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50 years of oral lipid-based formulations: Provenance, progress and future perspectives☆

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ABSTRACT

Lipid based formulations (LBF) provide well proven opportunities to enhance the oral absorption of drugs and drug candidates that sit close to, or beyond, the boundaries of Lipinski's 'rule-of-five' chemical space. Advantages in permeability, efflux and presystemic metabolism are evident; however, the primary benefit is in increases in dissolution and apparent intestinal solubility for lipophilic, poorly water soluble drugs. This review firstly details the inherent advantages of LBF, their general properties and classification, and provides a brief retrospective assessment of the development of LBF over the past fifty years. More detailed analysis of the ability of LBF to promote intestinal solubilisation, supersaturation and absorption is then provided alongside review of the methods employed to assess formulation performance. Critical review of the ability of simple dispersion and more complex *in vitro* digestion methods to predict formulation performance subsequently reveals marked differences in the correlative ability of *in vitro* tests, depending on the properties of the drug involved. Notably, for highly permeable low melting drugs e.g. fenofibrate, LBF appear to provide significant benefit in all cases, and sustained ongoing solubilisation may not be required. In other cases, and particularly for higher melting point drugs such as danazol, where re-dissolution of crystalline precipitate drug is likely to be slow, correlations with ongoing solubilisation and supersaturation are more evident. In spite of their potential benefits, one limitation to broader use of LBF is low drug solubility in the excipients employed to generate formulations. Techniques to increase drug lipophilicity and lipid solubility are therefore explored, and in particular those methods that provide for temporary enhancement including lipophilic ionic liquid and prodrug technologies. The transient nature of these lipophilicity increases enhances lipid solubility and LBF viability, but precludes enduring effects on receptor promiscuity and off target toxicity. Finally, recent efforts to generate solid LBF are briefly described as a means to circumvent the need to encapsulate in soft or hard gelatin capsules, although the latter remain popular with consumers and a proven means of LBF delivery.

Contents

Abbreviations: AP, Aqueous phase; API, Active pharmaceutical ingredient; b-r-o-5, Beyond rule-of-five; BS, Bile salt; DG, Diglyceride; FA, Fatty acid; GIT, Gastrointestinal tract; IL, Ionic liquid; LBF, Lipid based formulation; LCT, Long chain triglyceride; LFCS, Lipid formulation classification system; MCT, Medium chain triglyceride; MG, Monoglyceride; PL, Phospholipid; PWSD, Poorly water soluble drug; S, Supersaturation ratio; SEDDS, Self-emulsifying drug delivery system; SMEDDS, Self-microemulsifying drug delivery system; SNEDDS, Self-nanoemulsifying drug delivery; TG, Triglyceride; UWL, Unstirred water layer.

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1. Introduction

In spite of considerable efforts to reduce physicochemical liabilities, and to design-in reasonable 'developability' characteristics in prospective drug candidates, discovery programmes continue to identify drugs with low water solubility, limited cellular permeability and high metabolic clearance—properties that are expected to reduce oral bioavailability. The current theme issue is focused on the challenge of developing drug candidates with properties such as these and simplistically has been defined by reference to drugs that do not comply with the 'rule of 5' (r-o-5) suggested by Lipinski [1] *i.e.* 'beyond r-o-5' (b-r-o-5) drugs. In reviewing the available literature, however, it is apparent that most currently marketed drugs, even typical BCS class II/III/IV compounds with low solubility and/or low permeability, are largely r-o-5 compliant. This is especially the case if the requirement for compounds to violate two r-o-5 properties in order to sit within the b-r-o-5 chemical space is strictly applied. In the context of this article, therefore, much of the historical data that has been reviewed does not truly reflect the b-r-o-5 chemical space. Nonetheless, the approaches taken to address the solubility or permeability limitations of drugs that sit within, or close to the boundaries of r-o-5, are applicable to the increasing numbers of drug development candidates that are moving b-r-o-5.

Of the limiting factors to oral drug delivery described above, low water solubility is perhaps the most amenable to resolution based on the use of enabling formulation approaches [2]. In contrast, formulation approaches that markedly enhance intestinal permeability or reduce first pass metabolism, are much less common. Permeation enhancement for oral delivery has met with some moderate successes in early clinical development as described in a recent review by Aguirre et al. [3]. In the case of highly (first pass) metabolised compounds, strategies such as prodrugs, coadministration with inhibitors, or alternative routes of absorption, *e.g.* pulmonary, nasal and buccal administration are more commonly employed [4]. However, for many compounds with significant permeability or metabolic liabilities, parenteral administration is often required for efficient delivery.

For drugs where low aqueous solubility limits absorption, several formulation technologies have been developed and applied to support increases in dissolution rate and/or apparent solubility in the gastrointestinal tract (GIT). These include particle size reduction and nanomilling, salt formation, isolation as a cocrystal or high energy polymorph, the generation of solid dispersions, and formulation in lipid based formulations (LBFs). These approaches have been described in detail previously [5,6], and some are covered in the current theme issue [7,8]. This review focusses on the use of LBF to enhance exposure after oral administration. Whilst the major advantage of LBF, at least to this point, has been in increasing apparent gastrointestinal solubility, it is also becoming increasingly clear that they may provide advantages in permeability and, under some circumstances, in avoiding first pass metabolism.

LBF have been investigated as a means to enhance oral drug absorption for many years. Indeed, it is fitting that as the Journees Galeniques meeting (around which this theme issue is based), enters its 50th year, this retrospective analysis of the published literature shows examples of LBF development from at least 50 years ago [9,10]. Indeed, lipid suspension and emulsion formulations of sulphonamides were described as early as the 1950s [11,12]. It was probably not until the 1970s, however, that more detailed evaluations of the use of lipids to boost drug absorption were initiated [13–21]. Increasing application of soft gelatin capsule technologies further facilitated oral administration of undispersed LBF. The rationale for the initial exploration of LBF to promote the absorption of poorly water soluble drugs was the realisation that many compounds of this type exhibit significant positive food effects. Thus, coadministration with lipids recruits (or at least partially recruits) the physiological events that are initiated by food administration to promote dietary lipid absorption. Lipid administration results in pancreatic and gallbladder secretions that initiate the process of lipid digestion and subsequent solubilisation of lipid digestion products in bile salt/phospholipid/cholesterol mixed micelles. Ultimately, this leads to the development of a range of colloidal particles in the GIT that serve to solubilise dietary lipids, but that also significantly enhance the solvation capacity of the GIT for coadministered drugs.

The importance of lipid digestion in the processing of LBF has led to the development of *in vitro* models of digestion that can be used to mimic the likely pathways of formulation processing in the GIT. This in turn has allowed examination of the potential fate of a co-solubilised drug during formulation digestion. The first reports of these models emerged in the late 1980s [22–27], and accelerated significantly in the 1990s and 2000s [28–37]. These studies developed the hypothesis that for lipid formulations to be successful, drugs should remain in a solubilised state during formulation digestion and processing, as precipitation is likely to be detrimental to formulation performance. This concept was based on the expectation that drug precipitation during formulation processing would generate solid drug, and initiate the requirement for drug dissolution—a process that is typically slow for poorly water soluble drugs (PWSD). For many (but not all) compounds this general hypothesis appears to hold and good *in vitro–in vivo* correlation has been reported between drug solubilisation during *in vitro* lipid digestion and systemic drug exposure

after oral administration of some drugs [37–40]. This is discussed in more detail in Section 6.

Secondly, the perceived importance of the particle size of the dispersion formed on capsule rupture in the GIT, has driven the development of formulations that spontaneously emulsify to form lipid emulsions with particle sizes in the low nanometre size range. This hypothesis was propagated on the basis that endogenous lipid processing results in increasing degrees of lipid dispersion and solubilisation and that ultimately colloidal structures with small particle sizes are required to diffuse across the unstirred water layer (UWL) and present drug to the intestinal absorptive surface. Formulations that are pre-dispersed to form small emulsion droplets (microemulsions, nanoemulsions), or that spontaneously emulsify on contact with GI fluids (self-emulsifying drug delivery systems or SEDDS) have been suggested to provide improved performance. The complex series of interactions that take place in the GIT, however, including formulation digestion and interaction of digestion products with bile salt micelles, are likely to significantly change the nature of any SEDDS formulation. As such, it seems likely that the critical parameter in formulation assessment is not the nature of the initial dispersion, but rather the properties (including particle size) of the dispersion formed after interaction with biliary and pancreatic secretions that ultimately determine LBF performance.

To summarise, LBF continue to provide a robust option for delivery of drugs and drug candidates that do not conform to the r-o-5, as well as those that meet r-o-5 criteria, but still pose significant challenges with respect to formulation development. As experience with LBF grows, our understanding of the mechanisms by which they work, and therefore the design criteria required to optimise performance, also continues to evolve. This review provides an overview of the general composition, advantages, and basis for utility of LBF, and then provides more discussion of our evolving understanding of the relationship between solubilisation, supersaturation and absorption. We also discuss the role of drug lipophilicity in dictating the applicability of lipid formulations and the potential utility of lipophilic conjugates or complexes (for example; ionic liquids and lipophilic prodrugs) in enhancing the utility of LBF. Finally, we comment on the practicalities of LBF, including recent advances in solidification, in order to facilitate tabletting, and in the use of lipid multiparticulates.

2. Lipid formulation composition and classification

LBFs span a wide range of potential compositions and include diverse delivery systems ranging from lipidic subcutaneous depots, intravenous emulsions or liposomal formulations to topical creams or lotions. For the purposes of this review, discussion has been constrained to oral LBFs. The majority of these are ultimately filled into soft or sealed hard gelatin capsules for clinical and commercial application, but may also be dosed as the liquid fill material during preclinical development. Indeed, one of the significant advantages of LBF is that the same formulation can be scaled up from low volume liquid formulations gavaged to mice and rats, to encapsulated dose forms that can be hand filled into empty capsules for studies in dogs, through to soft or hard gelatin capsule products that can be manufactured on automated commercial filling lines. For an excellent overview of marketed products that employ LBF the interested reader is directed to the 2004 review by Strickley [41].

Physically, LBF are often liquids, but may also be solid or semi solid at room temperature when high melting lipids are employed or when lipids are adsorbed onto a carrier. They may also take the form of lipidic multiparticulates. Some examples of solid LBFs are described in more detail in Section 9.

Although some of the earliest examples of LBFs are lipid suspensions, and the feasibility and performance of these materials *in vivo* is often reasonable [42,43], suspension formulations pose additional challenges to robust material transfer and content uniformity in the finished product. These formulation types may also be prone to stability issues due to Ostwald ripening [44]. Consequently, excipients in LBF are typically optimised to maximise the chance of complete drug solvation in the formulation. For drugs with only limited lipid solubility this is not always possible and a range of approaches have been suggested to enhance lipophilicity and lipid solubility. This is discussed in more detail in Section 8.

The range of excipients employed to form LBF is wide, but is largely drawn from three broad categories of materials—lipids (the hydrophobic sink), surfactants (to aid emulsification/solubilisation) and cosolvents (to aid solvation/dispersion). The relative proportions of each of these materials dictate drug solubility, formulation dispersibility, transport and metabolic effects and also impact on formulation properties such as stability, capsule compatibility and viscosity. For a detailed overview of excipient properties and selection criteria, the interested reader is directed to ref. [45], and for previous reviews detailing LBF formulation design in the context of marketed formulations, self-emulsification and solubilisation capacity to ref. [41,46–53].

The least complex LBF comprise simple encapsulated solutions of drugs in oils and are typified by the many fat-soluble vitamin preparations. On capsule rupture in the GI fluids, these LBF are crudely emulsified by the shear associated with gastric emptying and GI segmentation, and digested by gastric and intestinal lipase enzymes to form more amphiphilic digestion products. These digestion products serve to stabilise the emulsions formed and are ultimately solubilised in bile salt/phospholipid/cholesterol micelles secreted in bile. The *in vivo* dispersion of these materials is therefore catalysed by the process of lipid digestion. In an attempt to circumvent the reliance on endogenous lipid processing for LBF dispersion, most contemporary LBF now contain surfactants and cosolvents. These materials are included to reduce interfacial tension to the point that emulsions are spontaneously formed on gentle mixing of the capsule content with the GI fluids (*i.e.* SEDDS formulations). Refinement of the SEDDS technology has subsequently resulted in formulations that disperse to generate colloids with smaller and smaller particle sizes, systems that were initially described as self-microemulsifying formulations or SMEDDS on the basis that the dispersions generated were microemulsions [54]. In reality, whether the colloids so formed are thermodynamically stable (a requirement for definition as a microemulsion) is questionable in many cases [55,56], and the 'micro'emulsion terminology is seemingly at odds with dispersions with dimensions in the nanometre size range. A recent study by Niederquell and Kuentz, for example, has shown in an exemplar series of 20 dispersed SEDDS, that the majority exhibited only kinetic stability and could not be accurately classified as 'microemulsions' [56]. In light of this, terminology based on particle size *e.g.* self-nanoemulsifying drug delivery systems (SNEDDS) has become increasingly popular. Alternatively, generic nomenclature that does not specifically refer to size (SEDDS) or terminology that prefixes the particle size of the dispersed formulation *e.g.* nanometre SEDDS (nSEDDS) or micrometre SEDDS (μSEDDS) may be more descriptive. For the purposes of this review, SEDDS, SMEDDS and SNEDDS nomenclature will be used where these were the terms employed by the original authors.

In an attempt to simplify classification of LBF and to group formulations on the basis of their composition, Pouton introduced the lipid formulation classification system (LFCS) (Table 1) in 2000 [47] and later updated the classification to expand the formulation groups [50]. The LFCS classifies LBF into four main types, based on the relative proportions of included lipids, surfactants and cosolvents (Table 1). Type I formulations are the simplest and comprise drug dissolved in triglyceride alone or in mixed glycerides. Type II formulations comprise combinations of glycerides and lipophilic surfactants (HLB <12) and are representative of some of the first SEDDS formulations that were described [57]. The original Type II formulations used polyethoxylated triglyceride-based surfactants (*e.g.* polyoxyethylene 25 glyceryl trioleate—Tagat TO). Similar, although not quite as efficient,

Table 1
The Lipid Formulation Classification System (LFCS) reproduced from Pouton et al. with permission [47,50].

Excipients	Increasing hydrophilic content →				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Typical composition (%)					
Triglycerides or mixed glycerides	100	40–80	40–80	<20	–
Water insoluble surfactants (*HLB* < 12)	–	20–60	–	–	0–20
Water soluble surfactants (*HLB* > 12)	–	–	20–40	20–50	30–80
Hydrophilic cosolvents	–	–	0–40	20–50	0–50
Particle size of dispersion (nm)	Coarse	100–250	100–250	50–100	<50
Significance of aqueous dilution	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required	Not required
Characteristics	Non-dispersing; requires digestion	SEDDS without water-soluble components	SEDDS/SMEDDS with water-soluble components	SMEDDS with water-soluble components and low oil content	Oil-free formulation based on surfactants and cosolvents
Advantages	GRAS status; simple; excellent capsule compatibility	Unlikely to lose solvent capacity on dispersion	Clear or almost clear dispersion; drug absorption without digestion	Clear dispersion; drug absorption without digestion	Good solvent capacity for many drugs; disperses to micellar solution
Disadvantages	Formulation has poor solvent capacity unless drug is highly lipophilic	Turbid o/w dispersion (particle size 0.25–2 μm)	Possible loss of solvent capacity on dispersion; less easily digested	Likely loss of solvent capacity on dispersion	Loss of solvent capacity on dispersion; may not be digestible

emulsification behaviour is possible with polyoxyethylene 20 sorbitan trioleate (Tween 85). Type II LFCS formulations have been largely superseded by Type III formulations, not least because of the limited range of available lipophilic surfactants that promote self-emulsification of Type II formulations and that have been used in registered products. Type III formulations comprise mixtures of glyceride lipids, more hydrophilic surfactants (HLB >12), commonly also polyethoxylated glycerides, but with larger quantities of ethylene oxide, *e.g.* polyoxyethylene 35 castor oil (Kolliphor EL) or polyoxyethylene 40 hydrogenated castor oil (Kolliphor RH 40) and may also include cosolvents (*e.g.* PEG400, Transcutol or ethanol). Many SMEDDS or SNEDDS formulations are typical Type III formulations. Type III formulations are further stratified into Type IIIA, that contain larger proportions of lipids, and lower proportions of surfactant and cosolvent, and Type IIIB formulations that contain relatively limited amounts of glyceride lipid (<20%) and larger quantities of hydrophilic components. A classification of Type IV 'lipid' based formulations was introduced later in response to the increasing use of formulations that contain no traditional lipids [50]. Type IV formulations comprise only a combination of surfactants and cosolvents. The general properties of the different types of LBF are summarised in Table 1. In brief, the lipid rich Type I formulations require digestion to increase amphiphilicity and dispersion into intestinal fluids whereas Type II-IV contain sufficient surfactant to promote spontaneous dispersion. Progression from Type I-IV decreases triglyceride content and formulation susceptibility to digestion, and in general also leads to reductions in particle size of the resulting dispersion. For example, Type IV formulations typically disperse to form micellar solutions with particle sizes of 20 nm or below. Increasing quantities of surfactant and cosolvent in Type IIIB and Type IV formulations usually increases drug loading, since, with the exception of the most lipophilic drugs, the majority of PWSD are more soluble in surfactants and cosolvents than they are in glyceride lipids. The downside to the more hydrophilic Type IIIB and Type IV formulations is that inclusion of larger quantities of water-miscible components increases the risk of drug precipitation on dispersion of the formulation in the GI fluids.

