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Application of Lipid-Based Formulations in Drug Discovery

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1. INTRODUCTION

An increasing number of drug candidates discovered in recent years are highly lipophilic compounds with poor aqueous solubility. This often leads to insufficient absorption in the gastrointestinal (GI) tract and low oral bioavailability. Among various solubilization strategies (salt formation, cosolvent solubilization, complexation, amorphous dispersion, particle size reduction, etc.), lipid-based formulations have been successfully applied to several commercial products including Sandimmune and Neoral (cyclosporine A), Fortovase (saquinavir), and Norvir (ritonavir).[1−5] It has also proven to be a viable option in improving oral exposure of paclitaxel,[6] halofantrine,[7] danazol,[8] and many other lipophilic compounds.[9−15]

Lipid-based drug delivery systems include lipid solutions, lipid emulsions, lipid dispersions, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS). In particular, SEDDS and SMEDDS are isotropic mixtures of lipids, surfactants, and cosurfactants that can disperse spontaneously in aqueous media and form fine emulsions (SEDDS) or microemulsions (SMEDDS).[16−19]

Lipid-based formulations enhance drug absorption and oral bioavailability via various possible mechanisms including (1) increase in drug solubilization in GI tract, (2) prevention of drug precipitation upon mixing with GI fluids, (3) potential modulation of P-glycoprotein (P-gp) mediated efflux transport, (4) intestinal lymphatic transport for potential target delivery to lymphatic systems or systemic delivery bypassing hepatic first-pass metabolism, and (5) prolongation of GI transit time.[4,5]

In this review, we will discuss the application of lipid-based formulations in drug discovery within discovery-based constraints of time (1−2 weeks) and limited drug (10−50 mg). We will focus on strategic assessment of formulation options at early discovery stages, design of lipid formulations with often poorly characterized solid forms, and in vitro and in vivo evaluation of lipid formulations in preclinical models. We will also share our perspectives on connecting the preclinical evaluation with clinical development plan early in the process, which will reduce the formulation development time and increase the overall potential success of a clinically viable formulation.

2. EARLY EVALUATION OF LIPID FORMULATION STRATEGY IN DRUG DISCOVERY

Until recently, lipid-based formulations have not been widely used in drug discovery. This is probably due to the complex nature of lipid formulations that makes formulation development and optimization a resource- and time-consuming process requiring significant amount of API. Another possible reason is the lack of pharmaceutical tools that can be easily adapted into discovery settings for formulation assessment with quick turnaround time and minimal material requirement. More importantly, because of the lack of early assessment of overall formulation strategy, discovery teams tend to defer nonconventional formulation options to later stages of the program when all commonly used formulations have failed. However, this could be too late in the program when the physicochemical properties and therapeutic doses of the drug candidate have already been defined and may not be suitable for lipid formulations. Thus, it is important in early discovery to evaluate both the chemical template and the biological target/ligand of the program and, in doing so, establish an overall formulation strategy and incorporate this strategy into drug design and lead optimization toward candidate selection.

2.1. Evaluation of Chemical Template: Is the Compound a Good Candidate for Lipid-Based Formulations?

The first step in strategic evaluation of lipid formulations in early discovery is to assess physicochemical properties intrinsic to the chemical template and the lead compounds in the program (Figure 1). The goal at this stage is to evaluate whether the compounds are good candidates for lipid formulations.

A quick check list of required properties for lipid formulations can be useful in the initial assessment. This includes (1) poor aqueous solubility (e.g., <10 ug/mL), (2) high lipophilicity (e.g., log P > 5), (3) good solubility in oils and lipids (e.g., >25 mg/mL), (4) relatively low melting point (low crystal packing energy), (5) good potency (low therapeutic dose), and (6) acceptable chemical stability (low potential for transacylation reactions between the drug and the excipients as well as oxidation in the presence of peroxides).[20,21]

2.2. Evaluation of Biological Target/Ligand: Is Lipid Formulation Strategy a Viable Option for the Program?

In addition to the assessment of physicochemical properties of the compound, it is also important to understand the intrinsic properties of the endogenous ligands for the biological target (Figure 1). Traditionally, discovery chemistry is driven toward a candidate that is sufficiently lipophilic for membrane permeation and receptor binding while polar enough for aqueous solubility and oral absorption. As a result, it is a common practice to improve aqueous solubility while maintaining binding via chemical modification in discovery chemistry. However, the endogenous ligands for some biological targets are highly lipophilic in nature, making chemical modification for solubility improvement inadequate to maintain receptor binding and biological functions. For example, cholesteryl ester transfer protein (CETP) is a plasma lipid transfer protein that facilitates the transport of cholesteryl esters and triglycerides between lipoproteins, and inhibitors of CETP have been developed for atherosclerotic cardiovascular

