AAPS PharmSciTech (2019) 20: 16
DOI: 10.1208/s12249-018-1239-1

Review Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
Guest Editor: Sanyog Jain

Critical *In Vitro* Characterization Methods of Lipid-Based Formulations for Oral Delivery: a Comprehensive Review

Nitin Kumar Swarnakar,[1,2] Natarajan Venkatesan,[1,2] and Guru Betageri[1,3]

Received 30 August 2018; accepted 8 November 2018; published online 19 December 2018

Abstract. Lipids have been extensively used in formulations to enhance dissolution and bioavailability of poorly water-soluble as well as water-soluble drug molecules. The digestion of lipid-based formulations, in the presence of bile salts, phospholipids, and cholesterol, changes the lipid composition *in vivo*, resulting in the formation of different colloidal phases in the intestine. Therefore, *in vitro* characterization and evaluation of such formulations are critical in developing a successful formulation. This review covers comprehensive discussion on *in vitro* characterization techniques such as solubility, drug entrapment, thermal characterization, dissolution, and digestion of lipid-based formulations.

KEY WORDS: lipids; solubility; *in vitro*; dissolution; lipolysis.

INTRODUCTION

Since centuries, oral route is the preferred route of administration for drugs because of efficacy, safety, patient compliance, and cost benefits. Formulation scientists are continuously developing new drug delivery technologies due to realization of factors such as poor permeability, low solubility and therapeutic window, rapid metabolism, and intra-subject variabilities. Among the new technologies, lipid-based drug delivery systems and its applications have taken new avenues in oral drug delivery. These systems have played a great role in improving the problems associated with poorly water-soluble, lipophilic drugs. The lipids employed to prepare the formulation are mostly biocompatible, biodegradable, and safe (1). With the understanding of physiochemical properties of lipids, formulators can modulate delivery features of lipid-based formulations which include enhanced absorption with sustained release to immediate release properties. Hence, oral delivery *via* lipid-based delivery systems can be made as solution, suspension, emulsion, microemulsions, self-emulsifying drug delivery systems (SEDDS), solid lipid nanoparticles (SLNs), nanostructured lipid carrier (NLC), liquid crystalline nanoparticles (LCNPs), and proliposomes.

Scientists are working intensely in this area and understating the factors governing the *in vivo* performance of the systems. Several successful formulations have been marketed using lipids as functional excipients (1,2). There is considerable interest in developing lipid-based formulations for oral route of administration. Poulton and his colleagues described the classification of typical properties for different types of lipid-based formulations (3). The classification was further modified and discussed in Table I. Lipid-based drug delivery system offers a large variety of options, and the success of these formulations depends on the suitable selection of the lipid composition. The current development of lipid-based formulations is mostly empirical, demand many animal studies, that turns to be expensive and time consuming. Therefore, it is very critical to develop *in vitro* characterization and evaluation of such formulations to optimize and develop successful formulation for *in vivo* evaluation in animal models that can be successful in human study. The aim of this review is to provide a comprehensive discussion on *in vitro* characterization techniques used for the lipid-based drug delivery systems and their preclinical and clinical relevance.

Particle Size

Particle size of the formulation is key for a successful formulation. It plays a critical role in encapsulation efficiency, drug release, both *in vivo* and shelf stability, bioavailability, therapeutic index, and clearance of the drugs upon administration. Various techniques such as extrusion, sonication, and

Guest Editor: Sanyog Jain

[1] College of Pharmacy, Western University of Health Sciences, Pomona, California 91766, USA.
[2] *Present Address:* TesoRx Centre of Excellence at Western University of Health Sciences, 3670 W. Temple Ave, Suite 270, Pomona, California 91768, USA.
[3] To whom correspondence should be addressed. (e–mail: gbetageri@westernu.edu)

Table I. The Lipid Formulation Classification System: Characteristic Features, Advantages, and Disadvantages of Lipid-Based Formulations Modified and Reproduced with Permission (3)

Formulation type	Materials	Characteristics	Advantages	Disadvantages
SNEDDS Type I	Oils without surfactants (*e.g.*, tri-, di-, and monoglycerides)	Non-dispersing, requires digestion	GRAS status; simple; excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic, Digestion is important
SNEDDS Type II	Oils and water-insoluble surfactants	SEDDS formed without water-soluble components	Unlikely to lose solvent	Turbid o/w dispersion (particle size 0.25–2 μm)
SNEDDS Type III	Oils, surfactants, cosolvents (both water-insoluble and water-soluble excipients)	SEDDS/SMEDDS formed with water-soluble components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
SNEDDS Type IV	Water-soluble surfactants and cosolvents (no oils)	Formulation disperses typically to form a micellar solution	Formulation has good solvent capacity for many drugs	Likely loss of solvent capacity on dispersion; may not be digestible
Proliposomes	Phospholipids, cholesterol matrix adsorbed on a carrier	Formulation disperses in GI fluid to form liposomes and other lipidic structures	GRAS status; simple; excellent capsule compatibility, drug absorption with or without digestions	Hydrophilic drug leakage in GI fluids, formulation has limited solvent capacity
Liquid crystalline nanoparticle	Polar lipid-based matrix stabilized by surfactants, solid or lipid	Formulation disperses to form dispersion	GRAS status; simple; drug absorption with or without digestions	Drug leakage in GI fluids, limited solvent capacity

homogenization are being employed to control the size and size distribution of lipid-based carrier (4,5). The prepared formulation must be characterized for particle size to assure their suitability for *in vitro* and *in vivo* applications. "Polydispersity index" (PDI) is the term used to define the particle size distribution and the degree of non-uniformity of size distribution of particles (6,7).

Various techniques available to determine the particle size are as follows:

- Microscopy (*e.g.*, optical microscopy, confocal microscopy, scanning electron microscopy, transmission electron microscopy (TEM), cryo-transmission electron microscopy (cryo-TEM), and scanning probe microscopy (SPM))
- Diffraction and scattering techniques (laser light scattering and photon correlation spectroscopy)
- Hydrodynamic techniques (gel permeation chromatography, Coulter counter, ultracentrifugation, field flow fractionation, and centrifugal sedimentation)

Microscopic methods are widely used tools to observe the size and shape of sample as well as its distribution in the sample, *i.e.*, presence/absence of any aggregation and/or fusion. This technique is used to establish the morphology, lamellarity, surface characteristics, size, and stability of nanocarriers. Other modern techniques based on different interactions between the tip and surface of the particle, to get three-dimensional images of the particle; are also being used to characterize the surface property, rigidity, and size of the carrier. Examples of such techniques are atomic force microscopy (AFM), scanning tunneling microscopy (STM), magnetic force microscopy (MFM), electrostatic force microscopy (EFM), and Kelvin probe force microscopy (KPFM). AFM and other associated techniques provide: (1) three-dimensional surface profile, (2) structural, (3) mechanical, and (4) topographical information about the particles. Cryo-TEM is also an ideal technique to visualize the carrier in a frozen state and prevents disruption of thermal-sensitive carriers like proliposomes and NLC by highly energized electron beam (8,9). Microscopic methods are considered as a more qualitative than quantitative technique because of the time required to analyze the particles in a sample.

