Mechanisms of drug solubilization by polar lipids in biorelevant media

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ABSTRACT

Despite the widespread use of lipid excipients in both academic research and oral formulation development, rational selection guidelines are still missing. In the current study, we aimed to establish a link between the molecular structure of commonly used polar lipids and drug solubilization in biorelevant media. We studied the effect of 26 polar lipids of the fatty acid, phospholipid or monoglyceride type on the solubilization of fenofibrate in a two-stage *in vitro* GI tract model. The main trends were checked also with progesterone and danazol.

Based on their fenofibrate solubilization efficiency, the polar lipids can be grouped in 3 main classes. Class 1 substances (n = 5) provide biggest enhancement of drug solubilization (>10-fold) and are composed only by unsaturated compounds. Class 2 materials (n = 10) have an intermediate effect (3-10 fold increase) and are composed primarily (80 %) of saturated compounds. Class 3 materials (n = 11) have very low or no effect on drug solubilization and are entirely composed of saturated compounds.

The observed behaviour of the polar lipids was rationalized by using two classical physicochemical parameters: the acyl chain phase transition temperature (T_m) and the critical micellar concentration (CMC). Hence, the superior performance of class 1 polar lipids was explained by the double bonds in their acyl chains, which: (1) significantly decrease T_m, allowing these C18 lipids to form colloidal aggregates and (2) prevent tight packing of the molecules in the aggregates, resulting in bigger volume available for drug solubilization. Long-chain (C18) saturated polar lipids had no significant effect on drug solubilization because their T_m was much higher than the temperature of the experiment (T = 37 °C) and, therefore, their association in colloidal aggregates was limited. On the other end of the spectrum, the short chain octanoic acid manifested a high CMC (50 mM), which had to be exceeded in order to enhance drug solubilization. When these two parameters were satisfied (C > CMC, $T_m < T_{exp}$), the increase of the polar lipid chain length increased the drug solubilization capacity (similarly to classical surfactants), due to the decreased CMC and bigger volume available for solubilization.

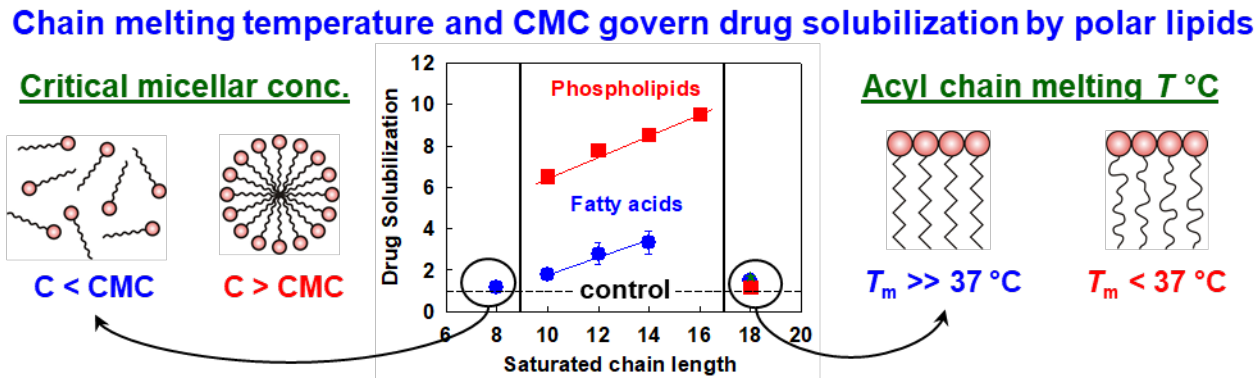
The hydrophilic head group also has a dramatic impact on the drug solubilization enhancement, with polar lipids performance decreasing in the order: choline phospholipids > monoglycerides > fatty acids.

As both the acyl chain length and the head group type are structural features of the polar lipids, *and not* of the solubilized drugs, the impact of T_m and CMC on solubilization by polar lipids should hold true for a wide variety of hydrophobic molecules. The obtained mechanistic insights can guide rational drug formulation development and thus support modern drug discovery pipelines.

Keywords (5-6 significant keywords (separated by semicolon))

oral drug delivery; lipid-based formulations; drug solubilization; poorly water-soluble drugs

Graphical abstract



1. Introduction

Modern drug discovery pipelines prioritize lipophilic targets,[1] resulting in new drug candidates with poor solubility in water.[2] The slow and incomplete dissolution of such drugs in the human gastro-intestinal (GI) fluids is one of the factors that limits their oral absorption and bioavailability, hence challenging the development of new oral drug products.[3–5] One of the approaches to improve the oral bioavailability of poorly water-soluble drugs, which has recently reappeared on the drug delivery landscape, are the lipid-based formulations (LBF).[6–8]

LBF have been used to develop at least 36 FDA-approved products[9] and are believed to enhance oral bioavailability by two main mechanisms: (1) increasing drug absorption by improving GI fluids solubilization capacity and sustaining drug supersaturation[10,11] and (2) partially circumventing first-pass metabolism via the lymphatic absorption route.[6,12] The excipient families most frequently used in LBF are glycerides (mono-, di-, tri- and their mixtures), phospholipids (PL), fatty acids (FA) and cosolvents (alcohols, PEG etc.).[13] The choice of excipients and their ratios determines the dispersibility of the formulation in aqueous media, its susceptibility to digestion in the GI tract and the drug loading capacity. The LBF classification systems, which provide a link between formulation composition, dispersibility and drug solvent capacity, were introduced to rationalize the application of the concept in drug development.[14–16]

The dispersion of an LBF in the intestinal fluids is usually accompanied with a loss of the solubilization capacity of the carrier formulation (due to dilution or/and digestion), leading to drug precipitation, the extent of which depends on the LBF type and composition.[17–19] The precipitation process is described by its kinetics, which defines the supersaturation window, and by the equilibrium solubility reached, which characterizes the extent of precipitation.[20–22] Both the kinetic and equilibrium aspects of precipitation are strongly affected by the drug solubilization capacity of the intestinal fluids, which is in turn influenced by the LBF composition. Hence, the type and concentration of excipients used in an LBF determine the drug supersaturation and precipitation behaviour in the GI tract.

The behaviour of LBF upon dispersion or/and digestion in GI tract models has been extensively studied, by using *in vitro* digestion (enzymes present) or dispersion models (no enzymes). These *in vitro* models attempt to mimic the conditions in the GI tract by using one or several components encountered in the intestine (e.g. bile salts, phospholipids, enzymes) with widely varying levels of complexity.[23–25] The most used digestion models are static, where the ratios of the components and the pH of the medium are constant,[26] as they are more accessible and easier to operate compared to dynamic, active feedback models.[27] Although several standardized digestion models have been defined,[24,26] each is characterized by its advantages and disadvantages, and a multitude of custom digestion models are still being used.[28–30]

In vitro studies have demonstrated that FA, PL and glycerides generally increase drug solubilization in simulated intestinal media, with the extent of the effect being determined by the lipophilicity (Log P/D) of the drug.[6,13,31–35] However, very few studies provide a link between the structure of the excipients used and the measured drug solubilization, which hinders mechanistic

understanding of drug solubilization and precipitation behaviour in the intestinal environment. Kossena et al. showed that the solubility of hydrocortisone increases significantly with increasing the concentration of C8:0, C12:0 or C18:1 FAs in the simulated intestinal fluids.[20] It was also found that the solubilization capacity of C18:1 was much greater than that of the short-chain FAs, which was attributed to the different type of solubilizing structures formed. In another study, phosphatidylcholine was shown to have bigger effect than monoolein on the solubilization of gemfibrozil in a 10 mM sodium glycocholate solution.[36] On the other hand, two studies using a design-of-experiments approach showed that sodium oleate increases FEN solubility in biorelevant media more strongly than soy phosphatidylcholine.[37,38] As can be seen, although significant data on LBF formulation behaviour has been accumulated, there is still no clear link between excipient structure (chain length, degree of unsaturation, type of hydrophilic moiety) and drug solubilization in biorelevant media.

Therefore, we aimed to determine the relationship between the structure of several polar lipids and drug solubilization in biorelevant media. The polar lipids selected for the study include FA, PL and monoglycerides (MG): compounds that are commonly used in LBF and that are also lipid digestion products (except for the phospholipids). We investigated the effect of the length, degree of unsaturation and hydroxylation of the hydrophobic chain, as well as the effect of the hydrophilic head group type. The model compound selected for the study was fenofibrate (FEN), as an example of a drug with physicochemical properties frequently encountered in LBF development.[39] Experiments with progesterone (PG) and danazol (DAN) were also performed, to verify the main trends observed in the study.

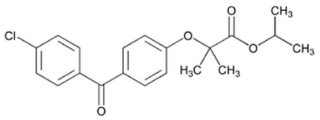
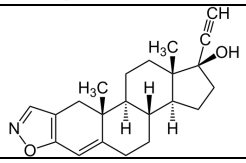
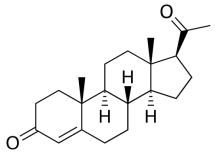
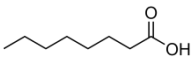
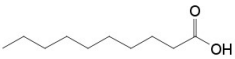
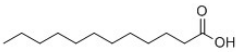
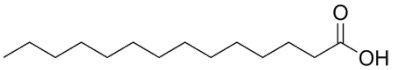
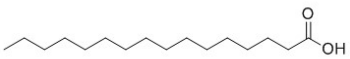
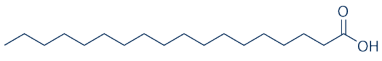
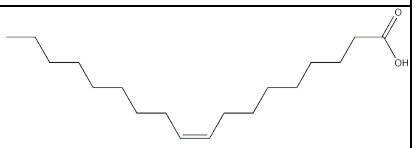
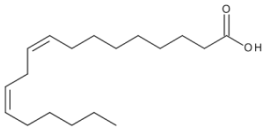
2. Materials and methods

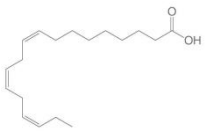
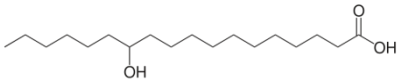
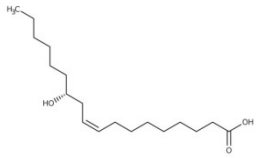
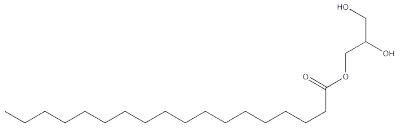
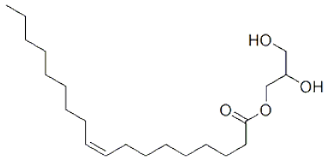
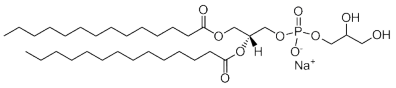
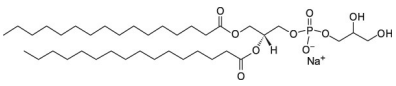
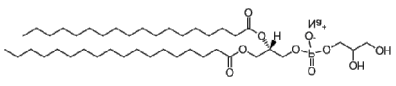
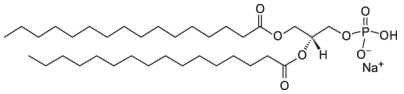
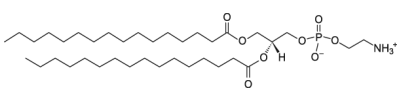
2.1. Materials.

