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Lipid based formulations as supersaturating oral delivery systems: From current to future industrial applications

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ABSTRACT

Lipid-based formulations, in particular supersaturated lipid-based formulations, are important delivery approaches when formulating challenging compounds, as especially low water-soluble compounds profit from delivery in a pre-dissolved state. In this article, the classification of lipid-based formulation is described, followed by a detailed discussion of different supersaturated lipid-based formulations and the recent advances reported in the literature. The supersaturated lipid-based formulations discussed include both the *in situ* forming supersaturated systems as well as the thermally induced supersaturated lipid-based formulations. The *in situ* forming drug supersaturation by lipid-based formulations has been widely employed and numerous clinically available products are on the market. There are some scientific gaps in the field, but in general there is a good understanding of the mechanisms driving the success of these systems. For thermally induced supersaturation, the technology is not yet fully understood and developed, hence more research is required in this field to explore the formulations beyond preclinical studies and initial clinical trials.

1. Introduction

It is scientifically clear that poor aqueous solubility of active pharmaceutical ingredients represents a significant risk factor for low oral bioavailability, yet increasingly lead drug candidates in drug discovery exhibit low aqueous solubility and a high hydrophobicity or lipophilicity. This has been driven by multiple factors, including how modern drug discovery technologies identify lead compounds (Williams et al., 2013a; Carrière, 2016), but also as a reflection of the targets selected. Use of large chemical libraries based on combinatorial chemistry or application of high throughput screening methodologies, often in non-aqueous media, have played an important role in this trend. Moreover, advances in potency increase of drug candidates based on their hydrophobic interactions with the target or exploration of novel drug targets involved in intracellular signaling pathways or lipid processing mechanisms, further determined the need for highly lipophilic, poorly water-soluble drugs (O'Driscoll and Griffin, 2008). The hydrophobicity – lipophilicity distinction has recently been reviewed by Ditzinger and co-workers (2018) with a focus on which physicochemical determinants that can be used to guide a more structured and scientifically-based formulation development of such poorly water-soluble compounds for oral delivery (Ditzinger et al., 2018).

As oral administration is preferred by most patients, an adequate oral bioavailability is a critical requirement for many drug development programs to achieve the projects target product profile. Therefore, pharmaceutical scientists in both industry and academia focus their collective research efforts on various formulation approaches to achieve the best possible oral absorption of poorly water-soluble compounds,

Abbreviation list: AUC, area under the plasma curve; DMSO, dimethylsulfoxid; FaSSIF, fasted state simulated intestinal fluid; FDA, Food and Drug Administration; GRAS, generally recognized as safe; HLB, hydrophile-lipophile balance; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; HPMC-AS, hydroxypropyl methylcellulose acetate succinate; HPMCP, hydroxypropyl methylcellulose phthalate; LCT, long-chain triglyceride; MC, methyl cellulose; MCC, microcrystalline cellulose; MCT, medium-chain triglyceride; Na-CMC, sodium carboxymethyl cellulose; PEG, polyethylene glycol; PPGAE, poly(propylene glycol) bis(2-aminopropyl ether); PVP, polyvinylpyrrolidone; PVP/VA, polyvinylpyrrolidone vinyl acetate; SEDDS, self-emulsifying drug delivery systems; SDS, sodium dodecyl sulphate; SMEDDS, self-microemulsifying drug delivery systems; SNEDDS, self-nanoemulsifying drug delivery system.

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using bio-enhancing or bioavailability enabling technologies. Standard approaches aimed at enhancing the oral bioavailability of poorly water-soluble compounds include salt formation, pH adjustment and particle size reduction, however, there are limits for the ability of technologies to enhance oral absorption of very low solubility drugs when integrated into of conventional solid formulations. The most challenging drug candidates therefore require enabling formulations such as either lipid-based drug delivery systems or amorphous solid dispersions (Buckley et al., 2013; Boyd et al., 2019).

Lipid-based drug delivery systems have been used to improve oral drug absorption for many years and the technology has a well-documented scientific, regulatory, and commercial path. As early as the 1950s, lipid suspensions and emulsion formulations of sulfonamides were investigated (Feeney et al., 2016). The field of lipid-based formulations truly flourished after the launch of Sandimmune® in 1983 and the subsequently improved and highly innovative formulation Neoral® in 1994. The Sandimmune® formulation was a self-emulsifying drug delivery system (SMEDDS) (consisting of mixture of olive oil, polyoxyethylated oleic glycerides and ethanol) that provided a crude emulsion with a droplet size in the μm range. In contrast, Neoral® consisted of corn oil mono- and di-glycerides, polyoxyl 40 hydrogenated castor oil, glycerol and propylene glycerol and upon dispersion, it produced a nano-emulsion, i.e., a self-nanoemulsifying drug delivery system (SNEDDS), which had multiple biopharmaceutical advantages over Sandimmune®, including a slightly increased drug absorption and substantially decreased *in vivo* variability (Feeney et al., 2016).

Poorly water-soluble compounds often have a positive food effect, i.e., showing an increase in oral bioavailability when taken with food (Feeney et al., 2016; Kuentz, 2011; O'Shea et al., 2019). Some of the early examples of poorly water-soluble compounds that benefited from co-administration with food include griseofulvin, danazol, halofantrine, atovaquone and troglitazone (Williams et al., 2013a). The food-induced increase in oral bioavailability were primarily due to lipids, which meant that co-administration with lipid-based excipients in formulations presented itself as a more controlled way of delivering these drugs (O'Driscoll and Griffin, 2008; Porter et al., 2007; Charman, 2000). Lipid-based drug delivery system can therefore mitigate a food-mediated variable oral bioavailability, while also harnessing the advantages of endogenous lipid processing pathways to enhance drug absorption (Williams et al., 2013a). A comprehensive overview of food effects on oral absorption and formulation strategies to overcome these effects has been recently reviewed elsewhere (O'Shea et al., 2019).

Lipid-based drug delivery systems for oral administration of poorly water-soluble compounds generally consist of a drug dissolved in a single component or a blend of two or more excipients, which may be triglycerides/oils, partially digested triglycerides (i.e. mono- and di-glycerides), lipophilic or hydrophilic surfactants, or cosolvents (Charman, 2000; Pouton, 2000). Classically, the pre-solubilization of the drug in the lipid vehicle was the assumed mechanism that promote oral bioavailability of challenging compounds, as the rate limiting dissolution of crystalline drug was thereby circumvented (Charman, 2000). In terms of predicting which classes of drugs that can be dissolved in lipids, "grease ball" molecules have been considered as good candidates for such lipid-based formulations because of their dominant lipophilic characteristics and relatively weak crystal lattice energy, which both tend to lead to improved solubility in a lipid vehicle (Mu et al., 2013). In contrast the solubility of "brick dust" molecules in lipids are often limited by a high crystal lattice energy (Ditzinger et al., 2018). Drugs with a melting point (T_m) $< 150^{\circ}$C have shown a reasonable solubility in glycerides, whereas a trend towards reduced solubility in lipid excipients with increased T_m values has been reported (Ditzinger et al., 2018). However, melting point is only one characteristic of the crystal lattice and a more refined consideration of other solid-state properties (i.e., enthalpy of fusion ΔH_f and entropy of fusion ΔS_f) has been recommended (Ditzinger et al., 2018). As a descriptor of lipophilicity, log P is often considered for grease ball compounds, whereas the mentioned solid-properties can be used to characterize brick dust molecules. Of the 36 lipid-based drug delivery systems (with 26 different drug compounds) approved by the Food and Drug Administration (FDA) by 2018, the range of log P is 0.8–7.5 with a median of 4.9. Among formulators, a rule of thumb exists in that a drug with a log P > 4 will be a good candidate for a lipid-based drug delivery system with high compositions of glycerides, while values between 2–4 may reflect a suitability for mixtures containing glycerides, surfactants and cosolvents (Ditzinger et al., 2018).

While lipid-based formulations have a proven track record, scientifically, clinically, and from a regulatory perspective, there are multiple cases where it has been difficult to dissolve the entire dose into the defined liquid fill-volumes of pharmaceutical capsules. With the success of amorphous solid dispersions, a hybrid between a lipid-based drug delivery system and an early generation solid dispersion has been described in the literature, whereby a polymeric precipitation inhibitor was incorporated withing the lipid-based system (Gao et al., 2003; Gao and Morozowich, 2006). The authors called their system supersaturable and use of the dispersed polymer addressed the *in situ* generated drug supersaturation to avoid or reduce undesirable drug precipitation following dispersion within the gastrointestinal fluids. This approach has demonstrated positive results with respect to oral bioavailability and the literature in this field has grown significantly (Park et al., 2020). From supersaturable formulations, it was a next step to go beyond thermodynamically stable mixtures to thermally induce drug supersaturation in the formulation itself and a review from 2015 (Joshi and Sangamwar, 2015) addressed this type of lipid-based approach, though the review included a very limited discussion on thermally induced supersaturated lipid-based formulations and important work has been published in the meantime. The present work therefore operates with two terms to generate a distinction between the types of formulations; 'supersaturated drug delivery systems (i.e., drug is present at concentrations above the thermodynamic solubility in the formulation induced by heating the formulation) and the 'supersaturating (or supersaturable) drug delivery systems' which are designed to generate transient drug supersaturation after dispersion/dissolution in gastrointestinal fluids. The present review will provide a full review of the published work with critical insights into the field of supersaturated lipid-based formulations regarding the different ways of how drug supersaturation can be generated, the physical stability, the biopharmaceutical learnings as well as a critical evaluation of potential applications in a pharmaceutical context for research, development, and commercial purposes as a supplement to the important scientific discussion in the field.

2. Types of lipid-based drug delivery systems

Drug absorption from lipid-based formulations is an interesting science that is both complex, dynamic, and highly affected by the digestion of many of the excipients used in lipid-based formulations. Insights into digestion biochemistry, lipid trafficking in the body including mixed micelles and intestinal lymphatic transport, are therefore critical to better understand the intestinal fate of a lipid-based formulation (Feeney et al., 2016; Mu et al., 2013). A detailed review of these topics is beyond scope of the current review, but the interested reader is referred to other reviews in the literature (e.g., Williams et al., 2013a; Feeney et al., 2016).

The *in vivo* performance of a lipid-based system may easily be affected by the composition of the formulation. Hence, a basic understanding is needed, as several critical aspects must be considered for the successful development of novel lipid-based formulations. These aspects include the physicochemical properties of the drug, the formulation type and its composition, as well as the physiological and biochemical mechanisms related to processing of the lipid-based formulation after oral intake. This includes the gastrointestinal physiology and composition of the liquid in the gastrointestinal tract. Finally, it is important to understand if drug precipitation may have a negative effect on the

biopharmaceutical performance of the formulations upon dispersion and/or digestion in the gastrointestinal tract. These different aspects of lipid-based formulations will be described further below.

2.1. The lipid formulation classification system

Lipid-based formulations are a diverse class of formulation systems, including solutions, emulsions, micellar systems, self-emulsifying drug delivery systems (SEDDS), and SMEDDS. To better compare different compositions, Pouton (2000; 2006) suggested a lipid classification system in which different formulations are classified into four main categories, as shown in Table 1.

Pouton's classification is meaningful in that the different types of lipid-based compositions also come with alternative formulation characteristics that have biopharmaceutical relevance (see Table 2). This categorization is helpful for formulators, not only from an academic perspective but also to identify the most relevant *in vitro* characterization in formulation development. Here, a brief introduction to the different classes of lipid-based formulations will be provided. However, it should be mentioned, that while Pouton's lipid-based classification system has been widely employed by scientists in the field, there have also been discussions of potential limitations and other conceptual considerations that should be kept in mind when using the system (Holm, 2019).

The type I is the simplest among the lipid-based formulations from a composition perspective as it only contains oils (triglycerides or mixtures of di- and monoglycerides). These lipid excipients must be digested in the intestine to facilitate the drug absorption process. Thus, digestion will transform the excipients into free fatty acids and monoglycerides, which will be incorporated into intestinal mixed micelles. Type I formulations are typically biocompatible and simple, containing excipients that are generally recognized as safe (GRAS). In general, lipid digestion does not lead to the loss of solubilization capacity after dispersion or digestion. Therefore, the drug would typically not undergo a phase of supersaturation within the intestine as a result of the bioconversion of the formulation.

Type II lipid-based formulations are composed of a mixture of lipids and water-insoluble surfactants with a low hydrophile-lipophile balance (HLB) number (HLB<12). These formulations may self-emulsify into crude oil-in-water (o/w) emulsions when they come into contact with gastrointestinal fluids. Pancreatic lipase may, therefore, digest the type II systems faster than the type I formulations due to the larger surface area. Since oil remains the main component in the formulation, digestion is important for the *in vivo* performance of the formulation. Depending on the surfactant used, there is a risk of a loss of drug solvation capacity after digestion. This could result in intestinal supersaturation, which may either drive an increased absorption or unfavorable crystallization and subsequent precipitation in the intestinal lumen (Williams et al., 2013a).

The two types of type III lipid-based formulations, class IIIA and IIIB, both contain water-soluble surfactant(s) with a high HLB number (HLB>12) as well as cosolvents. These formulations have the ability to self-emulsify spontaneously upon contact with intestinal fluids. They can either form SEDDS when the dispersion is a milky emulsion with a droplet size higher than approximately 200 nm, or as a SMEDDS when a transparent colloidal dispersion is formed in water. Some aspects of the biopharmaceutical difference between SEDDS and SMEDDS have already been mentioned earlier in this review, particularly in the discussion of the differences Sandimmune® and Neoral®. It should be noted that the term SMEDDS is often not differentiated in the literature from a self-nanoemulsifying drug delivery system (SNEDDS), which is understandable from a biopharmaceutical perspective even though some academic distinctions (Niederquell and Kuentz, 2013).

The type III formulation often contains higher amounts of cosolvents to facilitate the solubilization of the compound. However, this generates a risk of losing solvent capacity upon dispersion and digestion, as the cosolvents may rapidly migrate out of the formulation into the aqueous intestinal fluids. This, in turn, could lead to precipitation and drug crystal formation. Therefore, drug supersaturation is typically achieved with such formulations to create high concentration gradients for intestinal absorption. would be only biopharmaceutically advantageous in case of minimal to low drug precipitation.

Type IV systems do not contain oils and consist only of water-soluble surfactants, with optionally added cosolvents. When these formulations are dispersed in an aqueous medium, they form fine dispersions that can result in rapid drug release and absorption. The solvent capacity of these systems is often quickly lost upon dispersion, leading to pronounced drug supersaturation and an associated risk of precipitation in the gastrointestinal tract (Stillhart and Kuentz, 2016).

There are some lipid systems that do not readily align with the lipid formulation classification system, such as a recently reported type of mixture comprised drug, glycerides and fatty acid(s) (Wyttenbach et al., 2022). Fig. 1 displays components of such a mixture with the quantum-chemically calculated screening charge of surface segments (color-coded from blue to red with increasing values, i.e. blue being negative and red positive screening charge that is opposing the charge of electron density). In this context, the fatty acids are present here more as a co-former rather than a classical cosolvent, due to the strong and specific molecular interactions. Substantial solubility increase was observed for a range of drugs in this promising type of lipid-based mixtures.

Another type of lipid-based system that falls outside of the conventional lipid formulation classification system types are lipid-based suspensions, where the drug is present in crystalline form. This formulation type offers a scalable approach for mostly hydrophobic compounds, with the potential to enhance oral absorption via excipient-mediated effects on intestinal solubilization. Preclinical *in vivo* performance of several drugs, such as griseofulvin, atovaquone, phenytoin, diacerein, danazol, progesterone and fenofibrate was improved after oral administration as lipid suspensions (i.e., corn oil, sesame oil) relative to aqueous suspensions (Mu et al., 2013; Koehl et al., 2019). The lipid suspension studies that can be found in the literature have either been conducted with class I formulation (Mu et al., 2013) or with compositions closer to a class IV formulation (Larsen et al., 2008).

Table 1
Lipid formulation classification system, based upon Pouton (2006).

