1. INTRODUCTION

1.1. Lipid based formulations for poorly water soluble drugs

It was realized that the oral bioavailability of poorly water soluble drugs (lipophilic drugs) may be enhanced when co-administrated with a meal rich in fat[38]. This has led to increasing the interest in the formulation of poorly water soluble drugs in lipids in the form of suspensions, solutions, emulsions[39]. and as self-emulsifying formulations[40]. as an approach to enhance drug solubilization in the gastrointestinal tract (GIT)[14]. Hence relatively few lipid-based products have been commercialized (examples include Neoral® (cyclosporine), Norvir® (ritonavir), Fortovase® (saquinavir) and Agenerase® (amprenavir).

1.2. Lipid digestion processing

Digestion of exogenous lipids (i.e. food or formulation derived) begins in the stomach where triglycerides (TG_s) are hydrolyzed into di-glycerides (DG) and fatty acids (FA) by acid-stable lipases; lingual and gastric lipase leading to initial lipid emulsification. This finally results in lipid droplet size 1-100 μm facilitated by gastric agitation, gastric emptying and promoted by the presence of dietary phospholipids (PL), proteins and amphipathic products of partial TG lipolysis which act together to stabilize the oil/water interface in the GIT[41, 42].

In the duodenum, emulsified lipids stimulates the secretion of bile salts (BS), PL and cholesterol (Ch) from the gall bladder and pancreatic fluids (containing pancreatic lipase/co-lipase) from pancreas [43]leading to further lipolysis into one molecule of 2-monoglyceride (MG) and two FA molecules for each TG molecule[44] . In addition, 2-monoglycerides can undergo a non-enzymatic isomerization to form 1-monoglyceride which can be further hydrolyzed to glycerol and FA [45]where the relative prevalence of TG hydrolysis products has been reported to be 22% glycerol, 72% 2-MG and 6%1-MG[44]. Physical changes are accompanied with lipolysis including presence of mixed micellar phase containing BS and PL[44], unilamellar/multilamellar vesicles in the presence of BS[46, 47] and hexagonal liquid crystals during digestion in intestinal environment model [48, 49].

1.3. Drug solubilization

The formed lipidic micro environment from lipid solution to a series of colloidal species including micelles, mixed micelles, vesicles and emulsion droplets[50] into which co-administrated poorly water soluble drugs may partition thus provides a reservoir of solubilized drug at the absorptive site[51]. In addition, this generates a concentration gradient required to drive improved absorption (Figure 5). It was reported by Kossena *et al*[52] the impact of lipid concentration and colloidal structure on drug solubilization pattern was explored using a series of lipophilic steroid esters.

In recent years, self-emulsifying drug delivery systems (SEDDS) have been increasingly employed to enhance the oral bioavailability of poorly water soluble drugs. Self-emulsifying formulations comprise isotropic mixtures of natural or synthetic oils with lipophilic or hydrophilic surfactants and co-solvent(s) which spontaneously emulsify when exposed to the fluids of the GIT to form O/W emulsions or microemulsions[40, 53-55].

