molecular pharmaceutics

pubs.acs.org/molecularpharmaceutics

Article

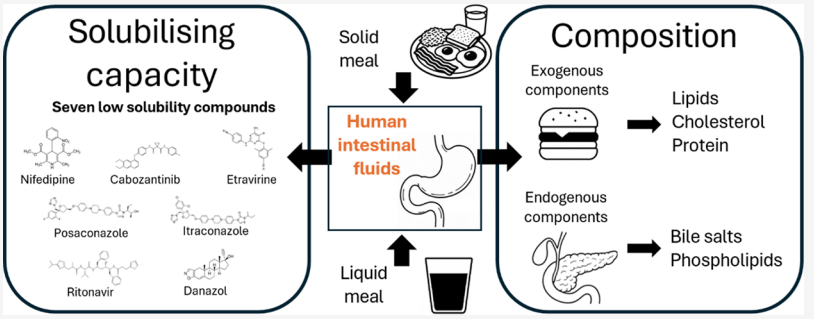
Characterization of Human Intestinal Fluids after the Administration of a Solid Meal

Brecht Goovaerts,[#] Mare Oja,[#] Sepanta Ehtemam, Joachim Brouwers, Álvaro López Mármol, Marlies Braeckmans, Tim Vanuytsel, Zahari Vinarov, Thomas B. Borchardt, Mirko Koziolek, and Patrick Augustijns*

Cite This: https://doi.org/10.1021/acs.molpharmaceut.5c01002

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Most available data on the composition and solubilizing properties of postprandial human intestinal fluid (HIF) are derived from studies involving liquid meals. These data inform the development of simulated intestinal fluids, widely used in in vitro assays for predicting intestinal drug behavior. However, the typical human diet primarily consists of solid meals, and the physical form of food has been shown to influence gastrointestinal transit and digestion, thereby affecting drug disposition and bioavailability. This study compares the characteristics of fed-state HIF collected after solid meal ingestion (SM-HIF) with previously published data on pooled liquid meal-derived HIF (LM-HIF) and newly generated data from individual LM-HIF samples. Time-dependent samples were analyzed over 180- and 90 min postprandial sampling periods to assess compositional changes following the administration of a solid and liquid meal, respectively. In addition, pooled samples were used to evaluate the solubilizing capacity for seven lipophilic model compounds. After intake of the solid meal, duodenal concentrations of exogenous (lipids, cholesterol, proteins) and endogenous (bile salts, phospholipids) components gradually increased to peak levels reached after 45−75 min. After 180 min, lipid and protein concentrations were still elevated compared to fasted state levels. In comparison to the liquid meal, the ingestion of the solid meal resulted in reduced concentrations of exogenous components, while endogenous components (bile salts and phospholipids) were relatively similar. For most compounds, the reduction in lipid content led to diminished solubilizing capacity of SM-HIF compared to LM-HIF when considering the combined micellar and lipid fractions. In contrast, the solubilizing capacity of the micellar fraction as such was largely independent of the meal type. Both the composition (particularly the micellar lipid concentration) and the solubilizing capacity of SM-HIF were highly variable between pools, albeit to a lesser extent than in LM-HIF. The findings of this study highlight that the physical form of the meal influences the composition and solubilizing capacity of HIF. These insights should be taken into account when refining biorelevant media for in vitro models to better predict food effects during drug product development.

KEYWORDS: *human intestinal fluids (HIF), solid meal, food effects, drug solubility, intestinal fluid characterization*

1. INTRODUCTION

Understanding the interaction between orally administered drugs and the gastrointestinal (GI) environment is essential for the targeted and successful development of new drug products as the dissolution, solubilization, and absorption of drugs are significantly influenced by the physicochemical conditions within the gastrointestinal (GI) tract.[1,2]

To achieve a comprehensive characterization of the luminal conditions in the human GI tract and to simulate these in preclinical in vitro models, human intestinal fluids (HIF) have been collected and characterized in different aspects.[3−10] In fed-state studies, liquid test meals are commonly used during HIF

