FORMULATION OF SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEM USING MIXED-TOCOTRIENOLS AS THE MODEL DRUG

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FORMULATION OF SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEM USING MIXED-TOCOTRIENOLS AS THE MODEL DRUG

by

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To my beloved family,

Lee Chong Yu, Tan Sok Ching,

You Ning, You Jia, Chang Xiu, Madam Lim Chew Mooi,

and supportive husband Ka Keat

In memory of Madam Tan Siew Keow

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LIST OF ABBREVIATIONS

ABC	ATP-binding cassette
ACE	Angiotensin-converting enzyme
ANOVA	Analysis of variance
AOR	Angle of repose
ARASC	Animal Research and Service Centre
ATP	Adenosine Triphosphate
AUC	Area under the plasma concentration-time curve
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero to infinity
AUC_{0-t}	Area under the plasma concentration-time curve from time zero to the last sampling point
$AUC_{t-\infty}$	Area under the plasma concentration-time curve from time t to infinity
BCS	Biopharmaceutics Classification System
C.V.	Coefficient of variation
CI	Carr's compressibility index
C_{max}	Maximum plasma concentration
CMC	Carboxymethylcellulose
Conc	Concentration
CYP3A4	Cytochrome P450 subfamily 3A4

CYP450	Cytochrome P450
FDA	Food and Drug Administration
GpIIb/IIIa	Glycoprotein IIb/IIIa
HbA1c	Hemoglobin A1c
HIV	Human immunodeficiency virus
HLB	Hydrophile-Lipophile Balance
HPC	Hydroxypropyl Cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
HR	Hausner ratio
IACUC	Institutional Animal Care and Use Committee
k_e	Elimination rate constant
Lab	Labrasol
LDL	Low-density lipoprotein
MCC	Microcrystalline cellulose
MDR	Multi-drug resistance
MELD	Model for end-stage liver disease
MRI	Magnetic resonance imaging
P-gp	P-glycoprotein
PCS	Photon correlation spectroscopy

PDI	Polydispersity Index
PEG	Polyethylene glycol
pKa	Dissociation constant
Pol	Poloxamer 188
PVP	Polyvinyl pyrrolidone
SD	Standard deviation
SE	Self-emulsifying
SEDDS	Self-emulsifying Drug Delivery System
SEM	Standard error of mean
SES	Self-emulsifying System
SLS	Sodium lauryl sulphate
T_{max}	Time to reach maximum plasma concentration
TPGS	D-alpha-tocopherol polyethylene glycol 1000 succinate
TRF	Tocotrienol rich fraction
USM	Universiti Sains Malaysia
v/v	Volume over volume
w/w	Weight over weight
ZAve	Z average diameter value

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FORMULASI SISTEM PENGHANTARAN DRUG PENGEMULSIAN SENDIRI BERJENIS PEPEJAL MENGGUNAKAN CAMPURAN TOKOTRIENOL SEBAGAI DRUG MODEL

ABSTRAK

Kajian ini dijalankan untuk membangun dan menilai formulasi sistem pengemulsian sendiri berjenis pepejal untuk meningkatkan biokeperolehan oral suatu bahan aktif jenis cecair yang kurang larut dalam air, iaitu tokotrienol. Pembawa jenis pepejal yang bersesuaian berserta dengan kapasiti pegangan cecair yang tinggi telah dipilih untuk pembangunan formulasi. Pelbagai ciri beberapa formulasi pengemulsian sendiri jenis pepejal termasuk kecekapan untuk mengemulsi, saiz titisan emulsi, sifat penyerapan dan aliran serbuk telah disiasat. Formula sistem pengemulsian sendiri jenis pepejal yang mempunyai ciri-ciri yang dikehendaki telah berjaya dibangunkan dengan menggunakan magnesium aluminosilikat (Neusilin US2) sebagai pembawa, dengan komposisi 65 hingga 70% TRF dan 30 hingga 35% surfaktan (poloxamer dan Labrasol) dengan natrium lauril sulfat sebagai ejen pembasahan. Formulasi tersebut menunjukkan kecekapan pengemulsian sendiri yang baik dengan pembentukan fizikal produk emulsi yang stabil, pengaliran serbuk yang baik, desorpsi tokotrienol dari pembawa yang lengkap, dan titisan emulsi yang bersaiz kecil dari 210 hingga 303 nm. Seterusnya, kajian *in vivo* menggunakan tikus Sprague-Dawley menunjukkan bahawa formulasi pengemulsian sendiri jenis pepejal dengan surfaktan kombinasi (poloxamer dan Labrasol) mempunyai kadar penyerapan yang lebih cepat berbanding dengan formulasi dengan hanya satu surfaktan (poloxamer atau Labrasol). Dalam kajian *in vivo* yang berikutnya, formulasi pengemulsian sendiri jenis pepejal dengan surfaktan kombinasi (D4) meningkatkan

biokeperolehan oral (3.4 hingga 3.8 kali lebih tinggi) berbanding dengan sediaan tokotrienol jenis bukan pengemulsian sendiri dalam tikus di bawah keadaan tanpa makanan. Formulasi tersebut dalam bentuk serbuk kemudiannya digunakan untuk pembentukan dos kapsul gelatin keras yang mengandungi 100 mg tokotrienol setiap kapsul. Granul disediakan melalui proses granulasi basah dengan penambahan PVP K30 sebagai ejen mengikat pada kuantiti 5.2 %w/w setiap kapsul. Formulasi granul menunjukkan ketumpatan pukal yang tinggi, pengaliran serbuk yang sangat baik dan kecekapan pengemulsian dengan indeks kebolehmampatan Carr, nisbah Hausner, serta sudut diam masing-masing dalam kategori "baik" hingga "cemerlang". Pelepasan tokotrienol daripada kapsul gelatin keras adalah lengkap dan emulsi yang terbentuk mempunyai saiz titisan dalam lingkungan submikron. Biokeperolehan tokotrienol daripada kapsul tersebut telah dinilai berbanding dengan produk pengemulsian sendiri cecair komersil Tocovid® Suprabio® dengan 8 sukarelawan manusia. Keputusan menunjukkan sediaan kapsul pengemulsian sendiri jenis pepejal mempunyai kadar dan tahap biokeperolehan yang setanding dengan Tocovid® Suprabio®, yang telah ditunjukkan dapat meningkatkan tahap biokeperolehan sebanyak 3 kali ganda berbanding dengan sediaan minyak tokotrienol jenis bukan pengemulsian sendiri. Kesimpulannya, formulasi tokotrienol dengan sistem pengemulsian sendiri jenis pepejal dalam sediaan kapsul gelatin keras dapat meningkatkan biokeperolehan oral tokotrienol, dengan kadar yang setanding dengan produk komersil yang telah menunjukkan peningkatan biokeperolehan tokotrienol.