In contrast, measurement of the size distribution using diffraction and scattering techniques is more rapid than microscopic techniques and provides a statistically meaningful result. This method is rapid, reproducible, and accurate hence routinely used to measure the particle size and distribution of lipid formulations. It measures a wide range of particle size starting from 3 nm to 3 μm and provides information about particle size distribution (PDI) within the sample. The value of PDI (ranges from 0.0 to 1.0) where 0.0 depicts a perfectly uniform particle size while 1.0 depicts polydisperse and multiple particle size distribution sample. Value of PDI (0.3 and below) is considered to be acceptable and indicates a homogenous population of lipidic formulation (10–12). However, it does not provide information regarding the morphology and shape of the lipid-based system and program to assume any aggregation of several particles as one single particle. Another technique, field flow fractionation, is used to measure size distribution and relative molecular mass of lipid-based carrier. This technique is

considered as combination of chromatography techniques (without stationary phase) and a field-driven method to separate the particles based on their nature in different bands such as small particle size band *vs* large particle band. The field-driven methods are of different types: (1) electric, (2) thermal, (3) magnetic, (4) gravitational, and (5) centrifugal forces (13). This method could be selected depending on the property of the particles and applied perpendicularly to the flow of the sample. Liposomes with sizes from 1 nm to 100 μm were measured and separated by using this technique (14).

Determination of size and PDI of individual nanoparticle are also possible by "scanning ion occlusion sensing" (SIOS). The mechanism of action of SIOS is based on the conventional Coulter counter, where individual particles are passed through a tunable pore, with each passage of particle a drop in ionic current occurs due to an increase in the electrical resistance (9,15). The extent of current reduction and the frequency of the pulses are related to the particle size and concentration of the nanocarrier sample, respectively. SIOS analysis is rapid, easy, and can determine the concentration of the particle and size in a range of 60 nm to a few micrometers. Furthermore, SIOS was successfully used to measure changes in the size and surface charge of phospholipid vesicles upon incubation in biological fluids (16,17). The practical problem with this technique is selection of suitable elastic pore for polydisperse samples and detects only one particle at a time (17). Others techniques like cryo-XRD (18), small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and their analogues can determine size distributions and resolve the size and shape of the samples and are widely used for liquid crystalline nanoparticles (19–22).

The effect of particle size on oral absorption has been shown by researchers who found that the emulsion droplet size affects the rate of absorption of cyclosporine A (23,24). Finer emulsion has shown more rapid absorption than the coarse emulsion. It is assumed that the droplet size of emulsion should be as fine as possible to increase the absorption of cyclosporine A. Marketed Neoral® formulation first-in-time designed to self-emulsify to form very small (sub-100 nm) droplets *in situ* as compared to Sandimmune® formulation (effective diameter 3.7 μm) (25), showed improved bioavailability. Neoral not only improved bioavailability but also had various benefits such as (1) reduced food effects, (2) reduced inter-subject variability, and (3) absorbed in liver transplant patients with disrupted biliary flow (26–31). It is also important here to know that a direct link between particle size of Neoral and improved *in vivo* bioavailability has never been established due to the presence of digestible lipids and surfactant (32). These components are expected to undergo digestion upon oral administration and would change the particle size of the formulation. In addition, Kolliphor RH40 has been reported to inhibit efflux transporters and metabolic enzymes which further increase the bioavailability (33). Notably, the fate of the drug after digestion of the formulation is very critical than the initial particle size. The drug could precipitate in the gastrointestinal tract (GIT) if the total solubilizing capacity of the formulation and its secondary structures are reduced after the *in vivo* lipolysis process. For example, esters and digestible surfactants are often rapidly hydrolyzed in the presence of pancreatic lipase and reduce solvent capacity of formulations.

In several cases, lipid formulations upon digestion coexist with other secondary structures such as micelles, mixed micelles, liquid crystalline, and liposomes. Hence, particle size characterization of these structures using techniques such as photon correlation spectroscopy and laser diffraction is challenging. These techniques have low resolution to detect multimodal distribution of structure as discussed earlier and need advanced modern techniques based on particle-to-particle size determination techniques such as Coulter counter and/or SIOS.

Solubility

Lipid formulations are generally formulated as three forms: (1) hydrophobic drug is dissolved in lipid-based formulations, (2) anhydrous drug-lipid matrix adsorbed on a solid carrier that can be a solid dosage form, and (3) drug is suspended in a lipid formulation (34). Lipophilic drugs with log *P* values greater than 5, for example halofantrine or cinnarizine, are good candidates for lipid-based formulations (35). Experienced researchers have suggested that the drugs which have high melting point and log *P* values of about 2 (example Griseofulvin) are poor candidate for lipid systems (35). These types of drugs are poorly soluble in glycerides and GI fluids such as micelle solution of lecithin and bile salts. There are many crystalline drugs which are difficult to formulate as the lipid-based system needs a different approach such as homogenization, sonication, or nanomilling to convert them into amorphous formulation. Researchers also used precipitator inhibitors (mostly are water-soluble polymers) in lipid matrix to reduce the rate of crystallization in the matrix as well as during its dilution *in vivo* (36–38). The examples of the inhibitors are poly (propylene glycol), poly (lactic acid), polyvinylpyrrolidone (PVP), and hydroxypropyl methyl cellulose (HPMC) and were reviewed and discussed in detail recently (39). A supersaturable self-emulsifying drug delivery system (S-SEDDS) of paclitaxel was prepared using HPMC and was reported to have fivefold higher oral bioavailability as compared with that of the orally dosed Taxol formulation and the SEDDS formulation without HPMC showed lower oral bioavailability (36). However, it is believed that precipitation inhibition is not a "one-size-fits-all" process. The molecular interactions between the polymer and the drug such as hydrogen bonds, polar and non-polar surface area, and dispersion forces need to be understood in-depth to make an educated choice of successful precipitator inhibitor-drug combinations.

For drugs with poor aqueous and lipid solubility, a suspension in lipid formulation might be beneficial to deliver greater amount of drug as compared to the drug in lipid solution (34). However, particle size of suspension, uniformity of dispersion, and permeability of drug suspension through intestinal wall and drug solubilization in intestinal fluids need to be understood in-depth during the development of these formulations.

Finally, weak acidic or basic drug containing lipid-based formulation also needs to be characterized for specific factors in relation to their bioavailability. For example, a free base presented in a lipid system can extract out into the acidic aqueous phase of the stomach. Authors also have experienced that the solubilization pattern for a salt of weak acid or

base is more likely to mirror that of the free acid/base. If the free drug has higher solubility in lipid formulation, then it is possible to improve their bioavailability *via* partitioning behavior of the drug. However, the solubilized drug could become insoluble in intestinal fluids, so fate of the drug will be dependent on re-solubilization of the drug in formulation or secondary structure of the digested formulation. Therefore, *in vitro* dissolution and gastric-emptying experiments must be performed to understand the precipitation of drug and bioavailability.