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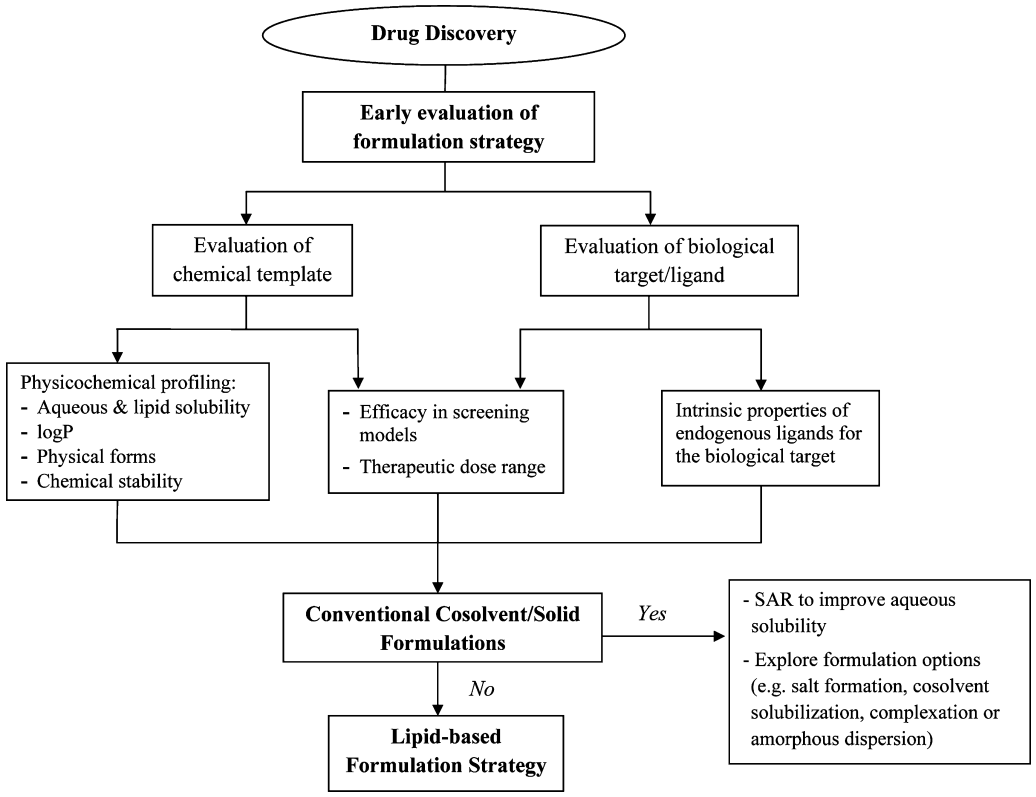

Figure 1. Early evaluation of formulation strategy in drug discovery.

diseases. The CETP inhibitors such as torcetrapib and anacetrapib are highly lipophilic with log $P > 7$, and lipid-based formulations have been applied to improve oral exposure of these compounds.[22,23]

Thus, on the basis of the initial evaluation of chemical and biological attributes of the program, the nonconventional formulation strategy needs to be carefully assessed early in discovery and strategically incorporated into drug design and lead optimization before candidate selection, i.e., actually design a drug to be effectively delivered by this formulation strategy (Figures 1 and 2). In this way the drug and the formulation are linked early in the discovery process, which will facilitate smooth transition down the road to the development. Specifically for highly lipophilic targets, a compelling strategy in discovery chemistry is to focus less on aqueous solubility and explore more lipophilic compounds. This would enable selection of candidates with better intrinsic potency and higher lipid solubility, both of which will facilitate successful development of a lipid-based formulation. More importantly, this would guide the medicinal chemists in building the structure–activity relationships (SARs) with appropriate properties (e.g., high lipophilicity and low melting points) and designing a drug molecule most amenable to the lipid-based drug delivery.[20]

3. DESIGN OF LIPID-BASED FORMULATIONS IN DRUG DISCOVERY

Figures 2 and 3 have outlined various aspects in designing and evaluating lipid-based formulations in drug discovery. The development of lipid formulations especially SEEDS and SMEDDS is often empirically based. The performance of a lipid formulation depends on the nature of lipid excipients and the physicochemical properties of the compound. A good lipid formulation should be able to solubilize the entire drug dose in a unit dosage form and maintain the drug in solubilized state with no precipitation in the GI tract. Formulation optimization is further challenged by many of the inadequacies of preclinical models and uncertain translation to complexities of lipid processing in humans.

3.1. Solubility Screen in Lipid Excipients. Table 1 shows the list of commonly used excipients for lipid formulations. One can clearly see the complex nature of these excipients which are mixtures of multiple components.[24,25] For example, Labrafil 1944 CS is defined as a mixture of mono-, di-, and trioleic acid esters of glycerol and mono- and diesters of PEG 300, and the fatty acid components include oleic acid (C18:1, 58–80%), palmitic acid (C16, 4–9%), stearic acid (C18, <6%), and linoleic acid (C18:2, 15–35%).[26]

The first and foremost step in designing a lipid formulation is to assess the solubility of the lead compound in various lipid excipients, cosolvents, and surfactants (solvent capacity). Since the clinical dose is usually unknown in early discovery, a general rule of thumb is that solubility in a range of 25–50 mg/mL in lipid excipients is needed to support future studies. The solvent capacity of lipids can be increased by addition of cosolvents and surfactants. The incorporation of cosolvents and surfactants can also help to reduce the interfacial tension and the oil–water partition coefficient and therefore can facilitate emulsification and effective absorption.[18–20,27,28]

The in silico prediction of drug solubility in lipid excipients could be very useful, since solubility screening at 25–50 mg/mL could be quite challenging in early discovery when the total supply of the compound may be in a range of 5–10 mg. However, predicting lipid solubility is complicated because of the complex nature of the lipid excipients and the interaction of drug molecules with the multicomponent lipids. In studies by Anderson and colleagues, linear free energy solvation relationships have been