FORMULATION OF SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEM USING MIXED-TOCOTRIENOLS AS THE MODEL DRUG

ABSTRACT

The present study was conducted to develop and evaluate the formulation of a solid self-emulsifying system for enhancing the oral bioavailability of a poorly water-soluble liquid active compound, namely tocotrienols. A suitable solid carrier with high liquid holding capacity was selected. Various properties of several solid self-emulsifying formulations including emulsification efficiency, emulsion droplet size, desorption and powder flow properties were investigated. A solid self-emulsifying formulation with the desired properties was successfully developed, using magnesium aluminosilicate (Neusilin US2) as the solid carrier, containing 65 to 70% TRF and 30 to 35% surfactants (poloxamer and Labrasol) with sodium lauryl sulphate as wetting agent. The formulation showed good self-emulsification efficiency with stable emulsion formed, demonstrated excellent powder flowability, complete desorption of tocotrienols from carrier, and small emulsion droplet size of 210 to 303 nm. Subsequent *in vivo* studies using Sprague-Dawley rats showed that the formulation with a combination of surfactants (poloxamer and Labrasol) had a faster rate of absorption compared to using only single surfactant (either poloxamer or Labrasol). In a following *in vivo* study, the solid self-emulsifying preparation with combined surfactants (D4) showed enhanced oral bioavailability (3.4 to 3.8 times higher) compared to the non self-emulsifying oily liquid preparation when administered at fasted state in rats. The formulation which was in a dry powder form was further developed into a hard gelatin capsule dosage form, containing 100 mg tocotrienols in each capsule. Granules were prepared by wet granulation with the

addition of PVP K30 as binder at 5.2 %w/w per capsule. The granules showed high bulk density, excellent flow properties and emulsification efficiency with Carr's compressibility index, Hausner ratio and angle of repose in the category of 'good' to 'excellent'. Release of the tocotrienols from the capsule was complete and the emulsion formed had droplet size in the submicron range. The bioavailability of tocotrienols from the capsules was evaluated in comparison to a commercial liquid self-emulsifying product, Tocovid® Suprabio® in 8 human volunteers. The results showed that the oral bioavailability of the capsules was comparable to that of Tocovid® Suprabio®, which has been demonstrated to enhance the bioavailability of tocotrienols by 3-fold compared to a non self-emulsifying oily preparation. In conclusion, the solid self-emulsifying formulation of tocotrienols in a hard gelatin dosage form was able to enhance the oral bioavailability of tocotrienols and was comparable to a commercial liquid self-emulsifying product which has been shown to enhance the oral bioavailability of the tocotrienols.

CHAPTER 1

INTRODUCTION

1.1 GENERAL PRINCIPLE OF ORAL BIOAVAILABILITY OF DRUGS

The oral route for dosage administration is generally the preferred option due to patients' ease of use and convenience for self-administration (Stuurman et al., 2013). Oral administration of dosage form also minimise pain and risk of infections associated with parenteral administrations like intramuscular and intravenous injections (Allen et al., 2011). For a drug to exert its therapeutic effect, it must first be absorbed into the systemic blood circulation. The blood then acts as a conduit for delivering the drug to the targeted site to elicit the intended response.

Oral bioavailability is defined as the rate and extent at which an active drug ingredient is absorbed and becomes available at its desired site of action (Cao et al., 2006; Chow and Liu, 2009). The complex process of oral absorption from a pharmaceutical dosage form typically involves four major factors (Lipka and Amidon, 1999): (i) drug dissolution from the dosage form; (ii) the drug's solubility as a function of its physicochemical characteristics; (iii) effective permeability of the drug across intestinal mucosa; and (iv) the drug's presystemic metabolism.

1.2 FACTORS AFFECTING BIOAVAILABILITY OF DRUGS

Factors affecting the bioavailability of drug can be broadly divided into three categories: physiological factors; physicochemical properties of a drug; and

formulation factors (Levine, 1970; Ashford, 2002a; Ashford, 2002b; Martinez and Amidon, 2002; Hurst et al., 2007; Mayersohn, 2009).

1.2.1 PHYSIOLOGICAL FACTORS

Oral dosage form, upon ingestion, will transit along the gastrointestinal tract prior to drug absorption. The physiological factors that may affect the oral bioavailability include gastrointestinal pH; gastric juice and luminal enzymes; food, physiological disorder and disease state; the gastrointestinal membrane; and pre-systemic metabolism; and transit time (Ashford, 2002b; Gerk et al., 2016).

Gastrointestinal pH

The pH of the fluids varies along the gastrointestinal tract; gastric fluid is extremely acidic, typically at pH 1 to 3.5 in fasted state and rising to pH 3 to 7 following a meal. Meanwhile, intestinal fluid has higher pH compared to gastric pH, and gradually rising from the duodenum (pH 4.9 to 6.4) to the ileum (6.5 to 7.4). The varying pH of the gastrointestinal tract may affect the drug bioavailability by altering the chemical stability, dissolution and absorption (Ashford, 2002b; Mayersohn, 2009).