Further amendments to the LFCS have been proposed to take into account the original classification system for lipids proposed by Small (rather than the combinations of lipidic excipients described by LFCS). These amendments were proposed in large part to better capture the properties of polar lipids that swell on contact with aqueous media and have markedly different properties to, for example, non-polar triglycerides [58] but that were grouped together as 'oils' in the LFCS.

Most recently, attention has switched to the classification of LBF based on *in vitro* performance, rather than solely on composition. This development was driven by the realisation that whilst excipient combinations that lead to useful self-emulsification are reasonably predicable, formulation performance for specific drugs is much more nuanced and the physicochemical properties of a drug alone are insufficient to inform *de novo* formulation design. As a result, preliminary formulation screening *via in vitro* dispersion and digestion testing is typically required to optimise LBF design. Work conducted under the Lipid Formulation Classification Scheme (LFCS) Consortium generated a large database describing the behaviour of Type I-IV formulations (containing a range of model drugs) during both dispersion in simulated GI fluids and on digestion under simulated intestinal conditions [59–64]. These data were then used to grade LBF based on *in vitro* performance in dispersion and digestion tests. In this lipid formulation—performance classification system (LF-PCS) [62], 'D' grade formulations were defined as those that resulted in rapid drug precipitation on formulation dispersion in model GI fluids. 'C′ grade formulations retained solvation capacity on dispersion but resulted in drug precipitation on initiation of digestion. 'B′ grade formulations retained solubilisation through dispersion and digestion challenges under normal conditions, but could be forced to precipitate under 'stressed' digestion conditions generated using *e.g.* high drug loads, increased dilution or longer time periods. 'A' grade formulations provided the most robust performance

and resisted precipitation under all dispersion and digestion challenges. In a more high-throughput approach, the use of simplified *in vitro* digestion models may also allow ranking of formulation performance and has been recently explored by multiple groups [65–67]. Notably, however, in all cases *in vitro* dispersion/digestion testing is based on the assumption that drug precipitation from lipid formulations *in vitro* provides an indication of hindered performance *in vivo*. This is not always the case, and the lack of an absorptive sink *in vitro* may overestimate the precipitation rate of some model drugs. Correlation between *in vivo* drug absorption and *in vitro* indicators of performance is discussed in more detail in Section 6.

3. Advantages of LBFs

LBF confer a range of biopharmaceutical, pharmaceutical and commercial advantages. Pharmaceutically, the ability to process LBF as solutions provides advantage for drugs with inherently low melting points (where solid dose forms may be impractical), for low dose compounds with potential content uniformity issues and for irritant and toxic compounds where dust control is a challenge. Commercially, LBF provide additional patient preference opportunities and in combination with a range of different finished dose forms (softgels, hard capsules or lipid multiparticulates) also provide a platform for evergreening and product life extension. The major advantages associated with the use of LBF, however, especially for b-r-o-5 compounds, are biopharmaceutical. These include changes to permeability, transit and metabolism, taste masking and for the great majority of reported applications, increases in intestinal solubility and avoidance of rate limiting dissolution. The latter are the major focal point of this review.

Lipids, and many of the other common components of LBF (surfactants and cosolvents), have been described to impact intestinal permeability, both *via* changes to passive permeability and *via* inhibition of efflux transporters. These effects have been extensively reviewed [68–72], and are not repeated in detail here. Briefly, a range of lipids (most notably medium chain fatty acids and lysophospholipids), surfactants (including bile salts) and cosurfactants have been shown to increase passive paracellular permeability by opening tight junctions [73,74], and to promote transcellular permeability by promoting membrane solubilisation and increasing membrane fluidity [70,75]. Conversely, apparent permeability may be reduced in the presence of colloidal species due to the formation of a competitive sink for solubilised drug and a reduction in thermodynamic activity [76–79]. Reductions in effective permeability have also been reported in the presence of polyethylene glycol and propylene glycol cosolvents [80]. More recently, attention has focussed on the ability of a range of surfactants and some endogenous species (including bile salts) to inhibit the activity of efflux transporters including p-glycoprotein [81,82], breast cancer resistance protein [83], multidrug resistance protein [84], and others. Almost all commonly employed surfactants have been suggested to show some inhibitory activity against efflux transporters, but perhaps the most compelling data has been generated with vitamin E TPGS and the Pluronic and Kolliphor surfactant families [79,85–87]. Surfactants are thought to inhibit efflux *via* changes to the structure and/or fluidity of membrane lipid domains leading to alterations in membrane protein/transporter structure, or by changes to transporter expression. Notably, although an increasing number of studies show compelling inhibitory effects *in vitro*, exemplification of efflux transporter inhibition *in vivo* is less widespread and often complicated by parallel effects on solubilisation. The effect of efflux transporters on *in vivo* absorption may also be limited in the presence of LBF by the attainment of luminal drug concentrations that are sufficiently high to saturate the transporter.

Lipid effects on metabolism are less well described, although some evidence of lipid and surfactant-mediated inhibition of presystemic metabolism is apparent, and again appears to be mediated by effects on enzyme activity and expression [88,89]. Lipids may also affect metabolism indirectly by changes to cellular and systemic drug distribution. For example, coadministration with lipids typically increases circulating lipoprotein levels, and for highly lipophilic drugs may increase drug association with plasma lipoproteins, reducing access to hepatic sites of metabolism. Drug abstraction into developing lipoproteins in the enterocyte has also been suggested to decrease enterocyte based metabolism [90]. However, effects on metabolism are hard to predict and increased lipoprotein association has been shown to both increase and decrease metabolism [91,92]. Furthermore, whether the quantities of lipid present in a typical LBF are sufficient to alter plasma lipoprotein levels to the point where changes in drug disposition are practically important is unknown [90].

Presystemic drug metabolism is also avoided by drugs that are trafficked to the systemic circulation *via* the intestinal lymph—a process that is supported by coadministration with lipids. Long chain lipids that are absorbed from the GI lumen into the enterocyte are re-esterified to triglyceride in the endoplasmic reticulum and subsequently assembled into lymph lipoproteins. The physical size of lymph lipoproteins (100–1000 nm) precludes diffusion across the continuous vascular endothelium and instead promotes selective uptake across the more permeable, and discontinuous, lymphatic endothelium. The intestinal lymphatics drain *via* the thoracic lymph directly into the systemic circulation and therefore circumvent the first pass metabolic events inherent in absorption *via* the portal blood. Drugs with high affinity for intestinal lymph lipoproteins typically have log D values greater than five and solubility in long chain triglycerides in excess of 50 mg/g [93], though exceptions have been reported [94]. These drugs may associate with lipoproteins in the enterocyte and can be delivered to the systemic circulation *via* the lymphatic system, resulting in reduced first pass metabolism [95,96]. The dependency of this absorption route on the presence of lipoproteins dictates that it is dependent on lipid re-esterification pathways, however, previous studies have shown that even a single capsule of lipid is able to support significant lymphatic transport in a dog [97]. Coadministration with lipids provides a source of lipids for lipoprotein assembly and this in turn provides the engine room for intestinal lymphatic lipid transport for drugs with inherent lipoprotein affinity. Oral lymphatic drug transport has been reviewed in more detail in a very recent review by Trevaskis et al. [98].

Finally, and perhaps most importantly, lipids and LBF significantly enhance the intestinal solubilisation of lipophilic PWSD. This increases exposure and in most cases also attenuates the large positive food effect commonly seen for PWSD after oral administration. These effects stem from integration of PWSD into the lipid digestion/absorption cascade and are described in greater detail in Sections 4 and 5.

4. Harnessing the potential of endogenous lipid digestion pathways

Endogenous lipid digestion and absorption pathways provide a highly dynamic and interactive conduit for drug delivery. Notably, dietary or formulation lipids (typically mixtures of glycerides), stimulate secretory processes in the GIT that profoundly alter the nature of the ingested lipid, resulting in altered GI conditions and significantly enhanced solvation capacity for the products of lipid digestion. In the context of drug delivery, these changes also (in the majority of cases) increase GI solvation capacity for a coadministered PWSD. Although many of the studies detailing GI response to lipid ingestion have been undertaken under post prandial conditions [99,100], and therefore under high lipid load, more recent studies have shown that lipid quantities of 2 g and lower are able to stimulate biliary secretion and elevate GI bile salt levels [101] as well as reduce gastric emptying [102]. Lipids and digestion products may also stimulate the ileal brake [103,104], thereby extending residence time in the proximal small intestine, ensuring maximal exposure to absorptive pathways in the duodenum and jejunum.

The biochemistry of lipid digestion has been described in detail on multiple occasions and will not be expanded on here. The interested reader is directed to the following reviews for a comprehensive overview of intestinal lipid absorption [2,105–109].

In the context of drug delivery, triglycerides are digested to diglycerides, monoglycerides and fatty acids by pancreatic lipases. Intercalation of these digestion products into colloidal biliary secretions generates a continuum of lipid reservoirs ranging from liquid crystalline phases at the oil:water interface, to multilamellar and smaller unilamellar vesicles, to mixed micellar species in bile salt rich areas of the GI fluids (Fig. 1) [108,110]. These lipid phases provide solubilising vehicles for poorly water-soluble lipid digestion products and are similarly able to solubilise PWSD. As dispersion and digestion proceeds, however, the changing nature of the colloids can result in changes in solvation capacity for PWSD, potentially resulting in supersaturation and precipitation of the drug as it transits the intestine. The complexity of the structural phases formed can be attributed to the unique physicochemical properties of hydrolysed lipid digestion products, molecules that are more amphiphilic than the parent triglyceride, but retain overall hydrophobicity. Hydration, swelling and self-assembly of these lipid digestion products results in the generation of liquid crystalline structures at the droplet interface and dispersion of these liquid crystal phases into intestinal fluids to form a range of structures, including lamellar, cubic and hexagonal phases, all of which have differing capacities to accommodate lipid digestion products or PWSD [100,111].

Evaluation of lipid phase behaviour *in vivo* is complex and is currently limited to *ex vivo* analysis. However, recent advances in computational models, microscopy and scattering techniques (particularly molecular dynamics (MD) simulations, atomic force microscopy (AFM), cryo-transmission electron microscopy (Cryo-TEM) and synchrotron small angle x-ray scattering (sSAXS)) are starting to provide much greater detail of the lipid nanostructures formed during digestion. Consistent with the general scheme described in Fig. 1, MD studies by Warren et al. have shown that as the water content of a LBF dispersion increases, the microstructure of the formulation changes from continuous phases, to reverse micelles to lamellar lipid dispersions

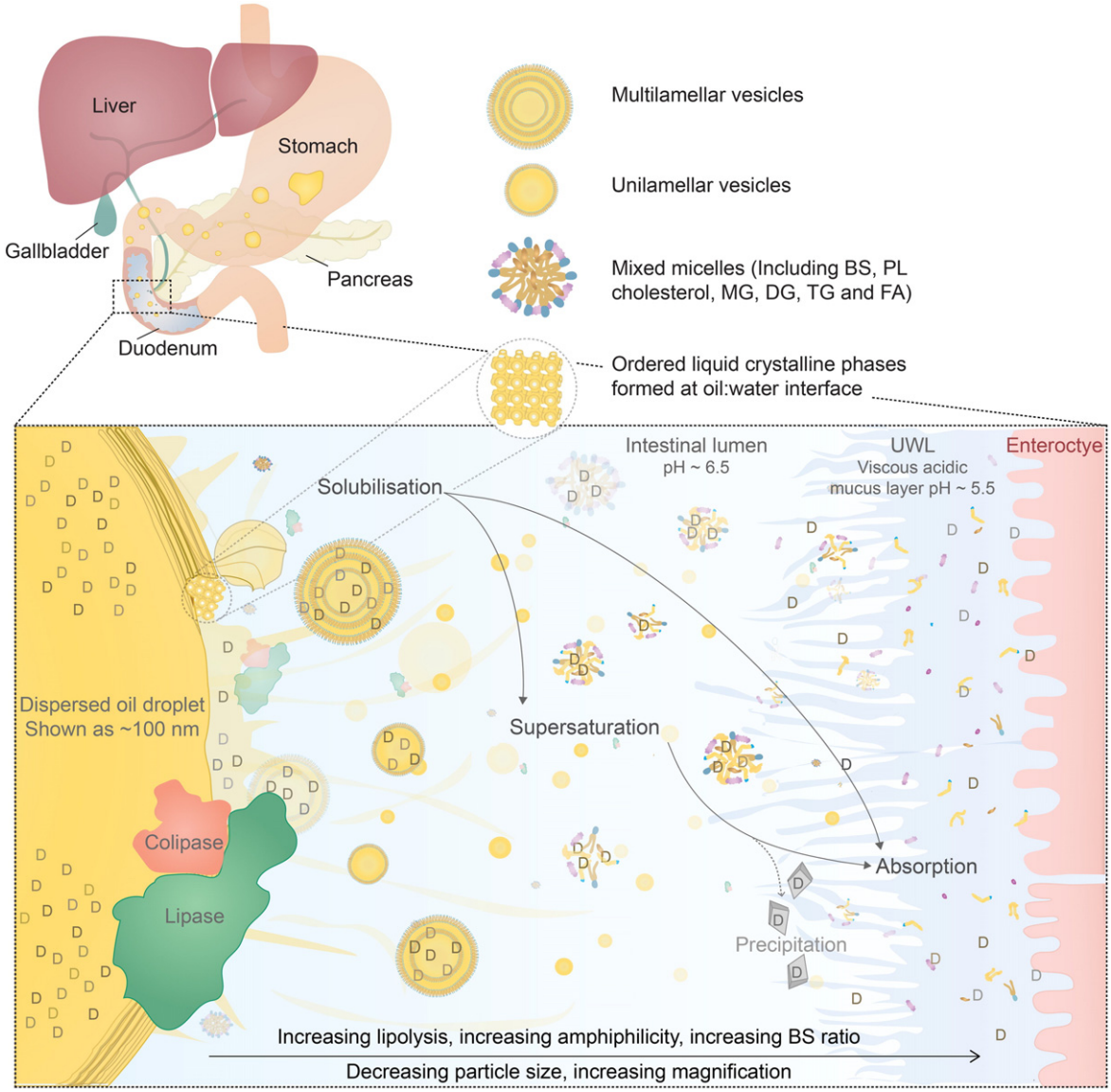

Fig. 1. Formation of a continuum of lipid reservoirs during triglyceride dispersion and digestion in the small intestine. Lipid species range from large digesting oil droplets to liquid crystalline phases, multi- and unilamellar vesicles, mixed micelles and finally to monomolecular species that are in equilibrium with the colloidal reservoirs and are absorbed at the enterocyte surface. Solubilisation of coadministered PWSD within these lipidic microdomains supports apparent drug solubility within the GIT, avoids traditional dissolution and typically enhances absorption. Figure adapted from Rigler et al. [112] and Porter et al. [2]. The following abbreviations are used: bile salts (BS), phospholipids (PL), monoglyceride (MG), diglyceride (DG), triglyceride (TG), fatty acid (FA), unstirred water layer (UWL).

with drugs situated at the interfaces of these structures [113]. Using TEM, Mullertz et al. have similarly shown that *ex vivo* post prandial human intestinal fluid (HIF) contains significant populations of micellar and vesicular species [114] and real-time SAXS studies of the *in vitro* digestion of milk have shown the transition of lipid structures from higher order liquid crystalline species to multi- and unilamellar vesicles in the presence of bile salts [115].

Digestion products (and coadministered PWSD) are ultimately solubilised into mixed micellar systems composed of fatty acids, monoglycerides, phospholipid, bile salts and cholesterol. These small, highly dispersed colloids provide an effective transport shuttle for hydrophobic species across the viscous unstirred water layer (UWL) to the absorptive surface of the intestine [116,117]. They also present a high surface area to promote free drug exchange between the solubilised reservoir and the GI environment [118]. Within the UWL, the slightly acidic environment results in protonation of solubilised fatty acid. This reduces fatty acid micellar affinity, increases saturation and thermodynamic activity and drives absorption of monomolecular digestion products [119]. In cases where lipid absorption is faster than drug absorption, and particularly where PWSD affinity for triglyceride digestion products is high, partitioning and absorption of lipids from intestinal mixed micelles appears to reduce micellar solvation capacity. This in turn may have the potential to generate transient drug supersaturation and effectively couple drug absorption to lipid absorption at the membrane [120].

Although a number of high profile papers have recently suggested the potential for absorption of oral particulates [121–123], evidence of absorption of intact lipid droplets including mixed micelles is less apparent. A recent study by Yeap et al. for example, revealed little difference in drug absorption from model colloids in the presence and absence of a range of inhibitors of putative lipid and particle uptake mechanisms. Subsequent studies examined drug absorption after administration in colloids with markedly different structure (vesicles *vs* micelles) but with matched thermodynamic activity and showed that drug absorption patterns correlated with thermodynamic activity and not structure [124]. The data are consistent with the suggestion that drugs that are solubilised in intestinal bile salt-lipid mixed micelles are absorbed *via* the free concentration that exists in rapid equilibrium with the solubilised reservoir, rather than *via* direct uptake of the colloid particle. These findings are in keeping with those reported by Shiau for the absorption of lipid digestion products [119].

To summarise, administration of PWSD in a LBF recruits a range of lipid processing pathways in the GIT that may be beneficial for drug absorption. These include enhanced initial GI solubilisation in intestinal colloids comprising mixtures of endogenous and exogenous solubilising components; improved transport across the UWL *via* micellar and mixed micellar transport shuttles; augmented absorption *via* increases in thermodynamic activity stimulated by lipid digestion and absorption, inhibition of intestinal efflux and potentially decreases in first pass metabolism (although these effects are largely driven by surfactants in LBF rather than lipids) and in some cases avoidance of first pass metabolism *via* stimulation of intestinal lymphatic transport [2,6,98].

