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EXPERT REVIEW

Lipid-Based Formulations and Drug Supersaturation: Harnessing the Unique Benefits of the Lipid Digestion/Absorption Pathway

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ABSTRACT Drugs with low aqueous solubility commonly show low and erratic absorption after oral administration. Myriad approaches have therefore been developed to promote drug solubilization in the gastrointestinal (GI) fluids. Here, we offer insight into the unique manner by which lipid-based formulations (LBFs) may enhance the absorption of poorly water-soluble drugs via co-stimulation of solubilization and supersaturation. Supersaturation provides an opportunity to generate drug concentrations in the GI tract that are in excess of the equilibrium crystalline solubility and therefore higher than that achievable with traditional formulations. Incorporation of LBF into lipid digestion and absorption pathways provides multiple drivers of supersaturation generation and the potential to enhance thermodynamic activity and absorption. These drivers include 1) formulation dispersion, 2) lipid digestion, 3) interaction with bile and 4) lipid absorption. However, high supersaturation ratios may also stimulate drug precipitation and reduce exposure where re-dissolution limits absorption. The most effective formulations are likely to be those that generate moderate supersaturation and do so close to the site of absorption. LBFs are particularly well suited to these criteria since solubilization protects against high supersaturation ratios, and supersaturation initiation typically occurs in the small intestine, at the absorptive membrane.

KEY WORDS absorption · bile salt · digestion · lipid-based formulations · polymeric precipitation inhibitors · solubility · supersaturation

ABBREVIATIONS

AP_{DIGEST}	The colloidal aqueous phase formed on digestion of a lipid formulation
AP_{MAX}	The maximum theoretical drug concentration that can be solubilized in the AP_{DIGEST} at a particular dose
FA	Fatty acid
LBF	Lipid-based formulation
LC	Long-chain
LFCS	Lipid Formulation Classification System
MC	Medium-chain
MG	Monoglyceride
PPI	Polymeric precipitation inhibitor
PWSD	Poorly water-soluble drug
S_{DIGEST}	Drug solubility in the colloids formed on digestion of a lipid-based formulation
S_{DISP}	Drug solubility in the colloids formed on dispersion of a lipid-based formulation
S_{free}	Drug solubility in the free (non-colloidal) phase

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SPF^{DIGEST}	The supersaturation promotion factor induced by digestion
SR	Supersaturation ratio
SR^{M}	The maximum supersaturation ratio formed on digestion
TG	Triglyceride
UWL	Unstirred water layer

INTRODUCTION

The oral absorption of poorly water-soluble drugs (PWSD) is frequently low and variable, but may be increased through a variety of formulation strategies designed to increase drug solubilization in the gastro-intestinal (GI) tract (1). For lipophilic PWSD with high solubility in lipids and/or surfactants, lipid-based formulations (LBFs) provide a relatively simple means by which oral exposure may be enhanced (2). The well recognized solubilization power offered by combinations of lipids, surfactants and/or cosolvents in LBFs has led to the historical view that LBFs enhance drug absorption by circumventing traditional dissolution processes and by boosting the solubilization capacity of the endogenous GI fluids – together resulting in the presentation of high solubilized drug concentrations for absorption across the intestinal wall (2–4). The value of solubilization enhancement and dissolution avoidance offered by LBFs is illustrated in Fig. 1a and is supported by several studies that show evidence of *in vitro – in vivo* rank order correlations between the concentrations of solubilized drug attained during *in vitro* dispersion and digestion tests and oral bioavailability (5–7).

It is becoming increasingly apparent, however, that many of the formulation approaches that enhance apparent drug solubility in the GI tract, do so via the generation of supersaturation. This in turn provides additional benefits for drug absorption via increases in thermodynamic activity (8–12). Thus, many 'enabling' formulations, including amorphous solid dispersions and mesoporous silica systems, are effective in generating drug concentrations in the GI tract that are above the solubility of the thermodynamically stable crystal form. In recent years, these discussions have extended to the potential for LBFs to promote *in situ* supersaturation. This has been prompted in part by the realization that dispersion and digestion can lower the solubilization capacity of a LBF (Fig. 1b), and that the unique manner in which LBFs integrate into endogenous lipid digestion/absorption pathways may promote supersaturation initiation and enhance drug absorption where supersaturation can be maintained (9,10,13–15). For solubilizing formulations such as LBFs, supersaturation provides a compelling means to combat the inevitable decrease in free concentration/thermodynamic activity that accompanies solubilization within colloidal species such as micelles.

The first studies to highlight the potential contribution of supersaturation to the performance of LBFs were performed by Gao and co-workers, and showed that the risk of drug precipitation on dispersion of self-emulsifying drug delivery systems (SEDDS) could be attenuated by co-formulation with polymeric precipitation inhibitors (PPIs). The data further showed that the reduced risk of precipitation afforded more sustained supersaturation and improved *in vivo* performance (9,10,16). More recently, it has become apparent that supersaturation may occur not only as a result of LBF dispersion, but also because of other factors including; initiation of

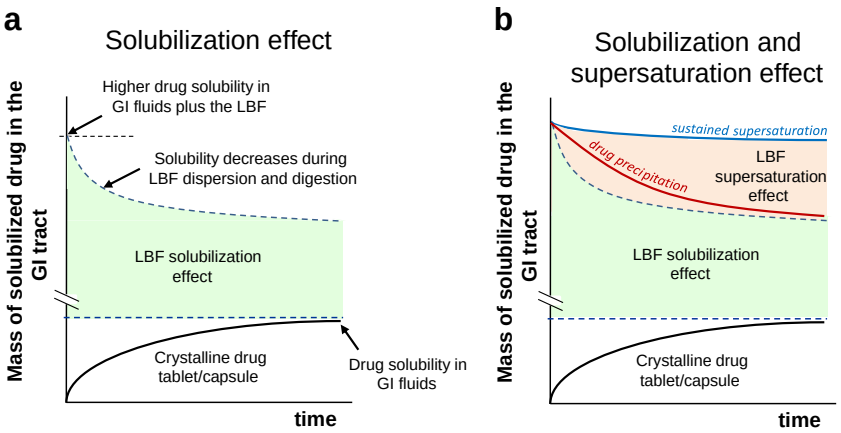

Fig. 1 Schematic representation of the solubilization and supersaturation effects afforded by lipid-based forumulations (LBFs). Lipids and surfactants (and to a lesser extent cosolvents) boost drug solubilization capacity in the GI tract following the dispersion and digestion of LBFs. This is described in (**a**) as the "solubilization effect", and as shown, this solubilization effect affords higher stable drug concentrations than traditional solid dosage forms containing crystalline drug. In addition, the changing nature of colloidal species formed as LBFs disperse and become integrated into lipid digestion and absorption pathways, and the progressive decreases in drug solubility in these colloids that typically occur as these processes continue, is such that LBFs have the capacity to generate supersaturation, described above in (**b**) as the "supersaturation effect". This effect is maximised in formulations where supersaturation is sustained (blue line) and limited where precipitation is rapid (red line)

digestion of formulation components such as glyceride lipids and fatty acid ester surfactants (13,17), by interaction with bile (18,35) and as a consequence of lipid absorption from intestinal micelles (15). When the degree of supersaturation generated is high, precipitation and decreased *in vivo* drug absorption is likely (particularly where drug permeability, and therefore absorption rate, is low) (5,7,19). However, where the degree of supersaturation is lower or where drug precipitation is inhibited by the inclusion of PPIs (and especially where drug permeability is high), kinetically stable periods of supersaturation may be generated that are sufficient to enhance drug absorption (9,13,16).

In light of the growing interest in the role of supersaturation in the performance of LBFs, our laboratory has recently focused on better understanding the mechanisms by which LBFs generate and maintain supersaturation *in vivo*. Our studies suggest that intercalation of LBFs into lipid digestion/absorption pathways provides a unique mechanism to facilitate both initial drug solubilization, and also to progressively promote supersaturation (13,15,17,18,35). Interestingly, since many of these processes are inevitable for LBFs containing digestible and/or absorbable formulation components, it seems likely that supersaturation contributes to the performance of many LBFs, including currently marketed products such as Neoral®, Agenerase® etc. This review provides a detailed account of the mechanisms by which lipid digestion and absorption can lead to supersaturation, and illustrates how this can be harnessed to promote drug absorption from LBFs. A brief account of approaches to mitigate the risk of precipitation from the supersaturated state is also provided.

MECHANISMS BY WHICH LBF GENERATE SUPERSATURATION

Most commonly, LBFs are administered orally in the form of a liquid/semi solid filled capsule containing a blend of lipids, surfactant(s) and, in some cases, cosolvent(s). Drug is typically pre-dissolved in the capsule fill material to circumvent the potential limitations to drug absorption that result from slow dissolution from the solid state. Drug solubility in the LBF fill material determines the maximum drug loading capacity (i.e., the unit dose), and is increased when the drug is highly lipid soluble or when the formulation contains high proportions of surfactant or cosolvent. The solubilization capacity of the formulation, however, usually decreases as included excipients are dispersed, digested and absorbed in the GI tract. Supersaturation occurs when drug concentrations in the GI fluids are transiently higher than equilibrium drug solubility in the same environment. A decrease in LBF solubilization capacity during *in vivo* processing creates the necessary imbalance between high initial solubilized drug concentrations and lower equilibrium drug solubilities post lipid dispersion and digestion, to stimulate drug supersaturation.

