# Drug Solubilization Behavior During *in Vitro* Digestion of Simple Triglyceride Lipid Solution Formulations

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***Purpose.*** The purpose of this study was to characterize the solubilization and precipitation characteristics of a range of poorly water-soluble drugs during digestion of either long-chain or medium-chain triglyceride (TG) lipid formulations.

***Methods.*** TG solution formulations of five selected drugs (griseofulvin, diazepam, danazol, cinnarizine, and halofantrine) were digested *in vitro* and drug distribution/solubilization behavior in the resulting digests assessed.

***Results.*** For the less lipophilic drugs, the mass of drug dissolved in either medium or long-chain TG was low and the drugs partitioned rapidly into the aqueous digestion phase. For the higher log *P* drugs, drug transfer to the aqueous phase was limited by accumulation in undigested long-chain TG. In contrast, medium-chain TG was digested completely producing a dispersed aqueous phase that was capable, at least in the case of the high log *P* drugs, of supporting supersaturated drug concentrations.

***Conclusions.*** The solubilization behavior of lipophilic drugs on digestion of simple TG lipid formulations is a function of the lipophilicity of the drug (which dictates the drug dose and the partitioning behavior), the nature of the colloidal phases produced on digestion of the different formulation lipids, and the kinetics of drug transfer between the digesting formulation and the colloidal phases produced.

**KEY WORDS:** dissolution; drug absorption; lipid-based drug delivery; lipid digestion.

## INTRODUCTION

The coadministration of poorly water-soluble drugs with lipids can increase drug absorption via enhanced solubilization and dissolution (1,2); however, the broader use of lipid-based formulations has been hampered by a lack of predictive *in vitro* formulation assessment tests. After ingestion and rupture of the dose form, poorly water-soluble drugs formulated in lipid solution formulations are typically assumed to partition from the lipid formulation into the intestinal aqueous environment. However, this is an overly simplified description as during lipid digestion, lipid-based formulations interact with gastric and/or intestinal fluids and endogenous biliary lipids to form a range of colloidal intestinal phases (3–6). Hence, issues associated with formulation performance (at least in terms of their impact on drug solubility) include the pattern of drug trafficking across these solubilized phases formed on digestion, the capacity of the solubilized phases to maintain drug in solution, and the propensity for poorly water-soluble drugs to precipitate during the digestion/dispersion process.

*In vitro* models to assess the kinetics of lipid digestion and the interaction of lipid digestion products with endogenous bile salts and phospholipids are well-recognized in the lipid biochemistry literature (reviewed in Ref. 7). Although some studies have examined the pharmaceutical consequences of lipid digestion on drug solubilization (8–12), there has not yet been a systematic study of the impact of different formulation lipids on a range of drugs with varying solubility and partition characteristics. In this report, we have explored the impact of digestion of different triglyceride lipid solutions on the solubilization patterns of a range of poorly water-soluble drugs (griseofulvin, diazepam, danazol, cinnarizine, and halofantrine) using an *in vitro* digestion model. The central hypothesis of this study was that the utility of lipid-based formulations arises from their ability to limit/prevent drug precipitation after interaction of the dose form with the intestinal environment and to promote drug transfer to the colloidal species that support the drug absorption process.

## MATERIALS AND METHODS

### Materials

Sodium taurodeoxycholate 99% (NaTDC), porcine pancreatin (activity 8 × USP specifications), Trizma maleate, soybean oil (SBO) (Sigma Chemical Co., St. Louis, MO, USA); sodium chloride, 1 M hydrochloric acid (Ajax Chemicals, Sydney, Australia); calcium chloride dihydrate (BDH Chemicals, Melbourne, Australia); 4-bromophenylboronic acid (4-BPB) (Aldrich Chemical Co., Milwaukee, WI, USA); Maisine 35-1 (Gattefossé, Saint-Priest, France); and Captex 355 and Capmul MCM (Abitec Corporation, Janesville, WI) were all used as received. Captex 355 is a medium-chain-length triglyceride consisting of 59% w/w caprylic acid ($C_8$), 40% capric acid ($C_{10}$), <1% lauric acid ($C_{12}$), and <1% caproic acid ($C_6$) (certificate of analysis, lot no 61202-6, Abitec Corporation) with average molecular weight 504. Capmul MCM is a blend of medium-chain mono-, di-, and triglycerides (58% monoglyceride, 36% diglyceride, and 5% triglyceride by TLC) consisting of 80% w/w caprylic acid ($C_8$), 20% capric acid ($C_{10}$), and 2% caproic acid ($C_6$) with average molecular weight 277 (lot no 71210-6, Abitec Corporation). Soybean oil is a long-chain triglyceride consisting of 54% w/w linoleic acid ($C_{18:2}$), 22% oleic acid ($C_{18:1}$), 11% palmitic acid ($C_{16}$), 9% linolenic acid ($C_{18:3}$), and 4% stearic acid ($C_{18:0}$) with average molecular weight 870.5 (lot no 038H0103, Sigma Chemical Co.). Maisine 35-1 is a blend of long-chain mono-, di-, and triglycerides (38% monoglyceride, 48% diglyceride, 13% triglyceride, and

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**ABBREVIATIONS:** 4-BPB, 4-bromophenylboronic acid; BS, bile salt; CIN, cinnarizine; DAN, danazol; DG, diglyceride; DIAZ, diazepam; FA, fatty acid; GRIS, griseofulvin; HF, halofantrine; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; MG, monoglyceride; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; PL, phospholipid; SBO, soybean oil; TBU, tributyrin units; TG, triglyceride.



