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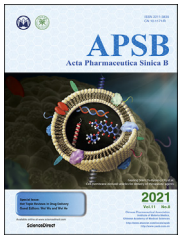

REVIEW

In vitro and *in vivo* correlation for lipid-based formulations: Current status and future perspectives

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Abstract Lipid-based formulations (LBFs) have demonstrated a great potential in enhancing the oral absorption of poorly water-soluble drugs. However, construction of *in vitro* and *in vivo* correlations (IVIVCs) for LBFs is quite challenging, owing to a complex *in vivo* processing of these formulations. In this paper, we start with a brief introduction on the gastrointestinal digestion of lipid/LBFs and its relation to enhanced oral drug absorption; based on the concept of IVIVCs, the current status of *in vitro* models to establish IVIVCs for LBFs is reviewed, while future perspectives in this field are discussed. *In vitro* tests, which facilitate the understanding and prediction of the *in vivo* performance of solid dosage forms, frequently fail to mimic the *in vivo* processing of LBFs, leading to inconsistent results. *In vitro* digestion models, which more closely simulate gastrointestinal physiology, are a more promising option. Despite some successes in IVIVC modeling, the accuracy and consistency of these models are yet to be validated, particularly for human data. A reliable IVIVC model can not only reduce the risk, time, and cost of formulation development but can also contribute to the formulation design and optimization, thus promoting the clinical translation of LBFs.

Abbreviations: ANN, artificial neural network; AUC, area under the curve; BE, bioequivalence; BCS, biopharmaceutics classification system; CETP, cholesterol ester transfer protein; C_{max}, peak plasma concentration; DDS, drug delivery system; FDA, US Food and Drug Administration; GI, gastrointestinal; HLB, hydrophilic–lipophilic balance; IVIVC, *in vitro* and *in vivo* correlation; IVIVR, *in vitro* and *in vivo* relationship; LBF, lipid-based formulation; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; SCT, short-chain triglyceride; SEDDS, self-emulsifying drug delivery system; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SLS, sodium lauryl sulfate; SMEDDS, self-microemulsifying drug delivery system; SNEDDS, self-nanoemulsifying drug delivery system; TIM, TNO gastrointestinal model; TNO, Netherlands Organization for Applied Scientific Research; T_{max}, time to reach the peak plasma concentration.

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1. Introduction

Oral route is the most popular way for drug administration. Currently, more than 50% of marketed drugs and 90% of drug candidates are poorly water soluble, and these proportions continue to grow because of the rapid progress in drug discovery[1−5]. Since dissolution is a prerequisite for drug absorption, poor solubility always leads to retarded dissolution rate and, thereby, poor bioavailability. The situation is even worse for drug candidates with poor solubility and poor permeability[6,7]. Great efforts have been made in the past to improve poor bioavailability of such compounds in an attempt to unlock their therapeutic potential as oral medicines and achieve some success[8−18]. The enhancement of dissolution and absorption is one of the enduring research topics in pharmaceutical researches[19−21].

Inspired by the positive "pharmaceutical food effect"[22], lipid-based formulations (LBFs) have been developed and demonstrated a great potential in enhancing the oral bioavailability of poorly water-soluble drugs[23,24]. Based on their components and contents, four main classes of LBFs have been evolved[25]. Type I LBFs are lipid solutions, which are non-dispersible in aqueous media but release co-formulated drugs upon digestion. Type II LBFs are self-emulsifying drug delivery systems (SEDDSs) comprising lipids and surfactants. The surfactants bear a hydrophilic−lipophilic balance (HLB) value of less than 12, and the type II LBFs generally form emulsions in aqueous media. Type III LBFs consist of lipids, hydrophilic surfactants with a HLB value larger than 12, and hydrophilic cosolvents. They are subdivided into types IIIa (SEDDSs) and IIIb [self-microemulsifying DDSs (SMEDDSs) and self-nanoemulsifying DDSs (SNEDDSs)], based on the size of the formed emulsions. Type IV LBFs only contain surfactants and hydrophilic cosolvents, without lipids, and form micelles when dispersed in water. All of the types of LBFs have been available in market, being shown in Table 1[26]. The first approval for each types of LBFs by the US Food and Drug Administration (FDA) is in 1941 (Drisdol®, type I), 1983 (Sandimmune®, type II), 1995 (Neoral®, type III), and 1999 (Agenerase®, type IV), respectively[23]. Numerous discoveries and substantial improvements have been achieved in the field of LBFs in the last 5 years, bringing this old technology back to the limelight[27]. Nonetheless, very few LBFs are available as commercial products on the market, while some have been discontinued (Table 1)[26,28]. On the one side, the problem is due to the scale-up and stability challenges. The majority of LBFs are filled in soft gelatin capsules for clinical application. However, in-house manufacturing capabilities of soft gelatin may be missing in a few countries, while soft gelatin capsules are not acceptable in all countries. In addition, incompatibility of the excipients with the shells of the soft gelatin as well as precipitation of the active ingredients during storage at a lower temperature are common stability issues for LBFs, which requires solidification of the formulation[29−31]. On the other side, the lack of *in vitro* tests that are able to predict the *in vivo* behavior of LBFs with much accuracy, is the crucial reason for the limited number of products[10,32].

In vitro and *in vivo* correlations (IVIVCs) are powerful tools for optimizing the formulation and dosage, setting dissolution limits, and reducing bioequivalence (BE) studies[19,33−39]. By definition, an IVIVC is a mathematical model bridging *in vitro* properties and an *in vivo* response of a preparation[40]. Dissolution is the most commonly used *in vitro* property, while the fraction of drug absorbed is the popular *in vivo* response. *In vitro* dissolution can be a surrogate for BE studies upon availability of an established IVIVC. Considerable interest in IVIVCs has been elicited in the pharmaceutical industry, academia, and regulatory sectors[20,38,41], while dosage forms have been extended from oral extended-release to oral immediate-release forms[19,35,42−47], modified-release parenteral dosage forms[36,47−51], and transdermal DDSs[33,34,52−56]. Similarly, a reliable IVIVC model could promote the development of LBFs. However, it is a significant challenge to establish IVIVCs for LBFs because of the complex *in vivo* process. Unlike normal dosage forms, lipid components in LBFs undergo extensive lipolysis in the gastrointestinal (GI) tract, while co-formulated drugs may precipitate or be dissolved during the intermediate phase of lipolysis[24,57−66]. The lack of mechanistic understanding of the *in vivo* behavior of LBFs hampers the possibility of obtaining an IVIVC[67].

This review briefly introduces the relationship between GI digestion of lipid/LBFs and enhanced oral drug absorption, as well as the concept of IVIVC. On this basis, the current status of establishing IVIVCs for LBFs is reviewed, and future perspectives in this field are discussed.

2. Lipid digestion and enhanced drug absorption

The development of LBFs was inspired by the phenomenon that a high-fat diet enhances the bioavailability of poorly water-soluble drugs. The underlying mechanisms are correlated with the digestion and absorption of lipids (Fig. 1), *i.e.*, the "pharmaceutical food effect" promotes physiological changes, assisting drug absorption. Lipid ingestion stimulates the secretion of gastric lipase, which partly breaks down triglycerides into diglycerides and fatty acids in the stomach. The process contributes to ∼15% of the overall lipid digestion in the GI tract[68]. In the meantime, dietary fat is converted into an emulsion of fine oil droplets. The transfer of these lipidic substances into the duodenum stimulates the secretion of pancreatic lipase and bile. Bile salts, phospholipids, and cholesterol coat and stabilize the emulsion droplets, which become more accessible to the action of pancreatic enzymes. The remaining lipids are completely digested in the small intestine *via* breakdown of triglycerides into a 2-monoglyceride and two fatty acid molecules. The lipolysis proceeds from the

Table 1 FDA-approved drugs utilizing lipid systems.