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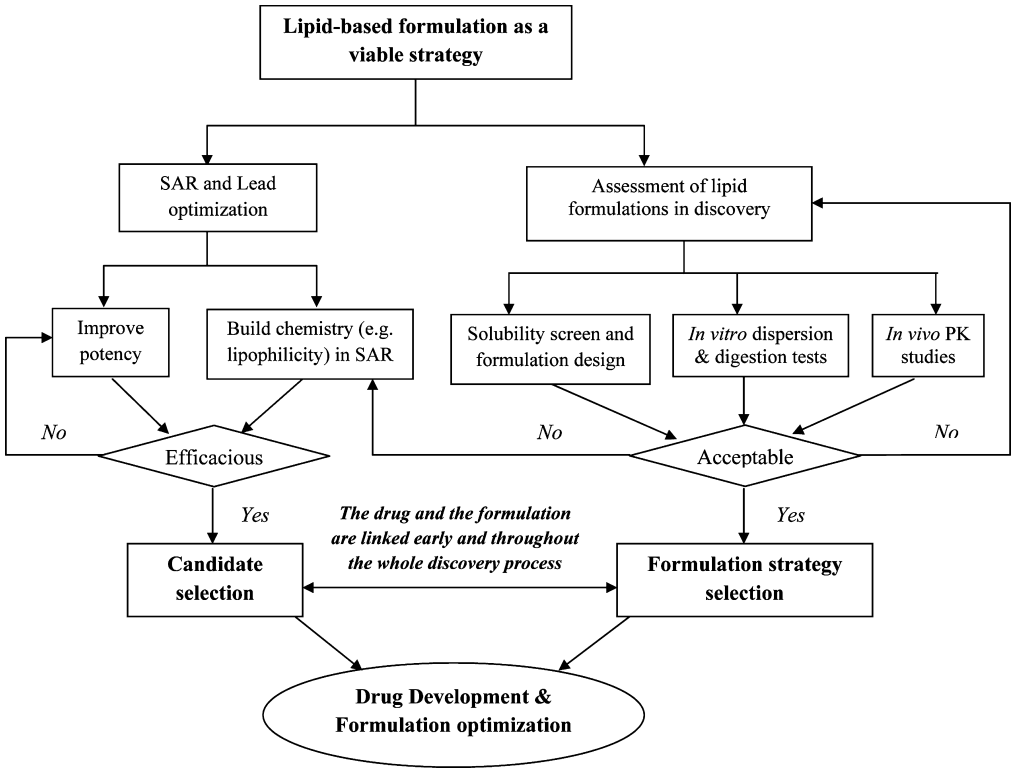

Figure 2. Application of lipid-based formulations in drug discovery.

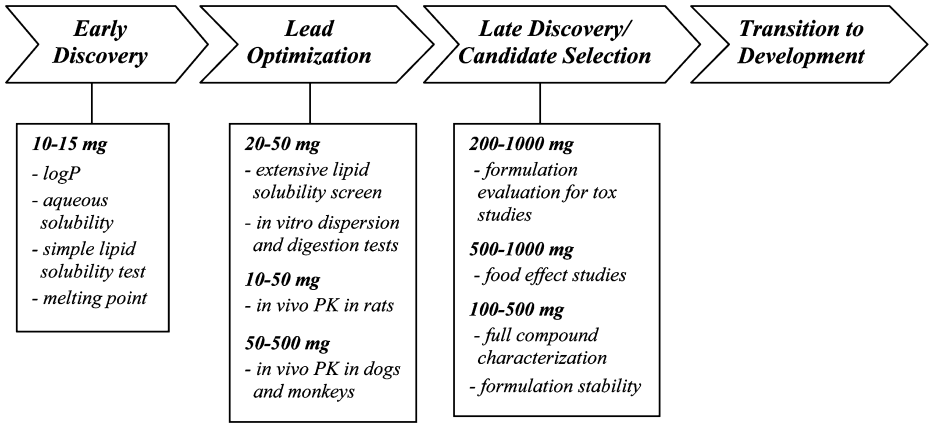

Figure 3. Timeline and minimum material requirement for lipid formulation development in drug discovery.

developed to obtain solvent coefficients of mono- and triglycerides. The authors have demonstrated that the solvent capacity of glycerides is a function of the molar concentrations of the individual functional groups such as ester moieties and hydroxyl groups.[29−31] Thus, on a weight basis the solubility of a lipophilic compound is higher in medium-chain triglycerides (MCT) than in long-chain triglycerides (LCT).

3.2. Design of Prototype Lipid Formulations in Drug Discovery. The design of prototype lipid formulations can be guided by the solubility screen results as well as the lipid formulation classification system proposed by Pouton and Porter.[18,27,32] On the basis of this system in which lipid formulations are classified into three categories, one can start with simple lipid solutions without surfactants (type I). If the whole dose cannot be solubilized in the lipid solution in a typical unit dosage form (e.g., 0.5−1.0 mL), then the option could be either type II formulations containing oils and water insoluble surfactants or type III formulations which are mixtures of oils, surfactants, and cosolvents.

3.3. Factors for Lipid Formulation Design in Drug Discovery. In addition to the solvent capacity discussed in section 3.1, a variety of other factors listed in Table 2 also need to be considered when developing lipid-based formulations in drug discovery.[25,27] For example, the properties of lipid excipients such as the fatty acid chain length have been shown to affect lipid digestion and drug solubilization.[15] In general, the medium-chain glycerides tend to work well with less lipophilic compounds that can be readily transferred to the highly solubilizing aqueous environment after lipid digestion.[33,34] The long-chain glycerides, on the other hand, prefer highly lipophilic compounds that would either remain in the undigested oil phase or partition into mixed micelles after digestion.[7,8]

Surfactants are needed for efficient emulsification as well as increasing solvent capacity and preventing drug precipitation.

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Table 1. List of Commonly Used Lipid Excipients in Drug Discovery