Type	Excipients in formulation (% w/w)			
	Oils:triglycerides or mixed mono and diglycerides	Water-insoluble surfactants (HLB[1] <12)	Water-soluble surfactants (HLB >12)	Hydrophilic cosolvents (e.g. PEG[2], glycerol, ethanol)
I	100	-	-	-
II	40-80	20-60	-	-
IIIA	40-80	-	20-40	0-40
IIIB	<20	-	20-50	20-50
IV	0	0-20	30-80	0-50

[1] HLB; hydrophile-lipophile balance.
[2] PEG; Polyethylene glycol.

3. Supersaturation of lipid-based formulations to increase drug loading

As mentioned in the introduction, it is important to distinguish between 'supersaturated drug delivery systems (where the drug is present at concentrations above thermodynamic solubility in the formulation) and the 'supersaturating drug delivery systems' which are designed to generate transient drug supersaturation after dispersion/dissolution in gastrointestinal fluids. The latter type of system has also been called a supersaturable formulation (Gao and Morozowich, 2006). Ideally, a supersaturating drug delivery system should generate and maintain drug supersaturation in the gastrointestinal fluids for a physiologically

Table 2
characteristics of different type of lipid classes classified by the lipid classification system (based upon Pouton (2006)).

Formulation type	Name	Typical composition	Significance of		Particle size of dispersion (nm)	Advantages	Disadvantages
			Dispersion/ Dilution	Digestion			
I	Oil(y) solutions	Oils (Triglycerides or blends of mono-, di-glycerides): 100%	Limited significance	Crucial importance	Coarse	GRAS status, simple, good capsule compatibility	Poor solvent capacity unless very lipophilic drug; slow digestion and slow "release" of the drug
II	Self- emulsifying drug delivery systems (SEDDS)	Oils - 40-80% and water-dispersible (HLB<12) surfactants: 20-60%	Unaffected solvent capacity	Likely to occur, essential	250-2000	Unlikely to lose solvent capacity *in vivo*	Turbid o/w dispersion: drug precipitation risk
IIIA	Self–emulsifying drug delivery systems (SEDDS)	Oils: 40-80% and water-soluble surfactant (HLB>12): 20-60%	Limited loss of solvent capacity	Likely to occur, important	100-250	(Almost) clear dispersion; drug absorption not dependent on digestion	Possible loss of solvent capacity; drug precipitation risk
IIIB	Self-miro or nano-emulsifying drug delivery systems (SNEDDS/SMEDDS)	Oils: 0-20%, water-soluble surfactant (HLB>12): 20-50%, hydrophilic cosolvents: 20-50%	Some loss of solvent capacity	Likely to occur, important	<100	(Almost) clear dispersion; drug absorption not dependent on digestion	Loss of solvent capacity, less easily digested, drug precipitation risk
IV	Oil-free systems	Water-soluble surfactants and cosolvents: 100%	Loss of solvent capacity	Unlikely to occur	<100	Good solvent capacity	Loss of solvent capacity, hardly digested, high drug precipitation risk

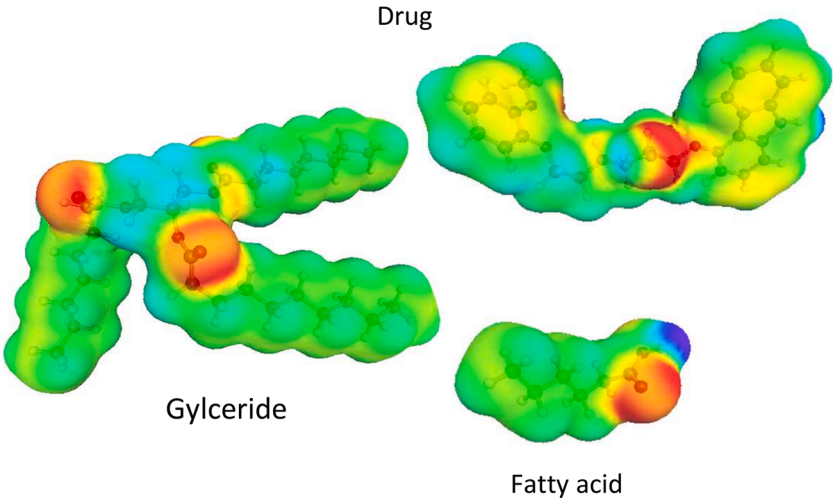


Fig. 1. Components of a lipid-based mixture are depicted with drug, fatty acid(s) and glycerides, which represents a type of system that does not fall into a classical category of lipid-based formulations. An example is shown for a medium chain triglyceride with the drug carvedilol and caproic acid. Quantum-chemically calculated screening charge densities suggest pronounced interactions between especially the drug base and the fatty acid. High electron density corresponds to a positive screening charge density, which is shown with highest positive values in red. This is related to Lewis basicity and the other end of the screening charge density is depicted in shades of blue according to how negative the values get, while green indicates a neutral charge.

relevant time, typically around 4 hours, which is equal to the upper gastrointestinal transit time) (Price et al., 2019). This concept is commonly referred to as the "spring and parachute" model (Guzman et al., 2007). A supersaturated solution or dispersion is typically generated from a higher energy form of the drug ("a spring") and is thermodynamically unstable, as mentioned previously. Lipid-based drug delivery systems, cosolvent systems, amorphous solid dispersions, nanoparticles, or co-crystals are considered spring generators and excipients like polymers, surfactants, and cyclodextrins are considered as precipitation inhibitors if they prolong the duration of supersaturation, which is also termed the "parachute" effect (Xu and Dai, 2013; Gao and Shi, 2012).

Specifically for the class III and IV in Pouton's lipid formulation classification system, a spring or a supersaturating delivery system is observed if a higher proportion of the formulation consists of organic cosolvents such as ethanol or low molecular weight polyethylene glycols (PEGs) that have a significant miscibility with aqueous media, as the intestinal fluids. These excipients are added to the formulation to enhance the solubility of the drug substance in the formulation, resulting in a simple solution is obtained. However, when these cosolvents come into contact with intestinal fluids, they quickly migrate into the aqueous phase, causing loses of dissolution potential in the lipid phase. Therefore, there is a risk that the compound may precipitate out of the oil phase in the worst case as crystalline material. As described later, there are simple formulation approaches to mitigate the negative impact of this mechanism on bioavailability.

The most frequently used approach for inducing supersaturation directly in lipid-based formulations is done thermally, i.e. by heating the lipids up to, for example, 60°C, saturating the system with the compound and subsequently colling it down to ambient temperature (Thomas et al., 2012; 2013; 2014; Michaelsen et al., 2016; 2019; Abo Enin and Abdel-Bar, 2016; Siqueira Jørgensen et al., 2018; Schultz et al., 2018; 2019; 2020; Meola et al., 2020; Koehl et al., 2020; Ilie et al., 2020a,b; 2021; Almasri et al., 2020). This approach takes advantage of increased solubilities at elevated temperatures. Thermally induced supersaturated lipid-based formulations offer an advantage in terms of the ability to administer highly concentrated drug solutions. Moreover, classical saturated lipid-based formulations, in some cases, do not offer a sufficient solubility in mostly preclinical dose escalation toxicity studies in which higher doses are administered compared to later clinical

dosage forms. Supersaturated lipid-based formulations can be easily manufactured without recourse to other advanced processing approaches, e.g. salt or co-crystal formation or drug amorphization in solid dispersions (Aungst, 2017; Ayad, 2015; Landis et al., 2018). Furthermore, when a safe dose range is defined in preclinical studies, first-in-human (FIH) clinical studies commonly start using similar 'simple' formulations such as solutions or suspension. Supersaturated lipid-based drug delivery systems are, therefore, highly suited to streamline the formulation process in early-stage drug development, allowing ease of administration as simple lipid solutions in rodent and non-rodent models, which can be readily scaled to clinical formulations such as liquid-filled capsules.

Supersaturated lipid-based formulations have a well-established use for topical and transdermal administration, with the advantage of increasing drug loading, especially for compounds that exhibit limited solubility in lipid vehicles (Cilurzo et al., 2015; Elkasabgy, 2014). More recently, supersaturated lipid-based formulations have increasingly become of interest for oral application, as described in detail below.

3.1. Degree of supersaturation obtainable in lipid-based formulations

The degree of supersaturation achievable in a formulation is important to know to define the drug loading and hence a realistic dose strength of the final dosage form. The chosen degree of supersaturation may influence the *in vivo* performance of the formulation but also the shelf-life of the product, which should not crystallize upon storage. For *in situ* supersaturation in the intestine, the exact degree of supersaturation that can be achieved is hard to define, as this will highly depend on the drug, the vehicle, as well as gastro-intestinal formulation processing. Therefore, it is comparatively simpler to study formulations that are supersaturated prior to their administration.

Thermal induction of supersaturation is the standard approach when inducing supersaturation in lipid-based formulations. Thomas et al. (2013) reported their supersaturated formulations with simvastatin to be stable for more than 10 months at 25°C in a sealed atmosphere systems at a 150% of the saturated solubility at the storage temperature. Schultz and coworkers (2018) investigated the solubility of ibuprofen in three different lipid vehicles at three different temperatures: ambient, 40, and 60°C. The solubility rank order observed at 25°C in the lipids did not change with increasing temperature – the increase in solubility versus temperature was log-linear and parallel for the investigated systems (Schultz et al. 2018). Almasri and coworkers (2020) investigated the solubility of fenofibrate in Capmul® PG8, Captex® 300, and Capmul® MCM at 25, 40, and 60°C (See Table 3 for a description of the lipid excipient tradenames). From the presented data, it was evident that the solubility of fenofibrate increased considerably with increasing temperature in all the tested vehicles. The rank order of the measured solubility in the three vehicles was constant across the three temperatures, although the extent of solubility increase was different for the vehicle. A 4.4- to 7.7-fold increase of fenofibrate's solubility was observed in the vehicles when comparing the solubility at 60°C to the solubility at 25°C (Almasri et al., 2020).

Ilie and coworkers (2020b) have reported investigations of saturated solubility of drug compounds in lipid vehicles at elevated temperatures. Ilie et al. (2020b) reported solubilities for three different compounds, celecoxib, cinnarizine, and JNJ-2A, in formulations of type I and II according to the lipid formulation classification system at 25, 37 and 60°C. Consistent with Schultz et al. (2018) and Almarsi et al. (2020), Ilie and coworkers (2020b) reported a constant rank order of solubilities in the vehicles across the investigated temperatures, as shown in Fig. 2. In agreement with the data from Schultz et al. (2018), Ilie and coworkers (2020b) also observed that the solubility increase did not deviate greatly from linearity. Therefore, both studies suggested that the solubility in lipid-based formulations at elevated temperature could be predicted based on simple extrapolation, at least for type I and II formulation. The possibility of extrapolating this observation to more complex systems also containing high HLB surfactants and/or cosolvents has, to the best of our knowledge, not been investigated. Furthermore, for the three drugs investigated by Ilie et al. (2020b), it was reported that the lines connecting the different data points for a lipid system were superimposable between the compositionally different lipid-based formulations, again in accordance with the data from Schultz et al. (2018). Collectively, this suggests a limited impact of the vehicle on the propensity to obtain drug supersaturation. In contrast, the slope of the lines representing the thermal-induced solubility increases was highly drug-dependent, meaning that some compounds exhibited a higher solubility enhancement as a function of the temperature than others.

For ibuprofen, a solubility enhancement of up to 230 % at 60°C was obtained when compared to the saturated solubility at 25°C (Schultz et al., 2018). Ilie and co-workers (2020b) reported average solubilities enhancements of 172.8%, 196.0% and 87.7% when comparing the solubility at 60°C with the solubility at ambient temperature for celecoxib, cinnarizine and JNJ-2A, respectively. Overall, this suggests that for classical lipophilic compounds, thermally induced supersaturation may potentially increase dose loading by up to 1.5 to 2.5-times higher than the saturated dose loading at ambient temperature. However, there will also be individual drug-specific exceptions that can either exceed this range or display lower solubility after exposure to elevated temperatures, such as the case of JNJ-2A, which inherently displayed a higher lipid solubility at ambient temperature (Ilie et al., 2020b).

Bennett-Lenane and coworkers (2021) investigated the solubility of 21 different compounds at ambient temperature and at 60°C in medium and long chain monoglyceride, i.e. Capmul® MCM and Maisine® CC. It

Table 3
Tradenames of lipid excipients, their composition and main function mentioned in the main text or tables.

Tradename	Potential synonym	Composition
Oils		
Capmul® MCM		Mixture of medium chain mono- and diglycerides
Captex® 300		Medium chain triglyceride
Labrafac™ lipophile WL1349	Labrafac™ CC	Medium chain triglyceride
Maisine® CC	Maisine® 35-1	Mixture of long chain mono-, di- and triglycerides
Miglyol® 812N		Medium chain triglyceride
Peceol™		Glyceryl monooleate
Surfactants		
Brij™ L4		Polyethylene glycol dodecyl ether
Capmul® PG8		Propylene glycol monocaprylate
Caproyl® 90		Propylene Glycol Monocaprylate
Gelucure® 44/14		Lauroyl polyoxyl-32 glycerides
Gelucure® 48/16		Polyoxyl-32 stearate
Kolliphor® EL	Cremophor EL	PEG-35 hydrogenated castor oil
Kolliphor® RH40	Cremophor RH40	PEG-40 hydrogenated castor oil
Kolliphor® HS15	Solutol HS15	Polyoxyl 15 hydrostearate
Labrafac® PG		Propylene glycol dicaprolate/ dicaprate
Labrafil® M1944CS		Oleoyl polyoxyl-6 glycerides
Labrasol® ALF	Labrasol®	Caprylocaproyl polyoxyl-8 glycerides
Lauroglycol™ FCC		Propylene glycol monolaurate
Pluronic® F-127	Poloxamer 407	2-[2-(2-hydroxyethoxy)propoxy] ethanol
Polysorbate 80	Tween 80	Polyoxyethylene (80) sorbitan monooleate
Polysorbate 20	Tween 20	Polyoxyethylene (20) sorbitan monolaurate
Vitamin E-TPGS		D-α-tocopheryl polyethylene glycol succinate
Cosolvents		
PEG		Polyethylenglycol
Tetraglycol		Tetraethylene glycol
Transcutol® P	Transcutol® HP	Diethylene glycol monoethyl ether

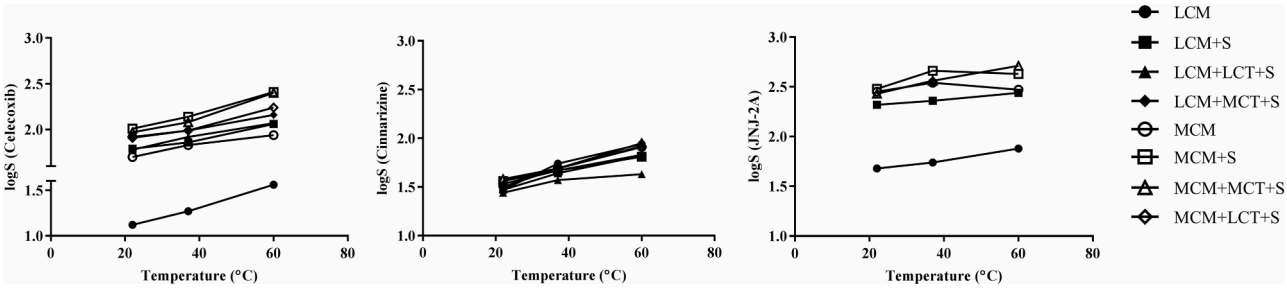


Fig. 2. Depicturing of the logarithm of drug solubility (provided as the logarithm of mg/mL) as a function of temperature for three different drug compounds in different lipid-based formulations. LCM: long-chain monoglyceride; S: surfactant; LCT: long-chain triglyceride; MCM: medium-chain monoglyceride; MCT: medium-chain triglyceride (reproduced from Ilie et al. (2020b) with permission).

was compound-specific whether the highest degree of supersaturation was observed in the medium- or long-chain monoglyceride. The increase in solubility from ambient temperature to 60°C was reported to be between 1- to 3.4-fold, providing a general range for the degree of supersaturation that may be achieved thermally. Bennett-Lanane et al. (2021) reported that no simple link was found between the achievable degree of supersaturation in the lipid and any solid-state property alone. Bennett-Lenane et al. (2021) used the dataset to generate artificial neural network-based models to predict the degree of supersaturation based upon molecular descriptors generated from ADMET Predictors 9.5. Overall, a wide range of drug descriptors reflecting topology, reactivity, structure and size, electrostatics, and thermodynamics, were reported to be significant in obtaining a high degree of supersaturation in the tested lipid vehicles. However, owing to the fact of elaborate experimentation, this pilot study only worked with 21 compounds and therefore a wider applicability of the models beyond the described test set may be limited. Nevertheless, the study indicates that within the defined chemical space studied, it is possible to generate in silico-based predictions on the potential to supersaturation in a lipid vehicle with a given compound. This approach can be extended with more data to evaluate the applicability of the formulation approach early on, based on simple input parameters, which is very well-suited for late-stage discovery/early stage development where compound availability is limited. In general, of the thermally induced supersaturated lipid-based formulations described in the literature, there is no systematic description nor solubility data presented in the articles. Instead, the articles generally stat that the used supersaturated solutions were visually free from particles when administered either *in vitro* or *in vivo*. Thus, identifying the degree of supersaturation is only possible for a very limited amount of the literature.