Drug Contained in Lipid-Based Formulation

The drug "content" covers both encapsulated and intercalated drug substance in the lipid carriers. "Encapsulated" refers to drug within the carrier system for example drug in aqueous compartment of liposomes or in lipid core of solid lipid nanoparticles. The "intercalated" refers to drug within a bilayer of lipid in a carrier-like liposome. In literature, the drug content is also referred as entrapment efficiency, defined as the percentage of drug bound to the carrier with respect to the total amount of drug present in the formulation. This parameter is determined generally by separation of free drug from the carrier and analysis of the drug and total drug used to calculate encapsulation efficiency (40). Various methods are used to separate free drug from the carrier. Table II summarizes the advantages and disadvantages of the various methods reported.

Thermal Characterization: DSC, TGA, and X-Ray Diffraction

Nature of lipid and drug, *i.e.*, amorphous, crystalline, or semi-crystalline, in the formulation affects *in vivo* performance of the formulation. Lipid crystallinity in the matrix of formulation has an effect on drug incorporation and its release rate; example for such type of formulations are SLN and NLC (41). SLN contains a matrix which is produced from a solid lipid for example tristearin, triacylglycerol mixtures (Dynasan bases), and mixtures of acylglycerols (such as Compritol®888 ATO). In contrast, the matrix of NLC is prepared from a lipid blend, consisting of a mixture of a solid lipid with a liquid lipid (oil). The ratio of lipid and oil in the mixture determines the melting point of matrix, drug-carrying capacity, and its release properties. It is reported that mixing of structurally different lipid molecules creates a "structured" matrix exhibiting imperfections in the lipid crystal which increases drug loading. For example, ketoconazole was entrapped between the fatty acid chains in the Compritol®888 ATO matrix of SLN and NLC. But NLC had better stabilized the drug due to the presence of α-tocopherol, which decreases the crystallinity of lipid matrix. During shelf-life, it was also reported that the expulsion of the oil from the matrix led to undesirable drug expulsion (42).

It is also reported that drug crystallinity changes the lipid digestion and its bioavailability. Furthermore, the bioavailability is also dependent on the state of the precipitated drug and on the re-distribution of the lipid-based formulations and its digested carriers. Drug with polymorphous nature can have different bioavailability. Interestingly, the drug precipitation in a metastable amorphous state is also possible, which can enhance dissolution rate and possible bioavailability of drug. For example, danazol and cinnarizine in lipid formulation showed time dependent but continuous precipitation of drug during lipolysis. The precipitated danazol release profile was comparable to danazol crystalline form. In contrast, precipitated cinnarizine had showed improved dissolution profile than crystalline cinnarizine (43). With hands-on experience in the field of lipid-based formulations, authors have observed that fast digestion of lipid formulation could induce fast precipitation of drug which resulted into amorphous state of drug. Hence, various factors in lipolysis test need to be elucidated to predict *in vivo* performance of lipid-based formulations. Solid state of the precipitated drug after lipolysis experiment can be characterized using tools such as X-ray powder diffraction (XRPD) and thermal analysis and polarized light microscopy (PLM) (44).

Generally, the transition temperature depends on the length of the fatty acid chain, unsaturation, charge, and headgroup. For example, phase transition temperature of the lipid lowered upon (1) decreasing hydrocarbon length, (2) introducing a *cis* double bond into the acyl group, and (3) introducing branched chain and bulky group. It is noteworthy to know that phospholipids showed phase transition temperature, *i.e.*, from a rigid gel to the liquid crystalline phase below 100°C upon thermal analysis in the presence of suitable solvent-like water (45,46).

A saturated fatty acid-containing phospholipid possesses a high (> 15°C) phase transition temperature as compared to phospholipids containing unsaturated fatty acids which

Table II. Advantages and Disadvantages of Various Free Drug Separating Methods Used for Lipid-Based Drug Delivery Reproduced from (40) with Permission

Method	Advantages	Disadvantages
Dialysis	Sample recovery, scalable	Slow process
Centrifree®	Rapid; requires a small sample volume	Expensive; applicable only to unilamellar liposomes; lipid concentration cannot exceed 5 mg/mL
Protamine aggregation	Economical; applicable to multilamellar liposomes	Slow with neutral and positively charged liposomes; contamination of liposome sample
Density gradient	Economical; rapid; sample recovery	Sample volume (0.5 mL), setup cost
Minicolumn (example Sephadex gel with different grades in column)	Economical; sample recovery	Tedious, small sample volume (0.1 mL)

generally have low (< 15°C) phase transition temperatures. An understanding of phase transition and fluidity of lipid membrane affects important properties of lipid-based carrier such as fusion, aggregation, deformability, permeability, and drug-carrying capacity and thus determines overall performance in biological systems. Especially, for proliposome-based technology, the temperature should be carefully optimized preferably near to the physiological temperature so that it can transform from matrix phase to liposomes as well as retain the drug during the transition. Authors have experienced that proliposomes composed of low transition temperature lipids (< 37°C) can immediately convert to liposomes but also more susceptible to leakage of encapsulated drugs in GIT fluid at physiological temperatures, hence require in-depth characterization.

In Vitro Release, Digestion, and Its Clinical Implication

In modern era, dissolution testing is not only a quality control test but can also be designed to determine the clinical performance of the formulation. It is a cost-effective and time-saving tool to predict bioavailability of drug by means of *in vitro* and *in vivo* correlation. This correlation can be achieved with the understanding of physiochemical properties of drug, formulation, and relevant *in vivo* conditions. *In vitro* release testing is recommended for the anhydrous formulations such as proliposomes and solid- and liquid-SNEDDS Type III and Type IV formulations using a standard dissolution apparatus with physiologically relevant conditions. The purpose of the test is to understand dispersibility of formulation upon hydration, the rate of drug dissolution, and to detect precipitation of drug with time. For example, SNEDDS Type III and Type IV formulations lose their solvent capacity for drug due to migration of water-soluble components from formulation into the bulk aqueous phase. Standard compendial dissolution testing is recommended to provide sink conditions during dissolution testing to demonstrate amount of drug available for absorption in solution form within the recommended time. Further, selection of suitable equipment and dissolution parameters is of great importance.

Water is an attractive medium but has low buffer capacity; alternatively, a diluted HCl/NaCl solution or a diluted acetate buffer with a final pH of around 5 can be used for initial feasibility studies. The USP recommends dissolution of drug or drug product in dissolution medium with different pH (1.2, 4.5, and 6.8) to understand the formulation properties. For poorly water-soluble drug, dissolution medium with suitable detergents like polysorbate 80 and sodium dodecyl sulphate (SDS) is used to increase the drug solubility in a target volume and can be used as one of the quality control tools. However, these simple dissolution or dispersion tests generally do not represent *in vivo* performance of the lipid-based formulation because lipids are generally prone to digestion in the gastrointestinal tract.

The performance of a drug and its lipid-based formulation after oral administration can be predicted only if the limiting factor to absorption can be modeled *in vitro* by accurately simulating the *in vivo* conditions such as composition, volume, and hydrodynamics of the contents in the gastrointestinal lumen. It is also well known that the enzymes present in the intestinal fluid are affected by the amount of food and may influence the bioavailability of lipophilic drugs and its formulations. Although pharmacopeias do not recommend any biorelevant media for drug development, characterization and quality control testing using simulated small intestinal biorelevant media are becoming important tool to understand stability, solubility, and dissolution of drug in the *in vivo* conditions.

