EFFECT OF GELUCIRE® 44/14 ON THE ORAL BIOAVAILABILITY OF CYCLOSPORIN A

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UNIVERSITI SAINS MALAYSIA

2007

EFFECT OF GELUCIRE® 44/14 ON THE ORAL BIOAVAILABILITY OF CYCLOSPORIN A

by

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Thesis submitted in fulfilment of the
requirements for the degree
of Master of Science

June 2007

To my dearest parents, Lim Kim Siew and Wong Keep Luan,

brothers, Vei Tat and Wei Jei,

and soul mates

ACKNOWLEDGEMENTS

My immense gratitude and appreciation go to my academic supervisors, Dr. Nurzalina Abdul Karim Khan and Professor Dr. Yuen Kah Hay for their invaluable advice, guidance and patience given throughout my postgraduate years. Their unfailing faith in me is one of the major sources of my strength and courage to motivate me in my intellectual pursuit.

My sincere thanks go to Dr. Wong Jia Woei for her advice and guidance. Also, I'm very grateful to all my colleagues and labmates, namely, Dr. Ng Bee Hong, Dr. Sharon Ling, Dr. Irene Yap, Wai Peng, Ai Beoy, Ching Khee, Hooi Ling, Yin Wai, Loon, Enrico, Sheau Chin, Pay Kim, Siew Siew, Bee Yean, Shaun, Sandy, Phaik Chin, Mei Mei, May Thing, Erena, Kamarul, Belle, Hwee Ching, Alan, Shah, Musab, Gamal, Samer, Sabiha, Mahmoud and Jiyauddin for providing an enjoyable working environment and help out in one way or another.

I would like to take this opportunity to thank School of Pharmaceutical Sciences, USM, Institute of Postgraduate Studies (IPS) as well as Hovid R&D Laboratory, Penang for providing me an excellent environment and their generous support of materials for the study.

My heartfelt appreciation also goes to my friends who never fail to be around whenever I need a helping hand or a listener. Last but not least, I am immensely indebted to my parents and brothers for their understanding, constant encouragement and boundless love throughout my academic years.

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LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

Abbreviation	Full description
σ	Interfacial energy
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
C8	Carbon chain length of 8
C10	Carbon chain length of 10
C12	Carbon chain length of 12
C18	Carbon chain length of 18
C_{max}	Peak plasma concentration
CN	Cyano
CsA	Cyclosporin A
CV	Coefficient of variation
DSC	Differential scanning calorimetry
D(*v*, 0.5)	Median volume diameter
∆G	Change in free energy
G44/14	Gelucire® 44/14
HCl	Hydrochloric acid

HIV	Human immunodeficiency virus
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
K_e	Elimination rate constant
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
$[M+H]^+$	Precursor ion
MS	Mass spectrometer
MTDSC	Modulated temperature differential scanning calorimetry
MRM	Multiple reactions monitoring
m/z	Mass over electric
N_i	Number of droplets
o/w	Oil-in-water
PEG	Polyethylene glycol
r_i	Radius of droplets
SD	Standard deviation
SEDDS	Self-emulsifying drug delivery systems
S.E.M.	Standard error of mean
SEM	Scanning electron microscopy
T_{max}	Time to reach peak plasma concentration
UV	Ultraviolet
v/v	Volume over volume
w/w	Weight over weight
XRD	X-ray powder diffraction

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KESAN GELUCIRE® 44/14 TERHADAP BIOKEPEROLEHAN ORAL BAGI CICLOSPORIN A

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesan Gelucire® 44/14 terhadap biokeperolehan oral bagi satu drug polipeptida lipofilik model, ciclosporin A. Formulasi Gelucire® 44/14 mengandungi ciclosporin A dengan nisbah drug kepada Gelucire® 44/14 1:4, 1:10, 1:14 dan 1:20 telah berjaya disediakan melalui teknik lebur-fusi. Kajian-kajian *in vitro* menunjukkan keterlarutan air and pelarutan bagi ciclosporin A meningkat secara berkadar langsung dengan jumlah Gelucire® yang digunakan dalam formulasi. Semua formulasi dapat membentuk produk emulsi yang stabil apabila dimasukkan ke dalam medium berakueus. Tambahan pula, apabila dimasukkan ke dalam medium berakueus, formulasi dengan nisbah ciclosporin A kepada Gelucire® 44/14 1:10, 1:14 dan 1:20 dapat membentuk produk emulsi yang mempunyai saiz titisan yang standing dengan formulasi yang Gelucire® 44/14 sahaja. Penambahan ciclosporin A ke dalam Gelucire® 44/14 juga tidak mengubah suhu peleburan tersebut.

Satu kaedah kromatografi cecair-spektrometri jisim bertandem telah berjaya dibangunkan untuk penentuan kepekatan ciclosporin A dalam plasma. Kaedah tersebut adalah ringkas, peka, dapat dihasilkan semula serta spesifik dan telah digunakan untuk menilai biokeperolehan formulasi ciclosporin A dalam Gelucire® 44/14.

Dalam kajian *in vivo* yang melibatkan penggunaan tikus Sprague-Dawley, formulasi yang bernisbah drug kepada Gelucire® 44/14 pada 1:10 dapat meningkatkan biokeperolehan oral ciclosporin A sebanyak 2.8 kali ganda berbanding dengan ampaian berakueus drug, dan setanding dengan satu formulasi pengemulsian kendiri yang diperolehi secara komersial, Sandimmun Neoral®.

