European Journal of Pharmaceutics and Biopharmaceutics 73 (2009) 1–15

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Lipid – An emerging platform for oral delivery of drugs with poor bioavailability

Subhashis Chakraborty, Dali Shukla, Brahmeshwar Mishra, Sanjay Singh *

Department of Pharmaceutics, Banaras Hindu University, Varanasi, India

ARTICLE INFO

Article history:
Received 6 May 2009
Accepted in revised form 2 June 2009
Available online 6 June 2009

Keywords:
Lipid-based oral drug delivery
Lipase activity
Lymphatic uptake
Bioavailability enhancement
Solid dosage form
Adsorption
Lipid digestion products
In vitro lipolytic model
In vitro–in vivo correlation
Solid lipid nanoparticles

ABSTRACT

The sole objective of pharmaceutical science is to design successful dosage forms which fulfill the therapeutic needs of the patients effectively. Development of new drug entities is posing real challenge to formulators, particularly due to their poor aqueous solubility which in turn is also a major factor responsible for their poor oral bioavailability. Lipids as carriers, in their various forms, have the potential of providing endless opportunities in the area of drug delivery due to their ability to enhance gastrointestinal solubilization and absorption via selective lymphatic uptake of poorly bioavailable drugs. These properties can be harvested to improve the therapeutic efficacy of the drugs with low bioavailability, as well as to reduce their effective dose requirement. The present communication embodies an in-depth discussion on the role of lipids (both endogenous and exogenous) in bioavailability enhancement of poorly soluble drugs, mechanisms involved therein, approaches in the design of lipid-based oral drug delivery systems with particular emphasis on solid dosage forms, understanding of morphological characteristics of lipids upon digestion, in vitro lipid digestion models, in vivo studies and in vitro–in vivo correlation.

1. Introduction

In the present scenario, oral drug delivery is continuously looking into newer avenues due to 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 failure of the conventional delivery system [1]. Since the last decade, the oral drug delivery has taken a new dimension with the increasing application of lipid as a carrier for the delivery of poorly water soluble, lipophilic drugs [2].

The unique properties of lipids viz., their physiochemical diversity, biocompatibility and proven ability to enhance oral bioavailability of poorly water soluble, lipophilic drugs through selective lymphatic uptake have made them very attractive candidates as carriers for oral formulations. With the above promises, the emerging field of lipid-based oral drug delivery system (LBODDS) has attracted considerable academic attention [3–6]. Perhaps, some of the reasons for this include the complexity of their physiochemical properties, challenges in stability and manufacturing at the commercial scale, limited solubility of some poorly water-soluble drugs in lipids, their pre-absorptive gastrointestinal (GI) processing, a lack of knowledge about the in vivo behavior and influence of co-administered drugs/lipids and finally, the lack of predictive in vitro and in vivo testing methodologies. In spite of these limitations, lipids definitely offer the potential for enhancing drug bioavailability, though the formulation opportunities are yet to be fully explored [7].

Formulation excipients capable of being digested in the GI tract play a major role in determining the rate and extent of absorption of drugs from the GI tract [8]. Formulators need to have an in-depth knowledge of the GI digestive process for interpretation of the biopharmaceutical properties of lipid-based oral formulations and design relevant in vitro tests to mimic the physiological environment for the formulation. Continuous efforts are being made towards the design of a biorelevant dissolution media as well as to understand the in vivo colloidal behavior of the lipid-based formulations in the presence of endogenous solubilizing species viz., bile salts (BS), phosphotidylcholine (PL) and cholesterol (CL) and enzymes (lipase). The present review is a consolidated approach towards understanding the role of lipids (both exogenous and endogenous) in the process of bioavailability enhancement of lipophilic drugs, mechanisms involved in the digestive process and transcellular transport, challenges involved in formulation development with particular emphasis on solid dosage forms and advances made till date in the development of morphological evaluation of lipid digestion products, in vitro lipid digestion models, in vivo studies and in vitro–in vivo correlation.

* Corresponding author. Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221 005, India. Tel.: +91 5426702712; fax: +91 542 2368428.
E-mail addresses: chakraborty_19@rediffmail.com (S. Chakraborty), drsanjaysingh@rediffmail.com (S. Singh).

0939-6411/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2009.06.001

2. Role of exogenous lipids in bioavailability enhancement

High solubility and permeability are considered prerequisites for oral absorption, and many drugs have been identified to exhibit poor and variable bioavailability due to high dose to solubility ratio. The bioavailability of such drugs is frequently increased by co-administration of food [9–13]. To our knowledge, Crounse [14] was the first to report the food-dependent bioavailability of drugs, wherein the absorption of a water-insoluble drug, griseofulvin, was significantly enhanced on administration with a high-fat meal. Among all the food constituents, lipid component of food is of particular importance in stimulating the physiological responses for the absorption of lipophilic or poorly water-soluble drugs [15–20]. Intake of a high-fat meal results in stimulation of biliary and pancreatic secretions, prolongation of GI tract residence time, stimulation of lymphatic transport, changes in mesenteric and liver blood flow, increased intestinal wall permeability and reduced metabolism and efflux activity which significantly contribute in improving bioavailability [21–22]. Studies on healthy human subjects have shown that in addition to already well-characterized parameters (such as pH and bile salt levels), some other parameters like buffer capacity, surface tension, osmolality and food components, which significantly change pre/postprandially, can also affect the intraluminal performance of dosage forms [23]. Ingestion of meals containing 10–25 g of lipid has been demonstrated to promote emptying of the gallbladder and its maximal contraction [17–18]. The presence of quantitatively most important lipid component in the human diet – triglycerides (TGs), which may amount to 100 g per day or more in the small intestine and long-chain (rather than medium-chain fatty acids) fatty acids (FAs) appear to be most effective in driving food-related inhibition of motility which would help in improving GI residence time [24–26]. For particularly lipophilic drugs or large molecular weight macromolecules, lymphatic uptake can be increased in the presence of a high-fat meal [27–28]. Gershkovich and Hoffman [29] suggested that changes in drug disposition for certain lipophilic compounds may occur when the drug interacts with TG-rich lipoproteins (TRL), the concentration of which elevate as a result of consumption of high-fat meals. It was perhaps the result of these interesting outcomes which infused the idea of lipid-based oral drug delivery system for bioavailability enhancement, among modern researchers.

The United States Food and Drug Administration (FDA) has given due attention to the ability of food to alter the pharmacokinetics of drug products and, therefore, have established standards for the design of clinical food effect studies. In December 2002, the FDA issued a guidance entitled, "Food-Effect Bioavailability and Fed Bioequivalence Studies" [30]. High-fat meals are recommended by the FDA for food effect studies; as such meal conditions (800–1000 cal; 50–65% from fat, 25–30% from carbohydrates, and 15–20% protein) are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. However, some drug products show an opposite effect on the extent of bioavailability and efficacy on co-administration with food [10]. Of course, there are many drugs for which food effects are non-existent or negligible.

Several cases of food effects on bioavailability of drugs have been reported in the literature in correlation to class of the drug as per biopharmaceutics classification system (BCS) (Fig. 1) [31]. It has been observed that the bioavailability of Class 1 drugs is not affected, while that of Class 2 and 3 drugs increases and decreases, respectively, in the presence of food. The probable reason for such observations can be explained on the basis of solubility, permeability and inhibition of efflux transporters in the presence of food [32,33]. Class 1 drugs being of high solubility and permeability can easily cross the membrane by passive diffusion and also

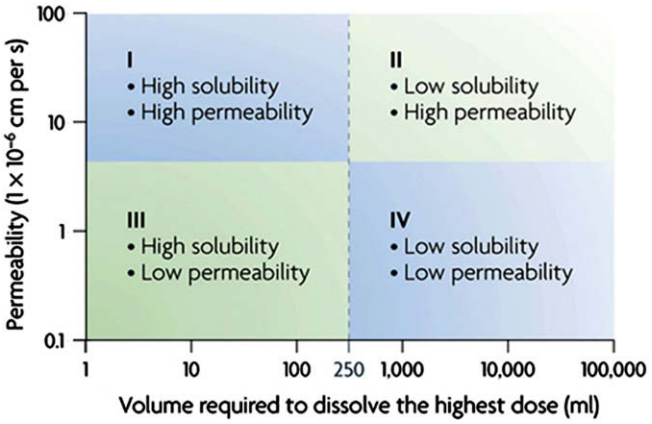

Fig. 1. Biopharmaceutical classification system. The *x*-axis shows the volume (ml) required to dissolve the highest dose strength of the parent drug at the lowest solubility over the pH 1–7.5. A parent drug is considered 'highly soluble' when the highest dose strength is soluble in <250 ml water over a pH range of 1–7.5, in which 250 ml reflects the so-called FDA glass of water. The *y*-axis shows the permeability, which is defined by various in vivo or in vitro assays, and a permeable drug is the one associated with 90% oral bioavailability or 90% absorption as assessed by urinary excretion data (adapted from ref. [36] with permission).

are capable of saturating any cellular transporter, both efflux and absorptive. As the absorption process is dominated by passive diffusion, transporter drug interaction is minimal and therefore, no significant effect on the extent of bioavailability is observed for Class 1 compounds in the presence of a high-fat meal. Similarly, for Class 2 drugs, absorption is primarily through passive diffusion due to their lipophilicity and high permeability. However, the low solubility of these compounds prevents saturation of the efflux transporters. Consequently, a dual effect of inhibition of efflux transporters and increase in the drug's solubility by micellar solubilization in the presence of food increases the extent of oral bioavailability and the rate of absorption of these drugs. Class 3 compounds, though are sufficiently available in the gut lumen due to good solubility, they are poorly metabolized and poorly permeable and therefore are majorly dependent on the cellular uptake transporters for penetration into the enterocyte. With high-fat meals, these drugs could show lower bioavailability due to inhibition of uptake transporters in the intestine [31,34,35]. Though it is difficult to predict the fate of Class 4 drugs, they may behave as Class 3 drugs due to increase in their solubility in the presence of high-fat meal.

The association of postprandial TG-rich lipoprotein with lipophilic drugs within the enterocyte has been found to be prone to both intestinal lymphatic transport and post-absorptive changes in disposition following a high-fat meal. This association of drug results in decrease in the volume of distribution and clearance and thus possibly changes the kinetics of the pharmacological action of the lipophilic drug [29]. Therefore, several challenges exist in the development of compounds that exhibit food effects. If a high-fat meal is required to obtain efficacious drug levels, there is serious concern of sub-therapeutic plasma drug concentration in patients taking the drug without food. The situation may worsen with compounds of narrow therapeutic index as changes in bioavailability, particularly in the positive direction, may precipitate unwanted side effects. As a result, the clinical plan may require control and/or monitoring of food intake in relation to dosing. However, the above issue may be addressed by administering such drugs as lipid-based formulations. Although the nature and quantity of lipids contained in a high-fat meal would be significantly different to what would be included in a pharmaceutical formulation, design of lipid-based formulations can reduce the inherent

limitations of slow and incomplete dissolution of poorly soluble drugs and facilitate the formation of solubilized phases from which absorption may occur. This can offer a prospective approach to reduce their food-dependent bioavailability and will also be functional in reducing the dose [7]. Some examples of drugs that exhibit enhanced bioavailability when administered in combination with food include griseofulvin [37], danazol [38], halofantrine [39], atovaquone and troglitazone [40–41].

3. In vivo fate of lipid in human body

A normal adult diet includes a daily intake of about 60–80 g of fat. Additionally, 40–60 g of fat is of endogenous origin, which consists of phospholipids, cholesterol and membrane lipids from desquamated intestinal cells and bacteria [42]. This indicates that an adult digestive system is powerful enough to hydrolyze approximately 100–140 g of lipid everyday. The solubilization of drug in the GI tract and its bioavailability depend predominantly on the intraluminal processing to which lipids are subjected prior to absorption. Therefore, knowledge of the journey of lipids from the GI lumen to the circulatory system in the presence of a powerful digestive system is of great significance for interpretation of the biopharmaceutical properties of oral lipid-based formulations and successful product development [43]. The processing of lipid-based formulations in the human body is highly complex, and the exact mechanism of drug absorption and its fate in association with the administered lipid are still not clear [5,6]. Therefore, the focus of this section is to simplify the understanding of the entire process by dividing it into three distinct phases:

(1) Digestive phase, (2) absorption phase, (3) circulatory uptake.

3.1. Digestive phase

The digestive phase initiates with the physical breakdown of lipid formulation into a coarse emulsion (lipid droplets ~0.5 μm) of high surface area due to shear produced by antral contraction, retropulsion and gastric emptying. This is accompanied with hydrolysis of the fatty acid glyceryl esters by gastric lipase secreted from chief cells in the stomach (capable of functioning in an acidic environment) which act at the oil/water interface. The enzymatic hydrolysis reduces the TGs into its more polar products monoglycerides (MGs) and FAs. Lipase cleaves the two ester bonds of the TG molecule, producing a molecule of diglyceride and one free FA first, and then two molecules of free FAs and one molecule of MG (Fig. 2). The dispersed lipid digestion products along with the undigested lipids then empty into the duodenum [44].

As the acidic gastric content reaches the duodenum, the low pH causes the release of secretin from the duodenal mucosa into the portal circulation, which drains the digestive organs, spleen, and pancreas and delivers the blood to the liver via hepatic portal vein. This stimulates the pancreas to produce and secrete bicarbonate (along with lipase and co-lipase) into the duodenum to create a pH-neutral environment, which in turn maximizes the activity of pancreatic lipase and co-lipase. In the presence of FAs, cholecystokinin is released into the portal circulation which additionally stimulates the pancreas to release TG lipase and co-lipase required to facilitate the TGs digestion within emulsified particles. Being partially ionized, FAs and MGs are also potent emulsifiers which promote binding of the co-lipase–lipase complex to the emulsion surface [45,46]. Thus, the lipolysis is an autocatalytic process capable of enhancing the emulsification when lipolytic products are produced. Both enzymes being water soluble act at the water/lipid interface of the particles and hydrolyze TGs to MGs and FAs [47,48]. The digestion phase ends with the formation of mixed micelles by the interaction of FAs and MGs with bile salts, while a part of the TGs and FAs may form vesicles after digestion in this preabsorptive phase [49–50]. It is at this phase that the drug released from the formulation due to either precipitation or dissolution into the gastric media is resolubilized as micelles or mixed micelles by emulsifications, which can play a significant role on the performance of the formulation [51–54]. The overall in vivo solubilization capacity depends on both the lipophilicity and chemical structure of the drug and the nature of the endogenous and exogenous lipids involved in the formation of colloidal species [55].