3.2. Physical stability of thermally induced supersaturated lipid-based formulations

As indicated by the term, supersaturated lipid-based formulations are not considered thermodynamically stable, which means the drug may crystallize over time in the formulation. Similar to solubility, this stability is judged visually and in general, the formulations are kept as short as possible to prevent physical instability from affecting the investigations. Ilie et al. (2020b) studied the physical stability of different type I and II lipid-based formulations for three compounds, where the degree of supersaturation was also investigated: celecoxib, cinnarizine, and JNJ-2A. Saturated solutions produced at 37 and 60°C were stored at ambient temperature, and the ability to maintain supersaturation during storage was assessed by monitoring the time to precipitate. Crystallization of drug was observed within 28 days for all the formulations investigated containing cinnarizine and for celecoxib when the degree of supersaturation was >1.35, whereas no visible precipitate was observed for JNJ-2A in any of the formulations. The lipid composition type was reported to have minor impact on the risk of precipitation, while a high degree of supersaturation was an important factor driving drug precipitation from the investigated supersaturated lipid formulations. Ilie et al. (2020b) also linked the risk of precipitation to the compound properties. The inherently poor stability of cinnarizine in supersaturated lipid-based formulations was in accordance with previous data reported by Siqueira et al. (2017), who investigated supersaturated self-emulsifying drug delivery systems. Cinnarizine was reported to have a pronounced tendency to crystallize, based upon the method described by Baird et al. (2010), whereas celecoxib and JNJ-2A were identified as slow crystallizers (Ilie et al., 2020b). This suggests that this approach may be used to identify compounds with a low risk of precipitation in the lipids from a supersaturated solution.

Alternatively, strategies to improve the stability of the supersaturated formulations could be considered. One approach could be addition of lipids that solidify at ambient temperature. Thereby significantly reducing the molecular mobility of the system. However, it is important that the solidification of the matrix is in a non-crystalline form, as crystallizing lipids would be expected to have limited drug loading. Schultz et al. (2018) proposed another approach based on their work with supersaturated lipid formulations containing ibuprofen. Schultz and co-workers (2018) overcame the instability of the supersaturated formulations using a silica-lipid hybrid system. In this system, the supersaturated lipid formulation was loaded into nanopores of porous silica microparticles, which was reported to inhibit the precipitation of ibuprofen and produced a solid-state lipid-based formulations (Schultz et al., 2018).

4. Biopharmaceutical evaluation of supersaturation in lipid-based formulations

As mentioned above, different kinds of supersaturated lipid-based formulations have been evaluated and described in the literature, specifically *in situ* supersaturated systems (or supersaturable systems) and mixtures with thermally induced supersaturation. These formulations are different and will hence be treated separately in the sections below.

The discussion in the sections below is based upon studies that have administered supersaturated lipid-based formulations *in vivo*. For completeness it has to be stressed that there is also a number of studies that have investigated the precipitation upon *in vitro* lipolysis without administering the formulations *in vivo* (e.g. Alskär et al., 2018; Devraj et al., 2014; McEvooy et al., 2017; Williams et al., 2013b; Crum et al., 2017). While these studies provide interesting data that may help understand some of the mechanistic elements important for absorption from a lipid-based formulation, they are considered out of scope for the present work, as it would require a critical review also of the applied method to provide a comprehensive and balanced overview. In general, *in vitro* studies in the field of supersaturated lipid-based formulations are conducted either by dispersing the formulation into fasted or fed simulated intestinal fluids or investigated in the lipolysis models to monitor precipitation kinetics. If the obtained solid phase is amorphous, then this would probably have a limited to no impact on the bioperformance of the formulation. However, if the compound crystallized

Table 4
Overview of *in vivo* studies reported in the literature testing the potential benefits of including a precipitation inhibitor into lipid-based formulations. The chemical composition of the lipid tradenames is described in Table 3.

Drug (Reference)	Precipitation inhibitor (w/w %)	Lipid-based formulation composition	Effect *in vivo/in vitro*
AMG-517 (Gao et al., 2009)	HPMC (5%)	Capmul® MCM, Tween 80 PEG 400	Monkey study: ~30% higher mean C_{max} and comparable exposure (AUC) as compared to an aqueous suspension
Benzimidazole (Rosso et al., 2021)	HPC (Klucel® LF or Klucel® EF) (1%)	Miglyol® 812N Kolliphor® RH40 Transcotol® HP Ethanol or DMSO	Mice study: A SMEEDS produced an AUC double as high as a suspension. The same SMEEDS added Klucel LF doubled the AUC. Replacing the ethanol with DMSO and using Klucel EF as the precipitation inhibitor led to a AUC at the same level as the SMEDDS without the precipitation inhibitor
Canagliflozin (Singh et al., 2021)	Poloxamer 188 (2.24%)	Lauroglycol™ FCC Tween 80 Transcutol® P	Rats study: The SMEEDS was solidified onto Neusilin® US2. The solidified SMEEDS with Poloxamer 188 produced a significantly higher AUC than an aqueous suspension and the commercial product
Carbamazepine (Zhang et al., 2011)	PVP-K90 (2%)	Miglyol® 812N Kolliphor® EL PEG 400	Dog study: bioavailability increased 5-fold relative to commercial tablet
Celecoxib (Shi et al., 2010) (Song et al., 2014)	PVP-12PF (~4%) + HPMC-E5 (~4%)	PEG 400 Eethanol Tween 80 Oleic acid Tromethamine	*In vitro* biphasic dissolution and correlation to human data, increase in AUC and C_{max} compared to Celebrex® and solution
	Soluplus® (4%)	Capryol® 90 Tween 20 Tetraglycol	Highest effective permeability coefficient and bioavailability increased 3.6-fold relative to aqueous suspension
(Chavan et al., 2015)	Soluplus (18%)	Capryol® 90 Tween 20 Transcutol® HP	Rats study: AUC increased six-fold when comparing an aqueous suspension with the SEDDS containing a precipitation inhibitor. When the SEDDS including the precipitation inhibitor was loaded onto a mesoporous silica C_{max} was reduced with a factor of about 2.7, while the AUC only tended to be slightly lower.
Curcumin (Jaisamut et al., 2018)	Eudragit® E PO (5%)	Cremophor EL Labrasol® Capryol® 90 Labrafac® PG	Rats study: Three formulations was tested in *vivo*, an aqueous suspension, a SMEEDS with and without a precipitation inhibitor. Relative to the suspension the absorptions increased 1.2- and 53-fold, respectively.
Danazol (Anby et al., 2012)	HPMC (5 %)	Captex® 300 Capmul® MCM Cremophor EL Ethanol	Dog study: A type IV and IIIA was investigated with or without HPMC. For the type IV formulation HPMC had no influence on the bioavailability. The type IIIA formulation was dosed with a dose equal to 40 or 80% of the saturated solubility in the vehicle. At low drug loads, addition of HPMC led to a 65% increase in exposure at high drug loading no effect from HPMC was reported
Docetaxel (Chen et al., 2011)	HPMC (2.5%)	Labrafac® Cremophr RH40 Transcutol® P	Rat study: The SMEEDS was solidified onto lactose with or without HPMC and compared to an aqueous suspension. Both SMEEDDS were reported to have a significantly higher C_{max} and AUC than the suspension. The SMEEDS contain HPMC had a 1.4-fold higher AUC than the SMEEDS without
Dutasteride (Kim et al., 2015; Kim et al., 2020)	Aerosil® 200 (33-50%) HPMC (33%) Soluplus® (33%)	Capryol® 90 Cremophor EL Transcutol® HP	Rats study: SMEEDS performed 4-fold better than an aqueous suspension, adding HPMC to the SMEEDS increased the AUC slightly adding Soluplus® a bit more, though not statistically significant Dog study: The SMEEDS with Soluplus® was tested in dogs and a slightly higher AUC was reported relative to the animals receiving the commercial formulation (Avodart, GSK)
(Lee et al., 2015)	Soluplus® (11.3%)	Capryol® 90 Cremophor RH40 Transcutol® HP	Rat study: A SEDDS formulation containing Soluplus® exhibited 3.9-fold greater AUC than that of the drug suspension and 1.3-fold greater than that of SEDDS without Soluplus®
Fenofibrate (Suys et al., 2018)	Eudragit® RL100 (1%), PPGAE 1%, HPMC (5%)	Kolliphor® EL Transcutol® HP	Rat study: A general trend towards higher drug absorption was obtained, which was statistically significant for PPGAE
(Suys et al., 2021)	PPGAE (1%) HPMC (1%) Eudragit® E100 (1%)	Captex® 300 Capmul® MCM Kolliphor® EL Transcutol® HP	Rat study: From a type IV formulation, a significantly higher AUC was reported when dosed in a vehicle added PPGAE than without, whereas addition of HPMC trended towards higher absorption. For the tested type IIIb vehicle, a similar absorption relative to the type IV system with the precipitation inhibitor was reported, however, addition of Eudragit® E100 tended to reduce the absorption
(Quan et al., 2017)	Soluplus® (15%)	Ethyl oleate Cremophor RH40 Transcutol® HP	Dog study: The *in vivo* study indicated that the SEDDS with Soluplus® improved the oral absorption of fenofibrate with approximately 40% when compared to SSEDDS
Indirubin (Chen et al., 2012)	PVP K17 (0.5%)	Maisine® CC Kolliphor® EL Transcutol® P	Rat study: bioavailability increased 1.3-fold relative to precipitation inhibitor free SEDDS
Paclitaxel (Gao et al., 2003)	HPMC 5%	Kolliphor® EL Glyceryl dioleate, PEG 400 Ethanol	Rat study: 10-fold higher maximum plasma concentration (C_{max}) and 5-fold higher oral bioavailability
PNU-91325 (Gao et al., 2004)	HPMC (20%) Pluronic L44 (18%)	Kolliphor® EL Glycerol monooleate and dioleate (2:8) PEG 400 Dimethyl acetamide	Dog study: bioavailability increased 6-fold relative to pure PEG 400 formulation,
Raloxifene (Jain et al., 2018)	HMPC (5%)	Capryol® 90 Cremophor RH40 Transcutol HP	Rat study: A significant increase in the bioavailability was reported for a SMEDDS relative to an aqueous suspension. When dosed in the cationic SMEEDS (2% (w/v)

(*continued on next page*)

Table 4 (*continued*)

Drug (Reference)	Precipitation inhibitor (w/w %)	Lipid-based formulation composition	Effect *in vivo/in vitro*
			oleylamine, zetapotential; 29.8 mV) with a precipitation inhibitor the fraction absorbed was significantly higher than the two other formulations investigated
Saquinavir (Jo et al., 2020)	HPMC 2910 (7%)	Capryl® 90 Labrasol® Propylene glycol	Rat study: Lymph was collected from anaesthetized rats after administration of an aqueous suspension, a SMEEDS without and with HPMC. The cumulative amount of saquinavir in the lymph after 8 hours was significantly different and ranked; SMEEDS with HPMC > SMEDDS > suspension
Silybin (Wei et al., 2012)	HPMC (5%)	Labrafac™ CC Cemophor RH40 Labrasol®	Rat study: AUC was increased a 3-fold when HPMC was suspended in the vehicle
Silymarin (Tung et al., 2019)	Poloxamer 407 (10%)	Labrafil M1944CS Kolliphor® RH40 Transcutol® P	Rabbit study: bioavailability increased 7.6-fold relative to commercial product (Legalon®)
Tacrolimus (Lee et al., 2016)	Soluplus® (6,3%)	Capmul® MCM Cremophor EL Transcutol® HP	Rat study: Two volumes of a SMEDDS formulation was tested – the low volume with and without Soluplus®. The SMEEDS with the high load had an AUC about half the AUC when a higher amount of vehicle was administered, which was at the same level as the formulation with Soluplus®
Telmisartan (Park et al., 2021)	Soluplus® (5 %)	Capmul® MCM Cremophor RH40 Tetraglycol	Rat study: 3 fold higher bioavailability when dosed in a SMEDDS relative to a suspension, which rose 1.25 fold further when Soluplus® was added
Trans-resveratrol (Singh et al., 2016)	HPMC (5%)	Lauroglycol™ FCC Transcutol® P	Rat study: $AUC_{0\text{-}8h}$ increased by 1.3-fold versus precipitation inhibitor free SEDDS
Valsartan (Yeom et al., 2017)	Poloxamer 407 (9%)	Capmul® MCM Tween 20 Transcutol® P	Rat study: bioavailability increased approximately 2,6 times relative to an aqueous suspension, but only 30% when the SMEEDS with or without the precipitation enhancer was added
(Shin et al., 2019)	Poloxamer 407 (9%)	Capmul® MCM Tween 20 Gelucire® 44/14	Rat study: SMEEDS was solidified into a granulate and produced an exposure approximately twice as high as an aqueous suspension of valsartan
(Goo et al., 2020)	Poloxamer 407 (9%)	Capmul® MCM Tween 20, 80 Kolliphor® EL Transcutol® P	Rat study: The surfactant was varied in the formulations and the highest absorption of valsartan was reported when Tween 80 or Kolliphor EL was used as the surfactant
Compound X (Gao and Morozowich, 2007)	HPMC (5%) or HPMC capsule	Kolliphor® EL Glyceryl dioleate Glycerol-monooleate PEG 400 Ethanol	Dog study: bioavailability was similar when HPMC was present either suspended in the lipid vehicle or the lipid formulation was encapsulated in a HPMC capsule

during the experiment, then this is in most cases interpreted as a limited effect from the precipitation inhibitor and the formulation would not be considered further. An extensive review of these studies is considered out of scope for the present review and the interested reader is referred to a recent review of the area (Kuentz, 2019). Studies on the influence of the colloidal structures on the supersaturated state could beneficially be further investigated in the in vitro lipolysis model investigating if the compounds from the supersaturated structures will be supersaturated in the colloidal or the aqueous phase – or if they will just precipitate out amorphous as described by Gao et al. (2009) for supersaturable systems and by Thomas and coworkers for thermally induced supersaturated formulations (2012).

4.1. *Lipid formulations that supersaturate upon dispersion in the gastrointestinal tract*

As mentioned previously, it is important to distinguish between supersaturated lipid-based formulations, where the drug is present at concentrations above the thermodynamic solubility in the formulation, and the supersaturating/supersaturable lipid-based formulations which are designed in a way so that supersaturation is only observed after dispersion/dissolution/digestion in gastrointestinal fluids. This section is about the latter type of formulations.

Supersaturating lipid-based formulations are designed to achieve and maintain drug supersaturation in gastrointestinal fluids for a physiologically relevant period, typically matching the transit time in the stomach and small intestine (around 4-5 hours) (Price et al., 2019). This concept is often referred to as a formulation containing a "spring and parachute" (Guzman et al., 2007; Brouwers et al., 2009; Augustijns and Brewster, 2012). Type III and IV lipid-based formulations have the potential to induce supersaturation upon dispersion in the gastrointestinal tract due to the presence of higher levels of water-soluble cosolvents. These formulations are thermodynamically stable on the shelf but tend to form thermodynamically unstable drug solutions in the intestine due to dilution of the added cosolvent.

