DEVELOPMENT OF FORMULATED EURYCOMA LONGIFOLIA AND PHYLLANTHUS NIRURI EXTRACTS FOR PHARMACOKINETIC AND BIOAVAILABILITY STUDIES

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2014

DEVELOPMENT OF FORMULATED *EURYCOMA LONGIFOLIA* AND *PHYLLANTHUS NIRURI* EXTRACTS FOR PHARMACOKINETIC AND BIOAVAILABILITY STUDIES

by

MA HAI QIU

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

February 2014

I dedicate this thesis to my parents Mr. Ma Xin Min and Mrs. Song Han Ying for their constant love, unconditional support and encouragement.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to Prof. Chan Kit Lam my supervisor, for his valuable supervision, guidance and support which enabled me to be grounded in my field of research.

I feel deeply grateful to my Co-Supervisor Dr. Nurzalina Abdul Karim Khan, the Deputy Dean of School of Pharmaceutical Sciences, Universiti Sains Malaysia, for her valuable advices and contribution to the satisfying working condition.

Many thanks are also extended to the technical officers in School of Pharmaceutical Sciences, Universiti Sains Malaysia, for their extended assistance in the laboratories.

Lastly, I must remember to thank my fellow colleagues and friends, Mr. Low Bin Seng, Dr. Teh Chin Hoe, Dr. Vikneswaran Murugaiyah, Dr. Kwok-Wen Ng, Miss. Liew Wai Lam, Miss. Forough Ebrahimi, Mr. Saifullahi Abubakar, Miss Cheng Lee Chuen, Mr. Liew Kok Fui, Miss. Lee Yuan-E, and Mr. Naveen Kumar H.S for their invaluable friendship and support that made the completion of this thesis possible.

Thank you all.

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LIST OF ABBREVIATIONS

A and B	Zero time drug concentration intercepts of biphasic disposition curve;
$AUC_{0–24}$	Area under the concentration–time curve from zero to 24 hours;
ADME	Absorbed, distributed, metabolized, and eliminated
α and β	Rate constants for distribution and elimination phases;
ANOVA	Analysis of variance
BCS	Biopharmaceutical Classification System
Caco-2	Human colon adenocarcinoma
CD	Cyclodextrin
C_{max}	Calculated maximum concentration
^{o}C	Degree of Celsius
CYP3A4	Cytochrome P450 3A4
Cl	Total body clearance.
DSC	Differential scanning calorimetry
EN	Eurycomanone
EP	13α(21)-epoxyeurycomanone
f1	Difference factor
f2	Similarity factor
F_{abs}	Absolute bioavailability
F_{rel}	Relative bioavailability
G44/14	Gelucire®44/14
GRAS	Generally Recognised as Safe
GIT	Gastrointestinal tract
HLB	Hydrophile-lipophile-balance
HPLC	High-performance liquid chromatography
IC_{50}	Median inhibition concentration
IV	Intravenous
IVIVC	*In vitro-in vivo* correction

k_a	Absorption rate constant;
kg	kilogram
K_m	Michaelis constant value
k_{10}	Elimination rate constant from central compartment;
k_{12}	Rate constants for the movement of drug from central compartment to peripheral compartment;
k_{21}	Rate constants for the movement of drug from peripheral compartment to central compartment;
LOD	Limit of detection
LOQ	Limit of quantification
Log P	*n*-octanol-water partition coefficients
mg	Milligram
mL	Milliliter
n	Sample size
NADPH	Nicotinamide adenine dinucleotide phosphate
OA	Orthogonal array
O/W	Oil in water
PEG	Polyethylene glycol
PAMPA	Parallel Artificial Membrane Permeability Assay
PT	Phyltetralin
PL	Phyllanthin
PDA	Photodiode array detector
P-gp	P-gp efflux and P450 metabolism
P450	Cytochrome P450
PNF5	Lignan-rich *P. niruri* extract
PNF5-PFC	PNF5 conventional Powder-filled Capsules
PNF5-SDC	PNF5 Gelucire® Solid Dispersion Capsules
P-gp	P-glycoprotein
QSAR	Quantitative structure activity relationship

RH	Relative humidity
RSD	Relative standard deviation
S.D	Standard deviation
T_{max}	Time corresponding to C_{max} calc.
$t_{1/2\_Ka}$	Half-life of the absorption phase.
$t_{1/2\ (\alpha)}$	Half-life of distribution
$t_{1/2\ (\beta)}$	Half-life of elimination
$t_{1/2\_K10}$	Half-life of K_{10}
TAF2	Quassinoid-rich *E. longifolia* extract
TAF2-PFC	TAF2 conventional Powder-Filled Capsules
TAF2-SDC	TAF2 Gelucire® Solid Dispersion Capsules
UV	Ultraviolet–visible spectroscopy
USM	University Sains Malaysia
µg	Micro gram
µL	Micro liter
µM	Micro molar
V_c	Volume of central compartment
WHO	World Health Organization
W/O	Water in oil
w/w	Weight over weight

PEMBANGUNAN FORMULASI BAGI EKSTRAK *EURYCOMA LONGIFOLIA* DAN *PHYLLANTHUS NIRURI* UNTUK KAJIAN FARMAKOKINETIK DAN BIOKEPEROLEHAN