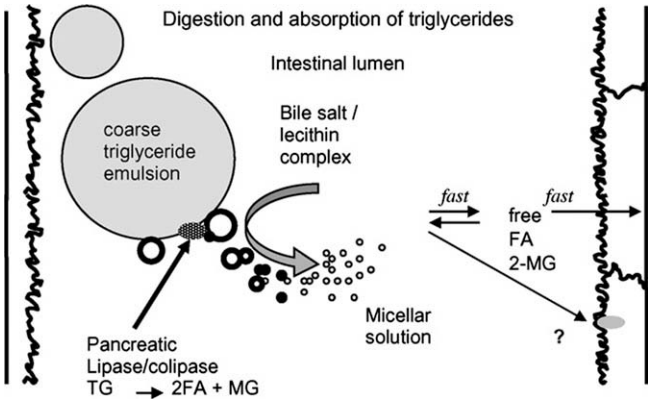

Fig. 2. A schematic representation of the role of lipase/co-lipase and mixed bile salt micelles in digestion of triglycerides and solubilization of the digestion products. Each triglyceride molecule is digested to generate two molecules of fatty acid and one molecule of monoglyceride which is solubilized in the lumen of the gut (from ref. [2] with permission).

Quantities even in the range of 2 g of long-chain lipid stimulate gall bladder contraction and elevate intestinal biliary lipid accumulation without any significant alteration in gastric emptying time. However, similar quantity of medium-chain lipid has been demonstrated to have little effect on gallbladder contraction and elevation in intestinal concentrations of biliary-derived lipids [53]. It has been shown that a lipid emulsion containing 10 g of glyceryl monooleate is capable of stimulating the same increase in drug absorption of danazol in healthy volunteers as that observed after administration with a large meal [38].

The enzymatic action being an interfacial process, the rate of lipolysis is enhanced in formulations with good dispersibility like self-nano/microemulsifying drug delivery systems. These types of formulations maximize the rate of drug partitioning into the aqueous intestinal fluids and provide consistent bioavailability, as seen in the case of Sandimmune and Sandimmune Neoral formulation [56,57].

3.2. Absorption phase

The colloidal species produced, in the form of micelles, mixed micelles, vesicles and free FAs as a result of lipid digestion, are taken up by passive diffusion, facilitated diffusion and active transport through the enterocyte membrane. In the cytosol, a fatty acid-binding protein transports them from the apical membrane to the smooth endoplasmic reticulum (ER). Thereby, a concentration gradient facilitates the uptake of FAs into the cell by a carrier-mediated process [58]. In the smooth ER, FAs and MGs are resynthesized into TGs and phospholipids, respectively, which are transferred to the golgi apparatus and stored into secretory vesicles to be released by exocytosis into the extracellular space via basolateral membrane. Another critical step is the association of the absorbed free drug with the intestinal lipoproteins (chylomicrons) within the enterocyte. These chylomicrons are relatively large (<1 μm in diameter) and colloidal in nature which eventually

lead to selective intestinal lymphatic transport of the lipophilic compound [59–63].

During the absorption phase, the drug molecules are usually exposed to the activity of major phase I drug metabolizing enzyme, Cytochrome P450 3A4 (CYP 3A4), present at high concentrations in enterocytes located at the villus tip of the small intestine in humans. Studies conducted across different laboratories have accounted the role of these enzymes in increasing the bioavailability of drugs when co-administered with lipid, which is indicative of an additional pathway by which lipids enhance oral drug bioavailability [64–70]. However, the exact mechanism behind this phenomenon is still unclear. Few workers are of the opinion that the lipids attenuate the expression and activity of these enzymes, while others have proposed that the lipid shields the drug molecules from the enzymes [71–74].

3.3. Circulatory uptake

The majority of orally administered drugs gain access to the systemic circulation by absorption into the portal blood. However, some extremely lipophilic drugs ($\log P > 5$, solubility in TG > 50 mg/ml) gain access to the systemic circulation via lymphatic route, which avoids hepatic first-pass metabolism. Therefore, highly metabolized lipophilic drugs may be potential candidates for lipid-based drug delivery. Compounds showing increased bioavailability in the presence of lipids (dietary or lipid-based formulation) are absorbed via the intestinal lymph as they are generally transported in association with the long-chain TGs lipid core of intestinal lipoproteins formed in the enterocyte after re-esterification of free FAs and MGs. Short-chain TGs are primarily absorbed directly in the portal blood. Drug transport via the lymphatics, therefore, requires co-administration of lipid to stimulate lipoprotein formation [75,76].

Direct uptake of TGs and phospholipids into the bloodstream is not possible, though the portal blood is approximately 500-fold higher than that of the intestinal lymph. This is because their large molecular size restricts them to pass through capillary fenestration spaces. The walls of lymphatic capillaries consist of a single layer of squamous epithelial cells, and this thin wall makes it possible for tissue fluid (interstitial fluid) from the interstitial space to enter the lymphatic capillary. Moreover, the endothelial architecture of the lymphatic vessels facilitates the size-selective transport of high molecular weight substances like chylomicrons for which facile access across the blood capillary endothelium is restricted [77]. Studies have shown that the free FA chain length and the composition and size of the lymph lipid precursor pool in the enterocyte play major role in lymphatic drug transport. In general, free fatty acids (FFA) of chain length $\leqslant$12 carbons are absorbed primarily by means of the portal blood, whereas FFA with chain lengths >12 carbons are re-esterified and transported via intestinal lymph [64]. Additionally, increase in the degree of unsaturation produces larger size lymph lipoproteins and selectively enhances lymphatic uptake [78–83].

The lymph fluid is then emptied (average 3 L per day) via thoracic duct into the subclavian vein [84], thus protecting the drug from hepatic first-pass metabolism. The lymphatic system, being the principal systemic transport pathway for B and T lymphocytes as well as the primary route of metastatic spread of a number of solid tumours and several viruses [2,4], is a potential drug delivery target for immunomodulatory, anticancer compounds and other related drugs [85–96]. The drug being transported in the circulatory system, in the form of either micelles or mixed micelles, may then be available in its free form, since upon dilution with a large volume of the lymph/blood, surfactant concentration may reduce below its cmc value and micelle may dissociate into mono-

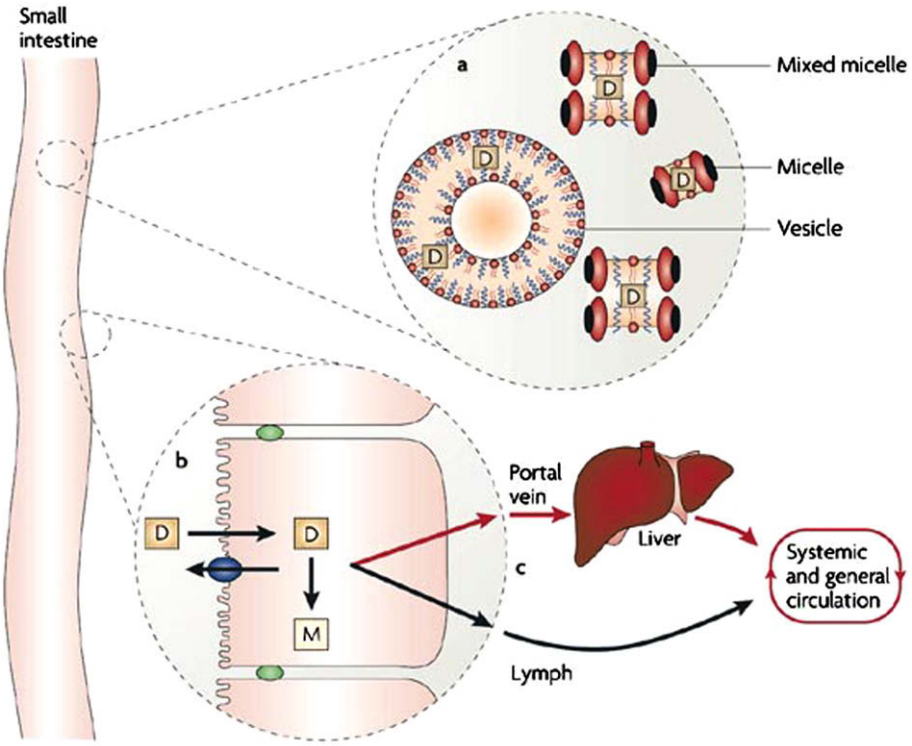

Fig. 3. Various mechanisms of enhancement of drug bioavailability in the presence of lipids: (a) solubilization of drug in the intestinal fluid by formation of colloidal species viz., vesicles, mixed micelles and micelles; (b) interference with enterocyte-based transport and metabolic processes, thereby potentially changing drug uptake, efflux, disposition and the formation of metabolites (M) within the enterocyte; (c) by selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation (adapted from ref. [99] with permission).

mers [97]. The drug transported as lipid vesicles may remain intact for extended periods and, thereby, can result in prolonged release of the encapsulated drug [98]. Fig. 3 is a diagrammatic presentation of the various mechanisms by which lipids enhance the bioavailability of drug.

4. Morphology of lipid digestion products

Lipid digestion in the intestine results in the formation of several colloidal species including vesicles, micelles and mixed micelles. Vesicles are self-assembled lamellar phases composed of water-insoluble phospholipids (such as phosphatidylcholine). Micelles are composed of surfactant molecules which solubilize as monomers in water up to the critical micellar concentration above which the monomers self assemble to form micelles. Mixed micelles are micelles composed of mixed surfactant systems (Fig. 3) [100,101]. The vesicles represent intermediate products of the interactions between oil droplets and bile salt media in the presence of lipase activity. During the digestive process, bilamellar vesicles are generated which usually transform into unilamellar vesicles. These spontaneously dissolve into micellar and/or mixed micellar phases with increase in the surfactant (bile salt)-to-lipid ratio [52,101,102]. The phase transition produces the thermodynamic condition most favorable for effective lipid absorption from the upper small intestine, while the lipolytic products dispersed as unilamellar and multilamellar vesicles are responsible for fat absorption along the later part of small intestine in bile salt deficiency states [52].

Quasielastic light scattering analysis has revealed that ex vivo mean hydrodynamic radii of micelles are $\leqslant$40 Å, whereas unilamellar vesicles are in the range of 200–600 Å, and lipids were largely solubilized by micelles than were dispersed as unilamellar vesicles [52]. Cryogenic transmission electron microscopy of in vitro digestion of a self-nanoemulsifying drug delivery system revealed the entire sequence of the phase changes in the digestive process, wherein micelles of around 10 nm were present at all time points [51]. The structures provided evidence to the previously proposed model in which unilamellar and multilamellar vesicles co-exist with micelles [102]. A liquid lamellar crystalline phase containing calcium and ionized FAs called calcium soaps followed by "a viscous isotropic phase" has been identified with light microscopy after hydrolysis of an oil emulsion [103]. Small-angle X-ray scattering measurements have also been employed efficiently as a screening tool to elucidate the processes encountered during the digestion of lipid-based formulations in an in vitro dynamic lipolysis [104]. Further investigations into the morphological characteristics of the structural changes occurring during lipid digestion process can help to understand the partitioning of the drugs to the lipolytic products and consequently the in vivo performance of the formulation.

5. Approaches in the design of lipid-based oral formulations

5.1. Liquid lipid-based formulations

The lipid-based dosage forms may be broadly classified into liquid or solid formulations depending on the physical state of the lipid at room temperature. Formulations using liquid lipid can be developed either as a simple emulsion or in the form of self-micro-emulsifying drug delivery system (SMEDDS). An emulsion (oil/water or water/oil) is a mixture of two immiscible phases, wherein an emulsifier (surfactant) is added in the external phase to stabilize the dispersed droplets. This system is thermodynamically unstable as the immiscible phases have a tendency to separate with time. Therefore, the proper choice and concentration of an emulsifier and production conditions are necessary to improve the shelf life of the product [105]. SMEDDS is an isotropic mixture of lipid, surfactant, co-surfactant and the drug substance. Its basic principle lies in its ability to spontaneously generate fine oil-in-water (o/w) microemulsions under mild agitation following dilution with aqueous phases. These conditions mimic the digestive motility in the GIT necessary to provide the agitation required for in vivo self-emulsification [106,107]. Unlike emulsions, self-nanoemulsified drug delivery system (SNEDDS) generates microemulsion with a narrow droplet size distribution of less than 50 nm due to which these systems have also been addressed as nanoemulsions [108]. The fine droplets of this dosage form have the advantage of presenting the drug in a dissolved form with a large interfacial surface area for drug absorption which results in more uniform and reproducible bioavailability as was observed in the case of cyclosporine [106,109,110]. Moreover, the drug is maintained in dissolved state throughout the gastrointestinal tract which helps in enhancing the bioavailability of drugs with poor aqueous solubility [107,111]. In addition, the fine droplets offer large surface area for pancreatic lipase to hydrolyze the lipids and thereby enhance the rate of drug release and/or generation of mixed micelles containing the drug [112]. The adequate solubility of the drug in lipid/surfactants blends, nature of the lipid/surfactant pair, the ratio between lipid and surfactant, the surfactant concentration and uniform droplet size distribution following self-emulsification are necessary components to be monitored during development of SMEDDS [106,109,113–116].

