INTRODUCTION
1. INTRODUCTION

In the present scenario, oral drug delivery is continuously looking into newer avenues due to the realization of the factors like poor drug solubility and/or absorption, rapid metabolism, high fluctuation in the drug plasma level and variability due to food effect which are playing major role in disappointing in vivo results leading to the failure of the conventional delivery systems (Vieth M et al., 2004).

Fig.1. Biopharmaceutical classification system (BCS). The x-axis shows the volume (ml) required to dissolve the highest dose strength of the parent drug at the lowest solubility at pH 1-7.5. A parent drug is considered ‘highly soluble’ when the highest dose strength is soluble in <250 ml water over a pH range of 1–7.5, in which 250 ml reflects the so-called FDA glass of water.

Therefore, producing suitable formulations is very important to improve the solubility and bioavailability (Tang B et al., 2008; Rasool et al., 2002) of such drugs. Formulation and development of poorly water soluble drug (PWS) candidates continue to be a challenge to formulation scientists (Yohei Kawabata et al., 2011; Zhao et al., 1999).
1.1 METHODS FOR ENHANCEMENT OF BIOAVAILABILITY

According to the definition of bioavailability, a drug with poor bioavailability is the one with-

- Poor aqueous solubility and/or slow dissolution rate in the biologic fluids,
- Poor stability of the dissolved drug at the physiologic pH,
- Inadequate partition coefficient and thus poor permeation through the biomembrane.
- Extensive presystemic metabolism.

There are three major approaches to overcome the bioavailability problems.

a) Pharmaceutics approach: Modification of formulation, manufacturing processes or physiochemical properties of the drug is done (Repka MA et al., 2008).

b) Pharmacokinetic approach: Pharmacokinetics of drug is altered by modifying its chemical structure (Stickerly RG et al., 1999, 2004).

c) Biological approach: In this, route of drug administration may be changed such as parenteral form instead of oral form. Rate dissolution and its solubility are very important factors in third approach.

The second approach of chemical modification has number of drawbacks such as being very expensive, time consuming, requires repetition of chemical studies, risk of precipitation and adverse effects. Moreover, the new chemical entity may suffer from another pharmacokinetic disorder or bear the risk of precipitating adverse effects. So generally only pharmaceutics approach is considered here.

The attempts, whether optimizing the formulation, manufacturing process or physiochemical properties of the drug, are mainly aimed at enhancement of dissolution rate as it is the major rate limiting step in the absorption of most drugs. There are several ways in which the dissolution rate of the drug can be enhanced. Some of the widely used methods, most of which are aimed at increasing the effective surface area of the drugs, are co-solvency (Rubino JT et al., 1987; KGH Desai et al., 2003; Seedher N et al., 2003), particle size reduction, hydrotrophy (Badwana A et al., 1982; Saleh et al.,1974), solid
dispersions (Chiou WL et al., 1971; Serajuddin et al., 1999; Wang L et al., 2006), micellar solubulisation (Carlota. O et al., 2005; C.H. Hsu et al., 2008; Dutt G.B. et al., 2003; Yua. B et al., 1998; Trochilin.V.P et al., 2001;), complexation, solubulising excipients, pH adjustment, colloidal drug delivery systems (Jones, M.C. et al., 1999) and lipid based drug delivery systems (Forbes B et al., 2005; Krishna G et al., 2001).

In all these methods either the drug characteristics are changed (crystalline to amorphous) or it becomes agglomerated (micronisation). Products of micronization and nanosuspension (Muller RH et al., 1995) are thermodynamically unstable, where the micronized particles eventually form agglomerates. Methods such as co-solubulization, solid dispersion, and inclusion complex involve altering of the physical property of the drug, which is not desirable.

Recent researches support the use of lipid –based formulations (Abdalla et al., 2008) to tackle the formulation challenges of poorly soluble drugs. There has been a considerable growth in the past 15 years on lipid based drug delivery system (LBDDS) as there is a growing need for novel drug delivery systems to deal with the vast majority of the new chemical entities (NCE) that has poor solubility or permeability (BCS Class II).

1.2 LIPID BASED DRUG DELIVERY SYSTEMS (LBDDS): (Pouton et al., 2000)

The lipid based formulation approach has attracted wide attention in order to enhance drug solubilization in the gastrointestinal tract (GIT) and to improve the oral bioavailability of BCS class II and IV drugs (Fig. 1) (Amidon GL et al., 1995; Gao D et al., 2007; Ljusberg-Wahren et al., 2005; Hulsman et al., 2000).

In LBDDS the most popular approach is the incorporation of drug compound into inert lipid vehicles such as oils, surfactant dispersions, liposomes, nanoemulsions (NEs). LBDDS range from simple oily solutions to complex mixtures of oils, surfactants, co-surfactants and co-solvents. The later mixtures are typically self-dispersing systems often referred to as self-emulsifying drug delivery systems (SEDDS) or self-micro emulsifying drug delivery systems (SMEDDDS). Formulations which disperse to form transparent
colloidal systems are usually referred to as SMEDDS. Lipid formulations are a diverse group of formulations which have a wide range of properties. These result from the blending of up to five classes of excipients ranging from pure triglyceride oils, through mixed glycerides, lipophilic surfactants, hydrophilic surfactants and water soluble cosolvents.

1.2.1 Classification of lipid delivery systems (LBDDS)

Type I

These formulations include the simplest formulations in which the drug is dissolved in triglycerides and / or mixed glycerides. Eg: oil soluble vitamins. Bioavailability from type I is likely to be very good because the triglycerides are rapidly digested to free fatty acids and 2-mono glycerides and these products are solubilized to form a colloidal dispersion within bile salt- lecithin mixed micelles. A hydrophobic drug in micelles results in a reservoir of drug in colloidal solution from which it can partition allowing passive absorption. Oil solutions are best option for potent drugs or compounds with log P>4. These systems are simple and biocompatible.

Type II

Type II formulations include a lipophilic surfactant (HLB value < 12) which improve the solvent capacity of the formulation. The distinguishing features of Type II systems are a) efficient self-emulsification b) absence of water-soluble components. These are best formulated with medium chain triglycerides or diglycerides blended with ethoxylated oleate esters with HLB values of approximately 11.

