AN INVESTIGATION OF LIPOSOMES FOR ORAL DELIVERY OF A POORLY BIOAVAILABLE MODEL DRUG: GRISEOFULVIN

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by

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To my wonderful parents, Ong Kok Chye and Lee Hoi Gat,

lovable sisters, Cindy and Wendy,

and charming little brother, Bernard

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LIST OF ABBREVIATIONS

Abbreviation	Full name
ANOVA	Analysis of variance
AUC	Area under the plasma concentration-time curve
AUC_{0-t}	Area under the plasma concentration-time curve from time zero to the last sampling time, t
BCS	Biopharmaceutics Classification System
BS	Bile salt
CLs	Conventional liposomes
C_{max}	Peak plasma concentration
CYP3A4	Cytochrome P450 subfamily 3A4
DDT	Dichlorodiphenyltrichlroethane
DG	Diglyceride
DSC	Differential Scanning Calorimetry
EDTA	Disodium ethylene diamine tetraacetic acid
FA	Fatty acid
FDA	Food and Drug Administration
GI	Gastrointestinal
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
LCLs	Long-circulating liposomes
LUVs	Large unilamellar vesicles
MDR	Multiple drug resistance
MFGM	Milk fat globule membrane
MG	2-monoglyceride
MLVs	Multilamellar vesicles
MVLs	Multivescular liposomes

MVM-FABP	Microvillus membrane fatty acid binding protein
NMR	Nuclear Magnetic Resonance
PC	Phosphatidylcholine
PCS	Photon Correlation Spectroscopy
PE	Phosphatidylethanolamine
PEGs	Polyethylene glycols
PI	Phosphatidylinositol
P-gp	P-glycoprotein
PL	Phospholipid
s.c.	Subcutaneous
SD	Standard deviation
SEM	Standard error mean
SUVs	Small unilamellar vesicles
TG	Triglyceride
TGA	Thermogravimetry
T_{max}	Time to reach peak plasma concentration
U.S.	United States
UWL	Unstirred water layer
v/v	Volume over volume
w/w	Weight over weight
ZAve	Z average diameter

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SATU KAJIAN LIPOSOM UNTUK PENGHANTARAN SECARA ORAL SATU DRUG MODEL BERBIOKEPEROLEHAN RENDAH: GRISEOFULVIN

ABSTRAK

Potensi formulasi liposom sebagai sistem penghantaran drug untuk pengambilan oral drug-drug berbiokeperolehan rendah telah dikaji menggunakan griseofulvin sebagai drug model. Satu teknik ekstrusi menggunakan alat yang dipasang sendiri telah dinilai untuk menano-saizkan liposom. Saiz partikel dan taburan saiz liposom yang dihasilkan dipengaruhi oleh pelbagai parameter pemprosesan. Namun demikian, kaedah ektrusi adalah ringkas, mudah dihasilkan semula dan lebih berkesan berbanding dengan kaedah pengurangan saiz lain yang dinilai. Kajian *in vitro* menunjukkan bahawa liposom yang disediakan daripada pelbagai pra-liposom, iaitu Pro-lipo duo®, Pro-lipo C® dan Pro-lipo S® semuanya mempunyai saiz berjulat nanometer dengan taburan saiz kecil. Liposom yang disediakan daripada Pro-lipo duo® mempunyai saiz yang paling kecil dan adalah paling stabil berbanding dengan pro-liposom yang lain. Kecekapan pemerangkapan griseofulvin dalam liposom dapat ditingkatkan dengan meningkatkan tempoh penyebatian dan suhu ataupun menggunakan pelarut-pelarut organik sebagai alat bantu pemelarutan. Pemerangkapan tertinggi griseofulvin telah dicapai dengan Pro-lipo duo® menggunakan kloroform sebagai alat bantu pemelarutan dan oleh itu, ia telah dipilih untuk penilaian *in vivo* yang seterusnya. Kajian perbandingan biokeperolehan *in vivo* mendedahkan bahawa biokeperolehan oral griseofulvin boleh ditingkatkan

sebanyak 2.7-3.2 kali apabila menggunakan liposom, akan tetapi, amaun drug yang terperangkap dalam liposom adalah penting untuk meningkatkan penyerapan sistemik. Walau bagaimanapun, peningkatan biokeperolehan griseofulvin dalam formulasi liposom yang diperolehi bukan disebabkan oleh promosi pengangkutan limfatik drug. Tambahan itu, saiz liposom didapati mempengaruhi takat biokeperolehan tetapi tidak menjejaskan tempoh penyerapan griseofulvin. Namun demikian, penyerapan atau biokeperolehan liposom didapati menjadi sama cekap untuk liposom-liposom bersaiz di bawah 400nm, di mana, pengurangan saiz liposom yang selanjutnya mempunyai kesan terhad ataupun tiada impak selanjutnya pada takat biokeperolehan. Berdasarkan penemuan-penemuan kajian ini, boleh disimpulkan bahawa liposom mempunyai potensi untuk menjadi satu sistem penyampaian drug yang berkesan untuk pengambilan oral drug-drug berketerlarutan air rendah, yang tanpa liposom adalah berbiokeperolehan rendah.