ABSTRAK

Ekstrak *Phyllanthus niruri* yang kaya dengan lignan (PNF5) menormalkan asid urik plasma tikus hiperurisemik, dan kesannya adalah setanding dengan allopurinol yang digunakan secara klinikal. Ekstrak *Eurycoma longifolia* (TAF2) yang kaya dengan kuassinoid dapat meningkatkan jumlah sperma tikus jantan secara signifikan, dan mempunyai potensi yang tinggi untuk merawati kemandulan lelaki. Namun demikian, biokeperolehan oral bagi lignan, phyltetralin (PT) dan phyllanthin (PL), dalam PNF5 dan kuassinoid, 13α(21)-epoxyeurycomanon (EP) dan eurycomanon (EN) adalah sangat rendah kerana keterlarutan lignan yang rendah dalam air dan kebolehtelapan membran yang rendah bagi kuassinoid. Kajian ini telah meningkatkan biokeperolehan oral PT dan PL dalam PNF5, dan EP dan EN dalam TAF2 yang dimasukkan ke dalam sebaran pepejal berdasar Gelucire 44/14 (SDC). Kajian *in vitro*, iaitu ujian pelarutan dan kaedah kantung usus tikus dalam ke luar, telah menunjukkan bahawa pelarutan dan kebolehtelapan PT dan PL dalam PNF5-SDC dan kebolehtelapan membran EP dan EN dalam TAF2-SDC dapat ditingkatkan berbanding dengan formulasi konvensional kapsul berisi serbuk (PFC). Parameter-parameter farmakokinetik bagi PT, PL, EP dan EN menunjukkan bahawa kadar pergerakan sebatian-sebatian ini dari bahagian pusat ke periferi adalah perlahan berbanding dengan kadar pergerakannya dari bahagian periferi ke pusat (P <

0.001). Kadar pengagihan sebatian-sebatian ini adalah lebih cepat ($P < 0.001$) berbanding dengan kadar penyingkiran. Justeru itu, farmakokinetik *in vivo* bagi PT, PL, EP dan EN itu digambarkan sebagai proses dwi-kompartmen. Kajian *in vivo* dalam tikus menunjukkan bahawa, biokeperolehan oral relatif PT dan PL dalam PNF5-SDC telah meningkat sebanyak 3 kali ganda, berbanding dengan PFC, manakala EP dan EN dalam TAF2-SDC telah meningkat sebanyak 1.7 kali dan 2.8 kali ganda masing-masing. Namun begitu, biokeperolehan mutlak bagi sebatian-sebatian yang dikaji dalam PNF5 dan TAF2 masih lagi rendah. Hasil daripada kajian *in vitro* menggunakan kantung usus tikus dalam ke luar dan kaedah pengeraman homogenate hati dan pemberian ketokonazol secara *in vivo* kepada tikus Sprague Dawley (SD), telah menunjukkan bahawa biokeperolehan mutlak yang rendah bagi PT dan PL dalam PNF5 berkemungkinan besar disebabkan oleh PT dan PL yang bertindak sebagai substrat dalam efluks P-gp. Penyerapan oral bagi EP dan EN dalam TAF2 pula didapati tidak dipengaruhi oleh efluks P-gp dan metabolisme CYP3A4. Disamping itu, fenomena puncak berganda juga dikesan dalam profil farmakokinetik bagi EP dan EN dalam sebaran pepejal TAF2. Kajian *in vivo* yang menggunakan tikus SD telah menunjukkan bahawa kelewatan dalam masa pengosongan gastrik dan penyerapan kolon telah mengakibatkan penghasilan fenomena puncak berganda pada farmakokinetik kuassinoid dalam sebaran pepejal TAF2.

DEVELOPMENT OF FORMULATED *EURYCOMA LONGIFOLIA* AND *PHYLLANTHUS NIRURI* EXTRACTS FOR PHARMACOKINETIC AND BIOAVAILABILITY STUDIES

ABSTRACT

The lignan-rich extract of *Phyllanthus niruri* (PNF5) normalises the plasma uric acid of hyperuricemic rats, and its effects are comparable to those of clinically used allopurinol. The quassinoid-rich extract of *Eurycoma longifolia* (TAF2) significantly improves the sperm count of male rats, and may be potentially suitable for managing male infertility. However, the oral bioavailability of the bioactive lignans, phyltetralin (PT) and phyllanthin (PL), in PNF5 and the quassinoids, 13α(21)-epoxyeurycomanone (EP) and eurycomanone (EN), in TAF2 were low due to the low aqueous solubility of the lignans and poor membrane permeability of the quassinoids. The present study led to the improvement of the oral bioavailabilities of PT and PL in PNF5, and EP and EN in TAF2 through incorporation in a Gelucire®44/14-based solid dispersion (SDC). *In vitro* studies, which were dissolution test and everted rat gut sac method indicated that PNF5-SDC formulation resulted in higher release of PT and PL, and TAF2-SDC resulted in higher permeation of EP and EN compared to the conventional powder-filled capsules (PFC) formulations. The pharmacokinetic parameters of PT, PL, EP and EN indicated that the rate of these compound movements from the central compartment to peripheral compartment were slow compared to the rate movement from the peripheral compartment to the central compartment ($P < 0.001$). The distribution rates of these

compounds were much faster ($P < 0.001$) than the elimination rates. The *in vivo* pharmacokinetics of PT, PL, EP and EN were thus being described as two-compartment process. The *in vivo* study in rats showed that the relative oral bioavailabilities of PT and PL in the PNF5-SDC increased by 3-fold compared to those in PFC, while the EP and EN in TAF2-SDC improved by 1.7-fold and 2.8-fold, respectively. However, the absolute bioavailabilities of the studied compounds in PNF5 and TAF2 were still low. The results from studies using the *in vitro* everted gut sac method and liver homogenate incubation method, and *in vivo* administration of ketoconazole to Sprague Dawley (SD) rats, revealed that the low oral absolute bioavailabilities of PT and PL in PNF5 were most likely due to PT and PL acting as substrates for P-gp efflux. On the other hand, the oral absorptions of EP and EN in TAF2 were not affected by P-gp efflux and CYP3A4 metabolism. In addition, a double peak phenomenon was also identified in the pharmacokinetic profiles of EP and EN in TAF2 solid dispersion. The result of an *in vivo* study using SD rats indicated that delayed gastric emptying time and colonic absorption accounted for the establishment of the double peak phenomenon observed for the pharmacokinetics of the quassinoids in the TAF2 solid dispersion.