However, a few issues limit the applicability of SMEDDS which includes critical fabrication technology, incompatibility with capsule shell, precipitation of drug during storage at low temperature and stability of the active ingredients in the aqueous medium [117,118]. It was for the above-mentioned reasons that an alternative method has been currently investigated by several authors, which involve the incorporation of liquid SMEDDS into a powder with free flowing and readily compressible characteristics in order to create a solid dosage form (tablets, capsules). Self-emulsifying pellets prepared by wet granulation of powder mixture consisting of drug, microcrystalline cellulose and lactose as carriers, and mono- and diglycerides and polysorbate 80 as the lipid-based excipients have shown to improve the solubility and permeability characteristics of the drug [119]. Pellets with similar composition have also been prepared using extrusion/spheronization process [120]. The drug release from pellet formulations can be controlled by polymer coating [121]. Recently, a novel eutectic-based SNEDDS of ubiquinone was incorporated into a tablet dosage form, which was termed "liquisolid" or solid–lipid compact with immediate drug release profile, using blends of maltodextrin, modified povidone, and microcrystalline cellulose [122–125]. Later, a solid-state microemulsion for the delivery of cyclosporine was prepared by coating a pre-microemulsion with an enteric coating material [126]. Similarly, tocopheryl nicotinate tablets were prepared by solvent evaporation method using calcium silicates as the adsorbing agent [127]. Solid SMEDDS of nimodipine was recently prepared by spray-drying, using water-soluble dextran 40 as the solid carrier. The process generated distinct and spherical particles without disturbing the self-emulsification performance of the liquid SMEDDS both in vivo and in vitro [128]. A novel solid SEDDS of dexibuprofen prepared by spray-drying liquid SEDDS with Aerosil 200 as an inert solid carrier suggests the potential of such formulation in the improvement of bioavailability of poorly water-soluble drugs [129]. A controlled release "liquisolid" compact that could release the lipid formulation from its solid carrier in a controlled release pattern over an extended period of time has also been fabricated [130]. This study reported the leakage due to lipid release and loss of hardness on storage of the formulation at higher

temperatures, and this issue needs to be addressed to develop a stable lipid-based formulation.

Similarly, the common problem of physical instability of simple emulsions has also been overcome by the formulation of dry powder emulsions. Several approaches have been used for the liquid-to-solid transformation which includes spray-drying [131,132], lyophilization [133–135] and solvent evaporation [136,137].

5.2. Solid lipid-based formulations

Formulations of solid or waxy lipid can be developed into multiparticulate system (powder, granules or pellets) containing drug either as fine dispersion or solid solution which could subsequently be filled into capsules or sachets. They may even be compressed into self-dispersing tablets using necessary tabletting excipients to overcome the technical difficulties (flow characteristics and sticking to the punches and die cavity) relating to the physical form of the waxes. A combination of lipid solid dispersion and surface adsorption technique using excipients with excellent flow property, compressibility, high specific surface area and high surface capacity (e.g. Neusilin, Florite, Sylysia) can be of potential use in the development of lipid-based formulation specifically to overcome the poor physiochemical characteristics of lipids (Table 1) [138]. Besides being simple and cost effective, this technique produces dosage forms which present the drug in its minimum possible particle size (even at the molecular state) with the maximum surface area which in turn improves the rate and extent of dissolution in the presence of GI fluids. In this technique, the lipid is required to be liquefied by melting, particularly in case when the lipid exists in solid state. The drug is then either dispersed or solubilized in the liquid/liquefied lipid, followed by addition of adsorbent which is then mixed properly to obtain a uniform blend. The final product is obtained by cooling the blend to room temperature, if required [139,140]. Considering the cost and convenience of production and convenience of patients, development of drug solution in liquid lipids and incorporating them onto suitable adsorbents to produce a solid dosage form by simple adsorption or melt granulation technique would also be advantageous. The adsorption efficiency of the adsorbents used is important, as the use of strong adsorbents to adsorb the molten lipid may slow down the drug release rate on solidification of the lipid, which may be explored to modulate the release of the drug [104].

The maximum advantage from a lipid formulation could only be drawn if the drug remains in lipid solution throughout its residence in the GI tract. However, the performance of lipid formulations and the fate of the drug in the GI tract depend on three Ds that occur simultaneously viz., dispersion, dilution and digestion of the formulation. Dispersion of the formulation due to gastric agitation results in increased exposure of the inner surfaces of the formulation, dilution in the gastric fluid results in partitioning of the drug in the external fluid phase and enzymatic breakdown of the lipid in the GI tract can result in change in their composition, structure and potential loss of their solvent capacity. The breakdown of the lipid vehicle in the presence of gastric lipase results in reduction in their solubilization capacity [142,143]. In case of lipid-based liquid formulations (solutions or emulsions), the drug may migrate into the bulk phase, while in the case of lipid-based solid formulations (solid solution), the solubilized drug may crystallize out on the surface on solidification of lipid and during storage [1,4]. This may be the probable reason why Gupta et al. [138] observed enhanced dissolution of drug from lipid-based dispersion granules upon storage. Altogether, these processes may cause precipitation of drug to occur and thus, the advantage of a lipid formulation is lost. However, the precipitated drug may be resolubilized by both the exogenous and the endogenous lipids in the form of colloidal species in the intestinal region which would depend on several factors, like, the lipophilicity of the drug, the nature of the colloidal phases produced on digestion of the different lipids and the kinetics of drug transfer between the digesting formulation and the colloidal phases produced [143].

Drugs with poor water solubility are suitable candidates for lipid-based formulation; however, this is a broad classification, wherein the drugs differ significantly in their physicochemical properties. Water insoluble and weakly basic drugs require special care in the design of lipid formulations [2]. These drugs administered in the solubilized form in the lipid vehicle may come out of the formulation due to solubilization in the gastric fluid and may precipitate in the intestinal fluid on gastric emptying. The bioavailability of such a system would then depend on how rapidly the precipitates can be resolubilized by the formulation or the intestinal fluid. An in vitro study was conducted in our laboratory using carvedilol as a model water insoluble, lipophilic and weakly basic drug (BCS Class II) and three different TGs (benefat, olive oil and caprylic acid). The drug was found to be soluble in benefat and olive oil while insoluble in caprylic acid. The drug was dissolved/dispersed in the TGs and adsorbed over Neusilin US2 (lipid adsorbent ratio used was 1:0.5) to obtain a free flowing solid multiparticulate system (Table 2). Unlike olive oil and caprylic acid, benefat being solid at room temperature was liquefied at 50 °C to dissolve the drug. The liquid/liquefied drug TG solution/suspension was mixed with the adsorbent, and finally, the liquefied mixture was cooled to the room temperature. The in vitro dissolution of the above-mentioned formulations was conducted in 900 ml simulated gastric fluid without enzymes using USP apparatus II at 75 rpm and 37 ± 0.5 °C. The rate and extent of drug release were found to be extremely fast (~90% in 10 mins) (Fig. 4) and almost similar for batches II, III and IV irrespective of the type and concentration of lipid, and moderately fast for batch V, with respect to the pure drug (Batch I). The reason for such a remarkable increment in the dissolution characteristic of the drug can be explained on the basis of interfacial tension between the drug and the medium. The pure drug particles being highly hydrophobic in nature offer high surface resistance which prevents its solubilization in the dissolution medium. In contrast, the formulations containing adsorbed lipid as drug carriers are capable of reducing the surface tension of the drug in the medium and improve its dissolution. The reason for a comparatively retarded dissolution profile of batch V than batch II–IV can be attributed to the solubility of the drug in the lipid. Being soluble, the drug is available in the molecular state in formulations containing benefat and olive oil, while being insoluble, the drug is dispersed in the particulate form in caprylic acid. Therefore, there is a significant increase in the surface area of the drug in the

Table 1
List of adsorbents to improve the flow properties of lipids [139,141].

Sl. no.	Adsorbent brand name	Adsorbent chemical name	Specific surface area (m^2/g)	Particle size (μm)	Company
1	Neusilin US2	Magnesium	280	75.0	Fuji Chemical Industry Co., Ltd., Japan
2	Neusilin S2	Alumino meta silicate	110	100	
3	Sylysia 320	Porous silicon dioxide	300	3.2	Fuji Silysia Co., Ltd., Japan
4	Sylysia 550		500	3.9	
5	Florite RE	Porous calcium silicate	120	26.1	Eisai Co., Ltd., Japan

molecular state than the particulate state which is responsible for the improved dissolution of batches II–IV than batch V. Thus, the high solubility of the drug in the acidic medium, results in its dissolution and dissociation from the lipid carriers irrespective of the type or concentration of the carrier. In such case, it may be proposed that an enteric-coated lipid-based formulation may be advantageous to harvest maximum benefit of the lipid vehicle. The concept of enteric-coated lipid carrier system is still premature, and there are many issues to be examined before actual use. However, our recent accomplishment implies the possibility to offer an improved utilization of lipid by intestinal release of drug in lipid formulations. At present, the work related to the above-mentioned concept is in progress.

The choice of lipid is crucial in the formulation design. Ideally, the drug is dissolved in lipid-based formulations as there is little evidence that suspending drug in a lipid formulation can reproducibly enhance bioavailability [144]. Lipids must be selected based on their ability to solubilize the drug. Although no standard method is available to determine the solubility of the drug in solid lipids, it may be done by dissolving the maximum amount of the drug in molten lipids (above the melting point) and then subjecting the cooled samples at ambient temperature to optical observation and X-ray diffraction (XRD) analysis to determine the presence of drug crystals. Alternatively, the drug (at different concentrations) and lipid may be dissolved in a common solvent, dried as a film and be analyzed using a microscope and/or with XRD [145]. This may not be possible for high melting lipids; therefore, the amount of drug to be added must be relatively less than the saturation solubility observed at temperature at and above their melting point, due to the fact that the solubilization capacity of the lipid may decrease with the decrease in temperature [4].

5.3. Lipid as colloidal drug carriers

Lipid-based colloidal drug carriers such as liposomes and nanoparticles have been used to improve the therapeutic efficacy of both established and new drugs. Liposomes are enclosed vesicles composed of one or more phospholipid bilayers enclosing an aqueous phase. They can be classified as large multilamellar vesicles (MLV), small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV), depending on their size and the number of lipid bilayers. They are capable of carrying either hydrophilic drug in their inner aqueous phase or hydrophobic drug in their hydrophobic lipid bilayers. As their composition is similar to the biomembranes, they are biodegradable and non-toxic [146]. The potential application of liposomes for oral administration of drug/protein has been widely studied for increasing their solubility or stability and absorption of poorly water-soluble drugs [147,148]. Some authors are of the view that liposomes are capable of protecting the drugs sensitive to the hostile environment in the gastrointestinal tract [149,150], while others have shown that the enzymatic activity in the duodenum and bile salts destroys the lipid bilayers of most types of liposomes, thus releasing the drug [151]. However, multilamellar liposomes prepared from phospholipids with phase transition temperature above 37 °C and those containing cholesterol as their component are most resistant to degradation. Though several techniques have been employed for the fabrication of liposomes, the popular methods used are film hydration technique, solvent injection method, reverse-phase evaporation technique, detergent removal technique and high-pressure extrusion technique [152,153]. The liposomes prepared by any of the above-mentioned methods can be lyophilized or freeze dried using suitable cryoprotectants to improve the shelf life of the product in case the drug is unstable in the aqueous phase.

Oral delivery of drugs incorporated in SLN has gained considerable interest since last two decades [154–156]. As they are derived from physiologically compatible lipids, SLN represent a safe and effective alternative in comparison to the conventional polymeric nanoparticles [157]. In vitro studies on Pgp-overexpressing human cancer cells using doxorubicin and paclitaxel-loaded lipid nanoparticles have shown to overcome multidrug resistance via Pgp inhibition and ATP depletion [158,159] which indicate their potential in improving the therapeutic efficacy of drugs. In vivo studies performed with orally administered lipid nanoparticles containing drugs such as cyclosporine A [160], camptothecin [161], idorubicin [162], tobramycin [163,164], rifampicin, isoniazid and pyrazinamide [165] and proteins such as chitosan-coated nanoparticles containing calcitocin [166] and lectin-modified insulin-containing SLN [167] have shown promising results in bioavailability enhancement. The technology for the production of SLN is well established which include high-shear homogenization and ultrasound, high-pressure homogenization (hot and cold homogenization), solvent emulsification/evaporation and microemulsion method. SLN may be either administered as an aqueous dispersion or after incorporation into a traditional dosage form, i.e. tablets, pellets, capsules or powders in sachets. The aqueous SLN dispersion may be used as the granulation fluid in the granulation process or can be spray-dried into a powder and mixed with the necessary excipients before compression into tablets. SLN dispersion can also be used as wetting agent in the extrusion process for the production of pellets [168]. Alternatively, SLN powders can be filled in hard gelatin capsules or incorporated in liquid PEG and filled into soft gelatin capsules. The spray-dried or lyophilized powders can even be filled into sachets. The physical characteristics of the resultant SLN powder like flow property, compressibility, bulk density, waxy nature and strength to withstand the compression force and temperature need to be carefully addressed before production of the final dosage form.

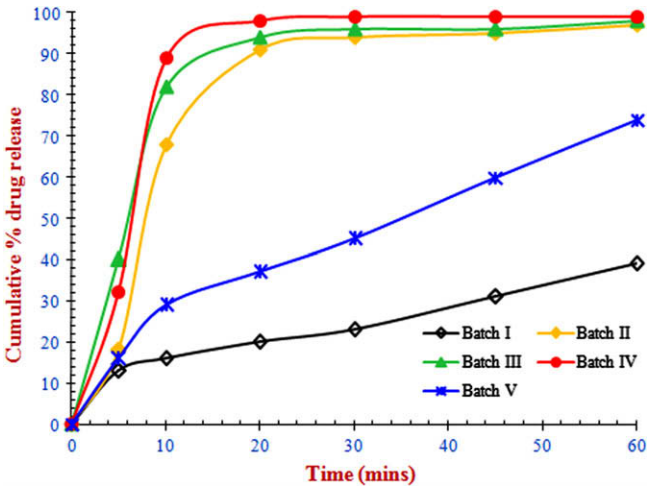

Fig. 4. Drug release profile of all prepared batches in simulated gastric fluid without enzymes.