AN INVESTIGATION OF LIPOSOMES FOR ORAL DELIVERY OF A POORLY BIOAVAILABLE MODEL DRUG: GRISEOFULVIN

ABSTRACT

The potential of liposomal formulation as a drug delivery system for oral administration of poorly bioavailable drugs was studied using griseofulvin as the model drug. An extrusion technique using a self-assembled instrument was evaluated for nanosizing the liposomes. The particle size and size distribution of liposomes produced was affected by various processing parameters. Nevertheless, the extrusion method was simple, reproducible and more effective compared to other size-reduction methods evaluated. *In vitro* studies showed that liposomes prepared from various pro-liposomes, namely Pro-lipo duo®, Pro-lipo C® and Pro-lipo S® were all in nanometer size range with a narrow size distribution. Liposomes prepared from Pro-lipo duo® were smallest in size and most stable compared to other pro-liposomes. The encapsulation efficiency of griseofulvin-loaded liposomes could be enhanced by increasing the mixing duration and temperature or using organic solvents as solubilisation aids. The highest encapsulation of griseofulvin was achieved with Pro-lipo duo® using chloroform as the solubilisation aid and hence, it was selected for further *in vivo* evaluations. The *in vivo* comparative bioavailability study revealed that the oral bioavailability of griseofulvin could be increased by 2.7 to 3.2 times using liposomes but the amount of drug encapsulated in the liposomes was important for enhancing the systemic absorption. However, the enhanced bioavailability of griseofulvin in liposomal formulations obtained was not due to the promotion of lymphatic drug

transport. In addition, the size of liposomes was found to affect the extent of bioavailability but has no influence on the duration of griseofulvin absorption. Nonetheless, the uptake or bioavailability of liposomes was found to be equally efficient with liposomes below 400nm, whereby, a further size reduction in the liposomes has limited or no further impact on the extent of bioavailability. On the basis of the findings of this study, it was concluded that liposomes have the potential of being an effective drug delivery system for the oral administration of poorly soluble drugs that are otherwise poorly bioavailable orally.

CHAPTER 1 INTRODUCTION

1.1 BIOAVAILABILITY

1.1.1 INTRODUCTION

The concept of bioavailability, at first called physiological availability, was first introduced in 1945 during the studies of relative absorption of vitamins from pharmaceutical products (Oser *et al.*, 1945). Over the years, the multiplicity of terms used to describe bioavailability had caused considerable ambiguity among the researchers. Confusion has arisen over the interchangeability of the terms used, namely, biologic availability (bioavailability), physiologic availability, generic equivalence and therapeutic equivalence (Wagner, 1975b). After much reviews and debates, bioavailability eventually became the mutually accepted term.

1.1.2 DEFINITION

The U.S. Food and Drug Administration (FDA) defines bioavailability as the rate and extent in which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action (Chen *et al.*, 2001). Since measurement of the drug concentration at the site of action is not always feasible, the bioavailability is more commonly defined as the rate and extent of which an active drug is absorbed from a dosage form and becomes available in the systemic circulation.

1.1.3 BARRIERS AFFECTING ORAL BIOAVAILABILITY OF POORLY WATER SOLUBLE DRUGS

The oral route has remained the preferred mode of drug administration, mainly due to its convenience and better patient compliance. However, poorly water-soluble drugs suffer low bioavailability when administered orally (Dahan and Hoffman, 2008, Humberstone and Charman, 1997). Various absorption barriers have been known to affect the oral bioavailability of these drugs (Figure 1.1). They can be categorized into pre-enterocyte, enterocyte and post-enterocyte barriers (Dahan and Hoffman, 2007a).

1.1.3(a) PRE-ENTEROCYTE BARRIERS

i) SOLUBILITY

Following oral administration, dissolution of the drug molecule in the intestinal milieu is a prerequisite for the absorption process. If the drug is insoluble or poorly water-soluble, it poses a problem of poor dissolution and/or absorption, since the flux of a drug across the intestinal membrane is proportional to its concentration gradient between the apical side and basolateral sides of gastrointestinal (GI) lumen (Panchagnula and Thomas, 2000).

According to the Biopharmaceutics Classification System (BCS) (Amidon *et al.*, 1995), poorly water-soluble compounds (with solubility less than 100µg/mL) are classified as either class II or class IV compounds, depending on their intestinal permeability. For BCS class II drugs that exhibit high permeability characteristics, drug absorption is governed by their dissolution

in the GI fluids. Whereas, BCS class IV drugs, characterized by both low solubility and poor intestinal wall permeability, are generally poor drug candidates for oral administration.

The aid of surfactants provided by biliary secretions is necessary to solubilize the poorly water-soluble drug and to enable its absorption (Dahan and Hoffman, 2008). These biliary secretions help by forming submicron mixed micelles with the drug and hence enable the solubilized drug to reach the absorptive membrane of the enterocyte. Limited in its capacity and considerably variable in different situations, this process is the rate-limiting step in the absorption of the poorly water-soluble drugs and often becomes a significant absorption barrier (Dahan and Hoffman, 2007a).

ii) LIMITED ABSORPTION SITE

The absorption of the poorly water-soluble drugs usually takes place along the small intestine, where drug solubilization occurs. The residence time of a drug in the upper GI tract has been shown to be relatively short and the transit time along the small intestine is about 3.0-4.5 hours in healthy volunteers (Yu and Amidon, 1999). Although fat can prolong the small intestinal transit time (by 30-60 minutes), it is not thought to be of any significance in drug delivery (Dahan and Hoffman, 2007a). As a result, the absorption of a poorly water-soluble drug may be poor due to limited site of absorption, and if the drug reaches the colon prior to solubilization, its bioavailability is expected to be even lower.