CHAPTER ONE: General introduction

1.1 Hyperuricemia and *Phyllanthus niruri*

1.1.1 Hyperuricemia and medicinal approaches.

Purines are heterocyclic aromatic organic compounds consisting of a pyrimidine ring fused to an imidazole ring. Catabolism of purine nucleotides ultimately leads to the production of uric acid, which is sparingly soluble in extracellular body fluids. The concentration of uric acid generally almost reaches the saturation level in the extracellular body fluids. Therefore, a slight change in this saturation level by purine metabolism may cause uric acid precipitation (Pennes and Martel, 1986). It was reported that the imbalance in the excretion and production rate of uric acid causes hyperuricemia which in turn results in gout (Sherman *et al*., 2008).

Currently, the clinical approaches for drug treatment of hyperuricemia are very limited (Murugaiyah and Chan, 2006). Allopurinol, benzbromarone, and probenecid are generally used in clinical treatments. Allopurinol is mostly used, but is associated with adverse drug reactions (Khoo and Leow, 2000). Some natural plants, such as *Larix laricina* (Owen and Johns, 1999), *Cinnamomum cassia*, *Polygonum cuspidatum* (Kong *et al*., 2000) and *Chrysanthemum sinense* (Nguyen *et al*., 2004) were reported to exhibit anti-hyperuricemic activity. Recently, a Malaysian plant, *Phyllanthus niruri*, was found to exhibit anti-hyperuricemic activity (Murugaiyah

and Chan, 2006).

1.1.2 *Phyllanthus niruri*

1.1.2 (A) Botanical aspects and traditional usage

Phyllanthus niruri L. (Euphorbiaceae) is known locally as "dukong anak." *Phyllanthus* has about 750 species (Hnatyszyn *et al*., 1999). *Phyllanthus niruri* is the most widely used plants of the *Phyllanthus* genus because of its worldwide distribution (Calixto *et al*., 1998). *P. niruri* is an annual glabrous herb that grows from 30 to 60 cm (Wright *et al*., 2007), and has small leaves and small off-white-greenish flowers (Bagalkotkar *et al*., 2006). *P. niruri* is very similar to *P. amarus* in appearance and chemical content, but recent studies have indicated that they are different but are very close species (Lee *et al*., 2006). The plant has been traditionally used as expectorant, anti-febrile, anti-diarrhoea, diuretic, and a remedy for colic and kidney problems (Murugaiyah and Chan, 2007, Wright *et al*., 2007).

1.1.2 (B) Chemical constituents and activities.

Several chemical constituents isolated from *P. niruri*, include alkaloids (Mulchandani and Hassarajani, 1984, Joshi *et al*., 1986, Petchnaree *et al*., 1986), benzenoids, coumarins (Ueno *et al*., 1988, Shimizu *et al*., 1989), flavonoids (Chauhan *et al*., 1977, Nara *et al*., 1977, Gupta and Ahmed, 1984b, a), lipids (Ahmad *et al*., 1981), phytallates (Singh *et al*., 1986), sterols (Mannan and Ahmad, 1976),

tannins (Ueno *et al*., 1988), triterpenes (Chauhan *et al.*, 1979, Singh *et al.*, 1989a, b), and lignans (Row *et al.*, 1966, Anjaneyulu *et al.*, 1973, Ganeshpure *et al.*, 1981, Huang *et al.*, 1992). Some of these compounds were found to possess pharmacological activities. Rutin has anti-inflammatory and analgesic functions (Santos *et al.*, 1995, Santos *et al.*, 1996). Ellagic acid was found to be an aldose reductase inhibitor (Shimizu *et al*., 1989). Geraniin is an ACE inhibitor, anti-allergy, and analgesic (Ueno *et al*., 1988). Quercetin has properties of an inhibitor of mitochondrial ATPase, phosphodiesterase, phosphorilase and tirosine kinase, and phopholipase A2. Niruside was found to be anti-HIV (Hussain *et al*., 1995). Repandusinic acid A, phillanthin, hypophillanthin, and hirtetralin were found to be anti-HIV, anti-hepatoxic, and are endothelin antagonists (Syamasundar *et al*., 1985). Phyllanthin and hypophyllanthin protected carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes. Triacontanal was protective against galactosamine-induced toxicity (Syamasundar *et al*., 1985). As a hepatoprotective herbal plant, the potential of the protein isolates from *P. niruri* was investigated against carbon tetrachloride (CCl_4)-induced hepatoxicity (Bhattacharjee and Sil, 2007).

1.1.2 (C) Toxicity and safety

Some toxicity studies of *P. niruri* and its compounds have been studied, but the lignans in *P. niruri* demonstrated no significant cytotoxic activity when evaluated against several cultured mammalian cells (Somanabandhu *et al*., 1993). Recently, *P.*

niruri MeOH extract was evaluated for cytotoxicity using human cancer cell lines following the MTT assay, but no evidences of acute cytotoxicity were found (Ng *et al*., 2010). Similar finding was also observed in another study (Srinivasulu, 1992), where no acute toxicity was observed at the pharmacological doses administered. The LD_{50} of the aqueous extract was > 5000 mg / kg body weight (Asare *et al*., 2011). In a later study, the ethanol extract of *P. niruri* was not cytotoxic or genotoxic and generally non-toxic on subchronic administration (Asare *et al*., 2012).

1.1.3 Lignans in *Phyllanthus niruri*

Modern scientific research indicated that the lignans are phenolic compounds existing in many plants (Willfor *et al*., 2006). Studies on dietary phytoestrogens also revealed that the lignans also existed in food (Kuhnle *et al*., 2008).