Molecule (trade name)	New drug application year	Biopharmaceutic classification system	Type of lipid-based formulation	Oil	Surfactant (HLB <12)	Surfactant (HLB >12)	Hydrophilic cosolvent
Ergocalciferol (Drisdol®)	1941	3	I	Soybean oil	–	–	–
Calcitriol (Rocaltrol®)	1978	2/4	I	Fractionated triglycerides of coconut oil	–	–	–
Valproic acid (Depakene®)	1978	1	I	Corn oil	–	–	–
Isotretinoin (Accutane®) Discontinued	1982	2	I	Beeswax, hydrogenated soybean oil flakes, hydrogenated vegetable oil, soybean oil	–	–	–
Cyclosporin A (Sandimmune®)	1983	2	II	Olive oil	–	Polyoxyethylated oleic glycerides	Ethanol 12.5%
Dronabinol (Marinol®)	1985	2/4	I	Sesame oil	–	–	–
Clofazimine (Lamprene®) Discontinued	1986	2	I	Beeswax	–	–	–
Cyclosporin A (Sandimmune®)	1990	2	II	Corn oil	Linoleic macroglycerides	–	Ethanol 12.7%
Ranitidine (Zantac®) Discontinued	1994	3	–	Medium-chain triglycerides	Gelucire 33/01	–	–
Cyclosporin A (Neoral®)	1995	2	III A/III B	Corn oil mono-di-triglycerides	–	Polyoxyl 40 hydrogenated castor oil	Ethanol 11.9%, glycerol, propylene glycol
Tretinoin (Vesanoid®) Discontinued	1995	–	I	Beeswax, hydrogenated soybean oil flakes, hydrogenated vegetable oil, soybean oil	–	–	–
Ritonavir (Norvir®)	1996	4	III A	–	Oleic acid	Polyoxyl 35 castor oil	Ethanol
Saquinavir (Fortovase®) Discontinued	1997	4	–	Medium-chain mono- and di-glycerides	–	–	–
Progesterone (Prometrium®)	1998	2	I	Peanut oil	–	–	–
Amprenavir (Agenerase®) Discontinued	1999	2	IV	–	–	Vitamin E TPGS	PEG400, propylene glycol
Bexarotene (Targretin®)	1999	–	IV	–	–	Polysorbate 20	PEG400
Doxercalciferol (Hectorol®)	1999	2/4	I	Coconut oil	–	–	Alcohol
Sirolimus (Rapamune®)	1999	–	III	Phosphatidylcholine, mono- and di-glycerides, soy fatty acids, ascorbyl palmitate	–	Polysorbate 80	1.5%−2.5% ethanol, propylene glycol
Cyclosporin A	2000	2	IV	–	–	Polysorbate 80,	Propylene glycol, alcohol

(*continued on next page*)

Table 1 (*continued*)

Molecule (trade name)	New drug application year	Biopharmaceutic classification system	Type of lipid-based formulation	Oil	Surfactant (HLB <12)	Surfactant (HLB >12)	Hydrophilic cosolvent
(Gengraf®)						Polyoxyl 35 castor oil	12.8% *v/v*
Cyclosporin A (Gengraf®)	2000	2	IV	–	–	Polyoxyl 40 hydrogenated castor oil, polysorbate 80	Propylene glycol
Ritonavir/lopinavir (Kaletra®) Discontinued	2000	4	III	–	Oleic acid	Polyoxyl 35 castor oil	Propylene glycol
Dutasteride (Avodart®)	2001	2/4	I	Mono-di-glycerides of caprylic/capric acid	–	–	–
Isotretinoin (Claravis®)	2003 (ANDA)	2	–	Hydrogenated vegetable oil, soybean oil, white wax	–	Polysorbate 80	–
Omega-3-acid ethyl esters (Lovaza®)	2004	–	I	Soybean oil	–	–	–
Tipranavir (Aptivus®)	2005	4	III A	Mono-/di-glycerides of caprylic/capric acids	–	Polyoxyl 35 castor oil	Ethanol, propylene glycol
Tipranavir (Aptivus®)	2005	4	IV	–	–	Vitamin E TPGS	PEG 400, propylene glycol, water
Paricalcitol (Zemplar®)	2005	4	I	Medium-chain triglycerides fractionated from coconut oil or palm kernel oil	–	–	Alcohol
Lubiprostone (Amitiza®)	2006	2/4	I	Medium-chain triglycerides	–	–	–
Fenofibrate (Lipofen®)	2006	2	III	–	–	Gelucire 44/14 (lauroyl macrogol glyceride type 1500)	–
Topotecan HCl (Hycamtin®)	2007	1	I	Hydrogenated vegetable oil	Glyceryl monostearate	–	–
Loratadine (Claritin®)	2008	2	–	Caprylic/capric glycerides	–	Polysorbate 80	–
Isotretinoin (Absorica®)	2012	2	–	Soybean oil, stearoyl polyoxylglycerides	Sorbitan monooleate	–	–
Enzalutamide (Xtandi®)	2012	2	I	Caprylocaproyl polyoxyglycerides	–	–	–
Nintedanib (Ofev®)	2014	2/4	II	Medium-chain triglycerides, hard fat	Lecithin	–	–
Calcifediol (Rayaldee®)	2016	2/4	II/III	Mixture of lipophilic emulsifier with a HLB <7 and an absorption enhancer with HLB of 13−18 Oily vehicle-mineral oil, liquid paraffins, or squalene			

–, not applicable; HLB, hydrophilic−lipophilic balance.

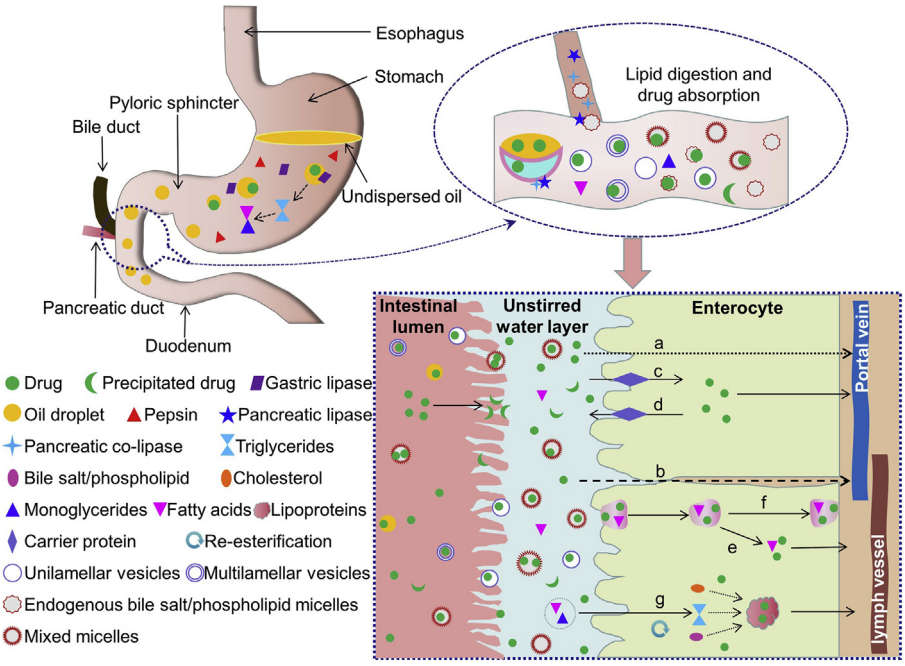

Figure 1 Illustration of gastrointestinal lipid digestion and enhanced absorption of co-administered drugs. Digestion of triglycerides in gastrointestinal tract liberates monoglycerides and fatty acids, which form unilamellar/multilamellar vesicles, mixed micelles, and micelles in combination with endogenous bile salts and phospholipids. Co-administrated drugs are solubilized in these colloidal structures, delivered across the unstirred water layer, and reach the enterocytes. The drug molecules may be released from the structures and diffuse to the basolateral side *via* either (a) transcellular or (b) paracellular pathway. Facilitated drug (c) influx by membrane proteins and (d) efflux of ingested drugs are also possible. In addition, the drug loaded vehicles or micelles may be absorbed *via* (e) endocytosis and (f) transcytosis pathways. The intracellular monoglycerides and fatty acids are re-esterified to form triglycerides, which are further (g) packed into chylomicrons. Drugs with high affinity to chylomicrons are then transported *via* the lymph route with chylomicrons.

outside with a continuously changing interface. Multilamellar liquid crystals are formed at the interface during hydrolysis and are further converted into diverse colloidal structures in combination with bile salts[68]. The identified structures include multilamellar and unilamellar vesicles, mixed micelles, and micelles. The lipophilic products of the breakdown of dietary fats (fatty acids and monoglycerides), as well as co-administered poorly soluble drugs, are solubilized in colloidal structures, which deliver the cargos across the unstirred water layer and reach the brush-border membranes of intestinal cells. The loaded drug may either leave the structures to diffuse across the epithelium or be absorbed as the cargo of the intact vehicles or micelles. The transepithelial pathways include passive diffusion of free drugs *via* either transcellular (Fig. 1a) or paracellular (Fig. 1b) way, facilitated drug influx by membrane proteins (Fig. 1c), and endocytosis (Fig. 1e) or transcytosis (Fig. 1f) of the colloidal structures. Efflux of ingested drugs is also possible (Fig. 1d). The intracellular monoglycerides and fatty acids are re-esterified to form triglycerides, which are further packed into chylomicrons and exocytosed to enter the central lacteal lymph vessels (Fig. 1g). Drugs that have a high affinity to chylomicrons may have a high potential to be transported *via* the lymph route, while others are mainly absorbed *via* the hepatic portal vein[32,59,63,69–72].