Type III

These systems incorporate hydrophilic surfactants (HLB value > 12) and water soluble cosolvents along with oils to produce self emulsifying systems. Particle size of < 100 nm can be produced under gentle agitation when the surfactant concentration is greater than 40%. The use of hydrophilic surfactants or cosolvents may also be to increase the solvent
capacity of the formulation for drugs with intermediate log $P(2 < \log p > 4)$. Type III systems are further divided into two types Type III A and Type III B to help identify very hydrophilic formulations (Type III B).

**Table 1: Classification of Lipid based drug delivery system (LBDDS)**

<table>
<thead>
<tr>
<th>Typical Composition (%)</th>
<th>Increasing hydrophilic content $\rightarrow$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Triglycerides or</td>
<td>Type I</td>
</tr>
<tr>
<td>mixed glycerides.</td>
<td>100</td>
</tr>
<tr>
<td>b) Surfactants</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>(HLB &lt; 12)</td>
</tr>
<tr>
<td>Hydrophilic Cosolvents</td>
<td>-</td>
</tr>
<tr>
<td>Particle size of</td>
<td>Coarse</td>
</tr>
<tr>
<td>dispersion (nm)</td>
<td></td>
</tr>
<tr>
<td>Significance of</td>
<td>Limited</td>
</tr>
<tr>
<td>aqueous dilution</td>
<td>importance</td>
</tr>
<tr>
<td>Significance of</td>
<td>Crucial</td>
</tr>
<tr>
<td>digestibility</td>
<td>requirement</td>
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</table>

The ability to efficiently deliver lipophilic drug molecules, especially in combination with lipid based delivery systems has led to renewed interest in intestinal lymphatic drug transport.

After absorption into the enterocytes, the vast majority of orally administered drugs rapidly diffuses across the cell are absorbed into the capillaries of portal vein and are thereby processed via liver to systemic circulation. Highly lipophilic drug molecules, however, may associate with lymph lipoprotein in the enterocytes and gain access to the mesenteric (intestinal) lymphatics, effectively bypassing the liver and gaining access to the systemic circulation via the thoracic lymph duct. (Porter CJH *et al.*, 2001).
1.3 SELF EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS)

SEDDS are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents. SEDDS upon mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can form fine oil in water (O/W) emulsions i.e. NEs (Li et al., 2007, 2009; Shaji et al., 2005). The poorly soluble drug can be dissolved in a mixture of surfactant and oil which is widely known as preconcentrate. These novel colloidal formulations on oral administration behave like oil-in-water microemulsions. Compared with ready-to-use microemulsions, the SEDDS and SMEDDS have been shown to improve physical stability profile in long term storage.

1.3.1 Advantages of SEDDS:

- Unlike microemulsions (which require a high surfactant concentration, usually about 20% and higher), NEs can be prepared using lower surfactant concentration, a surfactant concentration comprised between 3-10% may be enough.

- The nanosized droplets produced by SNEDDS on dispersion into water produce enormous interfacial areas that would influence the transport properties of drug.

- Efficient, convenient and more patient compliant approach as they can be filled in hard gelatin capsules (Unit dosage form).

- The drug can be delivered by lymphatic route restraining hepatic first-pass metabolism.

- Reduce or eliminate food effect.

- Easier to manufacture.

- SEDDS containing bioenhancers like cremophor, tween 80 are reported to improve the bioavailability by facilitating transcellular and paracellular absorption and inhibiting P-gp efflux (Basalious et al., 2010).
1.4 EXCIPIENTS USED IN NE/ME (Pouton *et al*., 2009; Li *et al*., 2007)

The self emulsification efficiency depends on the proper selection of Oil: Surfactant pair, concentration, ratio and the temperature at which self emulsification occur. In support of these facts, it has also been demonstrated that only very specific pharmaceutical excipient combinations could lead to efficient self-emulsifying systems.

**Oils**

Lipid is a vital ingredient of the SEDDS formulation not only because it can solubulized marked amounts of the lipophilic drug by it can also increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride. Both long and medium chain triglyceride oils with different degrees of saturation have been used for the design of self-emulsifying formulations (colin,w; *et al*.,2008). Edible oils, modified or hydrolyzed vegetable oils, novel semisynthetic medium chain derivatives are being used in NE.

**Surfactants**

A surfactant is obligatory to provide the essential emulsifying characteristics to NE. The surfactant involved in the formulation of NE should have a relatively high HLB and hydrophilicity so that immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous media (good self-emulsifying performance) can be achieved. Safety is a major determining factor in choosing a surfactant. The most widely recommended ones being the non-ionic surfactants as they are less toxic and offer better *in vivo* stability. Usually the surfactant concentration ranges between 30 and 60% w/w in order to form stable NE.
Co-solvents/ Co-surfactants:

The production of an optimum SEDDS requires relatively high concentrations (generally more than 30% w/w) of surfactants. The presence of cosurfactants decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form a NE over a wide range of composition (Shafiq et al., 2007). Organic solvents such as, ethanol, propylene glycol (PG), and polyethylene glycol (PEG) act as cosurfactants, and they enable the dissolution of large quantities of either the hydrophilic surfactant or the drug in the lipid base.

1.5 MECHANISM OF SELF EMULSIFICATION

The mechanism by which self-emulsification occurs is not yet well understood yet it is suggested that self-emulsification occurs when the entropy change that favors dispersion is greater than the energy required to increase the surface area of the dispersion. Emulsification occurs spontaneously with SEDDS because the free energy required to form the emulsion is either low and positive or negative.

The ease of emulsification was suggested to be related to the ease of water penetration into the various liquid crystal (LC) or gel phases formed on the surface of the droplet. The interface between the oil and aqueous continuous phases is formed upon addition of a binary mixture (oil/non-ionic surfactant) to water. This is followed by the solubilization of water within the oil phase as a result of aqueous penetration through the interface. This will occur until the solubilization limit is reached close to the interphase. Further aqueous penetration will lead to the formation of the dispersed phase. In the end, everything that is in close proximity with the interface will be LC, the actual amount of which depends on the surfactant concentration in the binary mixture. Thus, following gentle agitation of the self-emulsifying system, water will rapidly penetrate into the aqueous cores and lead to interface disruption and droplet formation. As a consequence of the LC interface formation surrounding the oil droplets, SEDDS become very stable to coalescence (Benita et al., 2004).
1.6 MECHANISM OF BIOAVAILABILITY ENHANCEMENT BY SEDDS

Presenting the drug in the dissolved form using lipid-based formulations provides significant improvement of oral absorption as compared to an oral solid or suspension dosage form. This advantage can be further improved in several cases by reducing the dispersion size of the dosage form. The reduction in dispersion size of Cyclosporine A (log P 4.29) SEDDS formulation, Sandimmune®, to its SMEDDS formulation, Neoral, improved its bioavailability by ~6.5-fold.