Most of the poorly soluble and weakly acidic drug dissolution is favored in the small intestine and is absorbed well. Hence, two types of biorelevant media, fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF), were proposed for BCS class I and class II drug (47). *In vitro* release data in FaSSIF and FeSSIF were well correlated with oral bioavailability data of some poorly soluble drugs (47,48). Later, the next-generation biorelevant media (49,50) were proposed in order to accurately simulate digested composition of the meal which enhances the solubility of lipophilic drugs. Hence, it is advisable to use the next-generation biorelevant media for dissolution and stability testing of lipid-based formulation. Dressman and colleagues recommended to use 300 mL of the fluid volume in the case of fasted state and 200 to 1000 mL in the case of fed sate condition (50). In addition, type of dissolution instrument and hydrodynamic conditions must also be considered to establish *in vitro* and *in vivo* correlation for lipid-based formulations (Table III). Recently, biphasic dissolution study using a reservoir of aqueous phase (300 mL of HCl 0.1 M) with an upper organic phase (200 mL of octanol) in USP apparatus 2 and release in USP apparatus 4 was studied for the evaluation of fenofibrate self-emulsifying formulations (see Fig. 1). The percentage of the drug dissolved in the biphasic dissolution medium (sum of two phases) was able to establish level A correlation based on FDA-recommended guideline (57). However, the established IVIVC was found to be dependent on the type of formulations and only able to predict *in vivo* profiles of the formulations produced by particles from gas-saturated solutions process and not by a common melt mixing process. This dissolution condition can be used for SEDDS because the formulation generally showed supersaturation in both *in vitro* and *in vivo* conditions. Moreover, some important practical issues that still need to be considered in the test include (1) octanol has a nauseating smell and the selection of another organic solvent would be dependent on the API solubility, volatility, and miscibility with the aqueous phase; (2) the emulsification of formulations with heavily surfactant-enriched aqueous media, such as FaSSIF or FeSSIF; and (3) formulation distribution inside the flow cells.

Reymond and Sucker have proposed *in vitro* digestion testing in 1980s which was further modified to understand the *in vivo* performance of lipid-based formulations (59–61). In this method, researchers have used (1) porcine pancreatin as a lipase source, (2) porcine bile extract containing various bile acids (34,62,63) or taurodeoxycholic acid (63) or taurocholic acid (64) as a bile species in the digestion medium with concentration ranges between 5 and 30 mM where the low levels simulate the fasted state and the higher level corresponds to the fed state, (3) phosphatidylcholine (34,62) or L-α-phosphatidylcholine (64) or lecithin (60% PC) (35,63) as phospholipid species with concentration four times less than

Table III. Summary of Various Dissolution Conditions Used for Lipid-Based Formulation to Establish *In Vitro-In Vivo* Correlation (IVIVC)

Drug	Main composition	*In vitro* test	*In vivo* test subject	IVIVC	Ref.
Cyclosporin	Composition similar to Neoral® Corn oil-mono-di-triglycerides, polyoxyl 40 hydrogenated castor oil NF, DL-α-tocopherol USP	Dissolution in 100 mL buffers (pH 1.2, 4.5, and 6.8); vigorous shaking	Dogs	Level A correlation between *in vivo* fraction absorbed *versus in vitro* fraction dissolved	(51)
JNJ-25894934	FI: fast-precipitation formulation PEG400/NMP (2.3/1) F2: slow-precipitation formulation Solutol® HS15/NMP (1/1) F3: no-precipitation formulation Solutol® HS15/NMP (3/1)	Test 1: in house designed precipitation test Test 2: precipitation test in USP apparatus 2 Media: SIF, FaSSIF, and FeSSIF	Mongrel dogs: fasted state and fed state	Good agreement between *in vitro* precipitation kinetics (test 1 and test 2) and *in vivo* pharmacokinetic profile	(52)
Ritonavir	Norvir®: butylated hydroxytoluene, ethanol, oleic acid, and polyoxyl 35 castor oil	900 mL of medium with 0.7% SLS + water and USP apparatus II at 25 rpm	Humans	Level A correlations were obtained in between percent dissolved *versus* percent absorbed	(53)
Lopinavir	Kaletra®: oleic acid, polyoxyl 35 castor oil, propylene glycol, sorbitol special	1000 mL of medium with 2.3% of sodium lauryl sulfate and pH 6.0 and USP apparatus 2 at 25 rpm	Humans	Level A correlation was obtained	(54)
Arundic acid or ONO-2506	Not available	900 mL of medium (a mixture of 50 mmol/l Na_2HPO_4 and 25 mmol/l citric acid pH = 8.0 or pH = 6.8) with 2% sodium dodecylsulfate (SLS)	Humans	Strong correlation was established by plotting *in vitro* dissolution time *versus in vivo* absorption time for dissolution media pH 6.8 with 2% SLS	(55)
Fenofibrate	Myritol 318/TPGS/Tween 80	250 mL of FaSSGF pH = 2 in a USP II mini-paddle apparatus and 500 mL of FaSSIF-V2 (PO4) in USP apparatus 2 at 75 rpm	Human and *in silico* software STELLA®	Human plasma profile was predicted by *in vitro* test and *in silico* simulations	(56)
Fenofibrate	Gelucire® 50/13	Biphasic dissolution reservoir 300 mL of aqueous phase (300 mL of HCl 0.1 M) with an upper organic phase (200 mL of octanol) in USP apparatus 2 (dual paddle − 50 rpm) and release in USP apparatus 4 (8 mL/min)	Pietrain crossed Landrace pigs; dogs	Level A correlations with dissolution obtained with the sum of both phases (aqueous phase + organic phase)	(57) (58)