Similarly, LBFs play a beneficial role in solubilization and absorption of co-administered poorly soluble drugs. The presence of LBFs in the GI tract also stimulates the secretion of endogenous lipases and bile[73]. The biliary lipids are combined with the exogenous lipids and lipid digestion products to form complex colloidal structures[74,75]. During this process, co-formulated drugs may either be solubilized in the intermediate colloidal phases or precipitate. It is reasonable to expect good IVIVCs for LBFs that keep solubilization of co-delivered drugs during lipolysis. However, recent studies on halofantrine and cinnarizine SNEDDSs have shown controversial results for general cognition[67,76]. The formulations that underwent rapid drug precipitation during *in vitro* lipolysis had similar bioavailability to those that did not show any precipitation. Although the reason was attributed to ready redissolution of the precipitated drugs, due to their amorphous state, the situation complicates the establishment of IVIVCs for LBFs. Nonetheless, the dispersion and digestion of formulation-derived lipids as well as the solubilization of co-administered drugs in the GI tract should be systematically considered in *vitro* models to obtain a more accurate prediction of the *in vivo* performance of LBFs.

3. A brief introduction to IVIVCs

In 1997, the FDA published guidelines concerning the construction of IVIVCs for development of extended-release oral preparations[40]. Four levels (A, B, C, and multiple C) of IVIVCs were proposed in the guidance based on the correlating relationships between *in vitro* data and the plasma drug concentration−time curve[77]. Level A is a point-to-point correlation between *in vitro* dissolution and *in vivo* drug absorption[78], wherein a straight line through the origin with a slope of one is obtained[79]. As the highest degree of correlation, level A is the only one that is recognized by FDA to grant a biowaiver from *in vitro* dissolution tests[80]. In addition, the level A correlation

helps control the quality of the formula and choose an appropriate formula[39,81,82]. The principles of statistical moment analysis are adopted in the construction of level B IVIVC, wherein the mean *in vitro* dissolution time correlates with the mean *in vivo* residence time. Due to the absence of a point-to-point correlation, level B IVIVC is unable to predict the *in vivo* performance of preparations. The level C is the lowest level of IVIVC, which shows a single-point correlation between *in vitro* parameters (*e.g.*, the time for 50% of the drug being dissolved or a dissolution percentage at 4 h) and pharmacokinetic (PK) parameters [*e.g.*, area under the curve (AUC), peak plasma concentration (C_{max}), and time to reach C_{max} (T_{max})]. Level C IVIVC is mainly adopted for formulation screening and development of quality standards. Multiple level C is a multiple correlation between drug dissolution *in vitro* at different time points (at least three points) and one or several PK parameters.

4. *In vitro* release/dispersion and IVIVC

Since the release of drug substance from dosage forms and the subsequent solubilization of the released drugs under physiological conditions are critical steps for drug absorption *via* oral route, *in vitro* dissolution is the main test for the prediction of the *in vivo* performance of oral solid preparations[83]. Similarly, *in vitro* release from SMEDDSs in enzyme-free aqueous media was first used to establish the IVIVCs for LBFs[84,85]. The process, which uses a USP type II dissolution apparatus, is rather simple. The rationale for this test is based on the recognition that the solubilized drug, instead of the precipitated one, is available for absorption. Hence, an IVIVC may be achieved using this *in vitro* release/dispersion test[86−89]. Inspired by a level A IVIVC for a cyclosporine SMEDDS, a biowaiver extension for a poorly water-soluble drug was claimed using a SMEDDS formulation[84]. However, during the *in vitro* release process, co-formulated drugs are not released to the media in the molecular form because SMEDDSs spontaneously form drug-loaded microemulsions, resulting in a dispersion process rather than drug release. The situation is completely different from that of solid dosage forms, wherein released drugs are solubilized in the media and available for absorption. In addition, this test ignores possible *in vivo* precipitation of dispersed drugs, due to the lipolysis of formulations in the GI tract, which leads to inconsistent results in terms of obtaining an IVIVC[90].

As a result of an insufficiently accurate simulation of the physiological environment in the GI tract by compendial media, biorelevant dissolution media were developed to achieve a better IVIVC for poorly water-soluble drugs[91−96]. Simulated gastric fluid (SGF) containing 0.5% (*w/v*) sodium lauryl sulfate (SLS) was adopted for *in vitro* drug release from an olmesartan medoxomil-loaded SMEDDS using a USP type II dissolution apparatus[97]. A high predictive power of the *in vitro* dissolution performance for the *in vivo* absorption was revealed by obtaining a level A IVIVC. Furthermore, a dialysis bag method was developed to understand the drug release profile, which was performed in SGF containing 0.5% (*w/v*) SLS for 1 h and simulated intestinal fluid (SIF) for another 2 h[69]. In a membrane with a cutoff of 12 kDa, >90% of the drug was released within 1 h, of which nearly 80% was released within 30 min. In a membrane with a cutoff of 1 kDa, only 13%−22% of the drug was released within 30 min, and a maximum of 54%−61% of the drug was released within 3 h. The reduced drug release profile of the 1 kDa membrane was due to the small cutoff, which only allowed a passage of free drug molecules. Nonetheless, in addition to a level A and a level B IVIVC, a level C correlation was achieved between *in vitro* drug release parameters ($t_{30\%}$, $t_{50\%}$, and $t_{90\%}$) and C_{max}, T_{max}, and AUC.

In some cases, an *in vitro* release in biorelevant media failed to produce an IVIVC[98]. The *in vitro* release of fenofibrate from LBFs was shown to be dependent on both biorelevant media and the LBF composition (Tween 80 with different lipids). In contrast to the *in vitro* results, the tested LBFs exhibited similar *in vivo* performance in rats in both fasted and fed states. The authors attributed these inconsistencies to incessant excretion of bile in rats, leading to the enhanced solubility of fenofibrate *in vivo*. Therefore, animal model may be crucial in the establishment of IVIVC.

The dissolution apparatus may also affect the construction of an IVIVC. The paddle (USP Apparatus 2) and Bio-Dis (USP Apparatus 3) methods were used to study the release of RZ-50 from lipid suspensions in compendial and biorelevant media, respectively[99]. The paddle method led to a very low drug release due to the poor dispersibility of the formulation, whereas the Bio-Dis method enhanced drug release by facilitating emulsification of the formulation. A level A IVIVC was obtained under fed gastric conditions using the Bio-Dis method.

5. *In vitro* digestion models and IVIVC

Despite attractive and simple, *in vitro* release/dispersion is not suitable to predict the *in vivo* performance of LBFs because of the inconsistency in achieving IVIVCs[66]. The primary drawback of the test is the lack of mimicking the complex *in vivo* digestion of LBFs and micellar solubilization[100]. Accordingly, *in vitro* lipolysis is more suitable for assessing the fate of LBFs by mimicking the intestinal lipid digestion process[101−103]. To obtain a strong IVIVC, it is crucial to simulate the complex physiological conditions that present in the human GI tract, such as pH, enzymes, transit times, and mixing[104,105]. However, none of the currently available models can simulate all of these complex multistage processes owing to technical challenges. Only simplified digestion models have been developed by capturing one or more key elements in human GI digestion. The pH-stat lipolysis model and the TNO (Netherlands Organization for Applied Scientific Research) GI model (TIM-1), which differ in the complexity, compartmental numbers, and physiological effects considered, are the most commonly used models for the evaluation of LBFs.

5.1. *pH-stat lipolysis model*

The pH-stat lipolysis model, which mainly simulates enzymatic digestion, is the most frequently used model in the evaluation of LBFs. Since retention of administered LBFs is negligible in the oral cavity, the model typically mimics the enzymatic conditions in the intestinal (one-compartment) or GI (one- or two-compartment) phase of digestion, while studies are all performed at a fixed pH.

5.1.1. *One-compartment intestinal digestion model*

The experimental setup mainly comprises a thermostated vessel (generally, at 37 °C), an overhead stirrer, a pH electrode, and a titrator (Fig. 2). LBFs are dispersed in a medium mimicking fasted- or fed-state intestinal digestive fluid. Initiation of lipid

digestion by addition of lipase and colipase leads to the liberation of fatty acids, causing a drop in the pH consequently. The pH variation is measured by the electrode, while the released fatty acids are automatically titrated with sodium hydroxide using the titrator. The extent of the digestion can be indirectly quantified using the rate of the addition of sodium hydroxide based on its stoichiometric reaction with fatty acids. Samples can be taken during the digestion process and ultracentrifuged to obtain three distinct phases, namely, an oil phase containing undigested lipids, a micellar phase containing a solubilized drug in colloidal structures, and a pellet phase comprising the precipitated drug. Quantification of the drug amounts in each phase enables prediction of the solubilizing capability of the formulation to co-formulated drugs in the GI tract. Furthermore, the solubilized amount of the drug in the micellar phase can be correlated with the *in vivo* PK parameters to construct an IVIVC. At the least, a rank order of the likely *in vivo* performances may be established for a series of LBFs, based on the hypothesis that the high percentage solubilized in the micellar phase results in a high bioavailability.