Polyoxylglycerides: Semisynthetic PEG Derivatives of Glycerides and Fatty Acids					
common name	chem name	chem description	hydroxyl group	main fatty acid chain	HLB
Labrafil M1944CS	oleoyl macrogol-6 glycerides, oleoyl polyoxyl-6 glycerides	long-chain mono-, di-, and triglycerides	PEG 300	oleic C18:1	4
Labrafil M2125CS	linoleoyl macrogol-6 glycerides, linoleoyl polyoxyl-6 glycerides	long-chain mono-, di-, and triglycerides	PEG 300	linoleic C18:2	4
Labrasol (Gattefosse), Acconon MC8-2 (Abitec)	caprylocaproyl macrogol-8 glycerides, caprylocaproyl polyoxyl-8 glycerides	medium-chain mono-, di-, and triglycerides	PEG 400	caprylic C8 and capric C10	14
Gelucire 44/14 (Gattefosse), Acconon C-44 (Abitec)	lauroyl macrogol-32 glycerides, lauroyl polyoxyl-32 glycerides	medium-chain mono-, di- and triglycerides	PEG 1500	lauric C12	14
Gelucire 50/13	stearoyl macrogol-32 glycerides, stearoyl polyoxyl-32 glycerides	long-chain mono-, di-, and triglycerides	PEG 1500	palmitic C16 and stearic C18	13
Ethoxylated Lipids, Polyoxyethylene Castor Oil					
common name	chem name	chem description	hydroxyl group	main fatty acid chain	HLB
Cremophor EL	polyoxyl 35 castor oil, macrogolglycerol ricinoleate	polyethoxylated castor oil	polyethylene glycol	ricinoliei C18 (castor oil), unsaturated	12−14
Cremophor RH40	polyoxyl 40 hydrogenated castor oil, macrogolglycerol hydroxystearate	polyethoxylated castor oil	polyethylene glycol	ricinoliei C18 (castor oil), hydrogenated	14−16
Solutol HS15	macrogol 15 hydroxystearate, polyoxyl 15 hydroxystearate	polyglycolester of 12-hydroxystearic acid	polyethylene glycol	12-hydroxystearic acid C18	14−16
Tween 80	polysorbate 80, polyoxyethylene 20 sorbitan monooleate	monooleate polyoxyethylene derivative	polyethylene glycol sorbitan	oleic C18	15
Tween 20	polysorbate 20, polyoxyethylene 20 sorbitan monolaurate	monolaurate polyoxyethylene derivative	polyethylene glycol sorbitan	lauric C12	16
Propylene Glycol Esters					
common name	chem name	chem description	hydroxyl group	main fatty acid chain	HLB
Capryol 90 (Gattefosse), Capmul PG-8 (Abitec)	propylene glycol monocaprylate	medium-chain monoglyceride	propylene glycol	caprylic C8	6−7
Lauroglycol 90 (Gattefosse), Capmul PG-12 (Abitec)	propylene glycol monolaurate	medium-chain monoglyceride	propylene glycol	lauric C12	4−5
Labrafac PG (Gattefosse), Captex 200P (Abitec)	propylene glycol dicaprylocaprate, propylene glycol dicaprylate/dicaprate	medium-chain diglyceride	propylene glycol	caprylic and capric C8, C10	2
Mono-, Di-, and Triglycerides					
common name	chem name	chem description	hydroxyl group	main fatty acid chain	HLB
Capmul MCM (Abitec), Imwitor 742 (Sasol)	glyceryl caprylate/caprate	medium-chain mono- and diglycerides	glycerol	caprylic and capric C8, C10	6
Capmul MCM C-8 (Abitec), Imwitor 988 (Sasol)	glyceryl caprylate	medium-chain mono-, di-, and triglycerides	glycerol	caprylic C8	6−7
Captex 300, Captex 355 (Abitec), Miglyol 812 (Sasol)	glyceryl tricaprylate/caprate	medium-chain triglycerides	glycerol	caprylic and capric C8, C10	11
Peceol (Gattefosse), Capmul GMO-50 (Abitec)	glyceryl oleate	long-chain mono- and diglycerides	glycerol	oleic C18:1	3−4
Maisine 35-1 (Gattefosse)	glyceryl monolinoleate	long-chain monoglyceride	glycerol	linoleic C18:2	4
Imwitor 491 (Sasol)	glyceryl monostearate	long-chain monoglyceride	glycerol	stearic C18	4
Captex 500 (Abitec), Triacetin (Sigma-Aldrich)	glyceryl triacetate	triacetic acid esters of glycerol	glycerol	acetic acid	
Pluro Oleique CC497 (Gattefosse)	polyglyceryl-3 dioleate	polyglycerol esters of oleic acid	polyglycerol	oleic C18	6

However, when the surfactant level is too high (e.g., >50%), a viscous gel-like structure may form at the water/surfactant interface, limiting water penetration and slowing lipid dispersion.[35,36] As a result, the coadministered drug can be sequestered in surfactant micelles, leading to low free drug concentration and poor intestinal absorption.[37,38] The lipophilicity of the surfactant has also been shown to impact the emulsion formed after lipid dispersion. In general, hydrophilic surfactants with HLB > 10 are found to provide finer, more uniform emulsion droplets compared to lipophilic surfactants with low HLB values. But in many cases it is the combination of low and high HLB surfactants that leads to the formation of stable microemulsions.[9,28,38]

The digestibility of the excipients may also affect drug solubilization and oral absorption. For example, Cuine et al. have reported that better solubilization and higher in vivo exposure of danazol can be achieved in the poorly digested Cremophor RH40 than in the readily digested Cremophor EL.[36] The use of Cremophor RH40 may be particularly beneficial for medium-chain glyceride formulations in which extensive digestion or reduction of lipid load would lead to precipitation of highly lipophilic compounds.

Table 2. Formulation Design Checklist: Factors To Consider in Lipid Formulation Design in Drug Discovery

factor	explanation
solvent capacity	- Solubility of compounds in lipid excipients - Needs to be high enough to support clinical doses

properties of lipid excipients	- Fatty acid chain length, degree of unsaturation, degree of esterification (mono-, di-, or triglyceride), etc.
	- Would affect lipid digestion and compound solubilization
	- Effect of chain length:
	  - medium chain glycerides: complete digestion, hydrophilic digestion products, good for less lipophilic compounds
	  - long chain glycerides: incomplete digestion, lipophilic digestion products, good for highly lipophilic compounds

amount and lipophilicity of surfactants	- Amount: formation of gel-like structures and drug sequestration in micelles at high surfactant levels (>50%) - Lipophilicity: hydrophilic surfactants (HLB > 10) provide finer emulsion droplets
excipient digestibility in GI tract	- May affect drug distribution and solubilization after lipid digestion
solvent miscibility	- Excipient mixture needs to be mutually soluble
excipient morphology at room temperature	- Solid vs liquid, melting point of solid excipients - Need to consider feasibility for dosing in preclinical species and practicality in handling during in vitro tests
safety and tolerability	- Need to be acceptable for preclinical toxicology studies

4. IN VITRO TESTS OF LIPID-BASED FORMULATIONS IN DRUG DISCOVERY

When a lipid formulation is introduced into an aqueous medium such as gastric or intestinal fluids, the incorporated drug would undergo complex partitioning processes. The drug may remain in the lipid, be solubilized in the aqueous phase, or precipitate as the insoluble drug substance. Small scale (<1 mL) in vitro tests can be used to assess the drug partitioning process in the GI tract and help formulators to evaluate various factors that control the fate of the drug after oral administration of the lipid formulation.