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<1% fatty acid) consisting of 57% w/w linoleic acid ($C_{18:2}$), 28% oleic acid ($C_{18:1}$), 11% palmitic acid ($C_{16}$), 2% stearic acid ($C_{18:0}$), <1% linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), and eicosenoic acid ($C_{20:1}$) with average molecular weight 540 (batch no. 20300, Gattefossé). Lecithin [approximately 60% pure phosphatidyl choline (PC) from dried egg yolk by TLC] was obtained from Pharmacia LKB (Uppsala, Sweden) and was used as received. 1 M sodium hydroxide (Titrisol, Merck, Darmstadt, Germany) stock solution was diluted with water to obtain 0.2 and 0.6 M NaOH titration solutions. Solvents were of HPLC grade (Mallinckrodt, Paris, KY, USA), and water was obtained from a Milli-Q (Millipore, Billerica, MA, USA) water purification system. Griseofulvin (GRIS, Sigma Chemical Co.), cinnarizine (CIN, Sigma Chemical Co.); diazepam (DIAZ, Alphapharm, Glebe, Australia); danazol (DAN, Sterling Pharmaceuticals, Sydney, Australia); and halofantrine base (HF, SmithKline Beecham Pharmaceuticals, Mysore, India) were all used as received.

### Solubility and Distribution Coefficient Determination

Drug solubility was determined in long-chain triglyceride (LCT, soybean oil), medium-chain triglyceride (MCT, Captex 355), glyceride mixtures representing partially digested triglycerides (Maisine 35-1 and Capmul MCM), and in low concentration (5 mM NaTDC/1.25 mM PC) and high concentration (20 mM NaTDC/5 mM PC) bile salt (BS) and phospholipid (PL) solutions. Equilibrium solubility of the probe compounds was also determined in digestion buffer (without BS/PL) and "blank" aqueous phases obtained after digestion of MCT and LCT under high and low BS/PL conditions. A lipolysis inhibitor was added to the aqueous phase immediately after collection to stop further digestion (9 μl/ml, 4-BPB 0.5 M in methanol) (4,9). Solubility determinations were performed at 37°C in triplicate using standard methodologies (6). Equilibrium solubility was defined as the solubility reached when consecutive samples varied by ≤5%. The partition coefficients of the drugs between the two triglycerides and a solution of 5 mM NaTDC/1.25 mM PC in digestion buffer were also determined using the shake flask method.

### *In Vitro* Digestion

Digestion experiments were conducted using a previously described *in vitro* lipid digestion model (13). Briefly, known quantities of lipid (containing drug at 50% of saturated solubility in the respective lipid) were dispersed in 9 ml of digestion buffer (tris maleate 50 mM, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5) containing either low (5 mM NaTDC/1.25 mM PC) or high (20 mM NaTDC/5 mM PC) concentrations of BS and PL, and experiments were initiated by the addition of 1 ml of a pancreatin extract [containing 10,000 tributyrin units (TBU) of pancreatic lipase activity] (13). Digestion experiments were conducted over 30 min, after which 2 × 4 ml aliquots of the postdigestion mixture were ultracentrifuged (334,000*g*, 30 min, 37°C, Optima XL-100K centrifuge, SW-60 rotor, Beckman, Palo Alto, CA, USA) to separate the digest into an oil phase, an aqueous phase, and a pellet phase. Blank aqueous phases were produced for equilibrium solubility measurements by repeating the digests with drug-free lipids.

### Sample Preparation

Lipidic phases obtained from the digestion experiments (oil phases and pellets) were dissolved in 5 ml of chloroform:methanol (2:1 v/v) and subsequently diluted at least 1 in 10 with acetonitrile before injection onto the HPLC column. Aqueous samples (including solubility determinations in buffer or BS/PL solutions and the aqueous phases obtained from lipid digests) were diluted at least 1 in 10 with acetonitrile before assay. Samples (~15 mg) obtained from experiments to determine drug solubility in the various formulation lipids were dissolved into 20 ml of acetonitrile and then diluted further in acetonitrile as required.

### HPLC Assays for Probe Compounds

HPLC analyses were performed on a Waters HPLC system comprising a Model 717 autosampler, Model 486 absorbance detector, and a 510 programmable pump (Waters, Milford, MA, USA). Data collection and peak integration was achieved using a Shimadzu CR6A Chromatopac chart recorder (Shimadzu Corporation, Kyoto, Japan). GRIS, DAN, DIAZ, and HF were assayed as previously described (6,14,15). The CIN assay was a modification of a previously reported assay (16) and used a Symmetry C18, 5 μm, 150 × 3.9 mm RP column (Waters) and UV detection at 253 nm. CIN eluted at 4.8 min using a mobile phase of 55% acetonitrile/45% 20 mM ammonium dihydrogen phosphate. The injection volume was 25 μl. Assay performance was validated using standard measures of linearity, precision, and reproducibility. Accuracy and reproducibility were better than ±15% across the working concentration range of the CIN assay (0.15–5.0 μg/ml). Validation data for the GRIS, DAN, DIAZ, and HF assays have previously been published (14,15).

## RESULTS

### Drug Solubility in Lipids

Drug solubility in the candidate lipids was initially determined to enable the drug–lipid solutions used in digestion experiments to be prepared at the same proportion of saturated solubility (i.e., 50%) to ensure the same thermodynamic activity. The solubility (w/w) of each drug was higher in the medium-chain triglyceride (MCT, Captex) when compared with the long-chain triglyceride (LCT, soybean oil) (Table I); however, in molar terms the solubility of each drug was similar in either MCT or LCT. For GRIS, DIAZ, and DAN, solubility in the monoglyceride/diglyceride vehicles (Capmul MCM and Maisine 35-1) was higher than in the corresponding triglyceride whereas for CIN and HF, the molar solubility was highest in the less polar triglyceride.

