INVESTIGATION OF THE POTENTIAL OF GELUCIRE 44/14 FOR ENHANCING ORAL BIOAVAILABILITY USING TWO MODEL DRUGS

by

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To Michelle Hunziker and Paolo Maldini

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P-gp	P-glycoprotein	2
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EPO	Erythropoietin	3
rhGH	Recombinant human growth hormone	3
NCE	New chemical entities	6
BCS	Biopharmaceutics classification system	6
PLGA	Polylactic-*co*-glycolic acid	8
SEDDS	Self-emulsifying drug delivery system	9
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HIV	Human immunodeficiency virus	10
t_{max}	Time to reach maximum plasma concentration	12
C_{max}	Peak plasma concentration	12
HLB	Hydrophilic-lipophilic balance	12
rhEPO	Recombinant human erythropoietin	13
BA	Bioavailability	15
VCM	Vancomycin	15
MIC	Minimum inhibitory concentration	18
$t_{1/2}$	Half-life	18
AUC	Area under the curve	18
PK-PD	Pharmacokinetic-pharmacodynamic	18
MRSA	Methicillin-resistant *Staphilococcus aureus*	18
PLA	Poly d,l-lactic acid	24
PLGC	Poly (D,L-lactide-*co*-glycolide)	24
VRE	Vancomycin-resistant enterococci	25
FDA	Food and Drug Administration	26
GF	Griseofulvin	28
DNA	Deoxynucleic acid	28
GI	Gastro-intestinal	28
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FTIR	Fourier-transform infrared	33
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T_g	Glass transition temperature	40
IR	Infrared	40
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MRT	Mean residence time	48
UV	Ultraviolet	51
SD	Standard deviation	57
TDM	Therapeutic drug monitoring	79
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ACN	Acetonitrile	80
FA	Formic acid	80
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MeOH	Methanol	80
TFA	Trifluoroacetic acid	80
MS	Mass-spectrometry	80
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KAJIAN POTENSI GELUCIRE 44/14 UNTUK MENINGKATKAN BIOKEPEROLEHAN ORAL DENGAN MENGGUNAKAN DUA DRUG MODEL

ABSTRAK

Dua drug model yang termasuk dalam dua kumpulan *Biopharmaceutics Classification System* (BCS) yang berbeza, iaitu vancomycin (VCM), satu sebatian kelas III (keterlarutan tinggi dan ketelapan rendah), dan griseofulvin, satu drug kelas II (keterlarutan rendah and ketelapan tinggi) telah digunakan untuk menilai potensi satu pembawa gliserida berpoliglikol, iaitu Gelucire 44/14, untuk meningkatkan biokeperolehan oral. Kedua-dua model drug tersebut berjaya digabungkan ke dalam pembawa berlipid tersebut dengan menggunakan teknik lebur-cantum, dengan menghasilkan penyebar pepejal dalam kedua-dua kes. Analisis termo yang dilaksanakan dengan menggunakan teknik DSC, telah mengesahkan ketiadaan kesan penuaan yang ketara bagi formulasi VCM-Gelucire 44/14 dan ketiadaan interaksi fizikal antara VCM dengan Gelucire. Kajian *Fourier-Transform Infrared* (FTIR) mengesahkan keserasian VCM dengan Gelucire 44/14. Kajian pelarutan yang telah dijalankan dalam media pH1, pH4 dan pH7 menunjukkan tiada perubahan yang signifikan dalam kadar ataupun takat pelepasan VCM daripada matriks Gelucire 44/14 dalam ketiga-tiga media yang telah dikaji. Analisis DSC ke atas formulasi GF-Gelucire 44/14 menunjukkan bahawa GF telah disebarkan dengan sekata dalam penyebar pepejal. Kajian *in vivo* ke atas formulasi VCM-Gelucire 44/14 tidak menunjukkan sebarang peningkatan dalam biokeperolehan VCM. Sebaliknya, kajian *in vivo* formulasi GF-Gelucire 44/14 menunjukkan peningkatan biokeperolehan sebanyak 45% apabila tikus diberi formulasi GF-Gelucire 44/14 dibandingkan dengan pemberian ampaian berair drug, dan

dengan ini, fungsi Gelucire 44/14 sebagai peningkat biokeperolehan/penyerapan untuk drug kelas II telah disahkan.

INVESTIGATION OF THE POTENTIAL OF GELUCIRE 44/14 FOR ENHANCING ORAL BIOAVAILABILITY USING TWO MODEL DRUGS

ABSTRACT

Two model drugs belonging to different groups of the Biopharmaceutics Classification System (BCS), which are vancomycin (VCM) as a class III drug (high solubility and low permeability) and griseofulvin (GF) as a class II drug (low solubility and high permeability), were employed to evaluate the potential of a polyglycolised glyceride carrier, namely Gelucire 44/14, to enhance their oral bioavailability. Each of the two model drugs was successfully incorporated into the lipid carrier using the melt-fusion technique, producing solid dispersions in both cases. The thermal analysis, performed using Differential Scanning Calorimetry (DSC) technique, confirmed the absence of significant aging of the VCM-Gelucire 44/14 formulation and physical interactions of VCM with Gelucire 44/14 were found to be not present. Fourier-Transform Infrared (FTIR) investigations confirmed the compatibility of VCM and Gelucire 44/14. The dissolution studies, performed in pH1, pH4 and pH7 media showed no significant changes in the rate or extent of release of VCM from the Gelucire 44/14 matrix in any of the three media investigated. DSC analysis of the GF-Gelucire 44/14 formulation suggested that GF was finely dispersed in the solid dispersion. *In vivo* investigations of the VCM-Gelucire 44/14 formulation did not show an increased bioavailability of VCM. On the other hand, *in vivo* studies of GF showed a 45% increase in bioavailability when fed to rats as GF-Gelucire 44/14 formulation compared to aqueous suspension of the drug, thus confirming the role of Gelucire 44/14 as bioavailability/absorption enhancer for class II drugs.