Sebagai kesimpulan, Gelucire® 44/14 dapat meningkatkan keterlarutan satu drug polipeptida lipofilik iaitu ciclosporin A dan seterusnya meningkatkan biokeperolehan oral drug tersebut. Dengan itu, Gelucire® 44/14 boleh dijadikan satu eksipien yang berguna dalam formulasi pengemulsian kendiri untuk meningkatkan biokeperolehan oral drug-drug berlipofilik.

EFFECT OF GELUCIRE® 44/14 ON THE ORAL BIOAVAILABILITY OF CYCLOSPORIN A

ABSTRACT

The present study was conducted to investigate the effect of Gelucire® 44/14 on the oral bioavailability of a model lipophilic polypeptide drug, cyclosporin A. Gelucire® 44/14 formulations of cyclosporin A at drug to Gelucire® 44/14 ratios of 1:4, 1:10, 1:14 and 1:20 (w/w) were successfully prepared using a heat-fusion method. *In vitro* studies showed that the aqueous solubility and dissolution of cyclosporin A were proportionally increased with the amount of Gelucire® used in the formulations. All formulations prepared could form stable emulsion products when introduced into an aqueous medium. Moreover, upon introduction into an aqueous medium, formulations with 1:10, 1:14 and 1:20 ratios of cyclosporin A to Gelucire® 44/14 formed emulsion products with comparable droplet sizes to that using Gelucire® alone. Additionally, incorporation of cyclosporin A into Gelucire® 44/14 did not alter the melting point of the base.

A liquid chromatography tandem mass spectrometry method was successfully developed for the determination of plasma concentrations of cyclosporin A. The method was simple, sensitive, reproducible and specific and was applied to evaluate the bioavailability of the 1:10 formulation of cyclosporin A in Gelucire® 44/14.

The *in vivo* study conducted using Sprague-Dawley rats revealed that at a drug to Gelucire® ratio of 1:10, the formulation was capable of increasing the oral bioavailability of cyclosporin A by approximately 2.8-fold compared to an aqueous suspension of the drug and comparable to that of a commercially available self-emulsifying formulation, Sandimmun Neoral®.

In conclusion, Gelucire® 44/14 enhanced the solubility of a lipophilic polypeptide drug, cyclosporin A, and subsequently increased the oral bioavailability of the drug. Thus Gelucire® 44/14 could be a useful excipient in self-emulsifying formulations for enhancing the oral bioavailability of lipophilic drugs.

CHAPTER 1

INTRODUCTION

1.1 SELF-EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS)

1.1.1 INTRODUCTION

The oral route is the preferred route of drug delivery because of its simplicity and convenience. However, oral absorption of some drugs is limited due to their unfavourable physiochemical properties. Nearly 40% of new drug candidates exhibit low solubility in water which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality (Robinson, 1996). One of the approaches to enhance the oral bioavailability of poorly water soluble drugs is to incorporate the lipophilic drugs into lipid delivery systems, such as surfactant dispersions (González *et al.*, 2002), emulsions (Kim *et al.*, 2002), liposomes (Lee *et al.*, 1999), lecithin vesicles (Guo *et al.*, 2001) and self-emulsifying drug delivery systems (Odeberg *et al.*, 2003).

Self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, one or more hydrophilic solvents and co-solvents which will form oil-in-water (o/w) emulsions instantaneously upon introduction into aqueous phase under gentle agitation (Pouton, 1985; Constantinides, 1995; Gursoy and Benita, 2004). Enhanced absorption of poorly bioavailable drugs can be achieved by presenting the drugs in small droplets of oil when the formulation emulsifies rapidly in the aqueous contents of the stomach. SEDDS are different from the conventional emulsion systems wherein the latter have several disadvantages such as their inherent

physical instability upon storage, the inconveniences due to their large volume and also poor precision of dosing (Humberstone and Charman, 1997).

SEDDS are usually formulated as oily formulations and they are encapsulated in soft gelatin capsules. They can also appear in the form of semi-solid when semi-solid oils or solid surfactants are employed in their formulation. These semi-solid formulations are encapsulated in hard gelatin capsules and they differ from the oily self-emulsifying formulations only in the physical appearance.

1.1.2 EXCIPIENTS USED IN SEDDS

Self-emulsifying drug delivery systems comprise oils, surfactants and solvents or co-solvents. There is a large variety of excipients available for the formulation of SEDDS, ranging from natural or synthetic oils, solid and liquid surfactants with varying hydrophilicity as well as co-solvents with varying solvation capacity. To formulate satisfactory SEDDS, the combinations as well as ratios of excipients employed are of utmost importance.

(a) OILS

Oil is an important component in the formulation of SEDDS. It is used as a carrier to dissolve the lipophilic drug. Naturally-derived oils are not frequently used as the oil fraction in SEDDS due to their poor ability to dissolve a large amount of lipophilic drug (Gursoy and Benita, 2004). Thus, synthetic or chemically modified oils, such as hydrolyzed vegetables oils are widely used in SEDDS. Besides having better drug solubility properties, chemically modified oils also have better ability to facilitate self-emulsification because they possess

surfactant properties of their own (Constantinides, 1995; Hauss *et al.*, 1998). Medium chain triglycerides were frequently used in earlier work to formulate SEDDS because of their higher fluidity, better solubility properties and self-emulsification ability (Charman *et al.*, 1992; Shah *et al.*, 1994). However, they have become less attractive when novel semi-synthetic oils with amphiphilic properties were introduced, such as the polyglycolized glycerides with varying fatty acid and polyethylene glycol chain lengths (Constantinides, 1995). In a study by Devani *et al.* (2004), which investigated the self-emulsification properties of six different types of polyglycolized glycerides, it was observed that C8/C10 polyglycolized glycerides (Labrasol) with the highest HLB value of 14 displayed the most satisfactory self-emulsification properties.