### Drug Distribution Patterns Obtained During *in Vitro* Lipid Digestion Experiments

The distribution of the selected drugs into the various lipid digestion phases was assessed by digesting 250 mg of a lipid solution of each drug in either LCT or MCT (drug present at 50% of equilibrium solubility in the lipid). The digests were subsequently ultracentrifuged during which they separated into a floating oil phase, a dispersed aqueous phase, and a pellet phase. The progress of lipid digestion (as re-

**Table I.** Lipid Solubilities of Griseofulvin, Diazepam, Danazol, Cinnarizine, and Halofantrine*

| Drug | Equilibrium solubility at 37°C (mg/g) | | | | Equilibrium solubility at 37°C (mmol/mol) | | | |
|---|---|---|---|---|---|---|---|---|
| | Soybean oil | Captex | Maisine | Capmul MCM | Soybean oil | Captex | Maisine | Capmul MCM |
| Griseofulvin | 0.48 ± 0.01 | 0.95 ± 0.03 | 1.86 ± 0.12 | 4.4 ± 0.1 | 1.18 ± 0.01 | 1.36 ± 0.04 | 2.85 ± 0.19 | 3.46 ± 0.08 |
| Diazepam | 18.3 ± 0.2 | 30.3 ± 0.5 | 44.0 ± 1.1 | 59.1 ± 8.8 | 56.0 ± 0.6 | 53.7 ± 0.9 | 83.4 ± 2.0 | 57.6 ± 8.6 |
| Danazol | 3.90 ± 0.05 | 8.7 ± 0.1 | 11.9 ± 0.2 | 21.6 ± 1.2 | 10.1 ± 0.14 | 12.9 ± 0.2 | 19.0 ± 0.3 | 17.8 ± 0.99 |
| Cinnarizine | 27.0 ± 0.6 | 40.7 ± 0.3 | 36.2 ± 1.1 | 36.4 ± 0.7 | 63.9 ± 1.45 | 55.7 ± 0.45 | 53.0 ± 1.6 | 27.4 ± 0.53 |
| Halofantrine | 47.3 ± 6.5 | 89.0 ± 2.7 | 49.1 ± 3.7 | 26.1 ± 2.4 | 82.4 ± 11.4 | 89.7 ± 2.8 | 53.0 ± 3.9 | 14.5 ± 1.33 |

* Mean ± SD, n = 3. Drug solubility in mmol/mol calculated using average molecular weights estimated from information provided by the manufacturer regarding the various constituents of each lipid and the proportion of each lipid component (refer to "Materials and Methods").

flected in the liberation of fatty acid) was monitored by pH stat titration and was essentially identical to the rate and extent of digestion for the same "drug-free" systems previously described (17). In these previous studies, the lipid content of the phases produced was further analyzed by high performance thin layer chromatography (HPTLC) to give absolute concentrations of FA, MG, DG, and TG in the digests with respect to time, and these data are reproduced in the current publication (Table II).

Intestinal BS/PL concentrations after oral administration of pharmaceutically relevant volumes of lipid have not been reported, and hence, digests were performed with concentrations of BS/PL spanning the likely physiological range. Digestion studies were performed using either 5 mM NaTDC/1.25 mM PC [reflecting typical fasting BS and PL concentrations (5,18)] or 20 mM NaTDC/5 mM PC [reflecting typical BS and PL concentrations postprandially or after administration of a lipid vehicle (3,18)].

The relative impact of lipid digestion on drug solubility and distribution patterns was determined after a nominal period of 30 min digestion. As previously reported, rapid digestion of MCT resulted in complete digestion of 250 mg of MCT over the 30-min period (17), and therefore drug distributed from the digested MCT into either a pellet phase or a dispersed aqueous phase. In contrast, digestion of LCT progressed more slowly, and after 30 min an oil phase remained. Drug distribution from the LCT digest was therefore into a pellet phase, a dispersed aqueous phase, or an oil/lipid phase. As the digestion of LCT is BS dependent, the digests performed under higher BS/PL concentrations were more extensive (60.6% digestion, expressed as loss of TG) compared with digests performed under the lower BS/PL concentrations (42.1% digestion) (17). Consequently, the mass of undigested lipid was lower, and the concentration of lipid digestion products solubilized in the aqueous phase was higher, for the digests conducted under the higher BS/PL concentration (Table II).

The distribution patterns of drug between the oil phase, aqueous phase, and pellet phase are shown in Fig. 1, and the concentration of drug present in the aqueous phase postdigestion is given in Table II. For the LCT digests, the drugs distributed across the physical phases present in the digests as a function of their partitioning behavior. For DAN, the percentage of the drug present in the aqueous phase (38.5% under low BS/PL, 65.3% under high BS/PL) was well correlated with the extent of digestion (42.1% under low BS/PL, 60.1% under high BS/PL) suggesting that trafficking of DAN from the lipid solution was related to the extent of digestion. In contrast, the more lipophilic compounds (HF, CIN) became more concentrated in the remaining undigested oil phase as digestion proceeded. The less lipophilic drugs (GRIS and DIAZ) readily partitioned out of the lipid phase into the dispersed aqueous phase and were less dependent of the extent of LCT digestion. In all cases, the mass of drug contained

**Table II.** Aqueous Phase Concentrations of Griseofulvin, Diazepam, Danazol, Cinnarizine, and Halofantrine After 30 Min Digestion of 250 mg of Long Chain Triglyceride or Medium Chain Triglyceride Containing Drug at 50% of Equilibrium Solubility of the Drug in the Lipid Under Low and High BS/PL Conditions*

| | Lipid concentration obtained in aqueous phase post digestion (mmol/l)† | | | Postdigestion drug concentration in aqueous phase (μg/ml) | | | | |
|---|---|---|---|---|---|---|---|---|
| | FA | MG | DG | Griseofulvin | Diazepam | Danazol | Cinnarizine | Halofantrine |
| LCT digest (5 mM BS/1.25 mM PL) | 22.6 ± 3.1 | 4.5 ± 0.4 | 1.1 ± 0.4 | 4.0 | 111.4 | 13.8 | 26.1 ± 8.8 | 72.0 ± 0.2 |
| MCT digest (5 mM BS/1.25 mM PL) | 96.1 ± 6.3 | 25.4 ± 1.1 | — | 9.2 | 271.9 | 83.1 | 279.5 ± 31.8 | 756.1 ± 45.6 |
| LCT digest (20 mM BS/5 mM PL) | 37.2 ± 2.8 | 12.0 ± 0.6 | 2.1 ± 0.2 | 5.6 | 143.2 | 23.8 | 35.9 ± 14.2 | 109.9 ± 5.7 |
| MCT digest (20 mM BS/5 mM PL) | 98.3 ± 11.7 | 16.2 ± 4.5 | — | 9.2 | 280.7 | 81.3 | 227.7 ± 10.5 | 401.8 ± 45.3 |

BS, bile salt; PL, phospholipid; FA, fatty acid; MG, monoglyceride; DG, diglyceride; LCT, long-chain triglyceride; MCT, medium-chain triglyceride.