Table 2
Lipid-based formulations of carvedilol.

Ingredients	Batch I	Batch II	Batch III	Batch IV	Batch V
Carvedilol	10	10	10	10	10
Benefat	–	100	250	–	–
Olive oil	–	–	–	250	–
Caprylic acid	–	–	–	–	250
Neusilin US2	–	50	125	125	125

Quantity expressed in mg. Carvedilol is soluble in benefat and olive oil and insoluble in caprylic acid.

6. Evaluation of LBODDS

6.1. Design of in vitro dissolution testing methodology

Design of an in vitro dissolution testing methodology for a formulation is necessary for the proper selection of excipients and to access its performance during the different phases of drug development process. It is also essential for quality control and to predict the product's in vivo performance post development. A meticulously designed in vitro dissolution test can be effectively used for regulation of post-approval changes and even to claim biowaivers and thus substitute clinical studies with in vitro dissolution studies. In case the in vitro results are inadequate to predict the in vivo performance of the dosage form, then clinical studies are required to evaluate the bioavailability of the product which adds substantial cost to the product [12]. Mimicking the complex physiological environment, which dictates the release of drug from the formulation and its absorption, is critical in the design of an in vitro dissolution method [13]. The matter becomes still more complicated when the components of the formulation join to decide the fate of the drug in the body. Therefore, it is important to obtain more comprehensive understanding of the physiochemical nature of the excipients used and the implication of the components of the dissolution medium on those excipients to establish an in vitro in vivo correlation for assessing absorption mechanisms [169].

As lipids undergo a complex series of events on interaction of lipid-based formulations with the GI environment, their use as carrier requires in-depth exploration of the dispersion properties of the formulation in aqueous media, drug diffusion mechanism and the impact of digestion of lipids on the solubilization and absorption of drug molecules [52,170–175]. Though no standard pharmacopoeial testing method for lipid-based formulations is available till date, special emphasis has been given to dispersion testing using a standard dissolution apparatus along with digestion test in order to design laboratory test methods capable of establishing sufficient in vitro–in vivo correlation, as dilution in the GI fluid and enzymatic breakdown of the lipid are potential reasons for loss of their solvent capacity [103,176,177]. The area of in vitro dissolution testing methodologies has been well reviewed in the past [3]; therefore, the aim of this section is not to revisit in detail many of the reports that have been already covered, rather to focus here on recent advances made till date.

The enzymatic hydrolysis of lipids has been studied using an instrument known as the pH-stat set (Fig. 5). The instrument, also known as Titra-stat or pH-stat titration system, has fully programmable multi-step titrations, real-time plotting and automatic documentation of entire titration data. The basic mode of operation of the instrument is to maintain a constant pH as hydrogen ions are released or consumed during the reaction. Any deviation in pH from the set value is quickly compensated by reagent addition. The speed, the volume of reagent addition and temperature of the reaction are also monitored to maintain the pH of the medium. The reaction medium for lipolysis should have an inherently low buffering capacity to ensure drop in pH with the liberation of fatty acids. The pH-stat titrates a measured volume of sodium hydroxide solution to maintain the initial pH which is equated with the fatty acid liberation. The activity of pancreatic lipase added for lipolysis is normally expressed in terms of Tributyrin Units (TBU), where 1 TBU is the amount of enzyme that can liberate 1 μmole of titrable FA from tributyrin per min in the presence of 5 mM $CaCl_2$ and 150 mM NaCl [178]. Calcium is added as it is reported to activate lipase and exert a significant influence on the rate of triglyceride lipolysis in the presence of bile, and sodium chloride is added to simulate the intestinal ionic environment [178]. The instrument along with a temperature-controlled vessel for the buffer solution

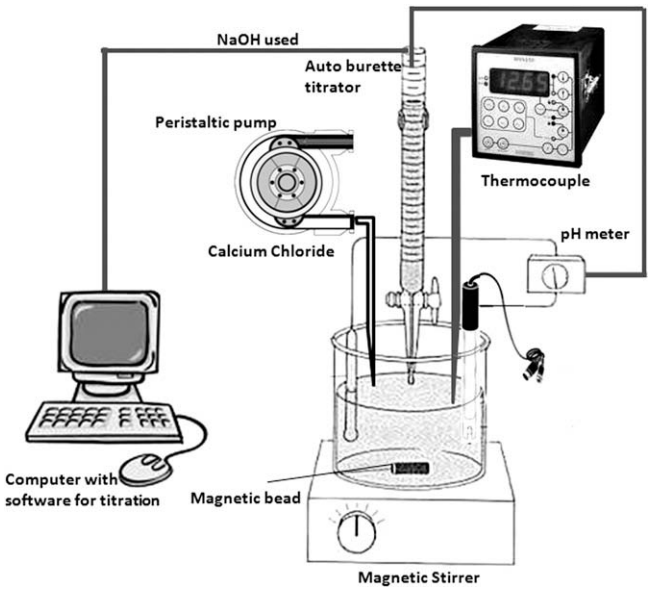

Fig. 5. Lipolysis set-up. It consists of a thermostated double-wall reaction vessel, the pH-stat with the auto burette for the addition of NaOH, a peristaltic pump for the addition of $CaCl_2$ and the computer with the software for the titration experiments. The temperature is monitored during the experiment with a thermocouple. The experiment is performed under continuous agitation at 37 °C.

and a stirring arrangement has been employed in many dynamic lipolysis models to mimic the intestinal conditions in terms of maintaining constant pH, presence of lipase/co-lipase and physiological concentration of bile salts and phospholipids [8,179–182]. A list of recent examples of the components used in the design of in vitro lipolytic model is presented in Table 3.

An attempt of Reymond and Sucker [179] to develop an in vitro model for cyclosporine by phase quantification method failed to simulate the dynamics of in vivo absorption events following administration of a poorly water-soluble drug in a lipid vehicle, probably due to oversimplification of the model. Zangenberg and co-workers [180–181] developed a dynamic in vitro lipolysis model, which was used to investigate the dissolution of poorly soluble lipophilic drug substances at controlled hydrolysis rates. In one of their studies [180], the effects of three experimental parameters—the bile salts concentration, calcium concentration and the lipase activity—were investigated. It was shown that all three investigated parameters influenced the initial rate of hydrolysis, whereas only the lipase activity and the concentration of calcium affected the subsequent stages. It was also shown that the rate of addition of calcium can be used to control the rate of lipolysis. In another study [181], they characterized and evaluated the model by investigating the composition of the aqueous phase and the concentration of two hydrophobic drugs in the aqueous phase. The analysis of the aqueous phase indicated that the concentration of lipolytic products was dependent on the bile salt concentration, while the concentration of the drugs was dependent on their lipophilicity. An investigation by Sek et al., [173] related to the digestion kinetics of TGs showed that the rate and extent of digestion of the medium-chain TG were greater than the long-chain TG and were independent of bile salt concentration, with complete digestion occurring within 30 min. Christensen and co-workers [183] have proposed an in vitro lipid digestion model which could be used to study food effects of poorly water-soluble drugs. This model was used to study the transfer of different poorly water-soluble drugs from fractionated coconut oil (MCT) and sesame oil (LCT) to the sampled aqueous micellar phase. It

Table 3
Recent examples of the components used in the design of in vitro lipolytic model.

Buffer	Lipase	Ions	Volume	Surfactants	Lipid substrate	Ref.
Trizma maleate (2 mM) pH 6.5	Pancreatin 270–1340 units/ml	Na^+ 150 mM Ca^{2+} 4–20 mM (0.135 mM/min)	300 ml	BS 1.5–6.6 mM	Soybean oil	[180]
Trizma maleate (2 mM) pH 6.5	Pancreatin 760 units/ml	Na^+ 150 mM Ca^{2+} 0.181 mM/min	300 ml	BS 5/10/20/30 mM	Soybean oil Oleic acid	[181]
Trizma maleate (2 mM) pH 6.5	Pancreatic lipase 800 USP units/ml	Na^+ 150 mM Ca^{2+} 0.105 mM/min	300 ml	BS 20 mM PC 4 mM	Sesame oil (S 3547) Fractionated coconut oil (Miglyol 812 N)	[183]
Trizma maleate (50 mM) pH 7.5	1 ml of Pancreatin extract (10,000 TBU/ml)	Na^+ 150 mM Ca^{2+} 5 mM	9 ml	NaTDC 5 mM PC 1.25 mM	Soybean oil (LCT) Captex 355® (MCT)	[176]
Tris maleate (50 mM) pH 7.5	1 ml of pancreatin extract (10,000 TBU)	Na^+ 150 mM Ca^{2+} 5 mM	9 ml	Low (5 mM NaTDC/1.25 mM PC) High (20 mM NaTDC/5 mM PC)	Soybean oil (LCT) Captex 355® (MCT), glyceride mixtures representing partially digested triglycerides (Maisine 35-1 and Capmul MCM)	[143,144]
Tris maleate (50 mM) pH 7.4	3.5 ml of pancreatin extract (1000 IU/ml)	Na^+ 150 mM Ca^{2+} 5 mM	35.5 ml	TC 5 mM PC 1.25 mM	Peanut oil (LCT), Captex 355 (MCT), Triacetin (SCT)	[173,182]
Phosphate buffer (1.5 mM KH2PO4, 7.4 mM NaHPO4) pH 6.5	5–20 μl of porcine pancreatic lipase solution (20000 units/ml)	Na^+ 150 mM	50 ml	–	Glycerol monooleate Glycerol dioleate Diglycerol monooleate Glycerol monocaprinate Diglycerol monocaprinate Glycerol trioleate (olive oil) Tributyrin Cremophor	[8]
Trizma maleate (2 mM) pH 6.5	Pancreatic lipase 800 USP units/ml	Na^+ 150 mM Ca^{2+} 0.045 mM/min	300 ml	BS-5 mM PC-1 mM	Sesame oil (LCT), Maisine 35-1, Mono-, di and triacylglycerides, Cremophor RH 40	[51]
Tris maleate (50 mM) pH 6.8	3.5 ml of pancreatin extract (1000 IU/ml	Na^+ 150 mM Ca^{2+} 5 mM	35.5 ml	5 mM TC 1.25 PC	Peanut oil (LCT), Triacetin (SCT) Triglycerides of caprylic/capric acid (MCT)	[197]

was found that differences in the physicochemical properties of the drugs resulted in differences in their distribution between the phases arising during lipolysis. Wahren and co-workers [43] have shown that the presence of drug reduces the initial rate of lipolysis of a self-microemulsifying formulation, compared with the formulation without drug. This indicates that the initial rate of lipolysis and hence, release of drug load from a self-microemulsifying drug delivery system is influenced by inclusion of drug as the drug bound in the water/emulsion interface limits the substrate availability for the lipase activity. The usefulness of in vitro lipolysis model for optimization of oral lipid formulations in the case of presystemic metabolism in the gut has been highlighted by Dahan and Hoffman [182]. However, the same in vitro lipolysis data may not be predictive for actual in vivo absorption when lymphatic transport is a significant route of absorption as the bioavailability of vitamin D3 was found to correlate with in vitro data only when the lymphatic transport was blocked. Brogard and co-workers [8] have designed a lipolysis method to study the degradation of lipids before the growing amounts of lipolysis products cause the retardation of the process. The study has emphasized on the use of lipase substrate in excess, and the amount of degrading enzyme is limiting for the degradation process. Under such conditions, as the amount of lipase substrate is held constant, an increase in enzymatic activity generates a proportional increase in the lipolysis rate which would enable to compare the results obtained from different enzyme batches and to correct for day-to-day variability. Based on the above findings, this model could be used for the investigation of dissolution of drug from the formulation during lipolysis which may be either a lipolysis of TG from the formulation or a concurrent TG emulsion simulating fed state.

6.2. In vivo studies

Appropriately designed in vivo studies of formulations, usually performed in the early phases of drug development, can provide important information about the impact of the excipients on the overall bioavailability and pharmacokinetic profile of the drug. Together with information from in vitro studies, these investigations can be a primary basis of labeling statements (e.g. to be taken with/without food) and can often help avoid the need for further investigations. As the lipid-based formulations are targeted to improve bioavailability by the lymphatic uptake of the drug, therefore a detailed investigation of the intestinal lymphatic absorption is required. However, due to significant differences in the methods and animal models used to study intestinal lymph drug transport and the lack of sufficient clinical studies has made the comparison of data very difficult [184]. Therefore, lot more work needs to be done to establish an in vivo method and model to understand the processes involved in the uptake of drugs via the intestinal lymphatic system. This section presents a summary of the relevant in vivo animal research and clinical studies of lipid-based formulations conducted so far.

Invirase® containing Saquinavir mesylate in a hard gelatin capsule was launched in the market in 1995. As its bioavailability was highly variable and as low as 4%, in 1997 Fortovase®, a new formulation of the free base of the drug was introduced in a lipid formulation consisting of medium-chain MG and diglycerides which displayed three times higher bioavailability than Invirase® in humans [185]. Subsequent exploration of the possible mechanisms of improved bioavailability of saquinavir on the mesenteric lymph duct cannulated rat model was carried out. The examination of both lymphatic transport and systemic bioavailability showed that

the increased bioavailability was most likely due to enhanced solubilization and/or permeability of the free base form in the lipid-rich pre-absorptive intestinal environment, as the extent of intestinal lymphatic transport of both forms was found to be similar [186]. This success story illustrates the need to formulate highly lipophilic drug candidates with low bioavailability in form of lipid-based formulations which are presently available in the conventional IR dosage forms in the market.

Sandimmune Neoral is a preconcentrate of cyclosporin in microemulsion of a surfactant, lipophilic and hydrophilic solvents and ethanol. The influence of a high-fat diet on the pharmacokinetics of the formulation was compared with Sandimmune (simple cyclosporine emulsion in soft gelatin capsule) in healthy human subjects [187]. The study showed that the effect of food was less pronounced in case of the former while was significant (37% increase in area under the curve) on the latter. This provides the patients on Sandimmune Neoral, flexibility with regards to their dietary schedule and the formulation is, therefore, more patient compliant.