Absorbed drug molecules entering the enterocyte are exposed to metabolizing enzymes, e.g., cytochrome P-450 3A4 (CYP3A4), or can be secreted back into the gastrointestinal lumen by P-glycoprotein (P-gp) efflux pumps on the enterocyte membrane. The impact of formulation ingredients on the biopharmaceutical properties of drugs is also illustrated by the inhibition of drug efflux pumps by certain formulation ingredients. For example, common pharmaceutical excipients used in SNEDDS like polyethylene glycol, tween 80, and cremophor EL, capryol90 have been shown to inhibit P-gp activity. Their inclusion in the formulation, therefore, can be expected to increase the bioavailability for drugs which are known substrates of P-gp efflux pumps.

Fig 2: P-gp protein as transmembrane efflux pump
Fig:3: Various mechanisms of enhancement of drug bioavailability in the presence of lipids: solubilization of drug in the intestinal fluid by formation of colloidal species viz., vesicles, mixed micelles and micelles; followed by selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation.

**1.7 NANOEMULSION (NE):**

NEs are oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm. The particles can exist as oil-in-water and water-in-oil forms, where the core of the particle is either oil or water, respectively. NEs are made from surfactants approved for human consumption and common food substances that are “generally recognized as safe” (GRAS) by the FDA. These emulsions are easily produced in large quantities by mixing a water-immiscible oil phase with an aqueous phase under high shear stress, or mechanical extrusion process that is available world-wide. (P.Shah *et al.*, 2010).

NEs are also referred to as miniemulsions, ultrafine emulsions and submicron emulsions. Phase behaviour studies have shown that the size of the droplets is governed by the
surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition. Their very large interfacial area positively (T.P.U.Ravi et al., 2011; N. Anton et al., 2009) influences the drug transport and their delivery, along with targeting them to specific sites.

Reducing droplet sizes to the nanoscale leads to some very interesting physical properties, such as optical transparency and unusual elastic behavior. In the world of nanomaterials, NEs hold great promise as useful dispersions of deformable nanoscale droplets that can have flow properties ranging from liquid to highly solid and optical properties ranging from opaque to nearly transparent. Moreover, it is very likely that NEs will play an increasingly important role commercially, since they can typically be formulated using significantly less surfactant than is required for nano structured lyotropic microemulsion phases.

1.7.1 Preparation of NE:

NEs are non-equilibrium systems of structured liquids (T.P.U.Ravi et al., 2011; N. Anton et al., 2009; T.G Mason et al., 2006), and so their preparation involves the input of a large amount of either energy or surfactants and in some cases a combination of both. As a result, high energy or low energy methods can be used in their formulation (N. Anton et al., 2009). The high-energy method utilizes mechanical devices to create intensely disruptive forces which break up the oil and water phases to form nano-sized droplets. This can be achieved with ultrasonicators, microfluidiser and high pressure homogenisers (TG Mason et al., 2006; S.Graves et al., 2005; S.M.Jafari et al., 2007). Particle size here will depend on the type of instruments employed and their operating conditions like time and temperature along with sample properties and composition (C.Quin et al., 2011). This method allows for a greater control of particle size and a large choice of composition, which in turn controls the stability, rheology and colour of the emulsion. Although high-energy emulsification methods yield NEs with desired properties and have industrial scalability, they may not be suitable for thermolabile drugs such as retinoids and macromolecules, including proteins, enzymes and nucleic acids.
NE can be prepared by a low-energy emulsification method, which has been recently developed according to the phase behavior and properties of the constituents, to promote the formation of ultra-small droplets (O. Sonneville Aubrum et al., 2004; C. Solans et al., 2005). These low-energy techniques include self-emulsification, phase transition and phase inversion temperature methods (L. Wang et al., 2007). The low energy method is interesting because it utilizes the stored energy of the system to form small droplets. This emulsification can be brought about by changing the parameters which would affect the hydrophilic lipophilic balance (HLB) of the system like temperature, composition, etc. (I. Sole et al., 2006(a), 2010(b)).

1.7.1.1 High Pressure Homogenization

This technique makes use of high-pressure homogenizer/ piston homogenizer to produce NEs of extremely low particle size (up to 1 nm). During this process, several forces, such as hydraulic shear, intense turbulence and cavitation, act together to yield NEs with extremely small droplet size. The resultant product can be re-subjected to high-pressure homogenization until NE with desired droplet size and polydispersity index (PDI) is obtained. However, very high phase volume ratios may result in coalescence during emulsification, but more surfactant could be added to create a smaller reduction in effective surface tension and possibly diminishing recoalescence.

1.7.1.2 Microfluidization

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 - 20,000 psi), which forces the product through the interaction chamber, consisting of small channels called “microchannels”. The product flows through the micro-channels on to an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is introduced into a microfluidizer where it is further processed to obtain a stable NE. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until the desired
particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform NE (SM Jafari et al., 2007).

1.7.1.3 Phase Inversion Temperature Technique

Phase inversion in emulsions can be one of two types: transitional inversion induced by changing factors which affect the HLB of the system, e.g. temperature and/or electrolyte concentration, and catastrophic inversion, which can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures (T. Tadros et al., 2004; C. Solans et al., 2005). Phase inversion temperature (PIT) method employs temperature-dependent solubility of nonionic surfactants, such as polyethoxylated surfactants, to modify their affinities for water and oil as a function of the temperature. It has been observed that polyethoxylated surfactants tend to become lipophilic on heating owing to dehydration of polyoxyethylene groups. This phenomenon forms a basis of NE fabrication using the PIT method. In the PIT method, oil, water and nonionic surfactants are mixed together at room temperature. This mixture typically comprises o/w microemulsions coexisting with excess oil, and the surfactant monolayer exhibits positive curvature. When this macroemulsion is heated gradually, the polyethoxylated surfactant becomes lipophilic and at higher temperatures, the surfactant gets completely solubilized in the oily phase and the initial o/w emulsion undergoes phase inversion to w/o emulsion. The surfactant monolayer has negative curvature at this stage.