5.1.2. *GI digestion model*

The one-compartment intestinal digestion model is simple and has been widely adopted in the evaluation of LBFs. The rationale of the model is that the intestine is the main site for lipid digestion and drug absorption. However, the model is inadequate for simulating GI physiology because it does not consider processes and conditions in the stomach. As mentioned above, lipid digestion in stomach contributes to ~15% of the overall lipid digestion in the GI tract. In addition, the effects of gastric emptying and sudden pH changes on the solubilization of co-formulated drugs are ignored[61]. Therefore, GI digestion pH-stat models, either two-step one compartment or two-step two compartments, were developed to simulate both gastric and intestinal digestion[106,107]. In the one-compartment model, the simulated gastric and intestinal digestion is performed in two sequential steps, respectively. LBFs are first dispersed in SGF, and gastric digestion is initiated by adding gastric lipases. After a period of time, the SGF was transferred to a medium similar to the intestinal fluid by addition of a concentrated SIF and pancreatic lipases. During both steps, automatic titration with sodium hydroxide maintains a constant pH, corresponding to the gastric and intestinal pH, respectively[106,107]. Two individual setups of the pH-stat model are used in the two-compartment model to simulate the stomach and small intestine, respectively (Fig. 3). SGF and SIF, as well as the corresponding lipases, are respectively added to the two reaction vessels, which are connected by a peristaltic pump. During the digestion process, the medium in the gastric compartment is continuously pumped to the intestinal one at a rate mimicking gastric emptying[107–109]. In this regard, the two-compartment model more closely mimics the *in vivo* conditions than does the one-compartment model.

5.1.3. *IVIVCs and the pH-stat lipolysis model*

The pH-stat lipolysis model is more reliable in the rank ordering of LBFs than in the construction of level A IVIVCs. The absolute bioavailability of danazol was found to increase with the dose of Labrafil® M2125CS, while the same rank order was obtained based on the percentage of solubilized danazol in the micellar phase following *in vitro* lipolysis of the formulations[110]. However, the release profile of danazol failed to correlate with the absorption profile in the *in vivo* study. Similar results were obtained for a lipid solution and suspension of halofantrine[102], supporting the potential utility of the model to evaluate and rank the *in vivo* performances of LBFs. Moreover, *in vitro* solubilization data for two cholesterol ester transfer protein (CETP) inhibitors, obtained using *in vitro* lipolysis of a series of SEDDSs, were plotted against *in vivo* drug exposure (AUC) with the same formulations (Fig. 4[111]). Although the plots were not linear, good rank orders between the *in vitro* and *in vivo* data were obvious.

In addition to typical LBFs, the rank ordering capability of the pH-stat lipolysis models was demonstrated in fenofibrate-loaded lipid particles[64]. Nanoparticles (100 nm) showed increased absorption than did microparticles and a crystalline suspension. The data correlated well with those of *in vitro* lipolysis, wherein a higher level of fenofibrate in the micellar phase was obtained from the 100-nm nanoparticles than from the microparticles and suspension.

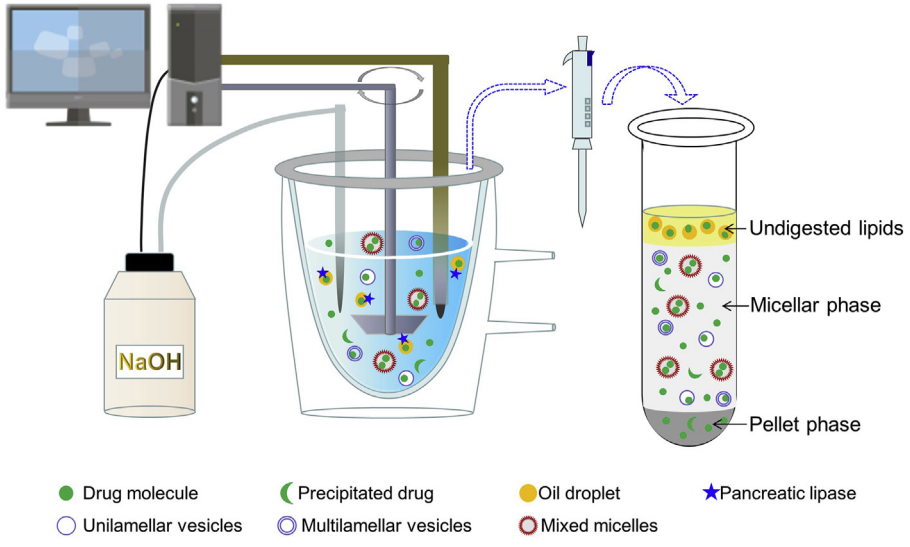

Figure 2 Schematic representation of the one-compartment pH-stat lipolysis model.

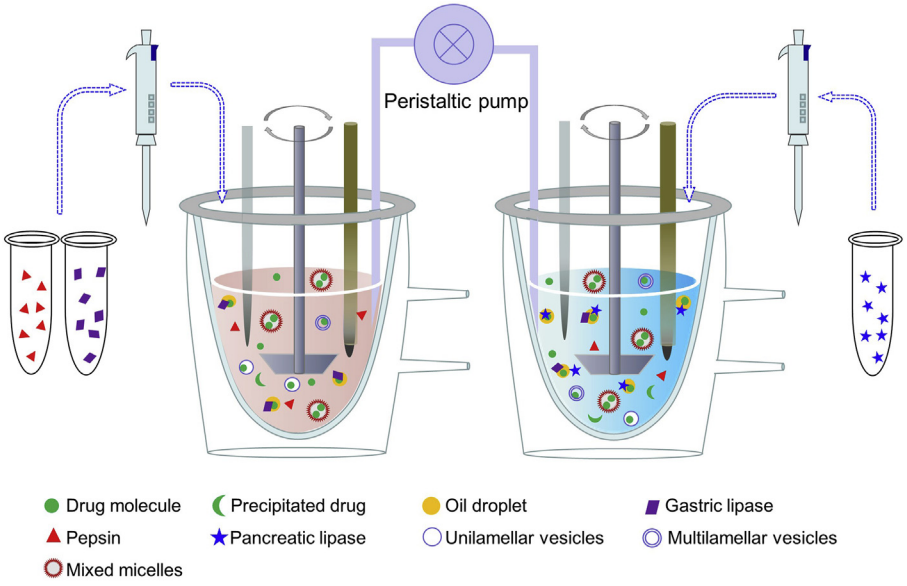

Figure 3 Simulation of the digestion process in the stomach and small intestine by a two-step two-compartment digestion model.

Consequently, the same rank order was observed between release and absorption, that is, 100-nm nanoparticle > microparticle > suspension.

Compared with a cell model of intestinal drug permeability, the pH-stat lipolysis model provided a superior simulation of oral absorption of LBFs, facilitating the establishment of a correlation with an *in vivo* output[63]. A SNEDDS significantly increased the solubility of four Biopharmaceutics Classification System (BCS) II drugs (griseofulvin, phenytoin, indomethacin, and ketoprofen), while their permeation through MDCK cell monolayers was lower than that of saturated water solutions. These results were attributed to differences in the drug states in the formulations. In saturated aqueous solutions, drugs are dissolved and transported in a molecular form, while in SNEDDSs, drugs are trapped inside oil cores and are transported as particles. The large size of the particles, relative to that of a molecule, hinders intestinal membrane permeability of drugs. However, *in vivo* absorption from the SNEDDS was significantly higher than that of free drug molecules, while an *in vitro* and *in vivo* relationship (IVIVR) was demonstrated between the drug content in the lipid phase and its oral bioavailability. Similar results were obtained for dexamethasone, griseofulvin, and progesterone solubilization from long (LCT)-, medium (MCT)-, and short (SCT)-chain triglyceride formulations[103,112]. Good correlations between the bioavailability and the drug contents in the micellar phase of *in vitro* lipolysis were obtained. The rank orders were LCT = MCT = SCT for dexamethasone, MCT > LCT > SCT > H_2O for griseofulvin, and MCT > LCT > SCT for progesterone. In addition, permeation of the drugs through the gut wall was tested using a modified Ussing chamber system following completion of the lipolysis. However, permeability did not correlate with the oral bioavailability. Even though the SCT formulation doubled the permeability coefficients of the drugs, the oral bioavailability of the formulation was more related to the solubilizing capability during lipolysis. More interestingly, a strong correlation with a correlation coefficient >0.99 was obtained between the griseofulvin concentrations in the