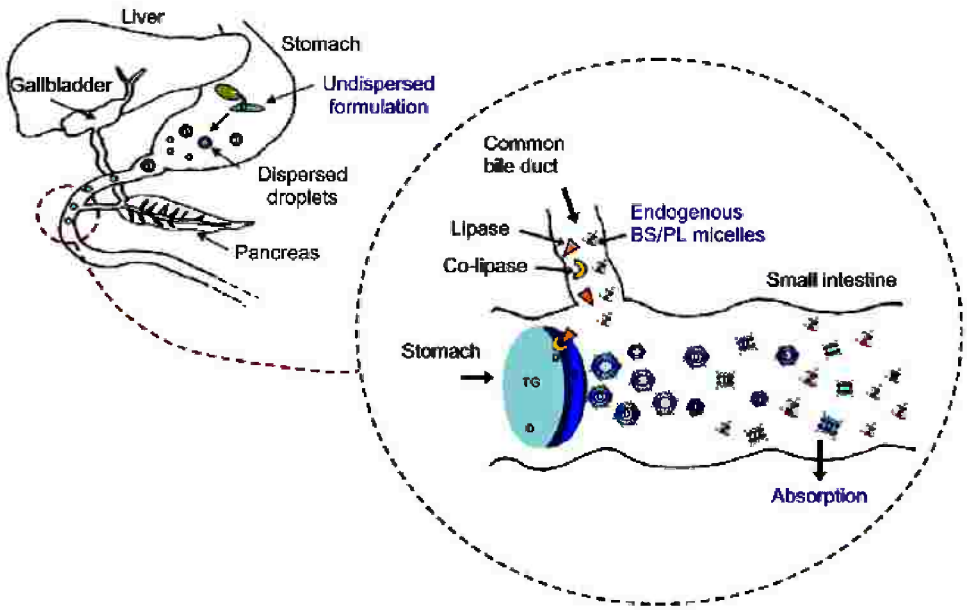

Figure 5: Lipid digestion and drug solubilization in small intestine.

Due to the large number of possible excipient combinations that may be used to assemble lipid-based formulations and self-emulsifying systems in particular, a classification system (the lipid formulation classification system LFCS) was established by Pouton[56] in 2000 and an extra 'type' of formulation was added in 2006[55, 56] to help in stratify formulations into those with similar component parts. LFCS is divided into 4 types; Type I, Type II, Type III (A, B) and Type IV. The characteristics for each class are illustrated in Table 2.

1.4. Formulation of lipid formulation classification system[57]

1.4.1. Formulation of Type I system

Typically they are blends of food glycerides derived from vegetable oils like long chain triglycerides (LCT) as soya bean oil and medium chain triglycerides (MCT) as triglycerides of caprylic/capric acid (i.e. Miglyol 812™), which are safe for oral ingestion, rapidly digested and absorbed completely from intestine. Because Type I formulations do not contain surfactant, they have very limited ability to self-disperse in water. Thus, they depend on digestion to facilitate colloidal dispersion by solubilization of digestion products in mixed micelles.

In vitro lipolysis experiments reveal that the increase in BS concentration enhances the lipolysis process for MCT and LCT[51]. Whether lipophilic drug is solubilized or suspended in the oily vehicle[58], Type I formulations are an excellent option in enhancing bioavailability as Type II and Type III formulations, and Type I formulations certainly have advantages in relation to safety and drug stability.

Table 2: Characteristics of lipid formulations[59].

Type	Materials	Characteristics	Advantages	Disadvantages
Type I	Oil without surfactants	Non dispersing, requires digestion	Stable and safe for oral ingestion, excellent capsule compatibility	Poor solvent capacity, Mostly suitable for lipophilic drugs
Type II	Oil and water insoluble surfactant	Self emulsifying drug delivery system (SEDDS) and may be formed without water soluble components	Retain solvent capacity	Coarser emulsion (particle size 0.2-2 μm)
Type III_a	Oil and water soluble surfactants	SEDDS/Self micro emulsifying drug delivery system (SMDDS) formed with water soluble components	- Almost clear dispersion (particle size approx: 100-250 nm)[53] - Drug absorption without digestion	Likely to lose solvent capacity and less easily digested[57]
Type III_b	Oil, water soluble surfactant with co-solvents		- Transparent dispersion with particle size : 50-100 nm)[56] - Drug absorption without digestion.	- Likely to lose solvent capacity on dispersion - May cause partial drug precipitation - Leases easily digested
Type IV	Water soluble surfactants and co-solvents (no oils)	Formulation disperses typically to form a micellar solution	This system has good solvent capacity for many drugs	- Likely loss of solvent capacity and increase risk of precipitation - May not be digestable and well-tolerated and chronic administration[55, 60]

1.4.2. Formulation of Type II system

This type of formulations is compromised from oils, polar oils and water-insoluble surfactants. It is suggested that the performance of these formulations as SEDDS relies on the formation of a dispersed lamellar liquid crystalline phase at low water contents (5-10%) which aids further penetration of water causing interfacial disruption.