The mechanisms that potentially trigger drug supersaturation in LBFs are highlighted in Fig. 2. Formulation dispersion (mechanism 1), may lead to a drop in solubilization capacity, especially where LBF contain high proportions of water-miscible cosolvents or hydrophilic surfactants. Where drug concentrations above the equilibrium solubilization capacity do not immediately result in precipitation, the solubilized concentration is supersaturated (9,20). A similar series of events unfold during the process of lipid (or surfactant) digestion where hydrolysis to form more polar digestion products (mechanism 2) typically decreases the solubilization capacity of the dispersed formulation (13,17,21). Further dilution of drug loaded intestinal colloids (comprising LBF digestion products and endogenous biliary components) also stimulates changes to colloidal structure (mechanism 3) and may lead to changes in drug solubility and the potential for supersaturation (especially for weak bases) (18,35). Finally, changes to colloidal structure triggered by entry into the acidic unstirred water layer and stimulation of the absorption of lipid digestion products (mechanism 4) can also induce supersaturation and promote drug absorption (15). These topics are developed in more detail in the sections below.

In each case, supersaturation may be quantified by the supersaturation ratio (SR):

$$SR = \frac{(C_{colloid} + C_{free})}{(S_{colloid} + S_{free})} \quad (1)$$

Where C is the drug concentration measured at any time in the GI fluids and S is the equilibrium solubility of drug in the same fluids. The total drug concentration in the GI fluids is the sum of drug concentration in the colloidal phases formed by the LBF (i.e., $C_{colloid}$) and the concentration in the non-colloidal phase or free drug in the surrounding fluid (i.e., C_{free}). Similarly, $S_{colloid}$ gives the equilibrium solubility of drug in the colloidal phases and S_{free} the solubility of drug in the non-colloidal phase of the GI fluids (essentially the aqueous solubility). As the nature of the colloidal phases changes during formulation digestion (by lipases) and dispersion (by bile), and as components of intestinal colloids are absorbed, the solubilization capacity of the GI lumen also changes and may reduce. Supersaturation occurs in instances where precipitation is not immediate and where the total drug concentration (i.e., $C_{colloid}$ plus C_{free}) remains transiently above the equilibrium solubilization capacity of the GI tract (i.e., $S_{colloid}$ plus S_{free}). In this instance, an increase in C_{free} above the aqueous solubility (S_{free}), and an attendant increase in thermodynamic activity, is expected to provide advantages in drug absorption. However, in instances where supersaturation cannot be sustained, drug precipitation leads

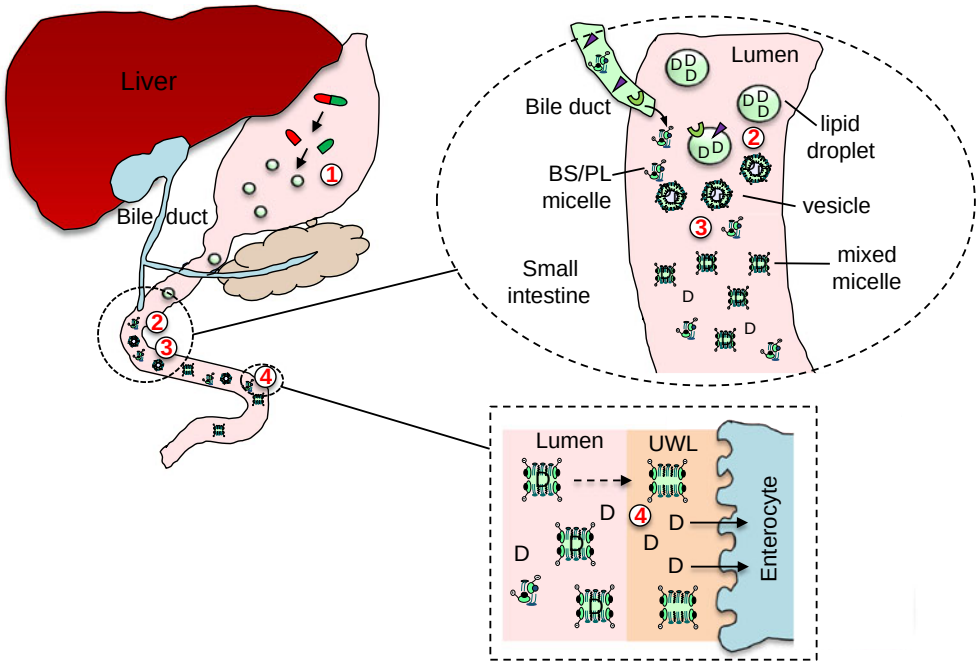

Fig. 2 Schematic showing the GI processing of an ingested lipid-based formulation (LBF) and the mechanisms by which supersaturation may be generated: 1. Dispersion-induced supersaturation. 2. Digestion-induced supersaturation. 3. Bile dilution-induced supersaturation. 4. Lipid absorption-induced supersaturation. Each of these mechanisms is discussed in greater detail in the main text. Figure modified from (1).

to decreased C_{free} and, therefore, decreased absorption. The likelihood of sustained increases in C_{free} (and therefore increases in absorption) *versus* precipitation (and decreases in absorption) reflect the degree of supersaturation where high SR values are expected to increase the risk of precipitation (13,17).

Supersaturation Generation on LBF Dispersion in GI Fluids

After oral administration, LBF capsule shells rupture rapidly in the stomach allowing LBF release and dispersion in the gastric fluids (mechanism 1 in Fig. 2). As described above, dilution of LBF especially LBF containing high proportions of surfactants and cosolvents, typically reduces solubilization capacity and has the potential to generate supersaturation. As most drugs are absorbed in the small intestine supersaturation in the stomach is typically undesirable and serves only to increase the risk of drug precipitation before the drug enters the absorption window. However, where supersaturation is moderate, such that it can be maintained for a period sufficient to allow gastric emptying prior to drug precipitation, absorption advantages may accrue. LBF design should therefore attempt to minimize supersaturation in the stomach or to provide a means to maintain supersaturation until entry into the intestine occurs.

The supersaturation ratio (Eq. 1) generated on dispersion is a function of the total solubilized drug concentration ($C_{colloid}$ + C_{free}), relative to the equilibrium solubilization capacity of the dispersed LBF and the intrinsic drug solubility in the stomach fluids ($S_{colloid}$ + S_{free}). The total drug concentration attained immediately after LBF dispersion is dependent on the drug dose and the volume of the gastric fluids (assuming no immediate drug precipitation). The SR generated on formulation dispersion will therefore increase proportionally with increased drug loading (unit dose).

The supersaturation tendency on dispersion is affected substantially by the proportional content of hydrophilic surfactants and cosolvents in the formulation. High HLB surfactants such as Tweens and Cremophors, and cosolvents such as PEG and ethanol, are often added to LBFs since they facilitate dispersion (or self-emulsification) of the formulation in the GI fluids. They also usually increase drug solubility in the LBF and, therefore, the maximum unit dose. On formulation dispersion, this increases the initial $C_{colloid}$ and C_{free}; however, high HLB surfactants and cosolvents are commonly miscible with aqueous media and, especially for cosolvents, dilution into the GI fluids results in non-linear decreases in drug solubilization capacity (i.e., decreases greater than that expected by simple dilution). The disproportional decrease in drug concentration and solubilization capacity on dispersion typically results in high SRs and a tendency toward drug precipitation.

Mohsin *et al.* (20) examined the relationship between LBF hydrophilicity and the precipitation tendency of an incorporated drug (fenofibrate) on dispersion, using a series of formulations containing varying proportions of surfactant and cosolvent. The data confirm a much greater decrease in solubilization capacity on dispersion of LBFs containing Tween 80 and propylene glycol when compared to LBFs containing only water immiscible lipids and surfactants. Figure 3 provides a further example of supersaturation generation following dispersion of a highly

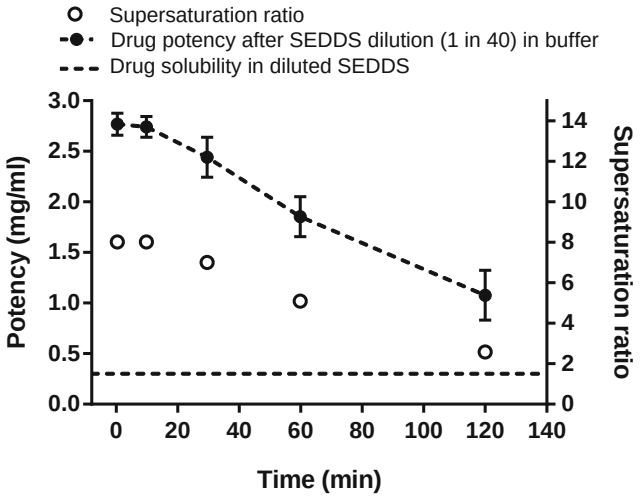

Fig. 3 The supersaturation ratio generated following dispersion of a self-emulsifying LBF containing GW4064, a commercially available agonist of the farsenoid X receptor. The dispersion medium was 0.05 M sodium phosphate buffer. In this example, supersaturation ratios (SR) were calculated using the dissolved drug concentration plotted on the y-axis (labeled "potency") minus the equilibrium solubility (the *dashed line*), divided by the equilibrium solubility, such that in the absence of supersaturation, SR = 0. Redrawn from (22).

hydrophilic LBF. In this example, 70% (w/w) of the formulation comprised cosolvent (propylene glycol/ethanol) plus hydrophilic surfactant (Tween 80 and Cremophor EL). The inclusion of high quantities of surfactant and cosolvent resulted in high drug solubility in the formulation and therefore high unit drug dose (the drug employed was GW4064, an agonist of the faresnoid X receptor). In contrast, the much lower equilibrium solubilization capacity of these excipients post dispersion resulted in an imbalance between the drug concentrations generated on dispersion (filled circles) and drug solubility in the same dispersed phases (dashed horizontal line), resulting in supersaturation (open circles). Supersaturation ultimately led to drug precipitation, however the precipitation rate was attenuated in this case by the inclusion of a polymer in the formulation (22).