A study of three lipid-based danazol formulations comprising of a long-chain TG solution (LCT) and two self-microemulsifying drug delivery systems based on long-chain (C18) (LCS) lipids and medium-chain (C8–C10) (MCS) lipids was conducted on fasted beagle dogs. The results showed significant increase in oral bioavailability of the drug due to LCT and LCS than MCS which showed significant drug precipitation in vitro than LCT and LCS [171]. In vivo study on fasted greyhound dogs has shown that even small amounts of lipid (approx. 880 mg of lipid in 1 g of microemulsion) can substantially support intestinal lymphatic transport in the fasted state. In addition, microemulsions containing long-chain glycerides enhanced lymphatic transport greater than those with equivalent amount of medium-chain glycerides [188]. Recently, Wasan and his

Table 4
Some lipid-based oral drug delivery systems available in market [196].

Sl no.	Generic name	Brand name/company	Dosage form	Use	Lipid components
1.	Amprenavir	Agenerase/ GlaxoSmithKline	Soft gelatin (SG) cpsule	HIV antiviral	d-alpha tocopheryl polyethylene glycol 1000 succinate
2.	Bexarotene	Targretin/Ligand	SG capsule	Anti-neoplastic	Polysorbate 80
3.	Calcitriol	Rocaltrol/Roche	SG capsule, solution	Calcium regulator	Fractionated medium-chain triglycerides of coconut oil, Fractionated triglycerides palm seed oil
4.	Carvedilol phosphate	Coreg CR/ Glaxosmithcline	Controlled-release hard gelatin capsule	Anti-hypertensive	Hydrogenated castor oil, hydrogenated vegetable oil
5.	Ciprofloxacin	Cipro/Bayer	Microcapsules for suspension	Antibiotic	Medium-chain triglycerides
6.	Clofazimine	Lamprene/Novartis	SG capsules	Antileprosy agent	Beeswax, butylated hydroxytoluene, citric acid, ethyl vanillin, gelatin, glycerin, iron oxide, lecithin, p-methoxy acetophenone, parabens, plant oils, propylene glycol.
7.	Cyclosporin A	Neoral/Novartis	SG capsule, oral solution	Immuno-suppressant	dl-α-tocopherol, corn oil-mono-di-triglycerides, polyoxyl 40 hydrogenated castor oil (Cremophor RH 40)
8.	Cyclosporin A	Sandimmune/Novartis	SG capsule, oral solution	Immuno-suppressant	Polyoxyethylated Linoleic glycerides (Labrafil M-2125CS) Olive oil, Polyoxyethylated oleic glycerides (Labrafil M-1944CS)
9.	Dronabinol	Marinol/Roxane and Unimed	SG capsule	Anorexia or nausea	Sesame oil
10.	Dutasteride	Avodart/GSK	SG capsule	For benign prostate hyperplasia	Mixture of mono- and diglycerides of caprylic/capric acid
11.	Enalapril maleate-Felodipine	Lexxel/Astra Zeneca	ER tablets	Anti-hypertensive	Polyoxyl 40 hydrogenated castor oil
12.	Fenofibrate	Lipofen/Kowa Pharmaceuticals America, Inc.	Hard gelatin capsule	Lipid regulating agent	Gelucire 44/14 (lauroyl macrogol glyceride type 1500)
13.	Isotretinoin	Accutane/Roche	SG capsule	Anti-comedogenic	Bees wax, hydrogenated soyabean oil flakes, hydrogenated vegetable oils, soyabean oil
14.	Lopinavir and Ritonavir	Kaletra/Abbott	Tablet, SG capsule	HIV antiviral	Sorbitan monolaurate (span 20) Oleic acid, polyoxyl 35 castor oil (Cremophor EL)
15.	Mesalamine	Pentasa/Shire US Inc	Controlled-release capsules	GI anti-inflammatory agent	Acetylated monoglyceride, castor oil
16.	Omega-3-acid ethyl esters	Lovaza/ GlaxoSmithKline	hard gelatin capsule	Anti-hypertriglyceridemia	α-tocopherol (in a carrier of partially hydrogenated vegetable oils including soybean oil)
17.	Paricalcitol	Zemplar/Abbott Laboratories	SG capsule	For secondary hyperparathyroidism	Fractionated medium-chain triglycerides of coconut oil or palm kernel oil
18.	Progesterone	Prometrium/Cardinal Health Encapsulation Tech.	Capsules	For endometrial hyperplasia	Peanut oil
19.	Saquinavir	Fortovase/Roche	SG capsule	HIV antiviral	Medium-chain mono- and diglycerides, dl-α-tocopherol
20.	Sirolimus	Rapamune/Wyeth-Ayerst	Oral solution	Immuno-suppressant	Phosal 50 PG (phosphatidylcholine, mono- and diglycerides, soy fatty acids, ascorbyl palmitate), polysorbate 80
21.	Tipranavir	Aptivus/Boehringer/ Ingelheim	SG capsule	HIV antiviral	polyoxyl 35 castor oil (Cremophor EL), Medium-chain mono- and diglycerides
22.	Tolterodine tartrate	Detrol LA/Pharmacia	ER hard gelatin capsule	Overactive bladder muscarinic receptor antagonist	Medium-chain triglycerides, Oleic acid
23.	Tretinoin	Vesanoid/Roche	SG capsule	Anti-neoplastic	Bees wax, hydrogenated soybean oil flakes, hydrogenated vegetable oils, soybean oil
24.	Valproic acid	Depakene/Abbott	SG capsule	Anti-epileptic	Corn oil

co-workers [189–191] studied the pharmacokinetics and biodistribution of amphotericin B (BCS class IV) in a mono/diglyceride–phospholipid (distearoylphosphatidylethanolamine)-based oral formulation and reported significant intestinal absorption in rats similar to a micellar i.v. preparation. The glyceride-based oral formulation of this drug was also shown to have less renal toxicity as compared to its micellar dosage form containing sodium deoxycholate with sodium phosphate as a buffer [192,193]. In one of these studies, the lipid-based excipients were found to significantly improve the stability of amphotericin B in simulated gastric fluid (relatively unstable at lower pH) which is also a contributing factor for enhancement in oral bioavailability of the drug [189]. These investigations add a new dimension in improving the current therapeutic treatment involving BCS class IV drugs using LBODDS.

The association of drug with chylomicrons in the enterocyte is an essential step in the lymphatic absorption pathway [194,195]. This was confirmed by a study [194] conducted on an experimental rat model with blocked chylomicrons flow to elucidate the lymphatic transport of Vitamin D3 and the results were compared with that of the mesenteric lymph duct cannulated rat model. The results showed that 75% of the absorbed Vitamin D3 is associated with lymphatic absorption and suggest that the drug association with the chylomicrons occurs at an early stage of its assembly process. The results also specify that lymphatic absorption and portal blood absorption are separate pathways and are independent of each other. Furthermore, Gershkovich and Hoffman [195] observed a linear correlation between the ex vivo uptake of lipophilic compounds by chylomicrons with the corresponding intestinal lymphatic bioavailability reported in rats, whereas log P and solubility in long-chain TGs showed only moderate correlation with lymphatic bioavailability. Thus, they developed a simple screening model based on the degree of association of lipophilic compounds with isolated chylomicrons which can be used for estimation of intestinal lymphatic transport potential of drug molecules. Some lipid-based oral drug delivery systems available in the market are listed in Table 4.

6.3. In vitro–in vivo correlation (IVIVC)

A key goal in the development of in vitro models is correlating the in vitro information of various drug formulations to the in vivo drug profile. A model capable of correlating in vitro and in vivo data aids in shortening the drug development period, economize resources and lead to improved product quality. A few studies have been carried out to evaluate the IVIVC of lipid-based formulations. An in vitro lipolysis and ex vivo intestinal permeability model was used to predict the corresponding in vivo oral bioavailability data of two model drugs, griseofulvin and dexamethasone. Though, the in vivo bioavailability of both drugs was found to have good correlation with their in vitro data, ex vivo permeation studies failed to predict the actual in vivo condition. Among the two, only the absorption of griseofulvin was found to be dependent on the nature of the lipid in the formulation probably due their large solubility differences. Griseofulvin being less soluble (5 μg/ml) than dexamethasone (100 μg/ml) is influenced by the presence of bile salts and phospholipids as well as the lipolytic products in the digestive media for its solubility enhancement while dexamethasone is sufficiently solubilized by bile salt and phospholipids and therefore independent of the formation of lipolytic products [197]. Another study on the relative oral bioavailability of halofantrine using medium- and long-chain TGs demonstrated a consistent correlation between the in vitro solubilization and digestion data to the in vivo data only when the in vitro studies were conducted using lower lipid masses. The long-chain TGs were found to be superior in improving the bioavailability of the drug. This study is indicative of the fact that the solubilization capacity of the lipid digestion products is highly dependent on the concentration of lipid present, and that the quantity of lipid utilized in lipid digestion experiments is, therefore, required to be taken into consideration during the design of in vitro lipolysis study [176].

Studies on Caco-2 cells have revealed a strong correlation between excipient-mediated inhibition of lipoprotein secretion and inhibition of P-glycoprotein (Pgp) efflux. Lipid excipients have shown to attenuate the activity of Pgp protein and multidrug resistance-associated protein 2 (MRP2) during treatment of Caco-2 cells [198–200]. Although the exact mechanism is not completely understood, the depressed activity of the efflux transporters upon addition of the lipid-based excipients may be due to decrease in the protein expression and an increase in cell membrane permeability. In addition to lymphatic uptake, this mechanism may also significantly contribute in the bioavailability enhancement of drugs formulated with lipid-based excipients. Common surfactants used in lipid-based oral drug delivery systems (Pluronic block copolymers and Cremophor EL) reversibly inhibited intestinal lipoprotein secretion and effects were found to be concentration dependent [201]. Therefore, the property of the excipients to alter the cellular process must be seriously considered during the selection of excipients for oral drug delivery system. Further studies revealed the lymphotropic property of polysorbate 80, wherein the excipients promoted chylomicrons secretion in Caco-2 cells which was in agreement with the response observed in the cannulated rat model [202]. As Caco-2 cells express P-glycoprotein, synthesize and secrete lipoproteins with similar characteristics to those found in vivo and also respond to FA stimuli similar to that reported in animals, therefore, they could be employed in the initial screening of pharmaceutical vehicle's effects on lipid metabolism as well as for studying absorption mechanisms from oral lipid-based formulations [203–206]. The different aspects of the role of Pgp in drug transport and absorption and the different formulation strategies to effectively inhibit Pgp have been well reviewed in the literature [207]. Computational model using molecular descriptors have been used to estimate the percentage of the absorbed dose to be transported lymphatically upon administration with a long-chain TG [208]. Unfortunately, the literature available for comparison of lipid formulation performance with any in vitro test is limited and lot more investigation is required including examination of more complex lipid-based formulations (containing surfactants and/or co-solvents).

7. Conclusion

Lipid carriers have bright future due their inherent property to enhance the bioavailability of lipophilic drugs with poor aqueous solubility. However, the limitations of these carriers like poor physiochemical properties of lipids, lack of drug solubility database in lipids and unavailability of standard methodologies for in vitro analysis, need to be addressed. The issues related to the development of LBODDS may be overcome by the application of the adsorption technique which has the potential to impart good flow and compressibility to the lipid-based granules. More human clinical studies are needed to be carried out to generate in vivo data and its correlation with in vitro dissolution data, which may help in understanding the solubilization mechanism of lipids in the formulation. All issues and important parameters mentioned herein should be considered for LBODDS, especially at the formulation development stage which would result into a concomitant improvement in the quality, efficacy and safety of drugs.