A wide range of excipients has been explored for their ability to inhibit precipitation in supersaturating lipid-based formulations, by either interference with nucleation and/or crystal growth or by enhancing the solubilization (Xu and Dai, 2013; Brouwers et al., 2009; Warren et al., 2010). Among the most studied precipitation inhibitors are polymers such as hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone vinyl acetate (PVP/VA). Even at low concentrations in the formulation can these polymers reduce drug nucleation and crystal growth rates through molecular interactions with the drug, i.e., hydrogen bonds, polar, or dispersion forces, thereby enhancing the bioavailability from supersaturated formulation systems (Price et al., 2019; Xu and Dai, 2013; Warren et al., 2010). Apart from these classical polymers, various other excipients have also been investigated as precipitation inhibitors for *in situ* supersaturated lipid-based formulations, including Poloxamers, D-α-tocopheryl polyethylene glycol succinate (vitamin E-TPGS), sodium dodecyl sulphate (SDS), PEG-40 hydrogenated castor oil (Kolliphor® RH40), and cyclodextrins (Xu and Dai, 2013). Several *in vivo* studies have assessed the influence of precipitation inhibitors in lipid-based formulations, for an overview please see Table 4. The field has seen extensive research in this area, and the following sections will discuss the use of different precipitation inhibitors in lipid-based formulations.

4.1.1. *Supersaturable system with HPMC as the precipitation inhibitor*

The approach of embedding a precipitation inhibitor in lipid-based formulations, was presented first in a pioneering study by Gao et al. (2003), using a SEDDS containing paclitaxel. The formulation contained

higher amounts of PEG 400 and ethanol, and through rat studies Gao and coworkers demonstrated that suspending HPMC in the lipid-based formulation resulted in a 10-fold increase in the oral bioavailability of paclitaxel when compared to the same SEDDS without HPMC. In a subsequent study by Gao and Morozowich (2007), it was suggested that the HPMC did not need to be suspended within the lipid-based formulation. Instead, encapsulating the lipid-based formulation into a HPMC capsule can be sufficient for HPMC to function as a precipitation inhibition. Gao and Morozowich (2007) reported that the absorption of compound X in dogs was similar when HPMC was suspended in the lipid-based formulation (encapsulated into a gelatin capsules) and when the formulation was just filled into a HPMC capsule.

Gao et al. (2004) reported a very low oral bioavailability in dogs for the compound PNU-91235 when administered as a solution in PEG 400, however, when 0.2% (w/w) HPMC was added to the PEG solution together with a low amount of water, the bioavailability increased by almost a factor of 5, to a level similar to the bioavailability obtained when the compound was dissolved in pure Tween 80. Additionally, a fourth formulation was tested, which was a SEDDS containing oil, cosolvents and HPMC, which provided a doubled bioavailability compared to the Tween 80 solution. These *in vivo* results were qualitative consistent with the predictions provided by the *in vitro* test reported in the study (Gao et al., 2004). In another study by Singh and Pai (2016), the oral bioavailability of resveratrol in rats was investigated using a vehicle consisting of LauroglycolTM FCC and Transcutol® P in nearly equal amounts. The addition of HPMC to the formulation resulted in approximately a 30% increase in the area under the plasma curve (AUC) compared to the formulation without HPMC. Similar observations were reported by Shi et al. (2010) when studying the possibility of improving the oral bioavailability of celecoxib. The reported in vitro dissolution data predicted both the obtained AUC and C_{max} for the three investigated formulations, where the SEDDS containing HPMC outperformed the other tested formulations by a factor of 4-5.

Anby et al. (2012) investigated two formulations with danazol, one containing just Cremophor EL and ethanol, i.e., a lipid classification system type IV formulation, and a formulation with Captex® 300, Capmul® MCM, Cremophor EL and ethanol, i.e. a type IIIa formulation. During in vitro lipolysis, precipitation was observed from both formulations, prompting the screening of various polymers as potential precipitation inhibitors. The tested polymers included hydroxypropyl cellulose (HPC), Eudragit® L100 (methyhacrylic acid-methyl methacrylate copolymer (1:1)) and E100 (butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate copolymer (2:1:1)), Aerosil® 200 (hydrophilic fumed silica with a specific surface area of 200 m^2/g), polyvinylpyrrolidone (PVP), HPMC, hydroxypropyl methylcellulose acetate succinate (HPMC-AS), methyl cellulose (MC) and HPC. Among these, HPMC, HPMC-AS, HPC, and MC demonstrated the greatest reduction in precipitation tendency during digestion, with HPMC being selected for further investigations *in vivo*. The type IV formulation contained danazol at a concentration equal to 40% of the compounds solubility in the vehicle. Rather surprising, for the type IV formulation there was no difference observed in the fraction absorbed with or without the HMPC added. For the IIIa formulation, two concentrations were tested based upon danazols saturated solubility in the vehicle. One where 40% of the saturated solubility was added and another with 80% saturation level. In both cases, a group with and without HPMC was evaluated. The SMEDDS with 40% of drug solubility in the vehicle produced an AUC at the same level as the type IV formulation and addition of HPMC significantly increased the drug absorption. When the administered vehicle had a danazol saturation of 80%, a slightly higher absorption was observed than with the 40% drug saturation with HPMC, however, adding HPMC to the 80% vehicle did not change the fraction absorbed (Anby et al., 2012).

In the study by Suys et al. (2021), the absorption of fenofibrate (a high permeability compound) and saquinavir (a low permeability compound) from lipid-based formulations was investigated. For fenofibrate, supersaturation was reported to be initiated by formulation interaction with biliary/pancreatic fluids, which was argued to drive the absorption. The addition of precipitation inhibitors, poly(glycidyl methacrylate) (PPGAE) and HPMC, was reported to reduce drug precipitation *in vitro*. When the precipitation inhibitors were added to the formulation, an increased fenofibrate supersaturation was reported, which lead to increased absorption from a lipid classification system Type IV formulation in an *in situ* intestinal perfusion model. For an investigated IIIb formulation, addition of the precipitation inhibitors did not affect the fraction of fenofibrate absorbed in the intestinal loop model. The impact of precipitation inhibitors on the absorption of the less permeable drug, saquinavir, was also evaluated in the *in situ* intestinal model. In this case the extent of drug absorption appeared to be related to the extent of supersaturation, although in this case it was reported that the precipitation inhibitor was unable to promote absorption from any of the tested vehicles. For both fenofibrate and saquinavir, drug absorption patterns obtained with the *in situ* perfusion model was reported to be correlated with *in vitro* supersaturation data and *in vivo* drug exposure data from oral bioavailability studies conducted in rats. The data were interpreted with a mechanism of drug absorption where rapid dilution of the lipid-based formulations occurs with biliary and pancreatic secretions at the absorptive site in the upper small intestine. This was suggested to promote transient supersaturation as a significant driver of drug absorption for both low and high permeability drugs and that precipitation inhibitors may delay drug precipitation, enhance supersaturation to promote drug absorption in a compound and formulation specific manner (Suys et al., 2021).

The lymphatic absorption of saquinavir was investigated from a SMEDDS containing Caproyl® 90, Labrasol® and propylene glycol with or without HPMC (Jo et al., 2019). The effect of precipitation inhibitors, specifically PVP K90 and HPMC 2910, on the dissolution of saquinavir was examined *in vitro*. HPMC 2910 was found to be the most effective precipitation inhibitor in inhibiting the precipitation of saquinavir. The compound undergoes extensive first pass metabolism, why the authors wanted to investigate the application of a lipid-based formulation to promote the intestinal lymphatic transport and thereby circumvent the liver metabolism (Griffin and O'Driscoll, 2006). Three formulations were dosed to rats: a suspension, a SMEDDS, and a SMEDDS with added HPMC. One hour after the formulation administration, the animals were anaesthetized, and their mesenteric lymph duct was cannulated. The lymph was collected over a period of eight hours, while the animals was unconscious. The cumulative amount of saquinavir collected from the animals was found to be significantly different among the three groups. The lowest amount of saquinavir was measured in the lymph from the animals dosed with the suspension, while the highest amount was observed in the animals dosed with the SMEDDS added HPMC.

Transcutol® P is a highly effective cosolvent that enhances the solubility for a range of compounds, and the excipient is frequently investigated in lipid-based formulations. Transcutol® is fully water miscible, why it will diffuse into the aqueous phase once the formulation dissolves in the stomach, thereby creating an *in situ* supersaturated formulation. Many researchers have investigated if addition of precipitation inhibitor to Transcutol® containing formulations may improve the bioavailability. In a study conducted by Chen et al. (2012), different precipitation inhibitors were examined in the development of a lipid-based formulation with a high cosolvent content (Transcutol®). I*n vitro* dissolution testing was performed when the lipid-based formulations was added PEG 4000, HPMC or PVP-K17. HPMC was found to be the most effective precipitation inhibitor among the three polymers tested, regardless of the polymer concentration (2%, 3%, and 5% w/w). However, PVP-K17 was also reported to prevent precipitation of indirubin effectively when dispersed with the lipid-based formulation. When administered to rats, a solid SMEDDS containing HPMC demonstrated a bioavailability improvement of approximately 30% compared to the same formulation without the polymer. (Chen et al., 2012).

Chen and coworkers (2011) developed a SMEDDS to improve the

bioavailability of docetaxel consisting of Labrafac™ lipophile, Cremophor RH40 and Transcutol® P. Precipitation was observed *in vitro*, why a number of precipitation inhibitors was screened, including PVP K30, SDS, HPMC and microcrystalline cellulose (MCC). HPMC was found to be most effective in preventing the precipitation of the compound *in vitro* so this polymer was selected for further investigation. The liquid lipid-based formulation was solidified by spray drying the SMEDDS formulation onto lactose. In rats, the solid SMEDDS was investigated with or without HPMC added relative to an aqueous suspension of docetaxel. Both SMEDDS formulations produced an AUC that was significantly higher than that of the suspension. Adding HPMC to the SMEDDS increased both the C_{max} and the AUC by approximately 45%, though no statistical difference was reported by the authors (Chen et al., 2011).

In their study, Jain et al. (2018) investigated the use of a cationic lipid-based system to enhance the absorption of raloxifene, a substrate for the efflux transporter P-gp. The cationic system was hypothesized to increase absorption through electrostatic interactions with the negatively charged cell membrane, as proposed by Gershanik and Benita (1996). The initial formulation consisted of Capryol® 90, Cremophor RH40, and Transcutol®, and various polymers, including 5% HMPC E15, E5, PVP K30, or K25, were added as potential precipitation inhibitors. Although none of the investigated polymers completely prevented precipitation of raloxifene over the three hours the dissolution was followed, HPMC E5 showed the best performance among the polymers investigated and was hence selected for further *in vivo* evaluation in rats. The *in vivo* study included three different formulations: an aqueous suspension of the crystalline compound, a SMEDDS, and a SMEDDS added 2% oleylamine and 5% HPMC E5. The SMEDDS had a reported zeta-potential on -22.4 mV whereas the SMEDDS containing oleylamine and HPMC had a zeta-potential on 29.8 mV (Jain et al., 2018). The *in vivo* study conducted by Jain and coworkers showed a statistically significant difference in the AUC obtained for all three formulations. The lowest absorption was observed from the suspension and the highest from the cationic SMEDDS with HPMC. Multiple factors could have contributed to this difference, including precipitation inhibition, P-gp inhibition, improved permeation due to the charge etc. The inclusion of the cationic component in the SMEDDS formulation was a novel approach and warrants further exploration to fully understand its potential benefits in enhancing drug absorption.

Wei and colleagues (2012) investigated a SEDDS formulation where silybin was dissolved in a SEDDS system consisting of Labrafac™ CC, Cremophor RH40 and Labrasol®. I*n vitro* release studies clearly demonstrated precipitation of the compound when the SEDDS was dispersed in simulated gastric fluid. When HPMC was dispersed into the SEDDS, a much higher amount of silybin stayed dissolved during the 1.5 hours study duration, which was supported by a 3-fold increase in the bioavailability of silybin when rats were dosed with the SEDDS contain HPMC relative to the SEDDS without HPMC (Wei et al., 2012).

Collectively, the above studies in general conclude that incorporation HPMC as a precipitation inhibitor in supersaturatable lipid-based formulations enhances the oral bioavailability. HPMC have been evaluated with different drugs and seems consistently to function *in vivo* making it a good candidate as a precipitation inhibitor provided that a suspension is acceptable.

4.1.2. Supersaturable system with polyvinylpyrrolidone (PVP) as the precipitation inhibitor

Zhang and coworkers (2011) investigated a SMEDDS with carbamazepine based upon medium chain triglyceride (MCT), PEG 400 and Cremophor EL (30:35:35) and studied the *in vitro* precipitation after addition of 2% (w/w) either HPMC, MC, sodium carboxymethyl cellulose (Na-CMC), PVP-K30, PVP-K60 and PVP-K90 suspended or dissolved into the lipid-based formulations. For the formulations containing HMPC, MC and Na-CMC, drug precipitation was reported to occur quickly *in vitro*, whereas PVP-K30 and PVP-K60 were able to prevent crystallization for four hours and PVP-K90 sustained supersaturation even longer. A commercial tablet containing carbamazepine was therefore compared to the lipid-based formulation containing 2% (w/w) PVP-K90 in dogs, which led to an improved AUC with a factor of four when dosed in the latter formulation.

4.1.3. Supersaturable system with cellulose as the precipitation inhibitor

Rosso and coworkers (2021) investigated the absorption of benzimidazole, where a formulation based upon MCT, Kolliphor® RH40, Transcutol® and ethanol was defined. Upon dispersion of the formulation, fine droplets were formed, but precipitation of the compound was also observed. To address this issue a precipitation inhibitor was added. Rosso and coworkers (2021) added HPC to a SMEDDS, but also developed a SMEDDS where ethanol was replaced with dimethylsulfoxid (DMSO). In the latter formulation, a different grade of HPC with higher viscosity was used as the precipitation inhibitor. When dosed to mice, the SMEDDS produced a 2-fold increase in AUC compared to a suspension after dose correction. The addition of HPC further increased the AUC by 3-folder (dose corrected), but replacing ethanol with DMSO and adding a more viscous HPC provided an AUC at the same level as the plain SMEDDS (dose corrected) (Rosso et al., 2021). As two compositional factors were changed, it is hard to single out the potential reason for this observed difference. DMSO has a higher dielectrically constant than ethanol, which may have led to a faster dilution into the aqueous phase upon dispersion, potentially resulting in a higher degree of supersaturation relative to the ethanol containing SMEDDS. The two HPC grades have the same chemical nature, however, the one used in the DMSO containing vehicle had approximately double the viscosity than the HPC grade added to the ethanol containing vehicle (Ashland, 2023). This may lead to a slower solubilization of the precipitation inhibitor in the first case and in combination with a faster dispersion of the solvent, a much lower AUC could be observed. Such factors of grade viscosity and solvent depletion from a dispersed formulation would merit further mechanistic study to facilitate a systematic development of an optimal *in vivo* supersaturated lipid-based formulation.

4.1.4. Supersaturable system with Soluplus® as the precipitation inhibitor

In a study by Song et al. (2014), the absorption of celecoxib from lipid-based formulations was investigated. The researchers found that a lipid-based formulation composed of Caproyl® 90, Tween 20, and tetraglycol (in a ratio of 1:4.5:4.5) improved the absorption of celecoxib compared to a suspension by a factor of 2.6., When Soluplus® (Polyvinyl caprolactampolyvinyl acetate-polyethylene glycol graft co-polymer) was added to the lipid-based formulation as a precipitation inhibitor this factor improved to 3.6, demonstrating the positive influence of the precipitation inhibitor (Song et al., 2014). Building upon the work of Song et al. (2014), Chavan et al. (2015) made a formulation for celecoxib containing Caproyl® 90 and Tween 20, while replacing tetraglycol with Transcutol® HP and by increasing the Soluplus® amount. Three formulations were investigated in rats: a suspension of the compound and two versions of the SEDDS containing Soluplus®, a liquid version and a version solidified onto a mesoporous silica. The reported bioavailability and C_{max} was significantly higher for the two SEDDS than for the compound suspension. When the SEDDS was solidified onto mesoporous silica, a significant reduction is C_{max} was reported, but the AUC was only insignificantly reduced (Chavan et al., 2015).