* The lipid concentrations in the aqueous phase have been previously determined and are included for information (mean ± SD, n = 3).

† Data recalculated from Sek *et al.* (17). Extent of LCT digestion, expressed as loss of TG, was 60.6% under high (20 mM NaTDC/5 mM PC) and 42.1% under low BS/PL conditions (5 mM NaTDC/1.25 mM PC). Digestion of MCT was essentially complete.

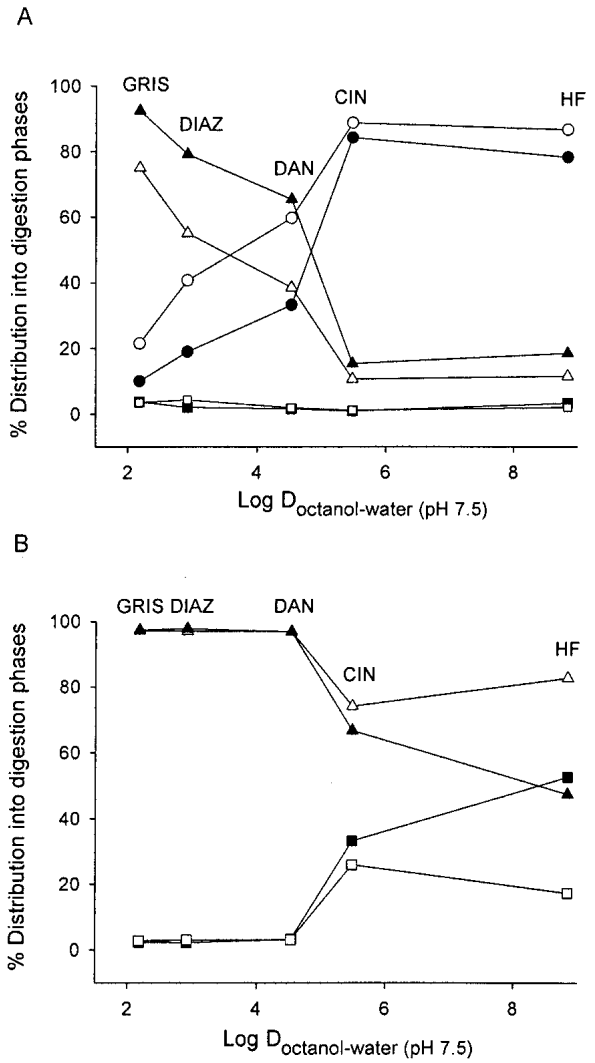


**Fig. 1.** Distribution of griseofulvin (GRIS), diazepam (DIAZ), danazol (DAN), cinnarizine (CIN), and halofantrine (HF) into a nondispersed oil phase (●, ○), dispersed aqueous phase (▼, ▽), and pellet phase (□, ■) after 30 min digestion of 250 mg of long-chain triglyceride (LCT, panel A) or medium-chain triglyceride (MCT, panel B) under high (20 mM NaTDC/5mM PC, filled symbols) and low (5 mM NaTDC/1.25 mM PC, open symbols) bile salt and phospholipid conditions. Drugs were dissolved in LCT and MCT at 50% of saturated solubility. Digestion and separation conditions were as described in "Materials and Methods." The extent of digestion of LCT, expressed as loss of TG, after 30 min was 42.1% under low and 60.6% under high BS/PL conditions. MCT digestion was essentially complete under both conditions.

in the pellet was low, and drug was present in either lipidic or aqueous solution.

In the case of the MCT digests, the low log *D* drugs were again fully solubilized in the aqueous phase of the digests. However, for the higher log *D* compounds (CIN, HF), the lack of a residual oil phase to maintain drug in lipid solution resulted in a substantial proportion of the drug being recovered in the pellet phase. Notwithstanding the increased drug recovery in the pellet, the absolute drug concentration attained in the aqueous phase was high (Table II) and significantly in excess of the aqueous phase concentrations attained in the corresponding LCT systems.

### Drug Partitioning Behavior Between Micellar Solutions and TG Lipids

To interpret the drug distribution/solubility patterns obtained during kinetic digestion experiments, the equilibrium partitioning characteristics of the five drugs between MCT or LCT and a 5 mM NaTDC/1.25 mM PC micellar solution were assessed (Table III). For comparison, the log *P*, p$K_a$, and log $D_{(\text{octanol-water})}$ at pH 7.5 are also included. As expected, micellar solubilization led to a reduction in the log $D_{(\text{MCT or LCT-micelle})}$ when compared to the log $D_{(\text{octanol-water})}$, and this effect was greatest for the more lipophilic drugs. To examine the impact of micellar solubilization on the partitioning characteristics, the difference between the log $D_{(\text{octanol-water})}$ and the log $D_{(\text{LCT or MCT-micelle})}$, ($\Delta$ log *D*) was also calculated, and a correlation between the log *D* of the drugs and the change to partitioning characteristics provided by micellar solubilization (as reflected in $\Delta$ log *D*) was evident. No large differences were seen in the partition characteristics of the probe drugs in soybean oil–micellar systems when compared with Captex–micellar systems.