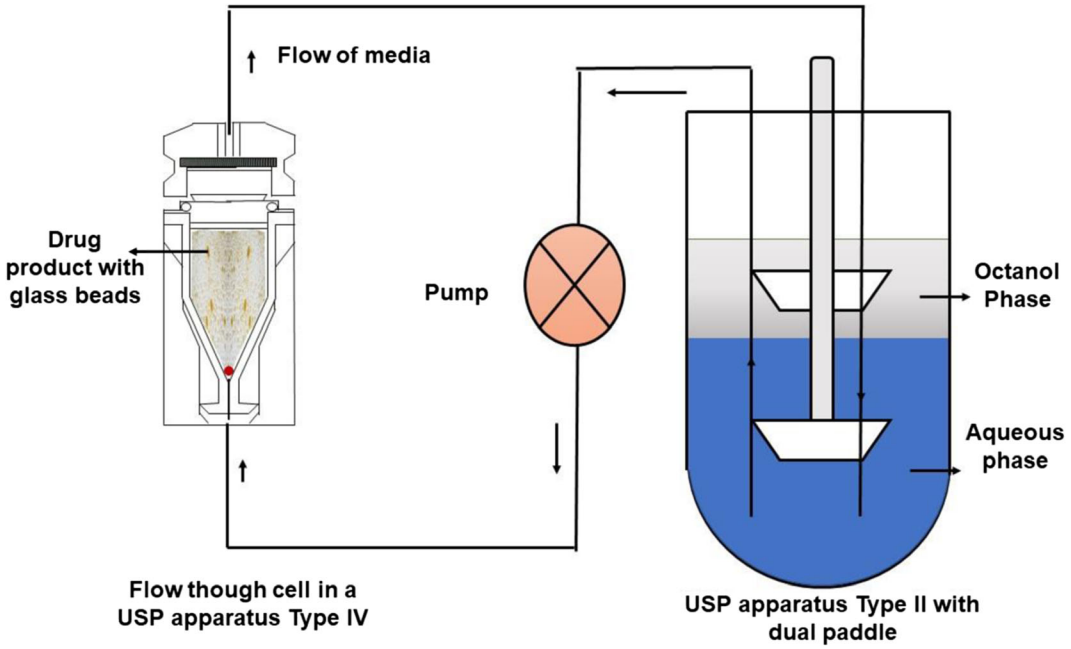

Fig. 1. Schematic representation of biphasic dissolution system: biphasic media in USP apparatus Type II combined with flow through cell in a USP apparatus Type IV

the bile salt concentration, (4) 5 mM calcium salt, and (5) 10 to 40 mL of 50 mM tris maleate buffer pH range (6.8 to 7.4) (63,64) or 300 mL of 2 mM tris maleate buffer pH range (6.8 to 7.4) (34,62). Details of various lipolysis models and conditions were previously reviewed and discussed (65).

In general, lipid-based formulation was exposed to micellar solution of bile salt containing lecithin and lipase enzymes at physiological temperature. The digested lipids release fatty acid and reduce the pH of medium. During lipid digestion test, the change in the pH is continuously monitored and maintained by auto titration with standard solution of NaOH using a PH stat system. Therefore, rate and extent of lipid digestion are indirectly determined by the stoichiometric titration for example 2 mol NaOH is needed for the hydrolysis of 1 mol triglyceride.

During *in vitro* lipolysis test, samples are withdrawn from the reaction vessel at different time points. Lipase activity in the sample is stopped by addition of a lipase inhibitor (commonly 4-bromobenzeneboronic acid) and different phases (oil/lipid/fatty acid salt layers) are analyzed following centrifugation of the samples. With the progress of the lipolysis, digestible lipid/oil layer diminishes and amount of pellet after the centrifugation increases due to precipitation of fatty acid-calcium soap (Fig. 2).

In vitro lipolysis test is a useful tool to quantify the rate and extent of lipolysis in lipid-based formulations and to determine the fate of the drug (solubilized *vs* precipitated) during or after the test. Precipitation of a drug compound (BCS classes II and IV) from a lipid-based formulation in the gastrointestinal tract can be caused by numerous different factors including hydrolysis of excipients present in the formulation. Many excipients used in lipid-based formulations contain ester bonds that are prone to hydrolysis by lipases or esterases present in the gastrointestinal tract and could precipitate the drug if the digested components have no solubilizing capacity for the drug (32,34,66). Hence, the test offers an opportunity to predict drug delivery potential of formulation in the intestinal lumen prior to absorption. This test is essential for evaluation of proliposomes, lipid-based nanoparticle, SNEDDS Type I, Type II, and Type III formulations, and for Type IV formulations (if surfactants are subject to digestion) (32).

The test has been used to predict fate of a series of drugs using different formulations (35). Researchers have found that the bioavailability of some drugs was effected by formulations containing medium and long chain triglycerides (63). *In vitro* lipolysis of the formulations, containing danazol with different volume of Labrafil M2125CS, was able to predict the rank order of the bioavailability from the formulations (34). Porter and his group also suggested that the amount of lipid in *in vitro* lipolysis test plays a great role in correlation of IVIVC of lipophilic drug halofantrine in beagle dog (67). They have observed that the correlation was good with lipid load at 5 mg/mL and not with 25 mg/mL lipid load in lipolysis media.

Similarly, *in vitro* lipolysis experiments suggested a rank order of Captex 355 (C8–10, medium chain triglycerides (MCT)) > peanut oil (C18, long chain triglycerides (LCT)) > triacetin (C2, short chain triglycerides (SCT)) for lipophilic molecules progesterone and vitamin D_3. The bioavailability of progesterone in the rat model was found to be correlated with the *in vitro* data, despite its significant pre-systemic metabolism. However, an *in vivo* performance rank order of LCT > MCT > SCT was obtained for vitamin D_3 due to its lymphatic absorption. Overall, the study suggested that if formulations showed a significant absorption through lymphatic transport then the *in vitro* lipolysis data may not be predictive for actual *in vivo* absorption (64).

Liposomes composed of unsaturated phospholipids and cholesterol showed instability in GIT upon oral administration. These liposomes are highly susceptible to gastric acid, bile salts, and lipases. Bile salt containing natural surfactants has a

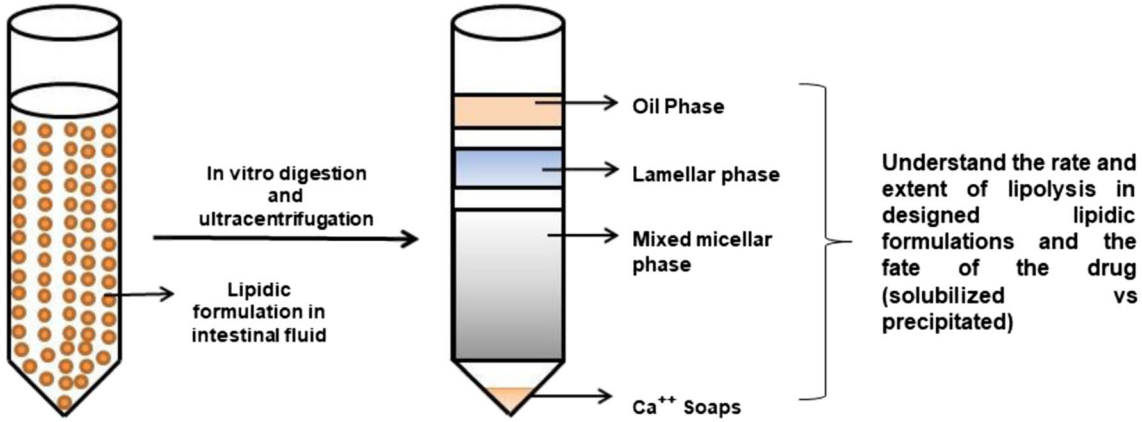

Fig. 2. A general fate of lipid-based formulation after *in vitro* digestion and its possible outcome