A range of LBF have been employed in order to harness these advantages and their progression has, in large part, followed increases in our understanding of the mechanisms of lipid and drug absorption. These are described below.

5. LBF provenance; from solubilisation to self-emulsification to supersaturation

The ability of lipids to enhance the absorption of PWSD was first reported in the context of coadministration with lipids in food [125] and unsurprisingly, most PWSD show significant positive food effects [125–128]. However, coadministration with food as a means of enhancing drug exposure, whilst effective, is inherently variable as factors including culture, gender, age and health status all play major roles in dictating the type and quantity of food consumed as part of a meal. As a consequence, the potential to coadminister drugs with LBF formulations in order to match (and therefore circumvent) variability in food effects is highly appealing.

5.1. LBFs to improve PWSD solubilisation

To the best of our knowledge, the earliest published advances in translating the solubilisation advantage of the fed state to a formulated LBF were made by Bates and coworkers, whose seminal studies showed that emulsions comprising postprandial concentrations of bile salts and phospholipids significantly enhanced the solubilisation behaviour of griseofulvin *in vitro* [129]. The authors postulated that PWSD "may be absorbed by a mechanism involving preliminary solubilisation of the drug by bile salt micelles present in the small intestine" [10,129].

Realisation that many of the beneficial events of coadministration of PWSD with food could be simulated by coadministration with a LBF drove a number of early studies that explored the use of simple dietary lipid-based suspensions or solutions [9,15,22,23,130], emulsions [13,20,131] and even non-digestible lipids to aid drug solubilisation [22,23,27,132]. In most cases, these formulations significantly improved exposure when compared to oral administration of crystalline drug. Interestingly, despite digestible lipid solution and emulsion formulations evolving over the years to become more complex SEDDS and SNEDDS [24,29,57,133], non-digestible LBFs have not been similarly developed. This potentially reflects the early use of poorly dispersible mineral and paraffin oil formulations that are unlikely to further emulsify *in vivo* and therefore performed poorly [22,23,27]. Single component lipid formulations of digestible triglycerides transform *in vivo* to more amphiphilic lipids. These digested lipids intercalate into bile salt micelles to form highly dispersed, solubilised vehicles that diffuse effectively across the intestinal unstirred water layer (regardless of the dispersibility of the initial formulation). In contrast, non-digestible lipids cannot incorporate into lipid digestion pathways and must therefore be pre-emulsified to form a fine colloidal dispersion in order to facilitate diffusion across the UWL and drug absorption. When formulated to generate highly dispersed micellar solutions, however, non-digestible lipids may well be highly effective, since non digestible formulations or formulations where digestion is inhibited avoid the variability and potential loss of solubilisation associated with lipid digestion [134–136].

5.2. Transition to self-emulsifying LBFs

In vivo transformation of lipids from the bulk oil phase to nanostructured emulsions and finally micellar species signals the potential benefit of pre-dispersing LBF to promote progression through the intestinal processing pathway [10,16,17,116,129,137–139]. Although lipid emulsions can be effective delivery systems for PWSD [140], the stability, volume and patient compliance challenges of two-phase oral emulsion formulations limit commercial application. To overcome these challenges, the first major step forward in the evolution of LBF was the development of SEDDS formulations. SEDDS were adapted from the herbicide and pesticide industries where lipophilic actives have been formulated for many years as preconcentrates containing surfactants. These preconcentrates were shipped at lower cost (due to lower volumes) and then readily dispersed *in situ* to form a fine emulsion prior to spraying [18,141].

The first examples of the potential pharmaceutical utility of SEDDS were described by Groves in the early 1970s [18,141] and expanded in the 1980s by Pouton and colleagues [24,57,142]. The field subsequently accelerated (Fig. 2) with the clinical and commercial success of the Sandimmune and Neoral™ formulations of cyclosporine [41,143], the properties of which led to increased focus on the particle size of the dispersion formed on capsule rupture as a possible indicator of *in vivo* LBF performance.

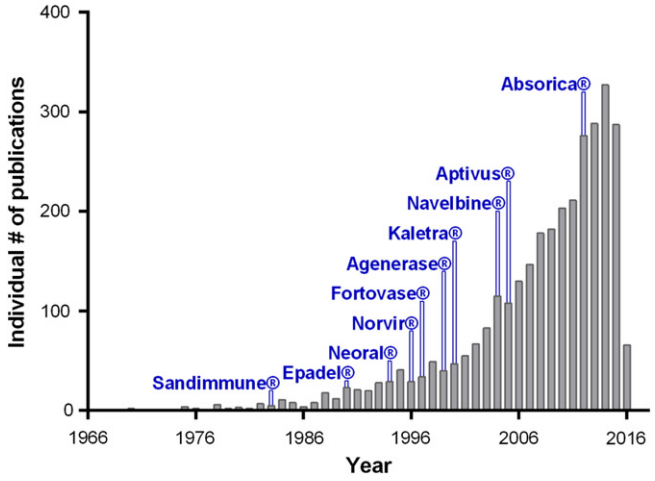

Fig. 2. Growth in number of LBF/SEDDS publications in PubMed, with marketed LBFs overlaid at corresponding dates of release onto market. Search terms: ((((oral) AND lipid formulation) OR SEDDS) OR SMEDDS) OR SNEDDS.

The Sandimmune Neoral formulation of cyclosporine was released in 1994 and was a reformulation of the original Sandimmune formulation, a relatively simple LBF that generated a crude lipid emulsion in the GIT on capsule rupture. Unlike the Sandimmune formulation, Neoral self-emulsified to form very small (sub-100 nm) droplets on capsule rupture and was arguably the first marketed SMEDDS formulation. Cyclosporine bioavailability was comparable or enhanced in the Neoral formulation, had the benefit of reduced food effects, reduced intersubject variability and was absorbed in liver transplant patients with disrupted biliary flow [54,144–149]. In many respects, Neoral has remained the 'gold standard' for self-emulsifying formulations ever since, although a causal link between particle size and *in vivo* bioavailability has never been proven and the relationship between the particle size of a dispersed lipid formulation and utility remains unclear. As described above, the action of lipolysis and the interaction of formulation components and lipid digestion products is likely to change the nature of any SEDDS formulation. Neoral contains digestible lipids and digestible surfactants [41] and might therefore be expected to undergo significant chemical and physical change *in situ*. Excipients contained within the formulation (or their digestion products) may also interact with drug transporters (or antitransporters) and metabolic enzymes. In the case of Neoral, Kolliphor RH40 is included in the formulation to generate finer emulsions, but has also been suggested to inhibit efflux transporters and metabolic enzymes [86]. The change in surfactant may also have improved solubilisation properties under conditions of intestinal digestion through changes in digestion rates or delaying drug precipitation. In this way, bioavailability may have been increased *via* mechanisms other than decreases in particle size (although effects on metabolism and efflux were specifically refuted by Choc et al., [150]). As such, direct correlation of the particle size of the dispersed Neoral formulation with its eventual utility is complex. However, it remains possible that the physicochemical properties of the formulation (including particle size) favourably impact downstream processing and in doing so, promote drug absorption.

In most cases, LBF digestion products have lower solvation capacities than the undigested parent formulation [35]. Where lipophilic drugs are predissolved in the undigested LBF, digestion therefore reduces solvation and increases drug saturation in the formulation. Ultimately, this may lead to drug supersaturation. This has two potential effects. Firstly, increasing saturation, and induction of supersaturation, may destabilise solubilised drug resulting in drug precipitation. Alternatively, increasing saturation will increase thermodynamic activity, effectively increasing the free concentration of drug in equilibrium with the solubilised reservoir and potentially increasing absorption. LBF development therefore involves a trade-off between maximising drug loading in the formulation, promoting moderate increases in saturation to drive increases in absorption and avoiding increases in supersaturation that are sufficient to promote drug precipitation. These aspects are discussed in more detail below.

5.3. Solubilisation versus supersaturation in LBF design

Coadministration of PWSD with lipids, or administration with a LBF typically enhances the overall solvation capacity of the GI fluids by creating additional, lipid swollen, colloidal species in which the solubility of a lipophilic PWSD is enhanced. However, to the best of our current understanding [119,124], these colloidal species are not absorbed intact and drug absorption occurs from the free concentration of drug that is in rapid equilibrium with the solubilised colloidal reservoir. Whilst the total solvation capacity of the GI fluids is therefore enhanced by the presence of mixed bile salt-phospholipid-lipid digestion product micelles, in the absence of supersaturation, the free concentration of drug is expected to be no greater than the drug solubility in intestinal fluid. Where drug is present as a saturated solution in intestinal colloids the free drug concentration in equilibrium with the solubilised reservoir is approximately equal to the saturated solubility of drug in the absence of the solubilising species (essentially the aqueous solubility). Under normal circumstances, therefore, although drug solubilisation in intestinal colloids increases effective solubility, the (free) concentration of drug, *i.e.* the concentration that drives absorptive flux, is not increased significantly above the aqueous drug solubility. In light of this limitation, but realising that LBF (or food) typically provide for significant increases in drug absorption, recent work has explored the hypothesis that lipid formulations intrinsically generate supersaturation during GI processing [35,120,151].

These studies suggest that for PWSD, there are three potentially complimentary routes by which supersaturation can be generated during LBF digestion. Firstly, solvation capacity is often lost during dispersion and digestion of drug loaded LBF [35,61,152]. Where this does not lead to immediate precipitation, supersaturation ensues. Secondly, absorption of lipid digestion products (that swell intestinal colloids and maintain drug solubilisation), also decreases drug solvation capacity and may lead to supersaturation in cases where lipid absorption is more rapid than drug absorption [120,153]. Thirdly, for ionisable PWSD, and in particular ionisable weak bases, supersaturation is also generated *via* the pH gradient encountered during gastrointestinal transit [154–156]. Thus, higher drug solubility is typically attained in the low pH environment of the stomach and this drops on transition to the more neutral pH conditions in the small intestine before encountering the acidic unstirred water layer. Supersaturated systems formed *via* one or more of these mechanisms have higher thermodynamic activity (and therefore higher absorption potential) when compared to colloids containing drug at (or below) equilibrium solubility. However, transition from a high-energy (supersaturated) state to the equilibrium point is energetically favoured, and therefore supersaturation also inherently predisposes systems to precipitation in order to re-attain equilibrium solubility. These precipitation events must first overcome the activation energy associated with crystal nuclei formation. Where the activation energy is high, crystallisation cannot proceed and a supersaturated metastable state may be maintained for a sufficiently long period to support drug absorption.

In the context of supersaturation on LBF dispersion, the nature of the formulation and the drug load are major drivers of precipitation. Formulations containing high drug loads and high proportions of amphiphilic excipients (surfactants and cosolvents) are most likely to result in rapid dissociation of water-soluble components, significant supersaturation and the greatest risk of drug precipitation [35,61]. Conversely, in formulations comprising more lipophilic components, excipient dissociation is less likely on dispersion and precipitation is often delayed for extended periods of time. In this case, drug concentrations in the GI tract may

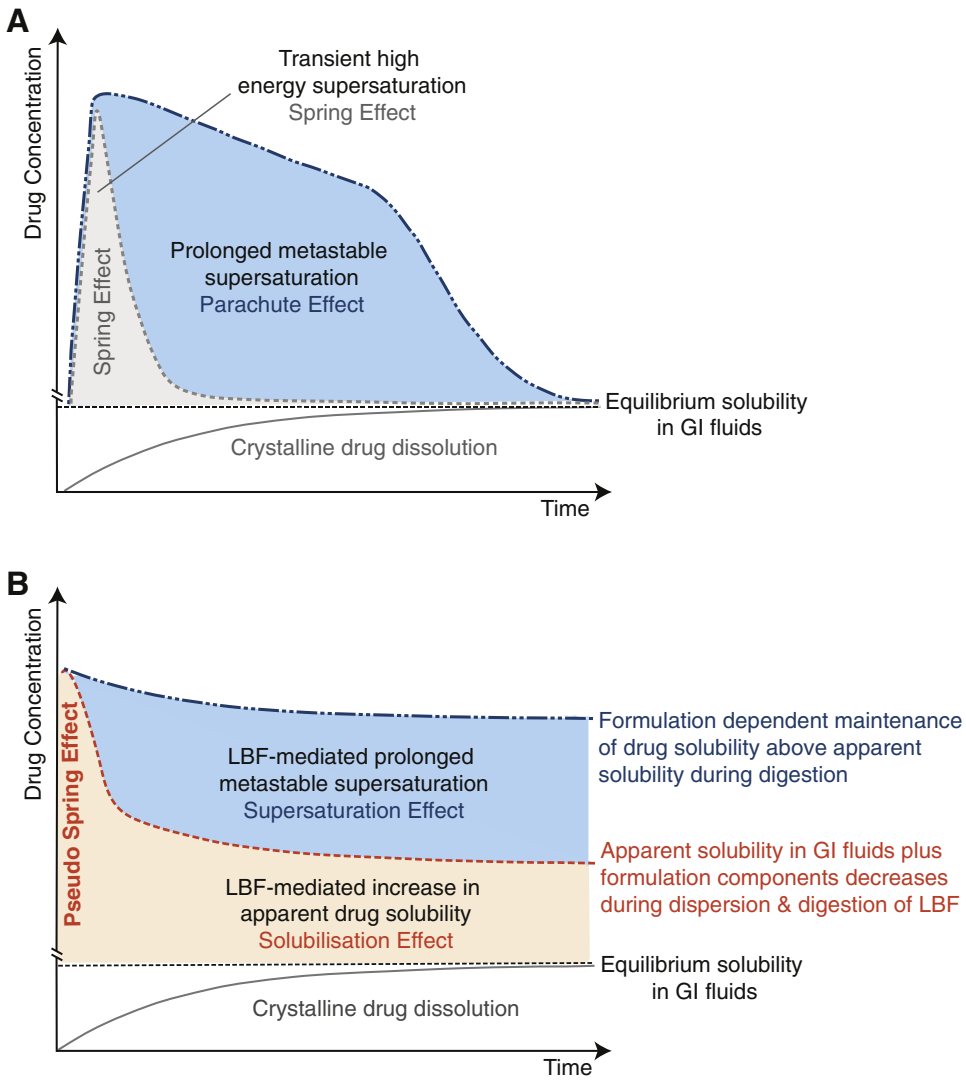

Fig. 3. Pathways to drug supersaturation during LBF dispersion and digestion. (A) The spring and parachute effect typically observed from solvent-shift or pH-shift methods used to evaluate drug supersaturation and precipitation kinetics or after dissolution of amorphous solid dispersions. Adapted from Browers et al. [157]. (B) The solubilisation and supersaturation effect generated by LBFs during formulation dispersion and digestion, adapted from Anby et al. [35].

be transiently elevated relative to apparent solubility in the GI fluids, leading to a metastable supersaturated state with higher thermodynamic activity (Fig. 3B).

On exposure to lipolytic enzymes, LBF are once again challenged and for digestible components, dissociation of more amphiphilic lipolysis products will likely reduce the solvation capacity of the colloid further, increasing supersaturation. Under these circumstances, the acyl chain length of the lipid(s) employed in the formulation may have a significant impact on the likelihood of drug precipitation. Lipase mediated digestion of long-chain lipids is slower than that of medium chain lipids [25,158], and the long chain digestion products produced appear to more readily maintain solvation capacity. Conversely, digestion of medium chain triglycerides is rapid and produces more water soluble digestion products, leading to higher supersaturation and increased potential for drug precipitation. Drug solubility in medium chain triglycerides is also often higher than that in long chain triglycerides allowing for significantly higher initial drug loadings in the medium chain based LBF [159]. Together, the higher drug loading and rapid digestion-induced reduction in solvation capacity in formulations based on medium chain triglycerides increases the likelihood of drug supersaturation and precipitation. The incidence of danazol supersaturation, triggered by digestion of medium chain triglyceride LBFs, has been described recently by Anby et al. [35]. In these studies, drug solubilisation or precipitation was related to the degree of initial supersaturation stimulated by dispersion and digestion. Thus, supersaturation above a certain threshold (in this case concentrations approximately 3 fold higher than equilibrium drug solubility in the colloidal species formed) resulted in precipitation. The concept that increases in the degree of supersaturation are likely to drive increases in the potential for precipitation is in agreement with the fundamental principles of nucleation and the realisation that nucleation rate and consequent precipitation is dependent on the extent of supersaturation [157,160]. Similar results (and similar threshold values) have subsequently been reported for a separate series of formulations of danazol, fenofibrate and tolfenamic acid [61], suggesting some degree of consistency in drug precipitation behaviour from SEDDS *in vitro* across a range of drugs and a range of different formulations.

Recognising that drug supersaturation may be a crucial driver for absorption, efforts have also focused on developing formulation strategies to stabilise or prolong drug supersaturation during the dispersion and digestion of LBF. This is analogous to the “spring and parachute” mechanisms of supersaturation generation and stabilisation widely described in the polymer literature (Fig. 3A). In the case of a LBF, dispersion and digestion events that promote supersaturation drive

either absorption or precipitation and form the 'spring', and polymeric formulation additives may be employed in an attempt to reduce drug precipitation (the "parachute") [35,161,162]. However, the choice of polymeric precipitation inhibitor is not trivial and requires careful balance of hydrophobicity, lipophilicity and compatibility with the PWSD under investigation [163,164].

In a somewhat related approach, formulations have also been developed that can slow or eliminate the digestion process, thereby attenuating decreases in solvation capacity, delaying initiation of supersaturation and reducing the drive towards drug precipitation [136,165]. Others have sought to allow precipitation but to promote precipitation of the amorphous forms of a PWSD, in the expectation that redissolution of drug will be enhanced from the high energy solid [34,153,166,167].