Received: July 11, 2025
Revised: October 14, 2025
Accepted: October 15, 2025

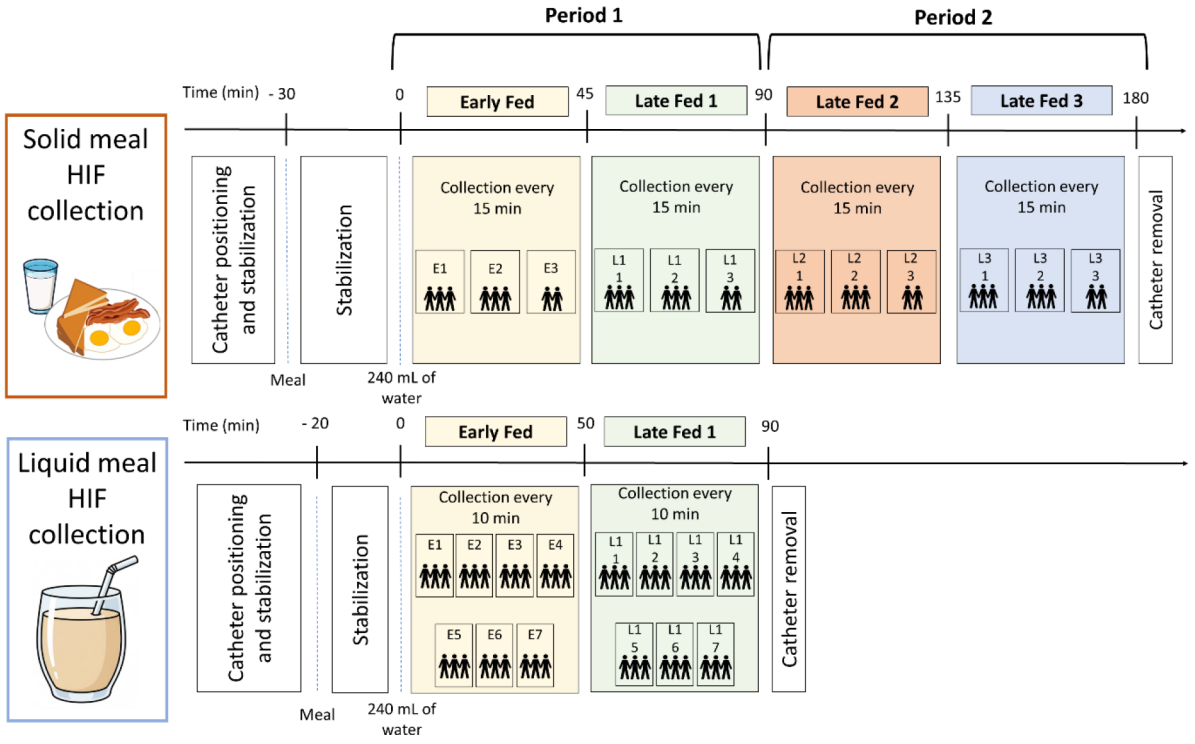


Figure 1. Graphical representation of intestinal fluid collection and pooling after the administration of a solid and liquid meal to healthy volunteers (HVs). Part of the aspirated fluids were combined into pools representing early and late fed states 1, 2, and 3 (solid meal) or early and late fed state 1 (liquid meal). Each pool consisted of fluids from two to three volunteers. E: early fed state, L1: late fed state 1, L2: late fed state 2, L3: late fed state 3. Image on HIF-collection reproduced from ref 26. Copyright 2024 American Chemical Society.

aspiration due to practical considerations, together with their ready-to-use nature, reducing experimental complexity. Consequently, most available data on the composition and solubilization properties of postprandial HIF have been derived from liquid meal studies. These data have been instrumental in the development of simulated intestinal fluids (SIF), which are widely employed in in vitro assays such as shake flask solubility studies, permeation tests, and dissolution experiments.[11] These simulations allow for the evaluation of drug solubility and permeability under physiologically relevant conditions.[7] The resulting parameters are critical inputs for in silico modeling tools, enhancing predictions of oral drug behavior in humans in different prandial states. This is particularly relevant for lipophilic, poorly water-soluble drugs, which often exhibit increased solubilization in the presence of lipidic components following high-fat meal intake.[12−14]

Despite the widespread use of liquid meals in aspiration studies, the typical human diet predominantly consists of solid meals. Unlike liquid meals, solid meals undergo partial particle size reduction through mastication but do not achieve the same degree of mixing and emulsification, which may significantly alter the physiological response of the gastrointestinal tract to food intake.[15] Multiple studies have demonstrated that the physical form of a meal influences GI transit and digestion, with potential consequences for intestinal fluid composition and solubilizing capacity. Solid meals delay gastric emptying due to the requirement for mechanical breakdown prior to transfer to the small intestine.[16−18] Additionally, solid meals, compared to liquid meals, prolong gastric acid secretion and stimulate gastrointestinal motility, which may, in turn, alter drug disposition and bioavailability.[19−21] Prolonged gastric retention following the coadministration of drugs with solid meals can increase or decrease the solubility of pH-dependent compounds, affecting dissolution rates and absorption.[22,23] For these reasons, the U.S. Food and Drug Administration (FDA) recommends a defined high-fat, high-calorie meal consisting of solid components such as eggs, bacon, toast, and potatoes for food effect studies.[24] This meal is commonly referred to as FDA standard breakfast and is used in most food effect studies.

Basic characterization of intestinal fluids after solid meal ingestion has been previously reported by Rubbens et al., who measured gastric and intestinal diclofenac concentrations following coadministration with a solid meal, and by Pentafragka et al., who assessed upper gastrointestinal characteristics after direct administration of a mixed solid meal to the stomach. However, neither study included a direct comparison with intestinal aspirates collected after liquid meal ingestion.[9,25]

Given these considerations, the present exploratory study aimed to compare the characteristics of fed-state HIF following the administration of a high-fat solid meal (half of the FDA standard meal), with previously published data of HIF after the ingestion of a high-fat liquid meal.[26] Individual aspirates were analyzed to provide a detailed characterization of HIF composition over an extended postprandial period. Additionally, pooled samples were used to assess the solubilizing capacity of HIF as a function of meal type and time postingestion, allowing for a more comprehensive understanding of how different food forms influence the solubility of lipophilic drugs.

2. MATERIALS AND METHODS

2.1. Materials. Sodium and potassium dihydrogen phosphate (NaH_2PO_4 and KH_2PO_4), taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC), glycoursodeoxycholic acid (GUDC), glycochenodeoxycholic acid (GCDC), glyco-

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

deoxycholic acid (GDC), glycocholic acid (GC), chenodeoxycholic acid (CDC), deoxycholic acid (DC), lithocholic acid (LC), cholic acid (C), sodium hydroxide (NaOH), cholesterol (Chol), cholesteryl oleate, cholesteryl palmitate, tripalmitin (TP), triolein, trilinolein, dipalmitin, diolein, dilinolein (DL), mono-oleate (MO), monopalmitin, monolinolein, palmitic acid, oleic acid (OA), linoleic acid, 1-octadecanol, L-tryptophan, ritonavir, danazol, nifedipine and orlistat were purchased from Sigma-Aldrich (St. Louis, MO). Tauroursodeoxycholic acid (TUDC), ursodeoxycholic acid (UDC), and taurocholic acid were acquired from Calbiochem (Darmstadt, Germany). Deuterated cholic acid (d4) was purchased from Cayman chemical (Ann Arbor, MI). The internal standards for itraconazole (d5) and chenodeoxycholic acid (d4) were bought from Alsachim (Illkirch Graffenstaden, France). Sodium choride (NaCl) and maleic acid were purchased from VWR chemicals (Leuven, Belgium). Hydrochloric acid (HCl) and acetonitrile (ACN, HPLC gradient grade) were purchased from Fischer scientific (Waltham MA) and methanol (MeOH, HPLC grade) from Acros Organics (Waltham MA). LC/MS grade MeOH and formic acid (FA) were acquired from Biosolve (Valkenswaard, The Netherlands). Isooctane (UV grade), ethyl acetate (LC-MS grade) and acetone (LC-MS grade) were purchased from Carl Roth (Karlsruhe, Germany). Acetic acid was bought from Chem-Lab analytical (Zedelgem, Belgium). Cabozantinib was purchased from Bionet Key Organics (Cornwall, UK). Itraconazole and etravirine were kindly provided by Johnson & Johnson Innovative Medicine (Beerse, Belgium). Posaconazole was bought from Biosynth Ltd. (Compton, UK). Ensure Plus was purchased from Abbott Laboratories B.V. (Zwolle, The Netherlands). Purified water was produced using a Purelab Flex water system from Veolia (Paris, France). All substances used for solubility experiments had a purity above 95%.