considerable variability ranging from 0.3 to 9.6 mM (68). It affects liposomal structure by solubilizing lipidic molecules while the lipase degrades phospholipids by hydrolysis (69). For example, lecithin is a substrate for the phospholipases and lipases secreted by the pancreas (70). It is hydrolyzed at the sn-2 position into the monoacylphospholipid of lecithin and one free fatty acid predominantly by the enzyme phospholipase-A2 (71). *In vitro* study showed that liposomes lose integrity within 2 h in simulated intestinal fluid which can potentially affect entrapment efficiency of water-soluble molecules (72). Formulators should be aware that phospholipids are chemically unstable and prone for hydrolysis. Generally, phospholipids possess four ester linkages (two ester linkages between hydroxyl groups of the glycerol and fatty acids and one ester linkage between the glycerol and the phosphate group and one ester linkage between the phosphate group and polar head group). These ester bonds can be hydrolyzed due to water, pH changes, and by enzymes *in vivo*. In practice, ester linkage between the hydroxyl group glycerol and carboxyl group of a fatty acid is susceptible to chemical hydrolysis by base or acid to form lysophospholipids. Depending on the type of phospholipid, the formed fatty acid may be saturated or mono- or poly-unsaturated. The unsaturated bonds of the fatty acids which sometime are dependent on the source of lipid are prone to oxidation. As an example, phosphatidylcholine obtained from egg yolk has a lower content of polyunsaturated fatty acids (less oxidation) compared to phosphatidylcholine from soy bean. Other examples of digestible lipids are triglycerides, diglycerides, phospholipids, fatty acids, cholesterol, and synthetic derivatives. Formulators should be aware of the source of lipid and its purity which could be tested by high performance liquid chromatography (HPLC) with mass spectrometry (HPLC-MS) or charged aerosol detection (HPLC-CAD) or evaporation light scattering detector (HPLC-ELSD), and thin layer chromatography (TLC) may be considered.

It is also reported that secondary structures such as mixed micelles and liquid crystalline structures are formed in the presence of digestive lipid, phospholipids, and their hydrolysis products, glycerol, free fatty acids, and cholesterol. These structures have a higher solubilization capacity which helps in the solubilization of poorly water-soluble compounds and have been reported to improve bioavailability of the compounds (73–75). Liposomes composed of saturated lipids such as distearoyl phosphatidylcholine with cholesterol was reported as stable at low pH and resistant to pancreatic lipase, while dipalmitoyl phosphatidylethanolamine, cholesterol, and dicetylphosphate composition was found to be unstable at low pH (69). These saturated lipids (non-digestible) generally cannot follow into all lipid digestion pathways but do form the secondary structure to facilitate diffusion across the mucosa and drug absorption. Other examples of non-digestible lipids are mineral oil and sucrose polyesters. Formulations containing no-digestible lipids may well be highly effective and can avoid the food effect variability in human.

Mooter *et al.* (76) have tested four different lipid-based formulations (Tween 80–Captex 200P, Tween 80–Capmul MCM, Tween 80–Caprol 3GO, and Tween 80–soybean oil) and one commercial micronized formulation (Lipanthyl Micronized®) of the lipophilic compound fenofibrate. The formulations were subjected to *in vitro* studies containing two biorelevant media and *in vivo* pharmacokinetic profile in rat model. In simulated gastric fluid without pepsin (SGFsp) and FaSSIF, Tween 80–Captex 200P system resulted in a stable fenofibrate concentration without forming supersaturated solution while rest of the lipid-based systems created fenofibrate supersaturation followed by precipitation. In contrast, no significant difference in bioavailability was observed among the four lipid-based formulations both under fasted and fed state. Authors have observed the conflicting situation due to the *in vitro* release studies in human biorelevant media and *in vivo* studies in rats. The poor *in vitro* and *in vivo* correlation was explained by continuous secretion of bile in the gastrointestinal tract of rats which led to enhanced bioavailability of the lipophilic drug. Similarly, precipitation of drug was observed in lipolysis model containing digestion media, but it was comparatively much lower in the rat intestine (44). Recently, researchers suggested that the propensity of drug precipitation during *in vitro* dispersion and digestion of lipid-based formulations can be used as a tool for the *in vivo* performance (77,78). For this, a maximum supersaturation ratio (SRM) has been suggested as a representation of the ratio of the theoretical drug concentration (in the absence of precipitation) and drug solubility in the aqueous phase (77,79). A threshold above (a value of SRM > 2.5) has been identified at which drug precipitation is likely to occur from formulation. However, the SRM precipitation parameter *in vitro* poorly reflected the *in vivo* minipig model for fenofibrate containing lipid formulation (79). Therefore, one must be careful in selecting a relevant animal model and lipolysis model for lipid-based formulations.

CONCLUSION AND FUTURE PERSPECTIVES

Current trends suggest that lipid-based formulations have tremendous potential in the field of oral drug delivery. However, the development of new lipid-based formulation is challenging due to the complex nature of the drug and fate of the formulation upon oral administration. We have discussed various characteristic features and *in vitro* characterization tools that are noteworthy in the development. The characterization tools can further be combined with cell culture and *ex vivo* permeation-based data in high-throughput screening of the lead formulation candidate. The prediction of *in vivo* performance of the formulations may be possible with the combination of particle size, biorelevant dissolution and lipolysis testing.

ACKNOWLEDGMENTS

The author (Nitin Kumar Swarnakar) expresses his sincere gratitude to Western University of Health Sciences, Pomona, California for awarding post-doctoral fellowship to carry out this project.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors report no conflict of interest.