Although digestion of Type II systems has not been studied adequately, it is believed that digestion of oily part in this type of formulations plays part in the fate of Type II systems inside the intestine. The influence of the surfactant component on phase behavior remains to be examined. As Type II system formulated with long chain triglycerides produces coarser emulsion by self dispersion.

1.4.3. Formulation of Type III system

This group has been further divided into Type IIIA and Type IIIB to distinguish between formulations which contain significant proportion of oils (Type IIIA) and those which are predominantly water-soluble (Type IIIB). The distinction between Types IIIA and IIIB was based arbitrarily as a starting point for discussion on the proportions of typical excipients in formulations.

Including co-solvents to the formulations of Type IIIB is to increase solvent capacity of the formulations for drugs which dissolve freely in co-solvents. However to enhance the solvent capacity significantly the co-solvent must be present at high concentration and this is associated with the risk for drug precipitation when the formulation is dispersed in water.

Such formulations have the potential to disperse quickly to form submicron dispersions. These submicron dispersions are often fine enough to form transparent dispersions. It was shown that Type IIIB formulations tend to lose its solvent capacity in in-vitro experiment upon dilution with water leading to drug precipitation. There is a good reason to expect that this formulation would be less successful in-vivo than Type III A formulations. However, it does not address the fate of the drug on digestion and this emphasis the need for detailed studies on digestion of Type III systems.

1.4.4. Formulation of Type IV system

Type IV systems are essentially compromised from pure surfactants or mixtures of surfactants and co-solvents. Type IV systems may be the chosen approach for drug candidate possess much more solubility in a given hydrophilic surfactant. Such disadvantages for applying such formulation type summarized in disability to dissolve rapidly upon dispersion due to formation of viscous liquid crystalline (or gel crystalline) phases at the surfactant-water interface and its irritant effect and poorly toleration in the gastrointestinal tract as adhesion of a partially dissolved viscous mass rich in surfactant to the wall of the stomach or intestine could do considerable local damage.

Blending of water soluble surfactants with co-solvents aids the dispersion of surfactant and reduces the loss of solvent capacity.

1.5. Assessment and evaluation of lipid based formulations using *in vitro* lipolysis

In recent years, in-vitro dispersion tests and in-vitro lipid digestion models are more reflective of the gastrointestinal environment. They have been developed to better predict the *in vivo* dissolution profile of poorly water soluble drugs[55, 61-63]. Assessment of the utility of lipid-based formulations is based on evaluation of the rate and extent of drug precipitation with respect to time (rather than solubilization). In this case, in-vitro dispersion tests appear to be more accurate description of the process of monitoring the ability of formulation to maintain the drug in a solubilized state during dispersion in the stomach and processing of the formulation in the presence of pancreatic and biliary fluids[44].

Porter *et al*[64] described the correlation between *in vitro* lipid digestion model and plasma profile of danazol after oral administration of SMEDDS formulations compromised of long chain lipids (LC-SMEDDS) and medium chain lipids (MC-SMEDDS), results revealed that about two thirds of the drug dose precipitated on in-vitro digestion of MC-SMEDDS formulations. However, the majority of the dose was maintained in solution in case of LC-SMEDDS. These findings were lined to the in-vivo performance for such formulations where the plasma level of danazol from LC-SMEDDS was much higher than MC-SMEDDS.

For Type II and Type III formulations, the highest concentration of surfactant and the lowest proportion of long chain lipid were least effective in maintaining drug solubilization. Thus increasing the proportion content of surfactant decreased drug solubilization[65].

Cuine *et al*[66] studied the impact of surfactant digestion on formulation performance. It was shown that all fatty acid-ester surfactants were to a lesser or greater extent digestible. Also, Cremophore RH 40 was considerably more resistant to precipitation on digestion.