2.2. Media Collection. In two independent study setups, HIF was aspirated from healthy adults after the administration of a liquid meal or a solid meal, using the timelines depicted in Figure 1. In line with the FDA guidance on food effect studies,[24] healthy volunteers (HV) consumed 240 mL of water ($t = 0$) at a standardized time following meal ingestion: 20 min after starting the liquid meal and 30 min after starting the solid meal. The 10 min longer interval for the solid meal reflected the additional time required for ingestion, due to the need for mastication and the greater difficulty of swallowing compared to the liquid meal. The composition of the meals used in this comparison is given in Table 1. In a previous study, duodenal fluids after the ingestion of a liquid meal (LM-HIF) were aspirated from 21 healthy volunteers over a 90 min period, with sampling every 10 min.[26] These aspirates were characterized, and the remaining fluids were pooled into 7 early and 7 late fed state pools, each consisting of fluids from 3 collections. For the solid meal-HIF (SM-HIF), duodenal samples were aspirated during 8 collections from 4 HVs over a period of 180 min, with sampling every 15 min. After characterization of the individual aspirates, the remaining fluids were pooled into an early (0−45 min) and late fed state pool 1 (45−90 min), similar to the LM-HIF pools, and two additional late fed state pools 2 and 3 (90−135 and 135−180 min). For each time window, 3 pools were made, consisting of fluids from 2 or 3 collections.

Table 1. Energy Content and Nutrient Composition of the Liquid and Solid Meal

	liquid meal[a]	solid meal[b]
amount administered	400 mL	295 g
energy	600 kcal	478 kcal
lipids	19.64 g (derived from canola and corn oil)	26.8 g
carbohydrates	81.44 g	39.4 g
protein	25.0 g	19.8 g

[a]The nutritional composition of the liquid meal was obtained from the manufacturer's specifications (Abbott Nutrition, www.nutritio-n.abbott). [b]The nutritional composition of the solid meal was determined using the products' label information and the quantities used, as detailed in Table 2.

2.2.1. HIF Collection after Intake of a Liquid Meal. The collection of HIF after the administration of a liquid meal and the characterization of the pools in terms of composition and solubilizing capacity was described previously in a study by Goovaerts et al.[26] The characterization of individually aspirated samples has not been described before. In brief, during a study at UZ Leuven, approved by the Ethics Committee Research UZ/KU Leuven (S53791), intestinal fluids from 21 healthy volunteers (9 women and 12 men, aged 21−56, BMI 18−26 kg/m^2) were collected. Volunteers fasted for 12 h before a catheter was placed in their duodenum (D2-D3) for fluid collection. Before fed state sampling, fasted state fluids were aspirated for 90 min, after which 400 mL of Ensure Plus was administered to simulate fed state conditions. As depicted in Figure 1, patients then drank 240 mL of water, marking the start of the fed state fluid sampling, where fluids were aspirated every 10 min for a duration of 90 min. Fluids were treated with orlistat (final concentration of 1 μM) to prevent postsampling lipolysis and stored at −26 °C. Due to insufficient volume, not all of the individual aspirates could be characterized. Therefore, results of samples from 5 to 12 HVs were included per time point. After characterization of individual samples, the remaining fluids were pooled into 7 early fed and 7 late fed state 1 pools for analysis and solubility testing, each consisting of fluids from 3 volunteers.

2.2.2. HIF Collection after Intake of a Solid Meal. Four healthy volunteers (one female and 3 males, age between 25 and 34, BMI 21−35 kg/m^2) were recruited for the clinical study. One male volunteer participated five times, resulting in a total of eight sample collections. The study was approved by the Ethics Committee Research UZ/KU Leuven (S53791). The female participant was not pregnant, and none of the volunteers had a history of gastrointestinal disorders. One volunteer took prescribed medication (thiamazole (Strumazol)), which is not known to affect human intestinal fluids. All volunteers provided written informed consent.

After being fasted for at least 12 h, volunteers were intubated with a PVC dual lumen catheter (Salem Sump Tube 14 Ch, external diameter 4.7 mm; Cardinal Health, Dublin, Ohio), which was inserted through the nose and positioned in the duodenum. The position of the catheter was confirmed using X-ray fluoroscopy. After a 20 min stabilization period, the meal was ingested.

A meal with the composition shown in Table 2 (half a portion of the FDA standard meal) was prepared before the study and briefly heated in a microwave before consumption. All meal ingredients were purchased from Colruyt market (Leuven, Belgium). Volunteers consumed the solid meal within 15 min. Thirty min after the start of the meal ingestion, 240 mL of water was administered and fed state sampling was initiated. Duodenal fluids were aspirated every 15 min for a total of 180 min through the catheter, using 50 mL catheter tip syringes (Terumo Europe,

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 2. Composition and Products Used for Preparation of the FDA Standard Meal (Half Portion)

item	amount (g)	product name
egg	50	BONI belgische scharreleieren L
bacon	30	BONI spekblokjes gerookt
butter	10	BONI melkerijboter ongezouten
hash brown potatoes	56.7	BONI aardappelblokjes
whole milk	113.4	BONI volle melk
toast	35	EVERYDAY wit brood gesneden

Leuven, Belgium). The catheter was removed after collection of the final sample.

Aspirated fluids were aliquoted immediately according to the characterization plan. The pH of the fluids was measured immediately after aspiration. For lipid analysis and solubility experiments, 250 mM of the lipase inhibitor 4-bromophenylboronic acid in methanol (1:100 ratio, final concentration 2.5 mM) was added to the aliquots. For protein analysis, 0.1 M of the protease inhibitor Pefabloc in water (1:200 ratio, final concentration 0.5 μM) was added to the aliquot. No inhibitor was included in the aliquots for bile salt and phospholipid analyses. After collection, all aliquots and remaining samples were kept on dry ice until storage at −26 °C. For the individual characterization, some samples could not be included due to insufficient aspirated volume; for each time point, 7−8 samples were included.