CHAPTER 1
INTRODUCTION

1.0 Oral Delivery of Drugs

An analysis of the drug delivery market reveals that oral products dominate more than half of it, reflecting their position as the preferred mode of drug administration (Brayden and O'Mahony, 1998). This preference stems from the ease of administration and convenience that this route imparts to the patients, leading to enhanced patient compliance. However, a drug that is delivered this way travels along the gastro-intestinal tract (GIT) and may or may not be absorbed into the systemic circulation depending on its fate after being acted upon by enzymes and other secreted substances, and its penetration through the epithelial cells.

The luminal surface of the GIT is covered by a single layer of columnar epithelial cells, which is interspersed with specialized cells such as goblet cells, endocrine cells and M cells. Spaces between adjacent epithelial cells are term the "tight junctions" and make up less than 1% of the intestinal surface area (Yeh *et al.*, 1998). Macromolecules such as peptides can be transported through this paracellular pathway via aqueous channels (Figure 1.1). Transcellular pathway, on the other hand, involves passive transcellular transport, carrier-mediated transport and endocytosis/transcytosis.

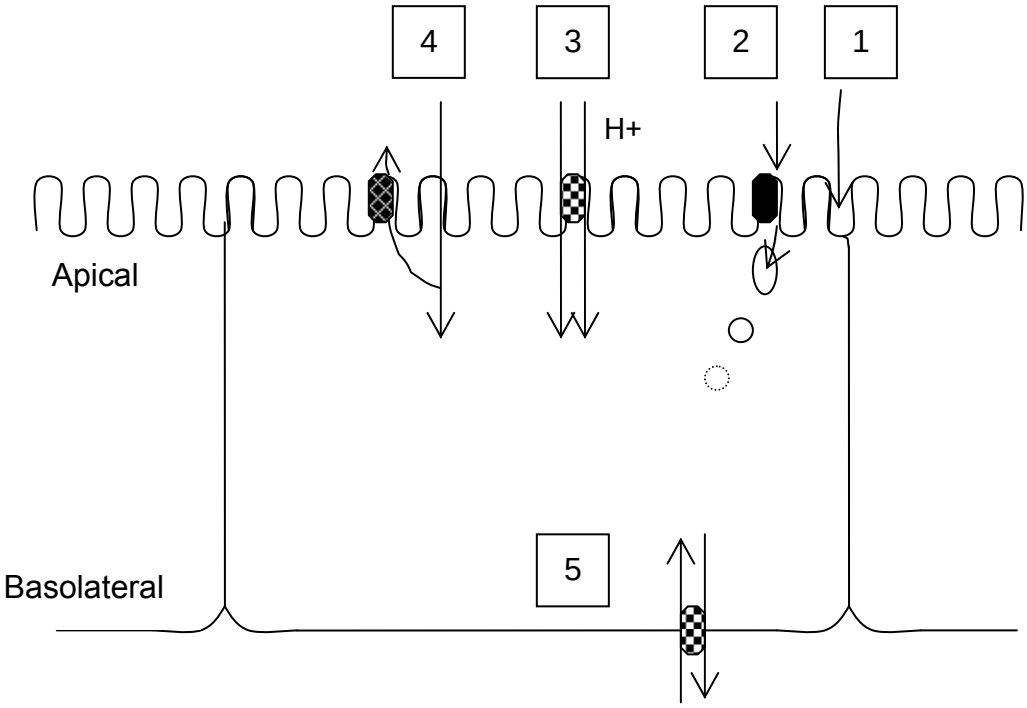

Figure 1.1: Potential pathways for peptide-like agents across intestinal epithelia.
1 Minor paracellular route, **2** Receptor-mediated transcytosis, **3** Carrier / transporter-mediated, **4** Passive absorption across apical membrane and may or may not be followed by P-gp efflux, and **5** Carrier / transporter-mediated basolateral efflux (adapted from Brayden and O'Mahony, 1998)

1.1 Delivery of Biopharmaceuticals

The importance of biopharmaceuticals is affirmed by their representation in approximately one in every four truly new molecular entities (NMEs), with sales going beyond the €30 billion mark annually (Walsh, 2005). Currently, biopharmaceuticals such as recombinant erythropoietin (EPO), human growth hormone (rhGH) and low molecular weight heparin are only available as injectables, and it is the target of drug delivery companies to produce novel oral formulations of these agents (Brayden and O'Mahony, 1998). Parenteral administration of peptides and protein drugs is associated with poor patient compliance in chronic conditions, which restricts its clinical usefulness (Lee and Sinko, 2000). There have been numerous investigations into viable routes of delivery for biopharmaceuticals as alternatives to the parenteral route. These include the pulmonary route through inhalation therapy, transdermal route through the usage of chemical permeability enhancers or electricity, or through the mucous membranes of the buccal, nasal, vaginal and rectal localities (Pettit and Gombotz, 1998). In spite of the availability of other delivery routes, the most popular way is through the oral route due to its ease of administration and better patient compliance to it during long-term therapy. Thus, in this introduction section, the review and discussion is focussed on the oral drug delivery route.