4.1. In Vitro Dispersion Test. Following oral administration, the lipid formulation is dispersed in the stomach and an emulsion is formed upon dilution with gastric fluid. During this process the coadministered drug needs to remain solubilized for long enough time to allow gastric emptying and subsequent transferring into small intestine for absorption.

The in vitro dispersion test offers a quick assessment of lipid emulsification and drug solubilization in the stomach. It can be used as the first-tier assay for screening lipid formulations at early stages of drug discovery when resources and compound supply are limited. It is conducted by diluting the lipid formulation in water or simulated gastric fluid at different dilution ratios. At predetermined time points, the dispersion is examined visually for the formation of an emulsion or microemulsion and the particle size of the oil droplets can be measured by laser light diffraction or photon correlation spectroscopy. The drug solubilization can be quantified by HPLC analysis of drug concentration in the aqueous phase.[28,39,40]

The in vitro dispersion test has been used in the literature to generate the phase diagram and to define the self-emulsification zone in which a microemulsion is formed spontaneously in water.[38,41,42] However, it is not practical in discovery to perform a full phase diagram, since it is time and resource consuming and, as indicated in section 6.2, requires careful interpretation regarding its relevance to in vivo data.

4.2. In Vitro Lipid Digestion Test. As the lipid formulation moves from stomach to small intestine following oral administration, the triglyceride (TG) lipids are digested into diglycerides (DGs), monoglycerides (MGs), and fatty acids (FAs). These lipid digestion products are subsequently solubilized into bile salts/phospholipids to form a series of colloidal structures. The formation of these colloidal species significantly expands solubilization capacity within the small intestine and therefore increases effective drug concentration for intestinal absorption.[5,40,43]

The in vitro digestion test is a useful tool for assessing solubilization and absorption potential of a lipid formulation in the small intestine.[7,8,33,34,43,44] It helps formulation scientists to better understand the efficiency of drug transfer from oil phase to aqueous phase, the amount of drug available in aqueous phase for absorption, and the precipitation potential of the compound in the GI tract. To conduct the test, the lipid formulation is first dispersed in a digestion buffer containing bile salts and phospholipids, followed by addition of pancreatic lipase and co-lipase to initiate the digestion. At a predetermined time, samples are collected and ultracentrifuged into a poorly dispersed oil phase (containing undigested TG and DG), a highly dispersed aqueous phase (containing solubilized drug as well as bile salts, MG, and FA) and a precipitated pellet phase (containing precipitated drug and undissolved bile salts). The drug concentration in each phase can then be quantified by HPLC analysis.[40]

Lipid digestion is a complex process in which multiple phases and various micellar species are formed in the GI tract.[43,45−50] Table 3 is a list of factors that may affect the lipid digestion process and the colloidal species formed after digestion. These factors need to be considered when designing the in vitro test and interpreting the digestion data and their relevance to drug delivery. For example, the nature of excipients plays an important role in lipid digestion, while the physicochemical properties of the compound dictate drug partitioning and solubilization in postdigested colloidal phases. Specifically, the medium-chain triglycerides (MCT) are more readily and

Table 3. Factors Affecting Digestion of Lipid-Based Formulations

factor	explanation
properties of lipid excipients	- Fatty acid chain length, type of surfactants, lipid class - Effect on types of colloidal species formed after digestion and their solubilization capacity - Effect on the extent of digestion (complete vs incomplete)
physicochemical properties of the drug	- log *P*, aqueous solubility and solubility in lipid excipients - Effect on drug partitioning in different colloidal phases
dilution factor	- Effect on types of colloidal species formed after digestion in different locations of GI tract
concentration of the drug (drug load)	- Depends on lipid solubility, potential precipitation at high drug load
amount of lipid in the formulation (lipid load)	- High lipid load facilitates formation of highly solubilizing colloidal phases and promotes efficient absorption - Potential tolerability and safety issues - Potential overestimation in preclinical studies with high lipid load
level of bile salts and phospholipids in digestion medium/GI tract	- Fasted vs fed state, food effect
volume of digestion medium	- Effect on dilution factor and the types of colloidal species formed - In vitro test ideally to mimic the volume of GI fluid in the preclinical species
duration for digestion	- Effect on the extent of digestion (complete vs incomplete)

completely digested than the long-chain triglycerides (LCT), and the digestion products of MCT are more hydrophilic than those of LCT. As a result, the less lipophilic compounds tend to favor aqueous phases containing hydrophilic digestion products of MCT,[33,34] while highly lipophilic compounds would readily partition into mixed micelles containing lipophilic digestion products of LCT or remain in undigested LCT oil phase.[7,8]

The dilution factor also plays a crucial role in the nature of colloidal species formed after digestion. At low dilution liquid crystals can be present at the surface of digesting lipid droplets, while additional dilution results in a phase change to large (multilamellar) vesicles. Upon further dilution, mixed micelles and unilamellar vesicles become dominant in the environment adjacent to the absorptive surface of enterocytes.[5,42] As these changes occur, the coadministered drug would migrate from the "oil-rich" lipid formulation to the increasingly "water-rich" intestinal colloidal species for effective absorption. The fate of the drug is dependent on its dilution in the GI tract and its partitioning to each colloidal species.

5. IN VIVO STUDIES OF LIPID-BASED FORMULATIONS IN DRUG DISCOVERY

5.1. Dosing Lipid Formulations in Preclinical Animal Species. When dosing lipid formulations to preclinical animal species in drug discovery, it is important to consider various factors such as animal models, dose volume, total lipid intake, administration method (capsule vs gavage dosing), and safety and tolerability of the lipid excipients.