1.7.1.4 Solvent Displacement Method

The solvent displacement method for spontaneous fabrication of NE has been adopted from the nanoprecipitation method used for polymeric nanoparticles. In this method, oily phase is dissolved in water miscible organic solvents, such as acetone, ethanol and ethyl methyl ketone. The organic phase is poured into an aqueous phase containing surfactant to yield spontaneous NE by rapid diffusion of organic solvent. The organic solvent is removed from the NE by a suitable means, such as vacuum evaporation. Spontaneous
nanoemulsification has also been reported when solution of organic solvents containing a small percentage of oil is poured into aqueous phase without any surfactant.

1.7.1.5 Phase Inversion Composition Method

This method is also known as self nanoemulsification method. Kinetically stable NEs with small droplet size (~50 nm) can be generated by the stepwise addition of water into solution of surfactant in oil, with gentle stirring and at constant temperature. The spontaneous nanoemulsification has been related to the phase transitions during the emulsification process and involves lamellar liquid crystalline phases or D-type bicontinuous micro-emulsion during the process. NEs obtained from the spontaneous nanoemulsification process are not thermodynamically stable, although they might have high kinetic energy and long-term colloidal stability.

1.8 LIPOLYSIS:

The ability of lipid vehicles (either in the pharmaceutical formulation or in food) to enhance the absorption of lipophilic drugs has been well known for many years. The mechanisms behind this augmented bioavailability include enhanced dissolution and solubilization of the co-administered lipophilic drug by stimulation of biliary and pancreatic secretions, prolongation of gastrointestinal tract (GIT) residence time, stimulation of lymphatic transport, increased intestinal wall permeability and reduced metabolism and efflux activity (A. Dahan et al., 2006; T. Gershanik et al., 2000).

In order to aid in the suitable selection of the lipidic vehicle composition and rationalize the formulation design for drug candidate, different in vitro lipolysis methods have been suggested. (H. Ljusberg Wahren et al., 2005; C.J.H. Porter et al., 2001; N.H. Zangenberg et al., 2001; G.A. Kossena et al., 2003). These methods were designed to mimic the GIT environment and reflect the intestinal conditions in terms of maintaining constant pH, the presence of lipase/co-lipase, and bile salts (BS) and phospholipid (PL) concentration. These models have also been shown to simulate other dynamic changes, including the formation of mixed micellar species that are generated throughout the interaction of the vehicle with the GIT environment (M. Hultin et al., 1994). A key goal in the
development of these in vitro models is correlating the in vitro information of various drug-formulations to the in vivo drug profile.

A model that reliably correlates in vitro and in vivo data shortens the drug development period, economizes resources and leads to improved product quality. A few studies have evaluated the in vitro—in vivo correlation (IVIVC) of the lipolysis model (V.H. Sunesen et al., 2005; J.B. Dressman et al., 2006; C.J.H. Porter et al., 2004) however, additional work is needed in order to verify the ability of this model to predict actual oral bioavailability of certain drug-vehicle combinations. Furthermore, data are needed on the potential influence of the vehicle on the permeability of the co-administered drug molecules through the gut wall. This issue does not take into account the in vitro lipolysis method.

The current development strategies in this area of Lipid based drug delivery system demands a large number of animal experiments and consumes time and money. Hence there was a need to simplify the in vitro method in order to aid in the suitable selection of lipid vehicle. To overcome these needs, a dynamic in vitro lipolysis model, which provides an excellent medium for in vivo lipid digestion process has been developed. A very important aspect during the development of lipolysis model has been correlating the in vitro data obtained to the in vivo drug profile. Having a proper IVIVC, the model may be used for shorting the product discovery time period, decrease the cost of production, decreases the wastage and improves the quality of the product (Ekarat Jantratid et al., 2008).

Lipid based drug delivery systems keep the drug in solution throughout GI tract. Thereby surpasses the dissolution step in GI tract, instead digestion of excipients and formation of different colloidal structures takes place. The drug is expected to be distributed between digestion products and colloidal structures before it is absorbed. If the drug is lightly to be solubulized in the colloidal structure which may leads to precipitation of the drug which may require dissolution step before absorption. The conventional dissolution media will not reveal the above process hence it is necessary to use a model which will simulate the intestinal GI tract. Currently 3 model is in use in addition to simulated
intestinal/gastric dissolution media namely Monash, Jerusulam and Copenhagen models (Anne T. Larsen et al., 2011).

Table 2: Different lipolytic models

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Copenhagen</th>
<th>Monash</th>
<th>Jerusalem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase source</td>
<td>Porcine pancreatin (3×USP)</td>
<td>Porcine pancreatin (8×USP)</td>
<td>Porcine pancreatin (8×USP)</td>
</tr>
<tr>
<td>Lipase activity in the digestion</td>
<td>300-800 USP units/ml</td>
<td>1000 Tributyrin units/ml</td>
<td>1000 tributyrin units/ml</td>
</tr>
<tr>
<td>Bile species in the digestion medium</td>
<td>Porcine bile extract containing various bile acids</td>
<td>Tauro deoxycholic acid</td>
<td>Tauro cholic acid</td>
</tr>
<tr>
<td>Concentration of bile in digestion media</td>
<td>5-30Mm</td>
<td>5-20mM</td>
<td>5mM</td>
</tr>
<tr>
<td>Phospholipid species in the digestion medium</td>
<td>Phosphotidyl choline</td>
<td>Lecithin(60%PC)</td>
<td>L-α-phosphatidyl choline</td>
</tr>
<tr>
<td>Bile acid to PC ratio</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Calcium addition</td>
<td>Continuous addition of calcium at 0.045-0.181mmol/ml</td>
<td>Initial addition of 5mM calcium</td>
<td>Initial addition of 5mM calcium</td>
</tr>
<tr>
<td>Ph (buffer)</td>
<td>6.5 (2mM tris maleate)</td>
<td>7.5 (50mM tris maleate)</td>
<td>6.8-7.4(50mM tris maleate)</td>
</tr>
<tr>
<td>Initial volume in the digestion medium</td>
<td>300ml</td>
<td>10-40ml</td>
<td>40ml</td>
</tr>
</tbody>
</table>