PUBLISHER'S NOTE

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Savla R, Browne J, Plassat V, Wasan KM, Wasan EK. Review and analysis of FDA approved drugs using lipid-based formulations. Drug Dev Ind Pharm. 2017;43(11):1743–58.
2. Mishra DK, Shandilya R, Mishra PK. Lipid based nanocarriers: a translational perspective. Nanomedicine. 2018;14(7):2023–50.
3. Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv Drug Deliv Rev. 2008;60(6):625–37.
4. Maherani B, Wattraint O. Liposomal structure: a comparative study on light scattering and chromatography techniques. J Dispers Sci Technol. 2017;38(11):1633–9.
5. Dong YD, Tchung E, Nowell C, Kaga S, Leong N, Mehta D, et al. Microfluidic preparation of drug-loaded PEGylated liposomes, and the impact of liposome size on tumour retention and penetration. J Liposome Res. 2017:1–9.
6. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9(2).
7. Jain AK, Das M, Swarnakar NK, Jain S. Engineered PLGA nanoparticles: an emerging delivery tool in cancer therapeutics. Crit Rev Ther Drug Carrier Syst. 2011;28(1):1–45.
8. Almgren M, Edwards K, Karlsson G. Cryo transmission electron microscopy of liposomes and related structures. Colloids Surf A Physicochem Eng Asp. 2000;174(1–2):3–21.
9. Ruozi B, Belletti D, Tombesi A, Tosi G, Bondioli L, Forni F, et al. AFM, ESEM, TEM, and CLSM in liposomal characterization: a comparative study. Int J Nanomedicine. 2011;6:557–63.
10. Jain S, Bhankur N, Swarnakar NK, Thanki K. Phytantriol based "stealth" lyotropic liquid crystalline nanoparticles for improved antitumor efficacy and reduced toxicity of docetaxel. Pharm Res. 2015;32(10):3282–92.
11. Jain S, Chaudhari BH, Swarnakar NK. Preparation and characterization of niosomal gel for iontophoresis mediated transdermal delivery of isosorbide dinitrate. Drug Deliv Transl Res. 2011;1(4):309–21.
12. Jain S, Heeralal B, Swami R, Swarnakar NK, Kushwah V. Improved oral bioavailability, therapeutic efficacy, and reduced toxicity of tamoxifen-loaded liquid crystalline nanoparticles. AAPS PharmSciTech. 2018;19(1):460–9.
13. Fraunhofer W, Winter G. The use of asymmetrical flow field-flow fractionation in pharmaceutics and biopharmaceutics. Eur J Pharm Biopharm. 2004;58(2):369–83.
14. Cölfen H, Antonietti M. Field-flow fractionation techniques for polymer and colloid analysis. New Developments in Polymer Analytics I. Berlin: Springer; 2000. p. 67–187.
15. Henriquez RR, Ito T, Sun L, Crooks RM. The resurgence of Coulter counting for analyzing nanoscale objects. Analyst. 2004;129(6):478–82.
16. de Vrij J, Maas SL, van Nispen M, Sena-Esteves M, Limpens RW, Koster AJ, et al. Quantification of nanosized extracellular membrane vesicles with scanning ion occlusion sensing. Nanomedicine (London). 2013;8(9):1443–58.
17. Yang L, Broom MF, Tucker IG. Characterization of a nanoparticulate drug delivery system using scanning ion occlusion sensing. Pharm Res. 2012;29(9):2578–86.
18. Chauhan H, Mohapatra S, Munt DJ, Chandratre S, Dash A. Physical-chemical characterization and formulation considerations for solid lipid nanoparticles. AAPS PharmSciTech. 2016;17(3):640–51.
19. Swarnakar NK, Jain V, Dubey V, Mishra D, Jain NK. Enhanced oromucosal delivery of progesterone via hexosomes. Pharm Res. 2007;24(12):2223–30.
20. Swarnakar NK, Thanki K, Jain S. Lyotropic liquid crystalline nanoparticles of CoQ10: implication of lipase digestibility on oral bioavailability, in vivo antioxidant activity, and in vitro-in vivo relationships. Mol Pharm. 2014;11(5):1435–49.
21. Swarnakar NK, Thanki K, Jain S. Bicontinuous cubic liquid crystalline nanoparticles for oral delivery of doxorubicin: implications on bioavailability, therapeutic efficacy, and cardiotoxicity. Pharm Res. 2014;31(5):1219–38.
22. Li T, Senesi AJ, Lee B. Small angle X-ray scattering for nanoparticle research. Chem Rev. 2016;116(18):11128–80.
23. Pouton CW. Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification. Int J Pharm. 1985;27(2–3):335–48.
24. Wakerly MG, Pouton, C.W. , Meakin, BJ , Morton, FS. Self-emulsification of vegetable oil-nonionic surfactant mixtures—a proposed mechanism of action 311: ACS Symposium Series. 1986. p 242–55.
25. Andrysek T. Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine. Mol Immunol. 2003;39(17–18):1061–5.
26. Mueller EA, Kovarik JM, van Bree JB, Tetzloff W, Grevel J, Kutz K. Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. Pharm Res. 1994;11(2):301–4.
27. Ritschel WA. Microemulsion technology in the reformulation of cyclosporine: the reason behind the pharmacokinetic properties of Neoral. Clin Transpl. 1996;10(4):364–73.
28. Trull AK, Tan KK, Uttridge J, Bauer T, Alexander GJ, Jamieson NV. Cyclosporin absorption from microemulsion formulation in liver transplant recipient. Lancet. 1993;341(8842):433.
29. Kovarik JM, Mueller EA, van Bree JB, Fluckiger SS, Lange H, Schmidt B, et al. Cyclosporine pharmacokinetics and variability from a microemulsion formulation—a multicenter investigation in kidney transplant patients. Transplantation. 1994;58(6):658–63.
30. Kahan BD, Dunn J, Fitts C, Van Buren D, Wombolt D, Pollak R, et al. Reduced inter- and intrasubject variability in cyclosporine pharmacokinetics in renal transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals. Transplantation. 1995;59(4):505–11.

