Toward the Establishment of Standardized *In Vitro* Tests for Lipid-Based Formulations, Part 4: Proposing a New Lipid Formulation Performance Classification System (LF-PCS)

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ABSTRACT

The LFCS Consortium looks to develop standardized *in-vitro* tests and to generate much-needed performance criteria for lipid-based formulations (LBFs). This article highlights the value of performing a second, more stressful digestion test to identify LBFs near a performance threshold and to facilitate lead formulation selection in instances where several LBF prototypes perform adequately under standard digestion conditions (but where further discrimination is necessary). Stressed digestion tests can be designed based on an understanding of the factors that affect LBF performance, including the degree of supersaturation generated on dispersion/digestion. Stresses evaluated included decreasing LBF concentration (↓LBF), increasing bile salt (↑BS) and decreasing pH. Their capacity to stress LBFs was dependent on LBF composition and drug-type: ↓LBF was a stressor to medium-chain glyceride-rich LBFs but not more hydrophilic surfactant-rich LBFs, while decreasing pH stressed tolfenamic acid LBFs but not fenofibrate LBFs. Lastly, a new Performance Classification System, that is LBF composition independent, is proposed to promote standardized LBF comparisons, encourage robust LBF development and facilitate dialogue with the regulatory authorities. This classification system is based on the concept that performance evaluations across three *in-vitro* tests, designed to subject a LBF to progressively more challenging conditions, will enable effective LBF discrimination and performance grading.

Keywords: *LFCS Consortium; lipid-based drug delivery systems; SEDDS; drug solubilization; in vitro digestion testing; Solubility; Bioavailability; In vitro models; Precipitation; Supersaturation. Performance Classification System*

INTRODUCTION

The Lipid Formulation Classification System (LFCS) was established to provide a standardized means through which the wide compositional range of lipid-based formulations (LBFs) could be classified into different formulation types (Table 1).[1] The LFCS also provided general descriptions of dispersibility and digestibility of different LBF types: Type I and II LBFs are those LBFs that form coarse/turbid emulsions when dispersed in aqueous fluids, and where digestion of the oil droplet phase is required for efficient transfer of a drug from the emulsion to the aqueous colloidal phase and ultimately into free solution (from where the drug can be absorbed); Type IIIA and IIIB LBFs, which are more hydrophilic, disperse to form fine dispersions in the nanometre particle size range. The resultant high surface area of the colloidal phase is such that digestion is not required to facilitate drug transfer into free solution. However, while digestion may not be required for good drug absorption, Type IIIA and IIIB formulations are still likely to be digested rapidly in the small intestine, to an extent where their physicochemical properties may change dramatically; Type IV LBFs disperse to form micellar dispersions and therefore do not require digestion for effective presentation of drug in a very fine colloidal state, though many commonly used non-ionic ester surfactants are also digested by enzymes found in the small intestine.[2-4]

While the LFCS is widely accepted and increasingly utilized by the lipid formulation community, it is apparent that LBFs of the same type can perform very differently. For example, certain Type IV LBFs have been shown to perform well both *in vitro* and *in vivo*[5] while in other cases Type IV LBFs have performed poorly.[6,7] Indeed, it is reasonable to expect that a formulation type may exhibit good performance *in vivo* at low drug loadings but show decreasing performance as the drug loading, and the likelihood of drug precipitation, is increased. As such, whilst the LFCS provides much-needed guidance in describing lipid formulations based on composition, it cannot (and does not) provide an indication of the likely *in vivo* performance of the formulation.

To this point, work performed within the LFCS Consortium has evaluated the fate of a diverse range of eight LBFs, containing different model poorly water-soluble drugs (PWSD), during the course of *in vitro* dispersion and digestion performance tests.[8-11] Significant progress has been made in identifying and understanding the key experimental and formulation variables that greatly impact LBF performance. For example, our results indicate that the supersaturation ratio generated within a LFCS digestion test overwhelmingly determines the likely patterns of precipitation of an incorporated drug. This finding will aid in the design and development of LBFs, but also provides a strong theoretical basis for defining new LBF performance criteria and in turn a performance classification system. Much like the Biopharmaceutics Classification System (BCS),[12] which classifies drugs based on GI solubility and intestinal permeability, a new performance classification system for LBFs should be based on measurable *in vitro* properties that relate to the biopharmaceutical properties that dictate *in vivo* LBF performance.

In this article, we take the first steps toward this objective and propose a new "Lipid Formulation Performance Classification System (LF-PCS)" for LBFs. The LF-PCS is based on data obtained using the dispersion and digestion tests previously described by the LFCS consortium, but has been expanded here to include an additional digestion test to provide for additional discriminatory power. The three tests employed are therefore (i) an *in vitro* dispersion test, (ii) an *in vitro* digestion test in "typical fasted" conditions and (iii) an *in vitro* digestion test in "stressed" conditions. Each test subjects the LBF to greater challenge than the preceding test. The *In vitro* dispersion test assesses the likelihood of drug precipitation as a LBF disperses within gastric fluid before entering the small intestine. Such tests are routinely performed prior to digestion tests in the development of LBFs[13] since they provide the opportunity to rapidly screen-out formulations that precipitate on simple dilution.[14] Digestion provides an additional means of discrimination over simple dispersion tests, and the standard model ("typical fasted") is employed here. A second tier digestion test that is more stressful to LBF performance and provides an additional level of discrimination between LBFs is

described here for the first time. These more stressful conditions have been designed using our growing understanding of the factors that can affect LBF digestion, lipid digestion product solubilization and intrinsic drug solubility, all of which can impact on the degree of supersaturation and the likelihood of drug precipitation. In the current studies, we investigated three modifications to the standard model which reflect exposure to alternative conditions that may be encountered in the intestine. Firstly, the quantity of lipid formulation in the test was reduced to assess LBF performance under more dilute conditions. Secondly, the bile salt (BS) and phospholipid (PL) concentrations were increased from 3 mM and 0.75 mM (respectively), which reflect typical concentrations in the fasted duodenum[15-17], to 10 mM and 2.5 mM (respectively), to represent the upper concentration range of BS and PL in the small intestine following administration of a LC lipid formulation.[18] Lastly, the pH within the digestion test was modified to challenge LBF performance because it is possible for pH to affect intrinsic solubility of electrolytes (and therefore alter the risk of precipitation) and also the solubilizing properties of colloids formed on digestion.

In the absence of this additional challenge (ie digestion under more stressful conditions), it is not always possible to distinguish between lipid based formulations. The current study therefore aimed to develop more stressful digestion tests in order to allow better discriminate between candidate formulations. Subsequently, the current studies utilised these extended data sets to develop a Performance Classification System that is based on LBF performance across all three of the *in vitro* tests described above. This performance-based classification system has the potential to (i) promote standardization of *in vitro* evaluation methods for LBFs, (ii) highlight key LBF performance criteria, (iii) encourage the development and progression of better performing LBFs into preclinical and clinical studies, (iv) provide greater opportunity to compare/discriminate different LBFs and (v) assist in dialogue with the regulatory authorities.

Table 1: The Lipid Formulation Classification System (LFCS). Adapted from [1]

	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils e.g. triglycerides or mixed mono and diglycerides	100	40-80	40-80	<20	0
Water-insoluble surfactants (HLB < 12) e.g. sorbitan tri/ monoesters, propylene glycol di/monoesters etc	-	20-60	-	-	0-20
Water-soluble surfactants (HLB > 12) e.g. PEG monoesters etc.	-	-	20-40	20-50	30-80
Hydrophilic cosolvents e.g. PEG, propylene glycol, glycerol, ethanol)	-	-	0-40	20-50	0-50
Dispersion and digestion properties	No/limited dispersion; Requires digestion	Turbid o/w dispersion (particle size 0.25–2 µm); Requires digestion	Clear or almost clear dispersion; Digestion not required for absorption	Clear dispersion; Digestion not required for absorption	disperses to micellar solution; May not be digestible
Risk	Slow digestion and slow "release" of the drug	Risk of ppt on digestion in the intestine	Risk of ppt on digestion in the intestine	Risk of ppt in the stomach and on digestion in the intestine	High risk of ppt in the stomach and intestine

MATERIALS AND METHODS

Materials

Maisine™ 35-1 and Transcutol® HP were from Gattefossé (Saint-Priest, France). Captex® 300 and Capmul® MCM EP were from Abitec Corp (Columbus, OH). Cremophor® EL was from BASF Corporation (Washington, NJ). Corn oil and Tween® 85 were purchased from Sigma Chemical Co. (St Louis, MO). Further details of the lipidic excipients used within LFCS Consortium can be found in earlier work.[8] Fenofibrate, tolfenamic acid sodium taurodeoxycholate >95 % (NaTDC), 4-bromophenylboronic acid and the porcine pancreatin extract (P7545, 8 x USP specifications activity) were also obtained from Sigma Chemical Co. Phosphatidylcholine (Lipoid E PC S, approximately 99.2% pure, from egg yolk) was obtained from Lipoid (Lipoid GmbH, Ludwigshafen, Germany. Sodium hydroxide 1.0 M solution, which was diluted to obtain 0.2 M and 0.6 M NaOH titration solutions, was purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

Selection of LBFs for Investigation in the LFCS Consortium

The compositions of the eight lipid-based formulations (LBFs) investigated by the consortium are shown in Table 2. The equilibrium solubility of fenofibrate and tolfenamic acid in each LBF was determined in our earlier work [10] and is also shown in Table 2 for reference purposes.