Drug degradation may occur in gastrointestinal fluid if the compound has limited stability in either acidic or alkali conditions. To minimise the degradation, drugs like omeprazole and erythromycin that degrade rapidly at acidic pH can be formulated as an enteric-coated dosage form, whereby the dosage remains intact in gastric region and dissolution only occurs when pH rises in the intestinal fluid.

In addition, the gastrointestinal pH affects drug dissolution especially when the compound is of weak acid or base, where its aqueous solubility is dependant on the surrounding pH. Basic drugs dissolve readily in acidic solutions and thus have higher dissolution rate in gastric fluids compared to that in intestinal fluids; on the other hand, acidic drugs have greater dissolution rate in intestinal fluids due to their higher solubility in alkali solutions. As small intestine is the major site of absorption and dissolution of drugs is a prerequisite process prior to absorption, hence poor solubility in the gastrointestinal tract may lead to low oral bioavailability.

Gastrointestinal pH further influence the absorption process if the ionisable drugs (weakly acidic or basic) become ionisable in gastrointestinal fluids and become impermeable to the gastrointestinal epithelia, preventing the occurrence of drug absorption (Ashford, 2002b; Gerk et al., 2016).

Luminal enzymes

The presence of luminal enzyme in gastrointestinal fluids may affect the drug absorption process. Pepsin is present in gastric juice while lipases, amylases and proteases are secreted from the pancreas into the small intestine. Certain drugs (e.g. peptides and esters) are susceptible to enzymatic degradation, rendering poor oral bioavailability. In addition, bile salts, which are highly surface-active, are secreted into the gut in response to the presence of food, especially those high in fats. Bile salts can enhance drug absorption by increasing the dissolution rate of poorly water-soluble compounds in the gastrointestinal fluids (Welling, 1977; Ashford, 2002b).

Gastrointestinal membrane

The gastrointestinal membrane, which separates the lumen of the stomach and the intestines from the systemic circulation, is the main cellular barrier for effective drug absorption. The complex bilayer structure of gastrointestinal membrane is constituted of lipids, proteins, lipoproteins and polysaccharides and provides a semi-permeable characteristic that allows rapid transit of certain compounds while hindering the passage of others (Ashford, 2002b; Mayersohn, 2009). The mechanism of transport across membrane will be further discussed in section 1.2.1.1.

There is also an aqueous boundary layer of nearly stagnant water, viscous mucus and glycocalyx that lines the intestinal wall, sometimes known as "the unstirred water layer". The layer becomes an added barrier for drug diffusion. Mucus may bind to certain drugs, thus reducing the drug bioavailability (Ashford, 2002b; Martinez and Amidon, 2002).

Disease state and physiological disorder

Certain disease states and physiological disorders related to the gastrointestinal tract have great potential to affect the drug absorption and bioavailability (Ashford, 2002b; Mayersohn, 2009). Disease states that resulted in a change of pH may cause changes in the stability, dissolution and absorption of the drug. For instance, subjects with achlorhydria have reduced gastric acid secretion, thus having elevated gastric pH may cause poor absorption of drugs that is most soluble in acidic pH, such as tetracycline. Surgical removal of sections of the stomach may result in a different absorption rate as the compound may reach the duodenum earlier compared to that of the normal subjects. Changes in gastric emptying as a result from disease conditions

can also alter drug absorption; such as in the absorption of riboflavin, which is reduced in hyperthyroidism and increased in hypothyroidism, conditions that affect gastric-emptying and intestinal transit time. Diarrhoea may also reduce drug absorption as the intestinal residence time is greatly shortened (Ashford, 2002b; Gerk et al., 2016).

1.2.1(a) Mechanisms of drug transport across the membrane

The mechanisms of drug transport across the gastrointestinal epithelium occur mainly by transcellular and paracellular pathways (Ashford, 2002b; Park and Chang, 2011). The paracellular pathway differs from the transcellular pathway as it involves the transport of materials in the aqueous pores between cells rather than across them. The cells are joined together by close-fitting tight junctions. This pathway of absorption is crucial for the transfer of small hydrophilic and charged drugs such as cimetidine and atenolol (Gerk et al., 2016).

The transcellular pathway includes passive diffusion, carrier-mediated transport and endocytosis. Passive diffusion is a process whereby drug molecules travel from a high concentration region in the lumen to a lower concentration region in the blood with no energy required. The rate of transportation is dependant on the physicochemical properties of the drug, the nature of the membrane and the concentration gradient of the drug across the membrane. This simple process is preferred by small lipophilic and unionised molecules. Very hydrophilic molecules lack sufficient lipid solubility to cross the gastrointestinal membrane thus requiring carrier-mediated transport, mainly active transport and facilitated diffusion.

Active transport involves the transfer of molecules against the concentration gradient, from a region of low concentration to a higher one and thus requires energy input, mainly generated from hydrolysis of adenosine triphosphate or from the transmembraneous sodium gradient and electrical potential. Each transporter has specific substrate (in terms of chemical structure of the substance) that it will transport, with some transporters having a wider specificity than others. Hence, if the drug molecules have similar structure to the natural substrate that the transporter actively transfer, the drug is probably carried across the membrane via the same mechanism. Penicillins, angiotensin-converting enzyme (ACE) inhibitors and renin inhibitors are peptide-like drugs that depend on the peptide transporters for absorption while antivirals and anticancer drugs of nucleoside analogues rely on nucleoside transporters (Ashford, 2002b; Park and Chang, 2011).

1.2.1(b) Cytochrome P450 3A4 and P-glycoprotein

Cytochrome P450

Prior to entering systemic circulation, drugs that are absorbed from the stomach, small intestine and upper colon will go through the hepatic portal system and are subjected to metabolism or degradation. Oral drug may be completely absorbed but remained low in bioavailability due to first-pass or presystemic metabolism by the gut wall or liver (Mayersohn, 2009).