Finally, drug loaded lipophilic colloids must diffuse across the acidic environment of UWL in order to reach the absorptive membrane. Recent work has suggested that collapse of the colloidal structures in the acidic microenvironment of the UWL and stimulation of lipid absorption may result in drug supersaturation and promote drug absorption [120]. The process of absorption-triggered supersaturation has subsequently been modelled by Stillhart et al. [153]. In these studies the authors suggest that supersaturation may be achieved by absorption of lipid digestion products but that concurrent drug absorption (in this case with fenofibrate as a model drug) may prevent attainment of high degrees of supersaturation and therefore prevent initiation of drug precipitation [153]. The latter studies illustrate that interpretation of supersaturation patterns using *in vitro* methodologies should ideally consider the role of absorption *in vivo*. Even in the presence of significant precipitation *in vitro*, drug absorption *in vivo* may reduce the drivers of precipitation and allow ongoing absorption, especially for highly permeable compounds. This is described in more detail in Section 6 alongside a discussion of altered *in vitro* assessment models that include an absorption sink.

6. Progress in developing *in vitro in vivo* correlations for LBFs

The complexity of the relationship between formulation dispersion and digestion, and absorption of solubilised drug and lipids, has long complicated the establishment of robust *in vitro-in vivo* correlations (IVIVC) for LBF. Early attempts to develop formulation performance evaluation tools focussed on correlation of the particle size of the dispersed LBF to *in vivo* absorption. These studies postulated that the dispersed particle size of the formulation was critical to the generation of micellar phases of sufficiently small particle size and high surface area to facilitate rapid drug diffusion to, and absorption at, the luminal surface [24,141]. These correlations were stimulated in large part by the success of the Neoral formulation that dispersed to form sub 100 nm lipid droplets [41,143]. As described above, however, the relationship between particle size and utility is complex and the impact of particle size is much less evident in many other cases. For example, in a recent study correlating droplet size with oral bioavailability, whilst Nielsen et al. reported a trend towards faster absorption with a SNEDDS compared to a SEDDS formulation of probucol, the 10-fold reduction in particle size (45 and 458 nm for SNEDDS and SEDDS respectively) did not result in significant differences in bioavailability [168]. In this case digestion (rather than particle size) was suggested to be have a greater influence on drug solvation and bioavailability [168]. Poor correlations have also been described between dispersion particle size and *in vivo* exposure for LBF of danazol and halofantrine [136,169]. Nonetheless, despite a lack of consistent correlation between dispersion particle size and bioavailability, the generation of a refined, uniform emulsion after dispersion or digestion of a LBF is generally considered desirable since, at the very least, there is evidence that these systems can reduce inter- and intra-subject variability after oral administration [57,143,147,168].

6.1. Does in vitro LBF dispersion data correlate with in vivo absorption?

Dispersion testing using more formal dissolution testing apparatus (rather than simple assessment of particle size) is commonly used to evaluate formulation performance *in vitro*. Traditional USP-like dissolution methods or modifications thereof, are a useful means to evaluate the dispersion/self-assembly properties of self-emulsifying LBF, and also provides an assessment of the likelihood of drug precipitation on LBF dilution. Dispersion tests are generally conducted in physiologically relevant media (simulated gastric/intestinal fluid) using USP type II apparatus (rotating paddles) [37,38,135,170,171], or at lower volumes in a pH stat apparatus prior to lipolysis testing [40,61]. Although there are exceptions, maintenance of drug solubilisation on formulation dilution and emulsification appears to be an important driver of formulation performance. Thus, formulations that show evidence of drug precipitation on formulation dilution/dispersion appear more likely to result in poorer *in vivo* drug exposure [172]. The precipitation phenomenon may be observed visually or quantified by separation of the solid precipitate from solubilised drug in the aqueous phase of the dissolution/dispersion media [40,61]. Real time characterisation of drug solubilisation and precipitation during both dissolution and digestion is becoming increasingly popular and provides important information on both the solubilisation and precipitation kinetics of PWSD. Surface UV imaging, in-line raman spectroscopy and focused beam reflectance are some of the techniques that have been used by Kuentz and coworkers to generate real time drug solubilisation and precipitation data [36,151,152] and have been recently reviewed in the context of small scale dissolution and precipitation testing [173].

To evaluate the potential for IVIVC based on the available literature, Figs. 4, 6, 7, 8 and 9 have been generated for this review by digitization of published data using Engauge (version 4.1) open source digitising software. The potential for (usually) linear correlations were subsequently calculated using GraphPad prism version 6.07. In all instances, the nomenclature of the x axes reflects the original nomenclature employed to describe the reported *in vitro* data. In some cases, therefore the resultant plots have different terms to describe PWSD solvation (% in solution, % dispersed, % drug in digestion aqueous phase (AP), digestion AP concentration, Supersaturation (S) during digestion). The nomenclature of the formulations in each IVIVC plot has been retained as published. Where full *in vitro* solubilisation profiles were available, data have been plotted as the AUC of the profile, calculated using the linear trapezoidal rule. Otherwise, data were plotted using reported solubilisation values at a fixed time point. *In vivo* AUC data was reported to t_{last}. Datasets that did not specify the time range used to calculate AUC do not have an AUC time range specified on the y axis.

Do and colleagues utilised *in vitro* dispersion data to examine the performance of four fenofibrate LBFs and a micronized crystalline formulation and compared this data to *in vivo* exposure after administration to Wistar rats (Fig. 4A) [174]. In these studies, the authors reported that the utility of dispersion experiments extended only to ranking LBF above the micronized formulation but did not discriminate between LBF administered in the fasted or fed state. Consistent with their suggestion, re-plotting the area under the curve of the *in vitro* solubilisation data shows no correlation between *in vitro* solubilisation and *in vivo* absorption (Fig. 4A). Indeed, in this case, a slight trend towards reduced *in vivo* exposure with increasing *in vitro* solubilisation is apparent for the LBF. In another study with fenofibrate, Griffin and coworkers assessed *in vitro* dilution and dispersion behaviour for three LBFs and attempted to correlate with *in vivo* exposure in landrace pigs (Fig. 4B), [170]. In this instance, the dispersion/release tests again did not discriminate between formulations since no drug precipitation was observed from any of the formulations during dispersion testing, but in this case the data were consistent with a lack of significant differences in oral bioavailability *in vivo*. Replotting the reported data in Fig. 4B confirms the author's suggestions and shows similar performance from all three LBF. More encouragingly, Larsen and colleagues

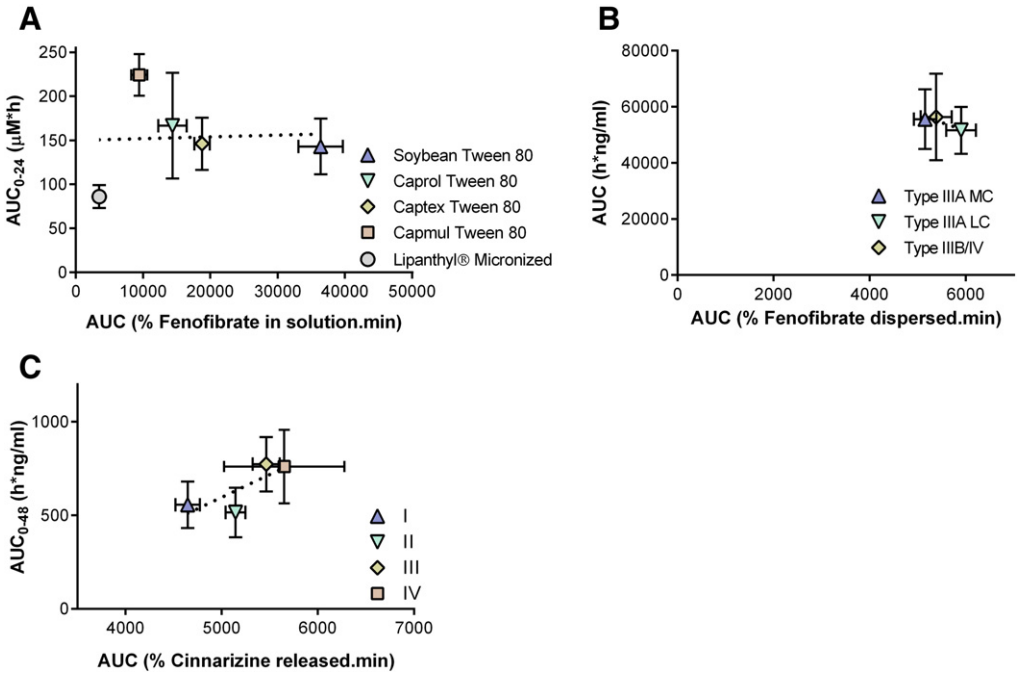

Fig. 4. *In vitro in vivo* correlation of drug absorption and drug solubilisation after formulation dispersion. (A) 'Dissolution' AUC (AUC of % fenofibrate 'in solution' (*i.e.* total fenofibrate in free solution plus solubilised in colloidal species) after 250 min) plotted against the AUC_{0-24h} of the plasma *Vs* time profiles after administration of four LBF formulations and one encapsulated crystalline formulation (Lipanthyl® micronized) to wistar rats. Data replotted from [174]. (B) 'Dispersion AUC' (AUC of % fenofibrate dispersed/released (*i.e.* total fenofibrate in free solution plus solubilised in colloidal species) after 120 min) plotted against the AUC of the plasma *Vs* time profiles after administration of three LBF formulations to landrace pigs. Data replotted from [170]. (C) 'Dispersion AUC' (AUC of % cinnarizine released (*i.e.* total cinnarizine in free solution plus solubilised in colloidal species) after 60 min) plotted against the AUC_{0-48h} of the plasma *Vs* time profiles after administration of four LBFs to Labrador dogs. R^2 0.64. Data replotted from [37].

reported a rank order correlation between solubilisation *in vitro* and *in vivo* absorption for the weak base cinnarizine from four LBFs [37]. These data are reproduced as reported in Fig. 4C, although the correlation of R^2 0.64 suggests only a moderate ability to discriminate between formulations based on dispersion data alone.

The studies outlined in Fig. 4 show that generation of IVIVC from dispersion data is not only a function of the drug investigated but is also a function of the formulations dosed. Notably, fenofibrate appears to benefit from most solubilisation strategies and to provide good exposure despite *in vitro* data that in some cases shows significant drug precipitation.

6.2. Does in vitro LBF digestion data correlate with in vivo absorption?

Maintenance of PWSD solubilisation on dispersion is expected to be important for the performance of a SEDDS formulation as precipitation prior to reaching the duodenum is likely to limit bioavailability for PWSD by reintroducing the need for dissolution from the solid state. However, it is not the only significant challenge to formulation viability, and drug precipitation may also occur on formulation digestion. Subjecting the formulation to an *in vitro* digestion challenge test has therefore become more common and allows some estimation of the GI disposition of drug after the lipid carrier has been hydrolysed.

In vitro digestion testing of LBF was pioneered in the 1980s and in general, lipolysis methods have not changed significantly since their inception by Reymond and Sucker and optimization by Alvarez and Stella [25,175,176]. In these models, *in vivo* lipid digestion conditions are mimicked *in vitro* by addition of a source of lipase enzymes (typically porcine pancreatin) into a temperature controlled reaction vessel containing the LBF dispersed in bile salt lecithin mixed micelles [30,177]. During lipid digestion, the fatty acid released reduces the pH of the reaction medium and is continuously monitored by a pH probe coupled to a pH stat system (Fig. 5). The fatty acid is titrated by autoburette against a known molarity of sodium hydroxide to both maintain pH and allow (indirect) quantitation of the rate and extent of digestion by assumption of stoichiometric titration.

In vitro lipolysis tests provide a relatively simple representation of the complexity of intestinal digestion conditions and the outcomes from *in vitro* lipolysis experiments are contingent on a number of experimental parameters that must be tightly controlled to provide reproducible data. These include buffer capacity [178], enzyme activity [64,65,176], bile salt and phospholipid concentration [60,178], stirring rate, calcium concentration [64,177,179] and pH. Relatively minor differences in the methods used for lipolysis studies can have a significant impact on formulation performance *in vitro*. In the context of LBF development, variability in the *in vitro* methodology employed therefore often prohibits facile inter-laboratory comparison of data sets.

To address this, attempts to standardise *in vitro* lipolysis procedures have been made to facilitate cross comparison of datasets between laboratories [59–64]. In parallel, the development of physiologically relevant media that better reflect the composition of human intestinal fluids remains an area of intensive research [180–182] and has been recently reviewed by Bergström and colleagues [183] and by Fuchs and Dressman [184].

The main advantage of *in vitro* lipolysis testing for analysis of drug-loaded LBF is that it allows estimation of drug distribution between the phases of a digesting formulation *i.e.* determination of the patterns of drug partitioning between an aqueous micellar phase containing bile salt, phospholipid, cholesterol and digested lipids (the 'aqueous phase' or AP); a solid precipitate (the 'pellet phase') or an undigested or partially digested oil phase (the 'oil phase') (Fig. 5).

Consistent with the rationale for formulation dispersion tests, increasing or maximising aqueous phase drug concentrations (and minimising drug precipitation) is expected to maximise the likelihood of robust drug absorption from LBF *in vivo*. It is increasingly apparent, however, that this is a conservative indicator. Thus, although formulations that provide for good post-digestion solubilisation almost always provide for good *in vivo* exposure, in some cases good drug absorption is possible from formulations where drug precipitation *in vitro* is

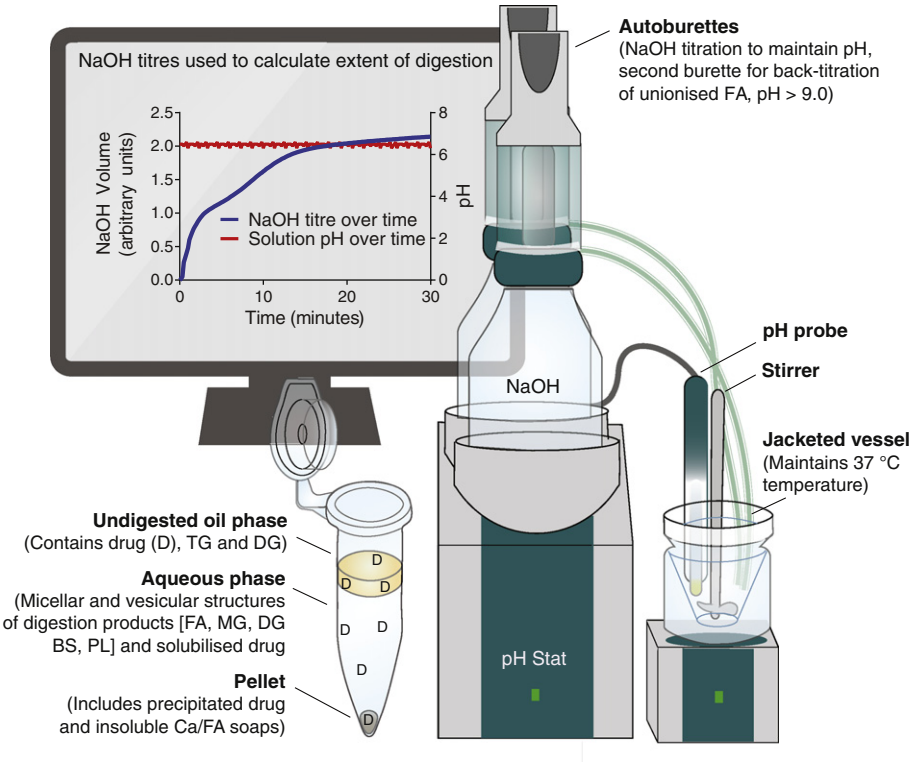

Fig. 5. Experimental model for *in vitro* assessment of lipid-based formulations. Abbreviations used are; sodium hydroxide (NaOH), drug (D), triglyceride (TG), diglyceride (DG), fatty acid (FA), monoglyceride (MG), bile salt (BS), phospholipid (PL), calcium (*Ca*).

significant. Digestion testing therefore provides a useful screening tool to identify formulations with a strong chance of good absorption *in vivo*—but may eliminate some formulation that might otherwise provide for useful absorption.

Analogous to the reanalyses presented in Fig. 4 for *in vitro* dispersion data, Fig. 6 shows a summary of published *in vitro–in vivo* correlation data using *in vitro* digestion tests.

From Fig. 6, it can be seen that IVIVC for differing LBFs is highly drug-dependent. Of the eight drugs examined four (50%) appear to show some degree of correlation (Fig. 6A, C, E and F) and four do not have strong correlations (Fig. 6B, D, G and H). Of those that show reasonable correlation between *in vivo* exposure and digestion AP solubilisation, two exhibit non-linear correlations (Fig. 6E and F) and two show clear linear correlation with the AP drug solubilisation after digestion (Fig. 6A and C). There is no one obvious physicochemical drug property (summarised in Table 2) that can be used to explain the likelihood of useful correlation, with the obvious exception of torcetrapib and its structural analogue CP-532,623 that as expected show similar correlation behaviours [40]. The two drugs that exhibit linear correlation (danazol and griseofulvin) are high melting point compounds with similar and relatively high logP. In contrast, dexamethasone which has a comparable melting point but lower logP appears to have formulation independent absorption [38,39] (possibly due to intrinsically higher aqueous solubility relative to dose). The logP of the drug alone clearly does not indicate the likelihood of IVIVC as danazol, cinnarizine and fenofibrate all have similar logP values but are starkly different performers *in vitro* and *in vivo* [37,38,170]. The data therefore suggest that simple physicochemical comparisons are not enough to deconvolute formulation performance *in vitro* and *in vivo*, although better correlations are apparent with poorly soluble, higher melting point drugs that presumably result in crystalline precipitates with slower rates of re-dissolution. Other physicochemical properties such as the glass-forming ability (*i.e.* the ability to form an amorphous state) may have an impact on the likelihood (or otherwise) of precipitation, the physical form of the precipitate (amorphous *vs* crystalline) and the redissolution kinetics of the PWSD in the lumen, but to this point have not been examined in detail in this context. To this end, early identification of PWSD glass forming potential through the use of computational models may allow more judicious selection of formulation approaches, and complementary *in silico* permeability modelling may help to further predict the behaviour of PWSD formulated in LBF. For an overview of the computational tools used to predict druggability, solubility, permeability and glass forming ability, the reader is referred to the review by Bergström and colleagues in this issue [186].