1.8.1 Effect of calcium:

Calcium as mentioned earlier helps in the removal of fatty acid which is being liberated during the lipolysis. Calcium ion is added to lipolysis medium for number reasons but in most cases it is added to remove fatty acids, since fatty acids inhibit the pancreatic lipase.
Calcium forms soap with fatty acid thereby promoting the free fatty acid absorption. Many literatures support the effect of calcium on the rate of lipolysis. There are two different approaches to the addition of calcium to the lipolysis medium; continuous and fixed addition. The Copenhagen model, also known as the Dynamic in vitro lipolysis model, uses the continuous addition of calcium. Continuous addition of calcium results in a controlled lipolysis rate. By adding more or less calcium the lipolysis rate can be regulated. The Monash and Jerusalem model uses the fixed addition of calcium, 5mM of calcium is added to the lipolysis medium prior to initiation of the lipolysis. With the fixed addition of calcium a very fast initial lipolysis is seen, almost all the lipolysis takes place within the first 5–10 min (Christopher J.H. Porter et al., 2004).

1.8.2 Bile and lecithin

Bile salts are bio surfactants which mainly have two important functions they play a crucial role in digestion and absorption of nutrients and also serve as a means for excretion of several waste products from the blood. Bile salts get absorbed thereby allowing lipase and co-lipase to absorb onto the lipid surface as it does not allow protein and other emulsifying agents to get absorbed onto it and thereby initiating lipolysis process (NH Zangenberg et al., 2001). Bile acids and phospholipids /lecithin plays a major role as they affect the pancreatic lipase activity. Bile acid and phospholipids should be added in order to resemble intestinal fluid composition. Their concentration changes during the fasted and fed condition. The concentration of bile in fasted and fed state is 2-5mM and 8 to 15mM respectively. Bile acid mixtures are available both from porcine and bovine origin. The porcine bile contains mainly glycine conjugated bile acids and therefore it will represent human bile well. Bile acid has got the capability of solubilizing the lipolytic product and thereby it avoids inhibition of pancreatic lipase by lipolytic products. The effect of bile on the rate of lipolysis has been studied by some author (Yvonne Elisabeth Arnld et al.). In vivo the concentration of phospholipids ranges from 0.1 to 0.6 mM in the fasted state and from 0.1 to 4.8 mM in the fed state respectively. A wide range of ratios between bile acids and phospholipids have been reported, from 1 to 38.9, but most studies report ratios ranging from 2 to 6. The ratio of 4 is usually employed in the lipolysis models. Phospholipids can inhibit the activity of pancreatic
lipase co-lipase complex in the presence of bile acids; in the presence of mixed micelles, containing bile acids and phosphatidyl choline, the pancreatic lipase can be displaced from the interface. Lipolysis of the lipid droplets is inhibited as a result. The inhibition is reversed in the presence of phospholipase A2, most likely due to phosphatidyl choline being hydrolysed to lyso-phosphatidyl choline, which does not inhibit pancreatic lipase.

1.8.3 Lipase source for *in vitro* lipolysis model

As mentioned above the source of lipase commonly used for *in vitro* lipolysis models is Pancreatin of porcine origin. Pancreatin is a good source of duodenal enzymes to use for *in vitro* lipolysis, due to its natural origin it should contain all the relevant enzymes, e.g. pancreatic lipase, phospholipase A2 and cholesterol esterase and possible also pancreatic lipase like protein 2, which represent a minor lipolytic activity in the pancreatic secretions. It is important to have a mixture of the enzymes present during *in vitro* lipolysis since LSBDDS contain various potential substrates. Many surfactants are also substrates for one or several of the enzymes in pancreatin, e.g. labrasol, gelucire 44/14, labrafil M2125CS and cremophor EL (Yvonne Elisabeth Arnold *et al.*, 2011). The lipolysis of the polyethylene glycol esters in labrasol is mainly carried out by cholesterol esterase and pancreatic lipase related protein 2. The lipase activity of pancreatin can be measured by different methods. The most commonly used method uses tributyrin as substrate and is expressed in TBU. Another method is given in the USP33/NF28 (USP33, 2010); in this case the substrate is olive oil emulsified with gum-arabic, which is a more relevant substrate for testing activity of lipase for digestion of LSBDDS, due to the widespread use of LCTs in the development of LSBDDS. Here the lipase activity is expressed as USP units; one USP unit of lipase liberating 1mol fatty acid per minute from the substrate. The mol fatty acids liberated is measured by pH-stat titration at pH 9. The USP method requires the use of a USP pancreatin lipase reference standard and therefore this method is very robust and results obtained from different laboratories can be compared. For *in vitro* lipolysis the lipase is usually added in excess as is the case in the *in vivo* situation. The lipase activity has been found to be of minor importance for the extent of lipolysis compared to other factors such as bile acids and calcium. Mac Gregor *et al.*, reported that the percentage lipolysis increased by a factor 3 when the lipase
activity was increased 50 times. The pancreatic lipase is inhibited by increasing levels of free fatty acids.

1.8.4 Precipitation of drug

The major concern of the formulator is the precipitation of the drug in vivo, since precipitated drug needs to be re-dissolved prior to absorption from the intestine and when working with BCS class II or IV drugs the solubility of the drug compounds in the gastrointestinal fluids is limited and precipitation might decrease bioavailability. Precipitation of a drug compound from a LSBDDS during passage of the GIT can be caused by numerous different factors. One of these factors is hydrolysis of excipients in the LSBDDS by enzymes present in the GIT (Sylvie Fernandez et al., 2008). Many excipients used in LSBDDS contain ester-bonds that are prone to hydrolysis by lipases or esterases present in the GIT. If the formed hydrolysis products have a lower solubilization capacity towards the drug, this can result in precipitation of drug. In addition simple dilution of a LSBDDS by the gastrointestinal fluids can also cause the drug to precipitate; this is due to a loss of water soluble excipients, primarily co-solvents, from LSBDDS to the gastro-intestinal fluids. This also results in a lower solubility of the drug in the dispersed LSBDDS and thus causing it to precipitate. Further, the degree of saturation of drug in a LSBDDS has an impact on precipitation as well, so the higher the concentration of drug, the less the solubility of the drug has to be reduced before initiation of precipitation. In vivo assessment of drug precipitation is complicated and would require intubation studies in humans or animals. Therefore in vitro lipolysis models have been used to predict drug precipitation during digestion of LSBDDS.

