary to dissolve large number of lipophilic drugs or hydrophilic surfactants. The homogeneity and stability of the formulation can be achieved in the oil phase by using co-solvent due to the enhancement of dispersibility of hydrophilic surfactants (Buya *et al.*, 2020; Cerpnjak *et al.*, 2013). SEDDS must have a large solvent capacity to dissolve a large number of lipophilic drugs or hydrophilic surfactants. Organic solvents such as propylene glycol and polyethylene glycol are typically used because they are non-toxic to humans. Alcohols and other volatile solvents, on the other hand, may not be suitable due to the risk of lipophilic drugs precipitating when these solvents migrate into the shell of hard and soft gelatin capsules (Gursoy and Benita, 2004).

1.2.2 IMPROVEMENT OF ORAL ABSORPTION BY SEDDS

For drugs that are poorly soluble in water, such as those in BCS II, the dissolution process of the drugs from their preparations is the rate limiting step in gastrointestinal absorption. According to the Noyes-Whitney equation, the rate of dissolution is proportional to the drug's exposed surface area. The surface area of a particle is increased by reducing its size or by increasing its dispersibility. SEDDS has been reported to improve the oral absorption of poorly water-soluble drugs, and partially avoid the additional drug dissolution step prior to absorption in the GI tract

(Kawabata *et al.*, 2011; Benameur, 2006). They increase the solubility of the drug in intestinal fluids, resulting in improved absorption. Additionally, absorption of the drug may be aided by the use of lipid-based excipients in the formulation. As example, the atorvastatin had been classified as member of class II drugs of Biopharmaceutics Classification System (BCS) (Czajkowska-Košnik *et al.*, 2015) and Shen and Zhong (2006) has reported that the selection of optimal composition to formulate the SEDDS formulation had improved its intestinal solubility and mucosal permeability, thus there is a significant increase in bioavailability of the formulations. Previous work has discovered through beagle dogs, that the relative bioavailability of carvedilol was increased by 4.1 times through SEDDS formulations compared to tablets (Wei *et al.*, 2005). Oral bioavailability of indomethacin was studied by Kim and Ku (2000) using rats and there was a significant increase when the drug was administered as a SEDDS compared to a methylcellulose suspension form. Araya *et al.* (2005) described a versatile novel SEDDS formulation that increased the aqueous solubility of poorly water-soluble compounds, thereby increasing their oral bioavailability. When six poorly water-soluble drugs, namely ibuprofen, ketoprofen, tolbutamide, AG041R, BO-653, and ER-1258, were formulated using the SEDDS, their aqueous solubility increased by 340 to 98000 folds. Additionally, it was determined that the oral bioavailability of the drugs is equivalent to that of a solution or an oil in water emulsion. The oral bioavailability of the drugs was increased by 1.5 to 78 times when compared to standard aqueous suspensions.

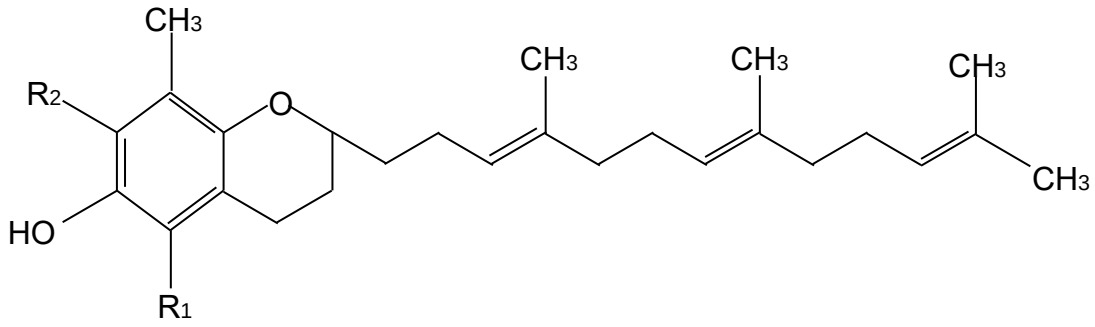
In summary, SEDDS formulations have a great potential to improve the oral bioavailability of poorly water-soluble drugs by presenting the drugs in a solubilized form within the oil phase which self-emulsifies in the gastrointestinal tract to form fine oil droplets.