The cytochrome P450 family (CYP450), mainly the CYP3A family, are the main phase 1 metabolising enzymes present in absorptive intestinal cells and liver. They serve as defences against ingested toxins or carcinogens, but also resulted in reduced bioavailability of many orally administered drugs (Watkins et al., 1987; Wacher et

al., 2001). The CYP3A enzymes were found to be high in the human small intestine (about 70% of the CYP content) whereas making up to 30% of total human hepatic CYP, suggesting that major metabolism occurs at the gastrointestinal mucosal prior to systemic absorption (Watkins et al., 1987; Shimada et al., 1994; Wacher et al., 2001). It was estimated that CYP3A is responsible for the metabolism of 50 to 70% of administered drugs (Wacher et al., 1998). The high concentrations of CYP3A in the villus tip of enterocytes contribute to larger surface area that allow the enzymes to interact with absorbed drug, expediting notable first-pass metabolism (Wacher et al., 2001).

The CYP3A4 has been determined to be the most important enzymes in drug metabolism (Wacher et al., 2001). The expression of CYP3A4 is most dominant in human liver and intestine where it accounts for 30 and 50% of the total CYP450 respectively in these tissues (Gibson et al., 2002). The expression varied markedly (10 to 100 fold) among humans (Shimada et al., 1994). It has been shown that CYP3A4 has wide variety of substrate and its activity can be induced by compounds such as glucocorticoids, macrolide antibiotics, phenobarbitone; or inhibited by ketoconazole and erythromycin (Gibson et al., 2002). Thus, its enzyme activities may result in drug interactions that could be of clinical significance. It has been reported that rifampicin (CYP3A4 inducer) increased the metabolism of cyclosporine (CYP3A4 substrate) and possibly affected the outcome of organ graft rejection (Lucey et al., 1990). On the other hand, cyclosporine administered together with ketoconazole (CYP3A4 inhibitor) resulted in an increase in its oral bioavailability, thus reducing the dosage of cyclosporine necessary to achieve sufficient immunosuppression by 60 to 80% (Gomez et al., 1995; Dresser et al., 2000). Another

study (Backman et al., 1998) reported that midazolam (CYP3A4 substrate) has much lower oral bioavailability when administered with rifampicin (CYP3A4 inducer) but significantly increased when administered with itraconazole (CYP3A4 inhibitor).

P-glycoprotein

P-glycoprotein (P-gp) is an ATP-dependent efflux pump that is part of the fifty human ATP-binding cassette (ABC) proteins (Chan et al., 2004). P-gp was first discovered when the colchicine-resistant Chinese hamster ovary cells with cross-resistance to multiple amphiphilic drugs showed over-expression of a membrane protein that decrease the amount of drug penetration (Juliano and Ling, 1976). Expression of P-gp was noted to be especially high in tumours of epithelial origin, such as that of colon, kidney and breast. P-gp was found in normal tissues, with high expression in kidney and adrenal glands; intermediate expression in liver, small intestine, colon and lungs; and low expression in prostate, ovary, spleen, heart, stomach, skeletal muscle and skin (Chan et al., 2004). P-gp was also found in the blood-brain and other blood-tissue barrier sites (Cordon-Cardo et al., 1989). The presence of P-gp suggest that its efflux function intend to protect body against intake or accumulation of toxic natural compounds or xenobiotics by actively excreting them into bile, urine and the intestinal lumen (Lin and Yamazaki, 2003). Due to their expressions in multiple sites, P-gp has substantial influence on drug absorption, distribution, metabolism and excretion of administered drugs. P-gp has been shown to be involved in the development of multi-drug resistance during chemotherapy in human cancers (Gottesman and Pastan, 1993).

The P-gp in the gastrointestinal tract is located at the villus tip of enterocytes, in close proximity to the lumen, which conveniently hinder the efficient absorption of compounds by actively transporting them back to the lumen (Chan et al., 2004). In the gastrointestinal tract, the distribution of P-gp appeared to be in increasing order: low in the stomach, intermediate in the jejunum, and high level in the colon (Fojo et al., 1987). One study demonstrated the uneven distribution of the P-gp when cyclosporine (P-gp substrate) was given to healthy volunteers via intubation tubes, directly delivered to various parts of the gastrointestinal tract. The AUC (area under the curve) of cyclosporine was noted to be highest when it was administered in the stomach, followed by the jejunum/ileum, and the lowest when given in the colon (Fricker et al., 1996). It has also been reported that the intestinal expression level of P-gp showed inter-individual variability of more than 8-fold (Lown et al., 1997).

More evidence of how intestinal P-gp affects oral drug bioavailability have been observed from experiments conducted in mdr1a(-/-) knockout mice, which lack P-gp expression. The plasma concentration of paclitaxel following paclitaxel administration was 6-fold higher in mdr1a(-/-) mice compared to mdr1a(+/+) mice with normal P-gp expression, indicating that intestinal P-gp had a role in restricting the absorption of drugs by expelling the drug back to the intestinal lumen (Sparreboom et al., 1997).

The influence of P-gp in drug absorption has been reported to cause drug-drug interactions. Plasma concentrations of digoxin (P-gp substrate) were increased by 60 to 90% and 200 to 300% when administered with verapamil and quinidine (both P-gp inhibitors), respectively (Bussey, 1982; Verschraagen et al., 1999). In another

study (Greiner et al., 1999), plasma level of digoxin was decreased by 30% in healthy subjects after pretreatment with rifampicin (P-gp inducer) for 10 days. The duodenal biopsies from before and after rifampicin treatment showed a rise in intestinal P-gp expression by 3.5-fold post-treatment. This indicated that the lower oral bioavailability of digoxin might be due to the induction and increased activity of intestinal P-gp (Greiner et al., 1999).

The function of the P-gp appeared to be saturable, much like that of CYP3A4. It was reported that plasma digoxin levels were low and variable when very low dose of 0.5 to 1 mg (intestinal lumen concentration well below saturation point) were administrated orally (Lin, 2003). At higher doses given, the activity of P-gp can be saturated. Indinavir (P-gp substrate) showed increased bioavailability by more than 60% in subjects when given orally at a high dose of 800mg (intestinal lumen concentration expected to be well above saturation point) compared to a dose of below 100 mg where P-gp were not saturated (Lin, 1999; Lin, 2003).