Table 2: Composition of the eight lipid-based formulations investigated in this study

Formulation	Composition (% w/w)	Fenofibrate / Tolfenamic acid solubility (mg/g)[a]	Solubility Ratio
Type I-LC	50.0% corn oil, 50.0% Maisine 35-1	98.1 / 8.8	11.4
Type II-LC	32.5% corn oil, 32.5% Maisine35-1, 35.0% Tween 85	99.9 / 20.6	4.8
Type IIIA-LC	32.5% corn oil, 32.5% Maisine 35-1, 35.0% Cremophor EL	106.8 / 26.0	4.1
Type I-MC	50.0% Captex 300, 50.0% Capmul MCM EP	149.6 / 17.3	8.6
Type II-MC	32.5% Captex 300, 32.5% Capmul MCM EP, 35.0% Tween 85	135.6 / 25.9	5.2
Type IIIA-MC	32.5% Captex 300, 32.5% Capmul MCM EP, 35.0% Cremophor EL	143.1 / 32.6	4.4
Type IIIB-MC	25.0% Capmul MCM EP, 50.0% Cremophor EL, 25.0% Transcutol	152.1 / 54.8	2.8
Type IV	50.0% Cremophor EL, 50.0% Transcutol	189.1 / 77.0	2.5

[a] Equilibrium solubility values are from Williams et al.[10] and were determined at 37°C

Drug Incorporation into the LBF

Formulations were loaded with fenofibrate or tolfenamic acid at concentrations between 40% to 80% of the solubility of the drug in the LBF (solubility values are presented in Table 2). The required mass of drug was weighed directly into clean screw-top glass vials and drug-free LBF was added up to the target mass loading. Vials were sealed, vortex-mixed and incubated at 37°C for at least 24 h prior to testing to ensure complete dissolution of the drug. The drug content in each manufactured LBF was verified (in triplicate) on the day of testing when accurately weighed samples were removed from the formulation, transferred to 5 mL volumetric flasks and made up to volume with chloroform:methanol (2:1, v/v). Aliquots (50-100 μL) were diluted >10 fold with acetonitrile and analysed for drug content by HPLC (see *HPLC detection of model drugs* section below for details).

In Vitro Evaluation of the LBFs

Digestion Experiments

In vitro digestion experiments were performed as described previously by Williams *et al*.[8-10] In brief, the experimental set-up employed by the LFCS Consortium consists of a pH-stat apparatus (Metrohm® AG, Herisau, Switzerland), comprising a Titrando 802 propeller stirrer/804 Ti Stand combination, a glass pH electrode (iUnitrode) and two 800 Dosino dosing units coupled to 10 mL autoburettes (Metrohm® AG, Herisau, Switzerland). The apparatus was connected to a PC and operated using Tiamo 2.0 software (Metrohm® AG). The formulation was dispersed in a thermostat-jacketed glass reaction vessel (Metrohm® AG, Herisau, Switzerland) using an overhead propeller stirrer 25 mm in diameter. After a 10 min dispersion phase, digestion was subsequently initiated via addition of 4 mL pancreatin from porcine pancreas extract prepared as described previously.[8] Sodium hydroxide titration solutions (titrants) of 0.2 M and 0.6 M were utilised for digests containing LC and MC LBFs respectively. Titrants were automatically added (controlled via the pH-stat controller) to the reaction vessel to maintain constant pH during digestion, with the rate of titrant addition reflecting the digestibility of the LBF.

Different digestion conditions were based on standard fasted conditions used previously within the LFCS Consortium. [8-10] In this standard condition (Table 3), 1.083 g of LBF is initially dispersed in 39 ml of a pH 6.5 medium containing 2 mM Tris-maleate, 150 mM NaCl, 1.4 mM $CaCl_2 \cdot 2H_2O$, 3 mM NaTDC and 0.75 mM PL (with NaTDC and PL concentrations at 10% in excess to account for the dilution effect on addition of pancreatin which is prepared using the same medium in the absence of NaTDC and PL). The addition digestion conditions explored to greater stress LBFs are also summarized in Table 3 and include a (i) a “high bile salt” digestion condition (↑BS), where the NaTDC and PC concentration was increased to 10 mM and 2.5 mM, respectively, (ii) a more “diluted LBF” digestion condition (↓LBF) where the initial formulation mass was

decreased to 0.173 g and (iii) a "low pH" digestion condition (↓pH) where the pH of the test was decreased to pH 4.5.

Table 3 lists certain other aspects of the tests which were kept constant, namely the test volume, and the number of samples that were removed.

Table 3: The three different digestion conditions utilized in this study and additional information regarding the lipid digestion protocol employed within the LFCS Consortium

	Digestion condition			
	Fasted	**↓LBF**	**↑BS**	**↓pH**
Test preparation				
LBF mass (g)	1.083	0.173	1.083	1.083
pH of digestion medium	6.5	6.5	6.5	4.5
BS : PL concentration (mM)	3 : 0.75	3 : 0.75	10 : 2.5	3 : 0.75
Digestion medium volume (mL)	39			
Dispersion phase				
Duration	10 min			
Samples	3 x 1 mL			
Digestion phase				
Pancreatin	4 mL added after 10 min dispersion			
Duration	30 – 60 min			
Samples	≥ 4 x 1 mL – for bench-top centrifugation 2 x 4 mL – for ultracentrifugation			

Back-Titrations and Calculating the Extent of Digestion

To determine the total amount of fatty acid (FA) released (both ionized and unionized at pH 6.5), back-titrations were performed at the end of the digestion experiments. [4,8] In these experiments, the pH-stat was programmed to add 1 M sodium hydroxide to the reaction vessel at the end of the experimental period to increase the pH to 9 to completely ionize all FA present. The volume of 1 M sodium hydroxide required to raise the pH (corrected for the amount required to increase pH to 9 in the absence of lipid substrate in separate control experiments) reflects the quantity of unionized FA present at the end of experimental

period. The quantity of ionized FA originally detected at pH 6.5 plus the concentration of FA (determined by back-titration) provides the total amount of FA released in response to digestion. This value was compared to the theoretical quantity of FA that could be liberated if the lipid excipients were completely hydrolyzed to provide an estimation of the extent of LBF digestion using Equation 1, and assuming that on digestion, one triglyceride molecule released two FA molecules, and that one molecule of diglyceride or monoglyceride (initially present in the formulation) liberated a single FA molecule.

$$\text{Extent (\%) of digestion} = \frac{\text{ionized fatty acid } + \text{unionized fatty acid}}{\text{theoretical maximum fatty acid from the LBF}} \times 100 \qquad \text{Equation 1}$$

Collection and Separation of Samples Removed During In Vitro Digestion Tests

Samples were removed during the initial dispersion phase (1 mL in volume) and during the digestion phase (1 mL or 4 mL volume depending on the centrifugation method). Digestion samples were immediately treated with a digestion inhibitor (5 μL per 1 mL of digestion medium of 1.0 M 4-bromophenylboronic acid in methanol) to arrest digestion.

In line with previous recommendations,[8] the centrifugation step was tailored to the LBF type and digestion conditions. An ultracentrifuge (400,000g, 37°C, Optima XL-100 K centrifuge, SW-60 rotor, Beckman, Palo Alto, CA) was used in instances where the formulation was only partially digested in the *in vitro* digestion tests and where the sample would form a poorly dispersed oil phase post centrifugation. In all other cases, samples were separated using a bench-top centrifuge (21, 000*g*, 37°C, Heraeus® Fresco 21, Thermo Scientific, Langenselbold, Germany). Samples were either immediately centrifuged after removal from the test in the case of the bench-top centrifuge, or incubated briefly at 37°C prior to centrifugation in the case of the ultracentrifuge.

Post centrifugation, samples contained a poorly dispersed oily phase, a dispersed aqueous colloidal phase (AP_{DIGEST}) and a precipitated pellet phase. The drug content in the digestion phases obtained post-centrifugation was analysed as described previously. [8-10]

Determination of Drug Solubility in Colloidal Aqueous Phases Post-Digestion (AP_{DIGEST})

The solubility of fenofibrate and tolfenamic acid in the colloidal aqueous phase (AP_{DIGEST}) generated by digestion of blank (i.e. drug free) LBF was evaluated in the manner described in our earlier work.[10] Solubility values in $AP_{DIGESTS}$ were used to calculate the maximum supersaturation ratio (SR^M)[9,10,19,20] which is the ratio between the maximum theoretical concentration of solubilized drug (in the absence of any drug precipitation; AP_{MAX}) and drug solubility in the AP_{DIGEST}:[9]

$$SR^M = \frac{\text{maximum drug concentration in } AP_{DIGEST} \ (AP_{MAX})}{\text{drug solubility in } AP_{DIGEST}} \qquad \text{Equation 2}$$

$$AP_{MAX} = \frac{Mass\ of\ drug}{Test\ volume}$$

HPLC Detection of Model Drugs

All HPLC analyses were conducted using a Waters Alliance 2695 Separation Module (Waters Alliance Instruments, Milford, MA) with a reverse-phase C_{18} column (150 x 15 mm^2, 5 µm, Waters Symmetry®) and C_{18} security guard cartridge (4 x 2.0 mm, Phenomenex, Torrence, CA). The UV detection for fenofibrate and tolfenamic acid was at 286 nm and 290 nm respectively. For both compounds, the injection volume was 50 μL, the mobile phase consisted of acetonitrile and water in a 80:20 v/v ratio with 0.1% (v/v) formic acid and was pumped through the column at a 1 $mL \cdot min^{-1}$ flow rate. Details of the method validation have been described elsewhere.[10]

RESULTS

The first aim of the studies described in this manuscript was to identify and characterise a 'stressed' digestion condition that could be used to better discriminate between LBF. To this end we initially explored the effects of decreasing lipid concentration and increasing bile salt concentration on lipid digestion, drug solubility in the resulting digests and the concentrations of drug attained in the colloidal aqueous phase of the drug digests during lipid digestion. These data suggested that combinations of these tests readily discriminated between the fenofibrate containing formulations, even where discrimination was not evident under standard digestion conditions. However, for tolfenamic acid this was not the case and good solubilisation was evident under all conditions, presumably due to ionisation of the weak acid under the digest conditions. Subsequent studies were therefore undertaken under conditions of varying pH in an attempt to identify more stressful digestion conditions for a weak acid such as tolfenamic acid.

Effect of Decreasing Lipid Concentration (↓LBF) on the Extent of LBF Digestion

The extent of LBF digestion within an *in vitro* digestion test is estimated from the total concentration of liberated FA (ionized plus unionized) relative to the theoretical concentration obtained if the LBF were completely digested (Equation 1). Figure 1 compares estimated digestion values for Type I, II and IIIA formulations containing either MC or LC lipids under fasted conditions (taken from our previous work,[8,9]) to values obtained here in the ↓LBF condition, where LBF concentration is reduced. Type II and IIIA formulations had the lowest apparent extent of LBF digestion. This can be explained with reference to the method used to estimate total available FA. Calculations are based on the assumption that all incorporated surfactant is completely digestible, which is unlikely, particularly in the case of PEG-rich surfactants such as Cremophor.[2] The results show that lowering the LBF concentration increases the digestion of Type I-LC and II-LC LBFs, but not the better dispersing Type IIIA-LC (Figure 1a). The unionized:ionized FA ratio for these LBFs was lower in the ↓LBF condition, indicating that the apparent pK_a of the FA was lower (i.e. increased FA acidity) in the more

dilute condition. Additional tests using a wide range of Type I-LC concentrations confirmed the trend of increasing FA ionization with increasing dilution (See Table S1, *Supplementary Material*).