After the characterization of the individual aspirates, the remaining samples were pooled into an early fed and three late fed state pools for further analysis and solubility testing. Per state, 3 pools were made (12 pools in total), each consisting of fluids from 2 or 3 aspirations.

Since the mean compositional profiles of the five aspirations obtained from a single male volunteer were not statistically different from the mean of the three aspirations from different volunteers (as determined using the method described by Hristova and Wimley), we decided to treat them as independent collections for the subsequent analyses and pooling.[27] In the pooled samples, the five aspirations from this one volunteer were divided across the 3 pooling groups (2-2-1).

2.3. Characterization of Intestinal Fluids. Both the individual samples and the pools of LM-HIF and SM-HIF were characterized for total lipids (triglycerides [TAG], diacylglycerides [DAG], monoacylglycerides [MAG], and free fatty acids [FFA]), bile salts, phospholipids, total cholesterol (cholesterol and cholesteryl esters), pH and total protein. For the pools, characterization was performed on (i) the micellar (aqueous) fraction, and (ii) the total sample consisting of both the micellar fraction and the lipid fraction. For the individual samples, only the total sample was analyzed due to constraints in sample volume. The total sample was taken as such, while the micellar sample was isolated by centrifugation (30 min, 20 000 g, 37 °C) on a benchtop centrifuge (Centrifuge 5804 R, VWR International, Leuven, Belgium) and subsequent removal of the upper lipid fraction using a glass pipet connected to a vacuum pump.

The analytical assays used to determine pH (glass electrode), total lipids and total cholesterol (liquid chromatography with charged aerosol detection), bile salts (liquid chromatography with tandem mass spectrometry), phospholipids (enzymatic kit), and total protein (tryptophan fluorescence assay) have been described in detail in a recent publication.[26]

2.4. Apparent Solubility of Selected Model Compounds. *2.4.1. Solubility Assay.* The equilibrium solubility of seven model compounds (i.e., ritonavir, nifedipine, cabozantinib, etravirine, itraconazole, danazol, and posaconazole) was determined in the SM-HIF pools, and subsequently compared to solubility data in LM-HIF pools, which were previously assessed using the same procedure.[26] These compounds were selected based on their poor water solubility, lipophilicity and variable food effect outcomes. The most important physicochemical properties are included in Table 3.

All solubility values were determined in triplicate and should be considered apparent, i.e., including both molecules freely dissolved in the aqueous phase and molecules solubilized in colloidal structures and lipid droplets.[35] Bacterial growth in the LM- and SM-HIF pools during the solubility experiments was prevented by the addition of a penicillin/streptomycin mixture in water (both 10,000 U/mL) using a 1/100 dilution ratio (final activity: 100 U/mL).

To an excess of crystalline drug powder (1 mg for all compounds, except for 0.6 mg for cabozantinib), 300 μL of SM-HIF was added. Subsequently, the suspension was incubated at 37 °C for 24 h under continuous shaking at 175 rpm (IKA KS 4000i control, Staufen, Germany) to reach equilibrium solubility, followed by centrifugation (30 min, 20,000 *g*, 37 °C). The 24-h incubation period was selected based on prior time-dependent solubility studies confirming that, for all compounds, equilibrium was reached within this time frame.

2.4.2. Sample Preparation. After incubation and centrifugation of the solubility samples (30 min, 20,000 *g*, 37 °C), multiple fractions were obtained: undissolved solid material at the bottom, an aqueous fraction containing colloidal structures, and a lipid fraction on top, the latter being visible in most samples. Since the aqueous colloidal fraction contained predominantly micelles, it will be further referred to as the micellar fraction. The solubility was determined in both the micellar fraction and the total sample (i.e., micellar and lipid fractions combined). In a first step, the isolation of the total sample required the transfer of both lipid and micellar fractions to a new tube, thus leaving the undissolved solid material behind. The micellar and lipid

Table 3. Physicochemical Properties of the Seven Model Compounds[a]

compound	molecular weight (g/mol)	acid/base/nonionizable	pK_a[b]	log *P*
ritonavir	720.9	base	4.46, 2.47	5.6[28]
nifedipine	346.3	base	1.28	2.50[29]
cabozantinib	501.5	base	5.3	5.3[30]
etravirine	435.3	base	2.77	>5[31]
itraconazole	705.6	base	3.7	6.2[32]
danazol	337.5	nonionizable		4.53[33]
posaconazole	700.8	base	3.6, 4.6	5.41[34]

[a]Table adapted from ref 26. Copyright 2024 American Chemical Society. [b]pK_a values were generated using ADMET predictor.

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 4. Sample Preparation and Analysis Conditions of Model Compounds[a]

compound	sample dilution	mobile phase	injection volume (μL)	flow rate (mL/min)	detection
ritonavir	MeOH:H_2O (1:20 v/v)	MeOH:buffer[b] (80:20)	50	1	UV: 241 nm
nifedipine	MeOH:H_2O (1:100 v/v)	ACN:buffer[b] (60:40)	50	1	UV: 340 nm
cabozantinib	MeOH:H_2O (1:100 v/v)	MeOH:buffer[c] (72:28)	50	1	UV: 322 nm
etravirine	MeOH:H_2O (1:100 v/v)	MeOH:buffer[b] (80:20)	50	1	UV: 312 nm
itraconazole	MeOH:H_2O 1:25 v/v (Fasted) 1:250 v/v (Fed)	MeOH:H_2O + 0.05% FA	2	0.6	MS/MS (m/z 705.3/392.3)
danazol	MeOH:H_2O (1:100 v/v)	MeOH:H_2O (82:18)	50	1	UV: 285 nm
posaconazole	MeOH:1%FA (1:10 v/v)	MeOH:buffer[b] (82:18)	50	1	Fluo: *ex* 240 nm *em* 385 nm

[a]Table adapted from ref 26. Copyright 2024 American Chemical Society. [b]Buffer: 25 mM Acetic acid in H_2O at pH 3.5. [c]Buffer: 40 mM formic acid in H_2O at pH 2.5.