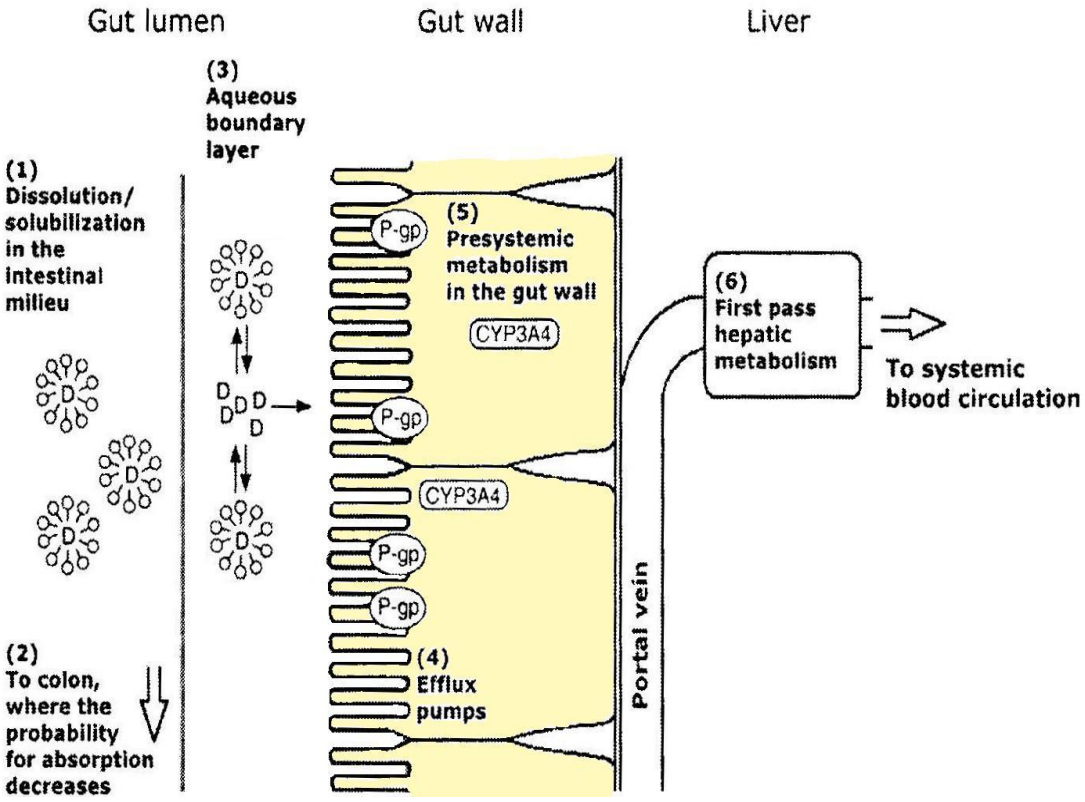

Figure 1.1 Barriers affecting oral bioavailability of poorly water-soluble drugs (adapted from Dahan and Hoffman, 2008)

iii) UNSTIRRED WATER LAYER

The unstirred layer refers to the stagnant layer of water adjacent to the absorptive membrane of the enterocyte (Ashford, 2002). It can be visualized as a series of water lamellas, each progressively more stirred from the gut wall toward the lumen bulk (Dahan and Hoffman, 2008). For BCS class II drugs, the rate of permeation through the brush border is fast due to large surface area and thus the diffusion across the unstirred water layer (UWL) becomes the rate-limiting step in the permeation process. A study by Read *et al.* (1977) found that the thickness of the UWL in human jejunum is over 500µm. Due to its thickness and hydrophilicity, the UWL may represent a major permeability barrier to the absorption of poorly water-soluble drugs. In addition, the effective surface area of the UWL also plays a role in limiting drug absorption. The ratio of the surface area of the UWL to that of the underlying brush border membrane is at least 1:500 (Westergaard and Dietschy, 1974). This low ratio value suggests that the effective surface area of the UWL available for the absorption of poorly water-soluble drugs is relatively small, and hence, their bioavailability are impaired.

1.1.3(b) ENTEROCYTE BARRIERS

Upon entering the enterocyte, a drug molecule faces another set of biochemical barriers that affect the magnitude of its absorption. The cytochrome P450 3A4 (CYP3A4) enzymes, located in the endoplasmic reticulum of the enterocyte, are responsible for most drug metabolism in the intestinal wall. A study by Wacher *et al.* (1998) found that this isoenzyme accounted for more than 70% of all small intestinal CYP450s. While some

transporters located in the apical wall of the enterocyte facilitate absorption, there are others that serve as efflux transporters. Efflux transporters play a major role in the disposition of many drugs, and thus, are regarded as the multiple drug resistance (MDR) transporters. The apical P-glycoprotein (P-gp) efflux pump is the most extensively studied MDR transporter. It reduces the fraction of drug absorbed by transporting the drug from the enterocyte back to the intestinal lumen (Gottesman *et al.*, 1996).

Synergism exists between the activity of the metabolic CYP3A4 enzymes and the P-gp system. A drug molecule that escapes the intra-enterocyte metabolism may either reach the blood circulation or be effluxed back into the GI lumen, and then may be reabsorbed (Benet and Cummins, 2001). Many studies have shown that these two pre-hepatic systems contributed to the limited oral bioavailability of many poorly water-soluble drugs (Lennernäs, 2003, Fitzsimmons and Collins, 1997, Thummel *et al.*, 1996, Kolars *et al.*, 1991).

1.1.3(c) POST-ENTEROCYTE BARRIERS

A drug molecule that manages to escape the intra-enterocyte metabolism and the MDR efflux systems will diffuse across the cell and be secreted from the basolateral membrane of the enterocyte into the lamina proporia. Following that, the drug is usually absorbed into the portal blood unless it is being incorporated into a chylomicron. However, before reaching the systemic blood circulation, the drug molecules will have to pass through the liver, and hence are exposed to the metabolic enzymes. This first-pass

hepatic metabolism has been shown to be a major barrier to the absorption of poorly water-soluble drugs, which are the most likely molecules to undergo oxidative metabolism.

1.1.4 APPROACHES TO ENHANCE THE ORAL BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUGS

Low oral bioavailability of poorly water-soluble drugs poses a great challenge during drug development (Lipinski *et al.*, 1997). Various approaches have been developed to improve the bioavailability by increasing the drug dissolution rate and solubility. A summary of the strategies and approaches is detailed in the following sections.