1.8.5 Other factors

Recent studies by different authors suggested that many other factors also influence the lipolysis of the drug other than the above mention factors. These factors which may influence the activity are HLB value, surfactants, viscosity and the critical packaging parameters. Article by (Yvonne Elisabeth Arnald et al.,) suggests that the drug has got an effect on the oil, HLB value, viscosity and the critical packaging parameters. Drugs
significantly lowered the apparent lipolysis rate of the oil. Study on the fundamental interfacial techniques such as interfacial tension and interfacial rheology to investigate the molecular changes occurring (Hazem Ali et al., 2008).

1.8.6 Cryo TEM

Cryo transmission electron microscopy provides the most vital information about the various colloidal phases formed during the in vitro digestion. It is a form of electron microscopy where the sample is studied at cyrogenic temperature (basically liquid nitrogen temperature i.e -196°C).

The material is spread on the electromagnetic [EM] grid and is preserved in frozen hydrated state by rapid freezing usually in liquid ethane near liquid nitrogen temperature. By maintain it at liquid nitrogen temperature; the samples can be introduced into high vacumm of EM column.

The main advantage of cyro TEM over other techniques are in this minimal sampler is needed and it can directly visualize the sample as compared to X-ray crystallography, which generally requires placing the samples in a non-physiological environment which can occasionally lead to functionally irrevelant conformational changes. As cyro TEM is thermal fixation it reduces molecular motion by vitrifying the sample in athin layer of solvents.

Elucidating the structural information of nanoscale material in their solvent exposed state is crucial as a result cryo TEM has become an increasingly popular technique.

1.8.7. pH Stat equipment

The pH equipment is the technique by which the pH of the medium could be maintained. It is fully a computerized system. The burette capacity in which the titrant is kept is only 10 ml. the temperature at which the experiment has to be carried out could also be maintained. The stirring speed is maintained according to the experimental requirement. Other than pH titration other titration could also be done in this equipment. The other
titration which could be done are acid based titration, non ionic titration, non aqueous
titration, etc.

1.9 LYMPHATIC FUNCTION

The lymphatic system is found in most tissues in the body, and plays an important role in
maintaining fluid balance (Dongaonkar, R.M. et al., 2009), immune cell trafficking from
the periphery to lymph nodes (Miteva, D.O. et al., 2010), and lipid transport from the
intestine to the circulation (Iqbal, J. and Hussain, M.M. et al., 2009). The lymphatic
vasculature comprises unique functional features that enable entry and transport of large
proteins, immune cells, lipids and fluid against a pressure gradient. Specifically, the entry
point of the lymphatic system is regulated by initial lymphatics (blind-ended
microvessels lacking smooth muscle), which have specialized junctions that prevent
backflow of fluid into the tissue after it has entered the vessel (Trzewik J. et al., 2001;
Baluk P. et al., 2007). The initial lymphatics merge into larger collecting vessels
composed of individually contracting units known as lymphangions. Each lymphangion
is lined with a functionally unique form of smooth muscle that provides vessel tone and
allows the vessel to contract down to as little as 20% of its resting diameter
(Muthuchamy M. et al., (2003); Von der Wei P.Y. and Zawieja, D.C. 2004). These
contractions, when combined with the valve leaflets that separate each lymphangion
(Bazigou E. et al., 2009), promote unidirectional propagation of flow (Dixon, J.B. et al.,
2006)

1.9.1 Lipid uptake into lacteals

Nearly all dietary lipid is absorbed by the enterocytes of the small intestine, packaged in
chylomicrons, and transported from the intestine to the bloodstream via the lymphatic
system (Iqbal, J. and Hussain, M.M. et al., 2009)). In general, lymphatic transport of
chylomicrons can be regarded as a two-stage process: (i) entry into the initial lymphatic
vessel of the small intestine, known as a lacteal and movement through the initial vessels
via the intrinsic motion of intestinal peristalsis (Collan, Y. and Kalima, T.V. et al.,1970),
and (ii) the subsequent movement of this lipid through the rest of the lymphatic system,
driven by the contractile activity of the larger collecting lymphatics (Dixon J.B. et al., 2006).

1.9.2 Digestion and absorption of lipids:

The process of digesting food derived lipids (predominantly in the form of triglycerides) starts in the stomach where pre-duodenal lingual (mouth cavity) and gastric acid lipase (gastric mucosa) that hydrolyze triglycerides to diglycerides and fatty acids (C.K.Abrams et al., 1988; M.Hamosh et al., 1981; M. Cohen et al., 1971). After the initial hydrolysis action of the acid lipase, the resulting by-products and remaining solid material migrate down the stomach where it passes to the pyloric antrum. At the pyloric antrum, gastric chyme is released and in combination with the peristaltic movements results in the emulsification of food-derived triglycerides as they empty into the duodenum (G. Pantaleo et al., 1994; P. Tso et al., 1994). The presence of the lipid in the duodenum stimulates the secretion of pancreatic fluids and bile (bile salts and bile lipids). The biliary lipids (phospholipid and cholesterol) adhere to the surface of the emulsion forming a colloidal like stable emulsion with smaller droplet size (higher surface area) that allows a better action of pancreatic lipase that allows the hydrolysis of triglycerides to 2-monoacylglycerol and two fatty acid molecules from each triglyceride (F.H.Mattson et al., 1956, 1964, 1968; Y.F. Shiau et al., 1981; A. F. Hofmann et al., 1963).