6.3. Do in vitro indicators of supersaturation improve IVIVC?

Although maintaining drug solubilisation, and reducing drug precipitation is a likely contributor to the absorption of PWSD from LBFs, the supersaturation events that precede precipitation are also important. Direct measurement of the free drug concentration (*i.e.* the fraction readily available for absorption) in a dynamic digestion environment is difficult as separation of free drug from solubilised drug is complex and takes finite time. As such the data obtained are unlikely to be able to track rapid changes in free and solubilised drug concentrations. A crude estimate of free concentration, however, may be made from measurement of the apparent solubility of the drug in a blank digested formulation and comparison of these values with the data obtained at the same timepoints during dynamic lipolysis experiments. This ratio provides an indication of the degree of saturation or supersaturation and therefore an indication of thermodynamic activity and free concentration. Supersaturation in the context of GI drug delivery, and with reference to LBF has been reviewed by both Bevernage and Williams respectively [187,188].

Despite increasing awareness of the importance of supersaturation in drug absorption from oral LBFs [7], relatively few *in vivo* studies have been published with accompanying correlations to

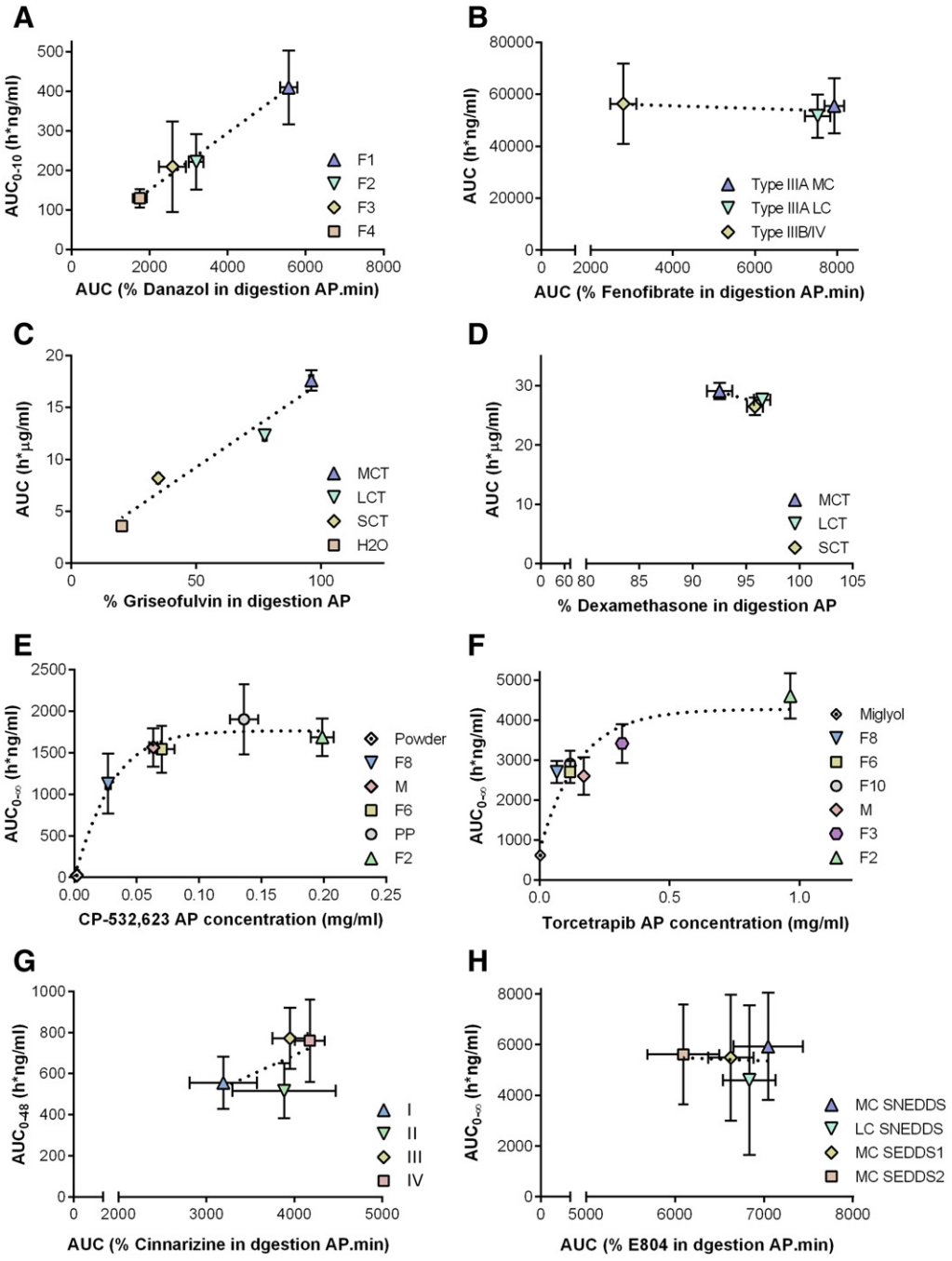

Fig. 6. *In vitro in vivo* correlation data of *in vivo* drug exposure and drug solubilisation after *in vitro* formulation digestion. (A) 'Digestion AP AUC' (AUC of % danazol in digestion AP after 60 min) plotted against the AUC_{0-10h} of the plasma *Vs* time profiles after administration of four LBF formulations to male beagle dogs, R^2 0.99. Data replotted from [38]. (B) 'Digestion AP AUC' (AUC of % fenofibrate in digestion AP over 80 min) plotted against the AUC of the plasma *Vs* time profiles after administration of three LBF formulations to landrace pigs, R^2 0.32. Data replotted from [170]. (C) % griseofulvin solubilised in digestion AP after 30 min *in vitro* digestion plotted against the AUC of the plasma *Vs* time profiles after administration of three LBF formulations and one suspension to male wistar rats, R^2 0.98. Data replotted from [39]. (D) % dexamethasone solubilised in digestion AP after 30 min *in vitro* digestion plotted against the AUC of the plasma *Vs* time profiles after administration of three LBF formulations to male wistar rats, R^2 0.68. Data replotted from [39]. (E) CP-532,623 concentration in digestion AP after 30 min *in vitro* digestion plotted against the $AUC_{0-\infty}$ of the plasma *Vs* time profiles after administration of five LBF formulations and a powder-in-capsule control to male beagle dogs. 4-PL curve fit (dotted line) shown as published, linear regression analysis ($R^2 = 0.60$, line not shown) was also conducted to allow comparison to other data in this figure. Data replotted from [40]. (F) Torcetrapib concentration in digestion AP after 30 min *in vitro* digestion plotted against the $AUC_{0-\infty}$ of the plasma *Vs* time profiles after administration of seven LBF formulations to male beagle dogs. 4-PL curve fit (dotted line) shown as published, linear regression analysis ($R^2 = 0.59$, line not shown) was also conducted to allow comparison to other data in this figure. Data replotted from [40]. (G) AUC of % cinnarizine solubilised in digestion AP after 60 min *in vitro* digestion plotted against the AUC_{0-48h} of the plasma *Vs* time profiles after administration of four LBF formulations to Labrador dogs, R^2 0.39. Data replotted from [37]. (H) AUC of % E804 solubilised in digestion AP over time plotted against the $AUC_{0-\infty}$ of the plasma *Vs* time profiles after administration of four LBF formulations to wistar rats, R^2 0.02. Data replotted from [185]. Data were extracted as described in Section 6.1.

supersaturation data during dispersion and digestion. This may be in part a function of the complexity of the measurement methods that are used to describe supersaturation during *in vitro* testing. Data analysis is also complicated by the potential to measure supersaturation at a fixed time point or to use an AUC measurement to estimate total supersaturation over a time period. Anby et al. first reported the impact of digestion-mediated supersaturation in relation to LBF and developed methodologies to quantify the degree of supersaturation over time [35]. In these studies, the AUC of drug solubilisation kinetics during digestion was divided by the AUC of drug apparent solubility in blank digested

Table 2
Physicochemical properties of the PWSD evaluated for IVIVC.

	Molecular weight	clogP[a]	clogD[a]	Melting point	Rule of 5 violations	Correlation[b]
Danazol	337.5	4.94	4.94 (pH 7.0)	225 °C	0	L
Fenofibrate	360.8	5.80	5.80 (pH 7.0)	81 °C	0	N
Griseofulvin	352.8	2.01	2.01 (pH 7.0)	219 °C	0	L
Dexamethasone	392.5	2.03	2.03 (pH 7.0)	262 °C	0	N
CP-532,623	598.5	6.77	6.77 (pH 7.0)	112 °C[c]	2	NL
Torcetrapib	600.5	6.64	6.64 (pH 7.0)	54 °C	2	NL
Cinnarizine	368.5	5.03	4.69 (pH 7.0)	118 °C	0	N
E804	365.4	1.65	1.65 (pH 7.0)	263 °C[d]	0	N

[a] clogP and clogD calculated values from ACD labs software V11.02, retrieved from SciFinder.
[b] Correlations classified as L—linear, NL—non-linear, N—no correlation.
[c] Mean (n = 3). Measured by hot-stage optical microscopy.
[d] Predicted melting point from US Environmental Protection Agency's EPISuite™ software. retrieved from RSC Chemspider.

formulation during the same time period and defined as the extent of supersaturation (S). This time resolved supersaturation ratio allows quick comparison of the precipitation potential of LBFs over the course of a dispersion/digestion experiment and is the method utilised to calculate supersaturation (S) in Figs. 7 and 8. However, this method requires *in vitro* dispersion and digestion data at multiple time points. In many published studies, full time-resolved profiles are not available and in these cases, supersaturation is typically calculated at a single time point by comparing the measured concentration in the aqueous phase at *e.g.* 30 min post digestion with equilibrium solubility in blank digested colloids at the same point. This method generates a supersaturation ratio defined as SR [60,189] to differentiate the calculation method from the time-resolved supersaturation (S) generated *via* AUC comparison as described above [35].

Gao and colleagues similarly described the development of supersaturable SEDDS (s-SEDDS) as early as 2003 [161,190]. In an approach analogous to the spring and parachute effect of prolonging metastable supersaturation, (as depicted in Fig. 3A), s-SEDDS formulations were prepared by incorporation of hydroxypropyl methyl cellulose (HPMC) as a polymeric precipitation inhibitor (PPI) into the SEDDS [190]. Formulation performance was evaluated using an *in vitro* precipitation (dispersion) test, where formulations were diluted in buffer at pH 2 to mimic release conditions in the stomach. Concentrations of the drug PNU-91,325 were defined as the total concentrations of both free drug and drug associated with the colloidal species formed on formulation dispersion. Addition of HPMC to the formulations retarded drug precipitation *in vitro* and subsequent *in vivo* administration of selected formulations showed that absorption from s-SEDDS was greater than from formulations that did not include a PPI. The IVIVC of the AUC of the reported *in vitro* precipitation (dispersion) data has been plotted against *in vivo* AUC in Fig. 7A using the methodology described in Section 6.1. Clear correlation between the dispersion AUC and *in vivo* absorption can be seen (R^2 0.90).

Interestingly, the authors reported the equilibrium solubility of the free drug in buffer, as well as dynamic drug precipitation data. These datasets allow calculation of a time resolved supersaturation ratio (achieved by calculating an AUC from the equilibrium solubility of free drug in solution (6 μg/ml) assuming that it remains unchanged for the duration of the three hour precipitation experiment). The resultant supersaturation ratios (S) are plotted against the reported *in vivo* AUCs in Fig. 7B. These data show the same trends as the solubilisation data in Fig. 7A since they are essentially the same data divided by a constant (the solubility AUC). Notably, unlike the supersaturation (S) or supersaturation ratio (SR) calculations described above, the methods employed by Gao et al. defined supersaturation relative to equilibrium solubility of drug in buffer alone, rather than drug solubility in the dispersed formulation. This leads to much larger values for the supersaturation ratio. Furthermore, the s-SEDDS and Tween formulations are likely to be digested *in vivo* leading to changes (potentially decreases) in solvation capacity. The combined effects of using equilibrium solubility in buffer and the absence of a digestion step, in this instance, probably overestimate absolute supersaturation, however the relative differences across formulations are likely to be similar. Subsequent studies by Gao et al. showed that the significant *in vitro* performance advantage of s-SEDDS extended to faster absorption of AMG 517 from formulations stabilised by PPIs, but in this case overall exposure in Cynomolgus monkeys was comparable to a conventional SEDDS [191].

In a similar manner to the studies by Gao et al., the supersaturation measures defined by Anby et al. [35] were employed to investigate the potential relationship between supersaturation during formulation digestion and drug absorption for lipid formulations containing PPIs. In this case the PPI significantly inhibited *in vitro* drug precipitation, thereby increasing supersaturation, but this did not lead to large changes in *in vivo* exposure in beagle dogs [35]. As such, no correlation was evident between supersaturation and absorption (Fig. 8A). In a more recent study using a rat model of digestion [136], and enhancing drug solubilisation by slowing MCT LBF digestion (therefore reducing supersaturation), improved drug solubilisation appeared to promote drug absorption after administration to Sprague Dawley rats. This led to a marginally negative correlation between drug exposure and supersaturation, Fig. 8B [136]. In contrast, Fig. 8C shows good correlation between

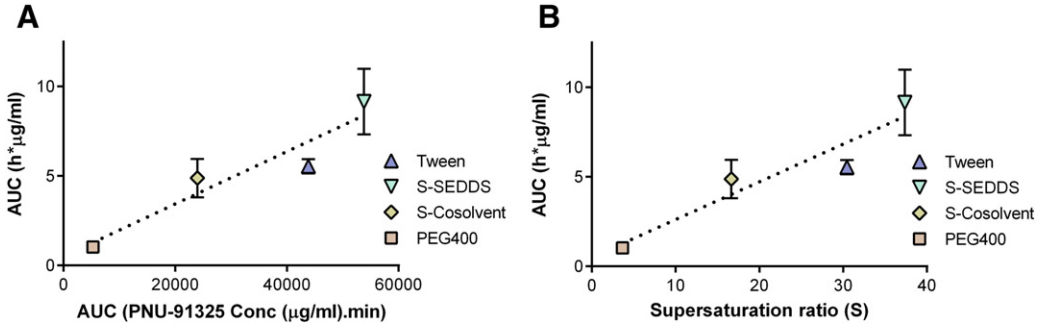

Fig. 7. IVIVC of *in vivo* exposure (y axis) of PNU-91325 with (A) AUC of *in vitro* drug concentration.min (R^2 0.90) and (B) *in vitro* supersaturation ratio (S) during *in vitro* precipitation tests (R^2 0.90). Supersaturation ratio (S) calculated using the method proposed by Anby [35]. Data recalculated and plotted from [190].

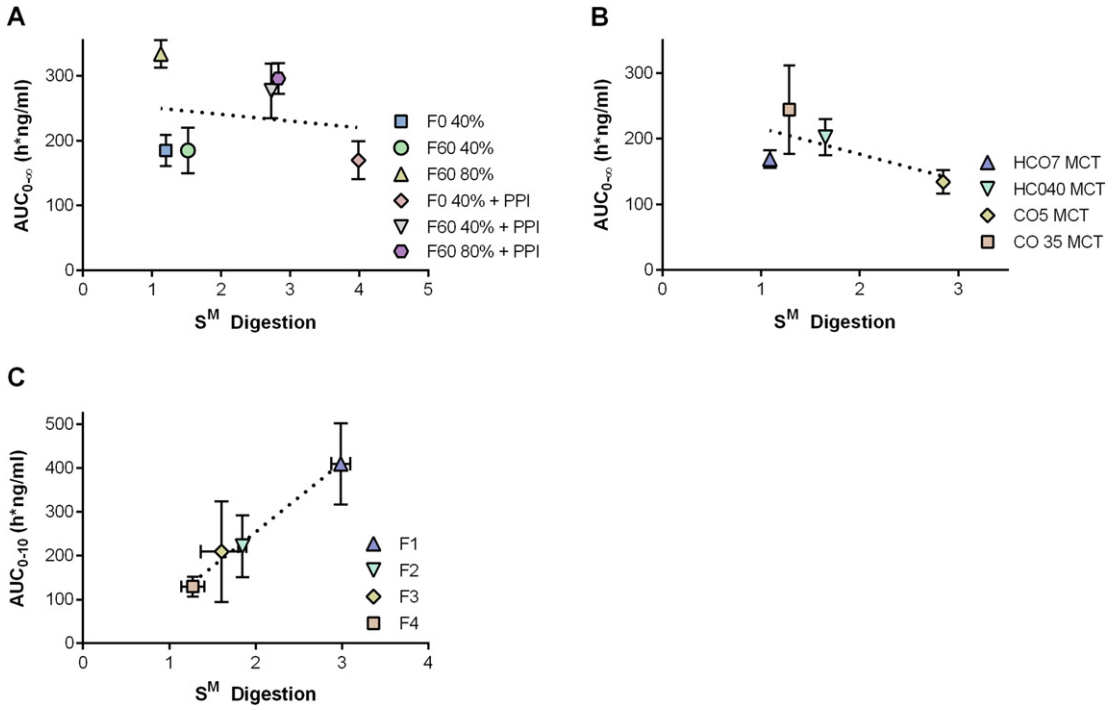

Fig. 8. IVIVC of danazol absorption and *in vitro* supersaturation (S) during digestion calculated using the method proposed by Anby et al. [35]. (A) Supersaturation ratio (S) in digestion AP plotted against the AUC of the plasma *Vs* time profiles after administration of four MCT LBF formulations to male beagle dogs. PPI = polymeric precipitation inhibitor. Data replotted from [35]. (B) Supersaturation ratio (S) in digestion AP (rat model) plotted against the AUC of the plasma *Vs* time profiles after administration of four MCT LBF formulations to Sprague Dawley rats. HCO = PEGylated hydrogenated castor oil surfactants. CO = PEGylated castor oil surfactants. Data replotted from [136]. (C) Supersaturation ratio (S) in digestion AP plotted against the AUC of the plasma *Vs* time profile after administration of four LCT LBF to male beagle dogs, R^2 0.99. LCT content of formulations decreases from F1 to F4. Data replotted from [38,192].

supersaturation during digestion and *in vivo* exposure for a series of long chain triglyceride based LBF administered to dogs. In this instance, the data from Cuine et al. was plotted against supersaturation data subsequently generated by Porter et al. [192]. A strong positive correlation of R^2 0.99 is evident for this data set. Interestingly, the three data sets in Fig. 8 have been generated from the same model drug (danazol) and from our research group, but do not appear to provide a consistent indication of utility. The data serve to underline the complexity of the relationship between digestion, supersaturation and drug absorption. It is evident that under some circumstances increases in saturation may promote absorption due to increased thermodynamic activity (Fig. 8C), whereas in others, increased supersaturation appears to reduce drug absorption, presumably since supersaturation is likely to be an indirect indicator of the likelihood of precipitation (Fig. 8B). Balancing the beneficial effects of supersaturation (increased thermodynamic activity) against the potentially negative effects (increased likelihood of precipitation) and identifying appropriate *in vitro* models to reflect these changes remains a challenge.

