Creating a Lipid Formulation Classification System

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The LFCS Consortium: 1 - Effect of bile salt concentration on *in vitro* digestion of a range of lipid-based formulations

Introducing the LFCS Consortium

- The LFCS Consortium is a non-profit organization consisting of both academic and industrial partners with the overall objective of developing standardized *in vitro* tests for lipid-based formulations (LBFs). Work presented here details some of the experiments undertaken in the first year of the LFCS Consortium**.**

STUDY AIM: In the present poster, we investigate how increasing the bile salt concentration in the in vitro digestion model affects (i) the digestion (rate and extent) of lipid-based formulations (see Fig 1a/2a), (ii) the fate of an incorporated model poorly soluble drug (danazol) and (iii) the degree of supersaturation in aqueous phase digests (see Fig 1b/2b).

- Formulations are grouped together according to their digestibility – formulations in the first results panel were only partially digested during the experiments while formulations in the second panel appeared to be completely digested (evidenced by no oil phase).

Methods

- **Table 1** below lists the composition of eight LBFs investigated within the LFCS. Formulations were incorporated with danazol at 80% of its equilibrium solubility in the formulation. One gram LBF (containing danazol) was initially dispersed in 36mL digestion medium (pH 6.5, 2mM tris-maleate, 150mM NaCl, 1.4mM calcium, 37 C) before digestion was commenced on addition of 4mL porcine pancreatin suspension (600 USP units/mL). Digestion was continuously monitored using a pH-stat titrator (Titrando®, Metrohm). Bile salt (sodium taurodeoxycholate) concentration in the test was either 0, 3, 5 or 10mM. Phosphatidyl choline (egg, Lipoid, Germany) was added to achieve a 4:1 bile salt:phospholipid ratio.
- Digestion samples were separated by centrifugation and danazol concentration in the poorly dispersed oil phase, aqueous phase and pellet was determined by HPLC. Danazol equilibrium solubility was determined in "blank" aqueous phase digests ($AP_{DIGESTS}$) obtained following the digestion of drug-free LBF.

Table 1: Composition of the eight LBFs investigated by the LFCS Consortium

** Danazol loading corresponded to 80% of its equilibrium solubility in formulation preconcentrate*

Formulation type	Composition (% w/w)	Danazol (mg/g)*
I-LC	50% corn oil, 50% Maisine™ 35-1	6.7
II-LC	32.5% corn oil, 32.5% Maisine™ 35-1, 35% Tween 85	13.5
III-LC	32.5% corn oil, 32.5% Maisine™ 35-1, 35% Cremophor EL	15.2
I-MC	50% Captex, 50% Capmul	19.8
II-MC	32.5% Captex, 32.5% Capmul, 35% Tween 85	21.8
IIIA-MC	32.5% Captex, 32.5% Capmul, 35% Cremophor EL	22.7
IIIB-MC	25% Capmul, 50% Cremophor EL, 25% Transcutol HP	39.0
IV	50% Cremophor EL, 50% Transcutol HP	52.4

Results: "Partially" digested LBFs

- The effect of increasing bile salt concentration on the *in vitro* digestion of Type I-LC, II-LC, III-LC and I-MC formulations, and on the fate of incorporated danazol after 30 minutes digestion is shown in **Fig. 1**.
- Increasing bile led to a progressive increase in digestion (rate/extent) of all these formulations, and the complete digestion of III-LC and I-MC formulations (**Fig. 1a**). Differences in the initial rate digestion of the formulations were most marked on increasing bile from 0 mM to 3 mM.
- For all formulations, increased digestion (in response to increasing bile) led to more drug entering the aqueous phase. For LC formulations, the "extra" drug was solubilized, evidenced by the low level of drug present in the pellet. Supersaturation of the aqueous phase (SSR values) were independent of bile salt concentration, suggesting that there was an enhancement in the solubilization capacity of the aqueous phase with increasing digestion. Conversely, the Type I-MC formulation showed evidence of increased drug precipitate in the pellet with increasing digestion of the formulation (increasing grey bars, **Fig. 1b**).