Despite the popularity of this route, the obstacles to successful oral delivery are numerous. Peptides can be degraded in the acidic environment of the stomach and metabolised by the luminal, brush border and cytosolic peptidases. The size, charge and hydrophilicity of peptides can adversely affect its permeability across the intestinal epithelium. Gastric transit time, dilution in GIT media and interaction with the contents of the intestine can interfere with

the contact between the peptides and the most absorptive part of the epithelium for it. The peptides can still be acted upon even after leaving the GIT, as the first-pass metabolism and entero-hepatic shunt elevates the risk of the peptides to be degraded. These factors contribute to the oral bioavailability of peptide drugs being typically less than 1-2% (Amidon and Lee, 1994; Zhou, 1994; Lagguth *et al.*, 1997). Due to these factors, oral drug delivery achievements associated with proteins and peptides have been limited, with the exception of cyclosporin (Haeberlin e*t al.*, 1996), a cyclic lipophilic peptide.

Some of the strategies adopted to circumvent these problems include applying enteric coating to the dosage form so that the drug remains protected from the harsh gastric environment by the intact polymer shell until it reaches the region of its absorption (Lee and Sinko, 2000). Proteolytic enzymes present in the small intestine will nevertheless limit the utility of this method (Lee and Yamamoto, 1990; Bai and Amidon, 1992; Woodley, 1994), unless protease inhibitors are delivered together, the local pH value is adjusted to the pH minima of the intestinal enzymes, or the enzymes are saturated by sustaining high drug concentrations (Friedman and Amidon, 1991; Bai *et al.*, 1995; Bai *et al.*, 1996). A reduction in intestinal pH by citric acid had led to the stabilisation of the administered salmon calcitonin, which in turn resulted in a higher oral absorption (Lee *et al.*, 1999). Absorption promoters that transiently and reversibly open the tight-junctions in the epithelium or that enhance delivery to the lymphatic system by forming chylomicrons could also be used. The use of absorption enhancers are not without risks as each class of agents has its associated adverse effects. Sodium taurodeoxycholate, lauroyl carnitine

chloride, Tween 80, myristoyl carnitine chloride among others, have been shown to promote the intestinal uptake of calcitonin but have also been linked to a reduction in transepithelial electrical resistance, indicating an acute toxic effect (Sinko *et al.*, 1999). Medium chain glycerides have also been shown to enhance intestinal permeability (Yeh *et al.*, 1994; Yeh *et al.*, 1995) but it was not clear whether the effect was via the paracellular pathway or the transcellular pathway. Tzan *et al.* (1993) have demonstrated that polypeptides with positive charge could cause increases in the tight junctional permeability but no similar effect was observed with negative or neutral polypeptides.

Other strategies include targeting receptors at the brush border of the intestine so that the biopharmaceutical would be uptaken upon the receptor activation. Enzyme-resistant peptide analogues could be synthesised to overcome the problem of peptide digestion. Bioadhesive formulations would allow peptides to be in contact with its most absorptive site without being prematurely cleared by gastrointestinal transit (Venkatesan *et al.*, 2006). Releasing the peptides in the colon would also be advantageous as the colon has a lower level of peptidases than ileum and the transit is slower in this part of the GIT. The peptides could be encapsulated in a polymeric casing that can be degraded by the bacterial flora in the colon, and this type of release offers the additional benefit of the time of release being more reliable as it is dependent on the local physiology, rather than timed release which could be affected by the variability of transit times between individuals. Pro-drugs, which are more hydrophobic and permeable could also be synthesised. Once inside the intestinal cells, the esterases in the cytosol would cleave particular linkages

to release the active peptides. More recently, the use of P-glycoprotein and cytochrome P450 3A inhibitors to suppress efflux and metabolism respectively has been investigated (Brayden and O'Mahony, 1998; Lee and Sinko, 2000).

1.2 Drug Delivery Systems

The importance of drug delivery strategies in formulating products with favourable properties has been heightened in the advent of biopharmaceuticals and other new chemical entities (NCE) with low solubility or permeability. Previously, work on drug delivery was centred on making existing drugs more effective or tolerable, as well as enhancing the ease of administration. More recently however, drug delivery techniques are incorporated at an earlier stage of the product development, with a view to turning the more "difficult" new drugs or compounds that traditionally would have been deemed as "failed" and discarded, into successful pharmaceutical products (Rosen and Abribat, 2005). With pharmaceutical research and development costs per approved agent estimated to be US$400-US$800 million (DiMassa *et al.*, 2003), it is of little surprise that the drug delivery approach is increasingly adopted to elevate the probability of success. In addition, formulating existing and classical drugs after the expiry of their patents, using new delivery technologies can further expand the drug market (Orive *et al.*, 2004). Such a potential can be illustrated by ceftriaxone, a third-generation cephalosporin antibiotic, which has properties of a class III drug according to the Biopharmaceutics Classification System (BCS), as defined by Amidon et al. 1995 (see Table 1.1). When the anionic ceftriaxone was physically complexed with a cationic analogue of deoxycholic acid and

formulated with propylene glycol, the oral bioavailability in rats was increased up to 70% due to the antibiotic being made more hydrophobic (Lee *et al.*, 2005).