Equivalent MC formulations were highly digestible, and digested to a similar extent in the fasted and ↓LBF conditions (Figure 1b). MC FA were also ionized to a greater extent than LC FA, consistent with our previous work.[8,9] However, similar to LC FA, MC FA acidity increased under more dilute conditions. The effect of decreasing LBF concentration on the digestion of Type IIIB-MC and IV LBFs and associated FA ionization were small (not shown), most likely due to the lower proportion of lipid in these LBFs (see Table 2).

The impact on the extent of digestion and the uinionized:ionized FA ratio caused by decreasing LBF concentration, described here, was largely consistent with the trends reported previously for increasing BS concentration.[9] The impact of the ↓LBF and ↑BS on drug solubility in digested LBF and fate of solubilized drug is explored in more detail below.

Effect of Decreasing Lipid Concentration (↓LBF) and Increasing BS Concentration (↑BS) on LBF Solubilization Capacity after Digestion

The measured crystalline solubilities of fenofibrate and tolfenamic acid in aqueous phase digests (AP_{DIGEST}) formed by digestion of the eight LBFs under ↓LBF and ↑BS conditions are plotted in Figure 2 as a percentage of the solubility in aqueous phase digests (AP_{DIGEST}) obtained under standard fasted state digestion conditions The dashed horizontal line shows an estimate of the solubility in the ↓LBF condition assuming that solubility in the AP_{DIGEST} is proportional to the LBF concentration. Solubilities in the fasted state are taken from our earlier study,[10] and complied in Table 4 for reference. In this table, corresponding solubilities in the same formulations in the dispersed state are also included to illustrate how digestion can significantly lower the solubilization capacity of LBFs. These reductions in solubilization capacity are most pronounced in the case of

fenofibrate when compared to tolfenamic acid, presumably reflecting the fact that fenofibrate is a more lipophilic molecule and that digestion converts oil-rich droplets into more highly-dispersed and hydrophilic micellar colloids. In the fasted state (Table 4), fenofibrate solubility in the digested LBF varied 10-fold across the various formulation types, reflecting its higher affinity for lipid digestion products over simple surfactant micelles.[21] The highest fenofibrate solubilities were obtained for Type IIIA-LC and IIIA-MC LBFs, since the high digestibility of these LBFs resulted in an AP_{DIGEST} rich in digestion products. In contrast, the solubility of tolfenamic acid, which will be substantially ionized at pH 6.5, varied only 2-fold across the LBF types.

In the case of fenofibrate, increasing BS concentration appeared only to impact solubility in the AP_{DIGEST} in instances where there was a corresponding increase in the extent of digestion (e.g. Type I formulations and Type II-LC, Figure 2a). Indeed in some cases, there is evidence that solubility decreased at the higher BS concentrations (e.g. Type IIIA-LC, IIIB-MC and IV LBFs), though this effect was modest. In contrast, tolfenamic acid solubility increased greatly in the presence of higher BS, by a factor of almost 2 and irrespective of whether the higher BS concentration affected the extent of digestion (Figure 2b). In-line with this observation, tolfenamic acid solubility in simple digestion media (i.e. in the absence of digestion products) increased from 72.5 ± 0.6 μg/ml to 201.4 ± 2.4 μg/ml on raising BS from 3 mM to 10 mM. Fenofibrate solubility in the digestion medium also increased with increasing BS (increasing from 7.1 ± 1.3 μg/ml to 26.0 ± 0.29 μg/ml). Interestingly, the absolute solubility of fenofibrate was considerably lower (10-fold) than that of tolfenamic acid in the fasted state digestion medium, regardless of bile salt concentration. In contrast, fenofibrate solubility in the presence of dispersed (AP_{DISP}) or digested (AP_{DIGEST}) lipid formulations was much higher and similar to that of tolfenamic acid, (Table 4). These data, (??) further confirm that fenofibrate solubility appears to be increased much more significantly by the presence of lipid digestion products, rather than increases in BS concentration, the latter being more important for tolfenamic acid.

The solubilities of both drugs in digested Type I-LC and II-LC LBFs in the ↓LBF condition were above the dashed line in Figure 2, and therefore greater than the solubility that might be expected if solubility in the AP_{DIGEST} was proportional to LBF concentration. In some cases, however, (for example, fenofibrate in Type I, II and IIIA-MC LBFs) solubility was below the dashed line, and therefore, lower than expected based on simple dilution

As described in the discussion below, the complex interplay between LBF solubilization capacity and LBF concentration/BS concentration is attributable to differences in LBF digestion, FA ionization and colloidal phase structure. As the potential impact of the ↓LBF and ↑BS conditions is highly dependent on LBF composition, and the solubilization capacity of resultant digested phases for each drug, different LBFs are expected to perform differently across these digestion conditions providing an opportunity for greater discrimination.

Table 4: Measured equilibrium solubility of fenofibrate and tolfenamic acid in colloidal aqueous phases formed following dispersion or digestion of the eight LBFs in fasted conditions. Values are expressed as means (n=3) ± 1 SD.

	Drug solubility in dispersed (AP_{DISP}) or digested (AP_{DIGEST}) lipid formulations (μg/ml)[a]							
	I-LC[b]	**II-LC[b]**	**IIIA-LC**	**I-MC[b]**	**II-MC**	**IIIA-MC**	**IIIB-MC**	**IV**
Fenofibrate, AP_{DISP}	129.2 ± 6.9	323.6 ± 38.3	1736.1 ± 180.8	1.7 ± 0.8	524.0 ± 75.5	1929.1 ± 83.1	899.5 ± 21.2	384.3 ± 3.0
Fenofibrate, AP_{DIGEST}	40.8 ± 1.5	134.0 ± 7.9	706.3 ± 153.6	231.9 ± 6.0	426.5 ± 11.3	506.3 ± 22.4	389.2 ± 5.9	329.8 ± 12.9
Tolfenamic acid, AP_{DISP}	nd	nd	nd	nd	446.6 ± 20.7	859.8 ± 7.6	863.4 ± 22.8	643.7 ± 16.7

Tolfenamic acid, AP_{DIGEST}	200.4 ± 1.5	228.4 ± 5.1	482.3 ± 18.1	308.1 ± 27.4	442.5 ± 6.2	408.9 ± 8.1	337.3 ± 6.5	343.8 ± 12.4

Formulation compositions are listed in Table 2

[a] Values are taken from Williams et al. [10]

[b] Formulations formed an oil-phase in the samples, hence drug solubilities in isolated AP_{DISP}/AP_{DIGEST} does capture the entire solubilization capacity of the LBF

Impact of *In Vitro* Digestion Conditions on the Drug Solubilization Properties of LC and Poorly Dispersing MC Lipid Formulations

Figure 3 compares the fate of incorporated fenofibrate and tolfenamic acid following 30 min *in vitro* digestion of Type I-LC, II-LC, IIIA-LC and I-MC LBFs in fasted, ↑BS and ↓LBF conditions. Fasted results are reproduced from Williams et al.[10] All formulations included drug at 80% solubility in each LBF. In this first comparison LBF that are relatively poorly dispersed are compared since there are similarities in behaviour. The more completely dispersing MC formulations and Type IV formulations are compared in the following section

In the fasted condition in vitro, Type I and Type II LC LBFs were poorly digested (<30%, see Figure 1), and as a result, centrifuged samples contain a phase-separated oil-phase. This oil phase had very high affinity for fenofibrate (Figure 3a). In spite of near complete digestion, the lack of formulation surfactant in the Type I-MC LBF also resulted in fenofibrate concentrating within a phase-separated oil phase. In contrast, the Type IIIA-LC formulation was nearly completely digested and the majority of the incorporated fenofibrate was solubilized within the AP_{DIGEST}. Realizing that there are likely to be differences in performance, depending on whether the drug is solubilized within an oil-rich phase or the AP_{DIGEST}, in this case it was not possible to discriminate between these formulations based on the level of drug precipitation, which was low in each case. Switching to the ↑BS or ↓LBF conditions leds to a modest increase in fenofibrate in the AP_{DIGEST} in the case of the Type I and II-LC formulations, andhad no effect on the performance of the Type IIIA-LC formulation. The ↑BS or ↓LBF conditions therefore did not significantly alter the performance of the the LC formulations of fenofibrate. In the case of the Type I-MC formulation, however, moving to ↑BS or ↓LBF conditions resulted in the removal of the oil-phase and a significant increase in drug precipitation.

Calculated SR^M values (Equation 2 and summarized in Table 5) for the Type IIIA-LC formulation are moderate and between 2.8–3.2 across the different test conditions. Calculated SR^M values for the Type I-MC formulation

were in contrast much higher at 9.2 and 24.0 in the ↑BS and ↓LBF conditions, respectively, and consistent with incidences of extensive drug precipitation. It should be noted that the precipitation of fenofibrate during digestion of the Type I-MC formulation in the ↑BS condition was in spite of an increase in fenofibrate solubility in the $AP_{DIGEST.}$ This seemingly contradictory result can be explained by the absence of an oil phase under the ↑BS condition. This results in an increase in drug concentration in the AP_{DIGEST} phase (since drug is not sequestered in the oil phase) and a subsequent increase in SR^{M} value according to Equation 2. The increase in SR^{M} ie the increase in supersaturation pressure, subsequently promotes drug precipitation.

In spite of a relatively high log P, tolfenamic acid is expected to be largely ionised at pH 6.5 and aqueous solubility is therefore relatively high in the digestion medium (72.5 µg/ml) As such tolfenamic acid was well solubilized in the AP_{DIGEST} for all LBF types (Figure 3b). A lower proportion of tolfenamic acid in the oil-phase was typically apparent under ↑BS or ↓LBF conditions. In contrast to fenofibrate, there was no increase in drug precipitation from the Type I-MC LBF on changing digestion condition. This was in-line with the low SR^{M} values generated by tolfenamic acid containing formulations (Table 5).