However, high clinical oral dose does not always assure higher bioavailability for P-gp substrates. The oral bioavailability of cyclosporin and paclitaxel (both P-gp substrates) administered at high doses remained low and variable (Fricker et al., 1996; Sparreboom et al., 1997). This might be attributed to their nature of being very poorly water-soluble that resulted in slow dissolution rates and low drug concentration in the intestinal lumen (below saturation point of P-gp). In addition, their large molecular weight may delay the rate of membrane diffusion, adding hindrance to the absorption process (Lin, 2003).

Interaction between CYP 3A4 and P-glycoprotein

CYP3A4 and P-gp may have synergistic roles in promoting or limiting oral bioavailability of drugs due to their many overlapping substrates specificities and locations (Wacher et al., 1995). Several potential mechanisms of how CYP3A4 and P-gp influence each other has been proposed (Watkins, 1997). P-gp may prolong the absorption process by repeatedly extruding substrates back to the intestinal lumen, thus increasing the exposure of the substrates to metabolism by CYP3A4. It was further postulated that the expression of P-gp and CYP3A are coordinated and regulated (Wacher et al., 1995; Lin and Yamazaki, 2003).

1.2.2 PHYSICOCHEMICAL FACTORS

The absorption and bioavailability of orally administered drugs are also influenced by the physicochemical properties of a drug, which determine its transition into solution and subsequent transport across membranes. These physicochemical factors include water solubility and dissolution rates, pKa, lipophilicity and chemical stability (Ashford, 2002a).

To be absorbed, the ingested drugs firstly have to dissolve in the gastrointestinal fluid and present in aqueous solution at the site of absorption. The aqueous solubility of drug is one of the most critical criteria in drug discovery and development (Panchagnula and Thomas, 2000). Many newly discovered and existing molecules showed excellent potency and activities *in vitro* but had poor aqueous solubility and oral bioavailability. Poorly water-soluble drugs have minimal dissolution in gastrointestinal fluid and lack the ability to establish a high concentration gradient to ensure transport of drug across intestinal membrane, thus leading to poor

bioavailability (Panchagnula and Thomas, 2000). As discussed in earlier sections, physiological factors such as pH and content of gastrointestinal fluids can affect the dissolution process and hence the drug's bioavailability.

Physicochemical properties of the drug molecule may be modified to improve the solubility. One approach is by introducing ionisable or polar group to the molecule. The discovery of indinavir came from addition of a basic amine to the previously poorly water-soluble molecule, and thus improving its aqueous solubility and bioavailability (Dorsey et al., 1994). Another example was the synthesis of fibrinogen receptor (GpIIb/IIIa) antagonists reported by Eldred et al. (1994), where the aqueous solubility and oral bioavailability was improved when the compound was added with a piperazine ring (Eldred et al., 1994).

Prodrug is another approach to improve the water-solubility of a drug. The physicochemical properties of a prodrug are altered by adding an ionisable or polar group onto the parent compound such that upon ingestion and absorption, it can revert back to the active parent compound upon hydrolysis or metabolism in body (Panchagnula and Thomas, 2000). Fosamprenavir is a water-soluble phosphate ester prodrug of the protease inhibitor amprenavir. The water solubility of the drug was increased by 10-fold when the phosphate group was added to the hydroxyl group of the drug (Chapman et al., 2004; Palombo et al., 2009). Following oral administration, fosamprenavir swiftly reverted back to the active amprenavir by the enzyme alkaline phosphatase at the intestinal epithelium (Palombo et al., 2009). The increased aqueous solubility using prodrug greatly enhanced the oral bioavailability of the

active amprenavir and thus reduced the high dosage burden usually required for patients (Chapman et al., 2004).

Particle size reduction is another effective method to improve drug bioavailability when the dissolution rate is the rate-limiting step for drug absorption. The smaller the particle size, the larger is the effective surface area, thus wettability is improved and the dissolution rate is increased (Pouton, 2006). Griseofulvin, a poorly water soluble compound with low oral bioavailability, showed a 1-fold increment in plasma level when the particle size was decreased from 10 to 2.7 μm and the specific surface area was increased from 0.4 to 1.5 m^2/g (Atkinson et al., 1962; Marvel et al., 1964). However, extensive particle size reduction, particularly in compounds that are very hydrophobic may result in aggregation of fine particles and decrease in the effective surface area. This may worsen the dissolution rate and bioavailability compared to compounds prior to treatment (Ashford, 2002a; Loh et al., 2015).

Another factor affecting the bioavailability is polymorphism of drugs. Polymorphism is the ability of a solid material to have more than one crystalline form. These different crystals of the same drug can have different physical properties. A metastable polymorph has a faster dissolution rate compared to a stable polymorph (Ashford, 2002a). The metastable polymorph B of chloramphenicol palmitate showed higher solubility and dissolution rate compared to the stable polymorph A. *In vivo* experiments showed consistent results of higher oral bioavailability from polymorph B compared to polymorph A (Aguiar et al., 1967; Aguiar and Zelmer, 1969).

Following the pH-partition hypothesis of drug absorption, lipid soluble drugs will be able to pass through the gastrointestinal membrane by passive diffusion. For weakly acidic or weakly basic compounds, the dissociation constant (pKa) and the pH of the gastrointestinal environment will govern the degree of ionisation of the compounds and subsequently absorption across gastrointestinal membrane (Ashford, 2002a). The barrier separating gastrointestinal lumen and blood showed preference to undissociated form of the drug (Jollow and Brodie, 1972). In theory, a weakly acidic drug is less unionised in an acidic environment (pH < pKa) and possibly better absorbed from the stomach (pH 1.2), whereas a weakly basic drug is less unionised in basic environment (pH > pKa) will be better absorbed from the intestine which has a higher pH. For instance, aspirin is a weak acid and remained unionised in gastric while the basic drug codeine remained unionised in the intestinal pH (Dowd, 2017). There are limitations to this theory as the degree of ionisation of a drug is not the only contributing factor influencing absorption (Ashford, 2002a). Weak acids have higher absorption rate in the small intestine compared to in the stomach, despite being unionised in the latter. The much greater surface area available for absorption and longer residence time in the small intestine may have made up for the extent of ionisation of weak acids at the higher pH of intestinal fluids (Ashford, 2002a).