1.1.4 Pharmacological properties of lignans

Attention was focused on the lignans because of the vast potential biological activities (Willfor *et al*., 2006). The lignans in *P. niruri* have anti-hepatitis B virus activities (Wei *et al*., 2012), anti-angiogenic activity (Ng *et al*., 2010), anti-tumour effects (Islam *et al*., 2008), anti-inflammatory and anti-allodynic actions (Kassuya *et al*., 2006), hepatoprotective effect (Nguyen and Tran, 2004), and anti-oedematogenic properties (Kassuya *et al*., 2003), etc. A recent research indicated that a lignan-rich fraction of *P. niruri* MeOH extract was able to reduce the plasma uric acid level of hyperuricemic rats comparable to that of clinically-used drugs. Phyllanthin,

hypophyllanthin, and phyltetranlin, were involved in the anti-hyperuricemia activity (Murugaiyah and Chan, 2006) due mainly to their uricosuric action and partly through the xanthine oxidase inhibition (Murugaiyah and Chan, 2006, 2009).

1.2 Male infertility and *Eurycoma longifolia*

1.2.1 Male infertility and medicinal approaches.

According to the World Health Organization (WHO), infertility is the "inability to conceive a child." A couple may be considered infertile if after two years of regular sexual intercourse without contraception, the woman has no development of pregnancy. Male infertility is the inability to achieve a pregnancy in a fertile female. In humans, male infertility accounts for 40 % to 50 % of infertility (Brugh and Lipshultz, 2004). More than 90 % of male infertility are due to low sperm count and quality (Bhasin *et al*., 1994). Social problems as well as psychological distress, according to Greil, may be caused by infertility (Greil, 1997, Schmidt *et al.*, 2005).

The combination of clomiphene citrate and vitamin E was used for the treatment of idiopathic oligoasthenozoospermia by improving sperm count and sperm motility (Ghanem *et al*., 2009). The semen count could be ameliorated by treatment with low doses of testosterone and estrogen (Sah, 1998). While assisted reproductive technologies, such as IVF (*in vitro* fertilization) are available, there are several disadvantages that are associated with these approaches, such as multiple birth, side

effects from fertility drugs, ectopic pregnancy, etc. Barriers also exist in terms of medical affordability.

1.2.2 *Eurycoma longifolia*

1.2.2 (A) Botanical aspects and traditional usage

E. longifolia Jack, from the Simaroubaceae family, is known locally as "Tongkat Ali". *E. longifolia* is a tropical herbal plant found in Indonesia and Vietnam and widely grows in Malaysia (Perry and Metzger, 1980). The four species of Tongkat Ali are *Eurycoma longifolia*, *Entomophthora apiculata*, *Polyathia bullata*, and *Goniothalamus* sp (Bhat and Karim, 2010). Amongst them, *Eurycoma longifolia* is the most commonly used species for traditional medicine (Athimulam *et al*., 2006). Traditionally, the plant was used in Malaysia to increase male virility and sexual prowess during sexual activities (Ang and Cheang, 2001). Scientific research has confirmed this curative effect (Ang *et al.*, 2000, Ang and Lee, 2002, Bhat and Karim, 2010).

1.2.2 (B) Chemical constituents and activities

Several constituents have been identified from the plant. These constituents are quassinoids (Ang *et al.*, 2002, Miyake *et al*., 2009, Teh *et al.*, 2009), canthin-6-one alkaloids (Mitsunaga *et al.*, 1994, Miyake *et al.*, 2010), β-carbolines alkaloids (Kuo *et al.*, 2003, Miyake *et al.*, 2010), tirucallane-type triterpenes (Itokawa *et al*., 1992),

squalene derivatives (Morita *et al*., 1993), biphenylneolignans (Morita *et al*., 1992) anthraquinones, anthraquinone glucosides and oxasqualenoid (Miyake *et al*., 2010). The alkaloids and quassinoids comprise the major constituents of this plant (Hou *et al*., 2011).

1.2.2 (C) Toxicology and safety

Although *E. longifolia* is widely used, published studies regarding its toxicity are scarce. The methanol, chloroform, n-butanol, and aqueous extracts of *E. longifolia* were studied for cytotoxicities (Chan *et al*., 1986). In another study, tests on normal cells indicated that these root extracts were not toxic or harmful (Nurhanan *et al*., 2005). A recent unpublished safety assessment was performed (Low, 2005) that included the acute, sub-chronic, chronic, reproductive toxicology, and teratological studies of the standardized quassinoid-rich *E. longifolia* extract in rats. The doses when extrapolated to humans were safe for use. In a most recent research, the *in vitro* evaluation of human spermatozoa samples for the toxicity study found that the therapeutic concentration of *E. longifolia* extract have no harmful effects (Erasmus *et al*., 2012).

1.2.3 Quassinoids in *Eurycoma longifolia*

Quassinoids are bitter constituents of Simaroubaceae, first structurally identified and isolated from *Quassia amar* (Curcino Vieira *et al*., 2006). These compounds are also isolated from *Ailanthus vilmoriniana* (Takeya *et al*., 1997), *Quassia indica* (Koike

and Ohmoto, 1994), and *Eurycoma longifolia* (Chan *et al.*, 1989, Jiwajinda *et al.*, 2001, Ang *et al.*, 2002, Miyake *et al.*, 2009).

1.2.4 Pharmacological properties of quassinoids

Quassinoids have anti-proliferative effects on cancer cell lines (Wong *et al.*, 2012). Quassinoids also possess activities that are anti-estrogenic (Teh *et al.*, 2011), anti-malarial (Kardono *et al.*, 1991, Chan *et al.*, 2004, Chan *et al.*, 2005), anti-ulcer (Tada *et al.*, 1991), anti-tumour promoting, anti-parasitic (Jiwajinda *et al.*, 2002), and anti-leukemic (Itokawa *et al.*, 1992).

A recent research found that a quassinoid-rich *E. longifolia* MeOH extract significantly improved the sperm count of male rats (Chan *et al.*, 2008a, 2009). This plant may potentially be suitable for the management of male infertility (Chan *et al.*, 2009). Another research group confirmed this finding. The study indicated that *E. longifolia* could be a potential agent for reversing the effects of estrogen by increasing spermatogenesis and sperm count in rats (Wahab *et al.*, 2010).