### Equilibrium Drug Solubility in Digestion Buffer, Micellar Solutions, and the Aqueous Phase Obtained from Lipid Digests

For each drug, solubility increases were observed in the presence of BS/PL mixed micelles when compared with solubility in digestion buffer alone, and this was most evident for the more lipophilic compounds (Table IV). The presence of lipid digestion products further increased drug solubility. The solubility enhancement afforded by the intercalation of MCT and LCT digestion products into the endogenous (fasted) BS/PL solutions was substantial and greater than that provided by increasing concentrations of BS/PL, even when the BS/PL concentrations were raised to levels typical of the upper physiological range (20 mM NaTDC/5 mM PC). Drug solubility in systems containing high BS/PL (20 mM NaTDC/5 mM PC) was generally higher than the corresponding low BS/PL (5 mM NaTDC/1.25 mM PC) systems.

Under low BS/PL conditions (5 mM NaTDC/1.25 mM PC), a drug-specific differential in the solubility-enhancing effects of MCT and LCT digestion products was evident. For the lower log *D* drugs (GRIS, DIAZ, and DAN), the solubility enhancement provided by the presence of MCT digestion products was greater than that of the corresponding LCT systems (Table IV). For the high log *D* drugs (CIN and HF), equilibrium drug solubility in the aqueous phase obtained from blank LCT digests was higher than the corresponding MCT system whereas the concentrations attained in the aqueous phase postdigestion were markedly higher after digestion of MCT. Importantly, the aqueous phase concentrations of HF and CIN obtained after digestion of the MCT systems (Table II) were in excess of the equilibrium drug solubility in identical "drug-free" aqueous phases (Table IV). The propensity for supersaturation of these aqueous phases appeared to correlate with the lipophilicity of the drug (Fig. 2). Under high BS/PL concentrations, the differences between the equilibrium solubility values determined in aqueous phases containing the products of MCT or LCT digestion were less evident than the data obtained under low BS/PL conditions. This applied for most of the studied compounds, although for HF,

**Table III.** p$K_a$ and Octanol–Water and Triglyceride–Micellar Solution Distribution Coefficients for Griseofulvin, Diazepam, Danazol, Cinnarizine, and Halofantrine*

| Drug | p$K_a$ | Log $P$† | Log $D$‡ | Log $D_{(LCT\text{-}micelle)}$§ | Log $D_{(MCT\text{-}micelle)}$¶ | Δ Log $D$ [Log $D$ – Log $D_{(LCT\text{-}micelle)}$] | Δ Log $D$ [Log $D$ – Log $D_{(MCT\text{-}micelle)}$] |
|---|---|---|---|---|---|---|---|
| Griseofulvin | N/A | 2.18‖ | 2.18 | 1.33 ± 0.04 | 1.73 ± 0.06 | 0.85 ± 0.04 | 0.45 ± 0.06 |
| Diazepam | 3.45** | 2.92** | 2.92 | 2.11 ± 0.01 | 2.36 ± 0.01 | 0.81 ± 0.01 | 0.56 ± 0.01 |
| Danazol | N/A | 4.53†† | 4.53 | 2.62 ± 0.03 | 2.92 ± 0.03 | 1.91 ± 0.03 | 1.61 ± 0.03 |
| Cinnarizine | 7.47‡‡,§§ | 5.77‖‖ | 5.48‡‡‡ | 3.29 ± 0.05 | 3.68 ± 0.03 | 2.19 ± 0.05 | 1.80 ± 0.03 |
| Halofantrine | 5.58*** | 8.86††† | 8.85‡‡‡ | 3.30 ± 0.03 | 3.57 ± 0.02 | 5.55 ± 0.03 | 5.28 ± 0.02 |

N/A, not applicable.
*Mean ± SD, n = 3.
† Octanol–water partition coefficients.
‡ Octanol–water distribution coefficients (pH 7.5).
§ SBO-5 mM BS/1.25 mM PC distribution coefficients (pH 7.5).
¶ Captex-5 mM BS/1.25 mM PC distribution coefficients (pH 7.5).
‖ Data from Leo *et al.* (20).
** Data from Taillardat-Bertschinger *et al.* (21).
†† Data from Mithani *et al.* (22).
‡‡ Data from Peeters (23).
§§ Cinnarizine has a further p$K_a$ of 1.95 (24).
‖‖ Data from Belsner *et al.* (25).
*** Data from Khoo *et al.* (26).
††† Calculated log $P$ (ACD Labs, Toronto, Canada).
‡‡‡ Calculated using the equation published in Ref. 27. Ion partitioning was not taken into account.

solubility in the blank LCT digest was substantially higher than in the corresponding MCT system.

## DISCUSSION

The broad application of lipid-based formulations has been limited by a number of factors including the inherent complexities associated with manufacturing and regulatory submission for these dosage forms and insufficient knowledge of the appropriate physicochemical descriptors for compounds where lipid formulations may be beneficial. In addition, as it has become more commonplace to attempt the use of lipidic formulations, the lack of effective *in vitro* tests that can be used to discriminate between lipidic formulations and excipients presents a further hindrance to the development process. The current study was conducted to address these issues by examining the impact of digestion of two common formulation lipids, MCT and LCT, on the solubilization of a range of poorly water-soluble drugs using an *in vitro* model of lipid digestion.

As drug solubility in a pharmaceutically appropriate volume of lipid is a key formulation consideration, the solubility of a range of poorly water-soluble drugs in simple glycerides of both long-chain ($C_{18}$) and medium-chain ($C_{8-10}$) fatty acids was determined. The solubility of each selected drug was higher in MCT compared with LCT (on a mg/g basis), although, in molar terms the solubilities of each drug in the MCT or LCT were essentially the same (Table I). This observation is consistent with the previously identified relationship between the molar concentration of the ester functional group of lipidic glycerides and their solubilizing capacity (19) indicating that the basis for solubility was largely independent of fatty acid chain length. The more polar MG/DG lipids provided a solubility benefit for the less lipophilic drugs (GRIS, DAN, and to a lesser extent DIAZ); however, for CIN and HF, drug solubility was substantially higher in the more nonpolar triglycerides (Fig. 3). Whereas drug solubility depends on additional factors such as the lattice energy of solid solute and the polarizability and hydrogen bond donor and acceptor capacity of the solute and solvent, the current data suggest that solubility of the more lipophilic drugs will likely be higher in nonpolar solvents such as triglycerides