Supersaturation Generation on LBF Digestion in the Small Intestine

On entering the small intestine, most of the lipid components of dispersed LBFs are readily digested by enzymes secreted by the pancreas (mechanism 2 in Fig. 2). As lipid digestion products are usually more polar and more water-soluble than the undigested material (e.g., fatty acids *vs.* glycerides), the solubilization capacity of the post-digestion colloidal phases is often lower than the dispersed and undigested colloids. The decrease in LBF solubilization capacity on digestion may be marked in the case of, for example, medium-chain TG (17,21) since medium-chain lipid digestion products are not very effective in boosting the solubilization capacity of post-digestion colloids (23). By rapidly lowering drug solubilization capacity, digestion can induce supersaturation, and unique to LBFs, this event is triggered by processes that are localized to the upper small intestine; the region of the GI tract where drug is most commonly absorbed. Similar to formulation dispersion, SRs generated on digestion are a function of the concentration of drug in digested LBF colloids and in free solution (i.e., $C_{colloid} + C_{free}$), relative to the solubilization capacity of the digested LBF and the intrinsic drug solubility in the intestinal fluids (i.e., $S_{colloid} + S_{free}$). As with events in the stomach, an increased risk of drug precipitation in the intestine is evident when SRs generated on digestion are high.

Digestion of lipids in food is initiated in the stomach by gastric lipase. However, in the fasted state, when gastric pH remains low, gastric lipase activity is low and the digestion of formulation lipids in the fasted stomach is likely to be more limited. Digestion of formulation-related lipids in the fasted state is therefore expected to be most significant after entry into the intestine (24). A detailed description of lipid digestion and the role of lipase and non-lipase enzymes in lipid and surfactant digestion is beyond the scope of the current submission, but has been well described recently by Bakala N'Goma *et al.* (25). In brief, glycerides present in the formulation are digested primarily by pancreatic lipase (triacylglycerol hydrolase) and co-lipase to diglyceride (DG), and subsequently to the more amphiphilic products; monoglyceride (MG) and free fatty acid (FA). Other pancreatic enzymes including phospholipase A2, carboxyl ester hydrolase and pancreatic lipase related protein 2 also play a role in the digestion of LBF and in particular in the digestion of MG and ethoxylated and pegylated surfactants (25). Lipid digestion in the human gut is very efficient, with the upper small intestine in a healthy individual reportedly capable of absorbing over 98% of ingested lipid (26). As expected for such an efficient process, the overall extent of intestinal digestion and absorption of lipids is independent of the initial droplet size (27). Inter-subject differences in LBF performance are therefore unlikely to result from variability in lipid digestion (since this is robust in healthy humans), but may be more evident in certain GI disorders, and are also possible as a result of inter-species differences in digestion capability.

Lipid digestion by pancreatic lipase occurs at the oil–water interface leading to transient accumulation of lipid digestion products (e.g., MG and FA) on the surface of dispersed LBF droplets. Lipid digestion products subsequently combine with bile salt, phospholipid and cholesterol mixed micelles secreted in bile to generate lipid-rich cubic, hexagonal and lamellar liquid crystals. The liquid crystalline phases so formed are diluted further with bile to form relatively lipid-rich vesicles and ultimately, bile salt-rich, lipid-poor intestinal mixed-micelles from where lipid and drug absorption is thought to occur (see Fig. 2).

The drug solubilization capacity of lipid-rich species such as vesicles is often high. Indeed, we and others have shown that drug solubilities in digested LBF can be 10 to 50-fold higher

than the respective solubilities in mixed bile salt/phospholipid micelles alone (17,18,23,28). Nonetheless, when compared to the solubilization capacity of the undigested formulation, the increased water solubility of the lipid digestion products reduces the solubilization capacity of the dispersed lipid phases and dictates that the drug solubilization capacity of the system is reduced (13,14,17). In many instances, lipid digestion therefore triggers supersaturation (13).

The difference in the solubilization capacity of the LBF in the dispersed and digested state reveals the extent to which digestion promotes supersaturation and can be captured by a digestion-mediated Supersaturation Promotion Factor (SPF^{DIGEST}). SPF^{DIGEST} is a simplified parameter that provides an indication of the maximum increase in supersaturation that might occur as a result of digestion. By taking variants of Eq. 1 for dispersed (i.e., SR^{DISP}) and digested LBFs (i.e., SR^{DIGEST}) and assuming no initial change in drug concentration on digestion (i.e., digestion drops equilibrium solubility (S), but kinetically the measured concentration (C) stays the same), SPF^{DIGEST} can be calculated as follows:

$$SPF^{DIGEST} = \frac{SR^{DIGEST}}{SR^{DISP}} = \frac{(S_{DISP} + S_{free})}{(S_{DIGEST} + S_{free})} \quad (2)$$

S_{DIGEST} and S_{DISP} represent the equilibrium drug solubility in the colloidal phases formed by dispersed and digested LBFs, respectively. Accordingly, SPF^{DIGEST} captures the propensity for digestion to induce supersaturation and can be quantified by the extent to which the equilibrium solubility of drug in the dispersed ($S_{DISP} + S_{free}$) and digested ($S_{DIGEST} + S_{free}$) LBFs differ. For example, a 2-fold decrease in solubilization capacity on digestion yields a proportional 2-fold increase in supersaturation ($SPF^{DIGEST} = 2$). The drop in drug solubility due to digestion ($S_{DISP} + S_{free} > S_{DIGEST} + S_{free}$) is highest for highly digestible lipid-rich formulations where digestion leads to the greatest change to the physicochemical properties of the lipids. As described above, this is particularly true of glycerides of medium and shorter chain fatty acids where drug solubility in the excipients is typically high [~ 2 fold higher on a mg/g basis in medium when compared to long chain TG, since molar solubilities are similar (29,30)], and therefore, where $S_{DISP} + S_{free}$ is usually high. At the same time, the water solubility of medium-chain digestion products (i.e., caprylic acid, capric acid, monocaprylin and moncaprin) limits the extent to which they incorporate into and augment the solubilization capacity of the colloid phase and, therefore, $S_{DIGEST} + S_{free}$ is low (and SPF^{DIGEST} is high). In contrast, drug solubility in long-chain TG is lower (i.e., $S_{DISP} + S_{free}$ is lower) and the lower water solubility of long-chain lipid digestion products promotes micellization and increases micellar solubilization capacity thereby increasing $S_{DIGEST} + S_{free.}$ Long-chain LBFs therefore typically show lower SPF^{DIGEST} values than their medium-chain comparators and are less susceptible to drug precipitation on digestion.

The greater propensity for drug precipitation from medium-chain LBFs is well illustrated in Fig. 4a–c. In this study (17), *in vitro* digestion of medium-chain LBFs containing danazol [Type IIIA and IIIB LBFs according to the Lipid Formulation Classification System (31)] resulted in drug precipitation in all cases, except at low drug loads. In contrast, similar long-chain LBF showed no evidence of precipitation at each of the investigated drug loadings. Measurement of drug solubility in the colloidal aqueous phase formed on LBF digestion revealed that the long-chain LBF exhibited the highest solubilization capacity (the dashed lines in Fig. 4d). As a result, digestion of the long-chain LBF resulted in SRs that were lower than those generated by the medium-chain LBFs at equivalent drug loadings, in turn resulting in decreased precipitation 'pressure' and superior performance (17). Whilst LBFs containing medium-chain lipids and high concentrations of surfactant and cosolvent provide for increases in LBF drug loading capacity, these data suggest that this also increases the likelihood of precipitation on digestion. A careful balance between drug loading, digestibility, supersaturation and precipitation must therefore be sought during the design of optimized LBF. Indeed, superior *in vivo* performance of LBFs containing long-chain lipid over medium-chain has been reported for danazol, with differences in performance attributed to a greater precipitation tendency on digestion of the medium-chain formulation (32). However, the induction of supersaturation on digestion of medium-chain LBFs may, in some cases, be beneficial if drug absorption occurs prior to drug precipitation since digestion increases thermodynamic activity. The balance between increasing supersaturation in order to promote drug absorption via increases in thermodynamic activity *versus* increasing the propensity for drug precipitation is described in more detail in the "Balancing the Risks and Rewards of Supersaturation Generation" section below.