Table 1.1: The Biopharmaceutics Classification System (adapted from Amidon *et al.*, 1995)

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

As previously discussed, biopharmaceuticals represent a challenge to the drug delivery field and various techniques have been devised to promote success especially in oral delivery. One example of such methods is the formation of biodegradable nano- and microparticles. The polymers used will slowly degrade, releasing drugs such as leuprorelin acetate or recombinant human growth hormone at a sustained rate, resulting in improved bioavailability. The minuteness of these systems promotes higher intracellular uptake, and covalently attaching folic acid to the surface to target folate receptors, can further enhance such uptake (Orive *et al.*, 2004). Being of such sizes could also allow the particles to move across the intestinal lining through the Peyer's

patches, which form part of the intestinal lymphatic system. It has been demonstrated that microspheres of about 100nm in size made from polylactic-*co*-glycolic acid (PLGA) could be taken up into Peyer's patches, with the efficiency of the uptake being 15-250 fold higher than larger particles. Peyer's patches was also found to have had 2-200 fold higher uptake than non-patch tissue from the same intestinal region, depending on the size of the particles (Desai *et al.*, 1996). When the microspheres were prepared using another biodegradable polymer, namely polyanhydrides, the particles demonstrated strong adhesive interactions with the mucous and cellular lining of the GIT. During their relatively long residency time at the surface of the intestinal epithelium, the particles were able to penetrate the cells, either paracellularly or transcellularly. This occurs both at the mucosal absorptive epithelium and the follicle-associated epithelium covering the Peyer's patches (Mathiowitz *et al.*, 1997). In addition to opening up an alternative route to drug uptake, encapsulation of biopharmaceuticals in the form of microspheres made from degradable polymers has permitted the drug to be released in a more sustained manner, with the carrier simply breaking down into biologically inert by-products.

Incorporating lipid materials into self-dispersing formulations has become a popular approach to enhance oral bioavailability especially for lipophilic drugs. These drugs fall into the class II category of the Biopharmaceutics Classification System (BCS), and poor dissolution would represent the rate-limiting step in the absorption process. Nevertheless, lipid based formulations have been found to be also useful in the oral delivery of class III bioactives, such as

biopharmaceuticals. New *et al.* (1997) designed and developed a water-in-oil emulsion, termed the Bridgelock formulation, which was capable of increasing the bioavailability of salmon calcitonin in surgically manipulated pig models. The peptide was incorporated into the aqueous phase that contained phosphatidyl choline and other phospholipids, whilst the oil phase was composed of oleic acid, lecithin, glycerol monooleate and cholesterol. The components used in the emulsion were selected for their ability to stimulate lymph flow and form chylomicrons. Thus, in addition to passing directly into systemic blood system via the microvasculature surrounding the intestinal tissue that in turns feeds into the hepatic portal system, the peptide can associate itself to the oil phase that is then uptaken via the lymphatics. This was also shown by Ling and co-workers (2006), where the lymphatic absorption of cefotaxime liposomal formulation was confirmed to play a role in the increased bioavailability of this peptidomimetic drug.

The lymphatics is an attractive system to target in drug delivery as an auxiliary absorption route. Lymph is estimated to flow from the intestine at a rate of about 100ml/hour in an active person, so that a substance contained within the lymph can reach the bloodstream in a short period of time due to the active circulation of interstitial fluid (New *et al.*, 1997).

1.2.1 Gelucire 44/14 as a Self-Emulsifying Drug Delivery System

Self-Emulsifying Drug Delivery Systems (SEDDS) have been defined as homogenous mixtures of natural or synthetic oils, solid or liquid surfactants or one or more hydrophilic solvents and co-solvents (Gershanik and Benita, 2000).

SEDDS require nominally no energy to emulsify. As a point of interest, the first self-emulsifying product to reach the market was Dettol by Reckitt&Colman (confidential internal report).

After few commercial successes, such as Sandimmune-Neoral (cyclosporin A), Solufen Lidose (ibuprofen), Fortovase (sequinavir) and Norvir (ritonavir), SEDDS received increased attention by formulation scientists as a useful tool to overcome the low oral bioavailability of newly discovered drugs characterised by low water solubility (Gursoy and Benita, 2004). In particular, a subset of SEDDS, which is Self-MicroEmulsifying Drug Delivery Systems (SMEDDS), show even better absorption due to smaller droplet sizes, as reviewed by Gursoy and Benita (2004). To date, one of the most common lipids to be associated with SMEDDS is Gelucire 44/14, which is a member of the polyglycolised glycerides family of excipients.

Gelucire 44/14 is derived from the reaction of hydrogenated palm kernel oil with PEG 1500 and conforms to the requirements of the European Pharmacopœia 4th Edition (2002) under the “lauroyl macrogolglycerides” monograph. Gelucire 44/14 comprises about 20% mono-, di- and triglycerides, 72% mono- and di- fatty acid esters of PEG 1500 and 8% of free PEG1500. A schematic view of the fatty acid components of Gelucire 44/14 is shown in Table 1.2 (Gattefossé, 1999).