(b) SURFACTANTS

Surfactants or surface-active agents are amphiphilic molecules and consist of both hydrophilic and lipophilic parts. They are used to facilitate the self-emulsification process of the SEDDS, which in turn help to improve or increase the bioavailability of poorly absorbable drugs. The mechanisms involved are complex and were described by Constantinides (1995) as “diffusion and stranding”.

Surfactants are classified according to their hydrophile-lipophile balance (HLB) values. This HLB value indicates the hydrophilicity of surfactants wherein surfactants which are more hydrophilic possess higher HLB values. O/w emulsions can be formed using surfactants which have HLB values in the range of approximately 8-18 (Constantinides and Scalart, 1997). It was reported that

surfactants with high HLB values will provide rapid emulsification, with excellent spreading properties and rapid cloud formation, leading to the formation of very fine o/w droplets (Gursoy and Benita, 2004).

Selection of the type of surfactant to be used in the formulation of SEDDS will depend on factors such as its emulsification performance, safety as well as the stability of the emulsion formed upon contact with aqueous medium. Naturally-derived surfactants are safe to consume but they display limited self-emulsification capacity. As for synthetic surfactants, non-ionic surfactants are widely recommended as compared to ionic surfactants because the former are less toxic. To form stable SEDDS, the concentration of surfactants to be used was suggested to be within the range of 30% to 60% (w/w) (Gershanik and Benita, 2000; Gursoy and Benita, 2004). The amount of surfactants to be used is critical as too large an amount may cause irritation of the gastrointestinal tract while insufficient amount of surfactants used may compromise the self-emulsification ability of SEDDS.

Surfactants commonly used in SEDDS are emulsifiers which include solid or liquid ethoxylated polyglycolized glycerides and polyoxyethylene 20 oleate (polysorbate 80) (Gursoy and Benita, 2004). Pouton (1982) and Wakerly (1986) screened a wide range of industrial non-ionic surfactants and found that the most efficient SEDDS could be formulated using surfactants with predominantly unsaturated acyl chains. Amongst these, the most efficient were oleates with HLB value of approximately 11.

(c) SOLVENTS/CO-SOLVENTS

Solvents/co-solvents are added into SEDDS to increase the solvent capacity of the formulations especially in those which contain a large amount (30% - 60%) of hydrophilic surfactants (Gershanik and Benita, 2000). High solvent capacity is required in SEDDS to dissolve large amount of lipophilic drugs or hydrophilic surfactants. Organic solvents such as propylene glycol and polyethylene glycol are normally used as they are safe for human consumption. However, alcohols and other volatile solvents may not be suitable because such solvents will migrate into the shell of hard and soft gelatin capsules, resulting in the precipitation of the lipophilic drugs (Gursoy and Benita, 2004).

1.1.3 MECHANISM OF SELF-EMULSIFICATION

To date, the mechanism of self-emulsification is yet to be fully elucidated. According to Reiss (1975), emulsification occurs when the entropy change that favours dispersion is greater than the free energy required to increase the surface area between the oil and aqueous phases of the dispersion. The change in free energy (ΔG) associated with the process of emulsification, ignoring the free energy of mixing, can be expressed by

$$\Delta G = \sum_i N_i 4\pi r_i^2 \sigma \qquad (1.1)$$

N_i is the number of droplets of radius r_i whereas σ is the interfacial energy. Self-emulsification will occur spontaneously only when the interfacial energy is low. However, emulsions are not thermodynamically stable as the oil phase and the aqueous phase will tend to separate with time to reduce the interfacial area and also free energy of the system. Therefore, the presence of emulsifiers will help

to reduce interfacial tensions by forming a barrier around the oil droplets and hence the free energy of the systems.

On the other hand, Groves *et al.* (1974) related emulsification with the formation of liquid crystalline phase. Liquid crystalline phase is the phase between liquid and crystal phase. A liquid crystal has both the properties of a crystal as well as liquid. When additional energy is exerted onto the liquid crystalline phase, it will turn into liquid phase. For self-emulsifying systems, when the oil phase is introduced into the aqueous phase with gentle agitation, the aqueous phase will penetrate through the interface into oil phase until the interface of the two phases is disrupted. Consequently, oil droplets are formed resulting in emulsification. Thus, the ease of emulsification is governed by the ease of water penetration into the various liquid crystal or gel phases formed on the surface of droplets. The liquid crystal formation surrounding the oil droplets will increase the stability of the emulsion. Nevertheless, the relationship between liquid crystal formation and emulsion formation could be more complicated as it appeared to be (Craig *et al.*, 1995). Various factors can affect the process of self-emulsification, such as the nature of oil/surfactant pair, the surfactant concentration used as well as the temperature at which self-emulsification occurs (Gursoy and Benita, 2004). Moreover, the presence of drug compound will alter the emulsion characteristics, probably by interacting with the liquid crystalline phase (Craig, 1993).