7. Recent developments in improving *in vitro* methods for LBFs

The disconnect in IVIVC that is evident in some cases (and exemplified above) has meant that efforts have redoubled to improve the physiological relevance of *in vitro* digestion tests. For example, gastric lipolysis is not typically addressed in the *in vitro* lipolysis model [37–39], and indeed, all the data shown in Figs. 6 and 8 was generated under simulated intestinal lipolysis conditions only. The development of gastric and intestinal lipolysis models may therefore provide a more comprehensive representation of the *in vivo* digestive environment [63,193–195]. To probe this possibility, Thomas and coworkers included gastric lipolysis in a recent evaluation of LBF of fenofibrate and attempted to correlate with *in vivo* exposure in Göttingen minipigs [189]. However, these data (Fig. 9) also failed to show a correlation between *in vitro* solubilisation and *in vivo* absorption. Three independent studies (Figs. 4B, 6B and 9) therefore suggest that fenofibrate exhibits robust *in vivo* absorption irrespective of formulation dosed, species used or whether dissolution or dispersion *in vitro* data is used to generate the IVIVC. In all cases the LBFs show improved absorption when compared to crystalline drug [170,174,189]. It seems likely that the lack of correlation in these studies reflects the choice of compound (fenofibrate), which appears to be well absorbed from most solubilised formulations (possibly due to very high permeability), rather than the intrinsic utility of the model evaluated.

The nature/form of the precipitate that is generated on LBF digestion may also impact on the likelihood of effective IVIVC. Thomas et al. recently reviewed the solid state characterisation techniques used to evaluate the nature of drug precipitates during *in vitro* digestion [196] and as such these will not be discussed in detail here. The most popular solid state characterisation techniques used in conjunction with *in vitro* digestion tests are summarised in Fig. 10. Precipitation of PWSD in the amorphous form might be expected to assist in drug redissolution

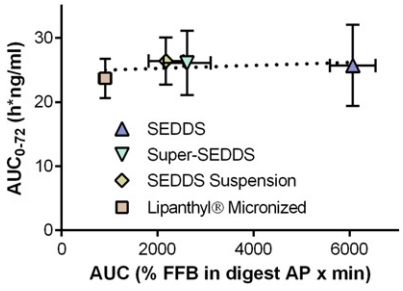

Fig. 9. *In vitro in vivo* correlation of drug absorption and *in vitro* digestion data. (A) AUC of % fenofibrate in digestion AP plotted against the AUC of the plasma *Vs* time profiles after administration of three LBF formulations and one encapsulated crystalline formulation (Lipanthyl® Micronized) to Göttingen Minipigs, R^2 0.12. Data replotted from [189]. Data were digitised and AUCs calculated as previously described.

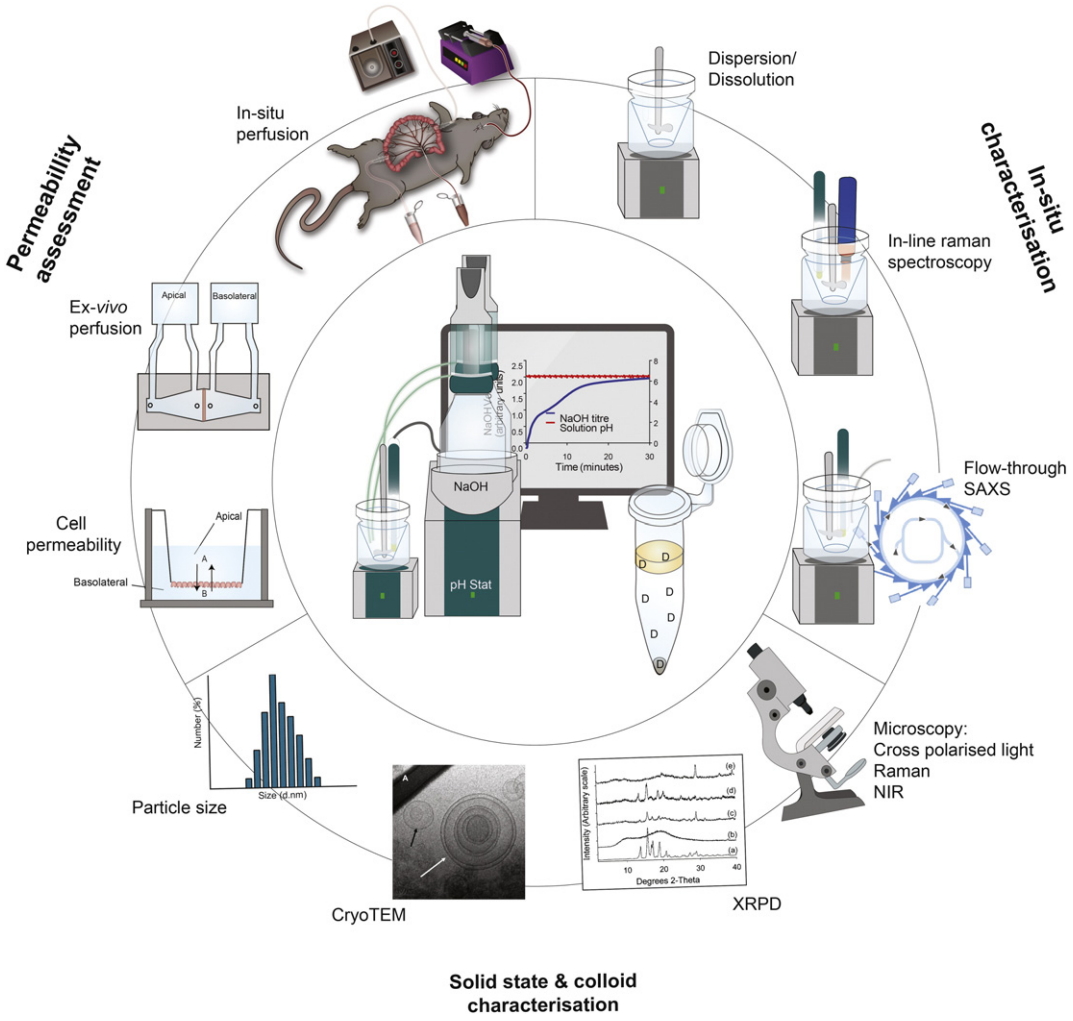

Fig. 10. Overview of the most prevalent characterisation techniques used in conjunction with *in vitro* lipolysis. In-line raman diagram adapted from [152], microscope adapted from Servier medical art, XPRD data for danazol reproduced from [35], cryo TEM of aspirated human intestinal fluid reproduced from [114], *in situ* perfusion diagram reproduced from [202]. Figures and data reproduced with permission.

when compared to precipitation in the crystalline form [167]. The addition of solid state property profiling of the precipitates formed during lipid digestion may therefore improve the quality of data interpretation in *in vitro* testing protocols [152,153,197]. To date, the formation of amorphous precipitates from dispersed or digested LBF has been reported most frequently for weakly basic drugs, possibly due to the potential to form amorphous complexes with fatty acid digestion products [34,167,198,199]. Amorphous precipitates have also been reported for drugs in formulations containing polymeric precipitation inhibitors [191,200]. For a recent review on the precipitation behaviour of PWSD in the presence of LBF the reader is referred to Khan et al. [201].

The species used for *in vivo* LBF evaluation may also have an impact on the applicability of the 'standard' *in vitro* lipolysis model. Rats are a convenient, economical and relatively high throughput *in vivo* model (when compared to dogs or pigs). For high cost drugs in particular, the use of a rat *in vivo* model may facilitate generation of early preclinical data without significant API outlay. An added benefit of this *in vivo* model is the potential for more facile inhibition of enzymes, transporters or efflux pumps to allow systematic evaluation of first pass metabolism or efflux on drug absorption from a LBF. *Ex vivo* or *in situ* analyses also tend to be conducted with excised rat tissue. In contrast, the majority of *in vitro* digestion experiments are run under conditions developed to reflect larger animals (dogs) or humans. To address this contradiction, an *in vitro* rat model of lipolysis which reflected the differences in volume and enzyme activity in the rat GIT was developed [65]. The model was subsequently used to evaluate the performance of a series of LBF in the presence and absence of 1-aminobenzotriazole (a CYP inhibitor) to investigate the impact of first pass metabolism on danazol absorption from LBFs. The data suggest that digestion in a rat is less efficient than in dog (and potentially in humans) and that danazol is very highly first pass metabolised, especially in the rat. An adaptation of the rat *in vitro* model with pH-stat control has since been utilised to explore IVIVC in rats for a series of digestion inhibiting LBFs [136]. In these studies, the mass of LBF administered to the rat was also scaled down to 30 mg per 300 g rat in order administer an approximately equal mg/kg formulation mass relative to dog (*i.e.* equivalent to 1500 mg formulation dosed to a 15 kg beagle). IVIVC of the adjusted rat *in vitro* digestion model and dose proportional *in vivo* model is presented in Fig. 8B.

7.1. Accounting for absorption in the in vitro digestion model

Perhaps most importantly, the IVIVC data described above rely on a closed system of *in vitro* lipolysis to generate solubility and supersaturation data that are then correlated with *in vivo* exposure. A limitation of these models is that they are unable to take into account the parallel process of drug (and lipid) absorption *in vivo*. As described above, lipid absorption might be expected to increase drug supersaturation in lipid swollen intestinal colloids (by reducing drug solvation capacity), whereas drug absorption is likely to reduce supersaturation. *In vitro*

tests that incorporate an adequate absorption sink might therefore be expected to better estimate both drug and digestion product absorption across the enterocyte [203].

Recognition of the limitation of closed *in vitro* systems is not confined to digestion testing. A far greater body of work has addressed the potential to integrate an absorption sink into *in vitro* dissolution apparatus to assess more traditional solid dose forms. In this case a number of different techniques have been employed to provide sink conditions. These include simple biphasic liquid dissolution models, transfer models, dialysis and filtration membrane systems and the coupling of dissolution models to Caco-2 monolayers and perfused intestinal segments in whole animals. These have recently been described in the excellent reviews by both McAllister and Kostewicz [204,205].

In the context of lipid-based formulations, a simplified biphasic liquid dissolution model utilising an aqueous (buffer) and an organic layer (1-octanol) has been employed by Pillay and Fassihi to evaluate release of nifedipine from a LBF [206]. In this case, the authors focused only on the release of drug from the formulation and chose not to investigate the impact of lipases on solubilisation and/or precipitation behaviour. There was also no *in vivo* data to allow correlation of the method with *in vivo* absorption. Shi and coworkers investigated a similar biphasic buffer–octanol system (where both the octanol and aqueous phases were stirred and coupled to a USP IV flow through cell) to evaluate the release of celecoxib from two LBFs [207]. The *in vitro* data were then compared to published clinical data to generate IVIVC. While the aqueous phase concentrations of celecoxib did not correlate with *in vivo* absorption, the AUC of drug concentration in the octanol phase (*i.e.* the 'absorption sink') showed rank order correlation to the clinical data (R^2 0.97). Unfortunately, the system lacked an *in vitro* digestion component and no attempts were made to generate or quantify supersaturation, which, in spite of the good correlation, may have better explained the comparatively poor *in vivo* absorption of, for example, a solution formulation of Tween 80 and ethanol. One significant drawback of biphasic systems for evaluation of LBF, however, is that the addition of media including solubilising species such as bile salt micelles and LBF derived surfactants to systems containing LBF components and digestion products is likely to cause some degree of emulsification at the octanol:water interface and may perturb efficient drug distribution between the two phases.

The addition of a more biologically relevant absorption sink to *in vitro* lipid digestion models is further complicated by the incompatibility of some simulated intestinal media, especially those containing bile salts and lipolysis enzymes, with many cultured cell monolayers [208]. Nonetheless, permeation studies of simple solutions of PWSD in buffers or SIF have been described and provide useful information as to the likelihood of dissolution, solubility or permeation limitations to *in vivo* exposure [209,210]. Kataoka et al., for example, evaluated the solubility-supersaturation-permeability paradigm for undigested SEDDS formulations of the permeable drug danazol and the poorly permeable drug pranlukast utilising a dissolution–permeation (D-P) model to predict absorption *in vivo*. Whilst acknowledging the limitation of the absence of lipolysis, and realising that the particle size of the undigested formulations was large in comparison to the colloids formed post digestion, the *in vitro* permeability studies did allow correlation between danazol absorption from the different LBFs in rats and absorption estimates from the D-P system. In contrast, pranlukast uptake appeared to be permeability limited, formulation independent and did not correlate to the D-P data [211].

These studies serve to reinforce the complexity in interpreting the data obtained from *in vitro* models such as the D-P model for drugs where absorption may be limited by solubility and/or permeability. Fig. 11 presents an adaptation of a figure from Ginski et al. [209] modified to incorporate the prospect of not only differentiation between drugs with low permeability, but also those that might have very high permeability. Thus, for drugs with low absorption and where absorption is dissolution or solubility limited, LBF are expected to enhance oral exposure when permeability is good (for example danazol [209]), but not when permeability is the primary limitation to absorption (as was the case with, for example, pranlukast [209]). Based on the published data for fenofibrate, however, it seems likely that further distinction might usefully be made between drugs with very high permeability, where LBF appear to enhance absorption, but do so in a relatively formulation independent manner. In this case even transient solubilisation appears to be able to drive significant membrane flux and absorption, and the rate of absorption may be sufficient to preclude *in vivo* precipitation. However, far more data is required to confirm this hypothesis and it remains possible that the data obtained for fenofibrate reflect differences in the drivers of precipitation *in vivo* when compared to the conditions employed in the *in vitro* digestion apparatus.

The GI mucous barrier may also play a role in PWSD absorption from LBF, but is rarely examined specifically [212]. Intestinal mucus forms a size, steric and molecular barrier to diffusion across the unstirred water layer and prevents rapid diffusion of protons away from the epithelial surface. Together these processes act to slow colloid transit towards the enterocyte, modulate exposure to bile salts and phospholipids and maintain the low pH of the UWL. The latter favours protonation of fatty acid digestion products and absorption of the unionised form of the lipid. Recent data shows that in spite of the potential permeability enhancing properties of some components of food, food ingestion actually enhances the barrier function of mucous (at least to the diffusion of model 200 nm nanoparticles), perhaps as part of a defence mechanism to avoid absorption of particulate contaminants in food. [213]. While the role of the GI mucosal barrier has been well explored in terms of nanoparticulate drug delivery [214–217], and has been extensively reviewed by Ensign and colleagues [218,219], the impact of GI mucus on cell-based permeability, stabilisation of drug supersaturation and UWL diffusion of PWSD solubilised in intestinal colloidal species, has been largely overlooked. In one of the few studies that have evaluated mucous effects, Preat and colleagues used Caco-2

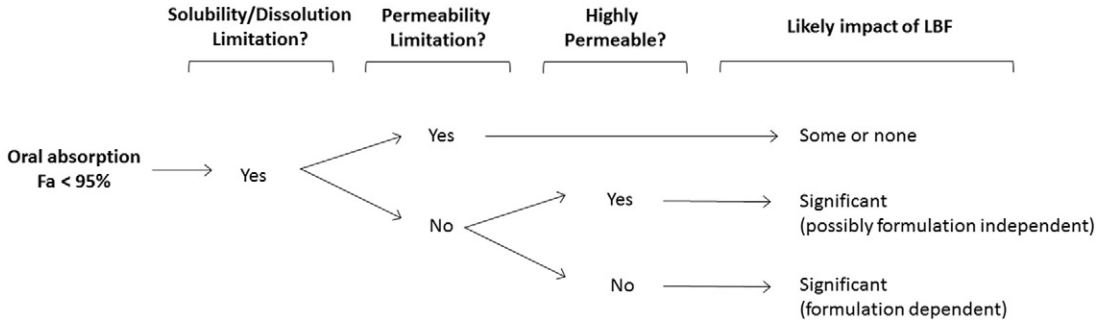

Fig. 11. Schematic presentation of the relationship between the limiting steps of oral absorption of poorly water-soluble drugs and the likelihood that LBF will promote absorption in a formulation dependent or independent manner.

cells and HT29-MTX/Caco-2 co-cultures (since HT29 cells secrete mucous) to evaluate the impact of mucus on the absorption of β-arteether (AE) from LBFs [220]. In this instance, the mucous layer appeared to reduce drug permeability slightly, however statistical significance was only reached for a tween 80 based SEDDS formulation, and not for a similar Cremophor based formulation. The more significant impact on absorption appeared to be lipid digestion and formulations that were partly resistant to *in vitro* lipolysis significantly increased the transport of AE across intestinal cell monolayers. Protection against lipolysis may have resulted from the use of PEGylated surfactants, which have been shown to inhibit lipase binding and lipid hydrolysis [136], but may also be attributable to the mucus penetrating properties of PEG [218,219]. A very recent study by Chang and McClements showed that fish oil droplets coated with a PEGylated surfactant (tween 80) were more stable in mucin than equivalent caseinate emulsions [221]. To better evaluate the role of mucous in drug absorption, Boegh and colleagues have established a caco-2/biosimilar mucus model as an alternative to co-culture for evaluation of protein and peptide absorption across cell monolayers [222,223] while Béduneau and coworkers have recently developed a tunable Caco-2/HT29-MTX co-culture model to better mimic human intestinal permeability [224]. In a subsequent study, Antoine et al. showed that the formation of a strongly adherent mucus barrier on the luminal side of the co-culture protected the cells from both bile salts and lipases when compared to a Caco-2 monoculture [225]. These studies exemplify the promise co-culture and *ex vivo* mucin models may hold as a more relevant *in vitro* model of the intestinal epithelium when compared to simple Caco-2 monolayers for the evaluation of LBF. Further work examining the potential impact of formulation excipients on the barrier properties of the GI mucosal layer could provide valuable insight into the impact of LBFs on both drug and formulation uptake. Cultured cell monolayer systems do, however, have one intrinsic drawback when assessing rates of drug absorption and that is the relatively low absorptive surface area when compared to the GIT.