1.1.4(a) PRODRUGS

The term prodrug may be defined as a chemical derivative of a drug that is bioconvertible into the active parent drug or an active metabolite responsible for the therapeutic effect (Kim and Singh, 2002). Prodrugs are formed by attachment of the active drug through a metabolically labile linkage to another molecule, the “promoiety’, to impart some desirable properties to the drug. Chloramphenicol for example, was chemically modified to produce a prodrug, chloramphenicol sodium succinate with enhanced solubility. As a result of this modification, its aqueous solubility was improved significantly from 2.5 mg/ml to 100 mg/ml (Maurin *et al.*, 2002). Besides that, prodrugs can be designed to improve the bioavailability via other means. L-Dopa for instance improved the bioavailability of dopamine by overcoming the blood-brain

barrier; whereas, propanolol hemisuccinate blocked the formation of glucuronide which resulted in a reduction of the first-pass metabolism to increase the bioavailability of propanolol (Anderson *et al.*, 1988).

1.1.4(b) COMPLEXATION

Cyclodextrins have been extensively utilized as complexing agents to improve the bioavailability of numerous poorly water-soluble drugs (Figeiras *et al.*, 2007, Larsen *et al.*, 2005, Green and Guillory, 1989). The cyclic oligosaccharides obtained from the enzymatic degradation of starch have a unique structure of an apolar cavity and a hydrophilic external part, which renders them to mask the physicochemical properties of the included drug molecule. The mechanism of bioavailability enhancement by cyclodextrins complexation has been attributed to the improvement of solubility, dissolution rate and chemical stability (Loftsson and Brewster, 1996). In addition to the inclusion of complexes formed by cyclodextrins, other types of molecular complexes, such as caffeine, sodium salicylate, sodium benzoate and nicotinamide have also been reported.

1.1.4(c) COSOLVENT APPROACH

A cosolvent is a water-miscible organic solvent that is used to increase aqueous solubility of a poorly water-soluble compound or to increase the chemical stability of a drug. Due to their low toxicity, ethanol, glycerol and polyethylene glycols (PEGs), are commonly selected for formulating poorly water-soluble drugs (Strickley, 2004). In a recent study, the cosolvent technique used for the preparation of carbamazepine nanosuspensions not

only increased the aqueous solubility of carbamazepine but also improved the stability of the formulations (Douroumis and Fahr, 2007).

1.1.4(d) PARTICLE SIZE REDUCTION

The dissolution rate of a drug can be proportionally increased by increasing its surface area as a consequence of comminution. The bioavailability of the antifungal, griseofulvin, was enhanced significantly by micronization and hence the effective dose was reduced by 50% (Atkinson *et al.*, 1962). Absorption of naproxen was faster and the plasma levels were higher when the drug was administered orally (to rats) as nanoparticles, compared to that administered as conventional drug particles (Liversedge and Conzentino, 1995). Besides that, several studies with a number of poorly water-soluble drugs, such as cilostazol, danazol and cyclosporine, have also demonstrated that particle size reduction can lead to an increased rate of dissolution and improved oral bioavailability (Jinno *et al.*, 2006, Liversidge and Cundy, 1995, Tarr and Yalkowsky, 1989). Therefore, nano-sized particles are now being considered to boost absorption of poorly water-soluble drugs.

1.1.4(e) PHARMACEUTICAL SALTS

Salt formation is a simple means to alter the biopharmaceutical properties of a drug substance, particularly useful to enhance drug solubility. Salt formation increases drug solubility by keeping the pH at which the drug is ionized (Perumal and Podaralla, 2008). Numerous studies have shown that the solubility of poorly water-soluble drugs such as piroxicam (Gwak *et al.*,

2005), meloxicam (Han and Choi, 2007) and diclofenac (O'Connor and Corrigan, 2001) could be enhanced via salt formation.

1.1.4(f) LIPID-BASED FORMULATIONS

The use of lipid as a potential formulation strategy for improving the oral bioavailability of poorly water-soluble drugs has generated much academic and commercial interest. Lipid-based formulations of poorly water-soluble drugs offer a large versatility for oral administration as they can be formulated as solutions, gels, suspensions, emulsions, self-emulsifying systems, liposomes and solid dispersions (Gershanika and Benita, 2000, Pouton, 2000). The role of liposomes in oral delivery of poorly water-soluble drugs will be reviewed in section 1.3.

Incorporation of the poorly water-soluble drugs into inert lipid vehicles could enhance the absorption and oral bioavailability via a combination of various mechanisms that are described in the following section. All these mechanisms could help to improve the oral bioavailability of drugs.

1.2 MECHANISMS OF LIPID-BASED FORMULATIONS IN ENHANCING THE ORAL BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUGS

The ability of lipid vehicles to enhance the bioavailability of poorly water-soluble drugs has been well known for many years. The presentation of the poorly water-soluble drug as a solution in oil, avoiding the complexities associated with solid state, is a major factor for this bioavailability enhancement (Dahan and Hoffman, 2008). The other mechanisms by which lipid based delivery systems enhance the bioavailability of poorly water-soluble drugs include:

i) Enhanced dissolution / solubilization

The presence of lipids in the GI tract stimulates an increase in the secretions of bile salts (BS) and endogenous biliary lipids such as phospholipids (PL) and cholesterol (Fleisher *et al.*, 1999). These biliary products, along with the gastric shear movement, form crude emulsion which promotes the solubilization of the coadministered poorly water-soluble drug (Tso, 1985). In addition, the exogenous lipidic component of the delivery system is subjected to enzymatic digestion. Esters are rapidly hydrolyzed in the presence of pancreatic lipase and the lipolytic products, upon interaction with BS or PL, form different micellar species that prevent precipitation of the coadministered poorly water-soluble drug (Dahan and Hoffman, 2008).