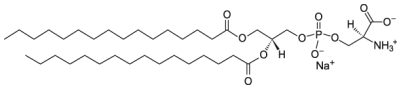
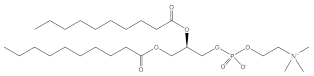
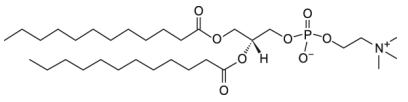
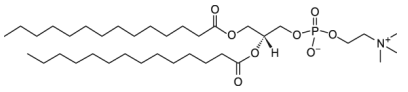
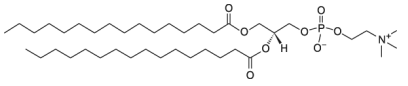
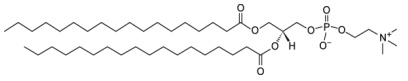
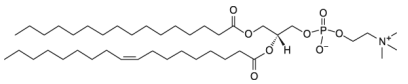
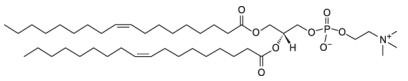
The relationship between FEN solubilization and polar lipid molecular structure was investigated by using a total of 26 lipid excipients: 13 PL, 11 FA and 2 MG. Two main groups of FA were studied: saturated FA with hydrophobic chain lengths between C8 and C18, and unsaturated FA with C18 chain and 1, 2 or 3 double bonds. Two hydroxylated FA were also used: hydroxystearic acid and ricinoleic acid. In respect to MG, we studied monoolein and monostearin. A set of 13 PL with chain lengths from C10 to C18 and different hydrophilic head groups were investigated. The abbreviations and the properties of the drugs and polar lipids used are summarized in Table 1. Methanol (99 %, HPLC grade, Honeywell) was used for HPLC analysis.

The materials used for the biorelevant medium were as follows: porcine bile extract (Sigma), which contains 50 wt % bile acids (average molecular mass of 442 g/mol)[40], 6 wt % phosphatidylcholine, less than 0.06 wt % Ca^{2+} 1.2 wt % cholesterol, and 6.7 wt % FA; hydrochloric acid (36%, Sigma); NaCl (99 %, Sigma); KCl (99 %, Valerus); $CaCl_2$ (99 %, Merck) and $NaHCO_3$ (99 %, Valerus).

Table 1. Properties of the drugs and excipients studied.

Name	Acronym used in text	Supplier, purity	Molecular mass, g/mol	Structure
Fenofibrate	FEN	TCI, 98 %	706	
Danazol	DAN	Sigma, 98 %	338	
Progesterone	PG	TCI, 98 %	315	
Fatty acids				
Caprylic acid	C8:0	Sigma, 99 %	144	
Capric acid	C10:0	Alfa aesar, 99 %	172	
Lauric acid	C12:0	Acros, 99 %	200	
Myristic acid	C14:0	Fluka, 98%	228	
Palmitic acid	C16:0	Sigma, 99%	256	
Stearic acid	C18:0	Across, 97 %	284	
Oleic acid	C18:1	TCI, 85 %	282	
Linoleic acid	C18:2	TCI, 91 %	280	

Linolenic acid	C18:3	TCI, 77 %	278	
Hydroxystearic acid	-	TCI, 80 %	300	
Ricinoleic acid	-	TCI, 80 %	298	
Monoglycerides				
Monostearin	-	TCI, 60 %	358	
Monolein	-	Danisco, 90 %	356	
Phospholipids				
Sodium dimyristoyl phosphatidyl glycerol	DMPG	NOF, 99 %	698	
Sodium dipalmitoyl phosphatidyl glycerol	DPPG	NOF, 99 %	745	
Sodium distearoyl phosphatidyl glycerol	DSPG	NOF, 99 %	801	
Sodium dipalmitoyl phosphatidic acid	DPPA	NOF, 99 %	670	
Dipalmitoyl phosphatidyl ethanolamine	DPPE	NOF, 99 %	691	

Dipalmitoyl phosphatidyl serine	DPPS	NOF, 99 %	757	
Didecyl phosphatidyl choline	DDPC	NOF, 99 %	565	
Dilauroyl phosphatidyl choline	DLPC	Avanti, 99 %	621	
Dimiristoyl phosphatidyl choline	DMPC	Avanti, 99 %	677	
Dipalmitoyl phosphatidyl choline	DPPC	NOF, 99 %	733	
Distearoyl phosphatidyl choline	DSPC	Avanti, 99 %	789	
Palmitoleyl phosphatidyl choline	POPC	Sigma, 65 %	759	
Dioleyl phosphatidyl choline	DOPC	Avanti, 99 %	785	

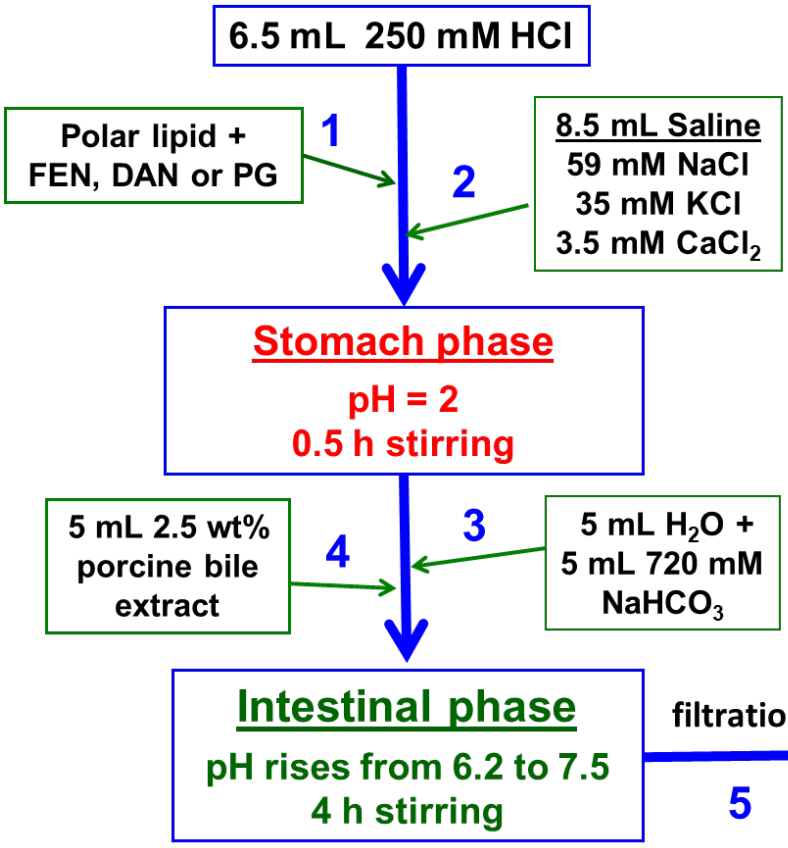
2.2. Drug solubilization at biorelevant conditions.

Drug solubilization was determined via an *in vitro* GI tract model, which was previously used by our group to obtain useful mechanistic information about the impact of food components on lipid absorption.[40–42] The concentrations of electrolytes and bile salts used are similar to the ones in the recently published INFOGEST *in vitro* digestion model.[26] Hence, the concentration of bile salts used (10 mM) is representative for fed state conditions (the median and mean values in post-prandial human intestinal fluids are 8 and 12 mM bile salts, respectively).[43]

Briefly, the protocol consists in two stages that simulate stomach and small intestinal conditions. One of the characteristic features of the model is that a physiological concentration of bicarbonate is used to gradually increase the pH in the intestinal phase from pH ≈ 6 to 7.5, mimicking the *in vivo* situation in the human small intestine.[44] Calcium ions are also present, which have been shown to be critical when assessing the solubilization of hydrophobic drugs and cholesterol by polar lipids, such as FA.[41,45]

The stomach phase was prepared by mixing 8.5 mL saline solution (59 mM NaCl, 35 mM KCl, 3.5 mM $CaCl_2$) with 6.5 mL 0.25 M HCl in a glass bottle with pre-weighted drug (45 mg

FEN, 45 mg PG or 30 mg DAN) and polar lipid. The amount of polar lipid weighed corresponded to a concentration of 10 mM polar lipid at the intestinal stage (for a volume of 30 mL). Then, the sample was stirred for 30 minutes. The shift from stomach to intestinal conditions was initiated by the sequential addition of 5 mL 0.72 M $NaHCO_3$, 5 mL bile extract (1.25 wt% in water, pre-dissolved for 30 min at T = 37 °C) and 5 mL of water (without any stirring), to obtain a final volume of 30 mL. The bottle was covered with a home-made glass cover and Teflon tape, on top of which the bottle cap was tightened. Then, the samples were stirred for 2 hours. Afterwards, the bottles were opened (to facilitate the release of CO_2 and the increase of pH) and the samples were stirred for 30 min. pH ≈ 7 was reached at this stage. Then, the bottles were closed again and stirred for 1.5 h. At the end of the experiment the pH of all the samples was measured (average pH of 7.5, range of 7.2 to 7.6). The total duration of the assay was 4.5 h and the temperature was kept constant at T = 37 °C, by a water bath. Exception was the proof-of-principle experiment with DPPC, which was performed at T = 30 °C. Finally, the suspensions were filtered via 200 nm NY syringe filter to remove any undissolved drug particles. If necessary, the filtrates were diluted in methanol and analyzed by HPLC-UV. The experimental protocol and the final concentrations of all components are schematically illustrated in Figure 1.