In an effort to incorporate more physiologically relevant sink conditions (with an intact, higher surface area mucosal barrier) into the *in vitro* lipolysis model, Dahan and Hoffman investigated drug permeability (dexamethasone and griseofulvin) from digested lipid vehicles (long, medium and short-chain triglycerides) across 5 cm^2 *ex vivo* jejunal segments in a side-by-side modified Ussing chamber [39]. The formulations underwent dynamic *in vitro* digestion testing and at the end of lipolysis stage, the media was transferred to the donor (mucosal) cell of the chamber and drug accumulation in the acceptor (serosal) cell was monitored over 3 h. However, data from the *in vitro* digestion–*ex vivo* jejunal model did not correlate to the *in vivo* data. The authors concluded that for permeable, yet solubility limited drugs the use of short chain triglycerides (which enhanced permeation in the *ex vivo* model but not *in vivo*) were unlikely to translate to an *in vivo* advantage. Again, knowledge of the rate limiting step in PWSD absorption (solubility limited or permeability limited) was highlighted as a critical factor in determining the likelihood of formulation mediated changes being capable of changing *in vivo* exposure.

Potential limitations to excised *ex vivo* absorption models include the lack of intact nervous system, lack of blood flow, changes to transporter and enzyme function or expression and incompatibility with complex solvent systems. To address this, *in situ* absorption models may be used to allow measurement of absorption in a relatively intact *in vivo* system. The intestinal single pass perfusion method, for example, first described in 1958 [226], has long been used to explore intestinal drug absorption mechanisms. Despite the experimental challenges associated with the model, it remains one of the most highly used systems for investigation of intestinal drug absorption mechanisms and transporter/metabolism/efflux studies. Further information on the *in situ* perfusion model in rodents may be obtained from the very recent, and comprehensive review by Stappaerts et al. [227].

The robustness of the *in situ* perfusion model to physiological concentrations of bile, fatty acids and lipases renders it an ideal absorptive sink to couple to *in vitro* lipolysis experiments. In light of this, a coupled *in vitro* digestion–*in situ* perfusion model for the assessment of PWSD absorption from LBFs was recently developed [202]. Perfusion of a dynamic, digesting LBF through an externalised jejunum segment of a rat with direct measurement of drug flux into blood *via* mesenteric vein cannulation provides a unique opportunity to simultaneously examine the solubility-supersaturation-permeability paradigm in a relatively controlled system. Due to experimental complexities, the model is unsuitable for use as a high throughput screening tool, and is intended as a means to provide mechanistic understanding of drug absorption from LBFs. In these studies, Crum et al. report on the absorption of fenofibrate from three different LBFs. Despite the *in vitro* lipolysis portion of the model showing clear discrimination between supersaturation of the three LBFs, drug flux into the mesenteric vein was not significantly different between lipid formulations and all LBFs showed significantly greater flux than crystalline drug. This data is in agreement with the robust *in vivo* absorption data for fenofibrate in pigs, rats and minipigs that was summarised in Figs. 4, 6 and 9. The data lend support to the hypothesis that for highly permeable drugs such as fenofibrate, solubility (and/or dissolution) limited drug absorption from the crystalline state may be overcome through lipid formulation strategies, but that differences between formulations that may be seen using current *in vitro* methods are less apparent *in vivo* due to the high permeability and rapid absorption from the GIT.

8. Lipophilicity, lipid solubility and LBF utility—a role for deliberate increases in drug lipophilicity?

As described above and elsewhere, LBF are, in almost all cases where they can be employed, effective in increasing GI solubilisation and absorption of PWSD. The relative benefits of different LBF inevitably vary with excipient choice and drug properties, and recent efforts have been directed at fine tuning *in vitro* methods to better predict optimal formulations. In most cases where LBF performance is suboptimal, rapid drug precipitation from the dispersed or digested formulation is the likely cause (although even rapidly precipitating formulations can provide good drug exposure where permeability is high). Rapid drug precipitation on dispersion often results from the need to include large quantities of surfactant or cosolvent in the formulation in order to dissolve the target drug dose. This observation introduces perhaps the most significant limitation to the more widespread application of LBF as a means of enhancing oral absorption for PWSD—that of drug solubility in the formulation. In most cases, formulations where drug is dissolved in the formulation/capsule fill material are preferred and limitations to acceptable capsule size therefore limit the possible dose that can be administered. Drug suspensions in LBF have been successfully employed [52] and can provide for very significant increases in drug exposure [37]. Lipid solution formulations are therefore not an absolute prerequisite—but two phase systems provide additional challenges in material transfer, content uniformity, viscosity and *in vivo* reproducibility and where possible lipid solution formulations are typically preferred.

Drug solubility in the formulation is therefore a key determinant of the utility of LBF. Simplistically, the assumption is often made that PWSD with high logP are 'lipophilic' and therefore that solubility in the excipients often employed in LBF will be high. However, log P is a poor indicator of absolute lipophilicity [159,228], and instead provides only an indication of 'relative' lipophilicity compared to water. Indeed, there are many drugs where relative partitioning between octanol and water is high (*i.e.* high log P), but where absolute lipid solubility remains low. These compounds are indicative of the typical 'brick-dust' type of poorly water soluble drug, that is essentially 'everything-phobic', but where relative affinity for octanol over water is often high. In this case solubility in both water and lipids is commonly limited by

intermolecular forces in the solid state—a property indicated by high melting point. In contrast, 'grease ball-like' poorly water soluble drugs may have similar log Ps, but in this case have intrinsically higher lipid solubility (and usually lower melting point). The importance of melting point in dictating lipid solubility is reflected in recent computational models for predicting lipid solubility, the utility of which is markedly improved by including melting point [186,228].

It is apparent therefore that distinction needs to be made between PWSD that have high log P, low melting point and high lipid solubility—and that are ideally suited to LBF, and those that have high log P, high melting point and low lipid solubility—and are a significant challenge to formulate in LBF. This raises the question as to whether drug molecules might usefully be designed proactively to match an appropriate formulation strategy. For example, the development of analogues with higher lipid solubility. Clearly, this is unlikely to be a 'first-approach' and traditional lead optimisation programmes that seek to identify leads with reasonable water solubility and membrane permeability will be the first option. However, it becomes increasingly clear that for some targets, the likelihood of identifying a novel lead that is a traditional BCS class 1 compound is extremely low [186]. Under these circumstances, re-purposing a lead optimisation programme to identify poorly water soluble drugs that have good lipid solubility (rather than water solubility), and that might take advantage of the absorption benefits of LBF may be appropriate.

It must be acknowledged, however, that there are many arguments against this general concept and several excellent papers have shown that highly lipophilic drugs typically have higher receptor promiscuity and toxicity, and increase the potential for metabolic elimination and the likelihood of metabolic drug–drug interactions. As such the 'b-r-o-5 space' has trended towards tighter definition with the 'rule of 4' (molecular weight < 400, log P < 4) suggested by Gleeson [229], and even tighter requirements—the 'rule of 3' (molecular weight < 300, log P < 3) for fragments [230]. Others similarly caution against higher lipophilicity showing that compounds with log P > 3 and polar surface areas (PSA) < 75 A^2 are 2.5 times more likely to be toxic [231]. These correlative analyses cannot be disputed, but suffer from simple correlation with log P (or log D) as a global indicator of 'lipophilicity' and perpetuate the generic 'lipophilicity is bad' concept. In contrast, others suggest that log P may be too broad and that more specific properties (albeit properties that also drive increases in Log P), for example increasing aromaticity may be more responsible for poorer developability [232].

From a formulators perspective these analyses suggest the possibility of distinguishing molecular properties that drive increases in lipid solubility (and therefore applicability for LBF) from other indications of lipophilicity that may be associated with less effective development outcomes. For example, addition of aliphatic carbons may drive increases in lipophilicity that are not as highly correlated with changes in receptor promiscuity as increases in aromatic carbons but will likely increase lipid solubility. Similarly, additional carbon centres will increase lipophilicity, but where these are SP^3 carbons rather than SP^2, this may also reduce intermolecular packing and therefore melting point and in doing so increase lipid solubility. To the best of our knowledge, published examples of lead optimisation strategies to deliberately increase lipid solubility to increase developability *via* increases in lipid (rather an aqueous) solubility are not evident. But, if this could be achieved in a manner that does not significantly enhance toxic liability, it may be beneficial.

An alternate approach to achieving the same ends, but at the same time reducing potential toxicity and metabolic liabilities even further, is to develop or isolate drug leads with 'temporarily' high lipophilicity. Strategies to increase transient lipophilicity such as lipophilic prodrugs and salts are further described in the following sections.

8.1. Lipid prodrugs and LBF

Lipophilic prodrugs have been widely employed to enhance membrane permeability and in doing so to promote oral bioavailability for polar, hydrophilic drugs; often *via* masking charged carboxylic acid groups [233,234]. Similarly, alkyl and aryl esters have been employed to cap metabolic sites and reduce presystemic metabolism and to reduce GI irritation [233]. In almost all cases, the prodrug moiety reduces polarity and adds lipophilicity. Rarely is this performed specifically to enhance lipid solubility and promote incorporation into LBF—however, in most instances this is likely to be the case, and as such may have potential utility in enhancing access to the advantages in exposure that LBF provide for PWSD.

One recent study, has described the deliberate synthesis of a range of lipophilic prodrugs of a poorly water and lipid soluble thumb pocket 1 polymerase inhibitor (HCVNS5B) in order to promote solubility in SEDDS. In this case, a glycolic amide ester of the parent drug showed the most favourable lipid solubility (>100 mg/g), and also resulted in rapid *in vivo* hydrolysis to liberate parent drug from the prodrug [235]. Subsequent comparative *in vivo* bioavailability studies examined systemic drug exposure after oral administration of the prodrug in combination with a self-emulsifying LBF in comparison to parent drug, and showed good increases in exposure in rats for the prodrug, but lower exposure in dogs and monkeys. Relative exposure in rats and monkeys was consistent with rates of drug liberation in the presence of liver microsomes. In dogs, exposure was very low and the authors suggested that this may be an outlier species for prodrugs of this type. A similar approach was also taken by Bala and colleagues who generated fatty acid esters of SN38 (a camptothecin derivative) as a means of increasing solubility in lipidic vehicles [236], although in this case additional benefits in terms of enhancing intestinal permeability and potentially promoting intestinal lymphatic transport were also envisaged. Solubility of SN38 in a long chain triglyceride (soybean oil) was low but increased significantly for the prodrugs. This was particularly apparent with the undecanoate ester and a diundecanoate ester with increases in lipid solubility of >400 fold.

Similar fatty acid esters have been widely employed to promote intestinal lymphatic transport, the best known being the current commercial oral formulation of testosterone—also an undecanoate ester (Andriol) [14,237]. In the case of testosterone undecanoate, formation of the lipophilic ester promotes drug association with intestinal lymph lipoproteins in the enterocyte and therefore enhances drug transport into the intestinal lymph after oral administration [238,239]. Since the lymph drains directly into the systemic circulation, *via* the major veins in the neck, and does not pass through the liver, stimulating intestinal lymphatic transport can lead to significant increases in bioavailability for drugs like testosterone where first pass metabolism is highly significant [98,240]. Formation of the fatty acid ester of testosterone also allowed ready incorporation into a lipid based soft gelatin capsule formulation. Recent studies have examined a range of lipophilic prodrugs with the principle intent of increasing intestinal lymphatic transport [241,242], but where synthesis of the prodrug increased lipid solubility and ease of incorporation into LBF. These prodrugs were either simple aliphatic esters or lipophilic glyceride esters employed to mimic dietary triglycerides. In all cases the prodrugs were able to promote lymphatic transport, with attendant benefits in increasing drug concentrations in the lymph and reducing first pass hepatic metabolism. However, in the current context, formation of lipophilic prodrugs also significantly elevated lipid solubility, thereby enhancing incorporation in LBF. In the case of lymph directing prodrugs this is critical as coadministered lipid is required to drive intestinal lipoprotein production. For a more detailed review of prodrug strategies in general the interested reader is directed to Stella et al. [233].

8.2. Ionic liquids and LBF

Ionic liquids (ILs) are defined as organic salts with melting temperatures below 100 °C [243]. As such they can exist in a liquid or solid form at room temperature and when in the solid form, ionic liquid

structure may be crystalline or amorphous. The unique physical properties of ionic liquids have been reviewed elsewhere [244].

ILs are a well-established class of materials with existing industrial applications in a number of areas including biomass processing, renewable energy, synthesis and analytical chemistry [243]. In comparison, the potential applications of ionic liquids in drug delivery have only recently been explored, but show great promise. These applications may be grouped into two general areas; (i) the use of ionic liquids as functional excipients or (ii) the transformation of drugs into ionic liquids in order to enhance drug properties.

Interest in the former largely stems from the "designer" solvent properties of ionic liquids since this can lead to higher drug loadings if ionic liquids are used in drug delivery systems [245]. For example, a commercially available ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate, shows a high degree of solvency towards both hydrophobic compounds (*e.g.* danazol and albendazole), and hydrophilic compounds (*e.g.* caffeine and acetaminophen) [246]. This may in part be attributable to favourable interactions between the hydrophobic portions of the drug and *e.g.* the alkyl chains in the IL. Additionally, hydrogen bonding between the drug and the ionic component of the IL may further enhance solvation properties for PWSD. Some ILs are also miscible with lipids and surfactants commonly used in LBF. For example, recent studies have shown that a series of N-alkylnicotinate ester ILs and N-alkyl-3-methylpyridinium alkyl sulphate ILs are able to increase solubility of danazol and itraconazole in LBF by up to 500-fold when compared to a standard LBF [247]. After oral administration to rats, the IL containing LBF resulted in similar exposure to a SEDDS formulation, however drug absorption from the IL containing formulation was sustained over 6–8 h.

Similarly, ILs have been described as having advantageous solvency properties in topical drug formulations. For example, ionic liquid-in-water emulsions have been explored for the topical delivery of etodolac [248], since etodolac solvency in the ionic liquids enabled much higher drug loading (374.3 mg/ml in 1-butyl-3-methylimidazolium hexafluorophosphate compared to <175 mg/ml in other lipids, surfactants and cosolvents).

A further application of ILs in drug delivery is the transformation of the drug itself into an ionic liquid form [249]. These resultant low melting temperature drugs are typically described as drug-ILs or active pharmaceutical ingredient (API)-ILs. The physicochemical properties of API-ILs have led to their use to avoid crystal polymorphism [250], to increase aqueous solubility and dissolution rate for oral [251,252] or parenteral delivery [252] and as a means to deliver stabilised cationic and anionic drug pairs [253].