ii) Prolongation of gastric residence time

Lipids in the GI tract delay gastric emptying and thereby increasing the gastric transit time. As a result, the residence time of the coadministered poorly water-soluble drug in the small intestine is prolonged. This enables longer exposure of the drug at the absorptive site, and thereby improves its absorption (Hunt and Knox, 1968).

iii) Stimulation of lymphatic transport

Bioavailability of poorly water-soluble drugs may be enhanced also by the stimulation of the intestinal lymphatic transport pathway. Further discussions are in section 1.2.2.

iv) Influence of intestinal permeability

Various combinations of lipids, lipid digestion products and surfactants have been shown to possess permeability enhancing properties (Aungst, 2000). In most instances, however, passive intestinal permeability is not thought to be a major barrier to the bioavailability of lipophilic drugs (Porter and Charman, 2001a).

v) Reduced metabolism and efflux activity

Certain lipids and surfactants have been shown to reduce the activity of the efflux transporters in the GI wall, and hence, increase the fraction of drug absorbed (Dintaman and Silverman, 1999). Because synergism exists between the activity of P-gp and CYP3A4, these lipids and surfactants may also reduce the intra-enterocyte metabolism (Dahan and Hoffman, 2008).

1.2.1 DIGESTION AND ABSORPTION OF LIPIDS FROM GASTROINTESTINAL TRACT

As mentioned in section 1.2, lipid-based formulations can reduce the inherent limitation of slow and incomplete dissolution of poorly water-soluble drugs, and facilitate the formation of solubilized phases from which absorption may occur. The attainment of a solubilized phase will not necessarily arise directly from the administered lipid, but most likely from the intraluminal processing to which lipids are subjected prior to absorption (Humberstone and Charman, 1997, MacGregor *et al.*, 1997). Therefore, an understanding of lipid digestion and the manner by which it contributes to drug solubilization is vital to the design of lipid-based formulations.

The lipid digestion and absorption process, and its direct association with lymphatic transport of lipophilic drugs, has been extensively reviewed (Humberstone and Charman, 1997, Porter and Charman, 1997, Tso, 1985, Carey *et al.*, 1983). Firstly, lingual and gastric lipases initiate hydrolysis of a limited amount of triglyceride (TG), forming the corresponding diglyceride (DG) and fatty acid (FA) within the stomach. These amphiphilic lipid digestion products, in combination with the shear mixing produced by antral contraction, retropulsion and gastric emptying, facilitate the formation of a crude emulsion which empties into the duodenum. In duodenum, the emulsion induces the secretion of bile salts, biliary lipids and pancreatic fluids into the duodenum. Biliary lipids adsorb onto the surface of the emulsified lipids, improving the colloidal stability and further reducing their particle size (Carey *et al.*, 1983). Subsequently, the hydrolysis of the emulsified lipids

takes place under the action of pancreatic lipase. This interfacial enzyme which requires co-lipase and bile salts to be active, acts at the surface of the emulsified TG droplets to quantitatively produce the corresponding 2-monoglyceride (MG) and two molecules of FA.

Polar digestion products such as short-chain and medium-chain FAs diffuse across the mucosa lining of the enterocyte and enter into the portal circulation (Kiyasu *et al.*, 1952). On the other hand, the non-polar digestion units such as the long-chain FAs (more than 12 carbons) and MG are emulsified by bile salts to form micelles. Micelles are not absorbed intact. The lipid monomers dissociate from the micelles and are subsequently absorbed from a monomolecular inter-micellar phase (Westergaard and Dietschy, 1976). In addition to passive uptake, long-chain FAs and their substrates may utilize an enterocyte-based carrier system, called the microvillus membrane fatty acid binding protein (MVM-FABP) (Stremmel, 1988, Stremmel *et al.*, 1985). Once inside the cell, the long-chain FAs are re-esterified to form triglycerides, which are incorporated into lipoproteins to form chylomicrons (Davidson, 1994). The chylomicrons cannot permeate the blood capillaries due to its large particle size (200-800µm) (Dahan and Hoffman, 2007a). Thus, they are absorbed into a porous mesenteric lymph vessel called lacteal and travel with the lymph until drainage into the systemic blood circulation.

1.2.2 INTESTINAL LYMPHATIC DRUG TRANSPORT

The majority of orally administered drugs gain access to the systemic circulation by direct absorption into the portal blood (Porter and Charman, 2001a). However, for some poorly water-soluble compounds, transport by way of the intestinal lymphatic system may provide an additional route of access into the systemic circulation (Porter and Charman, 2001a). Bypassing the liver, an alternative absorption pathway from the GI tract, provides an advantage over the portal blood route for avoidance of potential hepatic first-pass metabolism, as discussed in section 1.1.3(c). It has been shown to enhance the bioavailability of a number of lipophilic drugs including the fat-soluble vitamins (Dahan and Hoffman, 2007a), halofrantrine (Holm *et al.*, 2003), probucol (Palin and Wilson, 1984) and cyclosporine (Ueda *et al.*, 1983). Lipid-based vehicles and the presence of food often enhance the oral bioavailability particularly of poorly water-soluble drugs. In some cases, the lymphatic system plays a significant role in this enhanced bioavailability. Therefore, it seems likely that the physiological processes of lipid digestion and absorption are relevant to this enhanced drug delivery.

Lipid vehicles may enhance lymphatic transport of lipophilic compounds by stimulating the production of chylomicrons (O'Driscoll, 2002). Lipophilic drugs enter the lymphatic system in association with the triglyceride core of the chylomicrons. In many cases, for example with the antimalarial drug halofantrine (log P 8.5, TG solubility 50 mg/ml), a strong correlation has been established between the degree of lymphatic drug transport and the TG content of the lymph (Caliph *et al.*, 2000).The importance of the physiological

process of lipid digestion in stimulating drug lymphatic transport has also been highlighted by Liu *et al.* (1995). Following administration of a milk fat globule membrane (MFGM) stabilized soybean emulsion, 19.2% of the dose of vitamin D3 was transported via the lymph, but the degree of transport dropped to 0.27% in rats with pancreatic duct ligation. On the other hand, when the MFGM emulsion was administered with bile salt and pancreatic lipase, the extent of lymphatic transport was increased again to 20.4%.