5.1.1. Animal Physiology and Its Relevance to in Vivo Studies of Lipid Formulations. Physiology of preclinical animal species plays an important role in in vivo evaluation of lipid-based formulations. The lipid formulations are more prone to species dependence than are the cosolvent formulations because of the fact that physiological differences of animal models can affect lipid emulsification and digestion processes.[16,21,51,52] For example, the amount of gastrointestinal fluid in the rat may be insufficient to emulsify the administered lipids, resulting in formation of gel-like viscous structures and poor drug absorption.[5,42] It is therefore recommended to predisperse the lipid formulation with water for in vivo studies in rats. In addition, the bile secretion in the rat is continuous and not pulsatile as in other species because of the lack of gallbladder in rats, so simple bile salt micelles are constantly present in rat intestine. The bile acids composition also varies in different species, and the bile is substantially more diluted in rats than in other species that have gallbladder to release bile in response to food or lipids.[53] These factors would affect lipid digestion patterns in different animal models, which in turn may affect the solubilization capacity of the GI tract and drug absorption from lipid vehicles.

It is generally accepted that large species such as dogs are better animal models than rodents for in vivo evaluation of lipid formulations. However, because of the limited animal resources and compound supply at early stages of drug discovery, the rat model is often the only option for in vivo screening of discovery compounds. With a good understanding of the unique rodent physiology mentioned above and a careful study design (e.g., reduction of dose volume, predilution of the formulation, etc.), the rat model would still be a valuable tool for initial screening of prototype formulations and identifying potential for a lipid-based strategy. At later stages of discovery, the optimized formulation can be designed and further tested in large animal species to assess its oral absorption and feasibility for clinical studies.

5.1.2. Dose Volume and Lipid Load. In preclinical studies, the dose volume is typically >1 mL/kg and the total amount of lipid could be >1 g/kg, considerably higher than that used in humans. While high lipid load can facilitate the formation of highly solubilizing colloidal phases and promote efficient absorption in preclinical studies, it may potentially overestimate the performance of the formulation in humans when dosed at much lower lipid levels. Predilution of the drug concentrate in lipids with water can reduce the total lipid intake as well as facilitate adequate emulsification especially for the rat model. Alternatively, capsule dosing can be used to reduce the dose

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volume and the lipid load to better mimic clinical situations, although this may be limited only to large animal species.

5.1.3. In Vivo Studies with Solid or Semisolid Formulations. When solid or semisolid excipients are used in the formulation, gavage dosing at room temperature could be quite challenging because of the potential of quick solidification of the excipients. For large animal species, this can be overcome by filling the molten lipid formulation into hard-gelatin capsules and dosing together with small amount of water to facilitate dispersion and emulsification. For rodent studies, gavage dosing may be the only option and special procedures need to be applied to ensure no solidification during administration. These procedures include keeping the dosing formulation at 5 °C above the melting point, prewarming the syringes and cannulae, and dosing the animals as quickly as possible. While certainly feasible, it is not very practical for use of such solid formulations in the rat model especially when repeat dosing is needed.

5.1.4. Enablement of Toxicology Studies in Preclinical Species. Exploratory toxicology studies in preclinical species are usually initiated in late stages of discovery before candidate nomination. The goal of these studies is to assess tolerability of the drug candidate and identify key adverse effects, as well as to evaluate toxicokinetics of the compound and establish the safety window. To enable the studies, high doses (300−500 mg/kg or higher) are often required, preferably in solution formulations, and the exposure needs to be maximized to achieve adequate safety multiples.

For highly lipophilic drug candidates, lipid-based formulations could be very useful for exploratory toxicology studies. However, it needs to be kept in mind that high solubility (>100 mg/mL) is required in lipid excipients to accommodate the high doses. And as a result, there is a potential for drug precipition in the GI tract and solubility-limited absorption, leading to less than dose-proportional increase in exposure at high doses. In addition, the predilution for dosing in rodents will be very challenging (since it requires even higher solubility) and there is a potential for inadequate emulsification and drug sequestration in rats. To enable the discovery toxicology studies, it is recommended that TK/formulation assessment be conducted at elevated doses (e.g., 100 mg/kg) in the relevant animal species and that selection of the appropriate formulation be based on exposure and tolerability.

The safety and tolerability of the lipid excipients also need to be carefully assessed in preclinical species.[54,55] It needs to be noted that while a lipid excipient may be perfectly acceptable in humans, in fact consistent with levels in diet, the amount required for toxicology studies may potentially cause adverse effects. While some of the GI effects such as emesis and soft stools have been reported, there is still lack of complete toxicology profiles for many lipid excipients, especially at high dose volumes (high lipid load) and when combined with cosolvents and surfactants. It is therefore recommended to conduct vehicle tolerability studies prior to the exploratory toxicology studies of the drug candidate.

5.2. Factors Affecting Drug Absorption from Lipid Formulations. *5.2.1. Lipid Digestion.* Following lipid digestion, the digestion products (monoglycerides and fatty acids) and coadministered lipophilic drugs are solubilized into various micellar species present in the intestinal lumen. This significantly enhances the mass transfer of the drug across the unstirred water layer to the brush border membrane of enterocytes. From there the drug molecule may dissociate from micellar phases and get absorbed across the apical membrane by passive diffusion or carrier-mediated transport.[5,56,57]

5.2.2. Potential Effect on P-Glycoprotein and Other Transporters. The potential for lipid excipients to attenuate the activity of efflux transporters such as P-glycoproteins (P-gp) has been of considerable interest in recent years. A number of ethoxylated lipids and surfactants such as Gelucire, Labrasol, and Cremophor have been shown to inhibit P-gp modulated drug efflux in various in vitro studies.[58−60] However, it is oftentimes hard to compare the data from different laboratories with different P-gp substrates, various cell lines used in the in vitro systems, and different types and concentrations of excipients tested.