1.3 DEVELOPMENT OF SEMI-SOLID SEDDS USING LIQUID MODEL DRUG

Self-emulsifying drug delivery systems (SEDDS) refer to formulations that are emulsifiable and water-free, typically composed of oil and emulsifier with or without hydrophilic co-surfactant/solvent. The oil component in the formulations can be isotropic mixtures of drug, lipids (natural or synthetic oils), whilst the emulsifier component can be either solid or liquid, usually with one or more of hydrophilic co-surfactant/ solvents (Zhang *et al.*, 2018; Kollipara and Gandhi, 2014; Pouton, 2006). Encapsulation of liquid and semi-solid SEDDS is a straightforward method for converting liquid SEDDS to a solid oral dosage form. SEDDS in liquid form can be easily filled into capsules and sealed with a microspray or banding process (Jannin, Musakhanian and Marchaud, 2008). For a semi-solid SEDDS, it is a four step process: (1) heating the semi-solid excipients to at least 20°C above its melting point; (2) adding the drug in the molten mixture while stirring; (3) filling the drug loaded molten mixture into the capsule shell and (4) cooling the product to room temperature (Jannin, Musakhanian and Marchaud, 2008).

In this study, three main components were mixed in order to formulate the required semi-solid formulation. The liquid model drug was used and incorporated with the surfactant and co-surfactant/solvent to produce a semi-solid form which is suitable to be inserted into hard gelatin capsules without any spillages.

1.3.1 TOCOTRIENOLS AS LIQUID MODEL DRUG



Tocotrienols	R1	R2
α-tocotrienol	CH3	CH3
β-tocotrienol	CH3	H
γ-tocotrienol	H	CH3
δ-tocotrienol	H	H

Figure 1.1 Chemical structure of tocotrienols

Tocotrienols, a member of Vitamin E family, share similar structural features of a chroman head and a 16-carbon phytyl chain with tocopherols. Tocopherols and tocotrienols differs mainly in terms of their chain, whereby the former having a saturated phytyl chain, whilst the latter is unsaturated, with three double bonds at 3, 7 and 11 positions. Figure 1.1 shows the general structure of a tocotrienol. The main sources of tocopherols are generally present in nuts and common vegetables oils, whilst natural sources of tocotrienols are quite limited, with palm oil containing one of the highest concentrations of tocotrienols in nature (Sen, Khanna and Roy, 2007: Constantinides, Tustian and Kessler, 2004)

Tocotrienols have been gaining much interest in recent years and some reports highlighted their positive properties in terms of biological activities which were not found in tocopherols including cholesterol lowering activities (Chen and Cheng, 2006; Qureshi *et al.,* 2001), anticancer and tumor suppressive activities (Sen, Khanna and Roy, 2007), antiaggregating of blood platelets and neuroprotective properties (Khanna

et al., 2005, 2006; Sen *et al.*, 2004,2006). Structurally, tocotrienols consists of four isomers namely beta- (β), alpha- (α), gamma- (γ) and delta- (δ). The α-isoform has three methyl groups on the chromanol ring structure, whereas the -β and -γ isoforms have two methyl groups but at different positions and δ- isoform has only one, based on the tocotrienols studied. Thus, α-tocotrienol would be the most lipophilic followed by γ- and β-tocotrienols and lastly δ-tocotrienol. The higher lipophilicity of the α-tocotrienol might in part explain the higher bioavailability obtained with α-tocotrienol compared with γ- and δ- isomers, since lipophilicity can affect the passage of a molecule across biological membranes and its transport into the intestinal lymphatic system. Tocotrienols is classified as BCS Class II and thus has very low aqueous solubility and erratic oral absorption. Therefore, improving the solubility and dissolution of tocotrienols with the intention of enhancing its oral bioavailability had captured the interest of many researchers thus many approaches have been employed to achieve this goal such as using SEDDS.

1.3.2 GELUCIRE

Gelucire, a family of glyceride-based excipients has been widely used to enhance the oral bioavailability of drugs as well as controlled release matrices. The compound comprises of a mixture of mono, di and triglycerides and polyethylene glycol (PEG) ester of fatty acids which gives its amphiphilic property. Gelucire is inert, safe and non-toxic, typically existing as a semi-solid waxy material. Natural food-grade fats and oils are the sources where gelucire is derived from. Gelucire is typically synthesized via an alcoholysis reaction involving PEG (typically with a molecular weight of 300–1500) and hydrogenated vegetable oils (such as coconut, palm, or palm kernel oil) at 230 °C in a nitrogen atmosphere. Additionally, gelucire can be

synthesized directly from fatty acids (from coconut oils, palmitic acid, or stearic acid) using glycerol and PEG. Different degrees of esterification produce a wide range of gelucire grades (Gattefossé, 1999).