Even with lipid-based formulations, various factors were found to influence the extent of lymphatic transport. These include the chemical structure, digestibility and dispersed state of the lipid vehicle. One of the earliest reports of intestinal lymphatic transport by Palin *et al.* (1982) illustrated the importance of the digestibility of the vehicle on the extent of lymphatic transport. Arachis oil was found to significantly increase the lymphatic transport of dichlorodiphenyltrichloroethane (DDT) relative to non digestible liquid paraffin. In addition, long-chain unsaturated FAs were found to be more capable of enhancing lymphatic transport, and this has been attributed to their increased ability to stimulate chylomicron production (Charman et al., 1986a, Palin et al., 1982). Besides that, a study by Porter *et al.* (1996a) supported the hypothesis that formulations of lipids as dispersed systems may promote the lymphatic transport.

1.3 LIPOSOMES

1.3.1 INTRODUCTION

Liposomes are naturally occurring self-assembled structures that can also be synthesized in the laboratory (Gómez-Hens and Fernández-Romero, 2005). They are composed of one or several lipid bilayers enclosing aqueous compartments and may range in size from tens of nanometers to tens of microns in diameter. Liposomes are usually made of phospholipids, a class of amphiphilic molecules (possessing both hydrophilic and lipophilic properties) (Figure1.2) which is the main components of biological membranes. In aqueous medium, phospholipids arrange themselves into bilayers, by positioning their hydrophilic groups towards the surrounding aqueous medium, and their lipophilic chains towards the inner side of the bilayer.

The potential of liposomes as drug delivery systems has been widely recognized (Torchilin, 2005, Vemuri and Rhodes, 1995, Woodle and Papahadjopoulos, 1989, Fendler and Romero, 1977, Gregoriadis, 1976). Drugs with widely varying lipophilicities can be encapsulated into liposomes, either in the phospholipid bilayer, in the encapsulated aqueous volume or at the bilayer interface (Sharma and Sharma, 1997) (Figure 1.3). Hydrophobic drugs are incorporated into the lipid bilayers, while hydrophilic drugs are usually encapsulated in the aqueous compartments (Barenholz, 2003). For drug delivery, liposomes can be formulated as a suspension, an aerosol, or a semisolid preparation such as a gel or cream, or a solid preparation like a dry powder (Lasic, 1998).