References

[1] W. Mehnert, K. Mader, Solid lipid nanoparticles: production characterization and applications, Adv. Drug Del. Rev. 47 (2001) 165–196.
[2] C.W. Pouton, Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system, Eur. J. Pharm. Sci. 29 (2006) 278–287.
[3] C.J.H. Porter, W.N. Charman, In vitro assessment of oral lipid based formulations, Adv. Drug Del. Rev. 50 (2001) S127–S147.
[4] C.W. Pouton, C.J.H. Porter, Formulation of lipid-based delivery systems for oral administration: materials methods and strategies, Adv. Drug Del. Rev. 60 (2008) 625–637.
[5] C.J.H. Porter, W.P. Colin, J.F. Cuine, W.-N. Charman, Enhancing intestinal drug solubilization using lipid-based delivery systems, Adv. Drug Del. Rev. 60 (2008) 673–691.
[6] N.L. Trevaskis, W.N. Charman, C.J.H. Porter, Lipid-based delivery systems and intestinal lymphatic drug transport: a mechanistic update, Adv. Drug Del. Rev. 60 (2008) 702–716.
[7] A.J. Humberstone, W.N. Charman, Lipid-based vehicles for the oral delivery of poorly water soluble drugs, Adv. Drug Del. Rev. 25 (1997) 103–128.
[8] M. Brogard, E. Troedsson, K. Thuressona, H.L. Wahren, A new standardized lipolysis approach for characterization of emulsions and dispersions, J. Colloid Interf. Sci. 308 (2007) 500–507.
[9] W.N. Charman, C.J. Porter, S. Mithani, J.B. Dressman, Physiochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH, J. Pharm. Sci. 86 (1997) 269–282.
[10] P.A. Winstanley, M.L.E. Orme, The effects of food on drug bioavailability, Br. J. Clin. Pharmac. 28 (1989) 621–628.
[11] G.L. Amidon, H. Lennernäs, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (1995) 413–420.
[12] J.B. Dressman, G.L. Amidon, C. Reppas, V.P. Shah, Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms, Pharm. Res. 15 (1998) 11–22.
[13] D. Horter, J.B. Dressman, Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract, Adv. Drug Del. Rev. 46 (2001) 75–87.
[14] R.G. Crounse, Human pharmacology of griseofulvin: the effect of fat intake on gastrointestinal absorption, J. Invest. Dermatol. 37 (1961) 529–533.
[15] K.M. Cunningham, R.J. Baker, M. Horowitz, A.F. Maddox, M.A. Edelbroek, B.E. Chatterton, Use of technetium – 99 m (V) thiocyanate to measure gastric emptying of fat, J. Nucl. Med. 32 (1991) 878–881.
[16] C. Feinle, T. Rades, B. Otto, M. Fried, Fat digestion modulates gastrointestinal sensations induced by gastric distension and duodenal lipid in humans, Gastroenterology 120 (2001) 1100–1107.
[17] B.G. Stone, H.J. Ansel, F.J. Peterson, R.L. Gebhard, Gallbladder emptying stimuli in obese and normal-weight subjects, Hepatology 15 (1992) 795–798.
[18] F. Froehlich, J.J. Gonvers, M. Fried, Role of nutrient fat and cholecystokinin in regulation of gallbladder emptying in man, Dig. Dis. Sci. 40 (1995) 529–533.
[19] J.N. Hunt, M.T. Knox, A relation between the chain length of fatty acids and the slowing of gastric emptying, J. Physiol. 194 (1968) 327–336.
[20] S.D. Ladas, P.E.T. Isaacs, G.M. Murphy, G.E. Sladen, Comparison of the effects of medium- and long-chain triglyceride containing liquid meals on gall bladder and small intestinal function in normal man, Gut 25 (1984) 405–411.
[21] A. Dahan, A. Hoffman, Enhanced gastrointestinal absorption of lipophilic drugs, in: E. Touitou, B.W. Barry (Eds.), Enhancement in Drug Delivery, CRC Press, Florida, 2006, pp. 111–127.
[22] D. Wagnera, H.S. Langguth, A. Hanafy, A. Koggela, P. Langguth, Intestinal drug efflux: formulation and food effects, Adv. Drug Del. Rev. 50 (2001) S13–S31.
[23] L. Kalantzi, K. Goumas, V. Kalioras, B. Abrahamsson, J.B. Dressman, C. Reppas, Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies, Pharm. Res. 23 (2006) 165–176.
[24] H. Mu, T. Porsgaard, The metabolism of structured triacylglycerols, Prog. Lipid Res. 44 (2005) 430–448.
[25] M. Fried, E.A. Mayer, J.B. Jansen, C.B. Lamers, I.L. Taylor, S.R. Bloom, J.H. Meyer, Temporal relationships of cholecystokinin release, pancreatobiliary secretion, and gastric emptying of a mixed meal, Gastroenterology 95 (1988) 1344–1350.
[26] H.E. Raybould, J.H. Meyer, Y. Tabrizi, R.A. Liddle, P. Tso, Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation, Am. J. Physiol. 274 (1998) R1834–R1838.
[27] C. Li, D. Fleisher, L. Li, J.R. Schwier, S.A. Sweetana, V. Vasudevan, L.L. Zornes, L.H. Pao, S.Y. Zhou, R.E. Stratford, Regional-dependent intestinal absorption and meal composition effects on systemic availability of LY303366, a lipopeptide antifungal agent, in dogs, J. Pharm. Sci. 90 (2001) 47–57.
[28] M. Martinez, G. Amidon, L. Clarke, W.W. Jones, A. Mitra, J. Riviere, Applying the biopharmaceutics classification system to veterinary pharmaceutical products. Part II. Physiological considerations, Adv. Drug Del. Rev. 54 (2002) 825–850.
[29] P. Gershkovich, A. Hoffman, Effect of a high-fat meal on absorption and disposition of lipophilic compounds: the importance of degree of association with triglyceride-rich lipoproteins, Eur. J. Pharm. Sci. 32 (2007) 24–32.
[30] Food and Drug Administration, Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies, Food and Drug Administration, Rockville, Maryland, USA, 2002. <http://www.fda.gov/cder/guidance/5194fnl.htm>.
[31] D. Fleisher, C. Li, Y. Zhou, L.H. Pao, A. Karim, Drug, meal and formulation interactions influencing drug absorption after oral administration clinical implications, Clin. Pharmacokinet. 36 (1999) 233–254.
[32] L.Z. Benet, C.L. Cummins, C.Y. Wu, Unmasking the dynamic interplay between efflux transporters and metabolic enzymes, Int. J. Pharm. 277 (2004) 3–9.
[33] L.Z. Benet, C.L. Cummins, C.Y. Wu, Transporter–enzyme interactions: implications for predicting drug–drug interactions from in vitro data, Curr. Drug Metab. 4 (2003) 393–398.
[34] J.M. Custodio, C.Y. Wu, L.Z. Benet, Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption, Adv. Drug Del. Rev. 60 (2008) 717–733.
[35] Food and Drug Administration, Guidance For Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, Food and Drug Administration, Rockville, Maryland, USA, 2000. <http://www.fda.gov/cder/guidance/3618fnl.htm>.
[36] J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Järvinen, J. Savolainen, Prodrugs: design and clinical applications, Nat. Rev. Drug Discov. 7 (2008) 255–270.
[37] N. Aoyagi, H. Ogata, N. Kaniwa, A. Ejima, Effect of food on the bioavailability of griseofulvin from microsize and PEG ultramicrosize (GRIS–PEG) plain tablets, J. Pharmacobio-Dyn. 5 (1982) 120–124.
[38] W.N. Charman, M.C. Rogge, A.W. Boddy, B.M. Berger, Effect of food and a monoglyceride emulsion formulation on danazol bioavailability, J. Clin. Pharmacol. 33 (1993) 381–386.
[39] A.J. Humberstone, C.J.H. Porter, W.N. Charman, A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine, J. Pharm. Sci. 85 (1996) 525–529.
[40] E. Nicolaides, M. Symillides, J.B. Dressman, C. Reppas, Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration, Pharm. Res. 18 (2001) 380–388.
[41] L.E. Schmidt, K. Dalhoff, Food-drug interactions, Drugs 62 (2002) 1481–1502.
[42] A. Hinsberger, B.K. Sandhu, Digestion and absorption, Curr. Pediatr. 14 (2004) 605–611.
[43] H.L. Wahren, F.S. Nielsen, M. Brogard, E. Troedsson, A. Mullertz, Enzymatic characterization of lipid-based drug delivery systems, Int. J. Pharm. 298 (2005) 328–332.
[44] M.C. Carey, D.M. Small, C.M. Bliss, Lipid digestion and absorption, Annu. Rev. Physiol. 45 (1983) 651–677.
[45] B. Borgstrom, Importance of phospholipids, pancreatic phospholipase A2 and fatty acid for the digestion of dietary fat: In vitro experiments with the porcine enzymes, Gastroenterology 78 (1980) 954–962.
[46] S. Bernback, L. Blackberg, O. Hernell, Fatty acids generated by gastric lipase to promote human milk triacylglycerol digestion by pancreatic colipase–dependent lipase, Biochim. Biophys. Acta. 1001 (1989) 286–293.
[47] M.M. Kozlovm, W. Helfrich, Effects of a cosurfactant on the stretching and bending elasticities of a surfactant monolayer, Langmuir 8 (1992) 2792–2797.
[48] J.K. Embleton, C.W. Pouton, Structure and function of gastro-intestinal lipases, Adv. Drug Del. Rev. 25 (1997) 15–32.
[49] M. Ollivon, O. Eidelman, L. Blumenthal, A. Walter, Micelle-vesicle transition of egg phosphatidylcholine and octylglucoside, Biochemistry 27 (1988) 1695–1703.
[50] M.T. Paternostre, M. Roux, J.L. Rigaud, Mechanisms of membrane protein insertion into liposomes during reconstitution procedures involving the use of detergents. 1. Solubilization of large unilamellar liposomes (prepared by reverse-phase evaporation) by triton X-100, octyl glucoside, and sodium cholate, Biochemistry 27 (1988) 2668–2677.
[51] D.G. Fatourosa, B. Bergenstahl, A. Mullertz, Morphological observations on a lipid-based drug delivery system during in vitro digestion, Eur. J. Pharm. Sci. 31 (2007) 85–94.
[52] O. Hernell, J.E. Staggers, M.C. Carey, Physical–chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings, Biochemistry 29 (1990) 2041–2056.
[53] G.A. Kossena, W.N. Charman, C.G. Wilson, B.O. Mahony, B. Lindsay, J.M. Hempenstall, C.L. Davison, P.J. Crowley, C.J. Porter, Low dose lipid formulations: effects on gastric emptying and biliary secretion, Pham. Res. 24 (2007) 2084–2096.
[54] P. Borel, B. Pasquier, M. Armand, V. Tyssandier, P. Grolier, G.M. Alexandre, M. Andre, M. Senft, J. Peyrot, V. Jaussan, D. Lairon, B.V. Azais, Processing of vitamin A and E in the human gastrointestinal tract, Am. J. Physiol. Gastrointest. Liver Physiol. 280 (2001) G95–G103.
[55] G.A. Kossena, B.J. Boyd, C.J.H. Porter, W.N. Charman, Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs, J. Pharm. Sci. 92 (2003) 634–648.
[56] R.J. Ptachcinsky, R. Venkataramanan, G.J. Burckart, Clinical pharmacokinetics of cyclosporine, Clin. Pharmacokinet. 11 (1986) 107–132.
[57] J.M. Kovarik, E.A. Mueller, B.J.B. Van, W. Tetzloff, K. Kutz, Reduced inter- and intra-individual variability in cyclosporine pharmacokinetics from a microemulsion formulation, J. Pharm. Sci. 83 (1994) 444–446.
[58] W. Stremmel, Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein, J. Clin. Invest. 82 (1988) 2001–2010.