Phospholipids and cholesterol also get digested in the intestine. Phospholipid digestion occurs in the small intestine because gastric lipase is incapable of digesting phospholipids. The phospholipid that is mainly found in bile is phosphatidylcholine and is in mixed micelles with cholesterol and bile salts. Once bile is released in the small intestine, phosphatidylcholine is hydrolyzed by phospholipase A2 to fatty acids and lysophosphatidylcholine (B. Borgstrom et al., 1957; H. Van Den Bosch et al., 1965). Cholesteryl esters get hydrolyzed by cholesterol esterase in the small intestine to free cholesterol (B.K. Nordskog et al., 2001; N.McIntyre et al., 1976). Cholesterol esterase activity is enhanced by the presence of trihydroxy bile salts such as sodiumcholate, but also allows for the self-aggregation of the enzyme into a polymeric form (C. Erlanson, 1975).
The digestion products released from the triglyceride droplets in the colloidal emulsion form LCS that when combined with sufficient concentrations of bile salts produce unilamellar and multilamellar vesicles (JE. Staggers et al., 1990; O. Hernell et al., 1990). Accordingly, the postprandial intestinal lumen (having higher bile salts due to higher bile release) has a greater presence of unilamellar and multilamellar vesicles. The lipid digestion products contained in a mixed bile salt-phospholipid micellar phase first need to be dissociated from this phase in order to be absorbed and pass into the enterocyte (YF. Shiau, 1990; NE. Hoffman et al., 1970; W.J. Simmonds et al., 1972). It has been reported that the enterocyte surface might be in proximity to a low pH region that allows for a change in the colloidal structure leading to release and apical absorption of lipid digestion products (K. Chijiwa et al., 1984; YF. Shiau et al., 1990; K. Chijiwa et al., 1987). The transport of lipid digestion products across the apical membrane of the enterocyte has been reported to occur via passive transport and via active transport using specific membrane-bound carrier proteins. The passive transport predominates when the luminal lipid concentrations are high as will be the case post-prandially (A.B. Thomson et al., 1993; C. Scholler et al., 1995a, b). The membrane-bound carrier proteins involved in fatty acid uptake involve the microvillus membrane fatty acid binding protein and the fatty acid transporter (W. Stremmel et al., 1985, 1988; H. Poirier et al., 1996).

Once the lipid has been absorbed into the enterocyte, its chain length determines its subsequent intracellular processing. Short- and medium-chain lipids (C\textsubscript{b12}) generally diffuse across the enterocyte, while long-chain lipids (C\textsubscript{\geq12}) generally migrate to the endoplasmic reticulum where they get re-acylated and assembled into lipoproteins before secretion into the mesenteric lymph (B. Bloom et al., 1951a, b; L.L. Chaikoff et al., 1951; J. Y. Kiyasu et al., 1952). The re-acylation process of fatty acids and monoglycerides to triglycerides in the endoplasmic reticulum has been reported to be involved with two cytosolic fatty acid binding proteins (I-FABP and L-FABP) (N.O. Davidson et al., 1994; J. Storch et al., 2004; B.A. Luxon et al., 1999; R.K. Ockner et al., 1992; A. E. Thumser et al., 2000; D. H. Alpers et al., 2000; M. S. Levin et al., 1992; CJ. Porter et al., 2001). Furthermore, the re-acylation process appears to occur via two possible pathways. One pathway involves a two-step sequential direct acylation of \textit{2-monoacylglycerol} to triglyceride (JR. Senior et al., 1962) and accounts for the main
pathway of production for triglycerides destined for chylomicrons (CJ Porter et al., 2001). The second and minor pathway is the phosphatidic acid pathway or glycerol-3-phosphate that involves the sequential acylation of endogenous glycerol-3-phosphate with three molecules of activated fatty acid (E. Levy et al., 1995), which accounts for the production of triglycerides mainly destined for very low density lipoprotein (VLDL) (R.K. Ockner et al., 1969; M.M.Van Greevenbroek et al., 1998; D.A.Gordon et al., 2000; P. Tso et al., 1980, 1984)

1.9.3 Lymphatic pump function

After entry into the initial lymphatics, lymph must be transported against a pressure gradient, primarily through contractile lymphatics, by means of the periodic contraction of the lymphatic smooth muscle. Given that lymph flow rate increases after lipid absorption (Sharma, R. et al., 2007), tissue hydration enhances lipid absorption in the gut (Tso, P. et al., 1985) and lymphatic pump function is very sensitive to changes in mechanical load, it is likely that changes in these loads on the lymphatic system of the gut are an important regulator in lipid transport (L. Kagan et al., 2007).

In the presence of high flow rates under the same transmural pressure (i.e. same stretch but different shear stress), lymphatic contraction is inhibited by the upregulation of endothelial nitric oxide synthase (eNOS) and subsequent release of nitric oxide (NO) (Gashev, A.A. et al., 2002). This is not surprising as NO is known to be a shear-released vasodilator in the blood vasculature (Buga G.M. et al., 1991; Dimmeler S. et al., 1999). In lymphatics, NO not only alters the tone (resting diameter) of the vessel, but also inhibits the contraction frequency and amplitude of the vessel, which results in a further increase in the time-averaged diameter. This mechanism is thought to regulate whether the vessel should behave as a pump or a conduit, as lymphatic contraction in the presence of an exogenous flow source (e.g. lymph formation) increases resistance to flow (Quick C.M. et al., 2009).
1.9.4 Lymphatic absorption:

The majority of orally administered drugs gain access to the systemic circulation by direct absorption into the portal blood. However, highly lipophilic compounds may reach the systemic blood circulation via the intestinal lymphatic system (Dahan and Hoffman et al., 2006a). This alternative absorption pathway from the gastrointestinal tract (GIT) has been shown to be significant contributor for the overall bioavailability of a number of highly lipophilic drugs, including fat soluble vitamins, halofantrine (Karpf et al., 2004; Holm et al., 2003; Khoo et al., 2001), probucol (KJ. Palin and Wilson, 1984), ontazolast (DJ. Hauss et al., 1998), seocalcitol (Grove et al., 2006), mepiostane (T. Ichihashi et al., 1992 a, b) and others. In addition to increased overall bioavailability of lipophilic molecules, lymphatic transport of a drug provides further advantages, including avoidance of hepatic first pass metabolism, a potential to target the drug into the lymphatic system for the case of specific disease states known to spread via the lymphatics, and improved plasma profile of the drug. For these reasons, studies of the absorption of drugs via the intestinal lymphatic system have received increasing attention in recent years (O’Driscoll et al., 2002; Porter and Charman et al., 2001; A.B.Thomson et al., 1993).

Fig 4: Drug uptake through villi
1.9.5 Lymphatic system:

The lymphatic system plays an important role in the transport of drugs to the systemic circulation, given its extensive drainage network throughout the body. Some of the advantages of lymphatic transport of drug are avoidance of first-pass metabolism and targeting of specific diseases which are known to spread via lymphatics, such as certain lymphomas and HIV. The promising mechanisms include: I. facilitating transcellular absorption due to increased membrane fluidity; II, allowing paracellular transport by opening tight junctions; III, increased intracellular concentration and residence time by surfactants due to inhibition of P-gp and/or CYP450; IV, lipids stimulation of lipoprotein/chylomicron production.