1.1.4 CHARACTERIZATION OF SEDDS

Various approaches have been used to characterize SEDDS. Gursoy and Benita (2004) suggested that SEDDS could be characterized using the rate of emulsification, distribution of droplet size as well as polarity and charge of emulsion droplets formed. Groves and Mustafa (1974) measured the spontaneity of self-emulsification of oils by determining the rate of emulsification. Later, when Pouton (1984, 1985) investigated the emulsification efficiency of various compositions of Tween 85/medium-chain triglyceride systems, it was suggested that the particle size of the droplets formed was more important than emulsification rate for the assessment of emulsification efficiency. It is well known that the particle size of emulsion droplets formed will determine the rate and extent of drug release and subsequently affect the oral absorption of the drug. For this reason, Pouton (1985) suggested that the efficiency of SEDDS should be characterized by both emulsification rate and particle size distribution of emulsion products formed.

Formulations with small emulsion droplet size generally could increase the oral bioavailability of the drugs incorporated. It is because the small droplet size provides a large interfacial surface area for drug absorption (Charman *et al.*, 1992; Shah *et al.*, 1994). Khoo *et al.* (1998) pointed out that SEDDS typically produce emulsions with particle size ranging between 100 and 300 nm whilst transparent microemulsions will be formed when the particle size of the emulsion droplets is less than 100 nm. The droplet size of an emulsion is normally measured using laser diffraction method or photon correlation spectrometry method. The former method is used to measure droplet size in

micrometer size range whereas the latter is used to determine droplet size in nanometer size range. However, Devani *et al.* (2004) have highlighted the difficulties associated with the measurement of particle size for SEDDS. As the emulsion has to be diluted during the measurement, this would lead to a reduction in droplet size and an increase in the width of the size distribution. Therefore, low aqueous dilutions and a standardized method should be employed when measuring the emulsion droplet size in order to minimize inadvertent over-reduction of the droplet size and disparity between data sets.

Besides having the ability to form an emulsion with fine particles, Shah *et al.* (1994) pointed out that the SEDDS performance is also governed by the polarity of the resulting droplets. Favourable polarity permits an acceptable rate of drug release into the aqueous phase. The HLB value, chain length and degree of unsaturation of the fatty acid, molecular weight of the hydrophilic portion and concentration of the emulsifier have an impact on the polarity of the oil droplets (Shah *et al.*, 1994; Gursoy and Benita, 2004).

The presence of free fatty acids in the oil phase can cause the emulsion droplets formed to be negatively charged. It has been shown in some studies that positive charged emulsion droplets undergo electrostatic interaction with monolayer of Caco-2 cells and also the mucosal surface of everted rat intestine (Gershanik *et al.*, 1998; Gershanik *et al.*, 2000). Furthermore, positive charged droplets were observed to enhance the oral bioavailability of progesterone and producd higher blood levels of cyclosporin A compared to those with negative charge (Gershanik and Benita, 2000). Therefore, addition of a cationic lipid,

such as oleylamine, into the SEDDS formulation will produce a positive charged system which could facilitate the absorption of drugs.

1.1.5 IMPROVEMENT OF ORAL ABSORPTION BY SEDDS

Many studies have been conducted to investigate the potential of SEDDS in enhancing the oral absorption of poorly water soluble drugs. Tested using beagle dogs, the oral bioavailability of an antimalarial drug, halofantrine, was found to increase 6-fold when administered using a medium chain triglyceride SEDDS formulation compared to a tablet formulation of its hydrochloride salt (Khoo *et al.*, 1998).

Kim and Ku (2000) reported that the oral bioavailability of indomethacin in rats was markedly increased when administered as a SEDDS compared to a methylcellulose suspension of the drug. The increase in bioavailability was attributed to an increase in the water solubility of the drug when formulated as a SEDDS. More recently, Araya *et al.* (2005) reported a versatile novel SEDDS formulation which could enhance the oral bioavailability of poorly water soluble compounds by increasing their aqueous solubility. The aqueous solubility of six poorly water soluble drugs, namely ibuprofen, ketoprofen, tolbutamide, AG-041R, BO-653 and ER-1258, were shown to increase by 340 to 98000 folds when formulated using the SEDDS. Moreover, the oral bioavailability of the drugs was found to be equivalent to that of a solution or an o/w emulsion. Compared to normal aqueous suspensions of the drugs, the oral bioavailability was increased by 1.5 to 78 times.

In summary, SEDDS formulations could be utilized 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.

1.2 GELUCIRE®

1.2.1 INTRODUCTION

Gelucire® is a family of glyceride-based excipients which has been widely used as controlled release matrices and also for enhancing the oral bioavailability of drugs. It is amphiphilic, being composed of a mixture of glycerides (hydrophobic) and polyethylene glycol (PEG) ester of fatty acids (hydrophilic). Gelucire® is a semi solid waxy material which is inert, safe and non-toxic. It is derived from natural food-grade fats and oils and has been reported to be chemically stable to both temperature and humidity up to 50°C and 80% RH (Remunan *et al.*, 1992).

Generally, Gelucire® is synthesized through an alcoholysis reaction wherein PEG (usually of molecular weight range between 300–1500) is reacted with hydrogenated vegetable oils (such as coconut, palm or palm kernel oil) at 230 °C under a nitrogen environment. Besides alcoholysis, Gelucire® can also be manufactured through the direct esterification of fatty acids (of coconut oils, palmitic acid or stearic acid) with glycerol and PEG. A wide range of Gelucire® grades is produced from the different degrees of esterification, and the specification of each grade of the bases is showed in Table 1.1 (Craig, 1995).