**Table IV.** Equilibrium Solubilities at 37°C of Model Compounds in Digestion Buffer pH 7.5, in Digestion Buffer Containing Low and High Concentrations of BS/PL, and in Blank Aqueous Phases (AP) Obtained from Drug-Free LCT or MCT Digests*

| | Equilibrium solubility at 37°C (μg/ml) | | | | |
|---|---|---|---|---|---|
| | Griseofulvin | Diazepam | Danazol | Cinnarizine | Halofantrine |
| Digestion buffer pH 7.5 | 14.6 ± 0.6 | 59.7 ± 2.1 | 0.8 ± 0.1 | <0.15 | <0.1 |
| 5 mM NaTDC/1.25 mM PC micelles | 18.4 ± 0.4 | 115.6 ± 1.6 | 8.7 ± 0.1 | 8.3 ± 0.1 | 12.6 ± 0.1 |
| Aqueous phase from LCT digest (5 mM NaTDC/1.25 mM PC) | 53.6 ± 1.8 | 513.1 ± 16.4 | 61.4 ± 0.7 | 213.9 ± 11.3 | 354.6 ± 4.3 |
| Aqueous phase from MCT digest (5 mM NaTDC/1.25 mM PC) | 93.8 ± 2.1 | 774.4 ± 31.4 | 102.8 ± 1.6 | 188.6 ± 3.7 | 275.1 ± 16.2 |
| 20 mM NaTDC/5 mM PC micelles | 38.8 ± 0.1 | 318 ± 10 | 41.1 ± 0.2 | 30.2 ± 0.1 | 60.7 ± 0.02 |
| Aqueous phase from LCT digest (20 mM NaTDC/5 mM PC) | 115.8 ± 1.0 | 1037.5 ± 18.6 | 142.6 ± 1.1 | 187.7 ± 6.0 | 1072 ± 67.0 |
| Aqueous phase from MCT digest (20 mM NaTDC/5 mM PC) | 141.5 ± 0.3 | 1007.9 ± 27.2 | 134.1 ± 1.8 | 230.4 ± 10.8 | 317.9 ± 6.9 |

BS, bile salt; PL, phospholipid; LCT, long-chain triglycerides; MCT, medium-chain triglycerides; PC, phosphatidylcholine.
* Mean ± SD, n = 3.

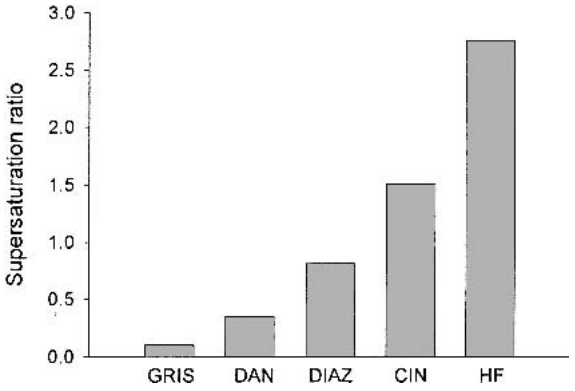


**Fig. 2.** Comparison of the drug concentration measured in the aqueous phase obtained after ultracentrifugation of medium-chain triglyceride (MCT) digests (from Table II) with equilibrium drug solubility measured in an identical "drug-free" aqueous phase obtained by digestion of blank MCT (from Table IV). The supersaturation ratio (digest aqueous phase concentration/equilibrium solubility) was obtained for griseofulvin (GRIS), diazepam (DIAZ), danazol (DAN), cinnarizine (CIN), and halofantrine (HF) after 30 min digestion under low (5 mM NaTDC/1.25 mM PC) bile salt and phospholipid conditions.

whereas for the less lipophilic drugs, enhanced solubility may occur using partial glycerides (MG/DG systems) of long- and medium-chain fatty acids.

### Drug Distribution Patterns After Digestion of LCT Formulations

The very low lipid solubility of GRIS limited to approximately 60 μg the mass of drug that could be dissolved (at 50% of equilibrium solubility) in the 250 mg of lipid introduced into the digestion vessel. Although the solubility enhancement for GRIS provided by solubilization in BS/PL solutions or the aqueous phase produced on LCT digestion was only moderate (~4–8-fold in equilibrium solubility studies) (Table IV), this was sufficient to solubilize the entire mass of GRIS introduced into the system. In combination with its relatively low distribution coefficient, this resulted in rapid partitioning of GRIS out of the digesting LCT into the aqueous phase (Fig. 1). At higher BS/PL levels, this trend was exacerbated and greater than 90% of the GRIS was solubilized in the aqueous phase after 30 min digestion. These data suggest that for poorly lipid soluble, hydrophobic drugs such as GRIS, the solubilization capacity of the dispersed phase produced on lipid digestion is likely to exceed the mass of drug that can be included in a simple lipid solution formulation, and, therefore, that formulation and delivery of these drugs (using lipid solution formulations) is limited by dose/lipid solubility considerations rather than luminal solubility.

Although the distribution coefficient for DAN was higher than GRIS or DIAZ, the absolute lipid solubility of DAN was still relatively low. In combination with the large increase in solubilization capacity provided by the products of lipid digestion (up to 150-fold compared with aqueous solubility) (Table IV), a substantial proportion of the small mass of DAN present in the LCT solution was therefore solubilized in the aqueous phase of the digest after 30 min digestion, although in contrast to GRIS, the higher log *D* of DAN led to a larger proportion of drug remaining within the undigested lipid phase.

The apparent aqueous solubility of DIAZ was substantially enhanced by the presence of lipid digestion products (Table IV), and although more DIAZ could be dissolved in 250 mg of LCT when compared with DAN, the solubility of DIAZ in the aqueous phase of the digest was still sufficient to solubilize all the drug present. The proportion of DIAZ associated with the oil phase, the pellet phase, and the aqueous phase was therefore dictated by its partitioning behavior, and the distribution profile was midway between that observed for GRIS and DAN.