Component	Concentration, mM
Bile salts	10
Fatty acids (from bile)	1.27
Cholesterol (from bile)	0.4
Lecithin (from bile)	0.67
HCO_3^-	66
Na^+	137
Cl^-	83
K^+	10
Ca^{2+}	1

Figure 1. Schematical representation of the used two-step *in vitro* upper GI tract model.

2.3. HPLC analysis.

HPLC analysis was carried out on a Shimadzu apparatus, equipped with two high-pressure mixing binary gradient pumps (LC-20AD), autosampler (SIL-10ADvp), four-line membrane

degasser (DGU-14A), wide temperature range column oven (CTO-10ASvp) and a dual-wave length UV-VIS detector (SPD-10Avp). We used Waters X-bridge C18 column (150 mm x 4.6 mm, 3.5 µm particle size with column guard VanGard (Waters), 3.9 mm x 5mm - C18, 3.5 µm). The eluent flow rate was 1 mL/min and the injection volume of the sample was 20 µL. Column temperature was set at 40 °C. The concentration of solubilized drug was determined by using a standard curve (R^2 = 0.999), which was prepared by dissolving a known amount of drug in methanol. The methods used for the 3 drugs studied are summarized in Table 2.

Table 2. Conditions for HPLC-UV analysis of the drugs studied.

Drug	Methanol, %	Water, %	UV detection, nm	t_R, min
Fenofibrate	75	25	286	10.1
Danazol	75	25	286	5.3
Progesterone	70	30	254	7.1

3. Experimental results

The results for the impact of the structure of FA on drug solubilization are presented first (section 3.1), followed by the results for the effect of MG (section 3.2) and PL (section 3.3). All experiments were performed in a two-stage *in vitro* upper GI tract model, which includes 10 mM bile salts, 1 mM Ca^{2+}, bicarbonate buffer and pH gradually increasing from 6 to 7.5 in the intestinal phase (see section 2.2 for a more detailed description).

3.1. Effect of fatty acids on drug solubilization.

The impact of 10 mM FA on the solubilization of FEN in simulated intestinal fluids (*S*) is presented in Figure 2A. The concentration of solubilized FEN passes through a maximum as a function of the saturated FA chain length at C14:0 (myristic acid, *S* = 22 µg/mL), whereas the solubilizaiton by the shortest (C8:0) and longest (C18:0) FA studied was close to the control (solubilization of FEN in absence of any added FA, *S* = 6.5 ±0.5 µg/mL).

The effect of chain saturation and hydroxylation was studied for a series of C18 FA homologues. The effect of the number of double bonds on FEN solubilization was non-linear (Figure 2B): the addition of one double bond increased FEN solubilization strongly, from *S* = 8 µg/mL for stearic acid (C18:0) to *S* = 49 µg/mL for oleic acid (C18:1). Further increase of unsaturation to 2 double bonds (linoleic acid) increased only slightly the solubilized FEN (*S* = 59 µg/mL), whereas a jump in FEN solubilization (*S* = 90 µg/mL) was observed when a FA with 3 double bonds was used (linolenic acid).

The effect of the naturally abundant hydroxy-stearic acid and hydroxy-oleic acid (ricinoleic acid) was also assessed, see the blue squares in Figure 2B. While the hydroxylation of

stearic acid increased only slightly FEN solubilization, a significant increase was observed when oleic acid was hydroxylated: *S* increased from 49 µg/mL for C18:1 to 71 µg/mL for C18:1-OH.

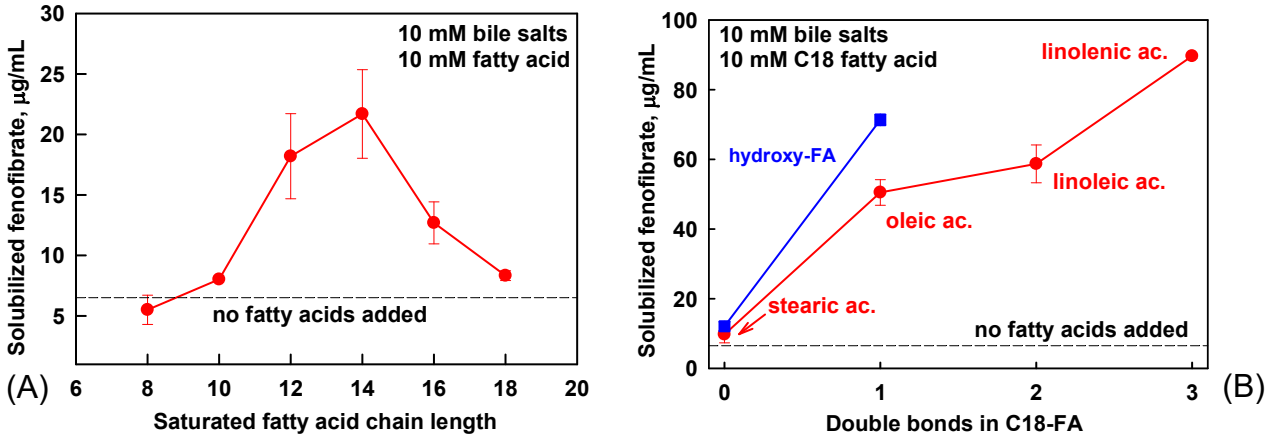


Figure 2. Solubilized fenofibrate as a function of (A) the saturated FA chain length or (B) the number of double bonds in C18 (red circles) or C18-OH (blue squares) FAs, $n = 2$, the error bars can be smaller than the symbols.

The obtained results demonstrated 2 interesting trends: (1) a chain length dependence that passes through a maximum and (2) a striking difference in the solubilization of FEN by saturated and unsaturated FA (note the different Y-scales in Figures 2A and 2B). To further investigate these phenomena, we studied the concentration-dependent solubilization of 3 hydrophobic drugs (FEN, DAN and PG) by selected saturated (C8, C10 and C18) and unsaturated FA (C18:1), see Figure 3. For all studied drugs, it was observed that in the range of FA concentrations from 0 to 20 mM: (1) the FA with the shortest and longest chain studied (C8:0 and C18:0) have small, concentration-independent effect on drug solubilization; (2) oleic acid increases very strongly drug solubilization and (3) the C10:0 FA increases slightly, in a concentration-dependent manner, drug solubilization.

While the lack of effect of C18:0 FA on drug solubilization could be explained by its high Krafft temperature (this is addressed in detail in the discussion section), that is not expected to be the case for the short chain C8:0 FA, which similarly did not show any effect in the range 0-20 mM. In this case, it was found that the CMC of C8:0 FA is very high (50 mM, see Figure S1 in the ESI) and lies outside the concentration range studied. Hence, additional experiments were performed at 100 mM C8:0, which showed that drug solubilization is significantly increased when the concentration of C8:0 FA is above its CMC (Figure 3A and 3B).

The obtained set of data can also be used to assess the effect of drug structure on solubilization by oleic acid, which showed superior performance to the saturated FA. A proper comparison between the different drugs can be achieved by using a solubilization ratio, obtained by scaling the drug solubilization in presence of oleic acid (S_{FA}) with the drug solubility in the intestinal medium in absence of additional polar lipids (S_{bile}). Such a comparison shows that oleic acid concentration had biggest effect for FEN (up to 18-fold solubilization enhancement), whereas the effect was much smaller for progesterone and danazol, see Figure 4.

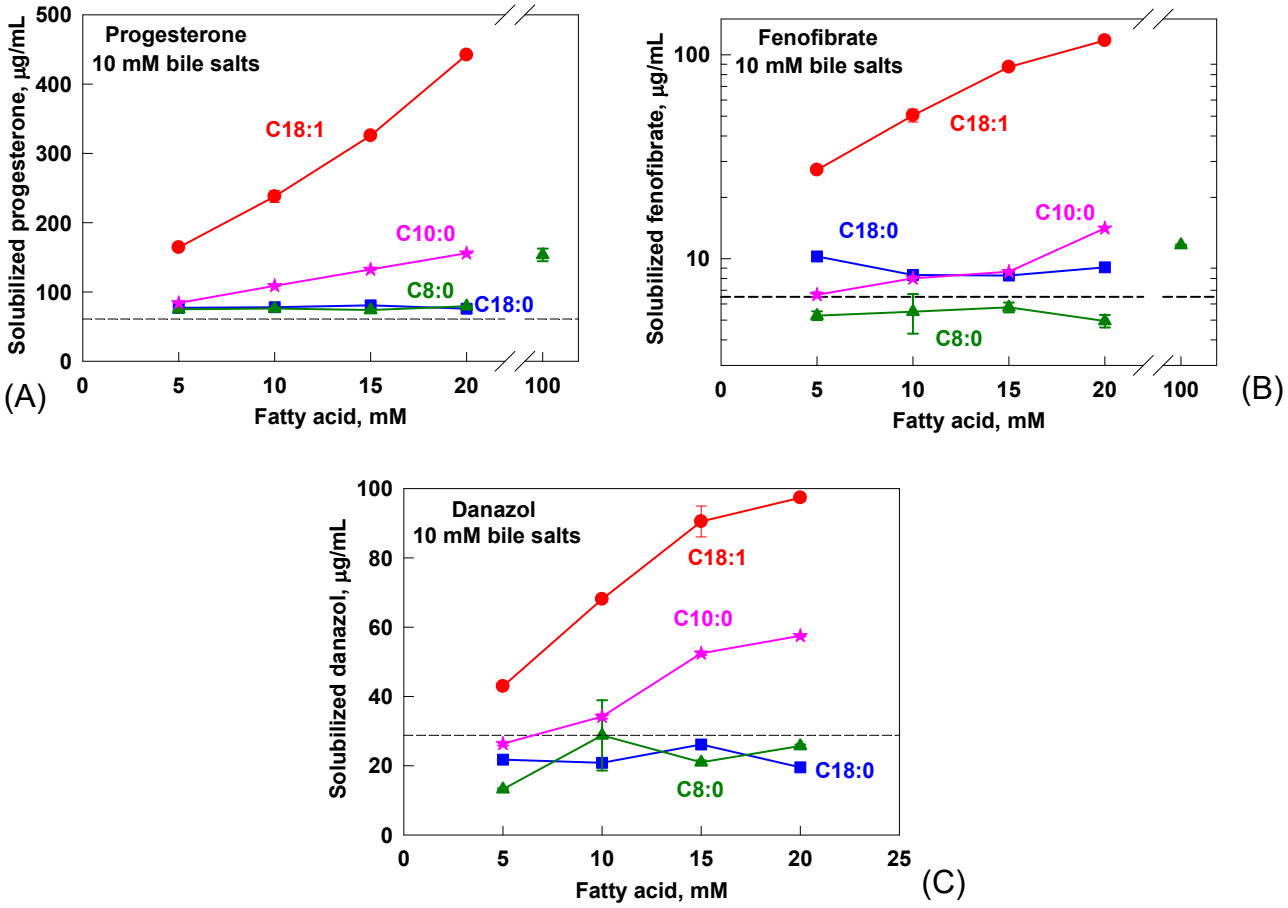


Figure 3. Solubilized (A) progesterone, (B) fenofibrate or (C) danazol, as a function of the concentration octanoic (green triangles), decanoic (pink stars), stearic (blue squares) and oleic acid (red circles). The dashed line represents drug solubilization at the intestinal conditions of the GI tract model, without any added FA.