Supersaturation Generation on Dilution of Colloidal Lipid Digestion Products by Bile

As described above, LBF digestion can trigger supersaturation as the solubilization capacity of the colloidal species that are formed is commonly lower than that of the dispersed and undigested formulation. The chemical transformation of e.g. TG into less lipophilic MG and FA is, however, only the first step in a series of sequential processes that continue to promote and maintain supersaturation. More specifically, under conditions of ongoing biliary secretion, the transition from lipid-rich/bile-poor colloids (usually liquid crystalline species such as lamellar structures, hexasomes and cubosomes) to progressively more bile-rich/lipid-poor mixed-micelles may also result in

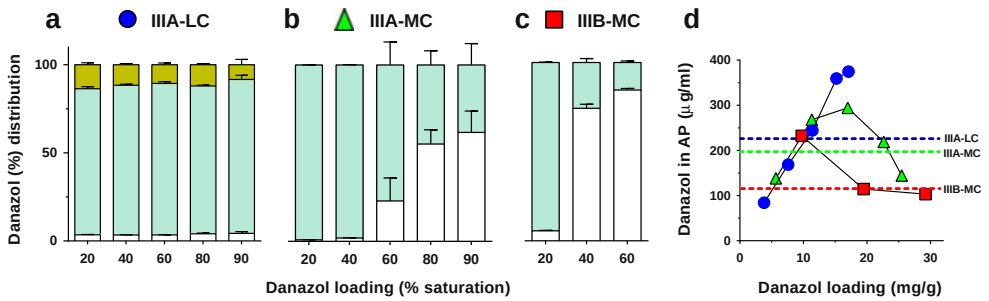

Fig. 4 The effect of increasing danazol loading in a long-chain (IIIA-LC) and two medium-chain lipid-based formulations (LBFs) (IIIA-MC and IIIB-MC) following *in vitro* formulation digestion for 30 min. In (**a**–**c**), the performance is plotted as the % of the danazol mass distributed across a partially digested oil phase (*yellow bars*), a colloidal aqueous phase [(AP) *light blue bars*] and a pellet phase (*white bars*) as a function of increasing danazol concentration in the formulation (expressed as a % of the saturated solubility in the formulation i.e. loading capacity). In (**d**), the performance is summarized by the danazol concentration solubilized in the AP after 30 min digestion as a function of absolute danazol loading (mg per g of formulation). Dashed lines in (**d**) represent the solubilization capacity of the AP following digestion of the three LBF which was in the order: IIIA-LC > IIIA-MC > IIIB-MC. Consistent with the rank order in solubility, digestion promoted drug precipitation from the MC formulations at lower drug loadings when compared to the LC formulation. Formulations were Type IIIA or IIIB according to the Lipid Formulation Classification System (31). Type IIIA LBFs consisted of 65/35 lipid/surfactant and Type IIIB LBFs consisted of 25/50/25 lipid/surfactant/cosolvent. Modified from (17).

a decrease in solubilization capacity with the potential to generate supersaturation (18) (mechanism 3 in Fig. 2).

During lipid digestion, densely packed amphiphilic lipid digestion products accumulate on the surface of digesting lipid droplets in a manner that favors the formation of enclosed bilayer liquid crystalline structures (i.e., lamellar liquid crystals). Lamellar liquid crystals subsequently slough off from the droplet surface and adopt a dispersed (vesicular) conformation. Due to their relatively large size, the rate of diffusion of the vesicles from the digesting TG droplets to the absorptive membrane is slow. In the presence of increasing concentrations of bile, however, liquid crystalline phases are solubilized into smaller mixed-micelles (containing bile salts, phospholipids, cholesterol and lipid digestion products). The micellar transformation that occurs following the penetration of bile salts and phospholipids into large liquid crystalline structures such as vesicles is essential for efficient absorption as the smaller colloids facilitate improved diffusion across the unstirred water layer to the absorptive site (33,34). During the transition from larger, lipid-rich species to smaller and less lipid-rich mixed-micelles and ultimately to bile-rich micellar colloids, the solubilization capacity for PWSD may decrease as the colloids become less lipophilic (23). Counterintuitively, therefore, the addition of bile salt micelles to lipid colloidal phases can, under some circumstances, lead to decreased drug solubility and, potentially, supersaturation (18). Where supersaturation does not immediately stimulate drug precipitation, this can enhance absorption (18).

Yeap and coworkers, for example, examined the solubilization properties of lipid-rich micellar colloids based on both medium-chain (18) and long-chain (35) lipid digestion products and showed that dilution with either simulated intestinal fluids (comprising bile salt, lyso-phospholipid and cholesterol), or bile collected from donor animals, resulted in a significant decrease in the solubilization capacity for cinnarizine, a poorly water-soluble weak base. Subsequent studies showed that, in some cases, the drop in solubilization capacity did not lead to immediate precipitation and instead supported periods of supersaturation of up to 1–2 h *in vitro* (Fig. 5a and b). Furthermore, co-infusion of cinnarizine loaded mixed-micelles containing biliary components and lipid digestion products (i.e., systems representative of initial post-digestion colloidal lipid phases) with bile in an *in situ* permeability model led to increases in drug flux that occurred coincidentally with the period of supersaturation (Fig. 5c and d).

Subsequent studies have shown, at least for colloids containing long-chain lipid digestion products, that supersaturation induction via interaction with bile appears to be more prevalent for weak bases solubilized in fatty acid containing colloids (35). It seems likely, therefore, that interaction with bile interrupts an association between weak bases and fatty acids in lipid-rich colloids leading to a drop in solubilization capacity and induction of supersaturation. In contrast, the solubilization capacity of similar colloids for model neutral drugs (fenofibrate, danazol) *increased* on addition of bile, more consistent with traditional solubilization paradigms (35).

The potential for bile dilution of lipid colloids to generate supersaturation may be studied directly using the methods described by Yeap *et al.* (18,35), or may be evaluated by incorporation into typical *in vitro* digestion protocols via the conduct of digestion tests at differing bile salt concentrations or in more dilute test conditions. Such tests have been termed "stressed" digestion tests by the Lipid Formulation Classification System (LFCS) Consortium (36) since they promote solubilization of lipid-rich colloids, result in higher SRs and lead to greater risks of precipitation. Several studies have reported increases in drug precipitation in *in vitro* digestion experiments in response to an increase in the bile salt:lipid formulation ratio (19,37). These

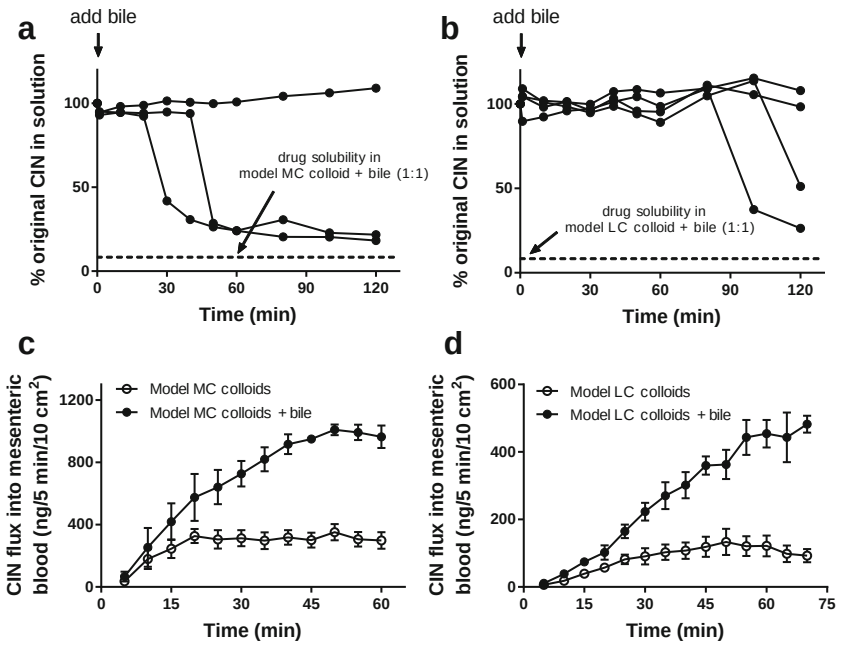

Fig. 5 The effect of bile on drug (cinnarizine) supersaturation generation in lipid colloids *in vitro* and drug perfusion across an isolated segment of rat small intestine. In the upper panels, the effect of bile on drug precipitation kinetics from model (**a**) medium-chain lipid or (**b**) long-chain lipid colloid systems is shown. The results of individual supersaturation experiments are shown (37°C). In both cases, the colloids contained cinnarizine at an equal thermodynamic activity (80% of the colloidal loading capacity). In (**c**) and (**d**), the positive effect of supersaturation generated on co-perfusion of the colloids with bile on drug flux is shown. Data shows absorptive drug flux into mesenteric blood following perfusion of lipid colloids containing cinnarizine at constant thermodynamic activity (80% of the colloidal loading capacity) through an isolated rat jejunal segment (~10 cm^2) with (*open symbols*) and without (*closed symbols*) 1:1 co-perfusion of bile from donor animals. Data represent mean ± SEM of $n = 3–4$ rats. (**a** and **c**) are from (18) and (**b** and **d**) are from (35).

data support the notion that increased exposure to bile salt micelles can generate supersaturation, but also serve to illustrate that supersaturation may promote both absorption and precipitation.