[59] W.N. Charman, C.J. Porter, Lipophilic prodrugs designed for intestinal lymphatic transport, Adv. Drug Del. Rev. 19 (1996) 149–169.
[60] E.H. Harrison, Mechanisms of digestion and absorption of dietary vitamin A, Annu. Rev. Nutr. 25 (2005) 87–103.
[61] T. Ichihsdhi, H. Kinoshita, Y. Takagishi, H. Yamada, Effect of oily vehicles on absorption of mepitiostane by the lymphatic system in rats, J. Pharm. Pharmacol. 44 (1992) 560–564.
[62] R.A. Myers, V.J. Stella, Factors affecting the lymphatic transport of penclomedine (NSC-338720), a lipophilic cytotoxic drug; comparison to DDT and hexachlorobenzine, Int. J. Pharm. 80 (1992) 51–62.
[63] G.W. Schmid-Schonbein, Microlymphatics and lymph flow, Physiol. Rev. 70 (1990) 987–1028.
[64] N.L. Trevaskis, C.J. Porter, W.N. Charman, The lymph lipid precursor pool is a likely key determinant of lymphatic drug transport, J. Pharmacol. Exp. Ther. 316 (2005) 881–891.
[65] V.J. Wacher, C.Y. Wu, L.Z. Benet, Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy, Mol. Carcinog. 13 (1995) 129–134.
[66] V.J. Wacher, L. Salphati, L.Z. Benet, Active secretion and enterocytic drug metabolism barriers to drug absorption, Adv. Drug Deliv. Rev. 46 (2001) 89–102.
[67] A. Parkinson, An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials, Toxicol. Pathol. 24 (1996) 45–57.
[68] N.L. Trevaskis, C.J.H. Porter, W.N. Charman, An examination of the interplay between enterocyte-based metabolism and lymphatic drug transport in the rat, Drug Metab. Dispos. 34 (2006) 729–733.
[69] P.A. Van Veld, R.D. Vetter, R.F. Lee, J.S. Patton, Dietary fat inhibits the intestinal metabolism of the carcinogen benzo[a]pyrene in fish, J. Lipid Res. 28 (1987) 810–817.
[70] R.D. Vetter, M.C. Carey, J.S. Patton, Coassimilation of dietary fat and benzo(a)pyrene in the small intestine: an absorption model using the killifish, J. Lipid Res. 26 (1985) 428–434.
[71] F.A. Reubsaet, J.H. Veerkamp, S.G. Bukkens, J.M. Trijbels, L.A. Monnens, Acyl-CoA oxidase activity and peroxisomal fatty acid oxidation in rat tissues, Biochim. Biophys. Acta 958 (1988) 434–442.
[72] N.H. Haunerland, Fatty acid binding protein in locust andmammalianmuscle. Comparison of structure, function and regulation, Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 109 (1994) 199–208.
[73] J.H. Veerkamp, H.T. van Moerkerk, Fatty acid-binding protein and its relation to fatty acid oxidation, Mol. Cell. Biochem. 123 (1993) 101–106.
[74] K.M. Wasan, The biological functions of lipid excipients and the implications for pharmaceutical products development, J. Pharm. Sci. 98 (2009) 379–382.
[75] W.N. Charman, V.J. Stella, Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules, Int. J. Pharm. 34 (1986) 175–178.
[76] A.B.R. Thomson, M. Keelan, M.L. Garg, M.T. Clandinin, Intestinal aspects of lipid absorption: in review, Can. J. Physiol. Pharmacol. 67 (1989) 179–191.
[77] L.V. Leak LV, The structure of lymphatic capillaries in lymph formation, Fed. Proc. 35 (1976) 1863–1871.
[78] D.M. Shehe, J.B. Green, M.H. Green, Influence of dietary fat saturation on lipid absorption in the rat, Atherosclerosis 37 (1980) 301–310.
[79] E.B. Feldman, B.S. Russel, R. Chen, J. Johnson, T. Forte, S.B. Clark, Dietary saturated fatty acid content affects lymph lipoproteins: studies in the rat, J. Lipid. Res. 2 (1983) 967–976.
[80] P.H. Green, R.M. Glickman, Intestinal lipoprotein metabolism, J. Lipid Res. 22 (1981) 1153–1173.
[81] M. Cheema, K.J. Palin, S.S. Davis, Lipid vehicles for intestinal lymphatic drug absorption, J. Pharm. Pharmacol. 39 (1987) 55–56.
[82] R.K. Ockner, J.P. Pittman, J.L. Yager, Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids, Gastroenterology 62 (1972) 981–992.
[83] S.E. Bergstedt, H. Hayashi, D. Kritchevsky, P. Tso, A comparison of absorption of glycerol tristearate and glycerol trioleate by rat small intestine, Am. J. Physiol. 259 (1990) G386–G393.
[84] J.E. Zuther, Anatomy, in: Lymphedema Management: The Comprehensive Guide for Practitioners, Thieme Medical Publishers Inc., New York, 2005, pp. 1–28.
[85] H.A. Cense, C.H. Van Eijck, H.W. Tilanus, New insights in the lymphatic spread of oesophageal cancer and its implications for the extent of surgical resection, Best Pract. Res. Clin. Gastroenterol. 20 (2006) 893–906.
[86] M. Arya, S.R. Bott, I.S. Shergill, H.U. Ahmed, M. Williamson, H.R. Patel, The metastatic cascade in prostate cancer, Surg. Oncol. 15 (2006) 117–128.
[87] S. Muranishi, Lymphatic delivery of drugs and its application to cancer chemotherapy, Yakugaku Zasshi 100 (1980) 687–698.
[88] A. Garzon-Aburbeh, J.H. Poupaert, M. Claesen, P. Dumont, G. Atassi, 1, 3-dipalmitoylglycerol ester of chlorambucil as a lymphotropic, orally administrable antineoplastic agent, J. Med. Chem. 26 (1983) 1200–1203.
[89] G. Pantaleo, C. Graziosi, J.F. Demarest, O.J. Cohen, M. Vaccarezza, K. Gantt, C.C. Muro, A.S. Fauci, Role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection, Immunol. Rev. 140 (1994) 105–130.
[90] G. Pantaleo, C. Graziosi, A.S. Fauci, The role of lymphoid organs in the immunopathogenesis of HIV infection, Aids 7 (1993) S19–S23.
[91] M. Lalanne, A. Paci, K. Andrieux, N. Dereuddre-Bosquet, P. Clayette, A. Deroussent, M. Ré, G. Vassal, P. Couvreur, D. Desmaële, Synthesis and biological evaluation of two glycerolipidic prodrugs of didanosine for direct lymphatic delivery against HIV, Bioorg. Med. Chem. Lett. 17 (2007) 2237–2240.
[92] M. Umeda, H. Marusawa, H. Seno, A. Katsurada, M. Nabeshima, H. Egawa, S. Uemoto, Y. Inomata, K. Tanaka, T. Chiba, Hepatitis B virus infection in lymphatic tissues in inactive hepatitis B carriers, J. Hepatol. 42 (2005) 806–812.
[93] V.V. Messling, N. Svitek, R. Cattaneo, Receptor (SLAM [CD150]) recognition and the V protein sustain swift lymphocyte-based invasion of mucosal tissue and lymphatic organs by a morbillivirus, J. Virol. 80 (2006) 6084–6092.
[94] N.T. Lan, R. Yamaguchi, A. Inomata, Y. Furuya, K. Uchida, S. Sugano, S. Sugano, S. Tateyama, Comparative analyses of canine distemper viral isolates from clinical cases of canine distemper in vaccinated dogs, Vet. Microbiol. 115 (2006) 32–42.
[95] M. Spiegel, K. Schneider, F. Weber, M. Weidmann, F.T. Hufert, Interaction of severe acute respiratory syndrome-associated coronavirus with dendritic cells, J. Gen. Virol. 87 (2006) 1953–1960.
[96] A. Kessel, E. Toubi, Chronic HCV-related autoimmunity: a consequence of viral persistence and lymphotropism, Curr. Med. Chem. 14 (2007) 547–554.
[97] M. Yokoyama, Block copolymers as drug carriers, CRC Crit. Rev. Ther. Drug Carrier Syst. 9 (1992) 213–248.
[98] K.J. Hwang, M.R. Mauk, Fate of lipid vesicles in vivo: a gamma-ray perturbed angular correlation study, Proc. Natl. Acad. Sci. USA-Biophys. 74 (1977) 4991–4995.
[99] C.J.H. Porter, N.L. Trevaskis, W.N. Charman, Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs, Nat. Rev. Drug Discov. 6 (2007) 231–248.
[100] R. Nagarajan, Molecular theory for mixed micelles, Langmuir 1 (1985) 331–341.
[101] D. Andelman, M.M. Kozlov, W. Helfrich, Phase transitions between vesicles and micelles driven by competing curvatures, Europhys. Lett. 25 (1994) 231–236.
[102] M.W. Rigler, R.E.R.E. Honkanen, J.S. Patton, Visualization by freeze fracture, in vitro and in vivo, of the products of fat digestion, J. Lipid. Res. 8 (1986) 836–857.
[103] J.S. Patton, M.V. Carey, Watching fat digestion, Science 204 (1979) 145–148.
[104] D.G. Fatouros, G.R. Deen, L. Arleth, B. Bergenstahl, F.S. Nielsen, J.S. Pedersen, A. Mullertz, Structural development of self nano emulsifying drug delivery systems during in vitro lipid digestion monitored by small-angle X-ray scattering, Pharm. Res. 24 (2007) 1844–1853.
[105] P.K. Gupta, J.B. Cannon, Emulsions and microemulsions for drug solubilization and delivery, in: R. Liu (Ed.), Water-Insoluble Drug Formulation, CRC Press, Florida, 2000, pp. 169–211.
[106] N.H. Shah, M.T. Carvajal, C.I. Patel, M.H. Infield, A.W. Malick, Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs, Int. J. Pharm. 106 (1994) 15–23.
[107] P.P. Constantinides, Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects, Pharm. Res. 9 (1995) 87–93.
[108] S.V.R. Rao, J. Shao, Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs I. Formulation development, Int. J. Pharm. 362 (2008) 2–9.
[109] S.A. Charman, W.N. Charman, M.C. Rogge, T.D. Wilson, F.J. Dutko, C.W. Pouton, Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound, Pharm. Res. 9 (1992) 87–93.
[110] E.A. Mueller, J.M. Kovarik, J.B. Van Bree, J. Grevel, P.W. Lucker, K. Kutz, Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation, Pharm. Res. 11 (1994) 151–155.
[111] P.P. Constantinides, J. Scalart, Formulation and physical characterization of water-in-oil microemulsion containing long- versus medium-chain glycerides, Int. J. Pharm. 158 (1997) 57–68.
[112] B.D. Tarr, S.H. Yalkowsky, Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size, Pharm. Res. 6 (1989) 40–43.
[113] C.W. Pouton, Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification, Int. J. Pharm. 27 (1985) 335–348.
[114] M.G. Wakerly, C.W. Pouton, B.J. Meakin, Evaluation of the self-emulsifying performance of a non-ionic surfactant–vegetable oil mixture, J. Pharm. Pharmacol. 39 (1987) 6P–9P.
[115] T. Gershanik, S. Benita, Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur. J. Pharm. Biopharm. 50 (2000) 179–188.
[116] T.R. Kommuru, B. Gurley, M.A. Khan, I.K. Reddy, Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment, Int. J. Pharm. 212 (2001) 233–246.
[117] I. Kovacs, M. Jusztin, E. Takacs, Z. Balazs, I. Kiss, Z. Varga, S. Jancso, C. Heim, I.K. Korcsmavos, E. Erdohati, M. Jarabin, Oral pharmaceutical preparation, U.S. Patent 5,58,3105 (1996).
[118] C.G. Wilson, B.O. Mahony, The Behavior of Fats and Oils in the Upper G.I. Tract, B.T. Gattefosse, vol. 90, 1997, pp. 13–18.
[119] E. Franceschinis, D. Voinovich, M. Grassi, B. Perissutti, J. Filipovic-Grcic, A. Martinac, F. Meriani-Merlo, Self-emulsifying pellets prepared by wet granulation in high-shear mixer: influence of formulation variables and preliminary study on the in vitro absorption, Int. J. Pharm. 291 (2005) 87–97.