Gelucire® is identified by two values, the first referring to the nominal melting point of the base and the second to the HLB value. The nominal melting point which ranges from 33°C to 70°C can only be used as a reference and does not represent the accurate melting point of the base. It is because each Gelucire® grade contains a mixture of many different components and as a result, it does not posses a single, sharp melting point. The melting point of Gelucire® can influence the release of the incorporated drug from the base. In the formulation of fast release dosage forms, Gelucire® grades with lower melting points are preferred. On the other hand, those with higher melting points are suitable for controlled release formulations. For example, Gelucire® 35/10 (Vicente *et al.*, 2000) and Gelucire® 44/14 (Dennis *et al.*, 1990) have been used to formulate fast release formulations whereas controlled release formulations have been prepared using Gelucire® 50/13 (Vippagunta *et al.*, 2002) and Gelucire® 64/02 (Charro and Jato, 1992). The release rate of potassium chloride from various Gelucire® bases has been investigated by Wu *et al.* (2002). They found that the release rate of potassium chloride was increased in the following order of the Gelucire® grades, namely 62/05, 53/10, 48/09, 46/07 and 44/14.

The HLB value of Gelucire® ranges from 1 to 18. It represents the hydrophilicity of the Gelucire® base and corresponds to the ratio of glycerides to the PEG esters in the base. Gelucire® bases with higher HLB values in general contain high portions of hydrophilic fractions (polyethylene glycol esters) than lipophilic fractions (glycerides) and are suitable for preparing fast release formulations. In contrast, Gelucire® bases with lower HLB values have higher portions of lipophilic fractions and are suitable for formulating controlled release

formulations. Dennis *et al.* (1990) observed that Gelucire® 50/13 released ketoprofen too rapidly to be useful for preparation of sustained release formulations. Gelucire® 50/02, another grade of Gelucire® which has the same melting point but different HLB value, was successfully used to prepare a controlled release formulation containing sodium salicylate (Aïnaoui and Vergnaud, 1998). Nevertheless, a mixture of two Gelucire® bases could be used to provide the desired lipophilicity. Dennis *et al.* (1990) prepared a controlled release formulation containing ketoprofen using a mixture of Gelucire® 50/13 and 50/02 at a ratio of 3:1 (w/w). The mixture has a final HLB value of 10.25.

1.2.2 GELUCIRE® 44/14 (G44/14)

Gelucire® 44/14 has a nominal melting point of 44°C and a HLB value of 14. It is derived from reacting hydrogenated palm kernel oil with PEG 1500. Gelucire® 44/14 consists of approximately 20% mono-, di- and triglycerides, 72% mono- and di- fatty acid esters of PEG 1500 and 8% free PEG 1500 (*Gattefossé*, 1999). The distribution of its fatty acids is shown in Table 1.2. Gelucire® 44/14 is an ideal excipient in self-emulsifying formulations due to its unique balance of short, medium and long chain fatty acids in the base (*Gattefossé*, 1999). It will form an exceptionally stable and fine dispersion when in contact with gastrointestinal fluids at body temperature (~37 °C).

Many workers have investigated the ability of Gelucire® 44/14 to promote rapid drug release and bioavailability via fast release formulations or self-emulsifying formulations. They have been reports of marked improvements in solubility, dissolution rate and bioavailability of poorly water soluble drugs when

formulated with Gelucire® 44/14. Yüksel *et al.* (2003) and Karataş *et al.* (2005) showed that the solubility and dissolution rate of piroxicam could be increased by formulating piroxicam as a semi-solid dispersion using Gelucire® 44/14 and Labrasol. These workers claimed that the increased dissolution of the drug from the semi solid dispersion could be attributed to a partial dissolution of the drug in the excipients, solubilization effects of Gelucire® 44/14 and Labrasol, as well as improved wettability of the preparation.

Schamp *et al.* (2006) found that a semi-solid lipid formulation of EMD 50733 using Gelucire® 44/14 with a solubilizing agent (2-vinylpyrrolidone) gave the best results with respect to dissolution, stability upon storage and bioavailability. They also concluded that to achieve adequate and reliable dissolution of poorly soluble drugs *in vivo*, lipid excipients used should not only have appropriate solubilizing properties for the drug in the formulation, but should also assist in maintaining the drug in solution during release in the gastrointestinal tract. It has been shown that the presence of Gelucire® 44/14 was able to prevent precipitation of EMD 50733 even during an extended dissolution period of 3 hours.

Gelucire® 44/14 can also be used to administer high doses of lipophilic drugs, for example DMP 323 (a HIV protease inhibitor) (Aungst *et al.*, 1997), without compromising oral bioavailability. When the solubility limit of DMP 323 in the gastrointestinal fluids is reached, increasing the dose results in decreased fraction of dose absorbed. It has been revealed that Gelucire® 44/14 improved DMP 323 solubility in dilute aqueous solution. In addition, Aungst *et al.* (1997)

also stated that amphiphilic vehicles with lower HLB values were much less effective solubilizing agents.

Moreover, Barker *et al.* (2003) had successfully prepared a dispersion of α-tocopherol in Gelucire® 44/14 which had dual advantages, namely incorporation of a liquid drug in a solid dosage form and improved bioavailability of the drug. The workers pointed out that the increase in bioavailability might be at least partially associated with emulsification or disintegration of Gelucire® 44/14 on contact with water, with evidence obtained that the drug was incorporated into the hydration layer of the Gelucire®.