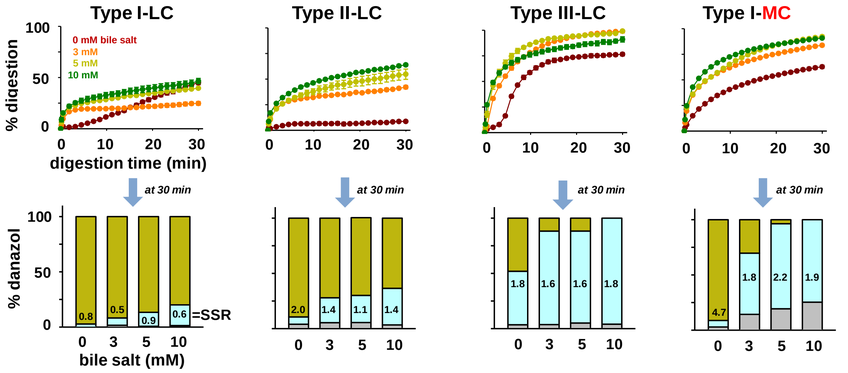

Fig. 1: Effect of increasing bile salt concentration on **(a)** the *in vitro* digestion of various LBFs and **(b)** on the distribution of incorporated danazol (80% saturation) between partially digested **OIL PHASE**, **AQUEOUS PHASE** and **PELLET** after 30 minutes digestion (n = 3). **Supersaturation ratio (SSR)** = $[danazol]_{aq}$ / $[equilibrium\ solubility]_{aq}$

Results: "Completely" digested LBFs

- Digestion profiles (**Fig. 2a**) and drug distribution plots (**Fig. 2b**) both indicate 3 mM bile was sufficient to achieve complete digestion of Type II-MC, IIIA-MC, IIIB-MC and IV formulations.
- Contrary to the performance of the LC formulations, however, there was evidence of greater drug precipitation. This was evident across the entire bile salt range for Type IIIB-MC and IV formulations. Formulations of the II-MC and III-MC type showed comparatively less precipitation, but when more than 50% of drug was retained in the aqueous phase, it was present in a supersaturated state (SSR > 1), thus thermodynamically unstable.

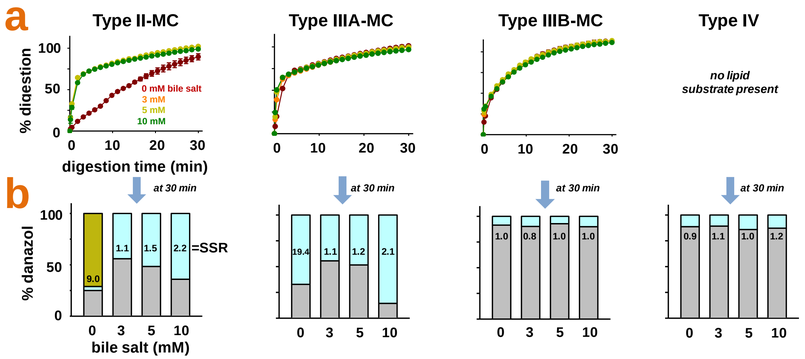

Fig. 2: Effect of increasing bile salt concentration on **(a)** the *in vitro* digestion of various LBFs and **(b)** on the distribution of incorporated danazol (80% saturation) between partially digested **OIL PHASE**, **AQUEOUS PHASE** and **PELLET** after 30 minutes digestion (n = 3). **Supersaturation ratio (SSR)** = $[danazol]_{aq}$ / $[equilibrium\ solubility]_{aq}$

Conclusions

The presence of bile had a significant effect on the digestion of several LBFs and the distribution of incorporated drug. Fasted state levels of bile (3-5 mM) were sufficient to completely digest the less lipophilic formulations, although these were more prone to drug precipitation when containing danazol at a 80% saturation level in the formulation**.**

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