For CIN and HF, the dominating factors of the distribution profile were the high log *D* and lipid solubilities. For these drugs, aqueous solubility was low, lipid solubility was high, and the enhancement in solubility provided by BS/PL solutions, and in particular the products of lipid digestion, was extremely large (>1000-fold) (Table IV). The mass of drug that could be introduced in solution in 250 mg of LCT was also higher than that of the other compounds, and exceeded, at least under low BS/PL conditions, the solubilization capacity of the aqueous phase containing the lipid digestion products. The extremely high partition coefficients of CIN and HF also resulted in drug accumulation in the oil phase as digestion progressed. This limited drug trafficking through to the aqueous phase, but also prevented precipitation into the pellet. The miscibility of MG/DG and TG lipids precluded assessment of the likely partitioning behavior of drug between undigested triglyceride and the DG/MG digestion products likely to be present on the surface of a digesting lipid droplet. However, the higher solubilities of HF and CIN in triglyceride when compared with DG/MG suggest that partitioning from TG environments to MG/DG environments would not be favored.

*In vivo* digestion of MCT and LCT is typically complete as a result of the improved sink conditions provided by lipid absorption from the various colloidal intestinal phases. The current studies therefore represent a "snapshot" of the environment likely to be present mid-way through the digestion process. Nonetheless, these intermediate scenarios allow discrimination of the two lipids on the basis of their digestibility and highlight the propensity for both drug solubilization and

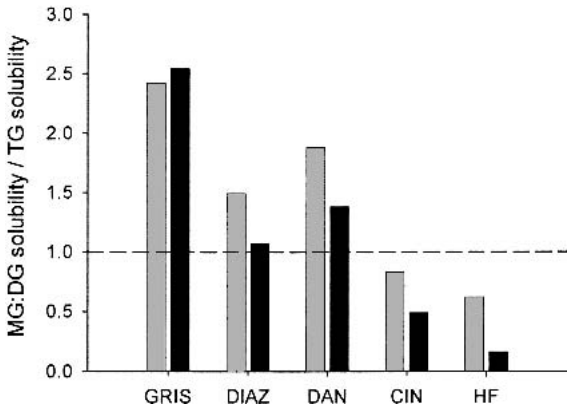


**Fig. 3.** Ratio of mol fraction drug solubility in monoglyceride/diglyceride (Capmul and Maisine) and triglyceride (MCT, LCT, respectively). Shaded bars depict data for long-chain lipid systems (Maisine/LCT); black bars depict data from medium-chain systems (Capmul/MCT).

precipitation, processes that in addition to the kinetics of drug transfer across the aqueous phase to the absorptive membrane are likely to dictate the absorptive aptitude of a coadministered drug. For highly lipophilic drugs (such as CIN or HF) where digestion of the LCT vehicle leads to drug accumulation within the lipid droplet, complete digestion *in vivo* will eventually "force" drug to be either solubilized in the dispersed aqueous phase or precipitate. In this regard, data obtained using the current model, but under conditions where LCT digestion was complete (50 mg lipid, 20 mM BS, 5 mM PL, 60 min digestion time), suggest that drug initially concentrates in the undigested lipid phase, but that on complete digestion of the formulation, drug precipitates (as opposed to becoming solubilized in the aqueous phase), even though the concentration of drug in the aqueous phase may be below the equilibrium solubility in that phase (data not shown).

Because the concentration of the drug across the different postdigestion phases appeared to be driven by partitioning behavior, the distribution coefficient [log $D_{(\text{LCT-micelle})}$] of each drug between TG (representative of the undigested formulation) and 5 mM BS/1.25 mM PC (representative of the aqueous phase) was determined (Table III). The difference between the log $D_{(\text{LCT-micelle})}$ and log $D_{(\text{octanol-water})}$ values was relatively small for the less lipophilic drugs, but increased proportionally for the more lipophilic drugs where the degree of solubility enhancement provided by the micellar solution was greatest. The net result of this trend was that the range of effective drug distribution coefficients in the systems under study was reduced when compared with log $D_{(\text{octanol-water})}$ values. Indeed, when examined using this more representative partitioning system, the apparent distribution coefficients for CIN and HF were remarkably similar, which is consistent with the similar distribution data obtained under digesting conditions. The improved ability of log $D_{(\text{LCT-micelle})}$ to predict distribution behavior in the *in vitro* digestion system is evident in Fig. 4, where the distribution profiles have been replotted against log $D_{(\text{LCT-micelle})}$. The implication of these findings is that the "effective" partitioning behavior of high log *D* drugs, such as HF, in the intestinal environment likely to be present after administration of a lipidic formulation may be less extreme than initially suspected as the solubilizing capacity of the dispersed lipid phase is greatest for the most highly lipophilic drugs.

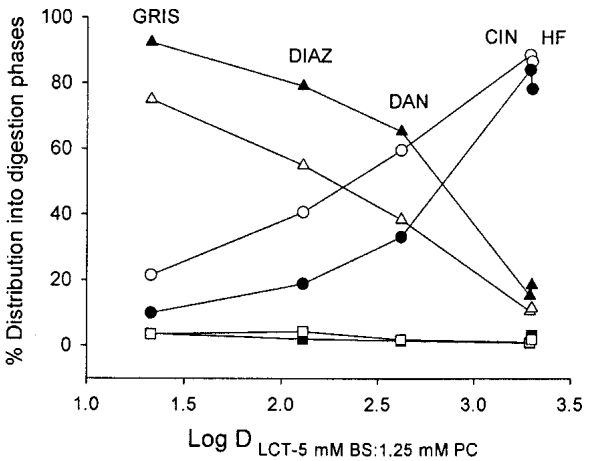


**Fig. 4.** Distribution data presented in Fig. 1A, replotted against drug distribution coefficients measured between soybean oil (SBO) and low concentration (5 mM BS/1.25 mM PL) bile salt and phospholipid micelles. All other details as per legend to Fig. 1.