Table 5: SR^M values and performance summary of eight LBFs containing fenofibrate and tolfenamic acid according to % solubilized after 60 min digestion

	Formulation Type							
	I-LC	II-LC	IIIA-LC	I-MC	II-MC	IIIA-MC	IIIB-MC	IV
Fenofibrate:								
Fasted, 80% sat			3.0		6.4	5.7	7.8	11.5
↑BS, 80% sat			3.2	9.2	6.2	5.3	9.1	15.0
↓LBF, 80% sat			2.8	24.0	15.3	10.0	8.0	11.4
Fasted, 40% sat	-	-	-	-	3.2	2.9	3.9	5.7
↑BS, 40% sat	-	-	-	-	3.1	2.6	4.5	7.5
↓LBF, 40% sat	-	-	-	-	7.6	5.0	4.0	5.7
Tolfenamic acid:								
Fasted, 80% sat			1.1		1.2	1.6	3.2	4.5
↑BS, 80% sat			0.6		1.1	1.3	2.2	3.0
↓LBF, 80% sat			0.5		0.7	1.0	1.9	2.5

Key: *= >75% solubilized* *= 75-50% solubilized* *= < 50% solubilized at 60 min*

Impact of *In Vitro* Digestion Conditions on the Drug Solubilization Properties of Efficiently Dispersing MC and Type IV LBFs

Figure 4 compares the fate of incorporated fenofibrate during 60 min *in vitro* digestion of Type II-MC, IIIA-MC, IIIB-MC and IV LBFs in fasted, ↑BS and ↓LBF digestion conditions. LBFs were loaded with drug at 80% and 40% saturation and fasted results are reproduced from Williams et al.[10] The decision to also investigate these four LBFs at a lower drug loading was based on the observation that these LBFs resulted in extensive drug precipitation at the high drug loads (80% saturation) and the hypothesis that greater discrimination between formulation types would be obtained at a lower drug loading.

In the fasted test condition, the onset of fenofibrate precipitation at the 80% loading occured during formulation digestion for Type II-MC and IIIA-MC LBFs, while for the Type IIIB-MC and IV LBF, which include more hydrophilic excipients, precipitation commenced during the 10 min dispersion phase prior to digestion. Aside from the onset of precipitation, there was evidence that the rate of precipitation was faster for Type IIIB-MC and IV LBFs compared to Type II-MC and IIIA-MC LBFs. In each case, however, <40% of fenofibrate remained solubilized after 60 min digestion irrespective of the digestion condition and at this time point there wass limited discrimination between the formulations. As expected, moving to more stressful ↑BS and ↓LBF conditions resulted in poorer formulation performance, and therefore, further limited opportunity to distinguish between the four formulations (Figure 4). This high propensity for precipitation of fenofibrate at high saturation levels in Type II-MC, IIIA-MC, IIIB-MV and IV LBFs irrespective of digestion condition was also associated with high SR^M values (i.e., >3, Table 5), and it is interesting to note that SR^M values for the lipid-rich Type II-MC and IIIA-MC formulations increased dramatically on moving to the ↓LBF condition, consistent with the data obtained with the Type I-MC formulation.

A reduction in SR^M and a lower precipitation propensity at the lower drug loading (40% saturation) nevertheless resulted in better discrimination between the performances of individual LBF. Indeed, in the fasted state condition, both Type II-MC (SR^M = 3.2) and IIIA-MC (SR^M = 2.9) LBF exhibited limited precipitation at the lower drug loading (Figure 4), and consequently out-performed Type IIIB-MC (SR^M = 3.9) and IV (SR^M = 5.7) LBF, both of which generated higher SR^M values and continued to show evidence of drug precipitation, even at the lower drug loading (Table 5). It proved more problematic to discriminate between the performance of Type II-MC and IIIA-MC formulations on changing BS or LBF concentration since the Type II-MC formulation best performed in the ↑BS condition while the Type IIIA-MC formulation performed the best in the ↓LBF condition. The II-MC>IIIA-MC performance difference in the ↑BS condition could not be accounted for by SR^M, possibly as values were similar (3.1 and 2.6, Table 5). In contrast, Type II-MC/IIIA-MC discrimination was much more consistent with SR^M values, which increased for both LBFs on moving to the ↓LBF condition (7.6 for II-MC and 5.0 for IIIA-MC, Table 5). The Type II-MC formulation and to a lesser extent the Type IIIA-MC formulation (both at 40% and 80% drug loading) showed a higher risk of precipitation as the LBF concentration in the digestion test was decreased. There was no substantial change in the performance of the Type IIIB-MC and IV formulations (irrespective of fenofibrate loading) in the ↓LBF condition (Figure 4). This can be rationalized by the proportional decrease in solubilization capacity on dilution of the formulation (Figure 2), and thus negligible change in the SR^M values (Table 5).

For fenofibrate, changing the drug loading in a LBF therefore allowed better discrimination between Type II-MC/IIIA-MC LBFs and more hydrophilic Type IIIB-MC/IV LBFs. At the lower drug loading, Type II-MC and IIIA-MC formulations exhibited good performance in the fasted condition while both were susceptible to precipitation when the digestion condition was changed.

Figure 5 compares the fate of incorporated tolfenamic acid during 60 min *in vitro* digestion of Type II-MC, IIIA-MC, IIIB-MC and IV LBFs in fasted, ↑BS and ↓LBF digestion conditions. LBFs were incorporated with drug at 80% saturation (fasted results from Williams et al.[10]). Tolfenamic acid formulations were only assessed at the 80% saturation level due to the low risk of precipitation exhibited by this drug.

Type II-MC, IIIA-MC and IIB-MC LBFs of tolfenamic acid were all insensitive to variation in the digestion conditions, showing no increase in precipitation despite changing LBF or BS concentrations. This low risk of precipitation was associated with SR^M values that were <3.3 (Table 5). In contrast, the Type IV LBF exhibited marked precipitation in the fasted state (SR^M = 4.5), but a reduction in precipitation under the ↑BS and ↓LBF conditions (SR^M = 3.0 and 2.5, respectively). These conditions, while able to stress the performance of fenofibrate formulations (most notably Type I-IIIA-MC LBFs), were therefore not suitable as a stress test for equivalent tolfenamic acid formulations.

Effect of *In Vitro* Digestion pH on the Drug Solubilization Properties of LBFs

Addition tests were subsequently performed to determine whether modulating digestion pH could lead to a change in LBF performance. As these tests were focussed on stressing the performance of the tolfenamic acid formulations, a more acidic digestion pH of 4.5 was used to increase the likelihood of precipitation of this weak acid by lowering its intrinsic solubility. The results of these tests performed under fasted conditions at pH 4.5 and 6.5 are shown in Figure 6 where the data for the Type IIIB-MC and IV LBFs is compared. Corresponding tests were also performed for fenofibrate (Figure 6). The performance of the LBFs was highly dependent on pH. For tolfenamic acid, lowering pH to 4.5 stimulated precipitation during dispersion and digestion of the Type IIIB-MC LBF. This more acidic pH also promoted tolfenamic acid precipitation in the case of the Type IV LBF. The opposite effect was observed for fenofibrate, a neutral compound, where there was a delayed onset and slower rate of precipitation at pH 4.5 compared to pH 6.5. These results confirm that a lower pH in the digestion test can be more stressful to the performance of LBFs where the drug is a weak acid but that it is less stressful for neutral compounds and most likely basic compounds where decreased ionisation of liberated fatty acid appears to swell fatty acid-BS mixed micelles, increasing solubilisation capacity for highly lipophilic drugs such as fenofibrate .

FA titration is severely attenuated at pH 4.5 indicating that the acidity of this medium is sufficient to quench the ionization of liberated FA, even for MC FA molecules (which are more acidic than LC FA due to a lower apparent pKa). Back-titrations performed at the end of the digestion test however revealed that the overall extent of digestion of LBFs containing MC lipid at pH 4.5 and pH 6.5 was similar despite this marked suppression of FA ionization (see Figure S1 in the *Supplementary Material*).

The impact of pH on the performance of more lipid-rich Type IIIA-MC LBF was also assessed. During the digestion of this LBF at pH 4.5, large coarse droplets were observed in the digestion vessel. These droplets were present for the duration of the test and rapidly settled at the bottom of the digestion vessel when stirring was stopped at the end of the test (see Figure S2 in the *Supplementary Material*). Post-centrifugation, the samples removed from the digestion vessel consisted of a floating oil phase, a clear AP_{DIGEST} and a large oily brown pellet. This was in contrast to the corresponding samples at pH 6.5, which consisted only of an AP_{DIGEST} and a predominantly white pellet due to the high concentration of precipitated fenofibrate.

The distribution of fenofibrate in samples removed after 60 min of digestion of the Type IIIA-MC LBF at pH 6.5 and 4.5 is compared in Figure 7. In samples removed at pH 6.5, over 80% of the fenofibrate was present in the pellet phase while the remaining fraction of the dose was solubilized in the AP_{DIGEST}. In contrast, at pH 4.5, the majority of the dose was recovered from the oily pellet phase and the floating oil phase. To better understand the relationship between pH and the type of colloidal phases formed, the pH in digestion medium after 60 min was progressively (but promptly to minimize on-going digestion/precipitation) increased from pH 4.5 to 6.5 by adding concentrated 5.0 M NaOH. Samples were removed at pH 5.5 and 6.5 and were then centrifuged. The pronounced brown oily pellet phase evident at pH 4.5 decreased in volume with increasing pH while the floating oil phase was also no longer visible and the AP_{DIGEST} was more turbid, indicating a higher concentration of solubilized digestion products. Notably, the incorporated fenofibrate followed the re-solubilization of the oily material into the AP_{DIGEST}.