Supersaturation Generation on Diffusion Across the Unstirred Water Layer and Stimulation of Fatty Acid Absorption

The final process by which endogenous lipid processing pathways can stimulate drug supersaturation after oral administration of LBFs takes place in the intestinal unstirred water layer (UWL) and, therefore, in the immediate vicinity of the site of drug absorption (mechanism 4 in Fig. 2). Supersaturation in this region is stimulated by a loss in solubilization capacity of bile salt-lipid digestion product micelles as the more acidic environment of the UWL encourages protonation of micellar fatty acids, promotes colloidal dissociation of fatty acids and ultimately increases fatty acid absorption [i.e., the "dissociation model" of fatty acid absorption (38)]. Decreasing the lipid content of mixed bile salt-lipid micelles via lipid absorption significantly lowers the micellar drug solubilization capacity and has recently been shown to promote supersaturation and enhance drug absorption *in vivo* (15).

As described previously, mixed bile salt-phospholipid micelles are effective in shuttling the products of lipid digestion from digesting TG droplets across the UWL to the intestinal wall (33). The action of the Na^+/H^+ antiporter at the apical surface of intestinal absorptive cells, and the combined effects of mucous and limited stirring in the UWL conspire to trap protons within the UWL and results in the generation of an acidic microclimate in the immediate vicinity of the absorptive site. The pH of the UWL acidic microclimate ranges from pH 5.3–6.2 (39,40) and plays a key role in fatty acid absorption (38). In brief, as mixed bile salt-phospholipid micelles enter the UWL, ionized fatty acids within the micelle convert to less amphiphilic, non-ionized species that have lower micellar solubilities than the ionized species. This stimulates fatty acid dissociation from the micelles. Fatty acid absorption is ultimately promoted by both micellar fatty acid dissociation, and enhanced fatty acid permeability due to protonation in the acidic microclimate of the UWL (38).

Fatty acid absorption progressively depletes bile salt-phospholipid-lipid digestion product micelles of lipid content and, in doing so, decreases drug solubilization capacity. The decrease in solubilization capacity therefore has the potential to generate supersaturation and to increase free drug concentrations (i.e., thermodynamic activity). Importantly, and uniquely to LBFs, supersaturation generation and augmentation of C_{free} occurs in immediate proximity to the intestinal wall, increasing the likelihood that supersaturation leads to absorption rather than precipitation.

These concepts have recently been exemplified by Yeap *et al.* (15) who studied cinnarizine absorption from colloidal systems in a single pass rat jejunum perfusion model and used co-administration of a Na^+/H^+ pump inhibitor (amiloride) to manipulate the acidic UWL. Using mixed-micelles containing bile components (bile salt, lyso-phospholipid, cholesterol) and oleic acid and monoolein as model lipid digestion products, and comparison to micelles constructed with a non-digestible and non-absorbable surfactant (Brij® 97), these studies showed that; (i) in the presence of the acidic microclimate of the UWL, drug absorption from the bile-lipid digestion product micelles was ~4–5 fold higher than from Brij® 97 micelles, even under conditions where initial thermodynamic activity was matched, (ii) attenuation of the acidic microclimate of the UWL had no impact on drug absorption from the Brij® 97 system, but in contrast, (iii) removing the acidic microclimate dramatically decreased drug absorption from the bile-lipid digestion product micelles such that drug absorption from Brij® 97 micelles and bile-lipid digestion product micelles was not significantly different (15). *In vitro* studies examined in further detail the impact of simultaneously decreasing pH and decreasing lipid (MG and FA) content on drug solubility in bile-lipid digestion product micelles. These studies demonstrated that a reduction in lipid content resulted in a significant drop in solubilization capacity for a range of drugs (acidic, neutral and basic) and thus confirmed this effect as a potential source of supersaturation generation regardless of drug charge (15,35). While supersaturation of the weak base cinnarizine was theoretically reduced by an increase in drug ionization (and therefore solubility) with decreasing pH in the UWL (15), the net effect of decreasing pH, was a reduction in solubility in the system as the impact of pH on lipid absorption (and therefore decreasing lipid concentration and drug solubilization capacity) was greater than the pH effect on solubility.

Collectively, the data suggest that micellar systems containing absorbable components (15), and in particular, systems where absorption of the micellar constituent results in a reduction in solubilization capacity, can provide significant absorption benefits when compared to micellar systems comprising non-absorbable components. In the former case, absorption of the micellar components results in progressive increases in drug thermodynamic activity (assuming absorption of the micellar component is faster than that of the drug), whereas in the latter, thermodynamic activity remains constant or decreases as drug is absorbed (while components of the micelle are not). Fatty acids provide a particularly powerful example of an absorbable micellar component that may generate supersaturation in the UWL, as absorption is specifically catalyzed by the acidic microclimate at the absorptive surface (see Fig. 6). Colloids containing absorbable lipids, and especially colloids containing lipids for which absorption is triggered by the acidic microclimate of the UWL, represent unique 'interactive' or 'triggerable' delivery systems where thermodynamic activity is increased at the absorptive site (15).

These data have significant implications for the design of LBFs and suggest that LBFs containing digestible lipids may have inherent advantages over formulations that do not [such as those comprising only surfactants and cosolvents and classified as Type IV according to the Lipid Formulation Classification System (31)]. The inclusion of digestible lipids is expected to result in the formation of pH sensitive micellar species that interact with the absorptive surface to increase drug thermodynamic activity. Interestingly, these suggestions are consistent with previous studies that reported higher drug

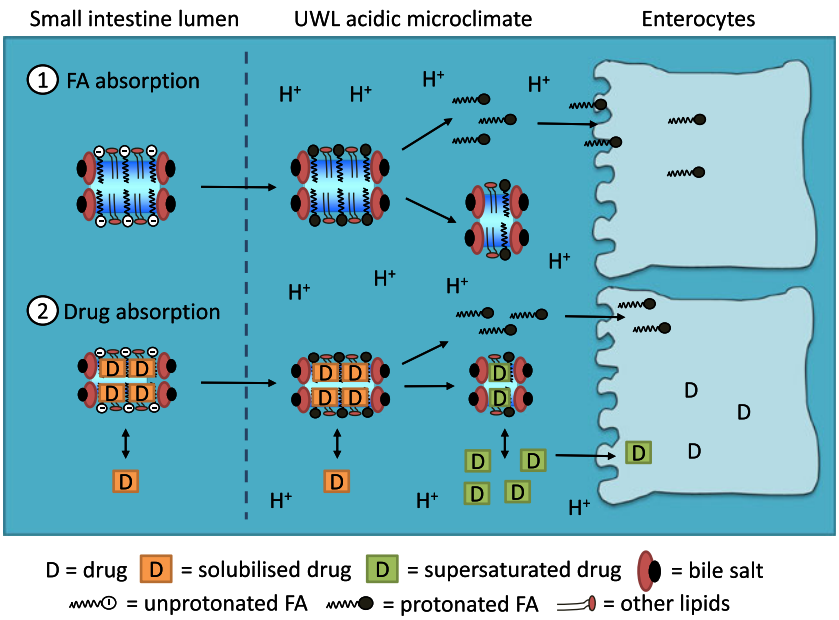

Fig. 6 Schematic of the proposed mechanisms by which the UWL acidic microclimate facilitates the absorption of solubilized long-chain fatty acids (FA) and poorly water-soluble drug (PWSD). In brief, decreased FA ionization in the UWL promotes FA dissociation from the micelle and absorption (process 1 in the schematic). Simultaneously, the solubilization capacity of micelle toward PWSD decreases resulting in supersaturation of drug in the immediate vicinity of absorption (process 2 in the schematic). Figure redrawn and modified from (15).

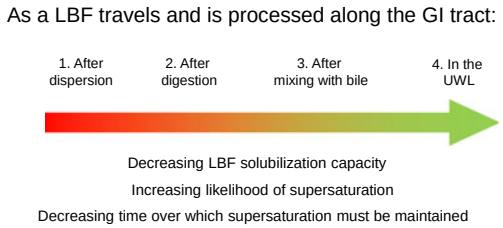

Fig. 7 The series of events that occur following oral lipid-based formulation (LBF) administration and the dynamic changes to LBFs properties that promote supersaturation and higher drug concentrations in free solution.

(danazol) exposure after administration of LBFs containing Cremophor RH40/soybean oil/Maisine 35-1/ethanol when compared with a similar system where the lipid constituents were substituted for a non-absorbable surfactant (Pluronic® 121), even though the latter system exhibited a lower tendency for precipitation on dispersion and digestion (41).

In summary, therefore, and unlike other enabling formulation approaches for PWSD, LBFs are uniquely capable of delivering high concentrations of solubilized drug to the intestine, and following a series of endogenous processes inherent to the lipid digestion/absorption pathway, are also capable of progressively generating supersaturation and increasing thermodynamic activity (Fig. 7). While these processes are inevitable in formulations containing digestible lipids, judicious LBF design is required to fully harness the beneficial effect of the lipid digestion/absorption pathway for enhanced drug absorption. This is particularly relevant in light of the supersaturation potential of LBFs since the formulation must maintain drug in the supersaturated state for a period of time sufficient for absorption to occur. The following section provides some discussion of the factors affecting the balance between enhancing supersaturation and simultaneously avoiding precipitation.