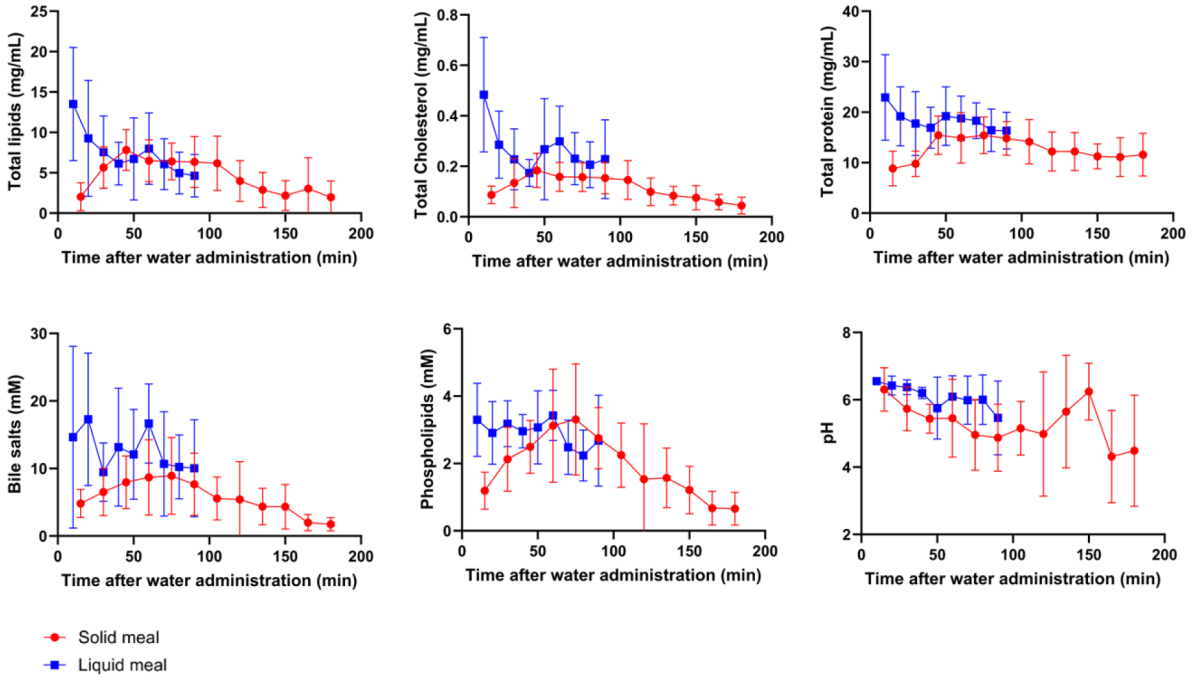


Figure 2. Composition of individual aspirates of LM-HIF (blue) and SM-HIF (red) with respect to total lipids (i.e., TAGs, DAGs, MAGs, and FFAs), total cholesterol (i.e., cholesterol and cholesteryl esters), total protein, bile salts, phospholipids, and pH. Characterization was performed on individual aspirates from 0 to 90 min after water intake following liquid meal ingestion and from 0 to 180 min after water intake following solid meal ingestion (see Figure 1). Data points represent the mean with standard deviations (SM-HIF: $n = 7-8$, LM-HIF: $5 \leq n \leq 12$ (depending on sample availability)).

fractions were subsequently rehomogenized to obtain the total sample, using a vortex mixer. After taking an aliquot of the total sample for analysis, the micellar fraction was isolated by centrifuging once again (30 min, 20,000 *g*, 37 °C), and removing the upper lipid fraction using a glass pipet connected to a vacuum pump. Aliquots of the total and micellar samples were diluted in either 50:50 MeOH/H_2O or in ice cold MeOH + 1% FA for protein precipitation, followed by a centrifugation step (10 min, 20,000 *g*, 4 °C) (see Table 4). As itraconazole was analyzed using tandem MS detection, an internal standard (itraconazole d5) was added to (50:50) MeOH/H_2O at a final concentration of 50 nM.

2.4.3. LC Analysis. The diluted samples were analyzed using (U)HPLC with UV absorbance, fluorescence or tandem MS detection, depending on the compound (Table 4). Detailed information about the analytical setup used to quantify the model compounds has been described by Goovaerts et al.[26]

2.5. Statistical Analysis. Mann−Whitney rank tests were used to test the similarity between LM- and SM-HIF pools both in terms of composition and solubilizing capacity. Spearman correlation coefficients were calculated to assess the relationships between the solubilizing capacity and the compositional factors of the HIF pools. These analyses were conducted using GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

In this study, fed state solid meal-HIF (SM-HIF) were collected over a period of 180 min after the administration of 240 mL of water, and compared to earlier collected liquid meal-HIF (LM-HIF), collected over a period of 90 min after the administration of water (see Figure 1). First, we looked at the composition of individual aspirates to study the food-, time-, and subject-dependent variability in HIF. Thereafter, the solubilizing capacity of different pools of SM-HIF and LM-HIF for low-solubility model drugs was evaluated, in relation to their composition.

3.1. Solid vs Liquid Meal HIF: Composition as a Function of Time. This section compares the composition of HIF samples collected over time following the administration of

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

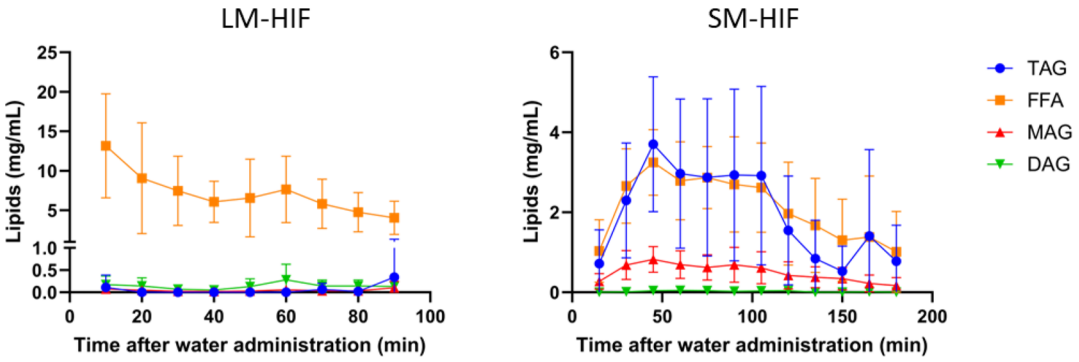


Figure 3. Lipid composition of individual aspirates (total samples) of LM-HIF (left) and SM-HIF (right). TAG (blue) and lipid digestion products FFA (orange), MAG (red), and DAG (green) are depicted in a time-dependent manner. Characterization was performed on individual aspirates from 0 to 90 min after water intake following liquid meal ingestion and from 0 to 180 min after water intake following solid meal ingestion (see Figure 1). Data points represent the mean with standard deviations (LM-HIF: $5 \leq n \leq 12$, SM-HIF: $n = 7-8$ (depending on sample availability)).