31. Trull AK, Tan KK, Tan L, Alexander GJ, Jamieson NV. Absorption of cyclosporin from conventional and new microemulsion oral formulations in liver transplant recipients with external biliary diversion. Br J Clin Pharmacol. 1995;39(6):627–31.
32. Cuine JF, McEvoy CL, Charman WN, Pouton CW, Edwards GA, Benameur H, et al. Evaluation of the impact of surfactant digestion on the bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to dogs. J Pharm Sci. 2008;97(2):995–1012.
33. Chiu YY, Higaki K, Neudeck BL, Barnett JL, Welage LS, Amidon GL. Human jejunal permeability of cyclosporin A: influence of surfactants on P-glycoprotein efflux in Caco-2 cells. Pharm Res. 2003;20(5):749–56.
34. Larsen A, Holm R, Pedersen ML, Mullertz A. Lipid-based formulations for danazol containing a digestible surfactant, Labrafil M2125CS: in vivo bioavailability and dynamic in vitro lipolysis. Pharm Res. 2008;25(12):2769–77.
35. Kaukonen AM, Boyd BJ, Charman WN, Porter CJ. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. Pharm Res. 2004;21(2):254–60.
36. Gao P, Rush BD, Pfund WP, Huang T, Bauer JM, Morozowich W, et al. Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. J Pharm Sci. 2003;92(12):2386–98.
37. Gao P, Guyton ME, Huang T, Bauer JM, Stefanski KJ, Lu Q. Enhanced oral bioavailability of a poorly water soluble drug PNU-91325 by supersaturatable formulations. Drug Dev Ind Pharm. 2004;30(2):221–9.
38. Gao P, Morozowich W. Development of supersaturatable self-emulsifying drug delivery system formulations for improving the oral absorption of poorly soluble drugs. Expert Opin Drug Deliv. 2006;3(1):97–110.
39. Price DJ, Ditzinger F, Koehl NJ, Jankovic S, Tsakiridou G, Nair A, et al. Approaches to increase mechanistic understanding and aid in the selection of precipitation inhibitors for supersaturating formulations—a PEARRL review. J Pharm Pharmacol. 2018.
40. Dipali SR, Kulkarni SB, Betageri GV. Comparative study of separation of non-encapsulated drug from unilamellar liposomes by various methods. J Pharm Pharmacol. 1996;48(11):1112–25.
41. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54(Suppl 1):S131–55.
42. Souto EB, Mehnert W, Muller RH. Polymorphic behaviour of Compritol888 ATO as bulk lipid and as SLN and NLC. J Microencapsul. 2006;23(4):417–33.
43. Sassene PJ, Knopp MM, Hesselkilde JZ, Koradia V, Larsen A, Rades T, et al. Precipitation of a poorly soluble model drug during in vitro lipolysis: characterization and dissolution of the precipitate. J Pharm Sci. 2010;99(12):4982–91.
44. Sassene PJ, Michaelsen MH, Mosgaard MD, Jensen MK, Van Den Broek E, Wasan KM, et al. In vivo precipitation of poorly soluble drugs from lipid-based drug delivery systems. Mol Pharm. 2016;13(10):3417–26.
45. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci. 2015;10(2):81–98.
46. Ali S, Minchey S, Janoff A, Mayhew E. A differential scanning calorimetry study of phosphocholines mixed with paclitaxel and its bromoacylated taxanes. Biophys J. 2000;78(1):246–56.
47. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res. 1998;15(5):698–705.
48. Nicolaides E, Galia E, Efthymiopoulos C, Dressman JB, Reppas C. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. Pharm Res. 1999;16(12):1876–82.
49. Arndt M, Chokshi H, Tang K, Parrott NJ, Reppas C, Dressman JB. Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state. Eur J Pharm Biopharm. 2013;84(3):633–41.
50. Jantratid E, Janssen N, Reppas C, Dressman JB. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm Res. 2008;25(7):1663–76.
51. Yang SG. Biowaiver extension potential and IVIVC for BCS class II drugs by formulation design: case study for cyclosporine self-microemulsifying formulation. Arch Pharm Res. 2010;33(11):1835–42.
52. Dai WG, Dong LC, Shi X, Nguyen J, Evans J, Xu Y, et al. Evaluation of drug precipitation of solubility-enhancing liquid formulations using milligram quantities of a new molecular entity (NME). J Pharm Sci. 2007;96(11):2957–69.
53. Rossi RC, Dias CL, Donato EM, Martins LA, Bergold AM, Froehlich PE. Development and validation of dissolution test for ritonavir soft gelatin capsules based on in vivo data. Int J Pharm. 2007;338(1–2):119–24.
54. Donato EM, Martins LA, Froehlich PE, Bergold AM. Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on in vivo data. J Pharm Biomed Anal. 2008;47(3):547–52.
55. Nishimura H, Hayashi C, Aiba T, Okamoto I, Miyamoto Y, Nakade S, et al. Application of the correlation of in vitro dissolution behavior and in vivo plasma concentration profile (IVIVC) for soft-gel capsules—a pointless pursuit? Biol Pharm Bull. 2007;30(11):2221–5.
56. Fei Y, Kostewicz ES, Sheu MT, Dressman JB. Analysis of the enhanced oral bioavailability of fenofibrate lipid formulations in fasted humans using an in vitro-in silico-in vivo approach. Eur J Pharm Biopharm. 2013;85(3 Pt B):1274–84.
57. Pestieau A, Lebrun S, Cahay B, Brouwers A, Streel B, Cardot JM, et al. Evaluation of different in vitro dissolution tests based on level A *in vitro*-in vivo correlations for fenofibrate self-emulsifying lipid-based formulations. Eur J Pharm Biopharm. 2017;112:18–29.
58. Pestieau A, Krier F, Brouwers A, Streel B, Evrard B. Selection of a discriminant and biorelevant in vitro dissolution test for the development of fenofibrate self-emulsifying lipid-based formulations. Eur J Pharm Sci. 2016;92:212–9.
59. Reymond JP, Sucker H. In vitro model for ciclosporin intestinal absorption in lipid vehicles. Pharm Res. 1988;5(10):673–6.
60. Alvarez FJ, Stella VJ. The role of calcium ions and bile salts on the pancreatic lipase-catalyzed hydrolysis of triglyceride emulsions stabilized with lecithin. Pharm Res. 1989;6(6):449–57.
61. Alvarez FJ, Stella VJ. Pancreatic lipase-catalyzed hydrolysis of esters of hydroxymethyl phenytoin dissolved in various metabolizable vehicles, dispersed in micellar systems, and in aqueous suspensions. Pharm Res. 1989;6(7):555–63.
62. Christensen JO, Schultz K, Mollgaard B, Kristensen HG, Mullertz A. Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols. Eur J Pharm Sci. 2004;23(3):287–96.
63. Porter CJ, Kaukonen AM, Boyd BJ, Edwards GA, Charman WN. Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation. Pharm Res. 2004;21(8):1405–12.
64. Dahan A, Hoffman A. 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. 2006;23(9):2165–74.
65. Thomas N, Holm R, Rades T, Mullertz A. Characterising lipid lipolysis and its implication in lipid-based formulation development. AAPS J. 2012;14(4):860–71.
66. Fernandez S, Chevrier S, Ritter N, Mahler B, Demarne F, Carriere F, et al. In vitro gastrointestinal lipolysis of four formulations of piroxicam and cinnarizine with the self emulsifying excipients Labrasol and Gelucire 44/14. Pharm Res. 2009;26(8):1901–10.
67. Porter CJ, Kaukonen AM, Taillardat-Bertschinger A, Boyd BJ, O'Connor JM, Edwards GA, et al. 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. 2004;93(5):1110–21.
68. Fuchs A, Dressman JB. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a

critical evaluation of the available data. J Pharm Sci. 2014;103(11):3398–411.
69. Rowland RN, Woodley JF. The stability of liposomes in vitro to pH, bile salts and pancreatic lipase. Biochim Biophys Acta. 1980;620(3):400–9.
70. Kossena GA, Charman WN, Boyd BJ, Dunstan DE, Porter CJ. Probing drug solubilization patterns in the gastrointestinal tract after administration of lipid-based delivery systems: a phase diagram approach. J Pharm Sci. 2004;93(2):332–48.
71. Caspary WF. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr. 1992;55(1 Suppl):299S–308S.
72. Liu W, Ye A, Liu W, Liu C, Han J, Singh H. Behaviour of liposomes loaded with bovine serum albumin during in vitro digestion. Food Chem. 2015;175:16–24.
73. Porter CJ, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6(3):231–48.
74. Wiedmann TS, Liang W, Kamel L. Solubilization of drugs by physiological mixtures of bile salts. Pharm Res. 2002;19(8):1203–8.
75. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, et al. Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 2013;65(1):315–499.
76. Do TT, Van Speybroeck M, Mols R, Annaert P, Martens J, Van Humbeeck J, et al. The conflict between in vitro release studies in human biorelevant media and the in vivo exposure in rats of the lipophilic compound fenofibrate. Int J Pharm. 2011;414(1–2):118–24.
77. Williams HD, Sassene P, Kleberg K, Calderone M, Igonin A, Jule E, et al. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 3: understanding supersaturation versus precipitation potential during the in vitro digestion of type I, II, IIIA, IIIB and IV lipid-based formulations. Pharm Res. 2013;30(12):3059–76.
78. Williams HD, Anby MU, Sassene P, Kleberg K, Bakala-N'Goma JC, Calderone M, et al. Toward the establishment of standardized in vitro tests for lipid-based formulations. 2. The effect of bile salt concentration and drug loading on the performance of type I, II, IIIA, IIIB, and IV formulations during in vitro digestion. Mol Pharm. 2012;9(11):3286–300.
79. Thomas N, Richter K, Pedersen TB, Holm R, Mullertz A, Rades T. In vitro lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery systems containing fenofibrate. AAPS J. 2014;16(3):539–49.