Gelucire® 44/14 can also be formulated as solid oral dosage forms. This semi solid base could be processed into a powder form such as pellets, tablets and hard capsules (Newton *et al.*, 2001). Chambin *et al.* (2004) produced a solid oral dosage form of Gelucire® 44/14 using cryogenic grinding. Gelucire® 44/14 was successfully prepared in powder form and the cryogenic grinding process did not modify the physical properties, self-emulsifying capacity or dissolution performance of the formulation tested which was made of Gelucire® 44/14 and ketoprofen at a ratio of 90:10.

1.3 CYCLOSPORIN A (CsA)

1.3.1 INTRODUCTION

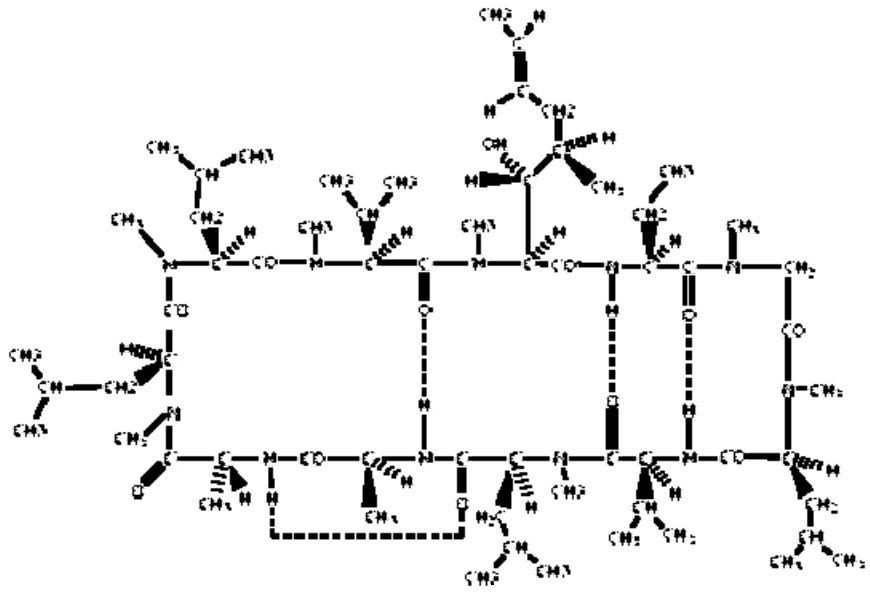

Figure 1.1 Chemical structure of cyclosporin A

Cyclosporin A is a hydrophobic cyclic undecapeptide with a molecular formula of $C_{62}H_{111}N_{11}O_{12}$. The molecular weight of cyclosporin A is 1202.6 g/mol. It is chemically designated as [*R*-[*R**,*R**-(*E*)]]-cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L-α-amino-butyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl). Cyclosporin A consists of 11 amino acids, including several N-methylated amino acids and the nonproteinogenic amino acids L-2-aminobutyric acid, (4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine, and D alanine. It is also known as ciclosporine in Europe and exists as white prismatic crystals. Cyclosporin A is poorly soluble in water with an aqueous solubility of 27.67 µg/ml at 25 °C (Ismailos *et al.*, 1991; Alfred, 1993) and a log P value of 2.92 (Taylor *et al.*, 1993). Cyclosporin A possesses good permeability through the

gastrointestinal membrane and is a Class II drug according to the Biopharmaceutics Classification System (BCS) (Amidon *et al.*, 1995).

Cyclosporin A is isolated from the fungus *Tolypocladium inflatum* Gams. Several reports have stated the isolation of cyclosporine from other fungal metabolites of the fungi *Tolypocladium inflatum* Gams (Dreyfuss *et al.*,1976; Traber *et al.*, 1977a; Traber *et al.*, 1977b; Traber *et al.*, 1982). Biosynthesis and synthesis of cyclosporin A have also been investigated by various researchers (Wenger, 1982; Wenger, 1983a; Wenger, 1983b; Zocher *et al.*, 1984). Discovery of cyclosporin began a new era in immunopharmacology. It is utilized clinically for immunomodulation such as prevention of rejection following transplantation of kidney, liver, bone marrow and pancreas (Matzke and Luke, 1988). Cyclosporin A is also used to delay or prevent disease progression in patients with autoimmune disease and inflammation (Miller *et al.*, 1992; Tibell and Norrlind, 1994; Noble and Markham, 1995). The immunosuppressive effect of cyclosporin A is due to a selective and reversible inhibition of T-lymphocytes (Ferron *et al.*, 1998). Cyclosporin A selectively inhibits interleukin-2 (IL-2) driven proliferation of activated T-lymphocytes.

In spite of the usefulness of cyclosporin A, its extended usage has often been limited by several disadvantages including low bioavailability, narrow therapeutic window, nephrotoxicity and hepatotoxicity (Kahan, 1989; Yee and Saloman, 1992; Gijtenbeek *et al.*, 1999). Nephrotoxicity may occur acutely, associated with reversible hemodynamic changes (Curtis and Laskow, 1988) or as a result of interstitial fibrosis after chronic dosing (Humes *et al.*, 1985).

Cyclosporin A is administered either orally or intravenously. It has poor absorption after intramuscular administration (Keown *et al.*, 1981) and was reported as a typical drug with the worst and non-regular absorption (Klompmaker *et al*., 1993). The oral absorption of cyclosporin A is slow and incomplete, regardless of in rats or in humans. The mean bioavailability is similar in rats and humans, which is about 30% of the administered dose (Ueda *et al.*, 1984; Wagner *et al.*, 1987; Kahan, 1989). The absorption site is the upper part of the small intestine and the mean time to get peak cyclosporin A blood level is around 4 hours (Margaret, 1989) but Kahan (1985) observed large inter-patient variations with the mean time to get peak cyclosporin A blood level ranging between 1 and 8 hours in renal transplant patients.