The absorption of a drug across the gastrointestinal tract is also influenced by its partition coefficient, which is a measure of its lipophilicity (Panchagnula and Thomas, 2000; Ashford, 2002a). The higher the partition coefficient, the higher is the concentration in the lipid layer and more likely for the lipid soluble drug to diffuse rapidly across membrane based on the greater concentration gradient between them. Prodrugs can be developed to increase the lipophilicity of compounds and enable

them to permeate the epithelial cell membranes of gastrointestinal tract. Pivampicillin and bacampicillin are examples of lipophilic prodrugs of the highly polar ampicillin (Panchagnula and Thomas, 2000).

As mentioned in the section on physiological factors, the pH in the gastrointestinal environment can affect the chemical stability of a drug. Drug instability in gastrointestinal fluid as a result of enzymatic hydrolysis or chemical degradation will result in low bioavailability. Enteric coating of tablets or pellets is used to protect sensitive drugs by preventing their release in the stomach that would lead to degradation when exposed. Polymers such as cellulose acetate phthalate and hydroxypropylmethylcellulose phthalate which are insoluble in gastric pH are used for the coatings. Once the dosage dorm reaches the higher pH in the intestine, the coating will dissolve and allows dissolution and absorption to occur, thus improving bioavailability (Porter and Ridgway, 1982). Omeprazole and erythromycin are among the drugs that have been delivered via enteric-coated dosage forms (Yakatan et al., 1985; Farinha et al., 2000).

In summary, there are many drug properties and physiological factors that affect absorption and bioavailability. It is clear that both the physiological and physicochemical factors are interrelated in influencing dissolution, absorption and bioavailability.

1.2.3 FORMULATION FACTORS: DOSAGE FORM AND EXCIPIENTS

The type of dosage form as well as excipients used in drug formulations can greatly influence the dissolution and absorption of a drug (Ashford, 2002a). As mentioned

previously, drugs are required to be dissolved in gastrointestinal fluids prior to absorption. Drugs in solutions and suspensions are in dispersed form when administered and are usually more readily absorbed compared to solid dosage forms. Solid dosage forms such as tablets and capsules which have to disintegrate prior to dissolution, may have a slower absorption process.

Excipients are usually added to the formulation to aid preparation and function of the dosage form, which include controlling the disintegration and dissolution of the contained drug, stabilising it from degradation, or as diluent to make up the volume of a feasible dosage form (Ashford, 2002a). Examples of excipients include diluents, disintegrating agents, lubricants, suspending and stabilising agents, flavouring and colouring agents. Excipients are generally considered inert and have no therapeutic and biological action; however, they may affect the rate and extent of absorption of a drug by affecting disintegration and dissolution of the dosage form (Panakanti and Narang, 2012).

Diluents are added to allow feasible formulation of practically size dosage forms, especially when the active ingredients have high potency (Jackson et al., 2000). In a case involving phenytoin, changing the diluent from calcium sulphate to lactose resulted in a significant rise in serum phenytoin concentration that led to toxicity. Calcium sulphate forms complexation with phenytoin that is poorly soluble and absorbable. Switching to lactose, which was freely water-soluble, resulted in a much higher phenytoin dissolution and bioavailability (Bochner et al., 1972).

Disintegrants are added to aid in the disintegration of the tablets and capsules into smaller fragments and particles upon ingestion, thus increasing the surface area of drug product to the gastrointestinal fluids to facilitate faster dissolution (Jackson et al., 2000). Dissolution rate of nifedipine tablet was faster with higher cumulative release when croscarmellose sodium was used as the disintegrant (Bolhuis et al., 1997). The type of disintegrant used can also affect dissolution and bioavailability. For example in one study, it was reported that using sodium starch gycolate as disintegrant increased the bioavailability of frusemide compared to when other disintegrants were used (Rubinstein, 1980).

Lubricants such as magnesium stearate are added to reduce adhesion between powder and metal surface during tablet manufacturing process. Only a small quantity is usually added as its hydrophobic characteristic can delay disintegration and dissolution, causing decrease in absorption and bioavailability (Jackson et al., 2000).

Surfactants are used in formulations as solubilising agents, emulsifiers, suspension stabilisers or wetting agents (Ashford, 2002a). Surfactants aid the dissolution of a poorly water-soluble drug by reducing the interfacial tension and improving the wetting of the solids, thus allowing more penetration of gastrointestinal fluids and subsequently more rapid disintegration. The increase in the total effective surface area of drugs in contact with the gastrointestinal fluids improves the dissolution and absorption rates (Jackson et al., 2000).

Surfactants have been shown to inhibit P-gp leading to increased substrates absorption. *In vitro* studies demonstrated that surfactants including D-α-tocopherol

polyethylene glycol 1000 succinate (TPGS), Cremophor EL and Tween 80 have inhibitory effect on cell lines overexpressed in P-gp (Hugger et al., 2002; Bogman et al., 2003). In a study by Tayrouz et al. (2003), administration of Cremophor RH40 prior to digoxin (P-gp substrate) dosing significantly enhanced the oral bioavailability of digoxin by 22% (Tayrouz et al., 2003).

Results from *in vitro* studies demonstrated that several surfactants (TPGS, polysorbate 20, polysorbate 80, Cremophor EL, Solutol HS 15) also have inhibitory effects on the metabolic CYP3A4 activities (Mountfield et al., 2000; Bravo Gonzalez et al., 2004; Christiansen et al., 2011). In an *in vivo* study by Ren et al. (2008), administration of Cremophor RH40 prior to midazolam (CYP3A4 substrate) dosing increased the serum midazolam concentration significantly, with levels similar to that obtained from dosing ketoconazole (CYP3A4 inhibitor) with midazolam (Ren et al., 2008).