1.9.6 Digestion and solubilization:

The balance between a drug's solubility in the aqueous environment of the gastrointestinal lumen and its permeation across the lipophilic membrane of enterocytes determines its rate and extent of absorption. After oral administration of lipid-based formulations, gastric lipase initiates the digestion of exogenous dietary triglyceride (TG) and formulation TG. Simultaneously, the mechanical mixing (propulsion, grinding and retropulsion) of the stomach facilitates formation of a crude emulsion (comprised of aqueous gastric fluid and lipid digestion products). Later in the small intestine, TG is broken down to diglyceride, monoglyceride and fatty acids by pancreatic lipase together with its cofactor co-lipase, acting primarily at the sn-1 and sn-3 positions of TG to produce 2-monoglyceride and free fatty acid. Pancreatic phospholipase A2 digests the formulation derived or biliary derived phospholipids (PL) by hydrolyzing at the sn-2 position of PL to yield lysophosphatidyl choline and fatty acid. The presence of exogenous lipids in the small intestine stimulates the secretion of endogenous biliary lipids from the gallbladder, including bile salt (BS), PL and cholesterol. Previously formed monoglycerides, fatty acids, and lysophospholipid (products of lipid digestion) are subsequently incorporated into a series of colloidal structures, including micelles and unilamellar and multi-lamellar vesicles in the presence of bile salts. The solubilization and absorptive capacity of the small intestine for lipid digestion products and drugs (D) is
significantly enhanced due to these formed lipid metabolites. In Fig 5, the oil droplet in the intestine is represented in different colors to indicate undigested TG in the core (orange) and digested products such as fatty acid (blue) and monoglyceride (green) on the surface of the droplet.

Fig 5: Schematic representation of lymphatic absorption

1.10 Recent advances in the understanding of lymphatic drug transport

Recent researches revealed the used of *in vitro* and *in vivo* simulation models help in estimating the fate of drugs which are also economical and less time consuming

1.10.1 *In vivo* models

Evaluation of intestinal lymphatic drug transport requires invasive and largely irreversible surgery to access and cannulate the intestinal lymphatic duct. As such, lymphatic drug transport cannot be studied directly in humans (Miura *et al.*, 1987) Various animal models have therefore been described in an attempt to quantitate the contribution of the lymphatic system to overall drug absorption (G.A Edwards *et al.*, 2001). In the majority of cases these pre-clinical models collect the entire volume of
lymph flowing through mesenteric or thoracic lymph duct cannulas and therefore provide an absolute indication of the extent of lymphatic transport. Other models have also examined the use of a lympho-venous shunt which has the advantage of allowing sampling of lymph over much longer periods, although in this case the relatively small database for lymph flow rates makes estimation of the absolute extent of lymphatic transport difficult. The majority of lymphatic transport studies described in the literature have utilised rats, reflecting the relative ease of sourcing and housing small laboratory animals, however, larger animal models such as dogs (S.M. Khoo et al., 2001, 2003; A. Lespine et al., 2006), pigs (D.G. White et al., 1991), sheep (M. Onizuka et al., 1997; A.M. Segrave et al., 2004) and rabbits (V.Bocci et al., 1986) have also been described. The advantages of larger animal models include the capacity to dose more clinically relevant full-sized human dosage forms and the ability to administer compounds under more representative fed and fasted states (rodents do not eat on command). The gastrointestinal tract, transit profile and biliary secretion patterns of dogs and pigs are also more similar to that of humans when compared with rats (in which bile is continuously secreted into the intestine). However, the complexity and cost of larger animal models typically limits widespread application.

Recently, an alternate in vivo approach to the estimation of intestinal lymphatic drug transport has been described in which the systemic exposure of drug is assessed after drug administration in the presence and absence of an inhibitor of intestinal chylomicron flow (e.g. Pluronic-L81 or colchicine) (A. Dahan et al., 2005). Comparison of systemic drug exposure profiles in the presence and absence of a functional intestinal lymphatic system provides an indication of the importance (or otherwise) of lymphatic drug transport to overall bioavailability. This approach has the advantage of not requiring the surgical interventions inherent in lymph duct cannulation, however, the broader implications of blocking chylomicron flow and intestinal lipid processing on drug exposure (and indirectly, lymphatic transport) are yet to be studied in detail.
1.10.2 *In vitro* models

The use of *in vitro* models as an alternate to *in vivo* models of assessment of lymphatic drug transport has also been described. For example, Caco-2 cells are well recognised in the pharmaceutical arena as an *in vitro* model of intestinal epithelium and are widely utilised to screen for intestinal permeability properties. However, Caco-2 cells have also been employed in the lipid biochemistry literature to examine aspects of intracellular lipoprotein assembly (E. Levy *et al.*, 1995) and have recently been evaluated as a prospective *in vitro* model to examine the influence of lipids and lipidic excipients on drug incorporation into lipoproteins and lymphatic transport (F. Seeballuck *et al.*, 2003, 2004; D.M. Karpf *et al.*, 2006). These data are reviewed in more detail elsewhere in this theme issue (C.M.O. Driscoll *et al.*, 2006).

Gershkovich and Hoffman (P. Gershkovich *et al.*, 2005) have also suggested that the degree of *ex vivo* association of drugs with chylomicrons harvested from plasma may be used as a simple predictive tool as to the likely extent of lymphatic drug transport. In these studies, a reasonable linear correlation ($r^2 =0.94$) was obtained between the extent of lymphatic transport of several lipophilic drugs and their degree of association with plasma chylomicrons *ex vivo*. Importantly, this correlation was substantially better than that between the extent of lymphatic drug transport and TG solubility or log P (Sakeda.T *et al.*, 2001). An in silico method aimed at developing a quantitative relationship between molecular structure and the extent of intestinal lymphatic drug transfer has also recently been described (R Holm *et al.*, 2004). The authors found that a relatively complex set of molecular descriptors was required to predict the likelihood of lymphatic transport, although once again the approach appeared to give more accurate predictions than that obtained using traditional descriptors (such as log P and TG solubility).