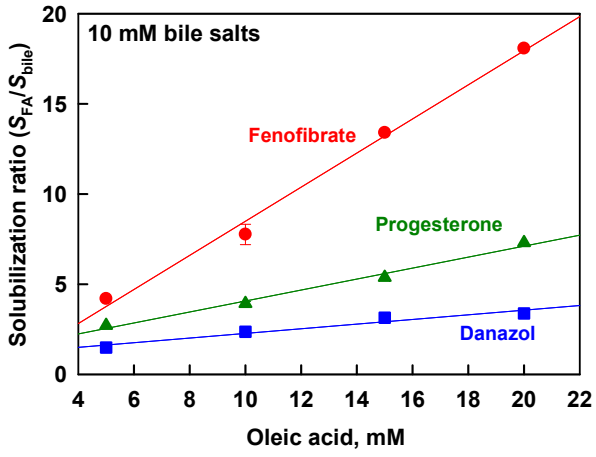


Figure 4. Solubilization ratio (S_{FA}/S_{bile}) of fenofibrate (red circles), progesterone (blue squares), or danazol (green triangles), as a function of the concentration oleic acid.

As the FA-based excipients used in drug formulation are usually composed of mixtures of FAs (e.g. natural fats and oils; technical FA products), we also studied FEN solubilization in binary FA mixtures at a ratio of 1:1. FA with high FEN solubilization and/or high abundance in

natural fats and oils (and their derivatives) were chosen for this set of experiments: the saturated C12, C14, C16 and C18 FA, and the unsaturated C18:1 FA. Near-ideal, linear behavior (R^2 > 0.90) was observed for all studied pairs of FA (see Figure S2 in the ESI): the solubilization of FEN in the 1:1 mixtures was close or identical to the average of the individual species. The only significant deviation from linearity (R^2 = 0.60) was observed for the mixture of palmitic and stearic acid, which nonetheless solubilized a very low concentration of FEN (13 µg/mL).

3.2. Effect of monoglycerides on drug solubilization.

We studied a MG frequently used in lipid-based formulations: monoolein (glycerol monooleate), and its saturated analogue, monostearin. The performance of these two MG was compared with the corresponding FA, see Figure 5. One sees that the addition of C18:1 FA or MG increases significantly FEN solubilization in a linear, dose-dependent manner. In contrast, the presence of their saturated analogues (C18:0) has a marginal effect on FEN solubilization.

A comparison between monoolein and oleic acid provides further valuable information about the effect of the hydrophilic head group of the polar lipid: the slope of the dependance for monoolein was higher, compared to oleic acid. Hence, the glycerol-esterified oleic acid has significantly better performance than free oleic acid.

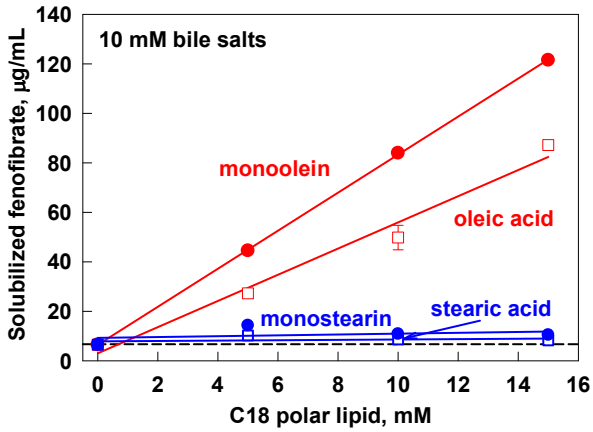


Figure 5. Solubilized FEN as a function of C18 polar lipid concentration for monoolein (full red circles), oleic acid (empty red squares), monostearin (full blue circles) and stearic acid (empty blue squares). The solid lines are a linear regression fit, whereas the dashed line represents drug solubilization at the intestinal conditions of the GI tract model, without any added FA.

3.3. Effect of phospholipids on drug solubilization.

The solubilization of FEN by the studied PL is presented in Figure 6. To determine the effect of the hydrophobic chain length, we compared sets of PL with the same hydrophilic head groups (Figure 6A): phosphatidylcholine and phosphatidylglycerol. For choline-PL, the increase of the saturated chain length from C10:0 to C16:0 leads to a gradual increase of FEN solubilization, reaching a maximum of S = 62 µg/mL. Further increase to C18:0 leads to a sharp drop in FEN solubilization, which reaches the value of the control (no PL added, S = 6.5 µg/mL).

In the case of the saturated phosphatidylglycerol PL, highest FEN solubilization (S = 62 µg/mL) was measured for C14:0 (DMPG). Increase of the hydrophobic chain length to C16:0 and C18:0 decreased gradually FEN solubilization, down to the control (no PL added).

The unsaturated DOPC and POPC showed considerably higher solubilization of FEN than all other PL studied: S = 125 and 168 µg/mL, respectively.

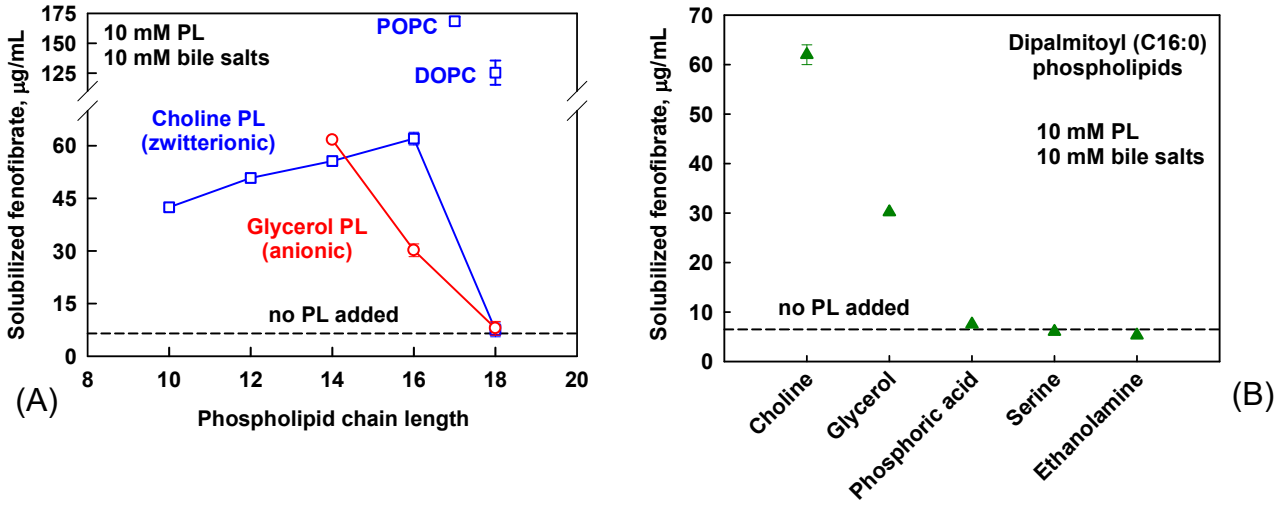


Figure 6. Solubilized FEN as a function of (A) the chain length of phosphatidylcholine (blue squares) and phosphatidylglycerol (red circles) phospholipids or (B) the hydrophilic head group type of dipalmitoyl (2×C16:0) phospholipids.

Next, we studied the impact of the hydrophilic moiety of the phospholipids by comparing phospholipids with the same hydrophobic chain length of C16:0 (Figure 6B). Highest FEN solubilization was measured for phospholipids with choline head group (S = 62 µg/mL), followed by the glycerol head group (S = 30 µg/mL). All other studied head groups (phosphoric acid, serine and ethanolamine) did not enhance FEN solubilization, compared to the control (no PL added).

4. Discussion.

The rank order of polar lipids is determined by acyl chain saturation

The studied 26 polar lipids can be arranged in 3 main classes, according to their FEN solubilization ratio (S/S_{Bile}), see Figure 7: class 1, high capacity ($S/S_{bile} \geq 10$); class 2, average capacity ($10 > S/S_{bile} \geq 3$) and class 3, low capacity ($1 < S/S_{bile} < 3$). Such classification provides a framework for interesting observations: the best performing substances (class 1, n = 5) are unsaturated, including representatives of all classes studied (PL, FA and MG); the average performers (class 2, n = 10) are composed primarily of saturated compounds (PL and FA) and two unsaturated FA (C18:1 and C18:2); class 3 contains only saturated compounds (n = 11).