It was proposed that the mechanism of CYP 3A4 and P-gp inhibition by surfactants was due to interactions between the surfactants with metabolic enzymes and transporters resulting in a decrease in the function of these proteins (Lo, 2003; Ren et al., 2008; Christiansen et al., 2011). Furthermore, a surfactant (Pluronic P85) was reported to cause inhibition of the enzyme ATPase, which led to a depletion of ATP that is needed in both P-gp and CYP functions (Batrakova et al., 2001; Christiansen et al., 2011). Recently, *in vitro* studies reported that the surfactants Labrasol, Solulan C24, and polysorbate 20 could increase drug transport via the paracellular pathway by disrupting the tight junctions (Dimitrijevic et al., 2000; Sha et al., 2005; Deli, 2009).

1.3 STRATEGIES TO IMPROVE ORAL BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUGS

It was estimated that 40 to 70 percent of newly discovered drugs suffer from poor aqueous solubility and consequently exhibit poor oral bioavailability (Gursoy and Benita, 2004; Hauss, 2007). Approximately 40% of the marketed oral drugs are categorised as practically insoluble (Kawabata et al., 2011). Poor water solubility is one of the critical limiting factor in affecting dissolution and consequently drug absorption and bioavailability (Kawabata et al., 2011). Various approaches have been used to improve the aqueous solubility of drug compounds.

1.3.1 CHEMICAL MODIFICATION

Modification of chemical structure with consequent alteration of physicochemical properties can help to enhance the aqueous solubility of drug molecules. Methods include adding polar or ionisable groups to the molecules. However, after chemical modification, a compound may be considered as a new chemical entity and thus may face more regulatory hurdles in the approval process (Vasconcelos et al., 2007).

1.3.2 FORMULATION APPROACHES

Chemical modification is not always feasible in improving the physicochemical properties of a drug. Formulation approaches can also be used to improve the drug properties for achieving better bioavailability (Gursoy and Benita, 2004).

Inclusion complexation of cyclodextrins with poorly water-soluble drugs has been shown to increase the solubility of some drugs (Brewster and Loftsson, 2007). The solubility and oral bioavailability of artemisinin and miconazole were greatly

enhanced after inclusion complexation with cyclodextrin (Tenjarla et al., 1998; Wong and Yuen, 2001). Solid dispersion is another method used to improve aqueous solubility where the poorly water-soluble drugs are distributed in inert hydrophilic carriers (Vasconcelos et al., 2007). Felodipine, albendazole and ritonavir formulated using such solid dispersion methods showed increased aqueous solubility and oral bioavailability (Kohri et al., 1999; Won et al., 2005; Sinha et al., 2010).

1.4 LIPID-BASED FORMULATIONS

Lipid-based formulation is another method to increase oral bioavailability of poorly water-soluble drugs by increasing their apparent gastrointestinal solubility (Feeney et al., 2016). Lipid-based formulations consist of the drug dissolved in lipid, surfactant and co-solvent mixtures (Feeney et al., 2016). Lipid suspensions or solutions are simple formulations, consisting of drug solubilised in triglycerides or mixed glycerides (Hauss, 2007). These formulations rely on the gastrointestinal lipid digestion mechanism to facilitate absorption of the drug (Hauss, 2007). Dutasteride (Avodart®), calcitriol (Rocaltrol®), valproic acid (Depakene®) are among the many commercially available lipid solution formulations available in soft gelatin capsules (Strickley, 2007).

More complex lipid-based formulations consist of solubilising the drug in triglycerides or mixed-triglycerides, surfactants and co-solvents. These formulations have been shown to enhance the bioavailability of poorly water-soluble drugs. One such formulation is the self-emulsifying drug delivery system, which will be discussed in section 1.4.2.

1.4.1 LIPIDS AND DRUG ABSORPTION

A general understanding of lipid digestion and absorption is essential to understand how the lipid-based formulations affect drug absorption (Porter and Charman, 2001a; Rezhdo et al., 2016). Digestion of lipid begins with dispersion of fat globules into coarse emulsion with increased surface area by shear generated from gastric emptying. Hydrolysis of fatty acid glyceryl esters by enzyme lipase takes place at the oil/water interface. The post-digestion products (monoglycerides and fatty acids) are solubilised and dispersed into a crude emulsion and ready for absorption (Humberstone and Charman, 1997; Porter and Charman, 2001b). Presence of lipid stimulates bile salts, biliary lipids and pancreatic juice secretion in the duodenum, which further reduce the emulsion droplet size. Biliary lipids (cholesterol and phospholipids) and bile salts help in stabilising the smaller particle-size emulsion formed by adsorbing at the surface of the crude emulsion, thus increasing the surface area for the action of pancreatic lipase/co-lipase complex (Humberstone and Charman, 1997; Porter and Charman, 2001b).

There are several possible mechanisms which lipids enhance the bioavailability of poorly water-soluble drugs (Porter and Charman, 2001a). Lipids increase the drug solubility in the gastrointestinal lumen by stimulating the secretion of bile salts and biliary lipids, which form intestinal mixed micelles and increase solubilising capacity. Subsequently, intercalation of administered lipids into the mixed micelles causes swelling of the micellar structures and further increase the solubilising capacity. Lipids reduce the gastric transit rate and thus increase the time available for dissolution. Various combinations of lipids and surfactants may have permeability enhancing properties that result in increase in absorption across gastrointestinal

membrane. Certain lipids and surfactants have been shown to reduce the activity of P-gp transporter and CYP3A4 metabolism, further contributing to the enhanced drug bioavailability. Lipids can also stimulate the intestinal lymphatic transport of drug, which minimise the effect of first pass metabolism and increasing the bioavailability of the drug.