1.3 Biopharmaceutical Classification System (BCS)

Previous studies on the bioactive lignans in *P. niruri* and quassinoids in *E. longifolia* extracts found poor oral bioavailabilities (Low *et al.*, 2005, Murugaiyah and Chan, 2007). In view of future formulation development, the bioactive compound-rich extracts could be first classified by using the biopharmaceutical classification system

(BSC) (Table 1-1). This system classified the drug substances in four classes according to membrane permeability and water solubility (Lobenberg and Amidon, 2000). With references to previous studies, the lignan-rich *P. niruri* extract could be classified as a BSC class Ⅱ type, possessing high permeability and low solubility. The quassinoid-rich *E. longifolia* extract could be classified as a BSC class Ⅲ type that has low permeability and high solubility. These categories provide a significant indicator for the formulation strategies that should be adopted to improve the oral bioavailabilities.

Table 1-1 Biopharmaceutical classification system.

Class	Permeability	Solubility
Ⅰ	High	High
Ⅱ	High	Low
Ⅲ	Low	High
Ⅳ	Low	Low

1.4 Conventional solid oral dosage forms

Oral drug delivery is a simple and easy way to administer drugs (Sugawara *et al.*, 2005). Solid oral dosage forms (such as powders, granules, tablets, and capsules) have several advantages compared with other oral dosage forms (Vasconcelos *et al.*, 2007), in terms of chemical stability, accurate preparation procedure, low manufacturing cost, effectively mask unpleasant taste, and easily be commercially used for oral delivery, etc. Although powders and granules have relatively faster dissolution rates compared with tablets and capsules, some disadvantages limit their

use. For instance, they are inconvenient for patients to carry, and masking their unpleasant tastes may be a problem as well. In addition, some drugs are not suitable for oral administration as powder and granules may be damaged by the acidic stomach. The tablet, as one of the most popular oral dosage forms, could overcome these problems. Firstly, the preparation procedure for tablets leads to accurate dose amount of the drug in each piece. Secondly, tablets are convenient to handle, and are prepared according to their use and delivery. In addition, tablets can be produced and provided as preparations of very low price with consistent quality.

Another popular dosage form is capsules. Capsules are usually made of gelatin and are filled with medicinal substances. The gelatin capsule could be easily dissolved in the stomach and has been scientifically proven as safe raw material (Jones, 2002). Gelatin capsules could be categorized as hard and soft. Variety of solid and semisolid materials could be filled in the hard gelatin capsules, whereas liquid and semisolid materials could be filled in soft gelatin capsules. Thus, modified release can also be achieved using capsules, and the most commercially available lipid-based oral formulations are capsules (Strickley, 2007). The extended shape and smooth surface of the capsule shell are made for improving patient compliances. In addition, the capsules could effectively mask unpleasant odour and taste, therefore suitable for clinical studies (Podczeck, 2004). Moreover, hard capsules require shorter manufacturing process compared with tablets.

1.5 Alternative dosage forms

Conventional formulations, such as physical mixing of actives with excipients, may not effectively increase the bioavailability of many active compounds belonging to BSC II, III, and IV drugs. Thus, other safe and effective formulation methods need to be considered. Cyclodextrin complexations, liposomes, nanoparticles, and solid dispersions could be used as alternative options because they efficiently improve the bioavailability of entrapped compounds, have less or non-toxic character and possess wider application.

1.5.1 Cyclodextrin complexation

Cyclodextrins (CD) are cyclic oligosaccharides of D-glucose residues attached by (R-1,4)-linkages, which can be described as a cone shape molecule. They consist of relatively hydrophobic interior and hydrophilic exterior (Clarke *et al*., 1988) (Figure 1-1). Out of many CDs and derivatives available, the three widely used are α-, β-, and γ-cyclodextrin (α-CD, β-CD, and γ-CD), which consist of six, seven, and eight D-glucose units, respectively.

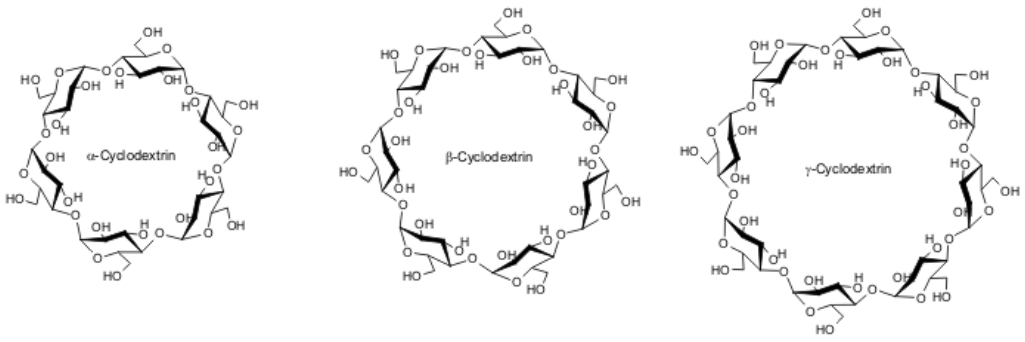

Figure 1-1. Cyclodextrins
(http://www.chemiedidaktik.uni-wuppertal.de/disido_cy/cyen/info/03_physical_cy.htm)