Margaret (1989) noted that gut motility, bile flow and food affect the absorption of cyclosporin A. Atkinson *et al.* (1984) pointed out that diarrhoea from any cause may reduce absorption of cyclosporin A sharply. The moderate increase in rate of gastric emptying induced by metoclopramide may increase the bioavailability of cyclosporin A (Wadhwa *et al.*, 1987a, b). Ericzon *et al.* (1987) showed that the administration of bile salts increased cyclosporin A absorption in dogs. In other studies, cyclosporin A absorption was shown to be reduced by wine (Ota, 1983; Tsunoda *et al.*, 2001) and slowed by a soybean nutritional supplement (Kahan, 1985) but increased by grapefruit juice (Yee *et al.*, 1995).

Due to the lipophilic nature of cyclosporin A, the drug accumulates in body fat. Waters *et al.* (1986) found that the area under the drug concentration/time curve

correlated not with total body weight but with skinfold thickness. In animal experiments, cyclosporin A was found in liver, kidney, adrenals, pancreas, thymus, thyroid and renal fat after administration of 3H-cyclosporine (Hassan and Al-Yahya, 1987). Using intravenous tritiated cyclosporin, the heaviest accumulation of radiation was in the liver and, in descending order, fat, kidney, reticulo-endothelial and endocrine systems, and blood (Maurer *et al.*, 1984). Another study revealed that from intravenous administration in human, cyclosporin A was found to be distributed according to a three-compartment distribution model (Hassan and Al-Yahya, 1987).

Cyclosporin A is extensively metabolized in the liver (Maurer *et al.*, 1984) and 90% is eliminated in the bile whilst around 6% is eliminated in the kidney (Kahan, 1989). Only 1% of an oral dose is excreted unchanged in urine or bile. Cyclosporin A elimination follows first-order kinetics (Kahan, 1985). It has been estimated that as much as 50% of a dose of cyclosporin A may be extracted from the blood during its first passage through the liver. Although cyclosporin A has more than 30 metabolites which could be quantified analytically (Christians and Sewing, 1993), cyclosporin A is still found to be more toxic than these metabolites (Hassan and Al-Yahya, 1987).

The first marketed oral formulation of cyclosporin A, Sandimmune® was formulated with olive oil, Labrafil (peglicol-5 oleate), and alcohol (Johnston *et al.*, 1986). This formulation will form a crude o/w emulsion upon dilution with an aqueous phase. Sandimmune® results in a comparatively low oral bioavailability along with a high inter-subject variability because of the presence of bile and

pancreatin is mandatory in promoting the absorption of cyclosporin A (Metha *et al.*, 1984; Friman and Bäckman, 1996). The mean absolute bioavailability of cyclosporin A is only approximately 30% when administered as Sandimmune®. Later, an improved formulation of cyclosporin A, Sandimmun Neoral® was commercialized. It is a microemulsion containing corn oil mono-, di- and tri-glycerides, polyoxyl 40 hydrogenated castor oil, propylene glycol and DL-α-tocopherol with cyclosporin A well dispersed within the mixture (Levy and Grant, 1994). Upon dilution with an aqueous phase, this SEDDS formulation produces a fine and homogenous microemulsion with no precipitation of the incorporated cyclosporin A. Sandimmun Neoral® has been shown to increase the oral bioavailability of cyclosporin A by 40% to 60% (Kovarik *et al.*, 1994) with less inter- and intra-subject variability in comparison with the earlier Sandimmune® product (Holt *et al.*, 1994).

1.3.2 ENHANCING THE ORAL BIOAVAILABILITY OF CYCLOSPORIN A

Cyclosporin A has very low oral absorption due to its BCS Class II characteristics. Its low aqueous solubility limits the dissolution rate in the lumen. Therefore, many approaches have been used to improve the solubility and dissolution of cyclosporin A with the intention of enhancing its oral bioavailability. It has been suggested that the oral bioavailability of cyclosporin A can be enhanced by increasing the drug permeability through the intestinal membrane, improving drug solubilization and reducing droplet size (Gao *et al.*, 1998). In most cases, drug solubilization effect could be increased by reducing the droplet size. Smaller droplet size means larger total surface area exposed which could lead to improvement in the drug dissolution. However, cyclosporin

A nanospheres prepared by Ford *et al.* (1999) were shown to posses a relative bioavailability of only 3% compared to Sandimmun Neoral® although the size of the nanospheres prepared were within the range of 250 nm to 900 nm.

Gao *et al.* (1998) reported on a formulation of a cyclosporin A microemulsion prepared by adding 20 mg cyclosporin A into 0.2 ml Oil-S_{mix} mixture containing Cremophor EL, Transcutol P and Captex 355 at a ratio of 10:5:4. The microemulsion droplets formed were only 22 nm in size when diluted with an aqueous phase and has comparable oral bioavailability to Neoral® microemulsion formulation. The workers concluded that the enhancement of the bioavailability of cyclosporin A loaded in this microemulsion system might be due to the small droplet size of the microemulsion formed.