Of relevance to the subject matter of this review, recent studies have explored the potential utility of generating lipophilic ILs from ionisable drugs in order to enhance solubility in LBF excipients. This approach was based on the hypothesis that reduction/removal of the crystalline drug lattice (manifest in a reduction in melting point), and the use of highly lipid soluble counter ions would enhance lipid solubility and facilitate the formation of LBF for drugs where lipid solubility was otherwise limiting [254]. An additional potential advantage of using an ionic liquid approach to improve lipid solubility is that it may not raise the same issues of receptor promiscuity and potential off-target toxicity that are commonly associated with highly lipophilic analogues since the lipophilicity of the drug remains unchanged, (although the fate and toxicity of many API-IL lipophilic counterions are as yet, unknown). Sahbaz et al. generated a series of ionic liquid drugs using a range of poorly water soluble weak bases and acidic lipophilic counterions [254]. For the PWSD cinnarizine, the IL form of the drug resulted in a 7-fold increase in solubility in LBF, which translated to a higher drug loading in the SEDDS without significant *in vitro* drug precipitation post dispersion and digestion. *In vivo* administration of solubilised IL drug in a SEDDS was achieved at doses significantly in excess of that which could be achieved using cinnarizine free base *i.e.* the equivalent free base formulation was a SEDDS suspension. Comparison of cinnarizine exposure after oral administration of SEDDS containing cinnarizine IL in solution or cinnarizine free base as a suspension, confirmed that exposure was higher after administration of the API-IL formulation. At matched doses to LBF where cinnarizine free base was in solution (which was only possible at low dose, 35 mg/kg) the cinnarizine IL formulation performance was comparable to the SEDDS formulation (Fig. 12A). Similar results were reported for itraconazole which is both water and lipid insoluble, with a docusate ionic liquid form showing a > 50-fold increase in solubility in lipid formulations. A 2–3-fold increase in exposure over the currently marketed amorphous drug formulation (Sporanox®) was observed after oral administration. Generation of lipid soluble API-ILs is also achievable for more hydrophilic drugs. A dextromethorphan decylsulphate IL, for example, was reported to be more soluble in medium chain SEDDS than the free base (93.3 mg/g for the API-IL and 23.5 mg/g for the free base) providing the potential to formulate relatively polar drugs in LBF.

A significant number of drugs currently on the market or in development are ionisable and are formulated as salts. There is therefore wide scope for the application of IL technologies to enhance drug solubilisation in lipids for oral drug delivery. Lipid soluble API-ILs may be synthesised using alkyl sulphates, docusate and fatty acid based anionic counterions, materials that have been widely used in oral drug products (either as counterions, excipients or excipient components), and therefore their toxicity risks are not anticipated to be significant.

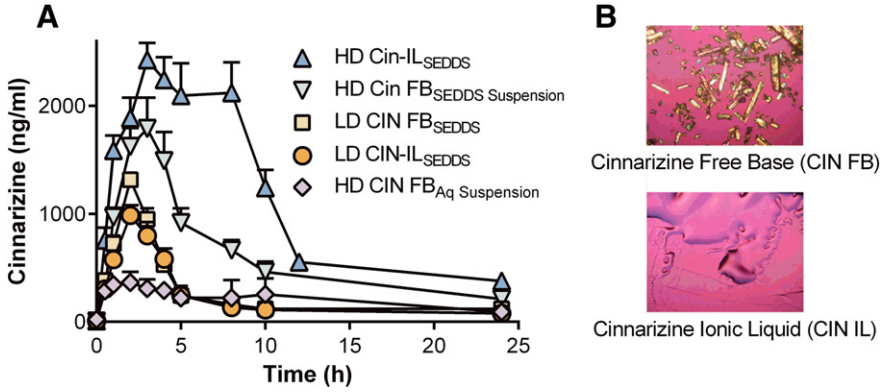

Fig. 12. (A) Pharmacokinetic profiles of cinnarizine free base formulated in suspensions, SEDDS or as an ionic liquid in suspensions and SEDDS. Cin-IL—cinnarizine ionic liquid, Cin FB—cinnarizine free base, HD—High dose (125 mg/g), LD—Low dose (35 mg/g) (B) differences in morphology of cinnarizine and cinnarizine ionic liquid as measured under cross polarised light. Data adapted from Sahbaz et al. [254].

9. Solid LBF development—generating alternative dosage forms for PWSD

Most LBFs are liquid or semisolid at room temperature and require encapsulation or solidification to facilitate ease of dosing. Multiple approaches have therefore been taken to achieve these ends and they are outlined below.

9.1. Encapsulation

Typically, encapsulated LBFs are filled into either soft or hard shell capsules with the choice of shell dependent on formulation compatibility and the mass of formulation to be dosed. Soft and hard shell capsules are typically gelatin based (though other polymeric alternatives to gelatin are also available [255]), and have their own inherent advantages and disadvantages. Soft gelatin capsules, due to their thicker walls, are more compatible with hygroscopic excipients such as PEGs or high HLB surfactants and can be filled almost to maximal capacity [51]. However the presence of plasticizers in soft gelatin capsules may also cause drug migration to the capsule shell affecting drug release [256]. In addition, soft gelatin capsules must be filled at comparatively low temperatures (40 °C) limiting their use for semisolid formulations and capsule filling is usually outsourced to specialised contract manufacturers. On the other hand, hard gelatin capsules are compatible with filling temperatures up to 70 °C, can be filled in-house and the absence of plasticizer reduces the likelihood of drug migration into the capsule shell. Additionally, hard gelatin capsules have lower water content which limits possible water exchange with the capsule fill [257]. The risk of leakage is higher from hard capsules but can be reduced through careful selection of filling parameters, or the use of semi solid fill materials, while capsule banding or sealing can be employed to provide further protection [257]. A common issue with both hard and soft gelatin capsules is moisture sorption either from the fill material or from the environment, which can significantly alter capsule properties [257], although these issues can be attenuated by appropriate secondary packaging. The presence of impurities (particularly peroxides [258]) in formulation excipients can cause crosslinking of gelatin shells, impacting drug release rates over time [51]. The chemical and physical stability of liquid LBFs must therefore be considered prior to encapsulation [258,259].

Despite these complexities, some of which are applicable to both solid and liquid fills, encapsulation is flexible, well tolerated in the clinic, widely employed and remains the primary marketed means of LBF delivery [52].

9.2. Solidification for powder fills or tabletting

Solid lipid formulations have gained interest over recent years, due in part to the desire to harness the benefits of lipids in a variety of different dosage formats, such as sachets, powder for suspension, multiparticulates and tablets. They are particularly attractive where conventional capsules are not suitable *e.g.*, where there are capsule compatibility challenges that cannot be solved using semi-solid formulations, or for clinical reasons, for example in patients with difficulty swallowing or where controlled release is required. Solidification is typically achieved by either combination with carriers or additives to form powders or by the use of high melting lipids to generate semi solid or solid formulations that may be encapsulated or processed to form lipid-based multiparticulates.

Adsorption onto solid carriers, spray drying, melt granulation, melt extrusion, freeze drying and solvent evaporation (Fig. 13) have all been used to convert LBF to solid forms and have been well described in recent reviews by Tan and coworkers [260] and by Jannin and colleagues [258].

Generation of powder or granular forms of LBF is an appealing solidification strategy as these types of solidified formulations are generally compatible with pre-existing tabletting, capsule or sachet filling procedures and equipment. Indeed the generation of solid state emulsions began over 50 years ago with spray drying and lypophilisation strategies [261,262]. In a more recent study by Hansen, Holm and Schulz [263], spray-dried emulsions of MCT were combined with a sugar, gelatine and Neusilin in an attempt to generate directly compressible LBF loaded powders. The study showed that the loading of the MCT solution as well as the particle size of the insoluble silica carrier and sugar directly affected tabletability. Subsequent *in vivo* studies did not show significant differences in oral absorption of the model drug Lu 28–179 when compared to a MCT solution and a HPMC dry emulsion indicating that the solidification process did not significantly impact LBF performance [264].

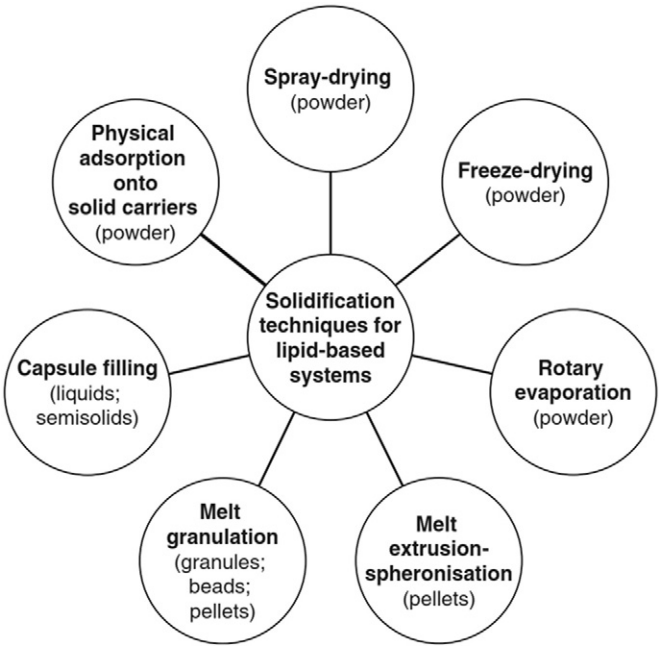

Fig. 13. Overview of solidification techniques commonly used for transforming liquid and semisolid lipid-based formulations into solid dosage forms. All methodologies (excepting rotary evaporation) are viable for scale-up to commercial manufacture. Reproduced from [260] with permission.

Pickering emulsions of LBFs stabilised by silica have been utilised by the Prestidge group to generate solid LBFs capable of controlling lipolysis rates, drug release and reducing food effects [265–267]. For example, a SLH formulation of celecoxib increased lipid hydrolysis when compared to an oil solution, and oral absorption of celecoxib in fasted beagles was significantly greater for the SLH formulation when compared to an oil formulation or when celecoxib was administered to fed dogs [266]. These studies led to a first in man study of SLH for oral PWSD delivery where a two-fold increase in bioavailability was evident relative to a commercial formulation. This was attributed to improved solubilisation of the model drug ibuprofen [268].

Silica finds further application in solidified LBF as a physical adsorbent. Studies utilising mesoporous silica as a carrier for LBFs have been described by multiple groups in recent years [269–271]. Mesoporous adsorbents increase the surface area of contact of a PWSD with solubilising media and may facilitate improved dissolution rates even in the absence of lipids [272,273]. In the context of LBF, these high surface area adsorbents can accommodate up to 100% *w*/w lipid formulation loadings, allowing tabletting of LBF, but this must be balanced with maintenance of adequate powder flowability and compressibility. Furthermore, studies by Van Speybroeck et al. have shown that incomplete desorption of SEDDS from uncompacted silica carriers may alter *in vitro* and *in vivo* performance [271]. In these studies, the model drug, danazol, was not found to strongly adsorb to the carrier and the lack of *in vivo* absorption was attributed to incomplete

desorption of the surfactant in the SEDDS which in turn altered the solvation capacity and increased the particle size of the redispersed formulation. Other studies have also shown that the formation of a gel layer may retard *in vitro* drug release from SEDDS adsorbed onto a Neusilin carrier [270]. Sander and Holm observed reduced bioavailability of adsorbed self-emulsifying formulations of cyclosporine after administration to dogs but found that the addition of a superdisintegrant markedly improved oral bioavailability with similar *in vivo* performance to an encapsulated LBF [274]. These differences in performance may be both drug-specific and formulation dependent and careful screening of adsorption and desorption of formulation excipients and the drugs used is advisable both before and after tabletting.

9.3. Solidification using thermoplastic excipients

Solid LBFs using thermoplastic (or meltable) surfactants and polymers and other solidifying matrices have also been widely explored. For a detailed review of solvent-free melt techniques for solid oral LBF preparation (extrusion, melt coating, granulation etc), the interested reader is directed to a recent publication by Becker et al. which comprehensively outlines the approaches available for generation of solid LBFs from excipient selection to manufacturing technique selection [275]. Shulka et al. have also reviewed the formulation of oral LBFs administered as multiparticulates (from liposomes to self-emulsifying pellets) [276]. However, the impact of these solidification processes on PWSD solubilisation or absorption has been less extensively explored. In a study examining hot melt LBF extrudates for oral delivery of propranolol [277], Mehuys and colleagues showed that oral bioavailability in humans was greater for the extruded system than for a comparable commercial formulation. In preceding studies [278,279], dissolution rates of the formulations were evaluated but the impact of digestion on drug solubilisation or precipitation was not evaluated. Hassan and Mader recently showed that PEG-30-di-(polyhydroxystearate) based semisolid SNEDDS protected the model drug progesterone from digestion-induced precipitation [280] and work from the Serajuddin group has explored the use of PEG and block copolymers to generate solid self-emulsifying formulations [281,282]. The latter formulations form spontaneous emulsions on redispersion, however, the impact of digestion on performance is as yet undescribed. Abdalla and Mader investigated the utility of solid self-emulsifying pellets as drug delivery vehicles for diazepam [283]. They showed that spheronzied solutol HS-15 containing pellets that self-emulsified resulted in faster drug release than formulations without solutol HS-15. However, the *in vitro* evaluation of drug release was limited to dissolution testing. Similarly, spheronised multiparticulate formulations based on gelatin have been reported by Aguirre et al. [284], where extrusion spheronisation was used to solidify an emulsion formulation containing permeation enhancers and salmon calcitonin, a poorly permeable peptide. *In vivo* absorption of sCT was evaluated in jejunal and colonic loop installations and absorption was greater in the presence of the permeation enhancers. Similar formulations for sustained release of cyclosporine are currently in clinical trials [3].

From the studies outlined above it can be seen that a number of different approaches to solidification of LBFs can be taken, although to this point formulation evaluation has been largely based on dissolution data alone. As with liquid LBFs, the performance of these solid LBFs might usefully be evaluated in the presence of hydrolytic enzymes and physiologically relevant concentrations of BS/PL in order to more fully understand their utility and to fine tune performance criteria.

10. Conclusions/perspectives

Drug discovery candidates that fall close to or outside the traditional 'rule-of-5' drug space remain an inconvenient reality. In spite of ever increasing efforts to minimising lipophilicity, molecular size and the numbers of rotatable bonds, h-bond donors and h-bond acceptors, these molecular characteristics appear to correlate with binding affinity for a range of drug targets. Indeed, as indicated by Bergström and colleagues in this issue [186], for some targets, highly lipophilic, and therefore poorly water soluble drugs appear to be a requirement. Fortunately, the challenge of low water solubility is significantly more tractable than, for example, low permeability and a range of formulation approaches have been developed to promote intestinal solubility [6]. Of these, lipid formulations and solid dispersion formulations have had perhaps the most commercial success and continue to be widely applied.

Lipid formulations provide an attractive option for enhancing the oral bioavailability of b-r-o-5 compounds. Unlike many formulation approaches, they have the potential to address solubility, permeability and presystemic metabolic liabilities, although the data base to support the latter two opportunities is both limited and equivocal. That lipid formulation can enhance apparent solubility in the GIT and promote the absorption of PWSD, however, is without question. Realising the limited data set, where LBFs can be employed they almost always appear to out-perform other enabling formulation technologies, including solid dispersion formulations.

Lipid formulations provide further advantage in terms of ease of assembly, flexibility (especially preclinically where varying doses must be orally gavaged to small animals) and scale, and several commercially and clinically successful examples are evident [52]. However, LBF do not provide a universal panacea and challenges remain. These include the need to access liquid filling capabilities, for either sealed hard gelatin or soft gelatin capsules, a situation that often requires outsourcing; the need to consider solution stability and capsule compatibility, particularly for drugs predisposed to oxidation and water sensitivity; and perhaps most importantly, the desire for single phase capsule fill materials, a situation that requires good drug solubility in the fill matrix. Whilst lipid suspension formulations are possible, and often effective, two phase liquid filling is complex and complicates material transfer and content uniformity. As such isotropic, homogeneous, lipid solution formulations are preferred. This typically limits the application of LBF to lipophilic, low melting drugs where solubility in non-aqueous vehicles is high (or the dose is low). To address this limitation, recent advances have identified alternative salt forms (ionic liquids), that reduce melting point and promote lipid solubility and provide new approaches to expand the potential applicability of LBF to drugs that are otherwise insufficiently lipid soluble. Lipophilic prodrugs or analogues are similarly able to increase solubility in non-aqueous vehicles, and in doing so, facilitate administration as a liquid fill capsule. Concerns over the development of highly lipophilic drug molecules due to the potential for increasing metabolic liabilities and receptor promiscuity dictate that ionic liquid or prodrug approaches that provide for 'temporary' increases in lipophilicity may be preferred over the generation of highly lipophilic analogues.

Alongside increasing focus on matched drug design and formulation design strategies, understanding of the mechanisms of drug absorption from LBF has increased, as have the technologies and models employed to assess formulation performance. For many years, the underlying paradigm for drug absorption from LBF was that their advantage stems from avoidance of classical solid–liquid dissolution (since the drug is in solution in the lipid vehicle) and enhancement in drug solubilisation in the GI fluids that are swollen with bile salts and lipid digestion products. This led to widespread use of *in vitro* lipid digestion models to evaluate formulation performance under simulated GI conditions and the overarching assumption that maximising the proportion of the drug that remained solubilised on GI digestion would maximise drug absorption. It is now apparent that whilst this holds true for many drugs, for some drugs, absorption may still be robust, even in light of significant precipitation on *in vitro* formulation digestion. Indeed, supersaturation rather than solubilisation is emerging as a significant driver of absorption and for highly permeable drugs, only transient periods of supersaturation may be sufficient to drive

absorptive drug flux. Nonetheless *in vitro* digestion testing remains a useful and simple challenge test and formulations that retain solubilisation capacity on *in vitro* digestion almost always perform well *in vivo*.

Finally, great strides are being made to develop LBF dosage forms that retain the advantage of traditional liquid fill materials, but that can be solidified to improve stability, manufacturability or ease of administration. The approaches employed range from the use of high surface area microporous materials which allow subsequent granulation or tabletting to the use of thermoplastic excipients for the generation of melt extrudates, pellets or multiparticulates compatible with different modes of administration (capsules, sprinkles, sachets *etc.*).

LBF therefore provide a flexible and highly effective means of enhancing exposure for b-r-o-5 compounds. The largest evidential data base supports the use of LBF to enhance apparent solubility in the GIT, but opportunities in permeability enhancement, controlled release and metabolic/transporter inhibition are increasing. As discovery programmes continue to identify increasing numbers of discovery candidates that are close to, or beyond the r-o-5, LBF provide a vital part of the drug delivery armoury.

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