The relative nonpolar cavity is the result of the framework of carbons and ethereal oxygens, which lined the cyclodextrin molecule (Loftsson and Brewster, 1996). Furthermore, the cavity is also lined with secondary dihydroxyl groups that are less polar than the outer primary alcohols. Thus, the inner less polar cavity promotes hydrogen bonding with polar compounds. The outer primary alcohols provide the aqueous solubility. Therefore, the CDs could be utilized as carriers to encapsulate bioactive nonpolar compounds to improve their solubility and absorption. As a strategic method, numerous formulation studies focus on the CDs. Nevertheless, cyclodextrins have some limitations. Firstly, some CDs especially β-CD are potentially toxic and has low solubility (Dilova *et al*., 2004). Derivates of CDs are normally applied by synthesis from natural CDs. The application may increase the cost of the development process. Secondly, the formation of CD complexes depends on the compatibility of the compound size and dimensions of the cavity of the CDs as well as the geometrical shapes of guest compounds (Cohen and Lach, 1963). No correlations exist between the physical or chemical properties of guest compound and the complexation ability with CDs. Thirdly, the complexation efficiency of CDs are low; hence, a relatively large amount of CDs is normally required to achieve the desired entrapment efficiency when preparing the complex, before the desired solubility could be achieved (Loftsson *et al*., 1999). Fourthly, highly water-soluble compounds cannot be complexed because of the interior and exterior polar properties of CDs. Fifthly, the high melting point compounds are generally entrapped weakly in CDs. Sixthly, only a limited amount of compounds can be practically carried because

CDs have large molecular weights. Finally, when preparing the complexation, the complexing agent may alter the toxicity of the drug. Thus, additional toxicity study needs to be conducted. The cost of drug development and manufacturing should also be considered as well (Wei, 2008).

1.5.2 Liposomes

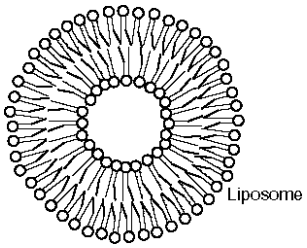

Figure 1-2. Liposome. (Liu, 2008)

As drug carrier, liposomes could improve the delivery of therapeutic compounds. They are micro-spherical vesicles that consist of phospholipid bilayers surrounding an aqueous space. They are biocompatible materials with varying diameter from 0.02 to 10 μm (Sharma and Sharma, 1997, Drulis-Kawa and Dorotkiewicz-Jach, 2009).

The liposomal drug could target the site of disease. This behavior can be attributed to the increased spaces of capillary endothelial cells of the disease states (Ostro and Cullis, 1989, Bangham, 1992). The drug compounds could efficiently target the disease tissue, and could increase drug potency and reduce toxicity (Liu, 2008).

The release of the entrapped drug compounds in liposomes is achieved by plasma proteins (example lipoprotein) that degrade the liposome. In this process, the lipid bilayer structure is destroyed by the removal of phospholipids molecules. The unstable lipid bilayer structure will then release the entrapped drug into the plasma circulation. Therefore, the liposome acts as a drug reservoir offering sustained release that is more suitable for parenteral dosage forms (Liu, 2008). The oral administrated liposomes are easily digested by phospholipids and bile salts in the GIT.

Although liposomes are mainly used for target release by parenteral administration, the oral administrations of liposomal formulation were also reported for both polar and nonpolar compounds because of the unique structure of the liposomes (Micklus, 1991). For the oral formulations, polar compounds can be entrapped in a central aqueous cave of liposomes, whereas nonpolar compounds are ensnared in the lipid bilayer (Figure 1-2). However, the mechanism of this oral administration of liposomes is similar to other lipid-based oral drug delivery system (Gregoriadis *et al.*, 1990, Liu, 2008). Thus, liposomes as oral delivery carrier have no particular superiority compared with other lipid-based oral drug delivery system.

Liposomes are excellent materials for drug formulation because they can encapsulate the wide range of active compounds regardless of size, shape, and polarity. However, liposomes normally possess comparatively lower entrapment capacity of water-

soluble compounds because of lower lipid/aqueous distribution ratio. In addition, the procedures to entrap the water soluble compounds in liposomes are more complicated than water insoluble compounds (Liu, 2008).

The stability of liposome formulation is another important issue that needs to be considered when designing the formulation because the phospholipids are the major materials in forming the liposome. Hydrolysis and oxidative degradation of phospholipids could significantly affect the liposome physical and chemical properties, and subsequently changes the bilayer fluidity, permeability, and encapsulation efficiency.

1.5.3 Nanoparticles

Nanoparticles can be achieved by using two methods, namely, mechanical and chemical processes. Mechanical processes produce the particles in a range of 100 to 1000 nm, whereas chemical processes can produce 10 to 100 nm particles (Acosta, 2009). Theoretically, the reduction of the particle size by mechanical processes results in an increase in the surface area, which subsequently improves the solubility of the drug and increase absorption. Nevertheless, for those insoluble and sparingly soluble compounds, the potency of this method is limited. In the case of chemical processes, chemical reactions or solubilizing agents are involved (Lee, 2008, Acosta, 2009). However, in some cases, the bioavailability may not be proportional to the particle size. Practical data reveal that the active ingredients in the range of 100 to

1000 nm could improve bioavailability (Acosta, 2009). In addition, materials with very small particle size (< 2 μm) are not easy to handle for manufacturing of dosage forms (Vasanthavada, 2008).

In the pharmaceutical field, nanoparticles are mainly applied as vehicles to improve the absorption of poor water soluble compounds and are also widely used as sustained, controlled, and target release formulations (Calvo *et al.*, 2001, Zhang *et al.*, 2006, Du *et al.*, 2010, Zhang *et al.*, 2011). Some researchers suggest in principle that nanoparticles could deliver polar nutraceutical compounds, such as isoflavones (Ratnam *et al.*, 2006, Acosta, 2009). Nevertheless, improvements of the absorption of these polar compounds may be due to the nanoparticles coat with lectins (Acosta, 2009), which are non catalytic sugar-binding proteins that act as bioadhesins in attaching to the epithelial lining at mucosal surfaces. Thus, this attachment could reduce the dilution, wash-off active compounds at the epithelial lining, and enhance the absorption (Clark *et al.*, 2000, Ohno *et al.*, 2005). Other researchers reported the preparation of water soluble drug nanoparticles by the precipitation method. However, the entrapment efficiency is poor (Cheow and Hadinoto, Barichello *et al.*, 1999, Govender *et al.*, 1999). Several applications of nanoparticles for the improvement of polar compounds are actually microemulsion-based, which could reduce the emulsified droplet size to less than 100 nm (Pouton, 2000). This procedure could also achieve high efficiency by designing a self-microemulsifying drug delivery system (SMEDDS) that uses lipids and/or surfactants.