a solid or liquid meal, as outlined in Figure 1. The measured factors include both exogenous components that are primarily derived from the meal (i.e., total lipids, cholesterol, and protein) and endogenous components that are predominantly secreted into the small intestine via physiological feedback mechanisms (i.e., bile salts and phospholipids).

In Figure 2, it is clearly visible that the overall time-dependency of these HIF components differ markedly based on the meal ingested. After intake of a solid meal (red curves), both exogenous and endogenous components gradually increased in concentration, followed by a gradual decrease. In contrast, after intake of a liquid meal (blue curves), initial peak concentrations were rapidly reached, followed by a rapid decrease for exogenous but not endogenous components.

3.1.1. Exogenous Components (Lipids, Cholesterol, and Protein). Exogenous components, predominantly entering the small intestine via gastrointestinal transfer, exhibit mean concentration−time profiles that were heavily influenced by the rate of gastric emptying, which varies remarkably based on meal consistency. In LM-HIF, the time to reach maximum concentration (Tmax) for total lipids, total cholesterol, and total protein was observed as early as 10 min post water administration (i.e., the first sampling point, Figure 2). In contrast, concentrations increased more gradually in SM-HIF, resulting in a later Tmax of 45 min. This difference likely reflected the faster gastric emptying associated with liquid meals, which typically follow first-order kinetics, compared to the slower, zero-order kinetics observed for solid meals.[36,37]

In line with the differences in gastrointestinal transfer, the average concentrations of exogenous components during the initial 90 min of aspiration (representing early and late fed stage 1), were higher in LM-HIF compared to SM-HIF: 7.38 mg/mL vs 5.79 mg/mL for total lipids, 0.27 mg/mL vs 0.15 mg/mL for cholesterol, and 18.5 mg/mL vs 13.2 mg/mL for proteins.

Due to the slow, zero-order gastric emptying after solid meal ingestion, exogenous component concentrations in SM-HIF were high at first during the last 90 min of aspiration (90−180 min after water intake) but then gradually decreased (Figure 2). However, by the final time point (180 min), most exogenous components, except cholesterol, had not yet returned to baseline fasted-state levels (as reported by Goovaerts et al.). Specifically, the average lipid concentration at 180 min was 2.0 mg/mL (compared to 0.75 mg/mL in the fasted state), and protein levels averaged 11.6 mg/mL (vs 6.4 mg/mL in the fasted state). In contrast, at 180 min, cholesterol levels had declined to 0.04 mg/mL, below the fasted-state average of 0.14 mg/mL. This discrepancy may be explained by the dual origin of cholesterol. While dietary cholesterol is primarily exogenous in the fed state, a significant fraction is also endogenously secreted via bile, making it an endogenous component in the fasted state.[38,39] As a result, cholesterol shows a relatively high baseline concentration, which may not follow the same postprandial decline pattern as other more pronounced exogenous components such as lipids and proteins (see Section 3.1.2).

Some noteworthy differences in the composition of lipids were observed between LM-HIF and SM-HIF. As shown in Figure 3, total lipids in LM-HIF consisted predominantly of FFA (on average 96%) with minimal traces of undigested TAG, MAG and DAG. In contrast, lipids in SM-HIF consisted of a mixture of FFA (52%), undigested TAG (36%), and MAG (11%). This discrepancy likely reflects differences in the rate of digestion related to the accessibility of lipid substrates for the digestive enzymes. Liquid meals typically contain finely emulsified lipid droplets, while solid meals consist of larger, coarse particles, potentially decreasing the lipid surface area, thus limiting pancreatic lipase adsorption and reducing the rate of lipid digestion.[40] In both LM-HIF and SM-HIF, the relative proportions of the lipid classes remained largely stable throughout the aspiration period.

3.1.2. Endogenous Components. Bile salts and phospholipids are predominantly endogenous bile components, secreted directly into the small intestine. Postprandially, bile secretion is triggered due to the release of cholecystokinin (CCK) and secretin upon the entrance of lipids and protein in the small intestine.[41] As can be seen in their concentration−time profiles (Figure 2), this feedback mechanism caused peak concentrations of bile salts and phospholipids to be reached later as compared to exogenous components. In LM-HIF, Tmax amounted to 20 min for bile salts and 60 min for phospholipids, as opposed to 10 min for exogenous components. In SM-HIF, Tmax values were delayed to 75 min for bile salts and phospholipids, as opposed to 45 min for exogenous components. These findings highlight the time lag inherent to secretory responses compared to the faster appearance of nutrient-derived exogenous components in the intestinal lumen.

Average concentrations of endogenous components during the first 90 min of aspiration were generally higher in LM-HIF compared to SM-HIF (Figure 2): 12.6 versus 7.5 mM for bile salts, and 2.9 versus 2.5 mM for phospholipids. This difference likely reflects the strength of the feedback response, which is

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

triggered by the presence of exogenous components in the duodenum. Given the higher concentrations of lipids in LM-HIF, a more pronounced stimulation of bile secretion is expected.