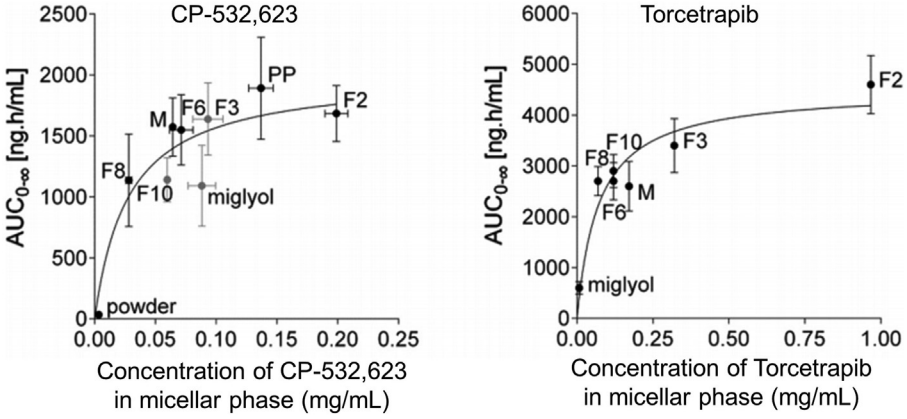

Figure 4 *In vitro* and *in vivo* correlations for two CETP inhibitors using diverse self-emulsifying drug delivery systems. The areas under the curves are plotted *vs.* the drug concentrations in the micellar phase during *in vitro* lipolysis. Reprinted with the permission from Ref. 111. Copyright © 2014 Elsevier B.V.

micellar phase following *in vitro* lipolysis and the AUC values of the corresponding formulations *via* oral administration.

In vitro lipolysis data may fail to construct IVIVC. In some cases, bioavailability from formulations that presented rapid drug precipitation following *in vitro* lipolysis was similar to that from formulations that did not show any drug precipitation[67,76,113]. Studies on halofantrine and cinnarizine SNEDDSs revealed that the precipitates were in an amorphous form, with a rather high dissolution rate, which may explain the enhanced absorption[67,76]. Therefore, the authors suggested that solid-state characterization of the pellet phase is essential in validating the predictive power of the *in vitro* lipolysis test. However, it is also possible that *in vitro* lipolysis failed to mimic physiological conditions. In addition, the theory would not work for BCS IV drugs, which are poorly permeable even in a solubilized form.

Of note, variations in the data obtained across different laboratories may be due to variable experimental conditions[114]. The complexity of the *in vivo* processing of LBFs has long interfered with the establishment of robust IVIVCs for LBFs. Variations of experimental conditions in the pH and the volume of the digestive medium, the employed concentrations of bile salts and calcium, and buffering capacity, may strongly affect the establishment of an IVIVC. To obtain consistent data across different laboratories, the Lipid Formulation Classification System Consortium was established to standardize the protocols of the *in vitro* digestion tests for the assessment of LBFs. The Consortium has published a series of papers, to which interested readers are referred, reporting the results of systematic studies of the factors affecting IVIVCs, including method parameters, effects of bile salt concentrations and drug loading, supersaturation *versus* precipitation potential, lipolysis by gastric lipase, and effects of varying pancreatin and calcium levels[114–119].

5.2. *TIM and IVIVC*

The TIM was developed to study food products under conditions close to GI physiology of human[120]. The dynamic process of the transit and digestion of a meal in the GI tract was simulated in the TIM. The simulated parameters include mixing, transit, pH variation, input of digestive media, and output of water and digestive products. A computer program was utilized to control and reproduce a specific digestive setting. Protocols have been developed to simulate physiologies of different species (*e.g.*, human, dog, pig, and calve) and different populations (*e.g.*, the young, the adult, and the aged).

TIM-1 (Fig. 5) is the most popular configuration of the TIM platform, which consists of four tubular compartments, *i.e.*, the gastric, the duodenal, the jejunal, and the ileal compartment, respectively. Peristaltic valve pumps connect the compartments for the passage of chyme in a controlled way. An alternating pressure is put on the flexible walls of the compartments to mix the contents. A water jacket outside the walls is used to control the temperature in the compartment. Gastric and duodenal secretions, containing bile salts, electrolytes, and digestive enzymes (pepsin, a fungal lipase as an alternative to gastric lipase, and pancreatin), are pumped into the individual compartments. The flow of all secretions is programmed in time as shown in Table 2[121]. The pH in the compartment is measured by an individual pH meter and is controlled *via* titration of hydrochloric acid or sodium bicarbonate to follow a physiologically relevant pH profile. The model also incorporates a hollow fiber membrane (cutoff size: 50 nm) on the jejunal and ileal compartments to mimic the absorption of dissolved/solubilized drugs. The pore size of the membrane has been verified to allow the passage of intermediate colloidal structures[122]. Approximately 80% of a nonprecipitating solute is recovered by filtration, at an aspiration rate of 3.9 mL/min, within 5 h[123]. The filtrates from the jejunum and ileum compartments can be collected to estimate the bioaccessibility of the formulation, which is defined as the percentage of the solubilized drug in both the oil and the micellar phases[123]. It is reasonable to predict the bioavailability of formulations using the bioaccessibility because solubilized drugs are readily absorbed.

By close mimicking the GI physiology, TIM-1 offers a promising tool to predict the oral bioavailability of most pharmaceutical compositions under one standardized experimental setting[124,125]. A systematic evaluation of the predictive power of TIM-1 was performed by researchers from AstraZeneca on nine model drugs of different BCS types and six formulations[126]. TIM-1 correctly predicted the *in vivo* rank order in 84% and 79% of cases for the AUC and C_{max}, respectively. A linear relationship with a correlation coefficient of 0.78 was observed between the bioaccessibility obtained in TIM-1 and the AUC. Owing to its strong predictive capability, TIM-1 has been deployed by AstraZeneca in the drug development for predicting the oral absorption of drug candidates and their formulations.

Until recently, TIM-1 has been used for the evaluation of Pickering emulsions because of a limited availability of the instrument[127]. The bioaccessibility obtained using TIM-1 showed a great potential for the rank ordering of Pickering emulsions in terms of their *in vivo* performance[128–130]. Compared with TIM-1, the pH-stat lipolysis model may overestimate the bioaccessibility of the formulations[128–130]. The difference was attributed to the differences in the designs and simulations of the models. In the pH-stat model, the formulations are fully exposed to the digestion media under continuous stirring until the digestion ends. By contrast, the transit of formulations in the GI tract is a peristalsis movement, which is mimicked by TIM-1, while the absorption of the formulation is concurrent with the lipolysis under realistic circumstances. Consequently, the unrealistic conditions in the pH-stat model lead to overestimation of bioaccessibility. However, it has also been noted that the adsorption of model drugs on the walls of the compartments in the TIM-1 digestive system causes loss of bioaccessibility.

Despite the superiority to the pH-stat lipolysis model, the TIM-1 shows obvious disadvantages. On the one hand, the setup of the model is rather complex, hindering its popularization and application. In addition, the complex process may greatly affect the accuracy and consistency of the data because one mistake may fail the process. A TinyTIM was designed to increase the throughput by simplifying TIM-1[121]. The simplified version retains the gastric compartment but only has one small intestinal compartment and no ileal efflux. On the other hand, the filter system is unable to mimic the active transport, efflux, and gut wall metabolism. A valid correlation between bioaccessibility and bioavailability cannot be obtained unless the transepithelial transport is not a limiting step. The combination of TIM-1 with a Caco-2 cell culture model or *in silico* modeling provides a solution to bridge the gap[131].

5.3. *Combined models and IVIVC*

As mentioned above, *in vitro* lipolysis studies may fail to accurately predict the oral bioavailability of LBFs because the model does not fully represent *in vivo* conditions. As a closed system,