1.5.4 Solid dispersions

Solid dispersions are robust methods to improve oral bioavailability of poorly soluble drugs (Vasconcelos *et al.*, 2007). Solid dispersion refers to the dispersion of active compounds in an inert matrix at solid state (Chiou and Riegelman, 1971). Recent studies show that the dispersion involves the forming of well-proportioned physical or molecular mixture of active drug compounds and water soluble carriers at eutectic temperature (Goldberg *et al.*, 1966, Vasanthavada, 2008). This method may also entrap the active compounds in carriers as amorphous materials. Often, it is a combination of amorphous, physical and molecular mixture (Mayersohn and Gibaldi, 1966, Vasanthavada, 2008, Newman *et al.*, 2012). Therefore, solid dispersions could form effective oral formulations without being fully solubilized in the excipient matrix. The theory of this system is to increase the dissolution rate. The increase may be due to reduction of drug particle size, absence of aggregation of drug crystallites, improvement of the wetting ability of the drug, and creation of the drug compound in an amorphous state, or the combination of these theories (Ford, 1986, Damian *et al.*, 2000).

For the poor water soluble drugs, the solubility and the permeability are the restraint steps for absorption. Although the nonpolar compounds in most cases possess high permeability, the small absorption window of the GIT in certain cases may restrict absorption in certain cases (Junghanns and Muller, 2008). Thus, using solid

dispersions in increasing solubility is one of the effective strategies in solving this problem. In the bench top scale, fusion and solvent evaporation are two commonly used methods.

The first generation of solid dispersions using crystalline carriers such as urea and sugars were thermodynamically stable, but did not release the drugs quickly (Kanig, 1964, Sekiguchi *et al.*, 1964). Instead, crystalline amorphous materials were used in the second generation, since these materials do not affect the dissolution process (Taylor and Zografi, 1997, Pokharkar *et al.*, 2006). In recent years, the third generation solid dispersion system was broadly applied in research and manufacturing. This generation contained surfactants or a mixture of amorphous polymers and surfactants as carriers, which have surface activities and self-emulsifying properties. Gelucire® is one group of carriers belonging to the third generation of solid dispersions (Karatas *et al*., 2005). Solid dispersions are widely used in the pharmaceutical formulations because of several advantages such as patient compliance for administration, convenient storage, more stable compared with liquid dispersions, and application to a wide range of compounds. The reduced particle size may reach molecular level as well.

Although many privileges are associated with solid dispersions, some disadvantages exist for this formulation. The disadvantages include less ability to scale up the formulation from bench top to manufacturing level. However, some pharmaceutical

companies (such as Fuji Chemical Industry) and techniques (such as hot melt extrusion and encapsulation, spray drying, and supercritical fluid) have already successfully scaled up some solid dispersion methods to some extent. Among them, hot melt encapsulation is a suitable method for Gelucires® to fill the capsules. Thus, this technique results in a simplified manufacturing procedure. In addition, the equipment to scale up this method to commercial-grade is already available (Vasanthavada, 2008).

1.5.5 Other formulation approaches

Some other pharmaceutical formulation methods to improve the bioavailability of active drug ingredients are also available such as emulsions, salt formation, and mechanical methods to reduce particle size. However, these methods all have limitations and may not be practical for using and manufacturing (Serajuddin, 1999, Neslihan Gursoy and Benita, 2004). For example, emulsions are not in solid forms, and have stability problems. Salt formations are not applied for neutral compounds, and they may be converted into acidic or basic forms *in vivo*. A reduced particle size may cause poor flow ability and adhesion during manufacturing.

1.6 Gelucire® solid dispersions

1.6.1 Gelucire®

Gelucire® is a group of excipients that are widely used in solid dispersions. The

drugs incorporated in these solid dispersions can have their bioavailabilities increased (Svensson *et al.*, 2004). They are semi-solid, waxy material with amphiphilic properties (Yuksela *et al*., 2003). Gelucire® are a family of polyglycolised glyceride bases, which consist of polyethylene glycol (PEG) esters of various fatty acids, tri-, di-, and mono-glycerides of the fatty acids, with some of the corresponding free fatty acids and PEGs present in small quantities (Khan and Craig, 2003). It is inert, safe, non-toxic, and has been reported to be chemically stable in temperature and humidity of up to 50 °C and 80 % RH, respectively (Remuan *et al*., 1992).

Gelucire® is characterized by two values, which are hydrophile-lipophile-balance (HLB) and melting point. The numeral values reflect a specific physical character, when solid dispersions of Gelucires are placed in the gastrointestinal fluids, such as hydrodispersibility, melting, and floatability (Doelker *et al*., 1986). The nominal melting point, which ranges from 33 °C to 70 °C, can only be used as a reference. This value does not represent the accurate melting point of the base. Many compounds exist in Gelucire® products; thus, a single and sharp melting point could not be identified for Gelucire®. The HLB value of Gelucire® ranges from 1 to 18. The Gelucire® product with a higher HLB value showing hydrophilic property is appropriate for the formulation of fast-release dosage forms. It tends to produce oil-in-water (o/w) emulsions, when the Gelucire® based product contact the GIT fluids. In contrast, the Gelucire® base with lower HLB values having higher portions of

lipophilic fractions are suitable for formulating controlled release formulations, and tend to produce water-in-oil (w/o) emulsions (Baykara and Yuksel, 1991, San Vicente *et al.*, 2000, Karatas *et al.*, 2005). The amphiphilicity of the base conferred by the long hydrocarbon chain and the alcohol moieties means that both hydrophilic and hydrophobic drugs can potentially be incorporated into these carriers (Choy *et al.*, 2005). In addition, extremely insoluble drugs tend to re-crystallize upon exposure to the gastrointestinal tract during dissolution (Serajuddin, 1999, Leuner and Dressman, 2000). Semi-solid surfactants, such as Gelucire®, have the potential to facilitate dissolution, and to inhibit precipitation in the GI tract because of their solubilizing properties (Yuksel *et al.*, 2003, Soliman and Khan, 2005). Gelucire® 44/14 and Gelucire®50/13 are two most commercially used products that are approved as food additives in the USA (Raymond C. Rowe *et al.*, 2012).