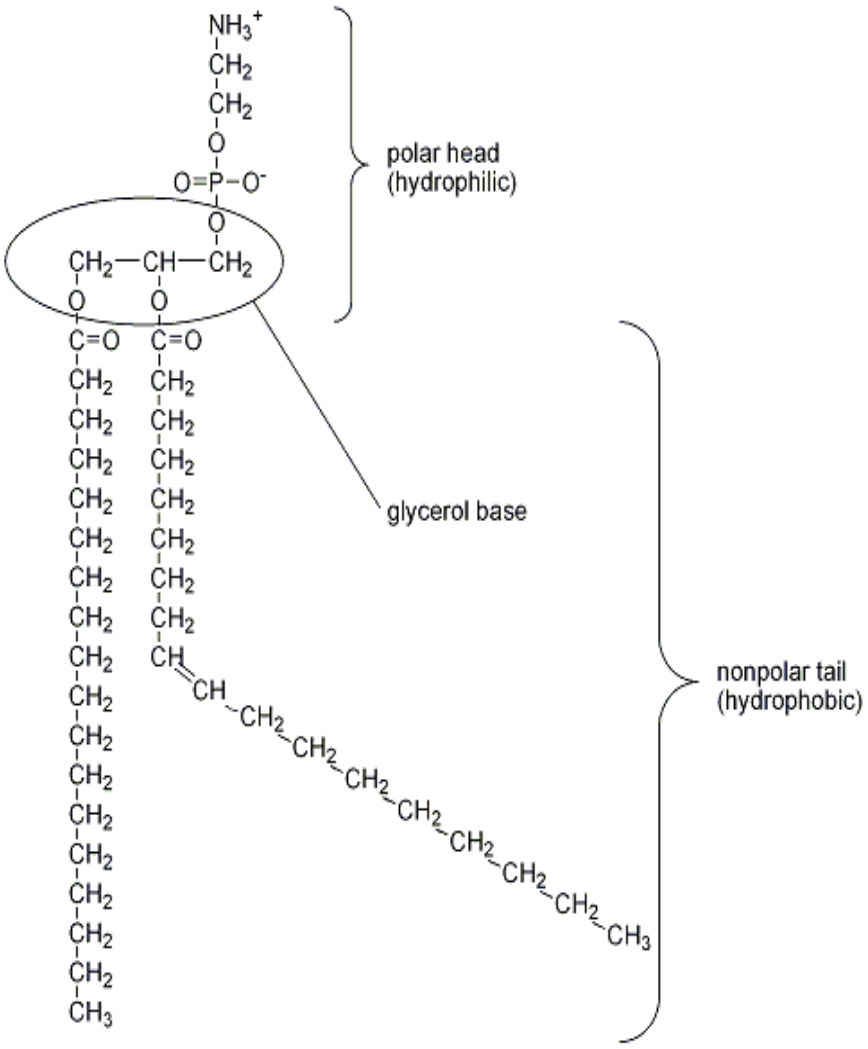

Figure 1.2 Structural formula of a phospholipid molecule

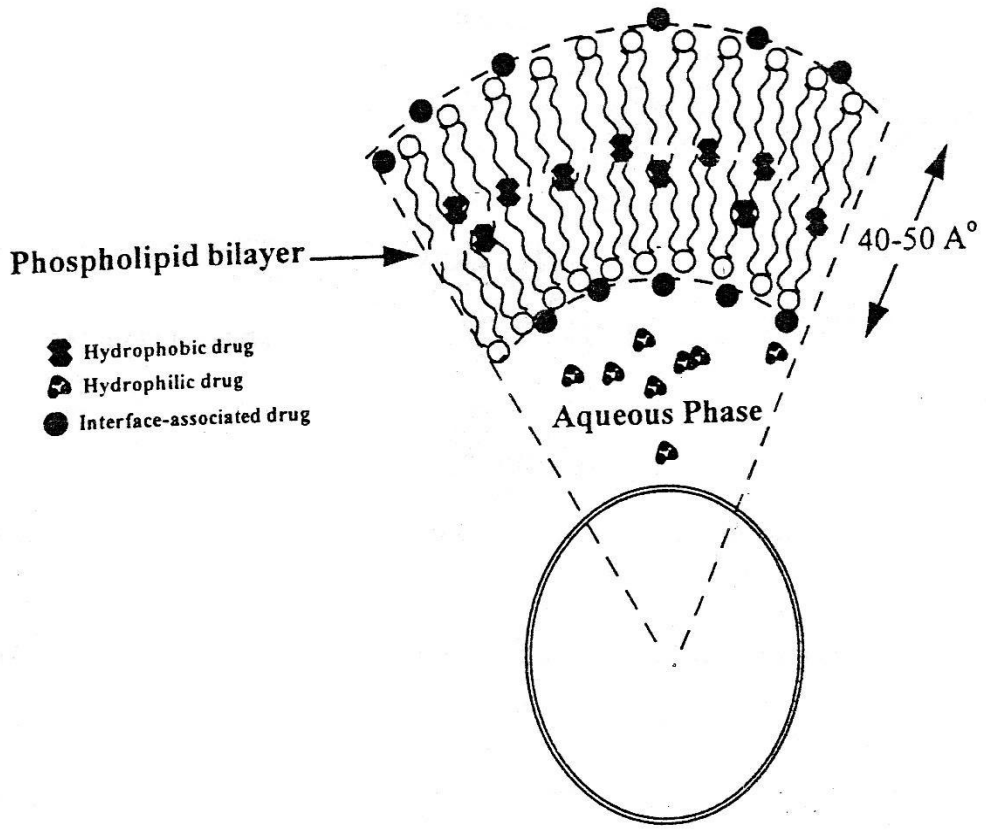

Figure 1.3 Distribution of drugs of different lipophilicities in liposomes (adapted from Sharma and Sharma, 1997)

Various routes of administration, namely parenteral, topical and oral, can be used for administration of liposomes (Betageri *et al.*, 1993a). Many studies on liposomes as drug delivery systems employed the parenteral route. In addition to intravenous (i.v.) administration, liposomes have been administered via the intraperitoneal (i.p.), intramuscular (i.m.) and subcutaneous (s.c.) routes (Crommelin and Schreier, 1994, Zonneveld and Crommelin, 1988). Topical administration of liposomes includes application to the skin, eyes, lungs or body cavities. Thus, liposomes may be used for either local or systemic delivery of drugs (Betageri *et al.*, 1993a). Besides that, liposomes have been used successfully as an oral drug delivery system. Examples of drugs that showed improved bioavailability when administered orally in liposomal formulations include heparin (Jiao *et al.*, 2002), insulin (Iwanagaa *et al.*, 1997), cyclosporine (Al-Meshala *et al.*, 1998), erythropoietin (Maitani et al., 2000a) and cefotaxime (Ling et al., 2006).

1.3.2 CLASSIFICATION OF LIPOSOMES

On the basis of their size and number of bilayers, liposomes can be classified as (Sharma and Sharma, 1997, Betageri et al., 1993b):

(a) multilamellar vesicles (MLVs)

MLVs usually consist of vesicles covering size range of 100 to 1000nm, each vesicle consisting five or more concentric bilayers. Vesicles, which are composed of less than five bilayers, are called oligo-lamellar vesicles.

(b) large unilamellar vesicles (LUVs)

LUVs have diameters larger than 100nm in size and consist of one lipid bilayer. Liposomes with sizes between 50 and 100nm are referred to as intermediate sized unilamellar vesicles.

(c) small unilamellar vesicles (SUVs)

SUVs are the lower sized liposomes. Their sizes depend on the ionic strength of the aqueous media and lipid composition, and are usually in the range 25 to 50nm. They possess only one lipid bilayer.

Besides classification based on the number of bilayers and size, liposomes can also be categorized in terms of their composition and mechanism of intracellular delivery (Sharma and Sharma, 1997). They are:

(a) Conventional liposomes (CLs)

CLs are composed of neutral and/or negatively charged phospholipids and cholesterol. As the contents of CLs are ultimately delivered to the lysosomes, CLs are therefore useful for reticuloendothelial system (RES) targeting.

(b) pH-sensitive liposomes

They consist of phospholipids with either cholesteryl hemisuccinate or oleic acid. At low pH, they fuse with cell membranes and release their contents in cytoplasm.

(c) Cationic liposomes

These usually compose of cationic lipid derivatives and neutral phospholipids. In most cases, they fuse with the cell membranes and are suitable for delivery of negatively charged macromolecules.

(d) Long-circulating liposomes (LCLs)

LCLs are also known as 'Stealth Liposomes'. They consist of neutral phospholipids with high transition temperature (T_c) and cholesterol with hydrophilic surface coating. The surface coating sterically hinders a variety of interactions at the bilayer surface, so that the liposomes can escape the rapid uptake by macrophage cells of the RES, and thus circulate in the blood stream for a long time and passively target onto sites of tumors, infection and inflammation, often characterized by the presence of a leaky vasculature.