Gelucire 44/14 has been studied as a lipid carrier for several drugs characterised by low solubility. UC 781, an anti-HIV compound, has intrinsically

poor solubility but this was increased by formulating it with Gelucire 44/14 (Damian *et* al., 2000; Deferme *et al.*, 2002; Damian *et al.*, 2002). However, problems of stability of UC 781 in the lipid matrix and aging effect of the formulations precluded further developments. Increased solubility was also obtained for the antiviral drugs DMP 323 (Aungst *et al.*, 1997), EMD 50733 (a lipophilic compound with intermediate log P) which *in vivo* bioavailability registered a 10-fold increment compared to the drug/lactose mixture (Schamp *et al.*, 2006) and for an undisclosed experimental compound (Joshi *et al.*, 2004).

Table 1.2: Fatty acids composition in Gelucire 44/14 (Gattefossé, 1999)

Fatty acid distribution	Gelucire 44/14
Caprylic acid (C8)	4-10%
Capric acid (C10)	3-9%
Lauric acid (C12)	40-50%
Myristic acid (C14)	14-24%
Palmitic acid (C16)	4-14%
Stearic acid (C18)	5-15%

SMEDDS formulations containing Gelucire 44/14 and incorporating numerous other drugs such as phenytoin and indomethacin (Kawakami *et al.*, 2006), a ceftriaxone complex (Cho *et al.*, 2004), piroxicam (Yüksel *et al.*, 2003; Karatas *et al.*, 2005), carbamazepine (Sethia and Squillante, 2004), griseofulvin (Yang *et al.*, 2007), and halofantrine (Khoo *et al.*, 2000; Abdul-fattah *et al.*,

2002) have been investigated. Khoo *et al.* (2000) found that even when the dissolution profile of halofantrine formulated with PEG 6000 was found to be better than the Gelucire 44/14 formulation, the latter formulation showed increased bioavailability compared to the former when investigated *in vivo*. In a study of α-tocopherol (Barker *et al.*, 2003), it was shown that the Gelucire 44/14 formulation was able to provide a 2-fold increase in bioavailability of this nutraceutical compared to the commercially available preparation.

In the previous instances, the aim of the researchers was to increase the oral bioavailability of these drugs mainly by increasing their solubility, as low solubility is reputed to be one of the main factors for the low bioavailability of poorly water-soluble drugs (Humberstone and Charman, 1997). Hauss *et al.* (1998), on the other hand, investigated a Gelucire 44/14 formulation containing ontazolast, also a poorly water-soluble compound, with the view to evaluate the lymphatic transport of the drug. Even though the SEDDS formulation provided the fastest absorption (lowest t_{max} and highest C_{max}) of ontazolast, the lymphatic transport, however, did not appear to play a significant role in the increased bioavailability, possibly due to the size of the formed micelles.

Koga *et al.* (2002) investigated the effect of Gelucire 44/14 and Labrasol at low concentrations on the membrane permeability of isolated rat intestine using cephalexin and cefoperazone as model drugs. Their results showed that Gelucire 44/14 did not enhance the membrane permeability of either drug as opposed to Labrasol, which enhanced the membrane permeability and intestinal absorption. Since the HLB for both Gelucire and Labrasol is 14, the effect of

HLB of non-ionic surfactants on membrane fluidity is not thought to be a factor here; instead, the enhancement by Labrasol was thought to be due to its promotion of the passive transport as well as the active transport via ion flux. The authors explained that the passive and active transports were in turn, affected by the particle size and polydispersity index of the emulsion droplets. It may be interesting to note that Labrasol is almost exclusively composed of caprylic and capric acids (approximately 58% and 41% respectively) while for Gelucire 44/14, the same two fatty acids are present as minor components (see Table 1.2).

Delivery of protein and peptide-like biopharmaceuticals have also been investigated using lipid based formulations. In fact, Mori *et al.* (2004) studied Labrasol and Gelucire 44/14 as absorption enhancers using low molecular weight heparin as model drug and showed that the formulation containing Labrasol resulted in higher bioavailability of the drug. Recombinant human erythropoietin (rhEPO) was prepared as nanoparticles for intra-jejunal administration (Venkatesan *et al.*, 2005) and patches for local delivery (Venkatesan *et al.*, 2006), using Labrasol and Gelucire 44/14 in both studies; in both cases Gelucire 44/14 enhanced the bioavailability of rhEPO, even though to a lesser extent compared to Labrasol. However, their studies confirmed the capability of Gelucire 44/14 to increase the bioavailability of protein-based compounds with a mode other than simply increasing the drug solubility. Even though the mechanism is still not clear, it is thought that microemulsions formed may protect the drug from degradation/denaturation in the gastric environment.

The method of dosage form preparation was also thought to influence the overall bioavailability of the model drugs employed. Chauhan *et al.* (2005) prepared physical mixture and solid dispersion of glibenclamide and Gelucire 44/14 and showed that the solid dispersion performed better in terms of the therapeutic efficacy of the drug. They related the favourable result of the solid dispersion to the presence of glibenclamide in the amorphous state. In another investigation, the cryogenically grinded powder of a solid dispersion containing ketoprofen and Gelucire 44/14 was evaluated against the non-grinded form (Chambin *et al.*, 2004). However, it was demonstrated that these two preparation techniques led to non-significant difference in the *in vitro* release of ketoprofen.