Nominal melting point of the base and HLB value are two criteria used to identify gelucire. The nominal melting point, which ranges from 33°C to 70°C, is merely a guide and does not accurately represent the base's melting point. This is because each gelucire grade is composed of a variety of different components and thus lacks a single, sharp melting point. The release of incorporated drug from the base can be influenced by gelucire's melting point. Gelucire grades with lower melting points are more favourable in the formulation of fast release dosage forms whilst higher melting points gelucire are better suited for controlled release formulations. Studies show that fast release formulations can be formulated utilizing Gelucire® 35/10 and Gelucire® 44/14 (Gattefosse, 1999) whereas controlled release formulations can be achieved by using Gelucire® 50/13 and Gelucire® 64/02. In a study conducted by Wu *et al.* (2002) on the release rate of potassium chloride from various gelucire bases, the rate of potassium chloride release increased in the following order of gelucire grades: 62/05, 53/10, 48/09, 46/07, and 44/14, while gelucire's HLB value ranges from 1 to 18. This is due to the gelucire base's hydrophilicity and the ratio of glyceride to PEG esters in the base.

Generally, gelucire bases with higher HLB values contain a greater proportion of hydrophilic fractions (polyethylene glycol esters) than lipophilic fractions (glycerides), making them ideal for preparing fast release formulations. In contrast, gelucire bases with lower HLB values have higher portions of lipophilic fractions and are better suited to formulate controlled release formulations. Nevertheless, instead of

using a single gelucire base, a mixture of two gelucire bases could be employed to provide the desired lipophilicity. For example, a controlled release formulation containing ketoprofen using a mixture of Gelucire® 50/13 and 50/02 was prepared by Dennis *et al.* (1990) at a ratio of 3:1 (w/w). It was found that the mixture has a final HLB value of 10.25.

1.3.2 (a) GELUCIRE® 44/14

Nominal melting point for Gelucire® 44/14 is 44°C whilst its HLB value is 14. Gelucire® 44/14 is a fatty acid derived from hydrogenated palm kernel oil and PEG 1500. Gelucire® 44/14 is composed of approximately 20% mono-, di-, and triglycerides, 72% mono- and di-fatty acid esters of PEG 1500, and 8% free PEG 1500 (Gattefosse, 1999). Typically, Gelucire® 44/14 possesses a unique balance of short, medium and long chain fatty acids in the base, making it an ideal excipient in self-emulsifying formulations. When in contact with gastrointestinal fluids at body temperature (37 °C), Gelucire® 44/14 exhibits an exceptionally stable and fine dispersion, which is a desirable property.

The properties of Gelucire® 44/14 have attracted researchers to investigate their ability to promote rapid drug release and bioavailability via fast release or self-emulsifying formulations. Positive reports indicate that when poorly water-soluble drugs are formulated with Gelucire® 44/14, their solubility, dissolution rate, and bioavailability improve (Charan *et al.*, 2017). For example, by formulating piroxicam as a semi-solid dispersion using Gelucire® 44/14 and Labrasol (Karataş *et al.*, 2005; Yüksel *et al.*, 2003), researchers showed that the solubility and dissolution rate of piroxicam could be increased. Few factors were identified as contributing to the increased dissolution of the drug from the semi-solid dispersion, including partial

dissolution of the drug in the excipients, the solubilization effects of Gelucire® 44/14 and Labrasol, as well as improved wettability of the preparation. The best results in terms of dissolution, stability upon storage and bioavailability was reported by Schamp *et al*. (2006) when they prepared a semi-solid lipid formulation of EMD 50733 using Gelucire® 44/14 and a solubilizing agent (2-vinylpyrrolidone). They identified two important factors required for adequate and reliable in vivo dissolution of poorly soluble drugs. To begin, lipid excipients should have the appropriate solubilizing properties for the drug in the formulation, and to assist in maintaining the drug in solution during gastrointestinal release. It was also found that precipitation of EMD 50733 can be prevented even during an extended dissolution period of 3 hours with the presence of Gelucire® 44/14 (Schamp *et al.*,2006).