DISCUSSION

One of the main objectives during LBF development for PWSD is to identify formulations that can maintain an incorporated drug in solution (or in a solubilized state) as the LBF is dispersed in the stomach and digested/further diluted in the small intestine.[22-25] The digestion process can positively contribute to the effective performance of LBFs by promoting drug transfer from a highly lipophilic colloidal phase into smaller BS/PL micelles that shuttle solubilized drug to the intestinal wall where it may be absorbed.[26] There is, however, an attendant risk that digestion of a some formulations will also trigger drug precipitation[9,10,27] and lower the level of exposure obtained when low drug solubility limits absorption.[28]

The LFCS Consortium has the objective of developing standardized *in vitro* tests for LBFs that assist in identification of robust lipid-based formulations prior to preclinical and clinical evaluations. The LFCS Consortium digestion test[8-11] is now used by several academic/industrial laboratories to screen-out formulations that show a high risk of precipitation during dispersion or digestion. In the current paper, these concepts have been extended to determine whether modifications to the digestion test can be used to alter LBF performance and to provide for more discriminating *in vitro* conditions. The rationale for this work came from the realization that: (1) developed lipid-based formulations that perform well in a single digestion condition may be near a performance threshold and, therefore, show variable performance *in vivo* and, (2) recent and historical evidence has shown that a single *in vitro* test does not always possess the power to discriminate between different LBF.[29-31]

Here, we conceive more “stressful” digestion test conditions based on a growing understanding of the factors that impact LBF digestion and LBF solubilization capacity, and in turn, the degree of supersaturation and likelihood of drug precipitation. These more stressful conditions were shown to be more effective in discriminating between the performances of different LBF and may therefore be used in combination with

data from 'standard' fasted digestion tests and less challenging dispersion tests as a third tier test in LBF formulation development. Together, these tests offer an improved system for rank order discrimination between LBFs, and allow the development of a LBF performance classification system, which is presented in the discussion that follows.

Stressing LBFs to Increase SR^M and Enable Better LBF Discrimination/Selection of Robust LBF

The solubilization capacity of a digested LBF is an important descriptor of performance since knowledge of this and the absolute drug concentration in the digestion mixture allows estimation of the mass of drug that is supersaturated, which in turn dictates likelihood of drug precipitation. Here and in our previous work,[9,10] we have used the maximum supersaturation ratio (SR^M) to capture this likelihood of precipitation. Importantly, the equation for SR^M (Equation 2) is analogous to the equation for Dose Number (D_O, Equation 3), which was used in the development of the BCS:[12]

$$D_O = \frac{M \times V_0}{C_S} \qquad \text{Equation 3}$$

Where M is the mass of drug (i.e. administered dose), V is volume of water administered with the dose (typically taken to be 250 ml) and C_S is the saturated drug solubility in water.[12] D_O has been historically been used to define the severity of the obstacle to absorption presented by the need to dissolve the entire drug dose in the available GI fluids. However, given its similarity to SR^M and the widespread use of enabling technologies to deliver PWSD, D_O may also be used to illustrate the challenge of *maintaining* the entire dose in a dissolved state in the GI tract when using supersaturating formulations such as lipid formulations or solid dispersions. Similarly, in our previous work, we showed that SR^M could accurately define the risk of precipitation, with formulations with SR^M values above 3 being highly predisposed to precipitation.[9,10]

It is well recognized that increasing drug loading in a lipid formulation will increase precipitation risk from the supersaturated state by increasing SR^M (or D_O). This was evident in the present study for fenofibrate (Figure

4) and has been widely captured in our previous studies.[9,28] It was also evident from the present study that, for some formulations, altering the digestion condition may lead to increased or decreased SR^M and, therefore, altered risks of precipitation. The capacity for different digestion conditions to alter SR^M provides the working basis for using this approach to better discriminate between lipid formulations.

The different digestion conditions explored here included decreased LBF concentration (↓LBF), increased BS concentration (↑BS) and decreased pH. The ↓LBF and ↑BS conditions were shown to dramatically reduce the performance of some fenofibrate formulations. Mechanistically, this decrease in performance can be firstly attributed to the increased BS/PL:lipid ratio in both conditions, and as a consequence, increased LBF digestion and/or solubilization of digestion products in the digestion milieu into small, well dispersed micelles rather than larger, more lipophilic vesicles. This phase-change presents a stress to the solubility of fenofibrate, which, due to its high lipophilicity, shows higher solubility in the presence of more lipophilic colloidal species, such as vesicles,[21] and thus may show an increased tendency towards precipitation in spite of increased bile salt concentrations. In contrast, the solubility of the less lipophilic (at least at neutral pH) tolfenamic acid, appears to be less dependent on the type of colloid present, but more dependent on the total concentration of solubilizing species present. As such itdoes not exhibit the same performance trends as fenofibrate.

It is important to note that these two conditions (↓LBF and ↑BS conditions) are highly relevant *in vivo* since they capture natural LBF processing events in the small intestine, namely the progressive dilution of slow moving lipid-rich colloids formed immediately after digestion with BS prior to trafficking the products of lipid digestion (and drug) across the UWL to the intestinal wall.[32,33]

Differences in colloidal phase behaviour in the digestion mixture can be identified in vitro either visually (i.e. a less cloudy AP_{DIGEST} indicates a greater proportion of smaller micellar structures, a measure that could also be quantified if required by particle sizing) or indirectly via a reduction in the solubilization capacity of the phase toward lipophilic drugs (evident in Figure 2a for fenofibrate). A decrease in the unionized:ionized FA

ratio (Figure 1) is also apparent as a vesicular-micellar transition promotes water access to FA in the colloidal phase and increases FA ionization.[34] To the best of our knowledge, the correlation between LBF concentration and FA ionization apparent in the present study has not been previously identified. The increase in FA ionization that occurred with increasing BS/PL:lipid ratio (stimulated by either a decrease in LBF concentration (evident here) or an increase in BS/PL (reported previously[9]) was evident across nearly all of the investigated LBFs irrespective of whether the fatty acids were LC or MC, or whether there were differences in the overall extent of digestion. However, the performance of the formulations only changes for Type I-MC, II-MC and IIIA-MC formulations and not LC formulations (Figure 2). For example, for the Type I-MC formulation containing fenofibrate, this formulation showed little or no precipitation in the fasted condition, but generated high SR^M values (>9.2) under ↑BS and ↓LBF conditions leading to extensive precipitation. The capacity for a change in digestion condition to alter the performance of select formulations highlights the value of using more than one digestion test condition to discriminate between robust and seemingly less robust lipid formulations.

While the use of ↑BS and ↓LBF conditions afforded better discrimination between fenofibrate containing LBFs (in accordance with changing SR^M values), this was not the case for tolfenamic acid where SR^M values generally remained <3 across the different conditions (Table 5). This reflects the fact that tolfenamic acid is predominantly ionized at pH 6.5 and exhibits a relatively low affinity for the colloidal phase. As such, effects of BS and LBF concentration on colloid structure had an lower overall impact on tolfenamic acid solubilization when compared to lipophilic drugs, such as fenofibrate, cinnarizine and halofantrine.[32] Based on this knowledge, it is clear that increasing the BS/PL:lipid ratio as a stress toward LBF performance is highly dependent on the drug, consistent with the findings in the present study where ↑BS promoted precipitation in the case of fenofibrate but decreasing precipitation in the case of tolfenamic acid (Table 5).

An alternative stress test for LBFs containing weakly acidic drug was however identified. For tolfenamic acid, there was a marked decrease in performance of the hydrophilic Type IIIB-MC and IV LBFs when the pH in the digestion test was decreased to pH 4.5 (Figure 6), in direct response to reduced intrinsic drug solubility (increasing SR^M) at the lower pH. Interestingly, the same change in pH led to a marked improvement in performance of analogous fenofibrate-containing LBFs (Figure 6). This improvement cannot be attributed to a change in extent of digestion at the lower pH as the overall digestibility of the investigated LBFs was unchanged between pH 4.5 and 6.5. Furthermore, differences in performance of both this LBF and the Type IV LBF were evident even during the initial dispersion phase (ie prior to digestion). Rather, since fenofibrate is a neutral and a highly lipophilic drug, the reduced tendency to precipitate at low pH reflects a decrease in FA ionization, and a change in the colloidal state formed on dispersion and digestion. Notably, at lower pH, decreased fatty acid ionisation reduces electrostatic repulsion between adjacent fatty acids[35] and promotes the formation of vesicular or emulsified phases (rather than micellar phases) that have greater affinity for highly lipophilic drugs such as fenofibrate. This is in direct contrast to the effect of increasing BS/PL:lipid ratio which favours a vesicle to micelle transition and promotes precipitation for fenofibrate.

It appears therefore that altering pH may be useful in increasing precipitation stress for LBF. However, there are additional experimental complexities when conducting digestion tests at lower pH. FA protonation at lower pH promotes the formation of more oil-rich species that may either phase separate as a floating oil phase or pellet as a dense lipidic phase. In these circumstances, great care is required during digestion phase isolation (for analysis) and subsequent data interpretation, as it possible that drug present within a dense oily phase may be mistaken for precipitated drug (see Figure 7). For weak acids, an additional factor that must be considered is the risk of precipitation on dispersion under acidic gastric conditions. This is currently under investigation within the LFCS Consortium.[14]

Overall, the present study suggests that slight modifications to digestion test conditions can yield useful differences in LBF performance. Critically, these differences in performance can be rationalized according to changes in the solubilization capacity of lipid formulations, and in turn, the SR^M value and risk of precipitation. In almost all cases, in instances where $SR^M > 3$ much higher precipitation tendency was evident, irrespective of the digestion test conditions. Identification of a more stressful condition for a given lipid formulation nonetheless requires knowledge of drug properties and the type of formulations being developed (e.g. is the drug ionizable? Is the lipid formulation rich in MC lipid?). From this position, the formulator should be able to identify an appropriate additional digestion test condition (in addition to a "standard" fasted condition) that can be used to better rank order LBFs according to *in vitro* performance so that the most formulation(s) can be identified.

Proposing a "Performance Classification System" Based on *In Vitro* LBF Performance

Initially proposed by Pouton[1] the LFCS was designed to classify LBFs based on composition, such as the percentage content of lipids, surfactant and cosolvent (Table 1). This classification system also provided some general performance-related guidance; however, it has become apparent that the performance of these formulations can vary significantly depending on the type of drug, drug loading and digestion test condition. For example, a Type IV formulation may in some cases perform very well *in vitro* and *in vivo*[5] (for example commercial Type IV products including Agenerase®, Targretin® and Advil®) while in other cases, this type of LBF has been shown to exhibit significant precipitation on dispersion/digestion[1,9,10,36-38] leading to decreased *in vivo* performace[7].