(e) Immunoliposomes

These are CLs or LCLs with attached antibody or recognition sequence. They participate in cell-specific binding (targeting) and release their contents extracellularly near the target tissue.

1.3.3 CHEMICAL CONSTITUENTS

Liposomes can be prepared from a variety of lipids and other amphiphiles such as nonionic surfactants. The vesicles prepared from the latter are referred to as niosomes and are mainly used for topical application. Phospholipids, the most commonly used lipid for liposome preparation, can

be divided into four groups (van Winden *et al.*, 1998, Barenholz and Crommelin, 1994):

(a) Natural phospholipids

The two main sources of natural phospholipids are eggs and soy beans. Natural phospholipids are usually present in a mixture of phospholipids with different chain length and varying degrees of unsaturation (New, 1990). Generally, plant-derived phospholipids have higher level of unsaturation in the fatty acyl chains compared to those of animal-derived. Natural phospholipids which are used for preparation of liposomes include phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin and phosphatidylinositol (PI).

(b) Modified natural phospholipids

These are natural phospholipids that are chemically modified to alter certain characteristics of the phospholipids. The acyl chains of natural phospholipids can be modified by partial or complete hydrogenation. Such modification reduces the degree of unsaturation (to different extents) and consequently improves the phospholipids' appearance and resistance to peroxidation (Barenholz and Crommelin, 1994). Another possible modification is conversion of head group of phospholipids with the aid of the enzyme phospholipase D.

(c) Semisynthetic phospholipids

They are obtained by removing the acyl chains of natural phospholipids by chemical replacement with defined synthetic acyl chains (Lichtenberg and Barenholz, 1988).

(d) Fully synthetic phospholipids

These compounds are prepared via complete chemical synthetic pathways. Fully synthetic phospholipids have the advantage of a defined fatty acid composition and can be tailored to specific needs.

Apart from phospholipids, other lipids used in liposome preparation include sterols, especially cholesterol. Cholesterol does not by itself form bilayer structures but can be incorporated into the phospholipid bilayer to provide greater stability (New, 1990). Cholesterol reduces the fluidity of the bilayer above the transition temperature, resulting in a corresponding reduction in the permeability to aqueous solutes. Cholesterol can be incorporated up to a level of 50 mol% (1:1 ratio), at which it displays its maximum stabilizing effect both *in vitro* and *in vivo* (New, 1990).

1.3.4 METHODS OF PREPARATION

Liposomes can be prepared according to three basic modes of preparation namely mechanical dispersion, solvent dispersion and detergent solubilization (Figure 1.4).

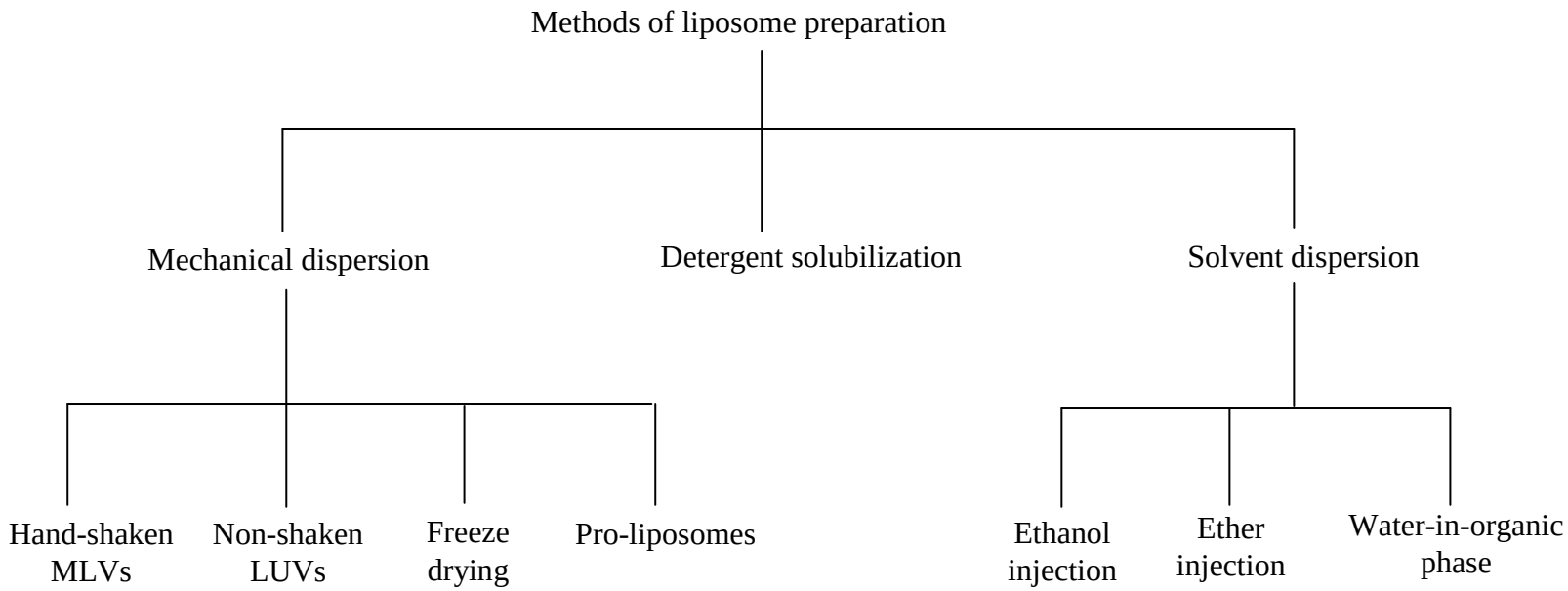

Figure 1.4 Classification of methods of liposome preparation (adapted from Jain 2001)