The definition of Gelucire®44/14 and Gelucire®50/13 can be found in the Handbook of Pharmaceutical Excipients. The chemical name of Gelucire®44/14 is lauroyl macrogolglycerides (polyoxylglycerides). Lauroyl macrogolglycerides is a mixture of monoesters, diesters, and triesters of glycerol, and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 1500. They are obtained by the partial alcoholysis of saturated oils, which mainly contain triglycerides of lauric acid, by using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils (Rowe *et al.*, 2012).

The chemical name of Gelucire®50/13 is stearoyl macrogolglycerides (polyoxylglycerides). Stearoyl macrogolglycerides are mixtures of monoesters, diesters, and triesters of glycerol, and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 4000. They are obtained by partial alcoholysis of saturated oils, which contain mainly triglycerides of stearic acid, by using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by a mixture of glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils (Rowe *et al*., 2012). Although similar in composition, Gelucire® 44/14 forms micro emulsions on contact with the aqueous phase, whereas Gelucire® 50/13 swells with water and give gradual drug release (Khan and Craig, 2003). Thus, Gelucire® 44/14 could be used for the formulation of fast release, and Gelucire® 50/13 for controlled or sustained release. Gelucire® could also be filled into the capsules, which will simplify manufacturing procedures (Tang *et al*., 2008).

1.6.2 Gelucire® 44/14

Gelucire® 44/14 (G44/14) refers to the Gelucire® excipients that are commercially used (Fernandez *et al*., 2008). G44/14 comprises about 72 % PEG esters, 20 % glycerides, 8 % pure PEG and 2 % glycerol (Svensson *et al.*, 2004). Gelucire® is known to be safe at LD_{50} (rat, oral) > 2004 mg/kg/day (Chambin and Jannin, 2005, Raymond C. Rowe *et al.*, 2012). The G44/14 is characterized by the hydrophile-

lipophile-balance (HLB) value (14) and melting point (44) which leads to a specific behavior when placed in the gastrointestinal fluids (Vial-Bernasconi *et al.*, 1995).

When G44/14 is released in the GIT, it will dissolve in the dissolution medium and form milky dispersions. Subsequently, an o/w micro or nano emulsion will form. Thus, the solubility of the nonpolar compounds could be increased. This condition may be explained by the increased wettability and micellar solubilization (Figure 1-3). However, G44/14 as a self-emulsifying microemulsion is superior to simple micellar solutions in terms of solubilization potential and their thermodynamic stability. G44/14 increased bioavailability was also reported to be associated with strong inhibition of the enterocytic efflux transporter (known as P-gp inhibition) and strong inhibition of the enterocytic drug metabolizing enzyme, CYP3A4 (Wandel *et al.*, 2003, Cornaire *et al.*, 2004, Jannin *et al.*, 2008).

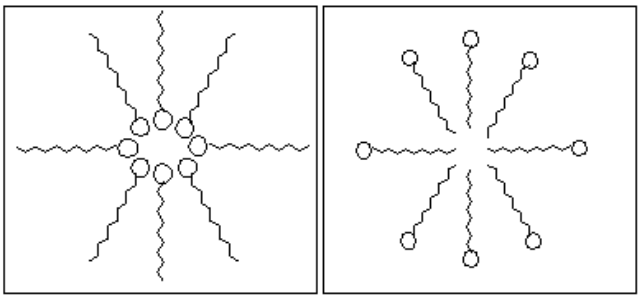

Figure 1-3. Micelles o/w and w/o
http://www2.umt.edu/medchem/teaching/medchem/mclect2.htm

For the microemulsions, the nonpolar compounds are suitable to prepare the o/w form, whereas polar compounds are appropriate for the w/o system. The HLB value

could be an indicator to demonstrate the tendency of w/o and o/w forms. The use of combined emulsifiers with low and high HLB values may produce a more rapid dispersion and finer emulsion droplet on contact with water. Cannon (2008) indicated that a w/o emulsion could be formed since the water would be taken up into the formulation at the beginning of dilution. Other researchers also reported the preparation of a mixture of emulsifiers to achieve certain release profiles (Tran *et al*., 2009). The incorporation of G50/13 and G50/02 by Dennis *et al*. (1990) increased the *in vitro* release of ketoprofen.

Nevertheless, the release mechanism of drug microemulsions (both w/o and o/w) from the lipid-based self-emulsifying drug delivery systems is not yet well understood. However, micelles facilitated delivery is generally accepted (Charman *et al*., 1997). Other possibilities include the inhibition of the pre-system metabolism, efflux of P-gp, and lymphatic transport (O'Driscoll, 2002, O'Driscoll and Griffin, 2008). For G44/14, it is difficult to predict the release behavior (Choy *et al*., 2005). The improved absorption may be due to the surface activities, or a combination of the self-emulsifying mechanisms (Tran *et al*., 2009).

1.7 Oral absorption and bioavailability

Drug bioavailability is the ratio of the amount of the unchanged drug reaching the circulation system after administration of a dosage form (Levy *et al*., 1969). Oral drug bioavailability is the extent that the drug molecules could be absorbed in the