The need to describe LBFs in terms of solubilization/supersaturation performance for a specific drug was recognized by the LFCS Consortium. Building on the platform of extensive *in vitro* studies described in this and previous publications, the LFCS Consortium therefore proposes in Figure 8 a new "Performance Classification System" for Lipid Formulations ("LF-PCS"). The theoretical basis for the PCS stems from an

increasing understanding of the factors that impact LBF performance. Much like the BCS, which classifies drugs based on biopharmaceutical properties measured *in vitro*, the PCS is based on three *in vitro* performance tests relating to *in vivo* GI processes: (i) *in vitro* dispersion in simulated gastric conditions, (ii) *in vitro* digestion under fasted intestinal conditions and (iii) *in vitro* digestion under stressed intestinal conditions. These three tiers of *in vitro* test are designed specifically to subject a LBF to progressively more challenging conditions while also increasing in complexity and throughput, in-line with the expectation that a decreasing number of formulations progress through each tier. In dispersion tests, the risk of drug precipitation on simple dispersion and dilution of a LBF in simulated gastric conditions is assessed, and such tests may be performed under relatively high throughput conditions using 6-station USP dissolution testers. Guidance for evaluating the dispersion properties of LBFs is currently underway within the LFCS Consortium[14] and preliminary results will be described in future publications.

As evident from Figure 8, LBFs can be graded A, B, C or D simply from their respective performance across these three tiers of *in vitro* tests. Grade A formulations are the most robust formulations *in vitro*, and from this, those working in the field would expect that progressing a grade A formulation would be associated with a lower risk of poor *in vivo* performance when compared to a grade C or D formulation. It is also important to realize that the same LBF (with respect to composition of excipients) may be grade A for a particular drug but may be a lower grade for another drug. For example, in this article, Type IIIB-MC formulations containing drug at 80% saturation were grade C for fenofibrate but grade B for tolfenamic acid. In addition, the same LBF containing the same drug could exhibit variation in grading with changing drug loading. For example, the Type IIIA-MC LBF with fenofibrate was grade C at the higher drug loading (80% saturation) but on halving the drug load, the same LBF performed according to B grade. Such LBF nuances have been highlighted by previous LFCS Consortium publications, but these differences in LBF performance cannot be captured with the existing LBF classifications and terminology. The LFCS Consortium also anticipates that sub-types of performance grades may be necessary to account for possible significant differences in dispersion and digestion properties

of LBFs of the same performance grade, for example, Type I vs Type IIIA-LC formulations for either fenofibrate or tolfenamic acid in the present study. Sub-classifications may therefore be based on dispersibility, speed of digestion, high/low supersaturation potential etc. This is in some ways analogous to the possible sub-division of Class II of the BCS into class IIa and IIb in recognition of the different challenges when absorption of BCS class II compounds is limited by dissolution rate or solubility. [39]

This grading system and related performance criteria will have several benefits to those looking to develop LBFs for both new chemical entities but also for existing drugs. These benefits are encapsulated as follows:

The LF-PCS:

- Provides a standardized scientific means of describing and comparing LBF *in vitro* performance
- Provides a facile means of identifying high *in vivo* performance risk and development risk
- Promotes robust LBF design and rational selection of lead formulations for preclinical/clinical evaluation
- Promotes clear and increased dialogue with regulatory authorities
- Promotes Quality by Design by encouraging the use of standardized in vitro methods, terminology and understanding of drug/formulation properties throughout lipid formulation performance testing

Of course, central to a performance classification system are specific and well defined performance criteria. Much like the BCS, where the boundaries for class I-IV drugs were established based on extensive *in vitro* and *in vivo* datasets relating to a wide range of drugs, LBF performance criteria may take several years and extensive feedback and discussions to fully refine. Nonetheless, some example criteria are now suggested. For example, for dispersion, a formulation is grade D if it cannot achieve *">80% incorporated drug solubilized after 2 hours dispersion in Simulated Gastric Fluid"* whereas a grade C LBF meets this criteria but would not achieve *">80% incorporated drug solubilized after 1 hour of digestion in fasted conditions"*. Once performance criteria such as these are defined and accepted, it will be possible to rapidly associate a description of an A-D grade LBF to performance. In addition to defining performance criteria, the conditions used for each tier of

the PCS must be defined. As stated earlier, conditions for *in vitro* dispersion are the subject of on-going work in the LFCS Consortium. The conditions of digestion in the 2nd tier reflect the "typical" fasted conditions used in this study and throughout the LFCS Consortium. From this study, it should be clear that some digestion conditions are stressful to some drugs but not to others. The most appropriate stressed digestion conditions, should be based on drug properties such as the pH dependent solubility of the drug and the lipophilicity of the drug, with the intent of promoting supersaturation in the test either by increasing drug concentration or decreasing drug solubility.

CONCLUSIONS

In this paper, modification of *in vitro* digestion test conditions was shown to lead to a significant change in the performance of lipid formulations, and thus, was able to identify those formulations that lie near a performance threshold. This highlights the value of using a more "stressful" digestion test in addition to a standard fasted-state to discriminate between robust formulations that show consistently good performance under changing conditions and seemingly good formulations where a drop-off in performance is apparent on moving to a slightly more challenging conditions. *In vitro* at least, the change in lipid formulation performance appears to be related to an increase in the maximum supersaturation ratio (SR^M), which increasingly is being shown to forecast the likelihood of precipitation irrespective of drug, formulation and digestion condition.

While SR^M provides a theoretical rationale for the use of multiple *in vitro* tests during lipid formulation development, this stress-testing approach also offers the opportunity to more effectively rank order lipid formulations according to a performance classification system (Lipid Formulation Performance Classification System, LFPCS). Presented here for the first time, the LFPCS provides a standardized means by which the *in vitro* performance of a lipid formulation can be classified and described. With additional work to better define the specific performance criteria that underpins this classification, the LFPCS has the potential to provide

much needed direction during lipid formulation development by outlining clear performance attributes.

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FIGURES

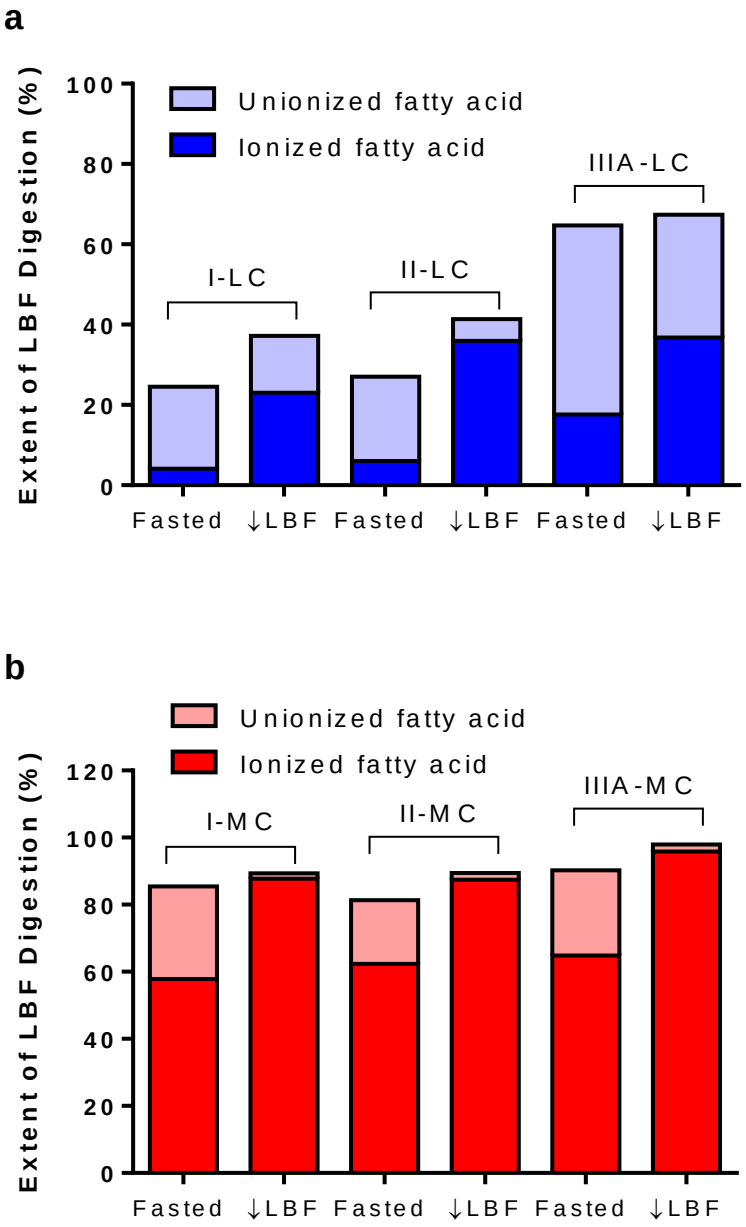

Figure 1: The estimated extent of *in vitro* digestion of Type I, II and IIIA lipid formulations containing (a) LC (long-chain) lipid or (b) MC (medium-chain) lipid. The extent of LBF digestion was calculated from Equation 1, where it is assumed that formulation surfactants (Tween® 85 in Type II systems and Cremophor® EL in Type IIIA systems) may be completely digested. Ionized FA was detected at pH 6.5 during the tests. Unionized FA was detected at pH 9 at the end of the tests. Digestions were performed under fasted conditions (1 g LBF/40 ml) and low lipid conditions (↓LBF, 0.16 g LBF/40 ml). Formulation compositions are listed in Table 2