More importantly, there is lack of sufficient evidence for the inhibition of P-gp by lipid excipients in in vivo studies. For example, Cornaire et al. reported that while a series of lipid excipients were found to enhance absorption of digoxin and celiprolol in vitro in the everted gut sac model, there was no increase in overall AUC of either compound when dosed in lipid and surfactant formulations to rats.[59] In studies with paclitaxel, while significant increase in exposure was found when cyclosporine A, a known P-gp inhibitor, was coadministered, the SMEDDS formulations showed only modest differences in PK parameters in rats.[6,61] The lack of in vivo effect could be due to the dilution of the excipients by the GI fluids or lipid digestion in the intestinal lumen prior to the absorption at enterocytes.

To assess the direct P-gp effect of an excipient, a careful design of the in vivo study is essential to rule out other possible contributing factors such as drug solubilization by lipid excipients, membrane permeation at high levels of surfactants, or inhibition of metabolism.[58,62] In particular, most P-gp substrates studied in the literature had low aqueous solubility, and in many cases it was most likely the solubilization by lipid excipients that ultimately improved the oral exposure of highly lipophilic, poorly water-soluble compounds. In a carefully designed clinical study by Bogman et al., the authors selected a P-gp substrate (talinolol) with good solubility and low affinity to CYP3A4 and tested the excipient (TPGS) at a level close to the IC_{50} for P-gp inhibition in vitro. However, only marginal increase in exposure of talinolol was found in the presence of TPGS, despite significant P-gp inhibition by TPGS in the Caco-2 cells in this study and many other in vitro systems in the literature.[59,60,62] It is apparent from these studies that the effects of lipids/surfactants on P-gp mediated efflux transport are complex with multiple mechanisms being involved.

5.2.3. Lymphatic Transport. The intestinal lymphatic system is a unique transport pathway for dietary lipids.[63] It also provides a route for target delivery of lipophilic compounds to lymphatic systems or systemic delivery for drug molecules to bypass hepatic first-pass metabolism.[64−69] Lymphatic transport occurs when lipid digestion products are resynthesized to triglycerides and assembled into lipoproteins (primarily chylomicrons) in the enterocytes. The lipoproteins are then exocytosed from enterocytes into interstitial space where they are preferentially transported across the highly permeable lymphatic endothelium rather than the portal blood capillaries with tight junctions.[70−72]

The intestinal lymphatic transport is dependent on physicochemical properties of the compound as well as the nature of lipid excipients. Highly lipophilic (e.g., log P > 5) compounds with high solubility in triglycerides (e.g., >50 mg/mL) are likely to be involved in intestinal lymphatic transport.[73,74]

Long-chain fatty acids are more prone to lymphatic transport than medium-chain fatty acids which are more water-soluble and readily absorbed by the portal blood.[65,75] The rate and extent of lymphatic transport is more profound in free fatty acids than in triglycerides which need to be hydrolyzed to free acids prior to lipid absorption.[70,76] In addition, the presence of food has been found to enhance lymphatic transport of lipophilic drugs.[77]

In recent years, lymph-directing lipid prodrugs have been reported to improve oral absorption of lipophilic compounds.[78] The lipid moiety (e.g., fatty acids, diglycerides, or phosphoglycerides) of the prodrug enables the incorporation of the molecule into the triglyceride resynthesis pathway within the enterocytes and therefore drives toward chylomicron production and lymphatic transport. For example, it has been shown that the lipophilic long-chain ester prodrug of testosterone is transported via intestinal lymphatic pathway and facilitates oral absorption of testosterone which would otherwise be limited by significant first-pass metabolism.[79]

It needs to be noted that the extent of intestinal lymphatic transport and its contribution to overall systemic absorption are compound- and excipient-dependent and may be rather limited in some cases. For example, while 28−54% of halofantrine has been found to be transported via intestinal lymphatic system in lipid formulations or with food, the lymphatic transport has been accounted for <10% of the total bioavailability for other lipophilic compounds such as saquinavir (0.3−1.3%),[68] seocalcitol (7.4%),[67] amphotericin B (7−10%),[69] and ontazolast (10%).[64] In a study with halofantrine, although the lymphatic transport was 3- to 5-fold higher from a long-chain lipid formulation than that from the medium-chain formulation, the overall absorption of the two formulations was not statistically different in a rat model.[75]

5.2.4. Food Effect. High fat meals can significantly enhance biliary secretion of bile salts and phospholipids, leading to an increase in solubilization capacity and drug absorption in the GI tract. The presence of food has also been found to alter the pharmacokinetic profile of the lipid-soluble compounds in which a large secondary peak may be present in the concentration−time profile because of the resolubilization of the remaining drug in the GI tract by postdose food intake.[7,8,80]

The food effect can be mitigated by well designed lipid-based formulations.[23,81] In a study with torcetrapib, it has been demonstrated that a self-emulsifying formulation in a mixture of MCT and surfactant/cosolvent is able to increase drug exposure at fasted state and thus reduce the food effect that has been observed in a simple MCT soft gel formulation.[23]

It needs to be noted that the in vivo evaluation of lipid formulations in discovery is usually conducted in fasted state in most preclinical models, and precautions are therefore needed in interpreting preclinical data and transferring to clinical development. If resource and time permits, the assessment of food effect can be initiated prior to candidate nomination, thus providing guidance for further evaluation in the development.