The CCK/secretin feedback mechanism is also visible in the last 90 min of SM-HIF aspiration, where endogenous components follow a similar pattern as exogenous components: initially present at relatively high concentrations, followed by a gradual decline. However, unlike the exogenous components, the final aspirates collected at 180 min post water administration showed concentrations of bile salts and phospholipids that were slightly lower than those observed in the fasted state: 1.7 vs 3.9 mM in the fasted state for bile salts, and 0.6 vs 0.7 mM for phospholipids. This may be attributed to the emptying of the gallbladder reservoir during earlier stages of digestion (as described by Mutirangura et al. where 93% of subjects had maximal contractions within 90 min after the ingestion of a liquid meal), resulting in a return to fasted state basal bile secretion levels.[42] Additionally, the increased intestinal fluid volumes, even in later stages of the fed state,[14] may further dilute the concentration of secreted bile constituents, contributing to the observed concentrations (similar to the pattern noted for cholesterol, see Section 3.1.1).

3.1.3. pH. The pH of intestinal fluids is influenced by both exogenous food components, and physiological mechanisms, including hydrochloric acid secretion in the stomach and bicarbonate release in the small intestine. However, the impact of meal consistency on pH was not pronounced, as both LM-HIF and SM-HIF exhibited similar pH profiles (see Figure 2). During the first 90 min of aspiration, the pH in both fluids declined from approximately 6.5 to a range of 5.0−5.5, accompanied by increasing variability. This variability continued to rise in the final 90 min of SM-HIF aspiration, with relative standard deviations (RSD) increasing from 15 to 27%.

3.2. Solid vs Liquid Meal HIF Pools: Composition and Solubilizing Capacity. As demonstrated in the previous section, distinct time-dependent compositional profiles were observed in HIF following ingestion of liquid versus solid meals. This section explores how these compositional differences impact the solubilizing capacity of the intestinal fluids, by performing equilibrium solubility tests using seven low-solubility model compounds in both the micellar and total samples (micellar and lipid fraction combined) of HIF.

To ensure sufficient volume for comparative solubility testing, individual HIF samples were pooled according to the scheme depicted in Figure 1. In addition to solubility measurements, the composition of both micellar and total samples of these pools was analyzed. LM-HIF pools, consisting of seven early and seven late fed state 1 pools, were previously characterized for composition and solubilizing capacity.[26] Following a similar strategy, three early and three late fed state 1 pools were generated for SM-HIF. Since SM-HIF was collected for 180 instead of 90 min in the case of LM-HIF, additional pools were created to represent late fed state 2 and 3 (three pools each).

In the following sections, we first compare LM-HIF with SM-HIF. To ensure a fair comparison, only the first 90 min of SM-HIF aspiration were considered, as this time frame aligns with the LM-HIF sampling window. This interval, referred to as **period 1** (0−90 min post water administration), includes both the early and late fed state 1 for LM-HIF and SM-HIF. Subsequently, we compare SM-HIF samples from **period 1** with those collected during the final 90 min of aspiration, referred to as **period 2**, which encompasses late fed states 2 and 3. While this approach reduces temporal resolution, it enables clearer visualization of general trends. Full data sets are provided in Figures S1 and S2.

3.2.1. Composition. After combining the samples aspirated during **period 1**, the general differences between LM-HIF and SM-HIF are similar to the ones observed in the individual samples (Figure 4). On average, total samples of LM-HIF pools

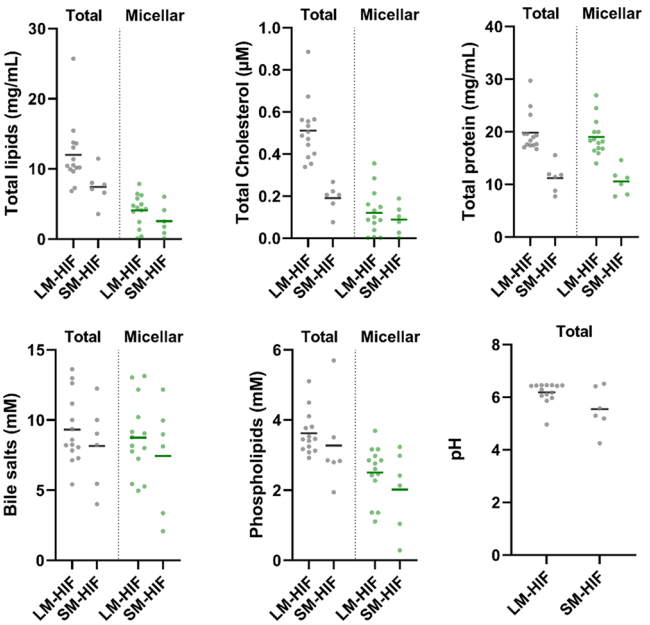


Figure 4. Composition of LM- and SM-HIF pools in period 1 (0−90 min after water administration), with respect to total lipids (i.e., TAGs, DAGs, MAGs, and FFAs), total cholesterol (i.e., cholesterol and cholesteryl esters), total protein, bile salts, phospholipids, and pH. Total samples (gray dots) and micellar samples (green dots) were displayed separately for all characteristics except pH. Data points represent a single pool, with the solid lines depicting the mean of the pools aspirated in period 1 (n = 14 for LM-HIF, n = 6 for SM-HIF).

(represented by gray dots) contained 40−60% higher concentrations of exogenous components (lipids, cholesterol and protein) compared to SM-HIF pools. In contrast, the difference in endogenous components was less pronounced, with LM-HIF pools containing only slightly more bile salts (6%) and phospholipids (12%). The average pH-value was 16% higher in LM-HIF pools.

A similar pattern was observed in the micellar fraction (represented by green dots in Figure 4), where LM-HIF pools contained higher average concentrations of exogenous components (27−44%) and, to a smaller extent, endogenous components (15−20%) than SM-HIF.

As shown in Figure 5, we compared the composition of SM-HIF pools collected during **period 1** with those collected in **period 2**. Consistent with the time-dependent profiles described in Section 3.1, the average concentrations of most components were lower in the later pools. In the total samples, the pools from **period 2** contained, on average, 50−59% less lipids, cholesterol, bile salts, and phospholipids compared to those from **period 1**. Protein was an exception, with only an 8% lower concentration in the late pools. A similar but even more pronounced trend was observed in the micellar fraction, where all components were 62−74% less abundant in **period 2**; protein was again a notable exception, showing only an 11% difference.