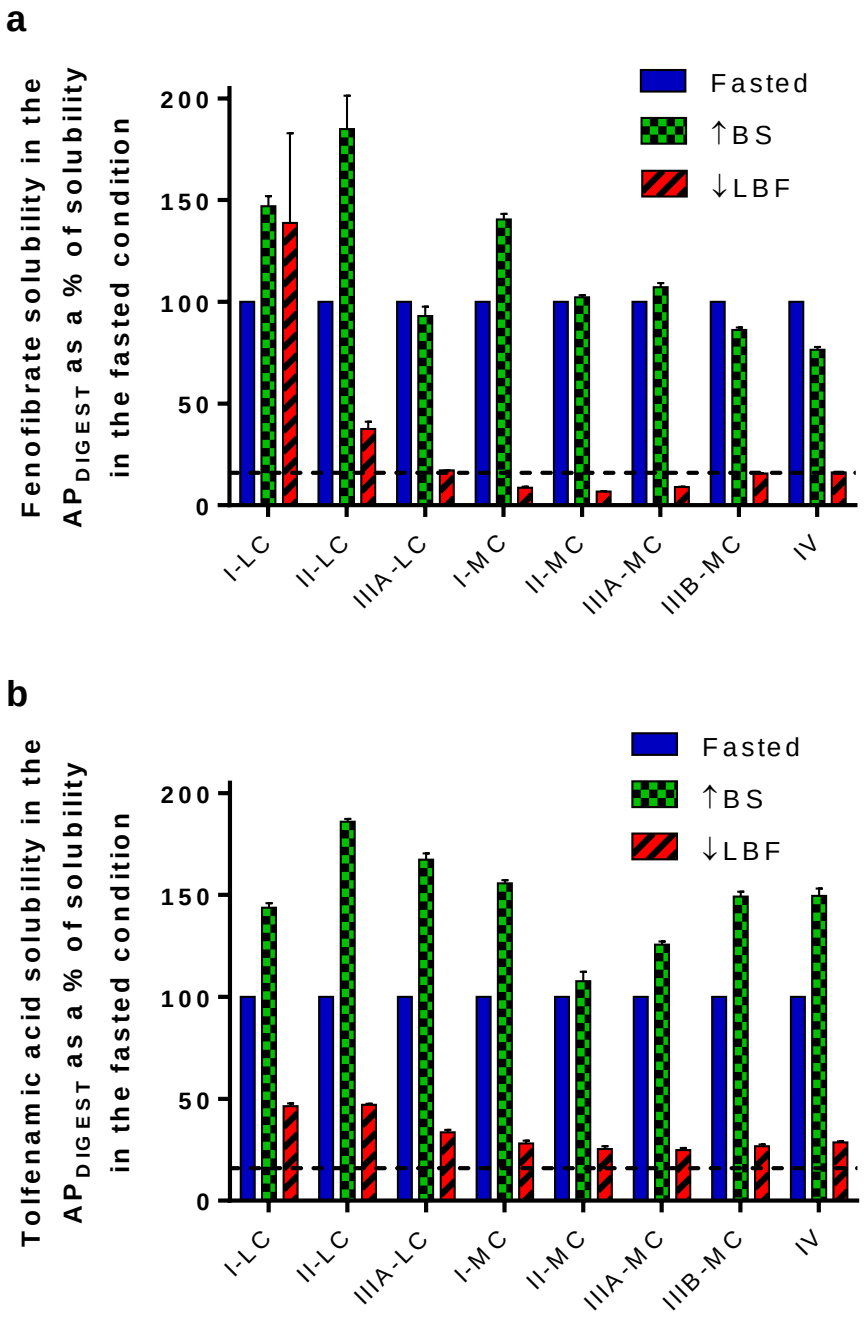

Figure 2: Measured equilibrium solubility of (a) fenofibrate and (b) tolfenamic acid in colloidal aqueous digests (AP_{DIGEST}) formed following digestion of the eight LBFs in fasted, ↑BS and ↓LBF conditions. Values are plotted as a % of the solubility in the fasted condition. Absolute solubility values in the fasted conditions are shown in Table 4. The dashed horizontal line denotes a proportional decrease in solubility with a decrease in lipid concentration to the low lipid condition. Values are expressed as means (n=3) ± 1 SD. Formulation compositions are listed in Table 2.

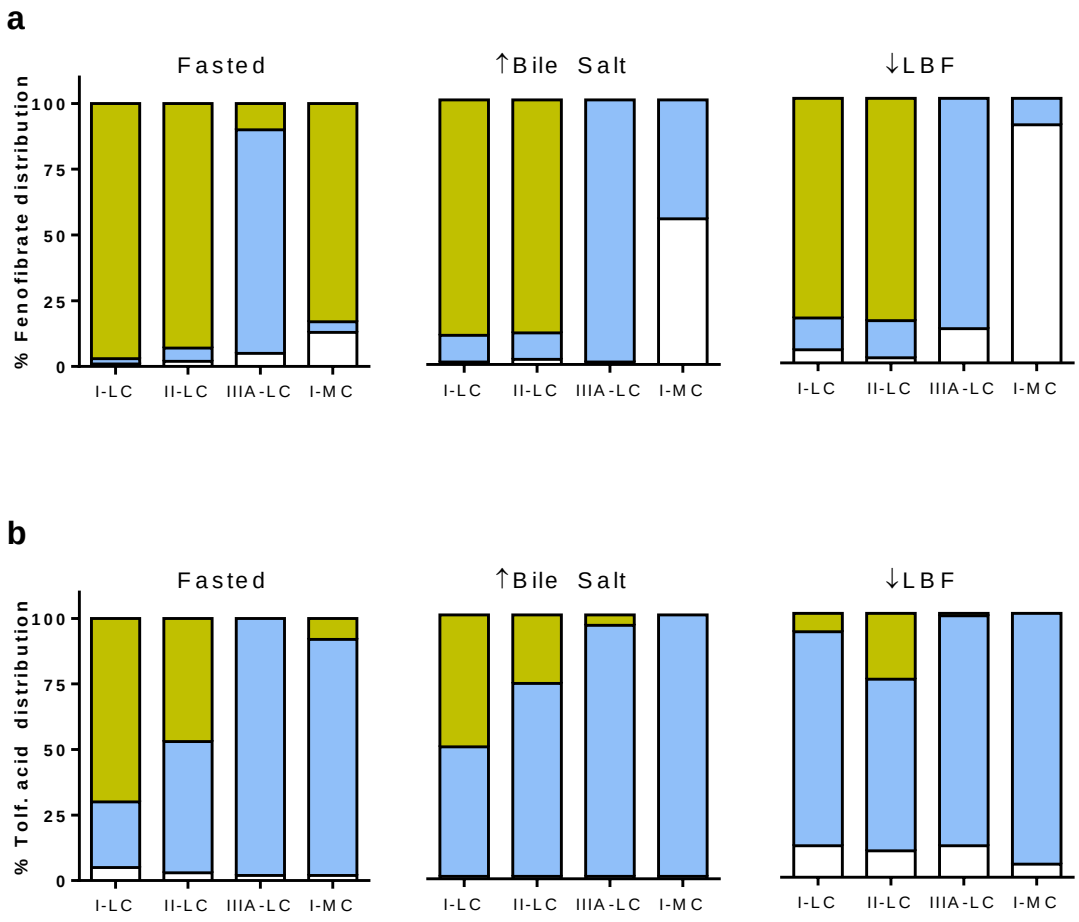

Figure 3: Figure showing performance of LC and partially digesting MC LBFs containing (a) fenofibrate or (b) tolfenamic acid (at 80% saturation) after 30 min *in vitro* digestion in fasted, ↑BS and ↓LBF conditions. Drug is distributed across a poorly dispersed oil phase (yellow bars), a colloidal aqueous phase [(AP_{DIGEST}) light blue bars] and a pellet phase (white bars). Formulation compositions are listed in Table 2. N=3

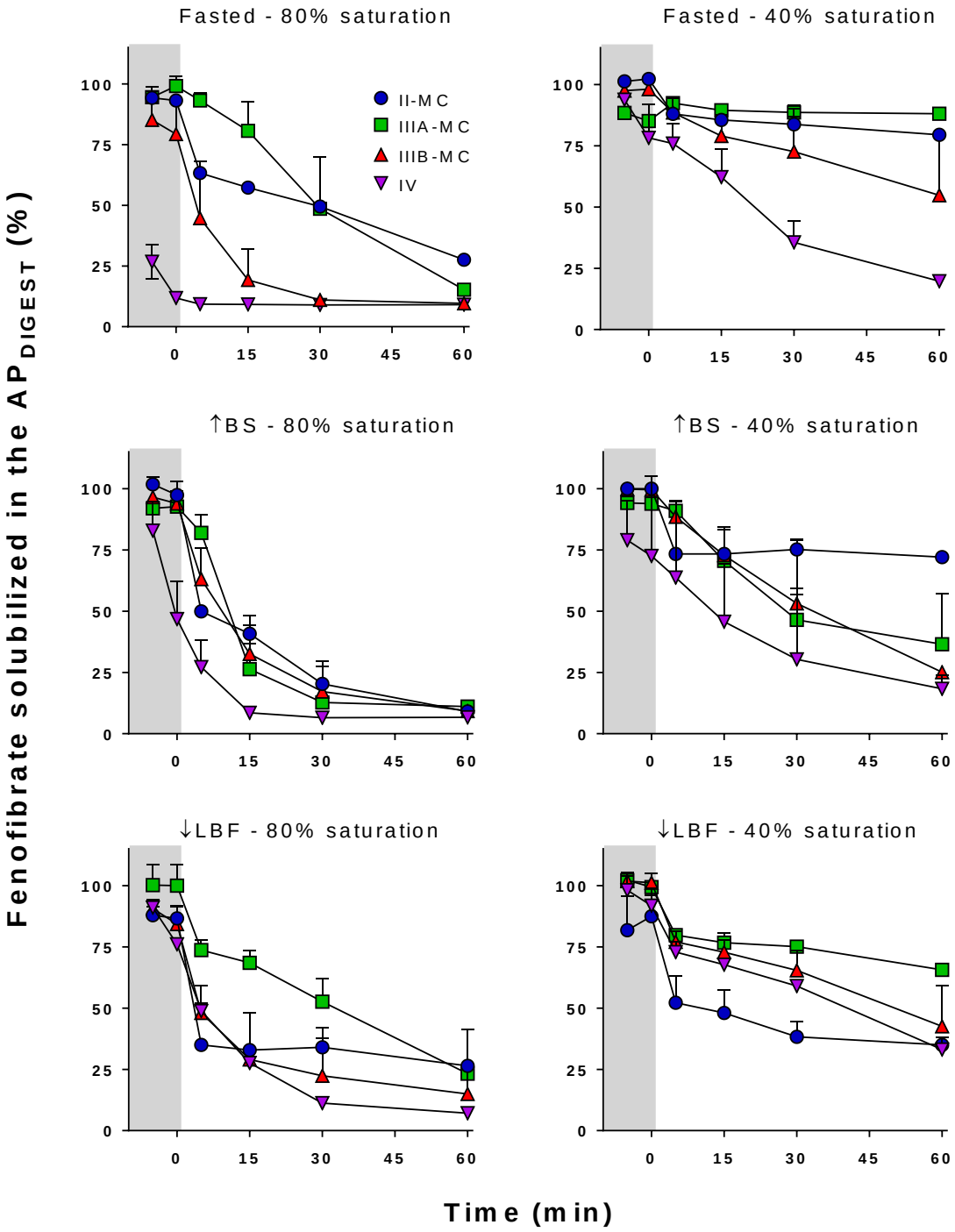

Figure 4: The performance of II-MC, IIIA-MC, IIIB-MC and IV LBFs containing fenofibrate at 80% saturation and 40% saturation in fasted, ↑BS and ↓LBF conditions. Formulation compositions are listed in Table 2. All values are expressed as means (n=3) ± 1 SD. The grey shaded region denotes the 10 minute dispersion phase after which digestion was initiated on addition of pancreatin.