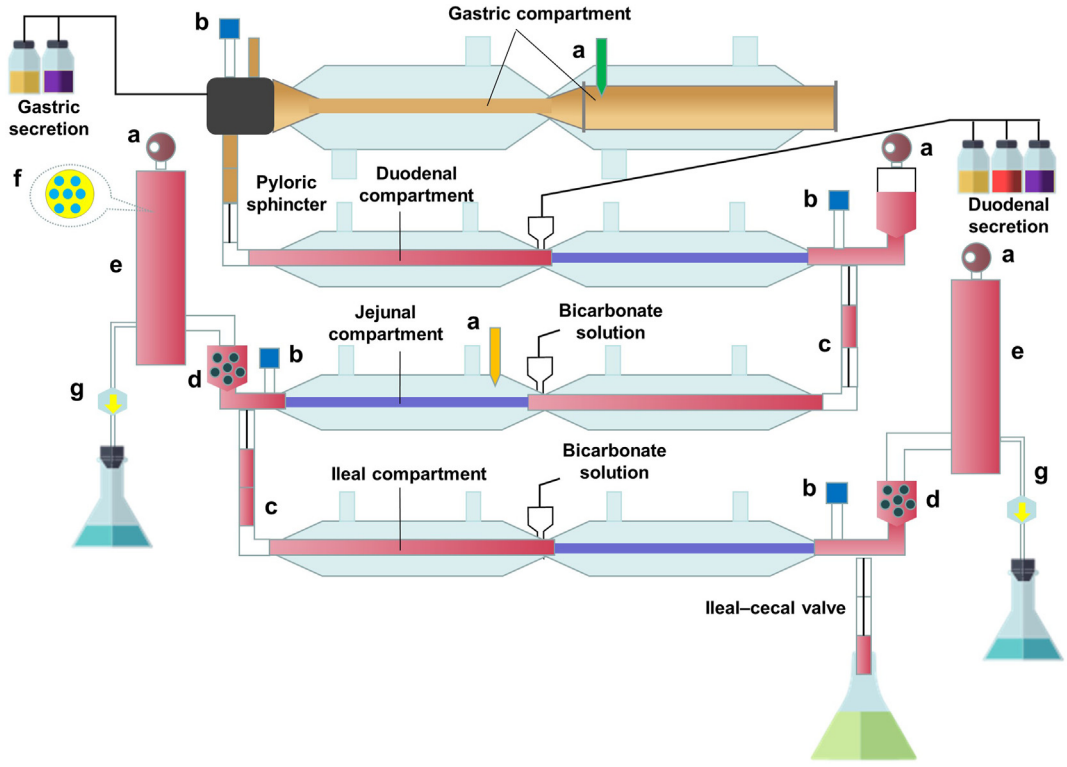

Figure 5 Schematic representation of the TNO gastrointestinal model (TIM-1). (a) Sensors; (b) pH meters; (c) Peristaltic valve; (d) Prefilter; (e) Filtration system; (f) Cross-set of the filtration system; (g) Filtrate.

Table 2 Typical parameter settings in the TNO gastrointestinal model (TIM-1) in response to the digestion of a high-fat meal.

Parameter	Setting
Volume (mL)	Stomach: 300, duodenum: 55, jejunum: 130, ileum: 130
Meal size (g)	300
Gastric secretion (mL/min)	1
Gastric emptying curve	$t_{1/2}$ = 80 min, β = 2
Gastric pH curve (time, pH)	(0, 5.2) (30, 3.2) (60, 2.2) (120, 1.7)
Bile secretion (mL/min)	0.5
Pancreatin/electrolytes (mL/min)	0.5
Ileal emptying curve	$t_{1/2}$ = 220 min, β = 2.2
Small intestinal pH	Duodenum: 6.2, jejunum: 6.5, ileum: 7.4

The table is adapted from Ref. 121 complying with the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

this model lacks the absorption sink that is present *in vivo* and may therefore overestimate the precipitation potential[98,113,132–134]. The intraluminal solvation capacity may be damaged because of the altered composition of GI fluids in the process of intestinal digestion, leading to supersaturation and consequent drug precipitation[32,135]. Meanwhile, *in vivo* absorption may lead to a rapid and sufficient drop in the luminal drug concentration to avoid precipitation. The absorption sink effect works even when the initial supersaturation is high, provided that the absorption is fast[136]. In addition to the absorption issue, absorbed drugs may undergo first-pass metabolism. In this case, the *in vitro* lipolysis model may overestimate the solubilization potential. Therefore, combined lipolysis–permeation and digestion–microsomal metabolism models were developed, respectively, to obtain a better IVIVC.

5.3.1. *In vitro lipolysis–permeation models*

In addition to the solubilization, supersaturation, and precipitation of co-formulated drugs during digestion of LBFs, permeation of model drugs is included in the lipolysis–permeation models. The original setup of the model consisted of two separate single compartments (Fig. 6). The lipolysis and permeation were performed in a consecutive way. Dispersion and digestion of LBFs were performed in a single compartment, utilizing the regular pH-stat lipolysis model. At predetermined intervals, samples were withdrawn and transferred to another compartment for the permeation study. A normal setup of the Transwell system (top to bottom) or Ussing chambers (side by side) can be adopted in this step. However, the absorptive membrane should resemble the intestinal epithelia and withstand the harsh lipolysis conditions, including pancreatic enzymes, diverse surfactants, excipients of LBFs, and digestion. Permeability through the Caco-2 cell (a human colon carcinoma cell line) monolayer represents the gold standard for the evaluation of oral drug absorption[133,137–141]. Differentiated Caco-2 cells resemble the epithelium of human intestine, which enables the assessment of drug transport mediated *via* different pathways, *e.g.*, passive *versus* active transport and paracellular *versus* transcellular routes[142–144]. Due to the intolerability of Caco-2 cells to the pancreatic enzymes, immobilized lipase was used in the digestion step and was shown to successfully digest LBFs and be tolerated by cell monolayers[133]. An

artificial membrane (PermeaPad®)[145] and intestinal rat tissue[112] are used as alternative membranes for Caco-2 cell monolayer. However, the model fails to establish the IVIVC for LBFs because of the lack of concurrence of the digestion and permeation[132,133,145–147]. As illustrated using griseofulvin LCT, MCT, and SCT LBFs, the consecutive lipolysis–permeation model failed to establish the IVIVC. Instead, the single lipolysis model was found to be useful[112].

To capture the simultaneous occurring of drug release and permeation during digestion, an *in situ* single-pass intestinal perfusion in rats was coupled with the *in vitro* lipolysis[148]. For *in situ* intestinal perfusion, the small intestine of an anesthetized rat was exposure by a midline incision in the abdomen; the jejunum (10 cm) was cannulated, while the intestinal contents were removed with saline flush. The mesenteric vein that drained the isolated region of the jejunum was cannulated to measure the drug absorption; donor blood was infused *via* the cannula to the jugular vein to maintain a consistent blood supply. The coupled model successfully predicted the *in vivo* performances of three fenofibrate LBFs, while the single *in vitro* lipolysis model failed[148]. In addition, the coupled model provided valuable mechanistical insights into the interplay among drug solubilization, supersaturation, precipitation, and absorption of LBFs during controlled digestion. However, due to the high technical threshold, the model is not a viable option.

Recently, a simple device consisting of two chambers, which are separated by a Caco-2 cell monolayer or an artificial membrane, was developed to simultaneously study lipolysis and permeation of LBFs[132,133,145–147] (Fig. 7). The upper chamber is used for digestion studies, while the lower one is for assessment of drug permeation. The presence of the absorptive monolayer allows reduction of drug concentration in the digestion chamber and thus maintains sink conditions, which facilitates improving the *in vitro* predictions[24,149]. Similarly, immobilized lipase was used in the digestion chamber for compatibility with the Caco-2 cell monolayer[132,146]. The accuracy of the prediction for *in vivo* drug exposure, based on drug amount in the acceptor chamber, has been validated with different fenofibrate- or carvedilol-loaded LBFs[132,146]. Conversely, absence of the absorption membrane led to fail of predicting the *in vivo* exposure of the formulations. It was intriguing to find that the mixture of lipids and carvedilol was as efficient as the carvedilol loaded LBF in oral bioavailability[146]. Alternatively, artificial membranes can be used to tolerate a porcine pancreatic extract. Screening with the membrane integrity marker Lucifer Yellow indicated that *n*-dodecane-coated polyvinylidene difluoride membrane supports (0.45 μm pore size, thickness 100–145 μm) were able to withstand the lipolysis with porcine pancreatin over a sufficient assay period[147]. The rank order of apparent permeability coefficients for different fenofibrate-loaded LBFs was similar to that obtained using the Caco-2 cell-based model. However, the IVIVR of the cell-free model is yet to be improved using alternative digestive agents.

In addition to the absorption membrane, an everted gut sac was recently combined with the pH-stat lipolysis model to better evaluate and predict the *in vivo* absorption of LBFs[150]. The everted gut sac model is efficient to study the mechanisms and kinetics of drug absorption[151], but it fails to evaluate LBFs because of the absence of lipolytic conditions. The issue was solved by incubating an everted gut sac in the medium of the pH-stat lipolysis model. The performance is similar to that of the original pH-stat model, except that samples are collected from the gut sac. Simultaneous lipolysis and absorption of LBFs are well simulated in this model. With optimized pH and concentrations of D-glucose and pancreatic lipase, the combined model showed a superior IVIVC ($r = 0.9772$) between the *in vitro* absorption percentages of an indomethacin LBF and the *in vivo* absorption fraction compared with that obtained using the single everted gut sac model[150]. However, the combined model has some drawbacks. Tissue viability represents one limiting factor. Another disadvantage is the presence of the muscularis mucosa, which may lead to underestimation of the absorption of compounds with a tendency to bind to muscle cells[151].