Effect of bile and lymphatic transport on drug absorption

Bile consists of bile salts, lecithin and cholesterol which are secreted from the gall bladder (Pouton, 2006). The concentration of bile salts ranges from 3 to 5 mM when fasted and rise to about 15 mM post-prandial (Pouton, 2006). The presence of bile increases the solubilisation and oral bioavailability of lipophilic drugs (Welling, 1977). The increased oral bioavailability of cyclosporine and albendazole has been attributed to the solubilisation activity of bile salts (Lange et al., 1988; Lindholm et al., 1990). The effect of bile on the absorption of cyclosporine was also evaluated in liver transplant patients, where a T-tube was inserted in their common bile duct for bile diversion (Mehta et al., 1988). The bioavailability of cyclosporine was shown to be 3 to 4 times higher in patients after clamping of T-tube (i.e. increase bile flow into the gut) compared to before clamping. Bile salts contributed to the increased absorption of lipophilic drugs by improving their dissolution and membrane permeability; or by increasing the residence time of the drug at absorption site (Mehta et al., 1988).

Orally administered drugs usually enter the systemic circulation through the portal vein. For very lipophilic drugs, absorption via the intestinal lymphatic pathway can occur as the drug enters the systemic circulation before reaching the liver

(Humberstone and Charman, 1997; Porter and Charman, 2001b). One advantage of lymphatic transport of drugs is the reduction of first pass metabolism (Porter and Charman, 2001b). This is beneficial especially if the drug is very poorly water-soluble and is a substrate to hepatic metabolism. The effect of testosterone is limited after oral administration due to extensive hepatic first-pass metabolism (Porter et al., 2007). Shackleford et al. (2003) showed that the systemic level of testosterone was enhanced after oral administration of lipophilic long-chain ester testosterone undecanoate, which was transported lymphatically and underwent hydrolysis to form testosterone (Shackleford et al., 2003).

1.4.2 SELF-EMULSIFYING DRUG DELIVERY SYSTEM

Emulsions are able to improve absorption of poorly water-soluble drugs by increasing the surface area for release of drugs from the vehicle (Humberstone and Charman, 1997). Griseofulvin administered in emulsion achieved 1.6 and 2.5 times higher oral bioavailability compared to oil suspension and aqueous suspension, respectively (Carrigan and Bates, 1973). However, conventional emulsions are thermodynamically unstable and tend to separate in order to reduce the interfacial area between the oil and water phases (Craig et al., 1995; Pouton, 1997). Due to their poor physical stability and bulkiness, routine oral use of emulsions is not so favourable (Humberstone and Charman, 1997; Gershanik and Benita, 2000).

A self-emulsifying system is an isotropic mixture of oil and surfactant(s), which emulsifies in aqueous medium under gentle agitation (Pouton, 1985). In contrast to conventional emulsions where the emulsification process requires input of energy, self-emulsifying systems are thermodynamically stable as the formation of emulsion

is spontaneous and requires low or negative free energy input (Craig et al., 1995). Self-emulsifying systems produce fine dispersion upon aqueous dilution in the gastrointestinal tract, aided by the agitation provided by the gut motility (Gershanik and Benita, 2000). Self-emulsifying systems have been applied in the herbicide and pesticide industries in the form of self-emulsifying concentrates (poorly water-soluble herbicides or pesticides dissolved in organic solvents with surfactants) that can be dispersed easily right before crop spraying (Pouton, 1985).

The emulsification mechanism involved was suggested to be related to the ease of water penetration into the various liquid crystal structures or gel phases formed on the surface of the droplets (Gershanik and Benita, 2000; Gursoy and Benita, 2004). Upon addition of a binary mixture of oil and surfactant, an interface between the oil and aqueous phase is formed. Aqueous penetration through the interface leads to solubilisation of water within the oil phase. Depending on the surfactant concentration added, further water solubilisation continues to form the dispersed liquid crystalline phase. Rapid penetration of water coupled with gentle agitation cause interface disruption and droplet formation, resulting in self-emulsified systems that have high stability (Wakerly et al., 1986; Gursoy and Benita, 2004).

Self-emulsifying systems provide the advantage of emulsions in oral bioavailability enhancement, with improved physical stability of the formulation as aqueous phase is only introduced in the gastrointestinal tract upon ingestion (Gershanik and Benita, 2000). One classic example is the commercial cyclosporine product, Neoral®, which consisted of the drug, surfactant, co-solvent and lipid. After oral administration, Neoral® formed finely dispersed microemulsion upon aqueous dilution by the

gastrointestinal fluid. This formulation was shown to enhance the bioavailability of cyclosporine by approximately 2-fold, with decreased inter- and intra-subject variability compared to the older cyclosporine (Sandimmune®) preparation (Kovarik et al., 1994; Mueller et al., 1994).

Emulsions with finer droplet size are absorbed more rapidly compared to a crude emulsion (Tarr and Yalkowsky, 1989; Pouton, 2000). Key example can be seen with Neoral® which self-emulsifies to form very small (approximately 100 nm) droplets upon ingestion whereas the Sandimmune® formulation relied on the digestion process to further disperse the crude lipid emulsion formed (Humberstone and Charman, 1997; Feeney et al., 2016). Another commercial self-emulsifying product, Agenerase® (Amprenavir, HIV protease inhibitor) contains d-alpha-tocopherol polyethylene glycol 1000 succinate (TPGS), polyethylene glycol 400 (PEG 400) and propylene glycol in soft gelatin capsules. The addition of TPGS as surfactant improved the aqueous solubility of amprenavir and enhanced the bioavailability by up to 80% (Yu et al., 1999; Strickley, 2007).

Self-emulsifying systems present and maintain the poorly water-soluble drugs in solubilised form throughout its transit along the gastrointestinal tract for absorption (Pouton, 2000). Optimum self-emulsifying systems will emulsify efficiently upon aqueous dilution and produce fine emulsion droplets, thus improving the dissolution and absorption rate of the poorly water-soluble compound (Pouton, 1985). The optimum formulation for a self-emulsifying system has been demonstrated to be dependent on the nature of the compound of interest and a proper selection of lipid and/or surfactant combination (Pouton, 1985; Craig et al., 1995). The following