Park et al. (2021) worked with telmisartan and reported that the compound dissolved in a SMEDDS consisting of 30% Capmul® MCM (oil), 23.3% Cremophor RH40 (surfactant) and 46.7% tetraglycol (cosolvent). This lipid mixture had a slightly better *in vitro* release profile, with delayed precipitation compared to the amorphous compound. To reduce the tendency of precipitation, Park and coworkers (2021) studied different precipitation inhibitors added to the SMEDDS; Soluplus®, HPMC, PVP/VA, Poloxamer 407 and vitamin E-TPGS. Soluplus® completely prevented precipitation of the compound from the SMEDDS for the two hours the dissolution study was followed, hence

this precipitation inhibitor was selected for *in vivo* investigations, together with an aqueous suspension of telmisartan, the amorphous compound produced as described by Chae et al. (2018), and the SMEDDS without the precipitation inhibitor. *In vivo* the suspension had both AUC and C_{max} that were significantly lower than the tree other formulations tested. The amorphous compound and the SMEDDS had similar AUC and C_{max}, whereas for the SMEDDS with added Soluplus® both AUC and C_{max} were approximately 1.25-folder higher (Park et al., 2021).

Lee and coworkers (2016) developed a formulation to deliver tacrolimus containing Capmul® MCM, Cremophor EL and Transcutol® HP. As precipitation was observed during *in vitro* dissolution studies, precipitation inhibitors, i.e. HPMC 2910, PVP K17 and Soluplus®, were evaluated. Soluplus® was demonstrated to be the best precipitation inhibitor *in vitro* (Lee et al., 2016). A pharmacokinetic study in rats was conducted with three different groups, all of which received a 5 mg/kg dose of tacrolimus. The evaluated SMEDDS was tested at a low and a high load of tacrolimus, i.e., the animals were dosed with 75 or 300 μL vehicle/kg, respectively. Additionally, the effect of adding Soluplus® to the high-load vehicle was investigated. The AUC of the low loaded vehicle was approximately double the AUC obtained after administering the dose in the high load vehicle. Adding Soluplus® to the high loaded vehicle, produced an AUC at the same level as the low loaded vehicle (Lee et al., 2016).

In a study reported by Quan and colleagues (2017), fenofibrate was dissolved in a vehicle containing ethyl oleate, Cremophor RH40, and 40% Transcutol®. The resulting liquid mixture was then solidified by applying it onto mesoporous silica (Quan et al., 2017). Solvent displacement studies, where fenofibrate was dissolved in DMSO and added into water, showed precipitate. Inhibition of the precipitation was therefore investigated by adding 0.05% (w/v) polymer into the aqueous phase. HPMC E3, E5, E15, PVP K30, PVP VA54 and Soluplus® were investigated. Soluplus® was clearly the best precipitation inhibitor for fenofibrate in this test, why it was also investigated in *in vivo*. Here addition of the polymer provided a 40% greater relative bioavailability in beagle dogs when compared to the formulation without the precipitation inhibitor at a 15% (w/w) optimal concentration 1:1 Soluplus®: fenofibrate (Quan et al., 2017).

Kim and coworkers (2015) also investigated a formulation containing Transcutol® at a higher amount (35% (w/w) in combination with Capryol® 90 and Cremophor EL to improve the bioavailability of dutasteride. The liquid formulation was loaded onto Aerosil® 200, which *in vitro* was demonstrated to improve the dissolution profile significantly. Moreover, it was investigated if the dissolution profiles could be further improved by adding HPC, HPMC, lactose, PEG 6000, PVP K30, PVP/VA, or Soluplus®, with the composition SEDDS:Aerosil® 200:polymer on 1:1:1 w/w/w. Adding Soluplus® ensured almost complete dissolution of dutasteride within the four-hour experimental time, with HPMC performing second best. *In vivo* studies in rats showed that the SMEDDS loaded to Aerosil® 200 performed significantly better than an aqueous suspension. Adding HPMC to the SMEDDS system increased the AUC slightly, while adding Soluplus® increased the AUC even further, in accordance with the results obtained *in vitro* (Kim et al., 2015). The formulation with Soluplus® was also evaluated in dogs and compared to the commercial formulation from GSK (Avodart®) (Kim et al., 2020). The commercial product is a soft gelatin capsule in which dustasteride is dissolved in mono- and diglycerides of caprylic/capric acid (Savla et al., 2017). A slightly, but statistical insignificant, higher AUC was reported for the animals receiving the experimental SMEDDS relative to the commercial product (Kim et al., 2020). Lee *at al.* (2015) investigated an almost identical formulation to improve the absorption of the same compound, though using Cremophor RH40, whereas Kim et al. (2015; 2020) used Cremophor EL in their formulation. Lee and colleagues (2015) investigated Soluplus®, PVP K90, HPMC 2910 and Kollicoat® MAE 30DP (methacrylic acid ethylacrylate copolymer) *in vitro* as precipitation inhibitors, which led to the selection of Soluplus® as the precipitation inhibitor in accordance with the results obtained by Kim and coworkers (2015). *In vivo*, the SEDDS with Soluplus® gave a 3.9-fold greater AUC than the drug suspension and a 1.3-fold greater AUC than that of SEDDS without Soluplus® (Lee et al., 2015). The same dose of 2 mg/kg dustasteride was used by both Kim et al. (2015) and Lee et al. (2015). Lee and coworkers (2015) dosed a liquid form of the formulation, whereas Kim et al. (2015) administered a solidified version of the SMEDDS added to Aerosil® 200 with a higher Soluplus® amount, whereas the lipid component was alike, as mentioned above. With the reservations of a comparison of obtained AUC's in different studies conducted at different locations, it is interesting to observed that Kim et al. (2015) obtained approximately doubled AUC than that observed by Lee et al. (2015). Therefore, it can be speculated if the Aerosil® 200 also had a positive effect in minimizing drug precipitation in combination with Soluplus® similar to other amorphous dispersions. A number of studies have investigated the use of Soluplur® as the precipitation inhibitor. In general, the polymer was reported well suited as a precipitation inhibitor as it consistently lead to a higher bioavailability in vivo.

4.1.5. Supersaturable system with nonionic surfactants as the precipitation inhibitor

Tung et al. (2019) described a larger dataset of *in vitro* work to identify the most suitable precipitation inhibitor for silymarin and in this context, Poloxamer 407, hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl-β-cyclodextrin and Eudragit L100 were evaluated. Based upon the *in vitro* investigations, Tung and coworkers (2019) tested a SEDDS containing Labrafil® M1944CS, Transcutol® P and Kolliphor® RH40 (2:1:2) and Poloxamer 407 as the precipitation inhibitor. An almost 8-fold higher oral bioavailability in rabbits was from the lipid-based formulation when compared to the commercial tablet. Similarly, Goo and coworkers (2020) worked with valsartan in lipid-based formulations containing high amounts of Transcutol®. Poloxamer 407 was used as the precipitation inhibitor in all the tested formulations together with a surfactant that was varied. Adding Tween 20 to the formulation significantly improved the absorbed fraction relative to valsartan suspension in rats. However, when Tween 80 or Kolliphor® EL was used as surfactant, the absorption increased even further. Valsartan is a P-gp substrate, hence the authors suggested that the used surfactants could be critical for inhibiting the efflux pump to obtain a higher bioavailability (Goo et al., 2020).

Yeom and coworkers (2017a) developed a SMEDDS with valsartan based on Capmul® MCM, Tween 20 and Transcutol® and screened for precipitation inhibitors *in vitro* enhancers. Soluplus®, HPMC, PEG 6000, PVP K90, vitamin E-TPGS, and Poloxamer 407 were investigated as precipitation inhibitors. Based upon the dissolution studies, Poloxamer 407 was selected as the polymer to be tested *in vivo* when incorporated into the SMEDDS. The formulation performed significantly better than an aqueous suspension of valsartan, however, the difference in the AUC without or with Poloxamer 407 was only 30%, with no statistically significant difference. In a follow-up study Yeom et al. (2017b) loaded the above lipid-based formulations into a granulate consisting of approximately 1:1:2 of MCC (Vivapur®): calcium silicate (Florite® PS-10) and tested the formulations in rats. Similar bioavailability was reported from the liquid and solidified formulation. The formulation was, however, reported to be unstable as it lost weight after storage at 25°C for one month. This was ascribed to loss of Transcutol® due to the excipient's low vapor point (Shin et al., 2019). A formulation was therefore tested where Transcutol® was replaced with Gelucire® 44/14. The obtained SMEDDS was solidified using a longer list of solid excipients and tested *in vivo* in rats. Gelucire® 44/14 is a completely other class of excipient than Transcutol® P and this change may mean that valsartan was not supersaturated with the formulation dispersed in the intestine or there was a more gradual increase of supersaturation triggered by lipolysis of Gelucire® 44/14. No *in vitro* release data were supporting these possible mechanisms, nor was the influence of Poloxamer 407 investigated in the study by Shin and coworkers (2019). AUCs

reported from the *in vivo* data was in accordance with the data obtained for the liquid SMEDDS with Transcutol® when dose corrected and slightly higher than the solidified Transcutol® containing SMEDDS (Shin et al., 2019). Goo et al. (2021) described a similar formulation system containing Gelucire® 44/14 and Brij™ L4 to improve the bioavailability of revaprazan. An improved absorption was also seen for this compound when a precipitation inhibitor (Poloxamer 407) was included in the formulation relative to the same formulation without Poloxamer 407.

In another study, Singh et al. (2021) developed a SMEDDS with Lauroglycol™ FCC, along with relative high amounts of Transcutol® and Tween 80 to dissolve canagliflozin in the formulatin. This formulation contained 2.24% (w/v) Poloxamer 188 and was solidified by adsorbing the formulations onto Neusilin® US2 (magnesium aluminometasilicate) by a spray drying step. The formulation was tested *in vivo* in rats and the SMEDDS formulation led to a significant higher AUC than the commercial formulation and an aqueous suspension of the compound.

4.1.6. Supersaturable system with Eudragit® surfactants as the precipitation inhibitor

Jaisamut et al. (2018) reported an influence of a precipitation enhancer from a system without cosolvents. A formulation containing high amounts of a surfactant mixture consisting of Cremophor EL and Labrasol® was mixed with an oil mixture consisting of Capryol® 90 and Labrafac® PG. Adding 5% (w/w) Eudragit® E PO (terpolymer based on N,N-dimethylaminoethyl methacrylate with methylmethacrylate and butylmethacrylate) to the SMEDDS formulation significantly improved the *in vitro* release profile The SMEDDS was tested *in vivo* with or without Eudragit® E PO relative to an aqueous suspension of curcumin. The absorption from the SMEDDS was significantly increased relative to the suspension. Adding Eudragit® E PO to the SMEDDS increased the AUC by a factor of 1.2 relative to the SMEDDS without the precipitation inhibitor (Jaisamut et al., 2018).

While most studies have reported the selection of the precipitation inhibitor based upon *in vitro* studies, Suys et al. (2018) reported a first screen for a formulation to promote the absorption of fenofibrate *in vitro* supplemented with an *in vivo* study in rats for which three different precipitation inhibitors were investigated, namely Eudragit RL100, PPGAE, and HPMC. Two different lipid-based formulations were tested *in vivo*, a type IIIB and a type IV formulation. The two formulations consisted of Captex® 300, Capmul® MCM, Cremophor EL, and Transcutol® in the proportions 1:1:2:0 and 0:0:1:1, respectively. The precipitation inhibitors tested were only added to the type IV formulation. *In vivo* the difference in the AUC between the type IIIIB and type IV formulation was approximately a factor of four, where the type IIIB produced the highest absorption. Adding the precipitation inhibitors to the type IV formulation increased the observed AUC to at least the same level as the type IIIB formulation, though no statistical difference was reported between the type IIIB formulation and the type IV containing the precipitation inhibitor. Nor was there any significant difference observed between the different precipitation inhibitors (Suys et al., 2018).

Taken together, these different studies suggest the potential benefits of adding precipitation inhibitors to lipid-based formulations, particularly in the case of type IIIB and type IV, to harness their biopharmaceutical potential. While some studies have investigated the use of different polymers as the precipitation inhibitor, the field still needs additional studies to define a rational approach in selecting the most relevant polymer for a specific compound and the selection of compounds for the formulation approach could benefit from additional studies.

As presented above, it is clear that many scientists have investigated lipid-based formulations that can generate a supersaturated state upon administration when in contact with aqueous media during dilution and digestion. Typically, these systems contained a cosolvent, such as ethanol, tetraglycol or Transcutol®, but some studies also worked in systems consisting of lipids and a surfactant without a cosolvent. The influence of the different types or mixtures of cosolvents have not been investigated in detail, though the data from Rosso and coworkers (2021) indicated that there could be an optimization possibility. In common for most of the studies described above, addition of a precipitation inhibitor was investigated and this addition in many cases increased the *in vivo* absorption of the investigated compound. The most frequently used precipitation inhibitors tested *in vivo* was HPMC and Soluplus® (see Table 4), however, the choice of precipitation inhibitor and its concentration was all based upon either *in vitro* release studies or *in vitro* lipolysis testing. Conductance of additional *in vivo* studies to confirm the choice of precipitation inhibitor (at the given concentration) based on *in vitro* methods would be highly valuable for the field.

4.2. Thermally induced supersaturated lipid-based formulations

The correlation between temperature and solubility in water and organic solvents is well described both from an experimental and theoretical perspective (see e.g. Mota et al., 2009; Domańska et al., 2011). Experiments have demonstrated that the same principles apply to lipids and lipid-based formulations, indicating that the solubility of most compounds increases with increasing temperatures. This approach has been used in all the studies with shelf supersaturated lipid-based formulations both *in vitro* and *in vivo*, see Table 5. Thomas et al. (2012) was to the best of our knowledge the first to report *in vivo* investigations of thermally induced supersaturated lipid-based formulations. Thomas and coworkers (2012) drew inspiration from previous in vitro lipolysis studies, particularly the finding that precipitated drug could exist in an amorphous solid state (Sassene et al., 2010). With this background, Thomas and colleagues (2012) investigated two different type IIIB lipid-based formulations based upon either long- or medium chain lipids, whereby dogs were dosed with either one or two capsules contain halofantrine equal to 75% or one capsule with halofantrine equal to 150% of the saturated solubility at 25°C in the given vehicle. For the medium chain lipid-based formulation, dose proportionality was not observed between the two saturated formulations and the supersaturated formulation outperformed the two saturated formulations with respect to bioavailability. For the long chain lipid-based formulation, the two capsules approach had an absorption that was similar to the thermally induced supersaturated formulation, which altogether outperformed the medium chain formulations investigated (Thomas et al., 2012). The study conducted by Kaukonen et al. (2004) previously suggested that the distinction between medium and long-chain lipid-based formulations plays a role in influencing the solubility of halofantrine within the mixed micelles formed after lipid digestion. More recently, Katev and coworkers (2021) reported supersaturation of fenofibrate after *in vitro* lipolysis studies when solubilised in an MCT formulation, which was not observed when a LCT vehicle was digested. Michaelsen et al. (2016) investigated the same long chain formulation in rats with halofantrine. Higher absolute bioavailability of halofantrine was reported when dosed in the supersaturated lipid-based formulations as compared to the same lipid-based formulation with half the drug amount added in a non-saturated case. Interestingly, Michaelsen and coworkers (2016) also demonstrated that inhibiting the lipase activity in the rats, by coadministration of orlistat, had no influence on the fraction absorbed from the non-saturated formulation and it even improved the bioavailability of the supersaturated formulation. Supersaturation in combination with administered orlistat nearly doubled the bioavailability when compared to the normal lipid-based formulation. A similar experimental setup was investigated in rats by Michaelsen et al. (2019) using fenofibrate as the model compound. For fenofibrate, an increased absorption was observed for the supersaturated system when compared to the non-saturated formulation. In accordance with the data reported for halofantrine (Michaelsen et al., 2016) orlistat had no influence on the fraction absorbed from the non-saturated system, but in this case, the

Table 5
Overview of *in vivo* studies evaluating supersaturated lipid-based drug delivery systems with thermally induced supersaturation. The chemical composition of the lipid tradenames is described in Table 3, MC; medium-chain; LC: long-chain; RC: Rosuvastatin calcium.