6. IN VITRO AND IN VIVO CORRELATION/DISCONNECT

6.1. Where Is the Drug after Oral Administration of a Lipid Formulation? To assess drug absorption from a lipid formulation, it is important to first understand the fate of the drug in the GI tract following oral administration. As lipid excipients are dispersed, digested, and intercalated into endogenous bile salts and phospholipids, the coadministered drug is distributed across various colloidal phases present in the intestinal lumen. Depending on its relative affinity for each colloidal phase, the lipid soluble drug would (1) be solubilized potentially as a supersaturated solution in the aqueous phase, (2) remain in the undigested oil phase, or (3) precipitate from the supersaturated solution. The drug distribution pattern is determined by the lipophilicity of the compound, the nature of the excipients, and other formulation factors listed in Table 3.[40,43,49,50]

6.2. In Vitro and in Vivo Correlation: What Matters the Most, Particle Size or Crash Resistance? The in vitro and in vivo correlation for lipid formulations depends on many factors including the properties of the compound and the excipients as well as the different types of in vitro assays. While good correlation has been reported between the particle size and the in vivo exposure in some studies,[82,83] an increasing body of evidence has indicated that the in vivo performance of a lipid formulation is poorly correlated to the physical state of the initial dispersion (i.e., particle size of the emulsion or formation of microemulsion zone in the phase diagram).[5,7,8,33−35,44] Rather, the drug absorption from a lipid formulation depends more on the solubilization/crash resistance capacity of the colloidal species formed postdigestion. This is probably because the initial lipid dispersion remains in the stomach for only a short period of time and would ultimately be altered upon lipid digestion in the small intestine. The key to successful delivery of a lipid formulation and effective absorption of the lipophilic drug is to keep the drug solubilized in the GI tract and prevent drug precipitation when transferring from oil phase to aqueous phase and ultimately to membrane.[7,8,44]

In a study by Porter and colleagues, while excellent dispersions with similar particle size of 40 nm were found in both long-chain and medium-chain lipid formulations of danazol, the in vivo exposure of the medium-chain formulation was significantly lower than that of the long-chain formulation.[8] It was suggested by the authors that the in vivo data corresponded well to a 69% of drug precipitation in the pellet phase for the medium-chain formulation vs 6% for the long-chain formulation in an in vitro digestion test.[8] In another study with atovaquone, while digestion test showed different drug distribution pattern for a LCT solution (30% in aqueous phase, 67% in oil phase) vs a LCT-surfactant formulation (97% in aqueous phase, no oil phase), the in vivo absorption was essentially the same for the two formulations. It was likely that the minimal drug precipitation in both formulations (~3% in pellet phase) played a crucial role in facilitating effective drug absorption.[44] It is also interesting to note that although digestion is incomplete in the case of the LCT solution and $^{2}/_{3}$ of the drug remains in the oil phase, the subsequent slow delivery to the aqueous phase may indeed help to prevent drug precipitation and ultimately improve drug absorption.

Drug precipitation upon lipid digestion is indeed one of the main reasons for the failure of lipid formulations, and maintaining the drug in a supersaturated, solubilized state in the GI tract is crucial for effective drug absorption. Drug precipitation can be minimized via formulation optimization such as adding cosolvents and surfactants to enhance solubility or choosing appropriate lipids based on the physicochemical properties of the compound (e.g., MCT for less lipophilic compounds and LCT for highly lipophilic compounds). Drug precipitation can also be prevented by using crash resistant polymers in lipid systems. Gao et al. showed that coadministration of polymers

such as hydroxypropyl methylcellulose (HPMC) can reduce the rate of crystallization and maintain the drug in a supersaturated state long enough for effective absorption.[61,83,84]

7. FEASIBILITY ASSESSMENT FOR POTENTIAL CLINICAL FORMULATIONS

When a lipid formulation is assessed in early discovery, it is important to keep in mind its feasibility for clinical use and its developability as a commercial dosage form. While achievement of adequate oral exposure is the main focus in discovery, other factors such as drug load, total lipid intake, formulation stability, and patient compliance (number of capsules to take) need to be considered for the development of a clinically relevant lipid formulation that can deliver the required clinical dose in a feasible dosage form.

As discussed in previous sections, a successful transition of a preclinical lipid formulation to a viable clinical dosage form relies on early establishment of lipid formulation strategy, SAR, and lead optimization for a candidate with required physicochemical properties for lipid formulations, as well as good design of in vitro and in vivo studies in drug discovery. In this way, the preclinical evaluation and clinical development goals are connected early in the process, which will significantly reduce the formulation development time and increase the overall success potential of a clinically viable formulation.

8. SUMMARY

Highly lipophilic, poorly water-soluble compounds are often subject to poor oral absorption and low bioavailability and present significant challenge in formulation development. Lipid-based formulations provide a viable option for enhancing oral absorption and bioavailability of lipophilic compounds. However, lipid formulations have not been widely used in drug discovery because of the complex nature of lipid systems as well as the lack of formulation tools and overall formulation strategy in discovery programs. This review summarizes various aspects in applying lipid-based formulations in drug discovery, with a goal to help discovery scientists to improve understanding of lipid excipients and apply lipid-based formulations in preclinical studies.

It needs to be kept in mind that while lipid-based delivery has proven to be a viable option for lipophilic compounds, there are limitations associated with lipid formulations and progression into a commercial setting. Compounds with low lipid solubility and high therapeutic dose would encounter problems such as low drug loading, high lipid intake, and poor patient compliance (with multiple capsules to take) and are often not suitable for lipid-based delivery. In addition, the chemical and physical stability over long-term storage, often in liquid or semisolid medium, could be quite challenging with respect to both the inherent stability of the drug and the potential interactions between the drug and the excipients. The tendency for greater batch-to-batch variability in many of the commonly used excipients only complicates matters. Of particular importance can be the change in solubility that comes with the appearance of a new physical form, hence making the characterization of solid forms critical for moving into a development setting. Early anticipation and evaluation of these limiting factors are essential to assessing the feasibility of the lipid formulations as a viable strategy for any program. Once the lipid formulation strategy is targeted, the potential exists for improvement of drug properties via SAR and lead optimization to further enable development of successful clinical formulations where the properties of the molecule end up being tailored to the delivery approach.

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ABBREVIATIONS USED

GI, gastrointestinal; SEDDS, self-emulsifying drug delivery systems; SMEDDS, self-microemulsifying drug delivery systems; P-gp, P-glycoprotein; API, active pharmaceutical ingredient; SAR, structure−activity relationship; MCT, medium-chain triglyceride; LCT, long-chain triglyceride; HLB, hydrophilic−lipophilic balance; BS/PL, bile salt/phospholipid; TG, triglyceride; DG, diglyceride; MG, monoglyceride; FA, fatty acid

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