1.2.2 Advantages of Gelucire 44/14

Lipid microemulsions have the potential to offer protection against enzymatic hydrolysis and may enhance absorption of drugs (Constantinides, 1995). A schematic view about which potential microemulsion could be prepared for drugs belonging to the different classes of the BCS is given in Table 1.3. For a definition of the BCS categories please refer to Table 1.1.

Table 1.3: Potentially useful microemulsion for biopharmaceuticals of different classes of the BCS (adapted from Constantinides, 1995)

Class	Potential microemulsion	Potential advantages
I	W/O	Stabilization and protection against chemical and enzymatic hydrolysis
II	SEDDS; O/W	Improved solubilisation and dissolution, increased BA
III	W/O	Stabilization and protection against chemical and enzymatic hydrolysis, increased BA
IV	SEDDS; O/W	Improved solubilisation and dissolution, increased BA

Gelucire 44/14, being a semisolid waxy material, offers an important advantage over Labrasol which is in liquid form in that can be loaded into hard gelatine capsules. In fact, from an industrial point of view, it is more economical and simple to prepare liquid filled hard gelatine capsules than liquid filled soft gelatine capsules.

1.3 Glycopeptide Antibiotics: Vancomycin

Vancomycin was the first compound discovered belonging to a new class of antibiotics: the glycopeptides. VCM was isolated in the 1950s by Eli-Lilly in a culture of *Nocardia orientalis* (later reclassified as *Amicolatopsis orientalis*). Scientists succeeded only in the 1980s to finalise the structure of VCM due to its complexity and presence of a significant number of functional groups (Figure 1.2). The general structure of glycopeptide drugs is characterised by the presence of a sugar linked to a polypeptide ring (Nagarajan, 1994). In particular,

VCM has an aminodisaccharide linked to a heptapeptide ring, which gives rise to the possibility of multiple charges once ionised.

VCM is an amphoteric molecule containing charged amino-, carboxyl- and phenolic groups, more specifically: three phenolic groups with pK_{a1} 10.6, pK_{a2} 10.3 and pK_{a3} 9.4; two amino groups (as N-terminal and aminosugar, respectively) with pK_{a1} 8.6 and pK_{a2} 6.8; one C-terminal carboxylate with pK_a 2.5 (Sitrin *et al.*, 1985).