Drug (Reference)	S_{eq} (37°C) or drug load	Formulations	*In vivo*	*In vitro*	Conclusions
Halofantrine (Thomas et al., 2012)	MC-SNEDDS: 47.0 ± 2.9 mg/g LC-SNEDDS: 64.0 ± 3.3 mg/g Drug load: 1: 35.2 mg/g 3: 70.4 mg/g 4: 48.0 mg/g 6: 96.0 mg/g	1 MC-SNEDDS 75% 1 caps 2 MC-SNEDDS 75% 2 caps 3 MC-supersaturated SNEDDS 150% 4 LC-SNEDDS 75% 1 caps 5 LC- supersaturated SNEDDS 75% 2 caps 6 LC- supersaturated SNEDDS 150% LC-SNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)MC-SNEDDS: Capmul® MCM + Captex® 300 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Dosing of supersaturated SNEDDS resulted in better plasma profiles compared to single capsule of SNEDDS 75% at different doses for LC and MC systems - values not dose-corrected For the same dose: no significant difference between formulations 2 and 3 (MC systems) and 5 and 6 (LC systems)	Yes, dynamic lipolysis; For both LC and MC-SNEDDS: drug concentration in aqueous phase higher from 2 capsules - formulations 2 and 5 (probably due to generation of more digestion products) compared to 1, 3 and 4, 6. Amorphous precipitate	*In vitro* model did not predict *in vivo* performance of supersaturated SNEDDS Better performance (not statistically significant) from LC systems relative to MC systems
Simvastatin (Thomas et al., 2013)	112.9 ± 2.7 mg/g	1 SNEDDS 75% 1 caps 2 SNEDDS 75% 2 caps 3 SNEDDS 150% 4 SNEDDS 200% (only *in vitro*)SNEDDS: Capmul® MCM + Captex® 300 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Increasing lipid dose with formulation 2 did not hamper the drug absorption Pharmacokinetic parameters better for supersaturated SNEDDS 150% (formulation 3) compared to equivalent dose in formulation 2	Yes, dynamic lipolysis; drug concentration in aqueous phase increased with the amount of preconcentrates and drug load in the first 30 minutes and decreased to equilibrium solubility afterwards; rank order for drug concentration in first 30 minutes: 4>2>3>1 Amorphous precipitate	*In vitro* dynamic lipolysis test was able to predict the *in vivo* rank order of 1 and 2, but underestimated the performance of supersaturated SNEDDS *in vivo*. Supersaturated SNEDDS superior to conventional SNEDDS for simvastatin
Fenofibrate (Thomas et al., 2014)	108.8 ± 4.1 mg/g	1 SNEDDS 75% 2 Supersaturated SNEDDS 150% 3 SNEDDS suspension 100+50% (solution 100% and 50% extra as suspension) 4 Lipanthyl® SNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Same dose administered (200 mg) No major differences between the 4 formulations in terms of plasma profiles, except for a high relative bioavailability (to Lipanthyl®) from formulation 3 (SNEDDS suspension), but no significant difference	Yes, combined gastric/ intestinal *in vitro* lipolysis protocol. Drug solubilized in aqueous phase: 1>2>3>4 Crystalline precipitate	No correlation between the AUC of solubilization - time curves and areas under the plasma concentration - time curves. Physical form (dissolved or suspended) did not impact the *in vivo* performance
Halofantrine (Michaelsen et al., 2016)	Not determined	1 SNEDDS 75% (+/- orlistat) 2 Supersaturated SNEDDS 150% (+/- orlistat) SNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Same dose administered (6.7 mg/kg) Super-SNEDDS resulted in a significantly higher C_{max} compared to SNEDDS; higher AUC, but not statistically different Orilistat increased t_{max}, but did not affect overall *in vivo* performance	Yes, *in vitro* dynamic lipolysis; the amount precipitated from the super-SNEDDS after 60 min of lipolysis was significantly higher than that of the SNEDDS. Addition of orlistat to the SNEDDS resulted in less drug precipitated at 60 min ($P<0.1$) Amorphous precipitate	The *in vitro* data did not fully explain the obtained *in vivo* data; The increased precipitation in the case of the supersaturated SNEDDS would lead to the expectation that the SNEDDS would perform better *in vivo* than the supersaturated SNEDDS, which was not the case
Rosuvastatin Calcium (RC) (Abo Enin and Abdel-Bar, 2016)	35.5 ± 2.01 mg/g (25°C) 49.87 ± 3.54 mg/g (37°C)	1 RC commercial tablet suspension in water 2 pure RC suspension 3 RC saturated SNEDDS 4 solid supersaturated SNEDDS 150% 5 solid supersaturated SNEDDS 200% (not tested *in vivo*) SNEDDS: Garlic + olive oil (22.8%), Tween 80 + PEG400 (77.2%)	Same dose administered 10 mg/kg. Results indicate that liquid or solid supersaturated RC SNEDDS were superior compared to conventional formulations, but limited differences between the two of them	Not the classical lipolysis model, but an *in vitro* precipitation test	Solid carriers were more efficient in sustaining the generated supersaturated state of supersaturated systems in an *in vitro* precipitation test
Cinnarizine (Siqueira et al., 2017)	25 ± 2 mg/g	1 SNEDDS 80% 2 Supersaturated SNEDDS 200% (solution) 3 Supersaturated SNEDDS 200% suspension 4 drug-free SNEDDS + aqueous susp (Chasing principle) 5 aqueous suspension SNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Same dose of cinnarizine. Inferior performance from 2, 3, 4 compared to 1, but 1 and 4 > 5 (statistically different); 2 and 3 < 5	Yes, *in vitro* dynamic lipolysis; A higher extent of cinnarizine in the aqueous phase was observed for all SNEDDS-compared to the aqueous suspension Amorphous precipitate	No rank order relation with the *in vivo* data
R3040 (Siqueira Jørgensen et al., 2018)	205 ± 2 mg/g	1 SNEDDS 80% 2 Supersaturated SNEDDS 200% (solution)	Same dose administered: 20 mg/kg Formulations 1 and 2	Yes, rat gastric and intestinal model and human intestinal model	The data from human-relevant the *in vitro* lipolysis showed no relation between the

(*continued on next page*)

Table 5 (*continued*)

Drug (Reference)	S_{eq} (37°C) or drug load	Formulations	*In vivo*	*In vitro*	Conclusions
		3 Supersaturated SNEDDS 200% suspension 4 drug-free SNEDDS + aq susp (Chasing principle) 5 aqueous suspension SNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	significantly different C_{max} relative to 5. For $AUC_{0\text{-}24h}$, formulations 2 and 3 significantly different relative to formulation 5	Human model: rank order 1>2>3>4=5 Rat model: rank order 1=2>3>4=5 Amorphous precipitate	solubilization of R3040 and the C_{max} or AUC obtained in the *in vivo* study. Rat lipolysis model, a rank order relation between $AUC_{0\text{-}1h}$ and C_{max} and the *in vitro* solubilization
Fenofibrate (Michaelsen et al., 2019)	Not determined	1 SNEDDS (75%) 2 Supersaturated SNEDDS (150%) 3 Supersaturated SNEDDS suspension All formulations with and without orlistatSNEDDS: Soybean oil + Maisine® 35-1 (55%), Kolliphor® RH40 (35%), Ethanol (10%)	Supersaturated SNEDDS had a higher C_{max} and $AUC_{0\text{-}30h}$ compared to SNEDDS and supersaturated SNEDDS suspension, both with and without orlistat. Statistically significant differences found for supersaturated SNEDDS without orlistat	Overall, lower drug concentrations in aqueous phases of digestion medium from lipolysis of supersaturated SNEDDS relative to SNEDDS	The *in vitro* lipolysis data could not predict the absorption of fenofibrate in SNEDDS, supersaturated SNEDDS and supersaturated SNEDDS suspension
Celecoxib (Ilie et al., 2020a)	Maisine® CC; 15.8 mg/mL Capmul® MCM; 57.4 mg/mL Maisine® CC + Labrasol® (4:1); 62.2 mg/mL Capmul® MCM + Labrasol® (4:1); 80.9 mg/mL	1 Maisine® CC (LC), 85% S_{eq} (37 °C) 2 Capmul® MCM (MC), 85% S_{eq} (37 °C) 3 Maisine® CC + Labrasol® ALF (4:1 V/V), 85% S_{eq} (37 °C) 4 Capmul® MCM + Labrasol® ALF (4:1 V/V), 85% S_{eq} (37 °C) 5 Supersaturated Maisine® CC, 85% S_{eq} (60 °C) 6 Supersaturated Capmul® MCM, 85% S_{eq} (60 °C) 7 Supersaturated Maisine® CC + Labrasol® ALF (4:1 V/V), 85% S_{eq} (60 °C) 8 Supersaturated Capmul® MCM + Labrasol® ALF (4:1 V/V), 85% S_{eq} (60 °C)	When dose normalized, the long-chain based formulations in general performed better than the medium chain systems. Adding the surfactant reduced the absorption and the supersaturated systems was at the same level as their saturated counterpart, except for the MCM system where the dose normalized AUC was almost double as high for the Supersaturated MCM when compared to the MCM	Yes, dynamic lipolysis coupled with an absorption step with an artificial membrane, (Permeapad). The flux obtained from the lipolysis of the different formulations followed the rank order of the formulations investigated	Long-chain systems performed better than medium-chain systems. Supersaturation dose normalized led to similar absorption levels in general. The *in vitro* lipolysis was coupled with the Permeapad to allow investigation of absorption during lipolysis, which was able to predict the differences
Venetoclax (Koehl et al., 2020)	Peceol™: between 2.9 ± 0.2 and 19.4 ± 2.0 mg/mL	1 powder capsule 2 Peceol™ suspension 3 Supersaturated Peceol™ solution	3.8-fold increase (statistically significant) in bioavailability from formulation 3 relative to 1	Yes, dynamic lipolysis; The highest venetoclax concentration in the aqueous phase of digestion medium was observed for formulation 3 (statistically significant relative to 1 and 2)	Supersaturated solid lipid-based drug delivery system led to an increased *in vivo* exposure of approximately 4-fold and 2-fold compared to a venetoclax powder capsule and lipid suspension respectively. The *in vitro* lipolysis provided a mechanistic basis for explaining the supersaturated solid lipid-based drug delivery system performance

lipase inhibitor did not influence the absorption from the supersaturated formulations. The authors provided no explanation for this phenomenon in neither of the papers.

Thomas et al. (2013) investigated the same medium chain formulation with simvastatin in dogs dosing again one or two capsules containing simvastatin at 75% of the saturated solubility in the formulation at 25°C or one capsule contain the same amount of compound as the two capsules, i.e., termed a 150% supersaturated formulation. A dose proportional AUC was reported between the animals dosed with one or two capsules. However, as reported for halofantrine, the supersaturated formulation system outperformed the saturated formulations with respect to the obtained AUC (Thomas et al., 2013). In a last study of this series of experiments, Thomas et al. (2014) investigated the performance in Göttingen mini-pigs using fenofibrate in a LCT type of lipid-based formulation based upon 24% long-chain triglyceride, 32.2% long chain monoglyceride, 20% Kolliphor® RH40 and 13.8% ethanol. In accordance with the results reported for cinnarizine, the bioavailability was similar when administering two capsules with fenofibrate below the saturation level compared to one capsule with the same composition containing the doubled amount of fenofibrate, i.e. corresponding to a supersaturated formulation. In accordance with these results Siqueira Jørgensen et al. (2018) reported similar absorption of an undisclosed experimental compound, R3040, when rats were given similar doses as a non-saturated and supersaturated long-chain based lipid formulation of type IIIA. These data altogether support that supersaturated lipid-based formulations may provide a similar bioavailability for lipophilic compounds when given as equal doses but in non-saturated or supersaturated lipid-based formulations The latter formulations would then have the advantage of a reduced pill count, i.e., number of capsules per given dose.

Ilie et al. (2020a) investigated type I and II formulation based on medium- and long-chain lipids in rats using the lipophilic compound celecoxib. In rats, the highest bioavailability was reported for the supersaturated type I formulation for both lipid types. Adding the surfactant, i.e., the type II formulation, reduced the bioavailability for the supersaturated formulations, but not that of the non-saturated formulations. Interestingly Ilie et al. (2020a) expanded the *in vitro* lipolysis model with a permeation step, which increased the predictability of the formulation relative to the results obtained *in vivo*, hence this approach could beneficially be explored further to investigate the supersaturated lipid-based formulations more mechanistically.

Table 6
Overview of *in vivo* studies evaluating supersaturated lipid-based drug delivery systems with thermally induced supersaturation and solidified with silica. The chemical composition of the lipid tradenames is described in Table 3.

Drug/reference	S_{eq} (37°C) or drug load	Formulations	*In vivo*	*In vitro*	Conclusions
Ibuprofen (Schultz et al., 2019)	S_{eq} (Capmul® PG8) = 211 mg/ml	1 Supersaturated silica-lipid hybrid A (10% drug, 45% liquid, 45% solid) 99.5% S_{eq} 2 Supersaturated silica-lipid hybrid B (20% drug, 40% lipid, 40% solid) 227% S_{eq} 3 Supersaturated silica-lipid hybrid C (30% drug, 35% lipid, 35% solid) 389% S_{eq} 4 Spray dried Supersaturated silica-lipid hybrid (9.5% drug, 60% lipid, 30% solid) 5 Nurofen® Lipid: Capmul PG8 + soybean lecithin (emulsifier),Solid: Nanoporoussilica microparticles	Suspensions, dose of 10 mg/kg Rank order:4 = 1 = 2 > Nurofen® > 3 Formulations 1,2 and 4 statistically significant different versus Nurofen®	Dissolution studies using USP Type II Paddle Apparatus The dissolution decreased with an increase in supersaturated drug load, which was associated with an increase in crystalline IBU content Rank order: spray-dried > A ≥ B > Nurofen® > C	The Pearson correlation coefficient confirmed a strong IVIVC (r = 0.9137, P < 0.0109) between *in vitro* dissolution and *in vivo*. The correlation suggests that the *in vitro* dissolution study in pH 2.1 media may offer a simple initial test to predict the performance of these formulations *in vivo*
Abiraterone acetate (Schultz et al., 2020)	Capmul® PG8: 147.5 mg/g Capmul® MCM: 113.0 mg/g	1 Silica-lipid hybrid 90 (6.2% drug, 46.9% lipid, 46.9% solid) 90% S_{eq} 2 Supersaturated silica-lipid hybrid P150 (10% drug, 45% lipid, 45% solid) 150% S_{eq} 3 Supersaturated silica-lipid hybrid P200 (12.8% drug, 43.6% lipid, 43.6% solid) 200% S_{eq} 4 Supersaturated silica-lipid hybrid P250 (15.6% drug, 42.2% lipid, 42.2% solid) 250% S_{eq} 5 Supersaturated silica-lipid hybrid M200 (10.2% drug, 44.9% lipid, 44.9% solid) 200% S_{eq} 6 Zytiga® 7 Unformulated drug Lipid: Capmul PG8 or Capmul MCM Solid: Nanoporous silica microparticles	Doses of 25 mg/kg, suspended in 0.4% CMC solution Formulation 1: %F increased 31-fold versus unformulated drug Formulations 2, 3, 4 %F increased 11, 10 and 7-fold, respectively versus unformulated drug Formulation 1: %F increased 1.43-fold versus 6 and Supersaturated silica-lipid hybrid had no improvement	*In vitro* dissolution (pH = 2) - rank order: 1=2=3=4>6> pure drug *In vitro* lipolysis (fasted state simulated intestinal fluid, FaSSIF + lipase): rank order: 1>2=3=4=5>Zytiga>pure drug	The F% of the Supersaturated silica-lipid hybrid P150, P200 and P250 were low (4.3, 4.0 and 2.9%, respectively) Correlations were made between the $AUC_{0\text{-}1h}$ of the *in vitro* solubilization-time curves for both dissolution and lipolysis studies, and the $AUC_{0\text{-}8h}$ of the *in vivo* plasma drug concentration–time curves, for each oral formulation
Simvastatin (Meola et al., 2020)	Capmul® PG8 80.3 ± 2.7 mg/g Capmul® MCM 69.3 ± 1.9 mg/g	1 unformulated simvastatin 2 Simvastatin Sandoz® 3 Silica-lipid hybrid-A@ (100% S_{eq}, Aerosil® 300) 4 Silica-lipid hybrid-A2x (200% S_{eq}, Aerosil® 300) 5 Silica-lipid hybrid A4x (400% S_{eq}, Aerosil® 300) 6 Silica-lipid hybrid-S2x (200% S_{eq} Syloid® 244)	Silica-lipid hybrid formulations enhanced the oral bioavailability of simvastatin up to 6.1-fold and 2.9-fold (greatest from formulation 6), in comparison to 1 and 2	Yes, a modified version of the USP dissolution monograph for simvastatin. During *in vitro* dissolution in pH 7.0 media, the silica-lipid hybrid formulations (3-6) performed up to 4.4-fold greater than formulation one	Silica-lipid hybrid technology was used as a promising solid-state lipid-based drug delivery system for reformulation and for the oral delivery of simvastatin

While all other studies investigating the use of supersaturated lipid-based formulations worked with lipophilic compounds having essentially a high lipid solubility, Koehl et al. (2020) investigated the possibility of using supersaturated lipid-based formulations for a brick dust molecule, i.e., venetoclax. Koehl and coworkers (2020) conducted *in vitro* lipolysis experiments with venetoclax in four supersaturated pure type I formulations: MCT and LCT as well as medium- and long chain monoglycerides. The medium monoglyceride was reported to provide the best dissolution in an *in vitro* dissolution, hence this system was chosen for *in vivo* investigations. Koehl et al. (2020) investigated three different formulations in landrace pigs, a powder filled capsule or two lipid-based formulation with the exact same composition, one in which venetoclax was suspended in the lipid and one where it was prepared as a supersaturated lipid-based formulation. The supersaturated lipid-based formulation showed a 2.1-fold higher bioavailability relative to the lipid suspension and a 3.8-fold higher bioavailability compared to the venetoclax powder capsule, which in the latter case was statistically significantly different ($p < 0.05$). Additionally, the supersaturated lipid-based formulation showed a faster absorption and shorter residence time in pigs (Koehl et al., 2020).