BALANCING THE RISKS AND REWARDS OF SUPERSATURATION GENERATION

In the preceding section, the capacity of LBFs to promote drug absorption via induction of drug supersaturation and increased thermodynamic activity following integration into the lipid digestion/absorption pathway is described. Although enhanced drug absorption is a desirable outcome of supersaturation generation, supersaturation also increases the potential for drug precipitation and this may lead to reduced drug absorption. In general, higher SRs (Eq. 1) increase the likelihood of drug precipitation, and this suggests the potential for a "supersaturation threshold" that marks the transition between "beneficial" supersaturation (i.e., that which enhances drug absorption) and "detrimental" supersaturation (i.e., that which promotes drug precipitation) (summarized in Fig. 8). The following section provides some discussion on the approaches that may be taken to ensure that supersaturation generated by LBFs is below the precipitation threshold.

Rational LBF Design for 'Sustainable' Supersaturation

Judicious LBF design has the potential to afford tight control over both the degree of supersaturation generated *in situ* and the GI location of supersaturation generation. For example, LFCS Type I LBFs comprising simple lipid solutions of drug in medium or long-chain lipids have relatively poor dispersibility properties, but present little risk of supersaturation or

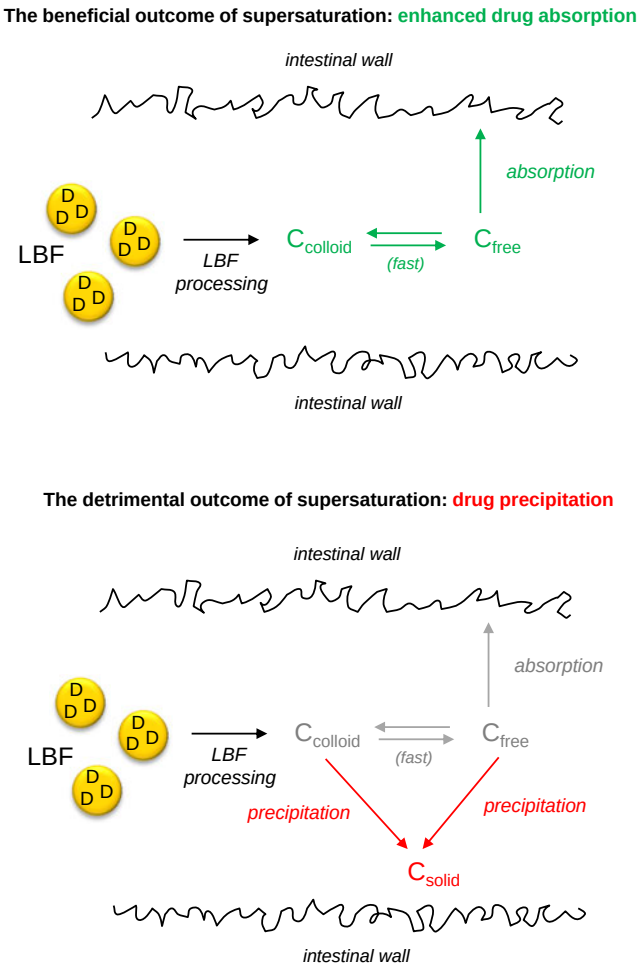

Fig. 8 A simplified schematic of the beneficial and detrimental consequences of supersaturation for drug absorption. In the upper panel, the attainment of supersaturation allows for higher solubilized drug concentrations (i.e., $C_{colloid}$), drug concentrations in free solution that are above equilibrium aqueous solubility in the absence of solubilizer (i.e., $C_{free} > S_{free}$), and therefore, enhanced drug absorption. In the lower panel, supersaturation drives nucleation, crystal growth and the formation of a solid drug precipitate (C_{solid}). The immediate decrease in $C_{colloid}$ and C_{free} and the requirement for re-dissolution of solid drug dictates that drug absorption may be reduced.

precipitation in the stomach. In contrast, the solubilization capacities of lipid solutions based on medium-chain lipids decrease significantly on digestion, potentially resulting in supersaturation and increased drug absorption in the intestine (via increases in thermodynamic activity), or conversely decreased drug absorption due to precipitation (13,17,21). Digestion to liberate long-chain fatty acids also provides the opportunity to generate supersaturation in the UWL by virtue of the inclusion of highly absorbable micellar components (15) (see above).

When comparing long and medium-chain lipids, lower drug solubilities in long-chain lipids often limit the maximum dose that can be administered, and therefore limit the solubilized drug concentration attained on formulation dispersion. Micelles comprising long rather than medium-chain lipid digestion products also typically have higher equilibrium drug solubilization capacities when compared to analogous medium-chain lipid containing colloids. Together, these factors conspire to limit the degree of supersaturation generated and to protect against the precipitation that may occur if the "supersaturation threshold" is breached (17,21). However, the lower degree of supersaturation generated also puts an upper limit on the possible benefits in thermodynamic activity that may be achieved. Medium-chain LBFs, in contrast, allow high drug loading and relatively low solubilization capacities in colloidal lipid digestion products, properties that combine to generate higher degrees of supersaturation. For highly permeable drugs, these higher degrees of supersaturation may be beneficial if absorption is sufficiently rapid to precede precipitation, but for moderately permeable or low permeability compounds, the higher degrees of supersaturation may provide a significant driver towards drug precipitation.

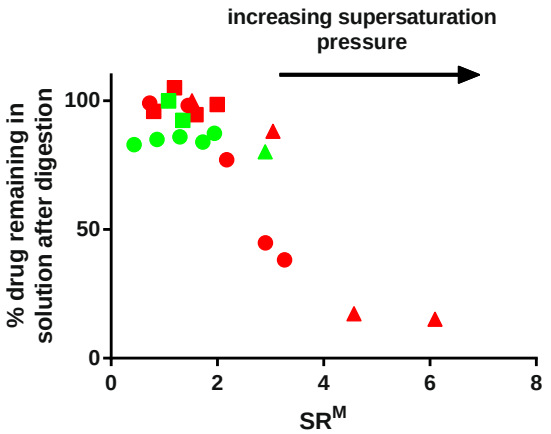

Fig. 9 The % drug solubilized after *in vitro* digestion of Type IIIA LBFs, comprising 65% (w/w) lipid and 35% hydrophilic surfactant, plotted as a function of the maximum supersaturation ratio (SR^M). SR^M is calculated from Eq. 3 and captures the maximum degree of supersaturation that may be attained in the digestion tests. Formulations contained either long-chain lipids (*green symbols*) or medium-chain lipids (*red symbols*) and increasing quantities of fenofibrate (*triangles*), danazol (*circles*) or tolfenamic acid (*squares*). Medium-chain formulations generate higher SR^M values, and as a result, show greater tendency towards precipitation. Data taken from (17) and (21).

Lower risks of precipitation following the digestion of long-chain LBFs are highlighted in Fig. 4 for danazol. This concept is further exemplified in Fig. 9 where danazol, fenofibrate and tolfenamic acid all remain predominantly solubilized in the colloidal aqueous phase (i.e., the "AP_{DIGEST}") formed following *in vitro* digestion of a long-chain lipid containing Type IIIA formulation (green symbols; Fig. 9). AP_{DIGEST} is the aqueous phase isolated after centrifugation of the digestion mixture and contains solubilized drug in micellar and lipid-swollen mixed-micellar species The AP_{DIGEST} may also contain larger and less well dispersed colloids (e.g. vesicles and lamellar liquid crystal phase). As described earlier, ongoing biliary secretions in the small intestine will promote the dispersion of these larger colloids into smaller micellar species.

For the drugs examined in Fig. 9, long-chain lipid containing formulations resulted in effective solubilization in AP_{DIGEST} despite drug incorporation at up to 90–100% of the LBF loading capacity. In contrast, digestion of equivalent medium-chain LBFs led to higher degrees of supersaturation and greater drug precipitation tendency (red symbols; Fig. 9) (17,21). Interestingly, the difference in supersaturation generated by long-chain and medium-chain LBFs was most marked for the neutral compounds danazol (circles; Fig. 9) and fenofibrate (triangles; Fig. 9), but was less obvious for the acidic drug tolfenamic acid, presumably due to drug ionization at intestinal pH increasing drug solubility in the intermicellar phase (21).

In Fig. 9 supersaturation is quantified using the maximum supersaturation ratio (SR^M). SR^M provides a measure of the maximum supersaturation "pressure" that is generated by LBF digestion (17) and is calculated from the ratio of the maximum concentration of solubilized drug that can be attained on formulation dispersion (i.e., immediately prior to digestion) in the absence of drug precipitation (AP_{MAX}), and the equilibrium solubility of drug in the AP_{DIGEST}:

$$SR^M = \frac{AP_{MAX}}{AP_{DIGEST}} \quad (3)$$

Consistent with well-established theories of the relationship between the SR and the thermodynamic and kinetic drivers to precipitation [e.g., classical nucleation theory (CNT) (42,43)], our recent studies suggest that the likelihood of drug precipitation during *in vitro* digestion of LBF is strongly governed by SR^M, with precipitation only evident in instances where a "SR^M threshold" of 2.5–3 is breached (13,17,21,44). Critically, the observation that sustainable supersaturation is most likely when $SR^M < 3$ draws on the performance of a range of LBFs and drugs, suggesting broad applicability. SR^M may therefore provide a means of calculating the maximum drug loading that may be tolerated by a LBF before precipitation occurs under

simulated intestinal conditions. Thus, by measuring drug solubility in the AP_{DIGEST}, and assuming a threshold SR^M of 3, AP_{MAX} can be calculated. From this, and a knowledge of the test volume, the maximum drug load may be calculated (since AP_{MAX} = drug load/test volume).