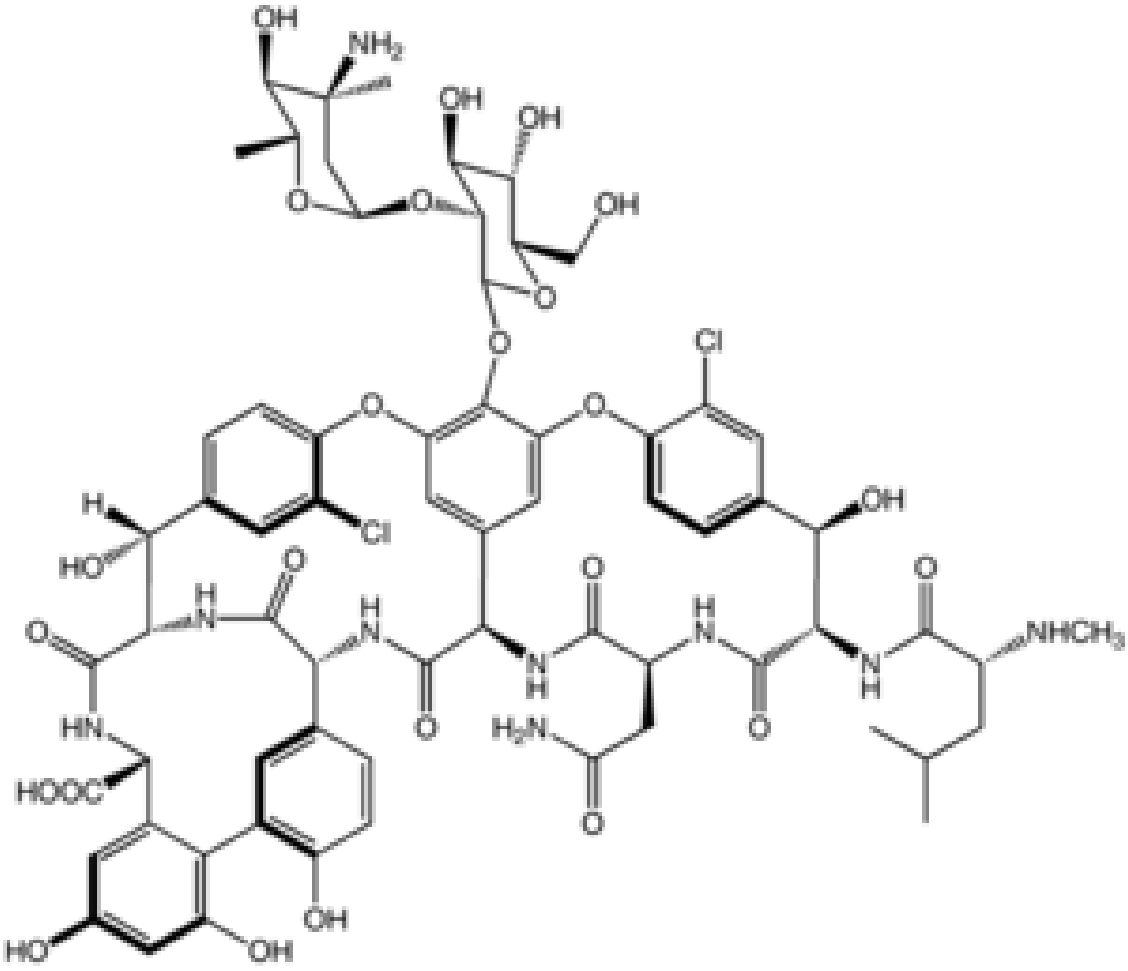

Figure 1.2: Diagrammatic representation of the structure of vancomycin

1.3.1 Properties of Vancomycin

At levels near its minimum inhibitory concentration (MIC), VCM is bactericidal to most of the bacteria that show susceptibility to it. The antibiotic achieves its function by inhibiting bacterial cell wall peptidoglycan synthesis, but targeting a location that is different from that of β-lactam antibiotics (Russell, 1998). In particular, VCM inhibits bacterial cell wall synthesis at a different site than penicillin and cephalosporins by binding to D-Ala-D-Ala portion of the cell wall precursor; this leads to destruction of bacterial cell by lysis; VCM may also alter the permeability of bacterial cytoplasmic membranes and may selectively inhibit RNA synthesis (USP Drug Information 14th Ed., 1994). VCM has a half life, $t_{1/2}$, of 2.9-9.1h (USP 24, 2000).

For systemic infections, the antibiotic must be administered via the intravenous route as it is poorly absorbed when taken orally. VCM penetrates well into most body fluids except possibly aqueous humour and cerebrospinal fluid, and is excreted mainly via the kidneys (Moellering, 1984). In humans, VCM efficacy is related to the 24-hour AUC-MIC as the important pharmacokinetic-pharmacodynamic (PK-PD) parameter (Craig, 2003).

1.3.2 Uses of Vancomycin

Currently, one of the important uses of VCM is for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. This intrinsic multidrug resistance expression by *S. aureus* has been postulated to be due to the activation of the *sarA* locus on the chromosome of the microorganism (O'Leary *et al.*, 2004). The number of outbreaks of MRSA, up to epidemic

proportions, are alarmingly increasing. These usually occur in hospitals and once MRSA is established in the high-dependency units such as intensive care, burns and cardiothoracic units, the pathogen then spreads readily to the rest of the patients and hospital staff. Together with teicoplanin, another glycopeptide antibiotic albeit about three times more expensive, vancomycin remains as the recommended treatment for these infections (Finch, 1998). Intravenous antimicrobials are generally more expensive than the oral forms, thus compounding to the factor that the glycopeptides are already more costly than aminoglycosides and beta-lactam antibiotics. Their increasing inclusion in treatment protocols will have a clear economic impact on health systems (Janknegt, 1997).

MRSA can cause complicated skin and soft tissue infections which treatment will most probably involve hospitalisation. In addition to the cost of the medications to be given to the infected patients, prolonged hospital stay will present another substantial economic burden to the health care system. Advocating earlier hospital discharge will greatly reduce the overall costs (Nathwani, 2003). A novel oxazolidinone antibiotic, linezolid, had been demonstrated in a clinical trial to be as effective as vancomycin in MRSA infections. The difference between the two antibiotics is that linezolid is 100% bioavailable in oral forms whereas vancomycin is not absorbed. When the two drugs were compared head to head in a randomised clinical study that evaluates the length of hospital stay of patients with complicated skin and soft tissue infections, linezolid was found to reduce the number of days in hospital significantly (Li *et al.*, 2003), with increased chances of being discharged within

the first week of hospitalisation (Nathwani, 2003). This could be due in part to the patients receiving linezolid being able to follow up on their intravenous drug administration with oral medication, which they may take at home, as opposed to the intravenous only vancomycin that often means confinement in the wards. It has been previously suggested that if patients were allowed to leave the hospital and complete their therapy with an oral formulation of a highly bioavailable antibiotic, a reduction in treatment costs could be achieved in addition to improving the efficacy of existing older antimicrobial agents (Niederman, 2001). This protocol was repeated in a multinational, multicentre, randomised clinical study and a comparable result to the above was found, with patients treated with linezolid significantly spending less time in hospitals compared to those treated with vancomycin and significantly more patients on linezolid being discharged within the first two weeks (Itani *et al.*, 2005).

VCM powder for injection dissolved in phosphate buffer (Itoh *et al.*, 2001), normal saline or phosphate buffered artificial tears (Fleischer *et al.*, 1986) was found to be effective against MRSA in eye infections. When formulated with Pluronic F-127 (ethylene oxide and propylene oxide), the drug was able to be applied into the ear for the treatment of chronic otitis media caused by MRSA (Lee *et al.*, 2004). The polymer solution exists as a liquid at room temperature allowing it to be drawn into a syringe, and once subjected to higher temperatures such as the body temperature inside the ear, the formulation changes into a gel from which VCM can be released in a sustained manner.

Another of VCM uses is for the treatment of osteomyelitis, which could have come about as a consequence of open fractures or surgery. Indeed, many of the more recent research involving VCM and formulation of delivery systems for the drug have been centred on this field of concentrating this anti-staphylococcal agent in the bone area. A localised VCM delivery system involving implantantion of drug loaded chitosan microspheres has been described (Cevher *et al.*, 2006). Biodegradable microspheres were obtained by mixing the chitosan solution with VCM and spray drying the mixture. By targeting the drug at the potential site of infection, side effects associated with systemic toxicity could be avoided and the biodegradability of the polymer used eliminates the need of surgical removal of the implant.