Apart from that, Gelucire® 44/14 can also be used to administer high doses of lipophilic HIV protease inhibitors, such as DMP 323 (Aungst *et al.*, 1997), and this was done without compromising oral bioavailability. Solubility of DMP 323 in dilute aqueous solution can also be improved when Gelucire® 44/14 is present. Additionally, Aungst *et al.* (1997) reported that amphiphilic vehicles with lower HLB values were significantly less effective solubilizing agents. A dispersion of tocopherol in Gelucire® 44/14 that has the dual advantage of incorporating a liquid drug into a solid dosage form and increasing the drug's bioavailability had been successfully prepared by Barker *et al*. (2003). It was evident that the drug was incorporated into the gelucire's hydration layer, and they explained that the increased bioavailability could be due to the emulsification or disintegration of Gelucire® 44/14 upon contact with water.

Alternatively, Gelucire® 44/14 can also be formulated as solid oral dosage forms and further processed into a powder form such as pellets, tablets and hard

capsules. Chambin *et al.* (2004), for example, used a cryogenic grinding technique to develop a solid oral dosage form of Gelucire® 44/14. Gelucire® 44/14 was successfully powdered in that study via a cryogenic grinding process and it was found that their physical properties, self-emulsifying capacity or dissolution performance were not altered when they tested a formulation consisting of Gelucire® 44/14 and ketoprofen at a ratio of 90:10.

1.4 PROBLEM STATEMENT

Oral administration has a number of advantages, including the fact that it can be self-administered, easy to handle, and highly accepted by patients. Oral administration is the preferred drug delivery system for a newly developed product for those reasons, in addition to more flexibility on formulation design that it affords (Zhong *et al.*, 2018). However, one of the frequent stumbling blocks that oral delivery needs to overcome is the poor water solubility of the incorporated active ingredient. Low water solubility results in a slower release rate and low bioavailability. Increasing the dose to a high enough level in the dosage form to achieve the required drug or active concentration in the body, is not an efficient drug delivery technique and leads to wastage of active ingredient with the accompanying high cost. A much worse outcome may even be drug toxicity (Argade *et al.*, 2013).

Moreover, when the active ingredient is in liquid form, another set of problems will be faced during preparation of dosage form. Liquid dosage forms such as solution, suspension or emulsion are not favoured due to bulkiness which will cause the product to occupy a lot of storage space, and difficulty in transportation due to potential breakage of the containers and spillage of the contents. In addition, leaving an oily active ingredient to remain in liquid form, especially in the presence of water such as

in liquid emulsion dosage form, could lead to stability issues which include separation of phases and microbial growth. An active ingredient could be formulated in its original oily liquid form as soft gelatin capsules if the active is potent enough to achieve satisfactory loading in the relatively smaller dosage form. However, soft gelatin capsules filling is expensive and is a more technically complex process compared to hard gelatin capsules. Although liquid filling into hard gelatin capsules is possible, there are issues related to this method of dosage form fabrication including incompatibility between capsule shell and filling formulations (Chen *et al.*, 2010; Cole *et al.*, 2008), leakage, suspension non-uniformity and Ostwald ripening (Jannin *et al*., 2008; Kesisoglou, Panmai and Wu, 2007).

The aim of this study is to convert liquid drug such as tocotrienols into semi-solid dosage forms that can be encapsulated in hard gelatin capsules. It is hypothesised that incorporating the oily active ingredient into an amphiphilic carrier such as gelucire that can be placed into hard-gelatin capsules, will allow it to be made into an oral semi-solid dosage form, circumventing the problems related to liquid formulations as stated above. Formulation with a gelucire that acts as a surfactant and the addition of a co-surfactant such as propylene glycol could result in a Self-Emulsifying Drug Delivery System (SEDDS) being obtained. SEDDS has been shown to be effective in increasing the oral bioavailability of drugs that are both lipophilic and poorly soluble in water (Kohli *et al.*, 2010). Therefore, a semi-solid SEDDS may combine the advantages of SEDDS (i.e. increased solubility and bioavailability) with the benefits of solidification for industrial and commercial applications (Joyce *et al.*, 2019).