The drug load required to exceed the SR^M threshold decreases with decreasing solubilization capacity of the digested LBF. Lipid-rich Type I, II or IIIA LBFs, and especially those containing long-chain lipids are therefore less prone to precipitation when compared to more hydrophilic Type IIIB-MC and IV LBFs at equal drug loading (17,21). At the same time, the use of highly lipophilic formulations (e.g., Type I, triglyceride solution formulations) runs the risk of poor dispersibility. When combined with relatively low drug loading, this poor dispersibility can entirely preclude supersaturation generation upon dispersion and digestion. The use of blends of triglyceride/diglyceride/monoglyceride lipids and the addition of hydrophilic surfactants is therefore increasingly popular since these LBFs — classified as Type IIIA according to LFCS (31) — exhibit excellent self-emulsification properties (i.e., better dispersibility), high digestibility and, more often than not, higher drug loading capacities when compared to simple lipid solutions. When constructed using long-chain lipids, Type IIIA formulations also result in attainment of moderate degrees of supersaturation that are less prone to precipitation. Historically, Type IIIA formulations often outperform related LBF (7,13,45,46).

Cosolvents such as ethanol, polyethylene glycol, propylene glycol and triacetin, are often incorporated into LBFs to boost drug loading capacity, especially for only moderately lipophilic PWSD. However, as the solvent capacity of cosolvents is rapidly lost on dilution, the use of high cosolvent concentrations creates a risk of generating very high degrees of supersaturation in the stomach and promoting drug precipitation prior to drug entry into the intestine. For weakly basic drug candidates, the risk of precipitation in the stomach may be attenuated by gastric acidity, however gastric emptying and exposure to more neutral pH in the intestine is subsequently likely to re-initiate the drivers for supersaturation and precipitation (1).

Does Intestinal Permeability Dictate the Degree of Supersaturation that can be Tolerated (and Therefore the Most Appropriate Formulation Strategy for PWSD)?

The generation of supersaturated solutions that show no evidence of precipitation over an extended period is desirable as it allows greater time to realize the absorption benefits of increased thermodynamic activity. However, extrapolation of the relationships that may be generated *in vitro* between supersaturation and drug precipitation, to the *in vivo* situation, is not straightforward. In particular, drug absorption *in vivo* decreases drug concentrations and reduces supersaturation pressure towards precipitation. Indeed, absorption and precipitation may be seen as competing endpoints for supersaturated solutions where the most efficient process is likely to sequester the greatest drug mass (Fig. 10).

In line with growing interest in formulation approaches to generate and sustain supersaturation, *in vitro* assays that allow supersaturation to be monitored in an "absorptive" environment are increasingly being developed (15,18,35,47,48). Most studies to this point have coupled a supersaturation generation step (e.g., dissolution of an amorphous solid or dilution of a co-solvent solution) with Caco-2 cell monolayers (48,49), artificial membranes (50) or a water-immiscible organic material such as octanol (47) to provide an absorptive sink. For LBF, these techniques present a challenge since bile salt and surfactant micelles are able to solubilize water immiscible organic phases, and the relatively high concentrations of bile salt, surfactant and fatty acids that are present in, or generated by, digestion typically undermine the integrity of cell monolayers.

In vivo, the absorptive barrier is protected by intestinal mucous, and is rapidly regenerated. As such the intestinal mucosa *in vivo* is more resistant to amphiphiles such as bile salts, irritants such as fatty acids and solubilizers such as surfactants. Techniques such as the *in situ* jejunal perfusion model described by Yeap *et al.* (18) employ an absorptive sink that is more consistent with the *in vivo* barrier than simple cell culture models, and one that has been shown to retain appropriate permeability properties after perfusion of bile salt-fatty acid micellar solutions (18). The complexity of this model, however, limits high throughput use.

The experimental factors that should be considered during supersaturation assessment with/without absorptive reservoirs have been recently reviewed (51), however, this experimental approach is relatively new, and as such, novel models continue to be described. The ideal combination of high throughput screens with appropriate GI barrier and resistance properties remains elusive – especially for studies where highly solubilizing conditions are appropriate such as the evaluation of LBF.

Supersaturation/permeability models have been used most frequently to illustrate the positive effects of supersaturation on the rate of drug permeation into/across an absorptive barrier by comparing drug permeation from supersaturated and non-supersaturated states (48,49). However, Bevernage *et al.* has also utilized Caco-2 monolayers and supersaturated solutions of loviride to show that "drug absorption" (in this case drug permeation into and across Caco-2 cells) may attenuate drug precipitation from supersaturated solutions by lowering drug concentrations and, therefore, the degree of supersaturation (48). The data suggest that the degree of supersaturation required to breach the precipitation limit *in vivo* is higher than the degree of supersaturation generated within *in vitro* experiments that lack an absorptive barrier.

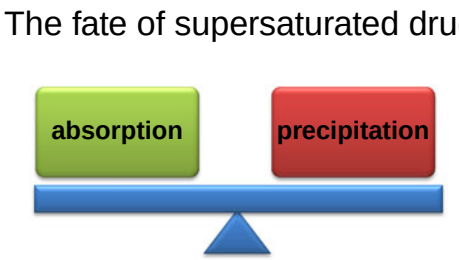

Fig. 10 The balance between the two supersaturation endpoints of supersaturated drug in the GI tract. With increasing degrees of supersaturation, thermodynamic activity and therefore the driver for absorption is also increased. Conicidently, however, increased supersaturation also increases the risk that drug will precipitate.

These findings raise the question as to how long supersaturation needs to be maintained in order to facilitate absorption, and suggest that in many circumstances, LBFs that generate supersaturation may only need to sustain drug in the supersaturated state for relatively short periods of time. This may be particularly the case when supersaturation is generated in the upper small intestine and even more so when supersaturation is generated in the UWL – that is, at the absorptive site. These arguments further suggest that supersaturation requirements/limits are likely to be compound specific and may be related to permeability. Thus, highly permeable compounds where absorption is rapid, may tolerate and indeed benefit from high degrees of supersaturation since rapid absorption attenuates the push of supersaturation toward precipitation (Fig. 10). In contrast, less permeable compounds where absorption is less rapid may require more stable supersaturation to allow absorption prior to initiation of precipitation. For LBFs, the prospect of certain formulation components altering permeability as well as solubility/supersaturation, for example, via inhibition of P-gp efflux, complicate this relationship (1,52). Further work is required to better understand the role played by drug absorption on the fate of supersaturated drug in the intestine and the relationship between *in vitro* and *in vivo* models of drug precipitation, especially for solubilizing LBF.

Importantly, *in vitro* models of drug precipitation, such as the *in vitro* digestion models described above, are inherently conservative. As such, the data obtained to this point suggests that formulations that are identified *in vitro* to provide for ongoing solubilization and supersaturation are expected to perform well *in vivo*. This does not preclude the possibility, however, that some formulations that lead to high degrees of supersaturation and precipitation *in vitro* may, in fact, "over-perform" *in vivo*, especially in instances where the drug molecule is highly permeable. That is, rapid absorption may dictate that only relatively brief periods of supersaturation/solubilization are required to support absorption. In the latter case, modification of *in vitro* endpoints such that the degree of supersaturation and drug fate is assessed at earlier time points (e.g., initially or within 5 min of interaction with intestinal fluids) may better assist in formulation discrimination.

Polymeric Precipitation Inhibitors (PPIs) for Kinetic Stabilization of Supersaturation

Polymers can stabilize supersaturated drug solutions and this concept has been applied widely in the design of polymeric amorphous solid dispersion formulations (1). In solid dispersions, the inclusion of polymers appears to stabilize crystallization tendency in both the formulation (where amorphous drug may be dissolved at concentrations above thermodynamic solubility) and also in the supersaturated solutions that are formed on formulation dissolution (1). PPIs reduce drug precipitation by kinetically stabilizing drug in the supersaturated state. PPIs therefore extend the duration over which the positive effects of supersaturation on drug absorption may be exploited (8,53,54). Although the use of PPIs in amorphous drug formulations has been widely described, fewer studies have evaluated the potential utility of PPIs in LBFs. In one of the first studies to exploit PPIs in LBFs, Gao *et al.* showed that the inclusion of 5% w/w hydroxypropyl methylcellulose (HPMC) E5LV (a low viscosity HPMC grade) in a self-

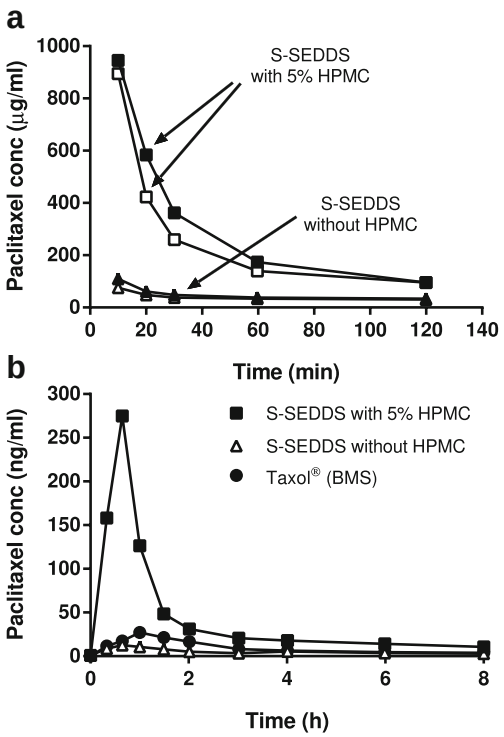

Fig. 11 Performance of paclitaxel-containing supersaturable self-emulsifying drug delivery systems (S-SEDDS) (**a**) during dispersion in simulated gastric media and (**b**) following administration to rats. Dispersion led to drug precipitation; however the addition of HPMC to the formulation significantly decreased the rate of drug precipitation and led to significantly improved performance *in vivo*. Redrawn from (16).