The use of an alternative polymer, poly(lactide-co-glycolide), has also resulted in a successful formulation of biodegradable microparticles loaded with VCM for bone implantation (Billon *et al.*, 2005). A sustained drug release over 10 days was reportedly achieved using this delivery system and the workers postulated that the microparticles could be injected together with biphasic calcium phosphate granules to form a bone substitute. Earlier investigations (Iooss *et al.*, 2001; Le Ray *et al.*, 2003) had resulted in the development of VCM loaded poly(ε-caprolactone) microparticles. The 200μm sized microparticles were obtained through simple emulsion evaporation/extraction process with manual dispersion of the drug, and together with biphasic calcium phosphate granules formed an injectable bone substitute that was capable of releasing the VCM at a controlled rate, hence eliminating the risk of infection at the site of implantation.

One of the commonest nosocomial infections are post-operative infections and VCM is the drug of choice to counter these surgical complication when methicillin-resistant *S. aureus* and *S. epidermidis* are implicated (de Lalla, 1999). Such complication becomes more pronounced in cases where a foreign object is introduced into the body and left there for an indefinite duration of time. A literature search with the keywords of “vancomycin” and “drug delivery” reveals a plethora of work focussing on prostheses or devices impregnated with VCM by Chilukuri and Shah (2005), Kelm *et al.* (2006) amongst others, various slow-release systems designed to be applied to the potentially affected site by Radin *et al.* (1997), Drognitz *et al.* (2006) amongst others, and matrices to treat osteomyelitis, a few of which have been outlined above.

The intensifying efforts in this field demonstrate that research laboratories are cognisant of the substantially increasing VCM use in the last few years due to colonisation of bone and prosthetic devices by MRSA. A sol-gel formulation of VCM in Poloxamer 407 (polyoxyethylene-polyoxypropylene) was found to release adequate amounts of the antibiotic to be effective against *S. aureus* even up to eight days *in vivo* in rats. At low temperatures, the formulation is in liquid form, allowing it to be drawn up into a syringe and administered to the tissue surrounding a foreign body. At body temperature, the formulation changes into a semisolid gel from which VCM is released in a sustained manner (Veyries *et al.*, 1999), not unlike the VCM in Pluronic F-127 previously described (Lee *et al.*, 2004). Moreover, the Poloxamer 407 formulation is thought to be capable of preventing the adhesion of bacteria to the foreign object in the body.

In paediatric medicine, VCM is still the treatment of choice for severe MRSA infections (Ladhani and Garbash, 2005). VCM is also given as an adjunct in the management of febrile neutropenia when first-line empirical therapy has failed (Ziglam *et al.*, 2005). The emergence of *Streptococcus pneumoniae*, which is resistant to many antibiotics, was thought to be in part due to over-exposure to these antimicrobials especially in relation to the respiratory tracts of children. Morbidity and mortality occurs significantly when the infection with this microorganism causes pneumonia and meningitits. Resistant pneumococcal infections occur at a higher rate amongst patients with malignancies, HIV co-infection and sickle cell disease. VCM is the only antimicrobial that the microorganism has not shown resistance to (Jacobs, 2004).

Since VCM is poorly absorbed when given via the oral route, the intravenous route has been the only option for treating generalised systemic infection (Moellering, 1984). Oral use of VCM has so far been restricted to the treatment of local infections in the gastrointestinal tract (GIT). A and B toxins, produced by *Clostridium difficile*, can cause pseudomembranous colitis and drug therapy indicated for this is oral vancomycin, 125mg four times daily for 10 days (Farthing *et al.*, 1994). Conversely, inflammatory bowel diseases such as the *Clostridium difficile* induced enterocolitis can actually lead to an increased absorption of oral VCM (Cadle *et al.*, 2006). Novel oral formulations, such as the one described by Musenga *et al.* (2005), frequently focuses on the delivery of VCM to a particular site of infection in the GIT. Albumin nanospheres loaded with the antibiotic and coated with stearic acid were found to give adequate

protection to the drug and release the drug at the targeted site, colon. On another note, oral VCM has been associated with an improvement in children with late-onset autism, as this condition may involve the presence of abnormal flora in the GIT (Finegold *et al.*, 2002).

1.3.3 Other Formulations of Vancomycin

Microcapsules made from poly d,l-lactic acid (PLA) and poly(*D*,*L*-lactide-co-glycolide) (PLGC) and loaded with VCM were prepared through the formation of water-oil-water (w/o/w) double emulsion and solvent evaporation technique. The bactericidal effect on *S. aureus* were found to be sustained for 4 days (Ozalp *et al.*, 2001) but it was unclear what the final route of administration or target site of the microcapsules would be. Other VCM w/o/w emulsions were prepared either from sesame oil and monoglycerolstearate (Shively and Thompson, 1995) or using Lipiodol-isopropyl myristate for IM administration (Okochi and Nakano, 2000). Even if the relative BA of VCM was significantly increased, these formulations were of complex preparation and stability issues were unresolved.

A self-micro-emulsifying (SMEDDS) formulation of VCM was prepared using Labrasol and D-α-tocopheryl-PEG 1000 succinate by Rama Prasad *et al.* (2003) and, once administered intraperitoneally, showed significant plasmatic levels of VCM compared to the saline solution. However stability data of the formulation are not available.