1.5 SCOPE OF STUDY

The aim of this study was to investigate the efficacy of Gelucire® 44/14 in enhancing the aqueous solubility and oral bioavailability of a model lipophilic drug that has poor aqueous solubility. Tocotrienols mixture was chosen as the model. Tocotrienols are categorized as a BCS Class II drug because their oral absorption is limited by their solubility. The present study was performed with the following objectives:

1. To formulate and evaluate self-emulsifying semi-solid formulation comprising mixtures of tocotrienols, Gelucire® 44/14 and propylene glycol.
2. To assess the stability of the formulation in terms of the release patterns of the tocotrienols upon storage.
3. To compare the bioavailability of a suitable self-emulsifying semi-solid formulation of tocotrienols with a commercial product of tocotrienols prepared in the form of soft gelatin capsules.

CHAPTER 2

FORMULATION STUDY AND MACROSCOPIC EXAMINATION OF SEMI-SOLID SEDDS CONTAINING TOCOTRIENOLS

SEDDS developed a stable formulation which can be produced either in the form of semi-solid soft capsules (e.g., Sandimmun®) (Kollipara *et al.*, 2014) or liquid-solid tablets. SEDDS can alleviate the problem of limited oral bioavailability inherent to lipophilic drug compounds by improving the dissolution rate and bioavailability, resulting in more consistent absorption profiles. SEDDS has the required properties to address the bioavailability challenges associated with BCS class II, III and IV drugs. One of the favourable properties of SEDDS is its ability to alter the solubility and permeability of these drugs. Thus, SEDDS is a promising strategy to formulate emulsions for orally administered for lipophilic types of drugs. SEDDS also can provide other benefits such as better physical stability and manufacturability (Gursoy and Benita, 2004; Tang *et al.*, 2008).

The present study aimed to formulate a semi-solid SEDDS dosage form contained in hard gelatin capsules. A lipophilic model active ingredient, tocotrienols complex, is formulated with Gelucire® 44/14 (surfactant) and propylene glycol (co-surfactant). Different ratios of the surfactant-co-surfactant was mixed with the model drug to develop a simple semi-solid formulation of tocotrienols with enhanced bioavailability.

2.1 MATERIALS

EVNol™ 50% (ExcelVite Sdn. Bhd., Ipoh, Malaysia) is an oily suspension containing tocotrienols complex (comprising of 6.4%, 21.6%, 10.7% and 10.9% of δ-, γ-, α-tocotrienols and α-tocopherol, respectively). Aside from tocotrienols and

tocopherol, EVNol™ 50% consisted of palm olein, plant squalene and phyto–sterol complex. Gelucire® 44/14 was obtained from Gattefossé (Cedex, France) and propylene glycol, from Sigma Chemical Co. (St. Louis, Mo, USA), respectively. All solvents used were either of analytical reagent grade or HPLC grade and were purchased from either Merck or Ajax Chemicals (Auburn, Australia).

2.2 METHODS

2.2.1 STANDARD PROCEDURE FOR PREPARATION OF SEMI-SOLID SEDDS

The formulations were prepared using a standard procedure as follows: a pre-determined amount of gelucire was first weighed accurately into a glass beaker and allowed to melt in an oven at 60 ˚C for 30 minutes to remove any crystalline part. Gelucire was then stirred for 30 minutes using a magnetic hot-plate stirrer at 300 rpm at 60°C. Subsequently, the required amount of EVNol™ 50% and co-surfactant was added at 1 to 1 ratio and continuously stirred for one hour at 60 ˚C to obtain a homogenous mixture (Jannin, Musakhanian and Marchaud, 2008; Magosso, 2007; Julianto, 2000).

The mixture was transferred using a pre-warmed pasteur pipette into hard gelatin capsules (size 0) until the fill weight achieved 515.5 mg. The capsules were kept upright for 30 minutes and allowed to cool to room temperature (approximately 25 °C).

2.2.2 FORMULATION STUDY

2.2.2 (a) EFFECT OF DIFFERENT GRADES OF GELUCIRE

Semi-solid SEDDS was prepared by mixing EVNol™ 50% (tocotrienols in oily suspension) with different grades of gelucire, namely grade 44/14 and grade 50/13. Various ratios of EVNol™ 50% to gelucire (oil:surfactant) were tested, with highest oil-surfactant ratio (9:1) to lowest oil: surfactant (4:6), as shown in Table 2.1. Lower oil content was not tested to ensure a minimum amount of active is contained in the formulation. Resulting preparations was selected for subsequent formulation study based on successful formation of semi-solid mixture.