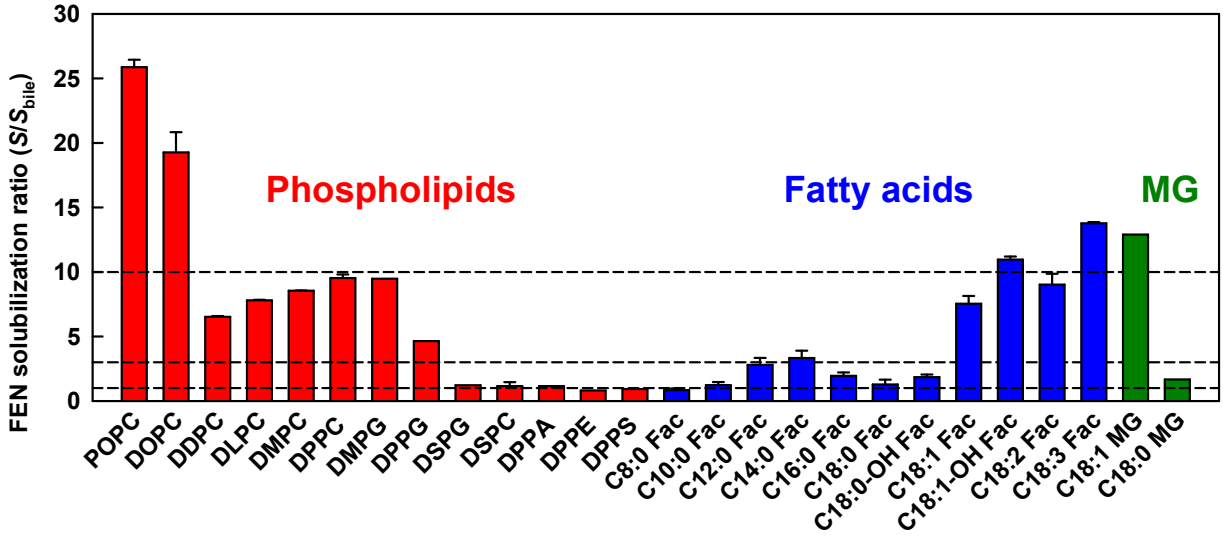


Figure 7. Fenofibrate solubilization ratio (S/S_{bile}), as a function of polar lipid type for phospholipids (red), FAs (blue) and monoglycerides (green).

Mechanisms of the acyl chain effects

The obtained results clearly demonstrate the poor drug solubilization properties of saturated-chain polar lipids. An obvious question to ask is what are the mechanisms that govern such a clear trend that spans over several classes of lipids and several drugs?

It would be reasonable to suggest that these mechanisms revolve around the ability of the polar lipids to form separate or mixed colloidal aggregates with the bile salts. Hence, two basic factors, known to be critical for classical surfactants, should be revisited:[46] (1) the concentration of polar lipid used, compared to its CMC and (2) the critical phase transition temperature above which the polar lipid can form colloidal aggregates (also referred to as the *Krafft point*).

If the concentration of the polar lipid is below its CMC, it will not form colloidal aggregates and thus will have no effect on drug solubilization. A typical case from the presented dataset is the short-chain C8:0 FA (Figure 3): it had no effect on solubilization in the initially studied concentration range (0 to 20 mM), due to its high CMC (50 mM). An experiment performed at a concentration of 2×CMC (100 mM C8:0) confirmed that drug solubilization is enhanced when C > CMC. This simple concept can also explain why low concentrations of C8:0 FA + C8:0 MG did not increase significantly the solubilization of hydrocortisone and 4 of its esters in the study of Kossena et al.[20] The lack of effect of C8:0 FA and C8:0 MG on the partitioning of α-tocopherol acetate in bile salt micelles observed by Takahashi & Underwood can also be explained in the same way.[47]

However, high CMC values cannot rationalize the lack of solubilization by the long-chain C18:0 FA (stearic acid): CMC decreases 2-3 fold for each CH_2 group added to the acyl chain (estimated CMC of 2.5 mM; solubilization was not observed up to 20 mM).[46] However, the increase of the hydrophobic chain length significantly decreases the solubility, which is reflected in the high Krafft temperature of sodium stearate (T_m = 50 °C), see Table 4. Note that at the conditions of the experiment (pH = 7.5), the fatty acids used should be in their ionized form (pK_a

in the range of 4.5 to 5)[48]. When the temperature of a soap solution is below the Krafft temperature, colloidal aggregates are not formed and solubilization cannot occur.[49] Therefore, stearic acid/sodium stearate does not increase drug solubilization because it simply does not form colloidal aggregates at the temperature of the experiments (T_{exp} = 37 °C).

Similarly to the CMC, the phase transition temperature concept described above can be used to explain results in other studies: for example, O'Reilly et al. stated that "Attempts to make mixed micelles with stearic acid were unsuccessful…", when attempting to measure the solubilizing effect of stearic acid in biorelevant medium at T = 37 °C.[50]

Table 4. Krafft temperatures of sodium FA* and acyl chain phase transition temperatures of PL.

Fatty acid sodium soaps[49]		**Phospholipids**[51]	
Type	**T_m, °C**	**Type**	**T_m, °C**
C12:0	20	DDPC	<< 37
C14:0	25	DLPC	<< 37
C16:0	38**	DMPC	24
C18:0	50	DMPG	24
C18:1	<< 20	DPPC	41
		DPPG	41
Monoglycerides		DSPC	54
Type	**T_m, °C**	DSPG	54
monostearin	57[52]	DPPS	54
monoolein	18[53]	DPPE	64
		DPPA	66

**Fatty acids are completely protonated at pH = 7.5 (pK_a in the range of 4.5 to 5)[48].*

***Interpolated based on the data for C12, C14 and C18.*

The lack of effect of most saturated PL and monostearin on drug solubilization can be rationalized in the same way (Table 4): the acyl chain melting temperature of these polar lipids is significantly above T_{exp}, which limits their association in colloidal aggregates. It should be noted, that both the Krafft temperature and the acyl chain transition temperature carry the same physical meaning in the context of solubilization: this is the critical phase transition temperature, above which the molecules of the polar lipid can form separate or mixed colloidal aggregates with the bile salts and enhance drug solubilization. For this reason, both will be referred to with T_m.

The role of the CMC and phase transition temperature for drug solubilization is illustrated in Figure 8A. One sees that the critical temperature rule is obeyed for all substances across the FA, PL and MG classes. The impact of the chain melting temperature for drug solubilization was highlighted also in our recent study on itraconazole solubilization in mixed PL-surfactant solutions.[54]

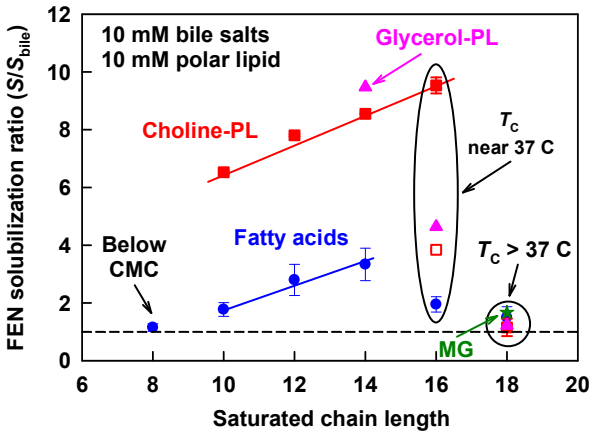


Figure 8. Fenofibrate solubilization ratio (S/S_{bile}), as a function of the saturated acyl chain length for phospholipids (red), fatty acids (blue) and monoglycerides (green). The lines are a linear fit. T_m indicates the Krafft temperature of fatty acid soaps and the acyl chain melting temperature of the phospholipids and monoglycerides. The empty red square corresponds to an experiment with DPPC at $T = 30$ °C.

Discrepancy is observed for the compounds with C16:0 chains (DPPC, DPPG and palmitic acid), as they all have critical transition temperatures near $T = 37$ °C, see Table 4. The increase from C14:0 to C16:0 increases the solubilization capacity in the case of choline PL, whereas a significant drop in solubilization was observed for glycerol PL and FA. The solubilization performance in this transition region requires a more complex explanation. It should first be considered that the 3 types of substances (DPPC, DPPG and C16:0 FA) have different ability to form mixed micelles with the bile salts. In our previous study, we showed that only 3 mM C16:0 FA can be solubilized in 15 mM of bile salts:[55] hence, the main fraction of C16:0 FA remains as crystals and cannot enhance drug solubilization in the bile micelles. On the other hand, DPPC is known to be solubilized completely by bile salt micelles in a wide range of concentrations,[56] leading to increased drug solubilization capacity of the mixed bile salt-phospholipid micelles.

To further confirm the role of temperature, a proof-of-principle experiment was performed with DPPC, which shows significant FEN solubilization, despite the fact its phase transition temperature is slightly higher (41 °C) than in the experiment. The experiment was performed at $T = 30$ °C and resulted in a significant drop in FEN solubilization. Although, the solubilization of FEN decreased to the level of DPPG at $T = 37$ °C, it remained ca. 4 times higher than the control. The latter can again be rationalized by considering the capacity of the bile salt micelles to at least partly solubilize DPPC,[56] which in turn increases drug solubilization.

When the conditions of C > CMC and $T_{exp} > T_m$ are met, the increase of saturated chain length has a *positive*, linear effect on drug solubilization (Figure 8). This effect has been widely documented for drug solubilization by classical surfactants in aqueous media and is explained by

the decreased CMC and the bigger volume available for solubilization in colloidal aggregates.[54,57–62]

Let us now focus on the mechanisms that drive the very strong drug solubilization in presence of the unsaturated polar lipids. The set of C18 FA homologues with varying degree of saturation (Figure 2A) provides useful data for such analysis. One way to explain the increase of FEN solubilization with the increasing number of double bonds in C18 FA is that the *cis* configuration of these bonds creates more space in the colloidal aggregates, thus facilitating the solubilization of FEN. One of the physicochemical parameters that is sensitive to such steric hindrance effects is the melting point of the crystalline substance: when the arrangement of the molecules in the crystal is hindered due to their structure, the melting point decreases. Indeed, Figure 9 shows that a reasonable correlation is obtained between these two parameters, confirming the impact of packing in the colloidal aggregates for drug solubilization.[54]

However, other factors might also be responsible for the observed effects: π-π interactions between the double bonds in the FA and the aromatic structure of FEN, could also play a role in the improved drug solubilization. For example, the solubilization of clofazimine, which contains multiple aromatic rings in its structure, was also found to increase with the increasing number of double bonds in the chain of C18 FA.[50]

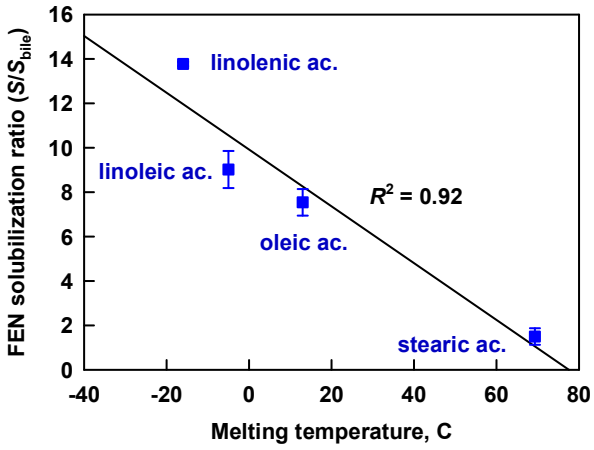


Figure 9. Fenofibrate solubilization enhancement (S/S_{bile}), as a function of the melting temperature of C18 fatty acid homologues with varying number of double bonds. The line is a linear regression fit.