emulsifying LBF was able to slow the rate of drug (paclitaxel) precipitation as the formulation dispersed in simulated gastric media (Fig. 11a). Consistent with inhibition of drug precipitation and stabilization of supersaturation, a 10-fold increase in drug exposure was evident after oral administration to rats when compared to a non-polymer containing control formulation (Fig. 11b) (16). Several studies have subsequently shown that HPMC can stabilize supersaturation on LBF dispersion (9,10,49,55,56). More recently, we have also demonstrated the potential utility of a range of polymers (e.g., HPMC, methyl cellulose, hydroxypropyl cellulose, HPMC acetate succinate, Eudragit E100) in stabilizing supersaturated solutions of danazol that form on *in vitro* digestion of a range of LBF (13,57). In this way, the addition of PPI allows for the incorporation of much higher drug loadings prior to initiation of precipitation (13).

Figure 12 provides an analogous representation of *in vitro* drug precipitation data as a function of SR, to that previously shown in Fig. 9, but in this case describes a series of formulations with and without the inclusion of HPMC as a PPI. It is apparent that consistent with other studies, in the absence of PPI (Fig. 9), the threshold SR marking precipitation/no precipitation on digestion is ~2–3, but that in the presence of HPMC this increases to ~4. PPIs are therefore effective in enhancing the resistance of formulations to precipitation after both *in vitro* dispersion (10,16) and digestion (13). In the study summarized in Fig. 12, however, bioavailability studies in beagle dogs subsequently indicated that the *in vitro* utility of the PPI (HPMC) was not always replicated *in vivo* and was seemingly limited in instances where higher drug loads led to higher degrees of supersaturation. The latter observation is consistent with data from the solid dispersion literature where the ability of polymers to influence drug crystallization appears to diminish with degree of supersaturation (58,59).

CONCLUSIONS AND FUTURE OUTLOOK

The challenge of low water solubility continues to complicate drug development. Many 'enabling' formulation approaches have been proposed to address these complications, and LBFs represent one of several strategies that can increase drug exposure after oral administration of PWSD by avoiding drug dissolution and promoting drug solubility in the GI lumen. Enabling formulations that promote drug solubilization via micellization (e.g. surfactants, LBFs) or complex formation (e.g. cyclodextrins), however, suffer from an inherent drop in thermodynamic activity as a consequence of solubilization. Although this drop in thermodynamic activity is unlikely to preclude increases in exposure of a PWSD above that of a crystalline dosage form (since beneficial effects on dissolution and solubility will likely dominate), thermodynamic activity considerations may be important when comparing different enabling formulations. As such, attention has recently turned to formulations that are able to promote GI supersaturation, as a means of attenuating the drop in thermodynamic activity that solubilization causes.

LBF are unique within the class of solubilizing formulations as their properties change significantly as they incorporate into endogenous lipid processing pathways. Specifically, the chemical nature of lipids is altered by lipid digestion, the physical nature of lipid and lipid digestion products is altered by interactions with biliary micellar secretions and the solubilizing volume of micellar digestion products is reduced by ongoing lipid absorption. Each of these processes is capable of decreasing the drug solubilization capacity of the solubilizing species, and where precipitation does not occur exactly in parallel with the decrease in solubilization capacity, supersaturation results. In comparison to other solubilizing strategies, therefore, LBFs are dynamic and transform in response to

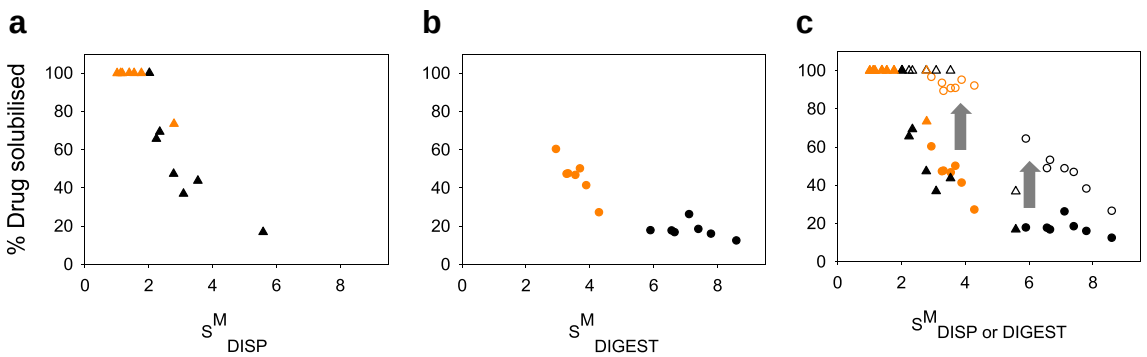

Fig. 12 Percentage drug (danazol) solubilized following (**a**) dispersion and (**b**) digestion of several lipid formulations plotted as a function of S^M (a parameter analogous to SR^M described in this article). Details of the formulation compositions can be found in (13). Formulations contained drug at 40% (*orange*) and 80% (*black*) of drug loading capacity. **c** Percent drug solubilized *versus* S^M in the presence (*open*) and absence (*closed*) of PPI. *Arrow* illustrates the effect of PPI on percentage of solubilized drug at constant S^M. Reprinted with permission from M.U. Anby, H.D. Williams, M. McIntosh, H. Benameur, G.A. Edwards, C.W. Pouton, and C.J.H. Porter. Mol Pharm. 9:2063–2079. Copyright 2012 American Chemical Society.

endogenous lipid processing events to progressively increase drug concentration in free solution. Importantly, these data challenge the recent suggestion that solubilization approaches to enhance PWSD absorption inherently reduce apparent permeability due to decreases in drug concentrations in the free fraction (11). It is now evident that this trade off can be overcome, at least in part, by changes to supersaturation as a result of lipid processing.

A better understanding of the mechanisms by which lipid formulations generate supersaturation and the formulation factors that determine the degree of supersaturation are therefore increasingly important in the effective design and evaluation of LBF. This includes an improved understanding of the drivers of drug nucleation/crystal growth from lipid colloidal systems, the kinetic stability of supersaturated drug, the patterns of drug absorption from these colloids and the potential utility of polymeric additives as precipitation inhibitors. Increased focus on these design criteria has stimulated a range of novel questions including, for example, whether it is more advantageous to design better solubilizing formulations or to kinetically stabilize supersaturation? (the identification of better solubilizing formulations will lower the risk drug precipitation, but will also decrease the potential benefits of an increase in thermodynamic activity whereas polymers in LBFs may lower the risk of drug precipitation but without decreasing thermodynamic activity). Alternatively, how important is the physical state (amorphous *vs.* crystalline) of any drug precipitate that ensues where supersaturation ratios are too high? Thus, does the phase separation of an amorphous solid permit sufficiently rapid drug re-dissolution that the barrier to absorption for PWSD presented by dissolution is overcome? If so, does LBF design impact on the propensity for drug precipitation in the amorphous or crystalline form?

More broadly, these questions also challenge the most appropriate design for *in vitro* models of assessment since drug absorption may reduce the push towards precipitation. Continued efforts seek to develop *in vitro* tests that contain an absorption "sink" in an effort to more appropriately reflect the *in vivo* scenario. For LBF, however, this is complicated by the irritant nature of high concentrations of bile salts and fatty acids, that collectively conspire, at least *in vitro*, to affect cell monolayer integrity. As such, modified dispersion/dissolution tests, such as the *in vitro* testing protocols currently being explored by the LFCS Consortium, will likely remain as a simple mainstay of LBF assessment (17,21,60). These protocols also present an inherently conservative approach to formulation assessment where the likelihood of false positives is low.

Finally, in a separate supersaturation approach, Mullertz and co-workers recently explored the potential utility of LBF where drug is present in the formulation in the supersaturated state (14). These authors showed that supersaturated LBF could be isolated and that supersaturation could be maintained for many months – thereby raising the prospect of LBF with higher drug loading capacity. Long term stability concerns for these thermodynamically unstable formulations will likely preclude application as commercially viable dosage forms, but as a means of increasing exposure in pre-clinical or early clinical development, where long term storage stability is not required, these approaches are an interesting new development.

In summary, LBF provide a robust formulation approach to enhance GI solubilization and to promote drug absorption after oral administration. In comparison to other solubilizing formulation approaches, integration into lipid absorption pathways has the potential to promote supersaturation and in doing so to enhance thermodynamic activity. When compared to non-solubilizing formulation such as solid dispersions, the solubilizing properties of LBF also limit the degree of supersaturation required to solubilize a significant drug mass. In doing so, LBF protect against supersaturation ratios that are sufficiently high that they promote drug precipitation. Future studies will usefully identify the formulation components that promote the generation of 'beneficial' degrees of supersaturation and simultaneously avoid supersaturation ratios that are extreme and propagate precipitation.

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