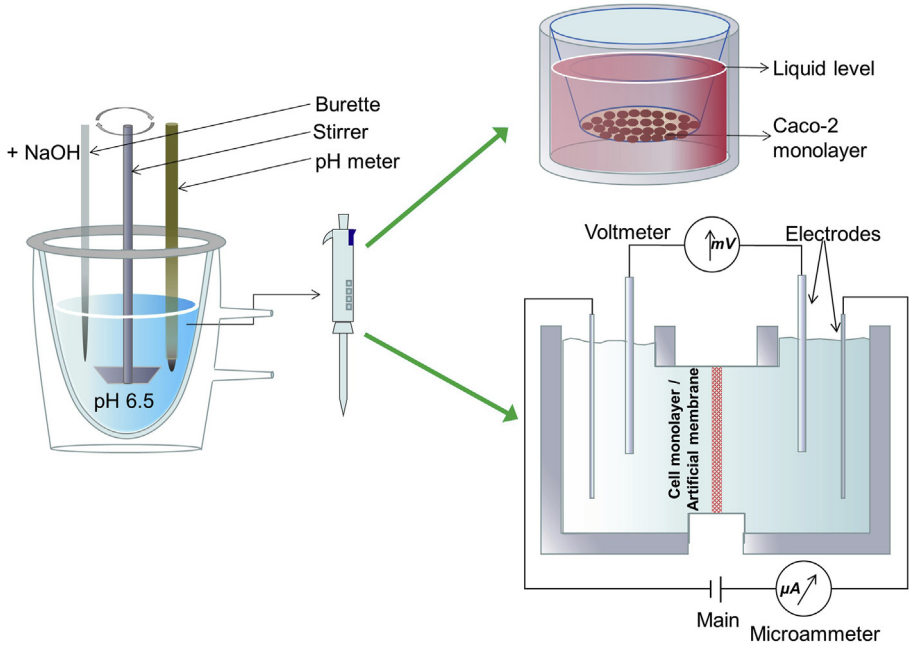

Figure 6 Consecutive use of combined *in vitro* lipolysis–permeation models.

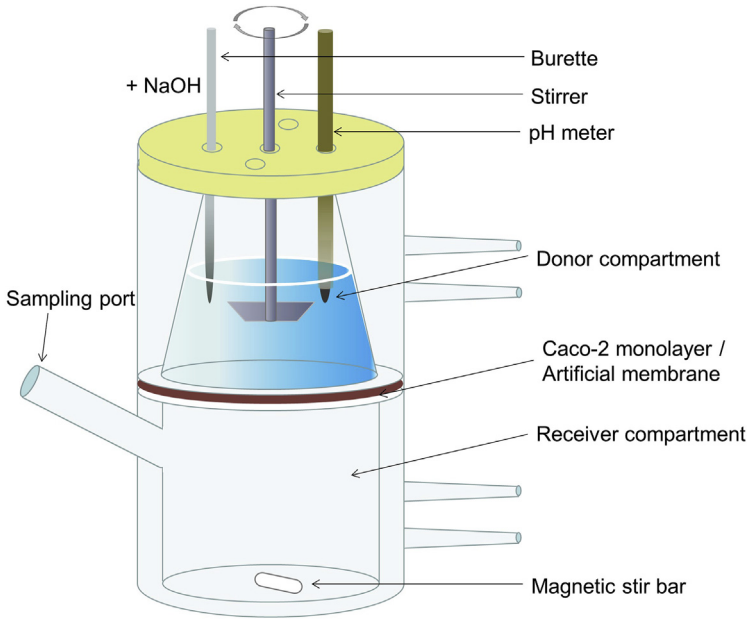

Figure 7 Simultaneous use of a combined lipolysis and permeation model.

5.3.2. Lipolysis−microsomal metabolism model

As far as the oral bioavailability of BCS II drugs is concerned, solubilization in GI tract as well as metabolism in enterocytes and liver, instead of permeability, are the main obstacles. Microsomal metabolism was thus coupled with an *in vitro* lipolysis to allow prediction of oral bioavailability of LBFs in human[152]. Marinol® (sesame oil solution of dronabinol) and Neoral® (SMEDDS of cyclosporine A) were used as model preparations. The *in vitro* lipolysis model enables an estimation of intraluminal solubility of delivered drugs, while microsomal stability assays provide the information on the first-pass metabolism ratio. The LBFs were digested in two separate lipolysis buffers, with different concentrations of sodium taurocholate and phosphatidylcholine. The absorption fraction (F_{abs}) was predicted by the drug concentration in the micellar phase following *in vitro* lipolysis, seeing that all solubilized drugs would be completely absorbed. Metabolism occurs both in the liver and within enterocytes. The fractions of the nonmetabolized drug dose in the liver (F_h) and in the gut (F_g) were determined by metabolism studies using human hepatic and intestinal microsomes, respectively. Subsequently, the predicted oral bioavailability ($F_{predicted}$) was estimated as shown in Eq. (1).

$$F_{predicted} = F_{abs} \times F_h \times F_g \quad (1)$$

A strong correlation between the observed and predicted oral bioavailabilities was verified by Pearson's correlation for both drugs at different doses. The composition of the digestion buffer affected the accuracy. More accurate predictions were obtained using the media with composition closer to physiological conditions. However, it should be noted that the predicted values disregard the effects of gastric metabolism and lymphatic transport, which facilitate the bypassing of hepatic metabolism.

6. *In silico* prediction of IVIVCs

The complex *in vivo* processing of LBFs hinders the predictability of the *in vitro* lipolysis model. Even a fairly complex model such as TIM-1 cannot simulate all of the complex, multistage *in vivo* processes, which involve the dispersion, digestion, solubilization, precipitation, absorption, and metabolism of LBFs and co-formulated drugs. However, *in silico* physiologically based PK (PBPK) modeling provides a possibility to predict the complex *in vivo* behavior *via* computational calculation based on the available *in vitro* data. Several commercial programs are now available for model generation, such as Gastroplus™, STELLA®, Simcyp™, and PK-Sim®[153]. Although the combination of *in vitro* solubility, dissolution, and precipitation testing with *in silico* modeling is still in its infancy, it has shown a great potential to predict the oral bioavailability of solid preparations[154,155].

An *in silico* approach was proposed to establish the IVIVC of fenofibrate LBFs[156]. Lipid excipients significantly enhanced the solubility and dissolution of fenofibrate in gastric and intestinal media, producing a high supersaturated state. Precipitation of the drug after dissolution in the GI media was detected and depended on the composition of the LBFs. The *in vitro* dissolution behavior of the formulations and the *in vivo* PK parameters were incorporated in a STELLA® software to set up the PBPK model. *In silico* simulation enables taking into account the possible precipitation and redissolution of co-formulated drugs during digestion of LBFs. Consequently, the simulated plasma concentration profiles were accurately fitted with the observed ones for all of the LBFs (Fig. 8[156]).

In addition to PBPK modeling, artificial intelligence, such as artificial neural networks (ANNs), has been adopted to deal with nonlinear *in vitro* and *in vivo* relationships and intrinsic variable parameters that may be faced during IVIVC modeling[157,158]. Recently, neuro-fuzzy modeling, a combination of ANNs and a fuzzy logic with a capability to treat nonlinear complex problems, has been introduced for IVIVC modeling of probucol LBFs[159]. In the study, the release of probucol from an oil solution, a SMEDDS, and a SNEDDS was tested using a lipolysis model[159]. The rank order of the rate and extent of probucol release (SMEDDS > SNEDDS > oil solution) was similar to that of the bioavailability in an *in vivo* study. A significantly high prediction

ability was achieved using the neuro-fuzzy model for different data formations, without employing complex configurations.

Both the *in vitro* and the *in vivo* data should be mathematically treated by either compartmental or linear methods to establish IVIVC, which can be facilitated by different modules affiliated to GastroPlus™. The PKPlus™ module provides the relevant PK parameters by analyzing the plasma concentration profiles using compartment methods. The IVIVCPlus™ module implements deconvolution using the Wager−Nelson (one-compartment), Loo−Riegelman (two- and three-compartment), and numerical deconvolution single and double Weibull methods to calculate the fraction of the drug absorbed for establishing a correlation (linear, power function, and second- and third-order polynomial). Based on the advantage of the powerful *in silico* GastroPlus™ simulation, good IVIVCs have been established for furosemide-loaded solid lipid nanoparticles[160], fenofibrate lipidic dispersions[161], and a rifampicin-loaded solidified SMEDDS[162].