For Type IV formulations, where the lipid content is minimized and surfactant content is maximized. It was shown that low oral bioavailability after in-vivo administration for Type IV formulations for donazol in Cremophore RH 40 and Cremophore EL[66].

For Cremophore RH 40, it was attributed to formation of liquid crystal on the surface of dissolving surfactants which in turns limit water penetration into the surfactant mass. Hence it reduces dispersion or dissolution of surfactant rich formulations[40]. On other hand Cremophore EL was known to be less resistant for drug precipitation. From this point of view, it was suggested that inclusion of co-surfactants or co-solvent will promote the dispersion for such formulations.

1.6. *In vitro* digestion assessment for suspension formulations in comparison with solubilized formulations

It was concluded by Kaukonen *et al*[58], those suspensions of drugs which are poorly soluble in water and TG lipid (i.e. Etodolac), may prove the same beneficial properties of drug in solubilized form. However for more lipid-soluble drugs, suspension formulations may offer little benefit as sufficiently high drug loadings can otherwise be achieved with simple solution formulations that still provide for adequate solubilization after TG digestion. From this point of view, Etodolac was selected for subsequent formulations according LFCS.

1.7. Etodolac as a model drug

Etodolac is a racemic mixture of [(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-6]indol-1yl)acetic acid. It is encountered as a weak acidic drug having a pKa of 4.65 as shown in Figure 6. It is one of a non-steroidal anti-inflammatory drugs ($NSAID_s$) which inhibits selectively cyclo-oxygenase-2(COX-2) enzymes (i.e. 10-fold selectivity over COX-1 enzymes)[67] knowing that COX-1 enzymes are responsible for the production of prostaglandins involved in cytoprotection of gastric mucosa and the renal blood flow regulation. Thus etodolac safely treats inflammatory disorders including rheumatoid arthritis, osteoarthritis and acute pain at oral dose of 200 mg twice daily; up to 1200 mg[68] without gastric irritation, ulceration, or bleeding[69].
Also etodolac is reported in the treatment of gout by lowering uric acid blood levels in humans[70, 71]. In addition, recent clinical studies revealed that etodolac chemically induced anti-carcinogenic effects on various human cancer cells[72-74].

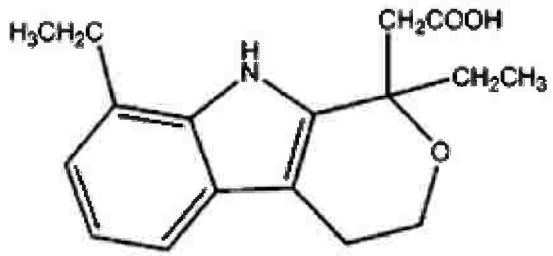

Figure 6: Chemical structure of Etodolac [67].

Although etodolac is practically water insoluble drug, it is absorbed from the gastro-intestinal tract with peak plasma concentrations being attained about 1 – 2 h after ingestion[75]. Thus, in accordance with biopharmaceutical classification system (BCS), etodolac is considered to belong to the class II drugs; which characterized by low solubility and high permeability[76, 77]. From this point of view, the rate limiting step in its absorption process is the dissolution rate in GIT fluids rather than the rapidity of their diffusion across the gut wall. Therefore, by improving the release profiles of etodolac, it is possible to increase its bioavailability and minimize its side effects[78, 79].

There were many attempts to enhance the dissolution rate of orally administrated drugs (i.e. Class II) by producing solid dispersion of the drug with additives[80],making liquisolid formulations[81], increasing surface area of drug particles via micronization and nanosizing[82, 83], complex formation of cyclodextrin[84] and water soluble prodrug formation[85].

For etodolac as a model drug of BCS class II, many approaches to enhance its dissolution rate via increasing its aqueous solubility as: etodolac-dextran conjugate (ED)[86], Solid dispersion of etodolac with PEG[75], Formation of melt dispersion with Gel 44/14 and TPGS[87]and Formation of self-emulsifying delivery system (SEDDS) of etodolac[88]