4.3. Thermally induced supersaturated lipid-based formulations adsorbed onto silica

Schultz et al. (2018) developed a hybrid supersaturated lipid-based formulation, where ibuprofen was firstly supersaturated into a lipid vehicle and subsequently added to mesoporous silica. This adsorbate enabled to get a solid dosage form and this delivery approach has been investigated in a number of *in vivo* studies from the same group, see Table 6. In a subsequent study Schultz (2019) and coworkers investigated how this formulation type increased the oral absorption in rats. The animals were administered with a commercial ibuprofen solution, a non-saturated spray dried emulsion or the hybrid supersaturated formulation with a saturation degree of 100, 227 or 389%. The highest oral absorption was seen in the spray dried formulations. The supersaturated formulations did not produce any additional absorption of ibuprofen, in accordance with *in vitro* dissolution conducted in simulated intestinal media (Schultz et al., 2019).

Simvastin was tested in a similar hybrid formulation, where the compound was dissolved in a mixture of medium chain mono- and diglycerides at 100, 200 or 400% of the compound solubility at ambient temperature by heating (Meola et al., 2020). To this lipid solution, lecithin was added, and the mixture was homogenized with a high pressure and subsequently mixed with a dispersion of one of two types of mesoporous silica. The obtained system was subsequent spray dried and redispersed into water before dispersing it into the dissolution media or administration to rats. The *in vitro* dissolution studies suggested that the system with a supersaturation of 200% would perform better that the other investigated systems, which agreed with the *in vivo* data. The formulation with 200% supersaturated performed better than both the 400% supersaturated system and the unsaturated system. There was some influence of the type of mesoporous silica, though it was not statistically different (Meola et al., 2020). Abiraterone acetate was tested in a similar system by Schultz et al. (2020) with either a MCT or a mixture of medium-chain, mono-, di- and triglycerides as the lipid phase with saturation degrees from 90 to 250%. Schultz and colleagues (2020) conducted some *in vitro* lipolysis experiments. The results suggested that the non-saturated formulation would perform as good if not better than the supersaturated formulations, which was in agreement with the *in vivo* data presented. While the mesoporous formulations enabled transforming a lipid-based formulation into a solid dosage form, there is no clear evidence that the silica at the same time can prevent precipitations from the supersaturated formulations (Schultz et al., 2020).

4.4. Thermally induced supersaturated lipid-based formulations containing precipitation inhibitors

Ilie et al. (2021) investigated the combination of thermally induced supersaturated lipid-based formulations with addition of precipitation inhibitors to the formulations, see Table 7. Ilie and colleagues (2021) made a first screen of relevant precipitation inhibitors using a high throughput approach with a solvent shift approach, i.e., the compound, cinnarizine, dissolved in N-Methyl-2-pyrrolidone (NMP) was added to a fasted state simulated intestinal fluid (FaSSIF) medium containing 1% (w/v) and using this approach, 21 different precipitation inhibitors were evaluated. The five precipitation inhibitors that performed best in a high throughput screening were selected and their influence on cinnarizine precipitation in pure LCT or MCT was tested, i.e., the investigated formulations represented a type I formulation according to the lipid classification system. The five tested precipitation inhibitors were Poloxamer 407, Kolliphor® HS15 and RH40, Vitamin E-TPGS and Soluplus®. *In vivo* studies in rats demonstrated an enhanced bioavailability (dose adjusted) for all added precipitation inhibitors in both the LCT and MCT vehicles, with a factor of 2-3 dependening on the given inhibitor. No statistical difference could be identified between the precipitation inhibitors, but across the two tested vehicles, there was a trend that Soluplus® provided the most consistent bioenhancement with the lowest variation (Ilie et al., 2021), see Fig. 3.

The study by Ilie et al. (2021) indicated a preference for precipitation inhibitors that were miscible with the tested vehicles. This was similar to the observations reported previously by Suys et al. (2018) who also screened excipients broadly.

Table 7
Overview of in vivo studies evaluating supersaturated lipid-based drug delivery systems with thermally induced supersaturation containing precipitation inhibitors. The chemical composition of the lipid tradenames is described in Table 3.

Drug	S_{eq} (37°C) or drug load	Formulations	*In vivo*	*In vitro*	Conclusions
Cinnrazine (Ilie et al., 2021)	Maisine® CC; 55.3 ± 2.5 mg/mL Capmul® MCM; 48.6 ± 3.3 mg/mL	1 Supersaturated Maisine CC (LC), 85% S_{eq} (60 °C) 2 Supersaturated Capmul® MCM (MC), 85% S_{eq} (60 °C) 3 Supersaturated Maisine® CC (sLC) + surfactant, 85% S_{eq} (60 °C) 4 Supersaturated Capmul® MCM (sMC) + surfactant, 85% S_{eq} (60 °C) Surfactants for formulation 3 or 4 was one of the following; Poloxamer 407, Kolliphor® HS15, Kolliphor® RH40, vitamin E-TPGS or Soluplus®	For both supersaturated long LCM and supersaturated MCM it was beneficial to add a precipitation inhibitor, as this increased the dose normalized AUC. Poloxamer 407 performed best in both supersaturated LCM and supersaturated MCM vehicles, though no statistical difference was observed in the dose normalized AUCs among the vehicles with added precipitation inhibitors	Yes, precipitation studies were conducted in FaSSIF media to identify the most interesting precipitation inhibitors. The rank order found *in vitro* did not match the rank order observed *in vitro*	Adding precipitation inhibitors, in the form of surfactants, to supersaturated lipid-based formulations in general increased the fraction of cinnarizine absorbed

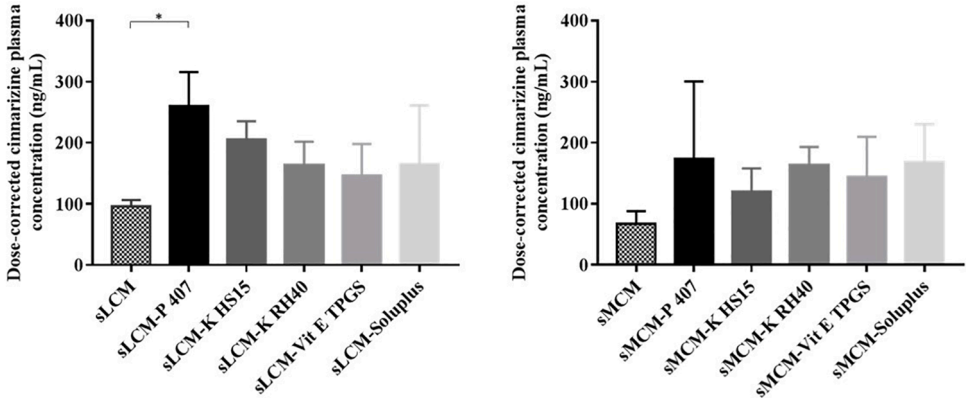


Fig. 3. Dose-normalized area under the concentration time curve for supersaturated lipid based drug delivery systems consisting of one lipid excipient (LCM or MCM) without or with precipitation inhibitors, reproduced with permission from Ilie et al. (2021).

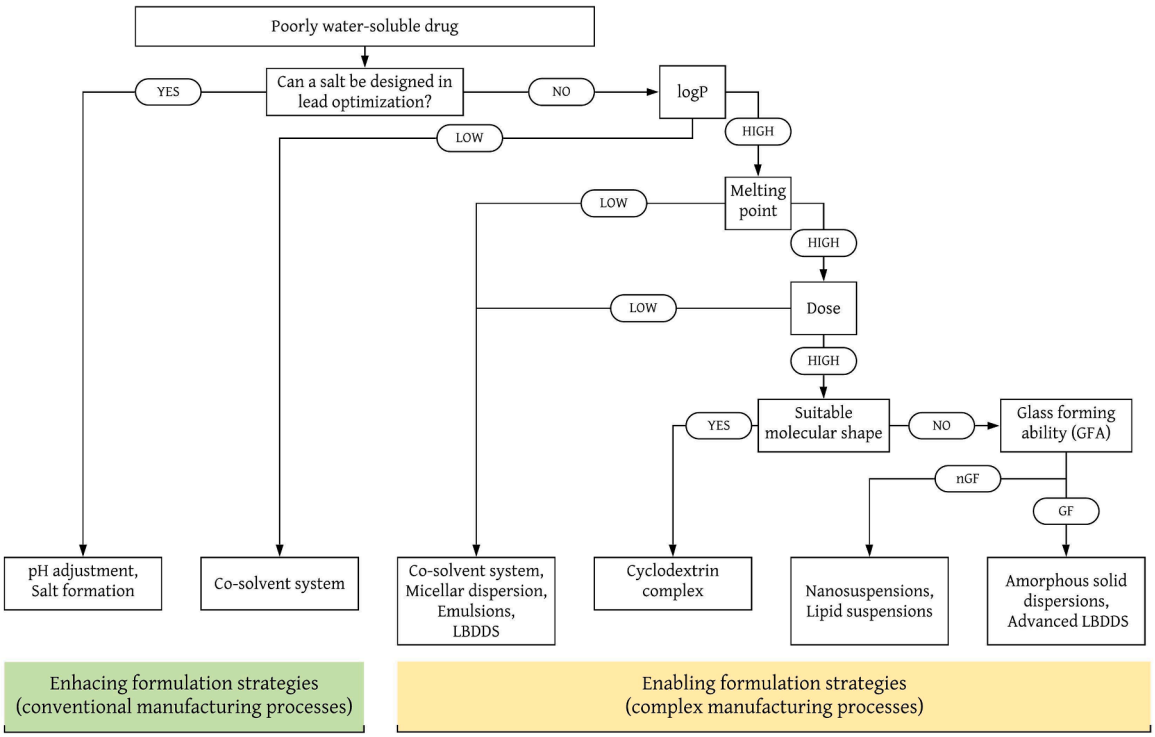


Fig. 4. Schematic representation of formulation selection strategies based on physico-chemical properties of poorly water-soluble drugs (reproduced from Kuentz et al. (2016) with permission); nGF = non glass former, GF = glass former.

5. Supersaturated lipid-based formulations in a development context

The formulation strategy for any new drug will depend on numerous factors, including the drugs physicochemical characteristics, pharmacokinetic and pharmacodynamic properties, shelf-life, manufacturability as well as company traditions and experience (Fridgeirsdottir et al., 2016). In the pharmaceutical industry, selection of a suitable formulation strategy also contains elements such as limited resources, reduced timelines, and stringent regulatory requirements (Kuentz et al., 2016). Several bio-enhancing (salt formation, particles size reduction) and bioenabling (cyclodextrins or cosolvent systems, lipid-based drug delivery systems or amorphous solid dispersions) technologies are available for the formulation scientist to ensure optimal drug exposure in early drug development where drug absorption is essential for pharmacokinetic and toxicokinetic assessment of drug molecules (Williams et al., 2013a; Van den Bergh et al., 2018). These technologies are shown in Fig. 4 as a decision tree based on the compound's physicochemical properties. It is important to differentiate between enhancing and enabling formulation strategies given that the development is influenced by the applied manufacturing technology, the time and resources invested in determining suitable excipient and optimal excipient ratios (Van den Bergh et al., 2018).

Generally, easy-to-produce conventional solutions or suspensions tend to be employed in early assessment of a new compound's *in vivo* characteristics. For preclinical screenings, molecules may be dissolved in a variety of vehicles that can match the required solvation capacity (Van den Bergh et al., 2018). Studies to determine the absolute bioavailability for the compound, if dosed as a solution relative to a suspension, are often performed to identify the need for conventional or bio-enabling formulations in later stages of development. Van Den Bergh and coworkers (2018) conducted a retrospective analysis of preclinical and clinical formulation strategies for Janssen's drug molecules and suggested that the dog was the best discriminating animal species for

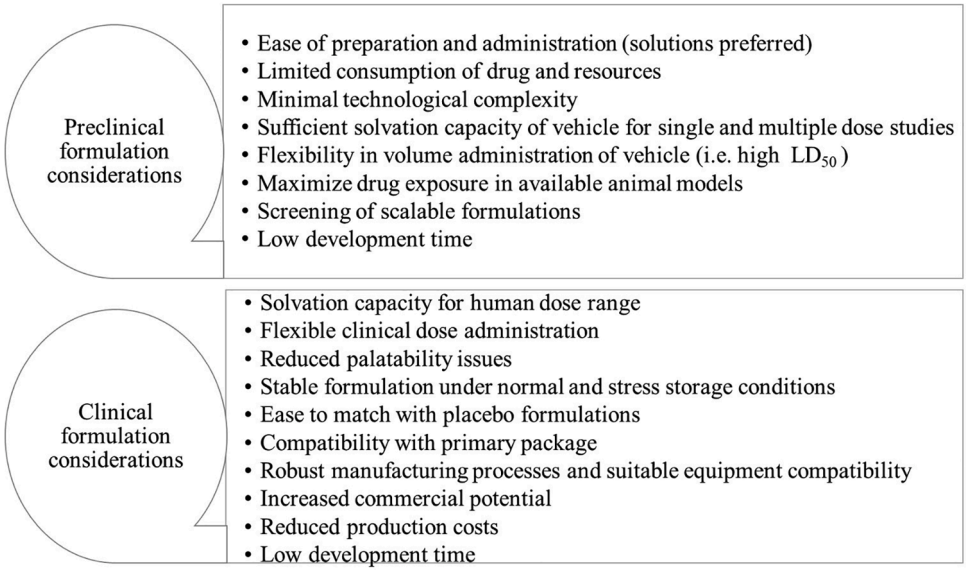


Fig. 5. Overview of considerations for formulation selection in preclinical and clinical development (non-exhaustive list).

these investigations to evaluate the need for solubility enhancing or enabling formulation strategy (Van den Bergh et al., 2018). Interestingly, the selected formulations in early preclinical development were not always the same as the ones used in late phases of clinical drug development. An overview of formulation selection considerations in preclinical and clinical studies is illustrated in Fig. 5.