Hydrophilic head group effects

The strong effect of the polar lipid head group on drug solubilization becomes apparent when polar lipids with the identical acyl chains are compared (see Figures 5-8). The performance of oleate derivatives decreases in the order choline-PL > MG > FA (*viz*. DOPC > monoolein > oleic acid, S_{FEN} = 125, 84 and 49 μg/mL, respectively). The same order is observed also for the homologue series of saturated FA and choline-PL (Figure 8): the PL always show significantly higher drug solubilization, compared to their FA analogues.

The effect of the diverse types of head groups in the PL class was displayed in Figure 6 and can be rationalized based on the differences in T_m (Table 4): choline- and glycerol-PL have the lowest T_m, and show significant drug solubilization, whereas the PL with ethanolamine, phosphoric acid and serine head groups have no effect on drug solubilization, due to $T_m > T_{exp}$. All stearate (C18:0) derivatives studied have $T_m > T_{exp}$ and do not enhance drug solubilization, so we cannot judge for the effect of the head group in this dataset.

Similar rank order (PL > MG > FA) of polar lipids on drug solubilization efficiency was observed also for tocopherol solubilization in mixed bile salt + polar lipid solutions.[47] The poor drug solubilization performance of free FAs, compared to their conjugated forms (MG or PL) observed in the current study, was also shown for gemfibrozil[36] and amphotericin B[63] by studies in mixed solutions of bile salts + polar lipids (oleic acid, monoolein and lecithin).

Therefore, the available evidence shows that the solubilization enhancement of poorly water-soluble drugs by polar lipids in biorelevant media decreases in the order PL > MG > FA.

Effect of drug structure

Drug structure can also influence the solubilization, due to the diverse molecular motifs and physicochemical properties that are common for drug substances. Based on the small set of 3 drugs investigated in the current paper, we can conclude only that oleic acid has bigger impact on the solubilization of the aromatic fenofibrate, compared to the steroidal danazol and progesterone molecules (Figure 4). In this case, additional interactions (e.g. π-π interactions between the double bonds in the FA and the aromatic structure of FEN), might explain the observed differences.

Specific drug-solubilizer interactions can have a significant impact on drug solubilization: for example, we have recently demonstrated that electrostatic attraction between oppositely charged phospholipids and drugs (albendazole and itraconazole) can significantly enhance solubilization.[54,61] Ion-dipole interactions can also promote the increase of the solubilization capacity, as shown for ionic surfactants and progesterone.[62] Thus, an expanded range of carefully selected drugs needs to be studied in order to clarify the effect of drug structure on solubilization by polar lipids.

Scope of validity of the observed trends and possible impact on oral absorption

The data presented in the current study demonstrates that typical physicochemical constraints (C > CMC, $T_{exp} > T_m$) control the ability of polar lipids to enhance drug solubilization in biorelevant media. Both parameters (CMC, T_m) relate to the ability of the polar lipids to associate in colloidal structures and are very sensitive to the acyl chain length and saturation, as well as to the hydrophilic head group type (this has been described in detail in classical literature).[46,64,65]

Polar lipid acyl chain unsaturation was identified as a major driver of drug solubilization, due to the following effects: (1) the considerably lower T_m of unsaturated chains, which allows long-chain polar lipids to associate in colloidal aggregates and (2) the *cis*-configuration of the

double bonds that prevents tight packing of the molecules in these aggregates, resulting in bigger volume available for drug solubilization.

It should be stressed that both the acyl chain and head group type are structural features of the polar lipids, *and not* of the solubilized drugs. Therefore, it can be expected that the impact of polar lipid CMC, T_m and chain unsaturation on solubilization, which was determined for 3 poorly water-soluble drugs in this paper, will hold true for a wider span of hydrophobic molecules.

Considering the implications of increased drug solubilization for intestinal absorption, the solubility-permeability interplay needs to be addressed. It has been shown that drug solubilization in classical surfactant micelles decreases the concentration of free drug, which decreases drug permeation and ultimately results in no cumulative effect on absorption or decreased absorption.[66,67] However, *in situ* rat perfusion studies show that the intestinal permeability of drugs solubilized in mixed bile salts + polar lipids micelles could actually be increased, rather than decreased.[50,63] In this context, the permeation-enhancing effects of short- and medium-chain FA are well-known and currently being used for the delivery of macromolecules.[68] Hence, systematic studies should be performed to clarify the impact of drug solubilization in mixed bile salts + polar lipids micelles on permeation.

5. Conclusions

We studied the effect of 26 polar lipids on the solubilization of the poorly water-soluble drugs fenofibrate, danazol and progesterone in a two-stage *in vitro* model of the upper GI tract. The following general trends and mechanisms of drug solubilization at biorelevant conditions were revealed:

1. General trends and rank-order of polar lipids:
   - Class 1 substances (n = 5) provide biggest enhancement of drug solubilization (>10-fold) and are represented by unsaturated polar lipids from all species studied (PL, MG and FA)
   - Class 2 materials (n = 10) have an intermediate effect (3-10 fold increase) and are composed primarily (80 %) of saturated polar lipids
   - Class 3 materials (n = 11) have very low or no effect on drug solubilization and are entirely composed of saturated compounds.
2. Acyl chain unsaturation drives the observed performance of long-chain unsaturated polar lipids (class 1), by the following mechanisms: the significantly decreased chain melting point, which allows these C18 lipids to form colloidal aggregates; and the *cis*-configuration of the double bonds that prevents tight packing of the molecules in these aggregates, resulting in bigger volume available for drug solubilization.
3. Typical physicochemical constraints (C > CMC, $T_{exp} > T_m$) control the ability of polar lipids to enhance drug solubilization in biorelevant media:
   - At concentrations below CMC, polar lipids do not change the solubilization capacity of the bile micelles, hence the drug solubilization remains unchanged.

- Polar lipids with acyl chain melting temperature (T_m > 41 °C) do not influence drug solubilization, because they do not form colloidal aggregates and are not solubilized by the bile salt micelles. Thus, the solubilization capacity of the bile micelles is not changed.
- The increase of chain length for polar lipids with T_m < 41 °C and C > CMC leads to the increased volume available for solubilization in the colloidal aggregates and increased solubilization capacity.

4. Drug solubilization enhancement by polar lipids with the same acyl chain decreases in the order PL > MG > FA.

The presented study provides an in-depth look at the performance of the widely polar lipid excipients in the context of poorly water-soluble drug solubilization at biorelevant conditions. The obtained mechanistic insights could be used for rational drug formulation development and thus support the modern drug discovery pipelines. Further research, which expands the set of studied drugs, includes the interplay with drug phase distribution (when an additional lipid phase is present) and considers drug permeation, is required to broaden the possible implications of the results obtained.

CRediT author statement

Vladimir Katev: Methodology, Investigation. **Zahari Vinarov**: Conceptualization, Methodology, Supervision, Writing - Original Draft, Writing - Review & Editing, Funding acquisition. **Slavka Tcholakova**: Conceptualization, Methodology, Supervision, Writing - Review & Editing, Funding acquisition, Project administration.

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References

(1) Keserü, G. M.; Makara, G. M. The Influence of Lead Discovery Strategies on the Properties of Drug Candidates. *Nat. Rev. Drug Discov.* **2009**, *8* (3), 203–212. https://doi.org/10.1038/nrd2796.

(2) Benet, L. Z.; Broccatelli, F.; Oprea, T. I. BDDCS Applied to over 900 Drugs. *AAPS J.* **2011**, *13* (4), 519–547. https://doi.org/10.1208/s12248-011-9290-9.

(3) Augustijns, P.; Wuyts, B.; Hens, B.; Annaert, P.; Butler, J.; Brouwers, J. A Review of Drug Solubility in Human Intestinal Fluids: Implications for the Prediction of Oral Absorption. *European Journal of Pharmaceutical Sciences*. 2014. https://doi.org/10.1016/j.ejps.2013.08.027.

(4) de la Cruz-Moreno, M. P.; Montejo, C.; Aguilar-Ros, A.; Dewe, W.; Beck, B.; Stappaerts, J.; Augustijns, P.; Tack, J. Exploring Drug Solubility in Fasted Human Intestinal Fluid Aspirates: Impact of Inter-Individual Variability, Sampling Site and Dilution. *Int. J. Pharm.* **2017**, *528* (1–2), 471–484. https://doi.org/10.1016/j.ijpharm.2017.05.072.

(5) Boyd, B. J.; Bergström, C. A. S.; Vinarov, Z.; Kuentz, M.; Brouwers, J.; Augustijns, P.; Brandl, M.; Bernkop-Schnürch, A.; Shrestha, N.; Préat, V.; et al. Successful Oral Delivery of Poorly Water-Soluble Drugs Both Depends on the Intraluminal Behavior of Drugs and of Appropriate Advanced Drug Delivery Systems. *Eur. J. Pharm. Sci.* **2019**, *137*. https://doi.org/10.1016/j.ejps.2019.104967.

(6) Porter, C. J. H.; Trevaskis, N. L.; Charman, W. N. Lipids and Lipid-Based Formulations: Optimizing the Oral Delivery of Lipophilic Drugs. *Nat. Rev. Drug Discov.* **2007**, *6* (3), 231–248. https://doi.org/10.1038/nrd2197.

(7) Bernkop-Schnürch, A.; Müllertz, A.; Rades, T. Self-Emulsifying Drug Delivery Systems (SEDDS) – The Splendid Comeback of an Old Technology. *Adv. Drug Deliv. Rev.* **2019**, *142*, 1–2. https://doi.org/10.1016/j.addr.2019.08.002.

(8) Feeney, O. M.; Crum, M. F.; McEvoy, C. L.; Trevaskis, N. L.; Williams, H. D.; Pouton, C. W.; Charman, W. N.; Bergström, C. A. S.; Porter, C. J. H. 50 Years of Oral Lipid-Based Formulations: Provenance, Progress and Future Perspectives. *Advanced Drug Delivery Reviews*. 2016. https://doi.org/10.1016/j.addr.2016.04.007.