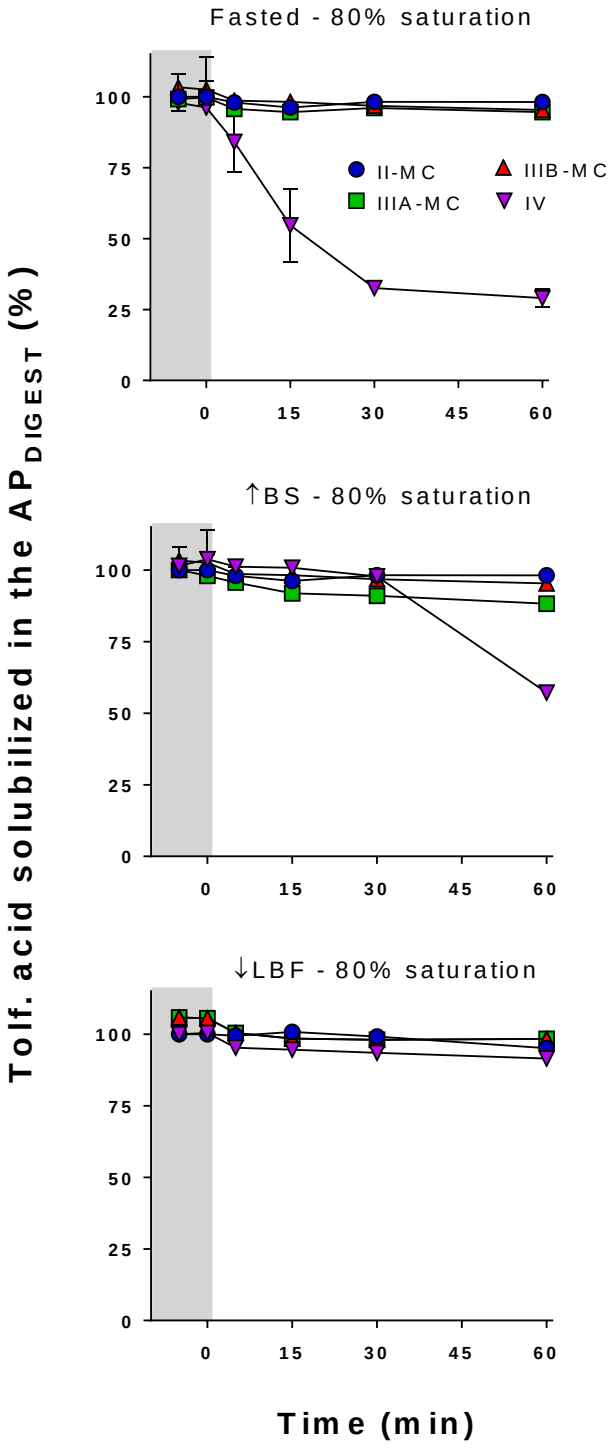

Figure 5: Figure showing performance of II-MC, IIIA-MC, IIIB-MC and IV LBF containing tolfenamic acid at 80% saturation in the fasted, ↑BS and ↓LBF conditions. Formulation compositions are listed in Table 2. All values are expressed as means (n=3) ± 1 SD. The grey shaded region denotes the 10 minute dispersion phase after which digestion was initiated on addition of pancreatin.

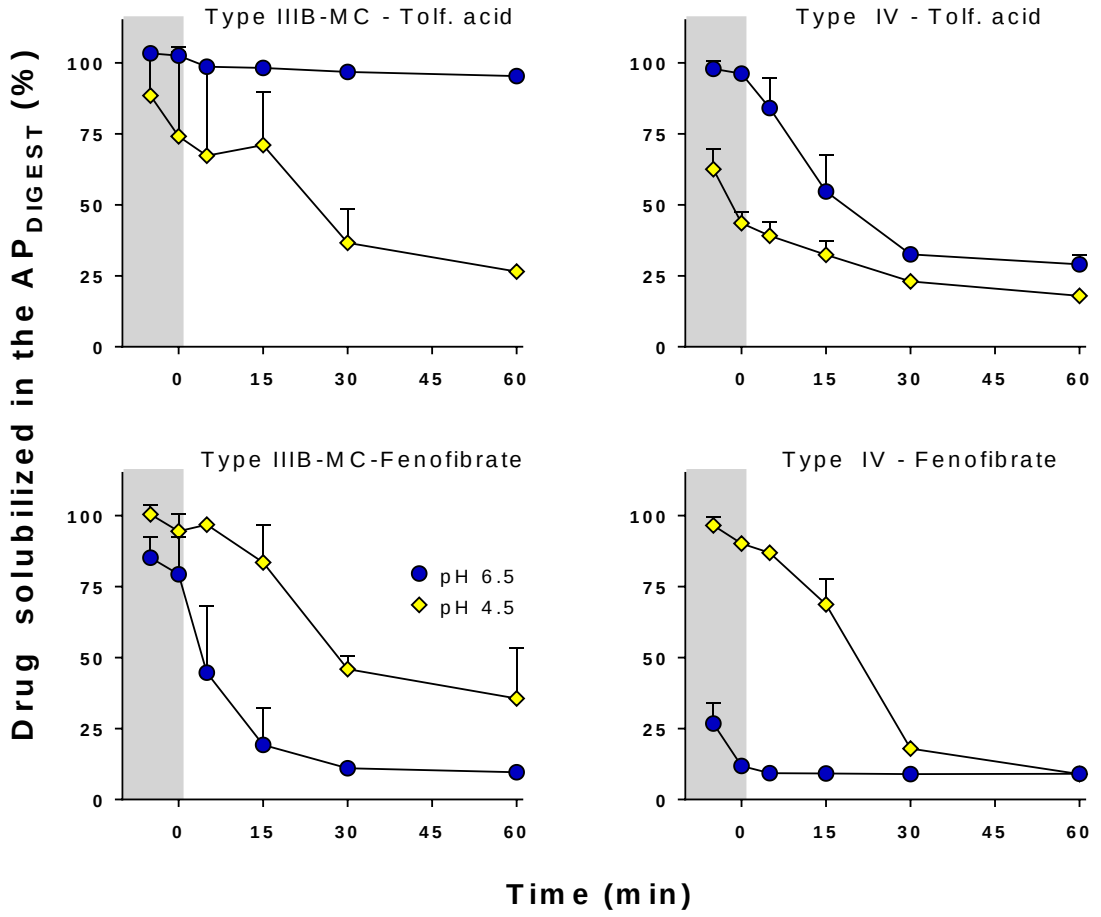

Figure 6: The performance of IIIB-MC and IV LBF containing tolfenamic acid and fenofibrate (both at 80% saturation) in the fasted digestion condition at pH 6.5 and pH 4.5. Formulation compositions are listed in Table 2. All values are expressed as means (n=3) ± 1 SD. Results at pH 6.5 are reproduced from Figures 4 and 5 for comparison. The grey shaded region denotes the 10 minute dispersion phase after which digestion was initiated on addition of pancreatin.

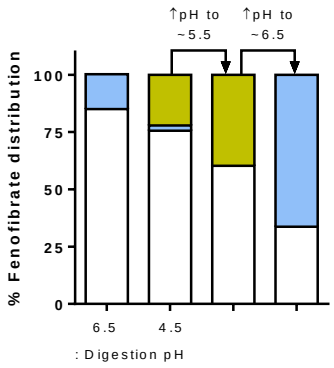

Figure 7: Fate of incorporated fenofibrate in the Type IIIA-MC LBF after 60 minutes of digestion at pH 6.5 and pH 4.5. At the end of the digestion test at pH 4.5, sodium hydroxide (5.0 M) was added to rapidly increase pH. The effect on fenofibrate solubilization is shown. Drug is distributed across a poorly dispersed oil phase (yellow bars), a colloidal aqueous phase [(AP_{DIGEST}) light blue bars] and a pellet phase (white bars).

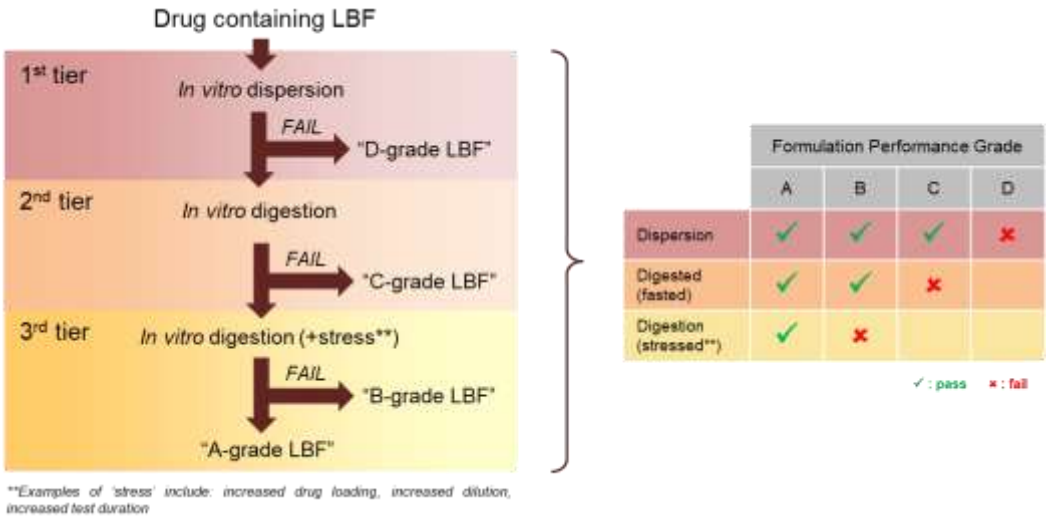

Figure 8: A proposed new Lipid Formulation Classification System based on *in vitro* performance evaluations.