[120] J.M. Newton, M.R. Pinto, F. Podczeck, The preparation of pellets containing a surfactant or a mixture of mono- and di-gylcerides by extrusion/spheronization, Eur. J. Pharm. Sci. 30 (2007) 333–342.
[121] M. Serratoni, J.M. Newton, S. Booth, A. Clarke, Controlled drug release from pellets containing water-insoluble drugs dissolved in a self-emulsifying system, Eur. J. Pharm. Biopharm. 65 (2007) 94–98.
[122] S. Nazzal, M.A. Khan, Response surface methodology for the optimization of ubiquinone self-emulsified drug delivery system, AAPS Pharm. Sci. Tech. 3 (2002) 23–31.
[123] S. Nazzal, M. Nutqan, A. Palamakula, R. Shah, A.A. Zaghloul, M.A. Khan, Optimization of a self-nanoemulsified tablet dosage form of ubiquinone using response surface methodology: effect of formulation ingredients, Int. J. Pharm. 240 (2002) 103–114.
[124] S. Nazzal, I.I. Smalyukh, O.D. Lavrentovich, M.A. Khan, Preparation and in-vitro characterization of a eutectic based semisolid self nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation, Int. J. Pharm. 235 (2002) 247–265.
[125] S. Nazzal, A.A. Zaghloul, M.A. Khan, Effect of extra-granular microcrystalline cellulose on compaction, surface roughness and in-vitro dissolution of a self-nanoemulsified solid dosage form of ubiquinone, Pharm. Technol. 26 (2002) 86–98.
[126] C. Kim, H. Shin, S. Yang, J. Kim, Y. Oh, Once-a-day oral dosing regimen of cyclosporine A: combined therapy of cyclosporine a premicroemulsion concentrates and enteric coated solid-state premicroemulsion concentrates, Pharm. Res. 18 (2001) 454–459.
[127] Y. Takashima, H. Yuasa, Y. Kanaya, I. Nomura, K. Shinozawa, Reduction of tablet coloration at tableting for oily medicine (tocopheryl nicotinate), Int. J. Pharm. 187 (1999) 125–135.
[128] T. Yi, J. Wan, H. Xu, X. Yang, A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs, Eur. J. Pharm. Biopharm. 70 (2008) 439–444.
[129] P. Balakrishnan, B.J. Lee, D.H. Oh, J.O. Kim, M.J. Hong, J.P. Jee, J.A. Kim, B.K. Yoo, J.S. Woo, C.S. Yong, H.G. Choi, Enhanced oral bioavailability of dexibuprofen by a novel solid SEDDS formulation, Eur. J. Pharm. Biopharm. 72 (2009) 539–545.
[130] S. Nazzal, M.A. Khan, Controlled release of a self-emulsifying formulation from a tablet dosage form: stability assessment and optimization of some processing parameters, Int. J. Pharm. 315 (2006) 110–121.
[131] H. Takeuchi, H. Sasaki, T. Niwa, T. Hino, Y. Kawashima, K. Uesugi, H. Ozawa, Redispersible dry emulsion system as novel oral dosage form of oily drugs: in vivo studies in beagle dogs, Chem. Pharm. Bull. 39 (1991) 3362–3364.
[132] G. Dollo, P.L. Corre, A. Guerin, F. Chevanne, J.L. Burgot, R. Leverge, Spray-dried redispersible oil-in-water emulsion to improve oral bioavailability of poorly soluble drugs, Eur. J. Pharm. Sci. 19 (2003) 273–280.
[133] M. Lladser, C. Medrano, A. Aranciba, The use of supports in the lyophilization of oil-in-water emulsions, J. Pharm. Pharmacol. 20 (1968) 450–455.
[134] I.S. Ahmed, M.H. Aboul-Einien, In vitro and in vivo evaluation of a fast-disintegrating lyophilized dry emulsion tablet containing griseofulvin, Eur. J. Pharm. Sci. 32 (2007) 58–68.
[135] I.S. Ahmed, M.H. Aboul-Einien, O.H. Mohamed, S.F. Farid, Relative bioavailability of griseofulvin lyophilized dry emulsion tablet vs. immediate release tablet: a single-dose, randomized, open-label, six-period, crossover study in healthy adult volunteers in the fasted and fed states, Eur. J. Pharm. Sci. 35 (2008) 219–225.
[136] S.L. Myers, M.L. Shively, Solid state emulsion: the effect of maltodextrin on microcrystalline aging, Pharm. Res. 10 (1993) 1389–1391.
[137] M.L. Shively, D.C. Thompson, Oral bioavailability of vancomycin solid-state emulsions, Int. J. Pharm. 117 (1995) 119–122.
[138] M.K. Gupta, D. Goldman, R.H. Bogner, Y.C. Tseng, Enhanced drug dissolution and bulk properties of solid dispersions granulated with a surface adsorbent, Pharm. Dev. Technol. 6 (2001) 563–572.
[139] Y. Itoa, T. Kusawake, M. Ishida, R. Tawab, N. Shibata, K. Takada, Oral solid gentamicin preparation using emulsifier and adsorbent, J. Control. Rel. 105 (2005) 23–31.
[140] Y. Ito, H. Araik, K. Uchino, K. Iwasaki, N. Shibata, K. Takada, Effect of adsorbents on the absorption of lansoprazole with surfactant, Int. J. Pharm. 289 (2005) 69–77.
[141] Y. Ito, T. Kusawakea, Y.V.R. Prasada, N. Sugioka, N. Shibata, K. Takada, Preparation and evaluation of oral solid heparin using emulsifier and adsorbent for in vitro and in vivo studies, Int. J. Pharm. 317 (2006) 114–119.
[142] K. Mohsin, M.A. Long, C.W. Pouton, Design of lipid-based formulations for oral administration of poorly water-soluble drugs: precipitation of drug after dispersion of formulations in aqueous solution, J. Pharm. Sci., in press, doi: 10.1002/jps.21659.
[143] A.M. Kaukonen, B.J. Boyd, C.J.H. Porter, W.N. Charman, Drug solubilization behavior during in vitro digestion of simple triglyceride lipid solution formulations, Pharm. Res. 21 (2004) 245–253.
[144] A.M. Kaukonen, B.J. Boyd, W.N. Charman, C.J. Porter, Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids, Pharm. Res. 21 (2004) 254–260.
[145] P. Chattopadhyay, B.Y. Shekunov, D. Yimb, D. Cipolla, B. Boyd, S. Farr, Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system, Adv. Drug Del. Rev. 59 (2007) 444–453.
[146] B.J. Aungust, Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism, J. Pharm. Sci. 82 (1993) 979–987.
[147] Y. Chen, Y. Lu, J. Chen, J. Lai, J. Sun, F. Hu, W. Wu, Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt, Int. J. Pharm., in press, doi: 10.1016/j.ijpharm.2009.04.022.
[148] A. Czogalla, Oral cyclosporine A – the current picture of its liposomal and other delivery systems, Cell. Mol. Biol. Lett. 14 (2009) 139–152.
[149] A. Arien, C. Goigoux, C. Baquey, B. Dupuy, Study of in vitro and in vivo stability of liposomes loaded with calcitonin or indium in the gastrointestinal tract, Life Sci. 53 (1993) 1279–1290.
[150] A. Arien, N. Henry-Toulmé, B. Dupuy, Calcitonin-loaded liposomes: stability under acidic conditions and bile salts-induced disruption resulting in calcitonin-phospholipid complex formation, Biochim. Biophys. Acta 1193 (1994) 93–100.
[151] J.F. Woodley, Liposomes for oral administration of drugs, Crit. Rev. Ther. Drug Carrier Syst. 2 (1986) 1–18.
[152] A. Jesorka, O. Orwar, Liposomes: technologies and analytical applications, Annu. Rev. Anal. Chem. 1 (2008) 801–832.
[153] S. Vemuri, C.T. Rhodes, Preparation and characterization of liposomes as therapeutic delivery systems: a review, Pharm. Acta Helvetiae 70 (1995) 95–111.
[154] C. Schwarz, W. Mehnert, J.S. Lucks, R.H. Muller, Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization, J. Control. Rel. 30 (1994) 83–96.
[155] R.H. Muller, K. Mader, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, Eur. J. Pharm. Biopharm. 50 (2000) 161–177.
[156] R.H. Müller, S.A. Runge, V. Ravelli, A.F. Thünemann, W. Mehnert, E.B. Souto, Cyclosporine-loaded solid lipid nanoparticles (SLN): drug-lipid physicochemical interactions and characterization of drug incorporation, Eur. J. Pharm. Biopharm. 68 (2008) 535–544.
[157] R.H. Muller, D. Ruhl, S. Runge, K. Schulze-Forster, M. Wolfgang, Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant, Pharm. Res. 14 (1997) 458–462.
[158] P. Ma, X. Dong, C.L. Swadley, A. Gupte, M. Leggas, H.C. Ledebur, R.J. Mumper, Development of idarubicin and doxorubicin solid lipid nanoparticles to overcome Pgp–mediated multiple drug resistance in leukemia, J. Biomed. Nanotech., in-press, doi: 10.1166/jbn.2009.1021.
[159] X. Dong, C.A. Mattingly, M. Tseng, M. Cho, Y. Liu, V.R. Adams, R.J. Mumper, Doxorubicin and paclitaxelloaded lipid-based nanoparticles overcome multidrug resistance by inhibiting P-gp via ATP depletion, Cancer Res. 69 (2009) 3918–3926.
[160] E. Ugazio, R. Cavalli, M.R. Gasco, Incorporation of cyclosporin A in solid lipid nanoparticles (SLN), Int. J. Pharm. 241 (2002) 341–344.
[161] S. Yang, J. Zhu, Y. Lu, B. Liang, C. Yang, Body distribution of camptothecin solid lipid nanoparticles after oral administration, Pharm. Res. 16 (1999) 751–757.
[162] G.P. Zara, R. Cavalli, A. Fundaro, D. Vighetto, M.R. Gasco, Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats, J. Pharm. Sci. 91 (2002) 1324–1333.
[163] R. Cavalli, G.P. Zara, O. Caputo, A. Bargoni, A. Fundarò, M.R. Gasco, Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration, Part I — a pharmacokinetic study, Pharmacol. Res. 42 (2000) 541–545.
[164] A. Bargoni, R. Cavalli, G.P. Zara, A. Fundaro, O. Caputo, M.R. Gasco, Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration, Part II — tissue distribution, Pharmacol. Res. 43 (2001) 497–502.
[165] R. Pandey, S. Sharma, G.K. Khuller, Oral solid lipid nanoparticle-based antitubercular chemotherapy, Tuberculosis 85 (2005) 415–420.
[166] M. García-Fuentes, C. Prego, D. Torres, M.J. Alonso, A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly(ethyleneglycol) as carriers for oral calcitonin delivery, Eur. J. Pharm. Sci. 25 (2005) 133–143.
[167] N. Zhang, Q. Ping, G. Huang, W. Xu, Y. Cheng, X. Han, Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin, Int. J. Pharm. 327 (2006) 153–159.
[168] J.F. Pinto, R.H. Muller, Pellets as carriers of solid lipid nanoparticles (SLN) for oral administration of drugs, Die Pharm. 54 (1999) 506–509.
[169] C.M. O'Driscoll, B.T. Griffin, Biopharmaceutical challenges associated with drugs with low aqueous solubility – the potential impact of lipid-based formulations, Adv. Drug Del. Rev. 60 (2008) 617–624.
[170] P. Tso, Intestinal lipid absorption, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, third ed., vol. 2, Raven Press, New York, 1994, pp. 1867–1907.
[171] C.J.H. Porter, A.M. Kaukonen, B.J. Boyd, G.A. Edwards, W.N. Charman, Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation, Pharm. Res. 21 (2004) 1405–1412.
[172] G.A. Kossena, W.N. Charman, B.J. Boyd, C.J.H. Porter, Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug, J. Pharm. Sci. 94 (2005) 481–492.

[173] L. Sek, C.J. Porter, A.M. Kaukonen, W.N. Charman, Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products, J. Pharm. Pharmacol. 54 (2002) 29–41.
[174] J. Borne, T. Nylander, A. Khan, Effect of lipase on monoolein-based cubic phase dispersion (cubosomes) and vesicles, J. Phys. Chem. B. 106 (2002) 10492–10500.
[175] G.A. Kossena, W.N. Charman, B.J. Boyd, D.E. Dunstan, C.J.H. Porter, Probing drug solubilization patterns in the gastrointestinal tract after administration of lipid-based delivery systems: a phase diagram approach, J. Pharm. Sci. 93 (2004) 332–348.
[176] C.J.H. Porter, A.M. Kaukonen, A.T. Bertschinger, B.J. Boyd, J.M. O'Connor, G.A. Edwards, W.N. Charman, Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine, J. Pharm. Sci. 93 (2004) 1110–1121.
[177] J.B. Dressman, C. Reppas, In vitro–in vivo correlations for lipophilic poorly water-soluble drugs, Eur. J. Pharm. Sci. 11 (2000) S73–S80.
[178] F.J. Alvarez, V.J. Stella, The role of calcium ions and BS on the pancreatic lipase-catalyzed hydrolysis of triglyceride emulsions stabilized with lecithin, Pharm. Res. 6 (1989) 449–457.
[179] J.P. Reymond, H. Sucker, In vitro model for cyclosporin intestinal absorption in lipid vehicles, Pharm. Res. 5 (1988) 673–676.
[180] N.H. Zangenberg, A. Mullertz, H.G. Kristensen, L. Hovgaard, A dynamic in vitro lipolysis model I. Controlling the rate of lipolysis by continuous addition of calcium, Eur. J. Pharm. Sci. 14 (2001) 115–122.
[181] N.H. Zangenberg, A. Mullertz, H.G. Kristensen, L. Hovgaard, A dynamic in vitro lipolysis model II: evaluation of the model, Eur. J. Pharm. Sci. 14 (2001) 237–244.
[182] A. Dahan, A. Hoffman, Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats, Pharm. Res. 23 (2006) 2165–2174.
[183] J.O. Christensen, K. Schultz, B. Mollgaard, H.G. Kristensen, A. Mullertz, Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols, Eur. J. Pharm. Sci. 23 (2004) 287–296.
[184] G.A. Edwards, C.J.H. Porter, S.M. Caliph, S.M. Khoo, W.N. Charman, Animal models for the study of intestinal lymphatic drug transport, Adv. Drug Del. Rev. 50 (2001) 45–60.
[185] C.M. Perry, S. Noble, Saquinavir soft-gel capsule formulation. A review of its use in patients with HIV infection, Drugs 55 (1998) 461–486.
[186] B.T. Griffin, C.M. O'Driscoll, A comparison of intestinal lymphatic transport and systemic bioavailability of saquinavir from three lipid-based formulations in the anaesthetized rat model, J. Pharm. Pharmacol. 58 (2006) 917–925.
[187] E.A. Mueller, J.M. Kovarik, J.B.V. Bree, J. Grevel, P.W. Lucker, K. Kutz, Influences of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation, Pharm. Res. 11 (1994) 151–155.
[188] S.M. Khoo, D.M. Shackleford, C.J.H. Porter, G.A. Edwards, W.N. Charman, Intestinal lymphatic transport of halofantrine occurs after oral administration of a unit-dose lipid-based formulation to fasted dogs, Pharm. Res. 20 (2003) 1460–1465.
[189] E.K. Wasan, K. Bartlett, P. Gershkovich, O. Sivak, B. Banno, Z. Wong, J. Gagnon, B. Gates, C.G. Leon, K.M. Wasan, Development and characterization of oral lipid-based Amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with *Aspergillus fumigatus* or *Candida albicans*, Int. J. Pharm. 372 (2009) 76–84.
[190] P. Gershkovich, E.K. Wasan, M. Lin, O. Sivak, C.G. Leon, J.G. Clement, K.M. Wasan, Pharmacokinetics and biodistribution of amphotericin B in rats following oral administration in a novel lipid-based formulation, J. Antimicrob. Chemother., in press, doi: 10.1093/jac/dkp140.
[191] S.J. Thornton, K.M. Wasan, The reformulation of amphotericin B for oral administration to treat systemic fungal infections and visceral leishmaniasis, Expert Opin. Drug Deliv. 6 (2009) 271–284.
[192] V. Risovic, M. Boyd, E. Choo, K.M. Wasan, Effect of various lipid-based oral formulations on plasma and tissue concentrations and renal toxicity of amphotericin B within male rats, Antimicrob. Agent. Chemother. 47 (2003) 3339–3342.
[193] V. Risovic, K. Sachs-Barrable, M. Boyd, K.M. Wasan, Potential mechanism by which peceol increases the gastrointestinal absorption of amphotericin B, Drug Dev. Ind. Pharm. 30 (2004) 767–774.
[194] A. Dahan, A. Hoffman, Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs, Eur. J. Pharm. Sci. 24 (2005) 381–388.
[195] P. Gershkovich, A. Hoffman, Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability, Eur. J. Pharm. Sci. 26 (2005) 394–404.
[196] Rxlist, The internet drug index, 2009. <http://www.rxlist.com>.
[197] A. Dahan, A. Hoffman, The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats, Eur. J. Pharm. Biopharm. 67 (2007) 96–105.
[198] K. Sachs-Barrable, A. Thamboo, S.D. Lee, K.M. Wasan, Lipid excipients peceol and gelucire 44/14 decrease p-glycoprotein mediated efflux of rhodamine 123 partially due to modifying p-glycoprotein protein expression within caco-2 cells, J. Pharm. Pharmaceut. Sci. 10 (2007) 319–331.
[199] J.X. Jia, K.M. Wasan, Effects of monoglycerides on rhodamine 123 accumulation estradiol 17 β-d-glucuronide bidirectional transport and mrp2 protein expression within caco-2 cells, J. Pharm. Pharmaceut. Sci. 11 (2008) 45–62.
[200] C.A. Barta, K. Sachs-Barrable, F. Feng, K.M. Wasan, Effects of monoglycerides on p-glycoprote modulation of the activity and expression in caco-2 cell monolayers, Mol. Pharm. 5 (2008) 863–875.
[201] F. Seeballuck, M.B. Ashford, C.M. O'Driscoll, The effects of pluronic block copolymers and cremophor EL on intestinal lipoprotein processing and the potential link with p-glycoprotein in caco-2 cells, Pharm. Res. 20 (2003) 1085–1092.
[202] F. Seeballuck, E. Lawless, M.B. Ashford, C.M. O'Driscoll, Stimulation of triglyceride-rich lipoprotein secretion by polysorbate 80: in vitro and in vivo correlation using caco-2 cells and a cannulated rat intestinal lymphatic model, Pharm. Res. 21 (2004) 2320–2326.
[203] P. Arturrson, J. Karlsson, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (caco-2) cells, Biochem. Biophys. Res. Commun. 175 (1991) 880–885.
[204] P. Saha, J.H. Kou, Effect of solubilizing excipients on permeation of poorly water-soluble compounds across Caco-2 cell monolayers, Eur. J. Pharm. Biopharm. 50 (2000) 403–411.
[205] E. Levy, M. Mehran, E. Seidman, Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion, FASEB 9 (1995) 626–635.
[206] M. Mehran, E. Levy, M. Bendayan, E. Seidman, Lipid, apolipoprotein, and lipoprotein synthesis and secretion during cellular differentiation in caco-2 cells, In Vitro Cell Dev. Biol. 33 (1997) 118–128.
[207] P.P. Constantinides, K.M. Wasan, Lipid formulation strategies for enhancing intestinal transport and absorption of P-glycoprotein (P-gp) substrate drugs: In vitro/in vivo case studies, J. Pharm. Sci. 96 (2007) 235–248.
[208] R. Holma, J. Hoest, Successful in silico predicting of intestinal lymphatic transfer, Int. J. Pharm. 272 (2004) 189–193.