7. Summary and future perspectives

Several *in vitro* models have been developed to construct IVIVCs of LBFs, which are summarized in Table 3. The pH-stat lipolysis model is the most popular one and forms the basis for the development of advanced models. Although a few early studies reported successful IVIVCs, a growing number of studies have demonstrated the inability of the pH-stat lipolysis model to generate level A IVIVCs. The absence of the absorption process is the main drawback in the design of the model. However, the pH-stat model is efficient in the rank ordering of formulations, which makes it an excellent tool in formulation screening. A simplified pH-stat lipolysis model adopted for 96-well plates may greatly increase the throughput and cost effectiveness of screening[163−166]. TIM-1 is preferable to the pH-stat model because of a closer simulation of the GI physiology in dealing with lipid digestion and removal of water and metabolites. Although pharmaceutical companies such as AstraZeneca have recognized the value of TIM-1, its application is limited by the high price and complex setup. However, TinyTIM may provide a practical option. The initial application of TIM-1 for the evaluation of Pickering emulsions shows a good potential in the rank ordering of formulations. The capability of the model to construct a level A IVIVC is yet to be confirmed. Meanwhile, the combined digestion−permeation model shows promise in constructing IVIVCs of LBFs. A model combined with a Caco-2 cell monolayer or everted gut sac is particularly promising for fulfilling level A IVIVC modeling because of the involvement of active transportation of solubilized drugs and metabolism inside the epithelia. None of the present models are able to provide full and consistent IVIVCs due to the inability to mimic fully the overall processes occurring *in vivo*. Yet some physiological and physicochemical parameters have not been touched, such as the hormonal and nervous control, feedback mechanisms, mucosal cell activity, realistic shape and motility of GI tract, mechanical forces from physiological contractions, and involvement of the local immune system[104]. It is also crucial to mimic the dynamic secretion of digestive enzymes/bile salts and changes in gastric emptying and GI transit time. The future perspective on the setup of the *in vitro* model is to closely simulate the physiological and physicochemical environments in the GI tract to increase the predictive capability.

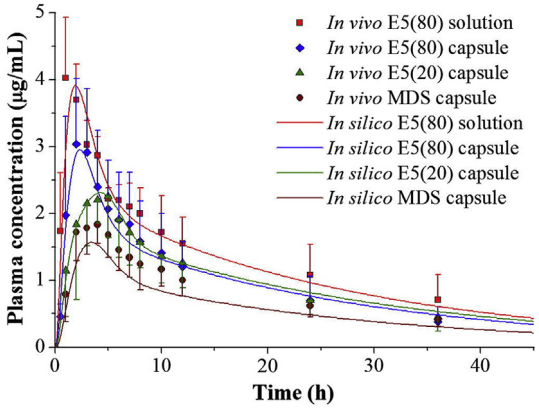

Figure 8 *In silico* approach facilitated establishment of *in vitro* and *in vivo* correlations of fenofibrate lipid-based formulations (LBFs). Simulated (solid lines) and observed (symbols with error bars) plasma fenofibric acid concentration profiles for the LBFs. Reprinted with the permission from Ref. 156. Copyright © 2013 Elsevier B.V.

In addition to the setup of an *in vitro* model, other issues should be considered in the construction of IVIVCs. Model drugs adopted in present studies, such as fenofibrate, griseofulvin, phenytoin, indomethacin, and ketoprofen, are typical BCS II drugs. They have poor water solubility but good permeability, which indicates a good probability of obtaining level A IVIVCs for BCS II drugs if they are solubilized during the lipolysis of LBFs. However, LBFs are overqualified for oral delivery of BCS II drugs and are more applicable to BCS IV drugs by increasing both their solubility and permeability. In this regard, the feasibility of an *in vitro* model for constructing an IVIVC should be determined for BCS IV drugs. Moreover, it should be noted that the ultimate goal of an IVIVC is to predict the *in vivo* behavior of LBFs in humans. The majority of the present studies are performed in rats, while the GI physiology of animals is different from that of humans. For example, bile is continuously secreted in rats, while bile secretion in humans is stimulated by food. It is crucial to verify the predictability of *in vitro* models using data obtained in humans. PBPK modeling may be promising in this regard. The PBPK platform provides equations describing the whole processes of administrated formulations in different compartments (*e.g.*, the gastric lumen, the intestinal lumen, the plasma, the liver, the glomerular filtration, and the periphery tissues) based on human physiological parameters. Combining with drug dependent parameters (*e.g.*, physicochemical properties, permeability, protein binding, and metabolism by hepatic enzymes) enables building a PBPK model to predict *in vivo* performance of formulations in human. For detailed concept of PBPK, please refer to recent reviews[167,168]. Lastly, the *in vitro* model should be conducive to understanding the mechanisms of action of LBFs. Present studies only measure the total drug amount for the construction of IVIVCs but do not discriminate between free drug molecules and those solubilized in formulations. It is unknown whether and to what extent the LBFs contribute to the absorption of drug molecules, particularly BCS IV drugs. Environment-responsive fluorescent probes, such as aggregation-caused quenching and Förster resonance energy transfer probes, may provide a powerful tool to answer this question. The environment-responsive fluorescent probes enable self-discrimination of LBFs *via* the fluorescent quenching (aggregation-caused quenching) or switching to different wavelengths (Förster resonance energy transfer) when the probes are released from the vehicles upon lipolysis. Theoretically, the fluorescent intensity can be utilized to quantify the intact LBFs. Since the hydrophobic cargos are not leaked from the LBFs unless the formulation is broken down upon lipolysis, the quantity

Table 3 Summary of the current *in vitro* models.

Model	Component	Simulated parameter	Advantage	Disadvantage
In vitro release/ dispersion model	USP type II or type III dissolution apparatus	Drug release from formulation; dispersion of formulation	Simple	Absence of the gastrointestinal situation.
One-compartment intestinal digestion model	A thermostatic vessel, an overhead stirrer, a pH electrode, and a titrator	Lipid digestion in intestinal track, solubilizing or precipitation of drugs during lipolysis	Simple, most widely adopted model in evaluation of lipid-based formulations	Ignoring lipolysis in stomach, gastric emptying, and pH changes in gastrointestinal tract; Absence of dynamic secretion of digestive enzymes and bile salts; Absence of the absorption process.
Gastrointestinal digestion model	Similar to the intestinal digestion model	Both gastric and intestinal digestion, pH changes in gastrointestinal tract, and gastric emptying	Mimicking both the gastric and the intestinal conditions; Gastrointestinal transit and pH changes are included.	More complex than one-compartment intestinal digestion model; Absence of dynamic secretion of digestive enzymes and bile salts; Absence of the absorption process.
TNO gastrointestinal model	Four tubular compartments (*i.e.*, the gastric, the duodenal, the jejunal, and the ileal compartment), peristaltic valve pumps connecting the compartments, gastric and duodenal secretions, pH meter, titration, filtration system	Lipid digestion in both gastric and intestinal tract, gastric emptying, pH changes in gastrointestinal tract, absorption of solubilized drugs	Closely mimicking the dynamic process of the transit, digestion, and absorption of formulations in gastrointestinal tract	Extremely complex setup, high price, poor reproducibility; The filtration system cannot provide active and facilitated transport processes and brush border enzyme activities.
In vitro lipolysis −permeation models	The lipolysis setup is similar to the one-compartment intestinal digestion model; The permeation study utilizes Transwell system, Ussing chamber, or diffusion cell; Caco-2 cell monolayer, artificial membrane, or everted gut sac is adopted as absorptive monolayer	Lipid digestion and permeation of model drugs in a consecutive or in a simultaneous way	Providing the absorption sink effect.	Absence of dynamic secretion of digestive enzymes and bile salts; Absence of transit in gastrointestinal tract.
Lipolysis −microsomal metabolism model	The lipolysis setup is similar to the one-compartment intestinal digestion model; microsomal stability assays	Solubilization of co-formulated drug following digestion; metabolism of the drug in enterocytes and liver	The first-pass metabolism is included in the model.	The model is limited to drugs with high first-pass metabolism; Absence of dynamic secretion of digestive enzymes and bile salts; Absence of absorption process.
In silico prediction	Physiologically based pharmacokinetic modeling	The dispersion, digestion, solubilization, precipitation, absorption, and metabolism of formulations and co-formulated drugs.	Computational calculation of the complex *in vivo* behavior based on the available *in vitro* data. Prediction of the *in vivo* performance in human is possible.	Accuracy of the model is yet to be validated.

of the intact LBFs may be converted to the drug amount still in formulation. Although these probes have been widely used for qualitative analysis, quantification is yet to be realized. Breakthrough in this technique will bring about critically important information for design of LBFs.

8. Conclusions

The feasibility of LBF use in oral drug delivery has been fully recognized by both academia and industry. Construction of IVIVCs is a prioritized research which provides a powerful tool to promote the development of LBFs. A variety of *in vitro* models have been developed to understand and predict the *in vivo* performance of LBFs. However, none of the present models are able to mimic fully the overall processes of LBFs occurring *in vivo*, leading to frequent failure in obtaining level A IVIVCs. Great efforts have been made to improve the predictive power of *in vitro* models by closely simulating the gastrointestinal physiology. A substantial improvement in this field will definitely promote the clinical translation of LBFs.

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Author contributions

Wei Wu, Quangang Zhu, and Yi Lu proposed the conception of the review. Yanping Huang and Qin Yu wrote the original manuscript. Zhongjian Chen, Quangang Zhu, and Yi Lu revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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