5.1. *Design of supersaturated lipid-based drug delivery systems*

Despite limitations in terms of commercial scalability, supersaturated lipid-based formulations have several advantages relevant for considerations, at least in the preclinical development or early-stage clinical development. The concept of supersaturated lipid-based formulations may be relevant in situations where high doses of poorly water-soluble compounds are needed. As outlined here supersaturated lipid-based formulations have shown promising results in terms of improving or matching drug exposure versus conventional lipid-based formulations or aqueous suspensions for drugs as celecoxib, venetoclax, simvastatin, halofantrine, fenofibrate and R3040, i.e., both grease balls and brick dust molecules. The advantages of supersaturated lipid-based formulations are ease of preparation, increased drug loading capacity and possibilities to reduce drug precipitation via inclusion of precipitation inhibitors or through solidification approaches. These systems can therefore be considered as an alternative to type IV lipid-based formulations or amorphous solid dispersions. A proposed development path of supersaturated lipid-based formulations is hence provided in the following sections. The focus in this section is on formulations where the formulation is supersaturated on the shelf, not

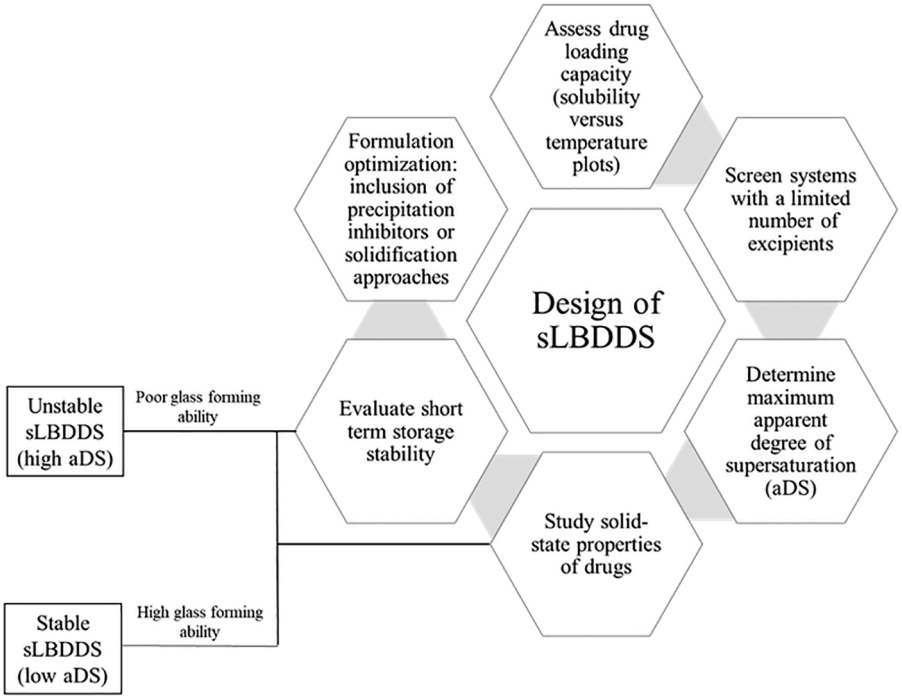


Fig. 6. Preformulation testing to guide design of supersaturated lipid-based formulations (sLBDDS).

on in situ formed supersaturated formulation, as existing literature in principle already exist for the latter.

5.1.1. Preformulation studies for supersaturated lipid-based formulations

The first step in the development of a supersaturated lipid-based formulation is to determine the drug solubility in lipid excipients and mixtures, as depicted in Fig. 6. Ideally, the whole dose should be dissolved by the tested lipid vehicle (Kuentz et al., 2016). It is recommended to limit the number of excipients added to the mixtures to facilitate the identification of the role and contribution of each excipient to the formulation's performance. Further, the drug propensity to supersaturate can be assessed by calculation of the apparent degree of supersaturation.

It should be emphasized again that critical preformulation experiments in developing a supersaturated lipid-based formulations are to evaluate short-term stability and compatibility with various excipients or mixtures to minimise the risk of failures in further development. Physicochemical properties of the drug, especially solid-state characteristics, seem to be very relevant for the drug stability in the supersaturated lipid-based formulations, whereby high T_m/T_g ratio may imply a higher crystallization tendency (poor glass forming ability) and hence a more unstable supersaturated formulation with a risk of drug crystallisation when the lipid-based formulation are stored at 25°C. In cases where the biopharmaceutical assessment of the supersaturated lipid-based formulation indicates a poor drug exposure as a result of increased precipitation tendency, formulation adjustments should be considered such as inclusion of precipitation inhibitors or even a formulation as solid dosage forms.

5.1.2. In vitro testing of supersaturated lipid-based formulations

Biopharmaceutical profiling approaches in early stages of drug discovery and development through solubility testing in biorelevant media, physiological based dissolution studies, permeability studies in cell lines, or *in silico* modelling provide an important part of compound selection, which constitutes a well implemented process in drug discovery. Biopharmaceutical profiling of lipid-based formulations includes assessment of dispersibility and droplet size measurements from dilution and dispersion in biorelevant media, *in vitro* dynamic lipolysis to mimic intestinal digestion, gastro-intestinal transfer models, *in situ* permeation studies or simultaneous dispersion/digestion-permeation models (Swarnakar et al., 2018; Butler et al., 2019; Berthelsen et al., 2019).

Extensive attention should be given to the *in vitro* assessment of new formulations and as such supersaturated lipid-based formulation are no different than other formulations. While the literature in general does not agree on how to evaluate lipid-based formulations *in vitro*, the general trend for supersaturated formulations is to conduct three types of experiments. Thus, solvent shift studies should be conducted to select precipitation inhibitors, dispersion studies to investigate the ability of the formulation system to stay supersaturated and finally, *in vitro* lipolysis to investigate the potential precipitation during digestion of the vehicle. The lipolysis model can potentially be linked to an absorption process, as suggested by Ilie et al. (2020a) with the application of the Permeapad model. As the literature provides no clear guidance on which approach that provides the best correlation between *in vitro* and in *vivo* data, it is suggested that precipitation inhibitors are selected by the solvent shift methodology and that at least some *in vitro* lipolysis experiments are conducted to select the most promising formulations for subsequent *in vivo* testing.

5.1.3. In vivo assessment of supersaturated lipid-based drug delivery systems

The literature has reported positive biopharmaceutical performance of the supersaturated lipid-based formulation in rats, dogs, mini-pigs and land race pigs. A relevant control to include in *in vivo* studies with supersaturated lipid-based formulations is an aqueous suspension and an unsaturated lipid-based formulation to verify any potential benefits both from the lipids as well as from the supersaturation. The *in vitro* studies and the characterization should indicate if a precipitation inhibitor should be considered, which could also be tested. This far, the literature does not provide clear general learnings on to which vehicle to select. Thus, whether or not medium chain lipids are better than long chain lipids, or if type I formulations are better than type IIIB etc., are still open academic questions so the experience of the formulator will at least for now have to drive the selection of formulations to test *in vivo*. The ability to generate supersaturated formulations seems to have driven the research into investigation of a few defined vehicles with a

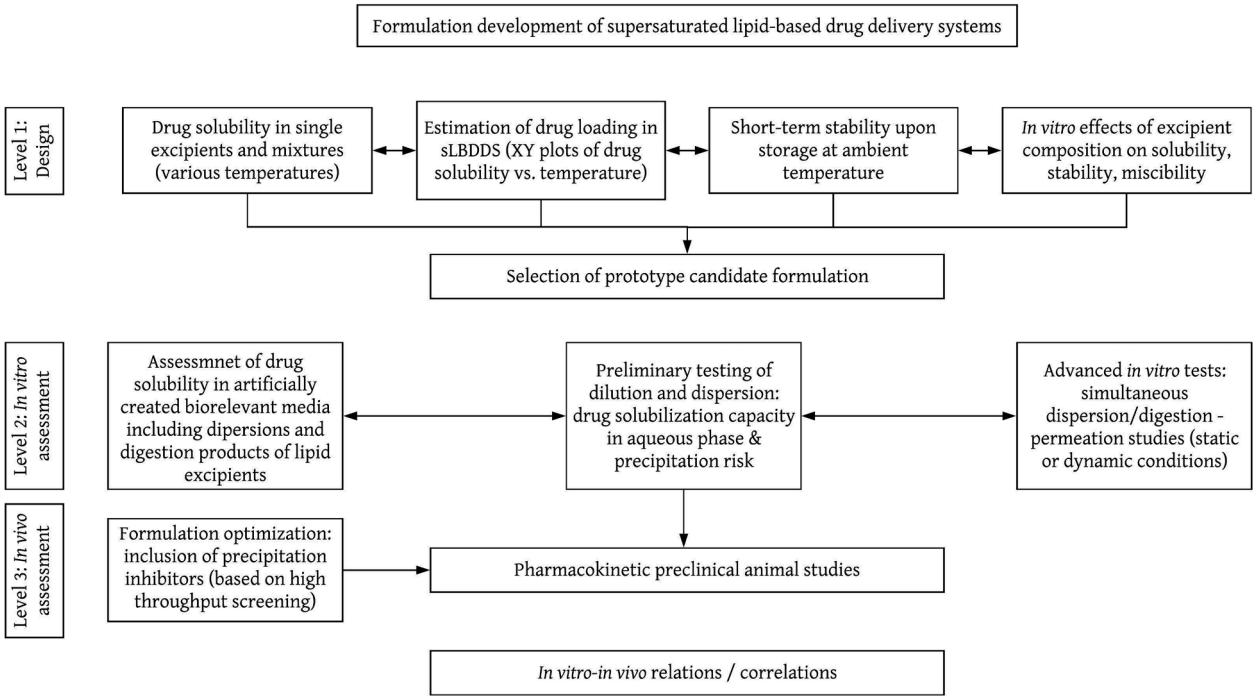


Fig. 7. Suggested development process for supersaturated lipid-based drug delivery systems.

range of different compounds to demonstrate that the concept is general applicable, however, the approach is now so established that systematic research into supersaturated lipid-based formulations would be beneficial. This could include elements as systematically investigate different vehicles within one class of the lipid classification system and well as better clarify when medium and when long chain lipids are beneficial. Also, more investigations into solvents used in the supersaturable systems and systematic precipitation inhibitor screens for both supersaturable and supersaturated lipid-based formulations combined with *in silico* predictions and subsequently *in vivo* studies, could help provide some generalized guidance in the formulation work. Despite the lack of general teachings from the literature, we still find it possible to suggest a schematic representation of high-level development of supersaturated lipid-based formulations including the different characterization models tested that could be considered when formulating a supersaturated lipid-based formulation, see Fig. 7. This scheme should be seen as a starting point for discussion among formulation scientists to investigate new drug molecules in supersaturated lipid-based formulations, where certainly a lot of scientific gaps can still be identified.

6. Future perspectives for supersaturated lipid-based formulations

While much potential of using supersaturated lipid-based system is obviously given in pre-clinical formulation applications, there is also the potential to obtain viable clinical or even market formulations. Overall, there are some challenges to further advancement of supersaturated lipid-based formulations to the market with respect to variability of excipients, availability of manufacturing and technology transfer processes, structured formulation design guidelines, and regulatory specifications for lipid excipients. However, for supersaturated lipid-based formulation a key limitation for clinical use is the risk of instability and therefore the lack of a commercially suitable long term shelf life. This limitation has guided recent studies towards exploration of novel solidification methods for supersaturated lipid-based formulation such as adsorption on silica or polymeric materials, melt granulation or 3D printing, though these have not truly demonstrated their benefit yet. Screening for excipients that could improve long-term storage stability of supersaturated lipid-based formulation should be a focus of further research. Formulation of the supersaturated lipid-based formulation into a solid system could be extremely advantageous from an industrial perspective.

In the pharmaceutical space, 3D printing offers the advantages of drug distribution control inside the dosage form, use of small drug amounts, reduced waste, fast production of various compositions for rapid screenings and the ability to manufacture patient-personalized dose strengths and drug combinations (Vithani et al., 2019). Although many challenges remain to be solved with 3D printing in manufacturing of solid dosage forms, proof-of-concept studies (with fenofibrate and cinnarizine) have already showed the potential for lipidic and polymeric excipients (i.e. Compritol® 888 ATO, Gelucire® 44/14, Gelucire® 48/16 and Poloxamer 188, PVP, MCC, PEG or HPMC) to serve as materials for such novel dosage forms and show the clear opportunities for 3D printing using lipids and polymers to satisfy unmet medical needs (Vithani et al., 2019; Siepmann et al., 2019).

Another way to advance development of supersaturated lipid-based formulation could be to focus efforts on simple compositions that can be rapidly screened in preclinical studies and offer mechanistic understanding into the role of each excipient in the *in vitro* and *in vivo* behavior of such lipid systems. Additionally, integration in early formulation development of computational techniques may also improve the understanding on the role of different excipients and their interactions, as well as guiding selection of optimized compositions with the desired profile (Mu et al., 2013; Alsenz and Kuentz, 2019). Moreover, compiling best practices for design and production of supersaturated lipid-based formulation should be of future interest, with a focus on assessment of initial storage conditions of drug and excipients prior to heating phase, time and frequency of heating-cooling cycles, minimum and maximum temperatures, etc. Availability of a standard design and characterization *in vitro* tools package for supersaturated lipid-based formulation is highly desirable.

With respect to formulation of supersaturated lipid-based formulation, early assessment of short-term stability, assessment of *in vitro* dispersibility and drug precipitation risk upon dilution in biorelevant media, simultaneous dispersion-permeation study, and high throughput screening of precipitation inhibitors to be included in supersaturated lipid-based formulation could be further explored to facilitate the use of the formulation option. Given their versatility in the preclinical space, ease of preparation and dose loading capabilities, supersaturated lipid-based formulation may have the advantage of streamlining the formulation from bench to clinics. Thus, not only it would be an easy and accessible option to assess *in vivo* behavior of poorly water-soluble compounds after administration of different doses, but it would also reduce the time invested in bridging preclinical and clinical formulation types and allow flexibility in administration of a dose range in clinical trials.

7. Conclusions

Lipid-based formulations are often generating drug supersaturation in situ and this approach has demonstrated to enhance the absorption of many drug candidates with poor biopharmaceutical properties. However, these formulations may have shortcomings with respect to the maximum dose loading capacity, hence supersaturated lipid-based formulations have been suggested as an alternative strategy to circumvent this limitation. The supersaturation is thermally induced by the formulator by heating the formulation to e.g. 60°C to dissolve the compound at this elevated temperature. The literature has suggested that up to 400% supersaturated formulations can be made in this way. Limited data is available on the physical stability of these supersaturated systems, but data available suggest that compounds with a high crystallization tendency may be less stable, whereas compounds with low crystallization tendency appear to make good candidates for this formulation approach.

The *in vivo* data for most of the supersaturated formulations investigated have shown an increased bioavailability relative to an unsaturated lipid-based formulation, but there are also examples of compounds for which the supersaturation approach did not increase oral bioavailability. This far the amount of data is too limited to provide general guidelines in the field of supersaturated systems, but the compiled data suggest a promising application especially in formulation supply for preclinical investigations of new drug compounds. The use of precipitation inhibitors was clearly demonstrated to be beneficial for the *in situ* supersaturated formulations and data also suggest that this may be the case for thermally induced supersaturated formulations, though this requires additional research to conclude on a more general term. Altogether, the present review has shown that supersaturated lipid-based formulations represent attractive oral delivery options, there are also several knowledge gaps and future research would help to provide further guidance to formulators based on a broader experimental basis than what is available today.

While supersaturated lipid-based formulations contain a huge potential, they are with the present scientific insights best suited for preclinical formulation supply or evaluations under very controlled clinical settings as simple phase I studies. While this may seem like a complicated task, the fact that the formulation approach holds promise for both grease ball and brick dust molecules provide extra arguments to continue the research in the field.

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RH, BG, MK and AI was involved in the conceptualization of the work and the review and editing of the manuscript. RH and AI each wrote parts of the original draft.

Declaration of Competing Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Data availability

No data was used for the research described in the article.

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