(9) Savla, R.; Browne, J.; Plassat, V.; Wasan, K. M.; Wasan, E. K. Review and Analysis of FDA Approved Drugs Using Lipid-Based Formulations. *Drug Development and Industrial Pharmacy*. 2017. https://doi.org/10.1080/03639045.2017.1342654.

(10) Anby, M. U.; Williams, H. D.; McIntosh, M.; Benameur, H.; Edwards, G. A.; Pouton, C. W.; Porter, C. J. H. Lipid Digestion as a Trigger for Supersaturation: Evaluation of the Impact of Supersaturation Stabilization on the in Vitro and in Vivo Performance of Self-Emulsifying Drug Delivery Systems. *Mol. Pharm.* **2012**, *9* (7), 2063–2079. https://doi.org/10.1021/mp300164u.

(11) Williams, H. D.; Trevaskis, N. L.; Yeap, Y. Y.; Anby, M. U.; Pouton, C. W.; Porter, C. J. H. Lipid-Based Formulations and Drug Supersaturation: Harnessing the Unique Benefits of the Lipid Digestion/Absorption Pathway. *Pharmaceutical Research*. 2013. https://doi.org/10.1007/s11095-013-1126-0.

(12) Trevaskis, N. L.; Charman, W. N.; Porter, C. J. H. Lipid-Based Delivery Systems and Intestinal Lymphatic Drug Transport: A Mechanistic Update. *Advanced Drug Delivery*

Reviews. 2008. https://doi.org/10.1016/j.addr.2007.09.007.

(13) Pouton, C. W. Formulation of Lipid-Based Delivery Systems for Oral Administration: Materials, Methods and Strategies. *Adv. Drug Deliv. Rev.* **2008**, *60* (6), 625–637. https://doi.org/10.1016/J.ADDR.2007.10.010.

(14) Pouton, C. W. Formulation of Poorly Water-Soluble Drugs for Oral Administration: Physicochemical and Physiological Issues and the Lipid Formulation Classification System. *Eur. J. Pharm. Sci.* **2006**. https://doi.org/10.1016/j.ejps.2006.04.016.

(15) Pouton, C. W. Lipid Formulations for Oral Administration of Drugs: Non-Emulsifying, Self-Emulsifying and "self-Microemulsifying" Drug Delivery Systems. In *European Journal of Pharmaceutical Sciences*; 2000. https://doi.org/10.1016/S0928-0987(00)00167-6.

(16) Williams, H. D.; Sassene, P.; Kleberg, K.; Calderone, M.; Igonin, A.; Jule, E.; Vertommen, J.; Blundell, R.; Benameur, H.; Müllertz, A.; et al. Toward the Establishment of Standardized in Vitro Tests for Lipid-Based Formulations, Part 4: Proposing a New Lipid Formulation Performance Classification System. *J. Pharm. Sci.* **2014**. https://doi.org/10.1002/jps.24067.

(17) Sassene, P. J.; Mosgaard, M. D.; Löbmann, K.; Mu, H.; Larsen, F. H.; Rades, T.; Müllertz, A. Elucidating the Molecular Interactions Occurring during Drug Precipitation of Weak Bases from Lipid-Based Formulations: A Case Study with Cinnarizine and a Long Chain Self-Nanoemulsifying Drug Delivery System. *Mol. Pharm.* **2015**. https://doi.org/10.1021/acs.molpharmaceut.5b00498.

(18) Mohsin, K.; Long, M. A.; Pouton, C. W. Design of Lipid-Based Formulations for Oral Administration of Poorly Water-Soluble Drugs: Precipitation of Drug after Dispersion of Formulations in Aqueous Solution. *J. Pharm. Sci.* **2009**. https://doi.org/10.1002/jps.21659.

(19) Williams, H. D.; Sassene, P.; Kleberg, K.; Calderone, M.; Igonin, A.; Jule, E.; Vertommen, J.; Blundell, R.; Benameur, H.; Müllertz, A.; et al. Toward the Establishment of Standardized in Vitro Tests for Lipid-Based Formulations, Part 3: Understanding Supersaturation versus Precipitation Potential during the in Vitro Digestion of Type I, II, IIIA, IIIB and IV Lipid-Based Formulations. *Pharm. Res.* **2013**. https://doi.org/10.1007/s11095-013-1038-z.

(20) Kossena, G. A.; Charman, W. N.; Boyd, B. J.; Dunstan, D. E.; Porter, C. J. H. Probing Drug Solubilization Patterns in the Gastrointestinal Tract after Administration of Lipid-Based Delivery Systems: A Phase Diagram Approach. *J. Pharm. Sci.* **2004**. https://doi.org/10.1002/jps.10554.

(21) Kaukonen, A. M.; Boyd, B. J.; Charman, W. N.; Porter, C. J. H. Drug Solubilization Behavior during in Vitro Digestion of Suspension Formulations of Poorly Water-Soluble Drugs in Triglyceride Lipids. *Pharm. Res.* **2004**. https://doi.org/10.1023/B:PHAM.0000016283.87709.a9.

(22) McEvoy, C. L.; Trevaskis, N. L.; Feeney, O. M.; Edwards, G. A.; Perlman, M. E.; Ambler, C. M.; Porter, C. J. H. Correlating in Vitro Solubilization and Supersaturation Profiles with in Vivo Exposure for Lipid Based Formulations of the CETP Inhibitor CP-532,623. *Mol. Pharm.* **2017**. https://doi.org/10.1021/acs.molpharmaceut.7b00660.

(23) Berthelsen, R.; Klitgaard, M.; Rades, T.; Müllertz, A. In Vitro Digestion Models to Evaluate Lipid Based Drug Delivery Systems; Present Status and Current Trends.

Advanced Drug Delivery Reviews. 2019. https://doi.org/10.1016/j.addr.2019.06.010.

(24) Williams, H. D.; Sassene, P.; Kleberg, K.; Bakala-N'Goma, J.-C.; Calderone, M.; Jannin, V.; Igonin, A.; Partheil, A.; Marchaud, D.; Jule, E.; et al. Toward the Establishment of Standardized In Vitro Tests for Lipid-Based Formulations, Part 1: Method Parameterization and Comparison of In Vitro Digestion Profiles Across a Range of Representative Formulations. *J. Pharm. Sci.* **2012**, *101* (9), 3360–3380. https://doi.org/10.1002/jps.23205.

(25) Verwei, M.; Minekus, M.; Zeijdner, E.; Schilderink, R.; Havenaar, R. Evaluation of Two Dynamic in Vitro Models Simulating Fasted and Fed State Conditions in the Upper Gastrointestinal Tract (TIM-1 and Tiny-TIM) for Investigating the Bioaccessibility of Pharmaceutical Compounds from Oral Dosage Forms. *Int. J. Pharm.* **2016**. https://doi.org/10.1016/j.ijpharm.2015.11.048.

(26) Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; et al. INFOGEST Static in Vitro Simulation of Gastrointestinal Food Digestion. *Nat. Protoc.* **2019**. https://doi.org/10.1038/s41596-018-0119-1.

(27) MINEKUS, M.; MARTEAU, P.; HAVENAAR, R.; HUIS IN 'T VELD, J. A Multicompartmental Dynamic Computer-Controlled Model Simulating the Stomach and Small Intestine. *ATLA. Altern. to Lab. Anim.* **1995**.

(28) Crum, M. F.; Trevaskis, N. L.; Williams, H. D.; Pouton, C. W.; Porter, C. J. H. A New in Vitro Lipid Digestion - In Vivo Absorption Model to Evaluate the Mechanisms of Drug Absorption from Lipid-Based Formulations. *Pharm. Res.* **2016**. https://doi.org/10.1007/s11095-015-1843-7.

(29) Tran, T.; Bønløkke, P.; Rodríguez-Rodríguez, C.; Nosrati, Z.; Esquinas, P. L.; Borkar, N.; Plum, J.; Strindberg, S.; Karagiozov, S.; Rades, T.; et al. Using in Vitro Lipolysis and SPECT/CT in Vivo Imaging to Understand Oral Absorption of Fenofibrate from Lipid-Based Drug Delivery Systems. *J. Control. Release* **2020**, *317* (November 2019), 375–384. https://doi.org/10.1016/j.jconrel.2019.11.024.

(30) Bannow, J.; Yorulmaz, Y.; Löbmann, K.; Müllertz, A.; Rades, T. Improving the Drug Load and in Vitro Performance of Supersaturated Self-Nanoemulsifying Drug Delivery Systems (Super-SNEDDS) Using Polymeric Precipitation Inhibitors. *Int. J. Pharm.* **2020**, *575* (December 2019), 118960. https://doi.org/10.1016/j.ijpharm.2019.118960.

(31) Müllertz, A.; Ogbonna, A.; Ren, S.; Rades, T. New Perspectives on Lipid and Surfactant Based Drug Delivery Systems for Oral Delivery of Poorly Soluble Drugs. *J. Pharm. Pharmacol.* **2010**, *62* (11), 1622–1636. https://doi.org/10.1111/j.2042-7158.2010.01107.x.

(32) Kleberg, K.; Jacobsen, J.; Müllertz, A. Characterising the Behaviour of Poorly Water Soluble Drugs in the Intestine: Application of Biorelevant Media for Solubility, Dissolution and Transport Studies. *J. Pharm. Pharmacol.* **2010**, *62* (11), 1656–1668. https://doi.org/10.1111/j.2042-7158.2010.01023.x.

(33) Kleberg, K. Biorelevant Media Simulating Fed State Intestinal Fluids : Colloid Phase Characterization and Impact on Solubilization Capacity. *J. Pharm. Sci.* **2010**, *99* (8), 3522–3532. https://doi.org/10.1002/jps.

(34) Christensen, J. Ø.; Schultz, K.; Mollgaard, B.; Kristensen, H. G.; Mullertz, A. Solubilisation of Poorly Water-Soluble Drugs during in Vitro Lipolysis of Medium- and

Long-Chain Triacylglycerols. *Eur. J. Pharm. Sci.* **2004**, *23* (3), 287–296. https://doi.org/10.1016/j.ejps.2004.08.003.

