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

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Received 7 March 2014; revised 1 June 2014; accepted 4 June 2014

Published online 1 July 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24067

ABSTRACT: The Lipid Formulation Classification System 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, 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, whereas 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. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2441–2455, 2014

Keywords: LFCS Consortium; lipid-based drug delivery systems; SEDDS; drug solubilization; *in vitro* digestion testing; solubility; bioavailability; *in vitro* models; precipitation; supersaturation

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 nanometer 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, although 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 nonionic ester surfactants are also digested by enzymes found in the small intestine.[2–4]

Although 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] whereas 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, although the LFCS provides much-needed guidance in describing lipid formulations based on composition, it

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This article contains supplementary material available from the authors upon request or via the Internet at http://wileylibrary.com.

Journal of Pharmaceutical Sciences, Vol. 103, 2441–2455 (2014)

Table 1. The Lipid Formulation Classification System

	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils, *for example, triglycerides or mixed mono- and diglycerides*	100	40–80	40–80	<20	0
Water-insoluble surfactants (<12), *for example, sorbitan tri/monoesters, propylene glycol, di/monoesters, and so on*	–	20–60	–	–	0–20
Water-soluble surfactants (HLB > 12), *for example, PEG monoesters, and so on*	–	–	20–40	20–50	30–80
Hydrophilic cosolvents, *for example, PEG, propylene glycol, glycerol, and 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

Adapted from Ref. 1.

cannot (and does not) provide an indication of the likely *in vivo* performance of the formulation.

To this point, the 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 (PCS). Much like the Biopharmaceutics Classification System (BCS),[12] which classifies drugs based on gastrointestinal (GI) solubility and intestinal permeability, a new PCS 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 (1) an *in vitro* dispersion test, (2) an *in vitro* digestion test in "typical fasted" conditions, and (3) 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] as 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 that reflect exposure to alternative conditions that may be encountered in the intestine. First, the quantity of lipid formulation in the test was reduced to assess LBF performance under more dilute conditions. Second, the bile salt (BS) and phospholipid (PL) concentrations were increased from 3 and 0.75 mM, which reflect typical concentrations in the fasted duodenum,[15–17] to 10 and 2.5 mM, respectively, to represent the upper concentration range of BS and PL in the small intestine following administration of a long-chain (LC) lipid formulation.[18] Lastly, the pH within the digestion test