Table 2.1 Formulation of tocotrienols mixed with different grades of gelucire

Oil: Surfactant	Formula	Gelucire® 44/14	Formula	Gelucire® 50/13
9:1	F1	Liquid	F7	Liquid
8:2	F2	Liquid	F8	Liquid
7:3	F3	Liquid	F9	Liquid
6:4	F4	Thick liquid	F10	Thick liquid
5:5	F5	Semi-solid	F11	Thick liquid
4:6	F6	Semi-solid	F12	Thick liquid

2.2.2 (b) EFFECT OF DIFFERENT AMOUNTS OF SURFACTANT & CO-SURFACTANT

Based on the observation obtained in section 2.2.2 (a), it was determined that Gelucire® 44/14 was better than Gelucire® 50/13, in forming a semi-solid preparation.

Therefore, formulation F5 containing 5 parts oil and 5 parts gelucire was further tested with the addition of labrasol (co-surfactant) at different amounts as listed in Table 2.2. Labrasol was added at a percentage of 2.5% up to 15% (w/w) of the total weight, displacing gelucire.

Table 2.2 Formulation of semi-solid SEDDS with different combinations of Gelucire® 44/14 and co-surfactant, Labrasol

Ingredients	Formula (%)				
	FGL1	FGL2	FGL3	FGL4	FGL5
Tocotrienols (oil)	50	50	50	50	50
Gelucire® 44/14	47.5	45	42.5	40	35
Labrasol	2.5	5	7.5	10	15
Oil:surfactant system ratios	100	100	100	100	100
G 44/14: Labrasol ratios	95:5	90:10	85:15	80:20	70:30
Physical form	Semi-solid	Semi-solid	Liquid	Liquid	Liquid

2.2.2 (c) EFFECT OF DIFFERENT TYPES OF CO-SURFACTANTS

From previous section, the incorporation of Labrasol as co-surfactant was not able to produce a stable semi-solid formulation. In this study, six other types of co-surfactants were tested, namely propylene glycol, glycerin, transcutol-P, pharmasolve, glycerol and PEG 400 at a maximum content of 5%. All combinations shown in Table 2.3 produced a stable semi-solid formulation, in terms of appearance (homogeneity of colour, physical appearance and separation layer).

Table 2.3 Formulation of semi-solid SEDDS with various co-surfactant

Ingredients	Ratios (%)						Formula
Tocotrienols	50	50	50	50	50	50	-
Gelucire® 44/14	45	45	45	45	45	45	-
Propylene glycol	5						FCS1
Glycerin		5					FCS2
Transcutol-P			5				FCS3
Pharmasolve				5			FCS4
Glycerol					5		FCS5
PEG 400						5	FCS6
Oil:surfactant ratios	100	100	100	100	100	100	-

2.2.2 (d) OPTIMIZATION STUDY ON SEMI-SOLID PREPARATION USING TOCOTRIENOLS, GELUCIRE® 44/14 AND PROPYLENE GLYCOL

From the previous section 2.2.2(c), propylene glycol was chosen as the most suitable co-surfactant as it gave the best emulsifying time compared with all other co-surfactants tested. In this part of study, different amounts of propylene glycol (0.5 – 5%) were tested, as listed in Table 2.4.

Table 2.4 Formulation of semi-solid SEDDS with different percentages of propylene glycol as co-surfactant

Ingredients	Ratios (%)									
	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10
Tocotrienols	50	50	50	50	50	50	50	50	50	50
Gelucire® 44/14	49.5	49	48.5	48	47.5	47	46.5	46	45.5	45
Propylene Glycol (PG)	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Oil:surfactant system ratios	100	100	100	100	100	100	100	100	100	100
G 44/14: PG ratios	99:1	98:2	97:3	96:4	95:5	94:6	93:7	92:8	91:9	90:10

2.2.3 ASSESSMENT OF SELF-EMULSIFYING PROPERTIES

To evaluate the self-emulsifying properties of the SEDDS formulations, a setup to test emulsifying time was used (Julianto, 2000). An aliquot of each formulation (0.5 ml) was injected, using a syringe, into 250 ml of distilled water, and stirred at 100 rpm using a paddle stirrer. The light source (100 Lux) is switched on and the paddle stirrer was set to rotate at 100 rpm. When the sample was introduced into the water, a stopwatch was activated simultaneously to capture the time it takes for the preparation to emulsify. The test was carried out at room temperature (25 °C) and performed in triplicates. The preparations were considered as self-emulsifying if an emulsion was formed within 180 seconds. The emulsions formed were observed and classified based