Lecithin vesicles were proposed to be promising carriers in the oral delivery of cyclosporin A in another study (Guo *et al.*, 2001). Cyclosporin A in lecithin vesicles were prepared by adding 0.375% w/w cyclosporin A and 4% w/w lecithin in a 1:1 mixture of methanol and chloroform. The oral bioavailability of cyclosporin A in the lecithin vesicles was compared to Sandimmun Neoral® using rabbits. The relative bioavailability of cyclosporin A lecithin vesicles versus Sandimmun Neoral® was 105 ± 21% (n = 6) and statistical analysis demonstrated that both preparations were bioequivalent. Therefore, the workers concluded that lecithin vesicles could facilitate the oral delivery of cyclosporin A, due to their absorption enhancement effect. Another formulation prepared by adding 3 mg/ml cyclosporin A in 2% egg lecithin and 10% soybean oil was reported by Kim *et al.* (2002). This oil-in-water emulsion showed comparable

pharmacokinetics and pharmacodynamics to the commercial products (Sandimmun Neoral® and CIPOL Inj®). Nevertheless, the workers found that this formulation was more effective via oral administration than intravenous.

There have been many other formulations reported for increasing the oral bioavailability of cyclosporin A using SEDDS. Cyclosporin A oral bioavailability from SEDDS formulations was found to be governed by a combination of different lipid excipients, ratio between lipid excipients and the percentage of drug incorporated (Odeberg *et al.*, 2003). González *et al.* (2002) prepared a cyclosporin A self-emulsifying formulation by adding cyclosporin A into Solutol HS15:Labrafil M2125:oleic acid (in a ratio of 7:2:1) to make a drug concentration of 100 mg/ml. This self-emulsifying formulation was shown to improve the oral bioavailability by 2-folds compared to a cyclosporin A microsuspension in rats. Moreover, Odeberg *et al.* (2003) successfully prepared a self-emulsifying drug delivery system based on natural lipid components [which consisted of 50% neutral lipids and 50% polar lipids (mixed phospholipids and galactolipids)] and medium-chain monoglycerides in a ratio of 1:1, with a cyclosporin A concentration of 10%. This formulation was bioequivalent with the Sandimmun Neoral® although the size of the emulsion dropets formed was nearly 100 times larger than that of Sandimmun Neoral®.

1.4 SCOPE OF STUDY

As reviewed above, Gelucire® which comprises a family of polyglycolized glycerides, has received acceptance as formulation excipient by the pharmaceutical industry. Among the members in the family, Gelucire® 44/14

has been investigated for enhancing the solubility and bioavailability of lipophilic drugs. This commercially available excipient can disperse spontaneously in aqueous media and thus can be utilized in self-emulsifying formulations. Lipophilic drugs could be incorporated into Gelucire® 44/14 to form solid solution or solid dispersion. The ability of Gelucire® 44/14 to increase the solubility of lipophilic drugs is due to its amphiphilic properties. Lipophilic drugs will be bound to the lipophilic part of the fatty acids contained in Gelucire® 44/14, whereas the hydrophilic part of the fatty acids will facilitate the dissolution or dispersion of the matrices in the aqueous phase. Consequently, the drugs incorporated will dissolve or disperse together with the matrices.

Thus, the aim of the present study was to investigate the potential of Gelucire® 44/14 in enhancing the aqueous solubility and oral bioavailability of a model lipophilic drug with poor aqueous solubility. Cyclosporin A, a lipophilic polypeptide, was chosen as model drug in present study. Cyclosporin A is categorized as a BCS Class II drug wherein the oral absorption of the drug is limited by its solubility. The present study was conducted in various stages with the following objectives:

1. To prepare and characterize self-emulsifying formulations of cyclosporin A using Gelucire® 44/14.
2. To develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for determination of cyclosporin A in plasma samples.
3. To evaluate the potential of Gelucire® 44/14 in enhancing the oral bioavailability of cyclosporin A in rats.

Table1.1: Specification of Gelucire® bases (Craig, 1995)

Type of gelucire	Acid value	Saponification value	Hydroxyl value	Iodine value	Melting point, °C
33/01	< 1	240 – 260	< 10	< 3	33 – 38
35/10	< 2	120 – 135	70 – 90	< 2	29 – 34
37/02	< 2	200 – 215	30 – 50	< 2	34.5 – 39.5
37/06	< 2	165 – 180	100 – 120	< 2	30.5 – 35.5
39/01	< 0.2	225 – 245	< 6	< 2	38 – 40
42/12	< 2	95 – 115	30 – 50	< 2	41.5 – 46.5
43/01	< 0.2	220 – 240	< 6	< 2	42 – 45
44/14	< 2	75 – 95	30 – 50	< 2	42.5 – 47.5
46/07	< 2	125 – 140	65 – 85	< 2	47 – 52
48/09	< 2	105 – 125	60 – 80	< 2	46 – 51
50/02	< 2	180 – 195	25 – 45	< 2	46.5 – 51.5
50/13	< 2	65 – 80	30 – 50	< 2	46 – 51
53/10	< 2	95 – 115	25 – 45	< 2	47.5 – 52.5
54/02	< 6	175 – 195	80 – 120	< 3	53 – 57
55/18	< 6	8 – 20	-	< 3	54.5 – 58.5
62/05	< 5	70 – 90	< 60	< 10	59 – 70
64/02	< 2	165 – 185	80 – 120	< 3	63.5 – 67.5

Table1.2: Distribution of fatty acids in Gelucire® 44/14 (*Gattefossé*, 1999)

Fatty acid	Chain length	Distribution
Caprylic acid	8	4 – 10 %
Capric acid	10	3 – 9 %
Lauric acid	12	40 – 50 %
Myristic acid	14	14 – 24 %
Palmitic acid	16	4 – 14 %
Stearic acid	18	5 – 15 %