The results of the current data are in qualitative agreement with the recent data of Zangenberg et al. (12) who compared the solubilization behavior of danazol and a more lipophilic drug (probucol, cLog *P* ~10) in a model of lipid digestion. Consistent with our findings, Zangenberg reported that the ability of LCT digestion products to increase solubility was most apparent for the higher log *P* drug probucol and that the limited appearance of drug in the aqueous phase under conditions of limited TG digestion was consistent with preferential partitioning of drug into the undigested oil phase.

### Drug Distribution Patterns After Digestion of MCT Formulations

In contrast to the LCT results, the rapid and complete digestion of MCT over 30 min resulted in the production of two phases: a dispersed aqueous phase and a pellet phase. Consistent with the LCT data, however, the distribution pattern of the less lipophilic drugs (GRIS, DIAZ, and DAN) was also dose-limited in the MCT systems; that is, the solubilization capacity in the aqueous phase was greater than the mass of drug that could be dissolved in the MCT (Table II and Fig. 1B). For the highly lipophilic CIN and HF, the mass of drug dissolved in 250 mg of MCT lipid was again greater than the maximum mass of drug that could be solubilized in the aqueous phase produced on lipid digestion (as predicted using equilibrium solubility measurements, Table IV). In the absence of a remaining (i.e., yet to be digested) oil phase that could retain drug, precipitation of drug from the fully digested MCT formulation was anticipated resulting in substantial quantities of drug in the pellet and aqueous phase concentrations approaching the equilibrium solubility. Surprisingly, the concentrations of HF and CIN present in the aqueous phase after 30 min digestion (Table II, Fig. 2) were supersaturated and substantially higher than the corresponding equilibrium solubilities under low BS/PL concentrations (Table IV), and the proportion of drug present in the pellet phase was lower than expected. The time dependence of this process was also studied, and when digestion of HF in MCT was interrupted at 5 min (extent of lipolysis around 30%), approximately 90% of the dose was still present in the oil phase (data not shown), indicating that distribution of the highly lipophilic compounds in the MCT digests was, at least initially, driven by partitioning behavior that was similar to that observed with the LCT systems.

For HF, both the absolute concentration attained in the aqueous phase postdigestion (Table II) and the degree of supersaturation were higher in digests conducted under low BS/PL conditions when compared to higher BS/PL conditions. A similar trend was seen for CIN although the differences were of borderline significance ($p < 0.055$). In all other cases, the presence of higher BS and PL levels had little impact on drug solubilization in the MCT digests, suggesting that the dominant factor in the enhancement of drug solubilization was the presence of the lipid digestion products (the concentration of which was the same in both systems) and not the enhanced BS/PL levels. This further suggests that appropriately digestible lipid formulations may facilitate substantially enhanced drug solubilization, even in the absence of raised BS/PL levels.

The nature of the physical phases formed on digestion of MCT has recently been examined in this laboratory, and the findings are consistent with the unusual solubilization trends observed under low and high BS conditions for the highly lipophilic compounds. These studies showed that a lipid-rich (and likely vesicular) phase with relatively high solubilizing power is formed on digestion of 250 mg MCT under low BS/PL conditions (6,17). At higher BS concentrations, a larger proportion of the lipid is contained in mixed micelles, and these mixed micellar systems have a lower solubilizing capacity for highly lipophilic drugs when compared with the lipid-rich vesicular phase (6).

In an attempt to probe the behavior of the supersaturated, high solubilization capacity phase formed on digestion of MCT more thoroughly, the stability of the system to dilution with buffer, or bile salt solutions, was examined because this situation would most likely occur *in vivo*. In all cases, drug precipitation did not occur even under relatively large dilution factors (20-fold) over a 30-min period. In contrast, during 24 h all systems exhibited substantial precipitation (data not shown). It appears therefore that rapid digestion of MCT leads to production of a lipid-rich (most likely vesicular) phase, and that this may be supersaturated with drug. Furthermore, this phase is relatively stable to dilution by either buffer or bile salt solution and may therefore persist *in vivo*.

In summary, as lipidic formulations are digested, dispersed, and intercalated into endogenous BS/PL structures, the distribution and trafficking properties of coadministered, poorly water-soluble drugs are dictated in large part by their relative affinity for each individual phase as reflected by partitioning indicators (Log *D*, *P*, and so forth). Under certain circumstances, however, drug concentrations in various phases can be attained that are substantially in excess of equilibrium solubility, demonstrating the importance of viewing the drug solubilization process as a kinetic event occurring in tandem with digestion and dispersion of the coadministered lipid and drug transfer across the absorptive membrane. For the range of poorly water-soluble drugs described, it is apparent that drugs with relatively low lipophilicity will always prove problematic as solubility in lipid solution is sufficiently low as to preclude administration of a reasonable dose for all but the most potent compounds. For more lipophilic drugs, a larger dose can be incorporated into lipid solutions, and under these circumstances the solubilization capacity of the phases formed on lipid digestion and the rate of lipid digestion will likely prove critical. Under the conditions described here, rapid and complete digestion of MCT leads to transfer of drug to a highly solubilizing aqueous environment that has the capacity to solubilize substantial quantities of drug (and which in some circumstances can transiently support supersaturated drug concentrations). In contrast, the digestion of a similar quantity of LCT is slower, and the concentration of drug attained in the aqueous phase may be limited by drug retention in the undigested oil phase (although the improved absorption sink present *in vivo* will help to transfer drug from the formulation). However, since the likelihood of drug precipitation is dictated by the solubilization capacity of the dispersed aqueous phase and the remaining lipidic phase and both the rate of delivery to these solubilisation sinks and the rate of removal from these phases (i.e., absorption), it is apparent that sequestration of drug in an undispersed oil phase and relatively slow delivery to the solubilized aqueous phase may help to prevent precipitation.

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