(35) Rezhdo, O.; Speciner, L.; Carrier, R. Lipid-Associated Oral Delivery: Mechanisms and Analysis of Oral Absorption Enhancement. *J. Control. Release* **2016**, *240*, 544–560. https://doi.org/10.1016/j.jconrel.2016.07.050.

(36) Luner, P. E.; Babu, S. R.; Radebaugh, G. W. The Effects of Bile Salts and Lipids on the Physicochemical Behavior of Gemfibrozil. *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists*. 1994, pp 1755–1760. https://doi.org/10.1023/A:1018967401000.

(37) Khadra, I.; Zhou, Z.; Dunn, C.; Wilson, C. G.; Halbert, G. Statistical Investigation of Simulated Intestinal Fluid Composition on the Equilibrium Solubility of Biopharmaceutics Classification System Class II Drugs. *Eur. J. Pharm. Sci.* **2015**, *67*, 65–75. https://doi.org/10.1016/j.ejps.2014.10.019.

(38) Dunn, C.; Perrier, J.; Khadra, I.; Wilson, C. G.; Halbert, G. W. Topography of Simulated Intestinal Equilibrium Solubility. *Mol. Pharm.* **2019**, *16* (5), 1890–1905. https://doi.org/10.1021/acs.molpharmaceut.8b01238.

(39) Ditzinger, F.; Price, D. J.; Ilie, A. R.; Köhl, N. J.; Jankovic, S.; Tsakiridou, G.; Aleandri, S.; Kalantzi, L.; Holm, R.; Nair, A.; et al. Lipophilicity and Hydrophobicity Considerations in Bio-Enabling Oral Formulations Approaches – a PEARRL Review. *Journal of Pharmacy and Pharmacology*. 2019. https://doi.org/10.1111/jphp.12984.

(40) Vinarov, Z.; Petrova, L.; Tcholakova, S.; Denkov, N. D.; Stoyanov, S. D.; Lips, A. In Vitro Study of Triglyceride Lipolysis and Phase Distribution of the Reaction Products and Cholesterol: Effects of Calcium and Bicarbonate. *Food Funct.* **2012**. https://doi.org/10.1039/c2fo30085k.

(41) Vinarova, L.; Vinarov, Z.; Tcholakova, S.; Denkov, N. D.; Stoyanov, S.; Lips, A. The Mechanism of Lowering Cholesterol Absorption by Calcium Studied by Using an in Vitro Digestion Model. *Food Funct.* **2016**, *7* (1). https://doi.org/10.1039/c5fo00856e.

(42) Vinarova, L.; Vinarov, Z.; Atanasov, V.; Pantcheva, I.; Tcholakova, S.; Denkov, N.; Stoyanov, S. Lowering of Cholesterol Bioaccessibility and Serum Concentrations by Saponins: In Vitro and in Vivo Studies. *Food Funct.* **2015**, *6* (2). https://doi.org/10.1039/c4fo00785a.

(43) Riethorst, D.; Mols, R.; Duchateau, G.; Tack, J.; Brouwers, J.; Augustijns, P. Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions. *J. Pharm. Sci.* **2016**, *105* (2), 673–681. https://doi.org/10.1002/jps.24603.

(44) Maurer, J. M.; Schellekens, R. C. A.; Van Rieke, H. M.; Wanke, C.; Iordanov, V.; Stellaard, F.; Wutzke, K. D.; Dijkstra, G.; Van Der Zee, M.; Woerdenbag, H. J.; et al. Gastrointestinal PH and Transit Time Profiling in Healthy Volunteers Using the IntelliCap System Confirms Ileo-Colonic Release of ColoPulse Tablets. *PLoS One* **2015**. https://doi.org/10.1371/journal.pone.0129076.

(45) Devraj, R.; Williams, H. D.; Warren, D. B.; Mullertz, A.; Porter, C. J. H.; Pouton, C. W. In Vitro Digestion Testing of Lipid-Based Delivery Systems: Calcium Ions Combine with Fatty Acids Liberated from Triglyceride Rich Lipid Solutions to Form Soaps and Reduce the Solubilization Capacity of Colloidal Digestion Products. *Int. J. Pharm.* **2013**. https://doi.org/10.1016/j.ijpharm.2012.11.024.

(46) Israelachvili, J. N. *Intermolecular and Surface Forces: Third Edition*; 2011. https://doi.org/10.1016/C2011-0-05119-0.

(47) Takahashi, Y. I.; Underwood, B. A. Effect of Long and Medium Chain Length Lipids upon Aqueous Solubility of α-Tocopherol. *Lipids* **1974**, *9* (11), 855–859. https://doi.org/10.1007/BF02532609.

(48) No Title https://pubchem.ncbi.nlm.nih.gov/.

(49) McBain, J. W.; Sierichs, W. C. The Solubility of Sodium and Potassium Soaps and the Phase Diagrams of Aqueous Potassium Soaps. *J. Am. Oil Chem. Soc.* **1948**. https://doi.org/10.1007/BF02645899.

(50) O'Reilly, J. R.; Corrigan, O. I.; O'Driscoll, C. M. The Effect of Simple Micellar Systems on the Solubility and Intestinal Absorption of Clofazimine (B663) in the Anaesthetised Rat. *Int. J. Pharm.* **1994**, *105* (2), 137–146. https://doi.org/10.1016/0378-5173(94)90459-6.

(51) Cevc, G. *Phospholipids Handbook*; 2018. https://doi.org/10.1201/9780203743577.

(52) Batte, H. D.; Wright, A. J.; Rush, J. W.; Idziak, S. H. J.; Marangoni, A. G. Phase Behavior, Stability, and Mesomorphism of Monostearin-Oil-Water Gels. *Food Biophys.* **2007**. https://doi.org/10.1007/s11483-007-9026-7.

(53) White, S. H. Phase Transitions in Planar Bilayer Membranes. *Biophys. J.* **1975**. https://doi.org/10.1016/S0006-3495(75)85795-X.

(54) Vinarov, Z.; Gancheva, G.; Burdzhiev, N.; Tcholakova, S. Solubilization of Itraconazole by Surfactants and Phospholipid-Surfactant Mixtures: Interplay of Amphiphile Structure, PH and Electrostatic Interactions. *J. Drug Deliv. Sci. Technol.* **2020**. https://doi.org/10.1016/j.jddst.2020.101688.

(55) Vinarova, L.; Vinarov, Z.; Tcholakova, S.; Denkov, N. D.; Stoyanov, S.; Lips, A. The Mechanism of Lowering Cholesterol Absorption by Calcium Studied by Using an in Vitro Digestion Model. *Food Funct.* **2016**, *7* (1), 151–163. https://doi.org/10.1039/c5fo00856e.

(56) Almgren, M. Mixed Micelles and Other Structures in the Solubilization of Bilayer Lipid Membranes by Surfactants. *Biochim. Biophys. Acta - Biomembr.* **2000**, *1508* (1–2), 146–163. https://doi.org/10.1016/S0005-2736(00)00309-6.

(57) Ong, J. T. H.; Manoukian, E. Micellar Solubilization of Timobesone Acetate in Aqueous and Aqueous Propylene Glycol Solutions of Nonionic Surfactants. *Pharm. Res. An Off. J. Am. Assoc. Pharm. Sci.* **1988**. https://doi.org/10.1023/A:1015903827042.

(58) Bhat, P. A.; Dar, A. A.; Rather, G. M. Solubilization Capabilities of Some Cationic, Anionic, and Nonionic Surfactants toward the Poorly Water-Soluble Antibiotic Drug Erythromycin. *J. Chem. Eng. Data* **2008**. https://doi.org/10.1021/je700659g.

(59) Krishna, A. K.; Flanagan, D. R. Micellar Solubilization of a New Antimalarial Drug, B-arteether. *J. Pharm. Sci.* **1989**. https://doi.org/10.1002/jps.2600780713.

(60) Vinarov, Z.; Katev, V.; Radeva, D.; Tcholakova, S.; Denkov, N. D. D. Micellar Solubilization of Poorly Water-Soluble Drugs: Effect of Surfactant and Solubilizate Molecular Structure. *Drug Dev. Ind. Pharm.* **2018**, *44* (4), 677–686. https://doi.org/10.1080/03639045.2017.1408642.

(61) Vinarov, Z.; Gancheva, G.; Katev, V.; Tcholakova, S. S. S. Albendazole Solution Formulation via Vesicle-to-Micelle Transition of Phospholipid-Surfactant Aggregates. *Drug Dev. Ind. Pharm.* **2018**, *44* (7), 1130–1138. https://doi.org/10.1080/03639045.2018.1438461.

(62) Vinarov, Z.; Dobreva, P.; Tcholakova, S. Effect of Surfactant Molecular Structure on Progesterone Solubilization. *J. Drug Deliv. Sci. Technol.* **2018**. https://doi.org/10.1016/j.jddst.2017.09.014.

(63) Dangi, J. S.; Vyas, S. P.; Dixit, V. K. Effect of Various Lipid-Bile Salt Mixed Micelles on the Intestinal Absorption of Amphotericin-B in Rat. *Drug Dev. Ind. Pharm.* **1998**. https://doi.org/10.3109/03639049809082364.

(64) Rosen, M. J.; Kunjappu, J. T. *Surfactants and Interfacial Phenomena: Fourth Edition*; 2012. https://doi.org/10.1002/9781118228920.

(65) Small, D. M. *The Physical Chemistry of Lipids*; 1986. https://doi.org/10.1007/978-1-4899-5333-9.

(66) Miller, J. M.; Beig, A.; Krieg, B. J.; Carr, R. A.; Borchardt, T. B.; Amidon, G. E.; Amidon, G. L.; Dahan, A. The Solubility-Permeability Interplay: Mechanistic Modeling and Predictive Application of the Impact of Micellar Solubilization on Intestinal Permeation. *Mol. Pharm.* **2011**. https://doi.org/10.1021/mp200181v.

(67) Dahan, A.; Miller, J. M.; Hoffman, A.; Amidon, G. E.; Amidon, G. L. The Solubility-Permeability Interplay in Using Cyclodextrins as Pharmaceutical Solubilizers: Mechanistic Modeling and Application to Progesterone. *J. Pharm. Sci.* **2010**. https://doi.org/10.1002/jps.22033.

(68) Maher, S.; Mrsny, R. J.; Brayden, D. J. Intestinal Permeation Enhancers for Oral Peptide Delivery. *Advanced Drug Delivery Reviews*. 2016. https://doi.org/10.1016/j.addr.2016.06.005.