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

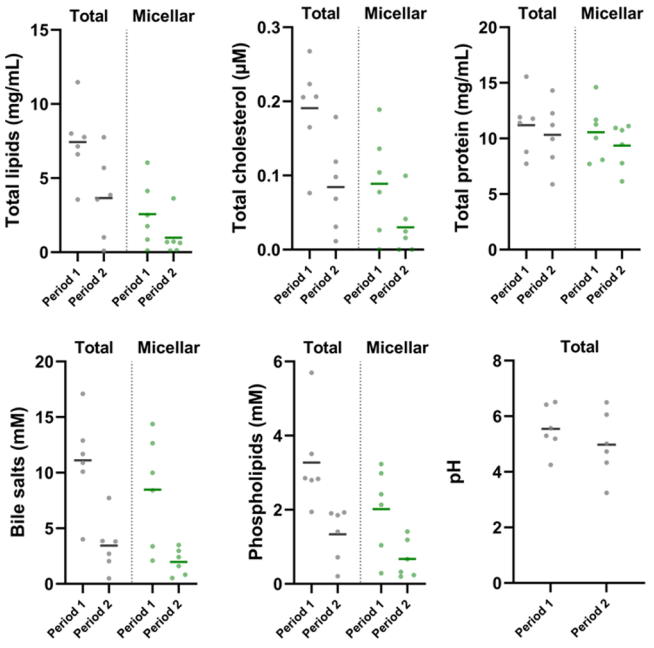


Figure 5. Composition of SM-HIF pools representing period 1 (0−90 min after water administration) versus period 2 (90−180 min) with respect to total lipids (i.e., TAGs, DAGs, MAGs, and FFAs), total cholesterol (i.e., cholesterol and cholesteryl esters), total protein, bile salts, phospholipids, and pH. Total samples (gray dots) and micellar samples (green dots) were displayed separately for all characteristics except pH. Data points represent a single pool, with the solid lines depicting the mean of the different pools per period (n = 6).

In summary, both total and micellar samples of SM-HIF pools representing **period 1** contained, on average, less lipids and proteins compared to LM-HIF pools. The differences in average bile salt and phospholipid concentrations were less pronounced. In SM-HIF pools representing **period 2**, a decrease in the average concentration of all components except proteins, was observed.

Although clear trends in the average composition of the fed state HIF pools were apparent from the obtained data, it is important to interpret these findings with caution due to the substantial variability between the pools, as illustrated by the individual dots in Figures 4 and 5. Indeed, the observed differences were only statistically significant ($p < 0.05$, Mann−Whitney rank test) when comparing total concentrations of exogenous components between LM-HIF and SM-HIF pools (i.e., total lipids, cholesterol and protein).

The variability in the composition of the LM-HIF pools has been addressed in detail in our previous study using the ratio of the maximum and minimum concentration (MMR) of a specific component in a set of pools.[26] For SM-HIF pools representing **period 1**, extremely high variability was observed for micellar lipid concentrations with an MMR of 51. Also for the other components, considerable variability was seen in both total and micellar samples, with MMR values ranging from 2 to 11. Albeit to a lesser extent, this mirrors the trend seen in LM-HIF, where the highest variability was also observed for the micellar lipid concentration with an MMR of 77. During **period 2** of SM-HIF aspiration, variability in the total samples increased notably, particularly for lipids, cholesterol, and bile salts, with MMR values all exceeding 15. This increase in variability was not observed in the micellar samples.

3.2.2. Solubility. Similar to the compositional data presented earlier, Figures 6 and 7 illustrate the equilibrium solubility of seven poorly water-soluble model compounds in the LM-HIF and SM-HIF pools. Figure 6 first compares solubility differences

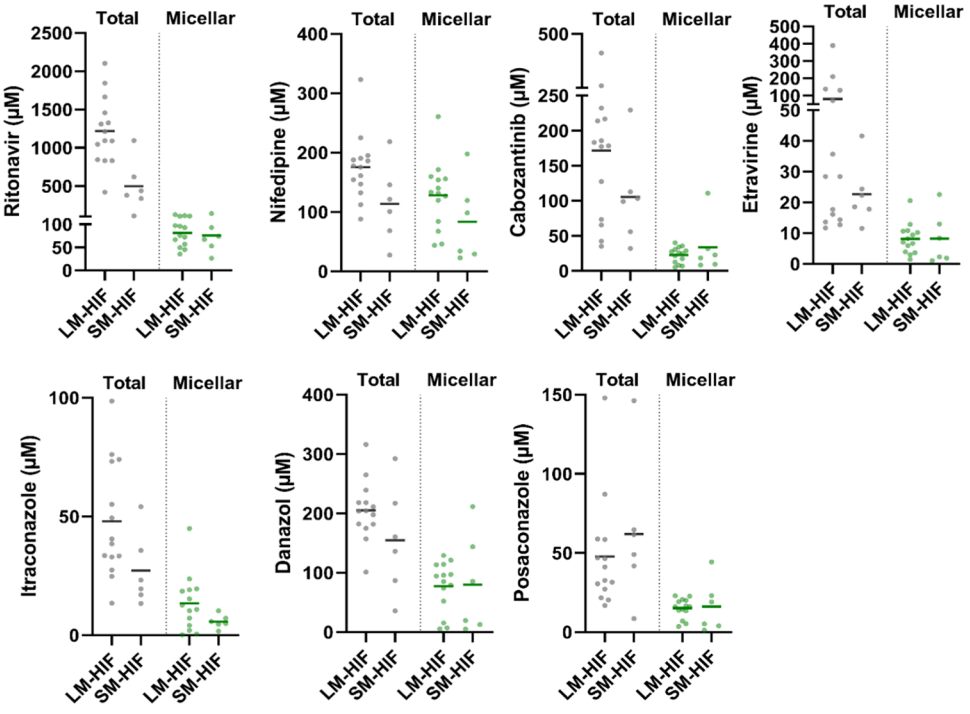


Figure 6. Equilibrium solubility of seven poorly water-soluble model drugs in LM- and SM-HIF pools representing the period 1 (0−90 min after water administration). Data points represent the mean of an experiment in triplicate, with the solid lines depicting the mean of the solubility values in the different HIF pools representing period 1 (n = 14 for LM-HIF, n = 6 for SM-HIF). Total samples (gray dots) and micellar samples (green dots) are displayed separately.

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

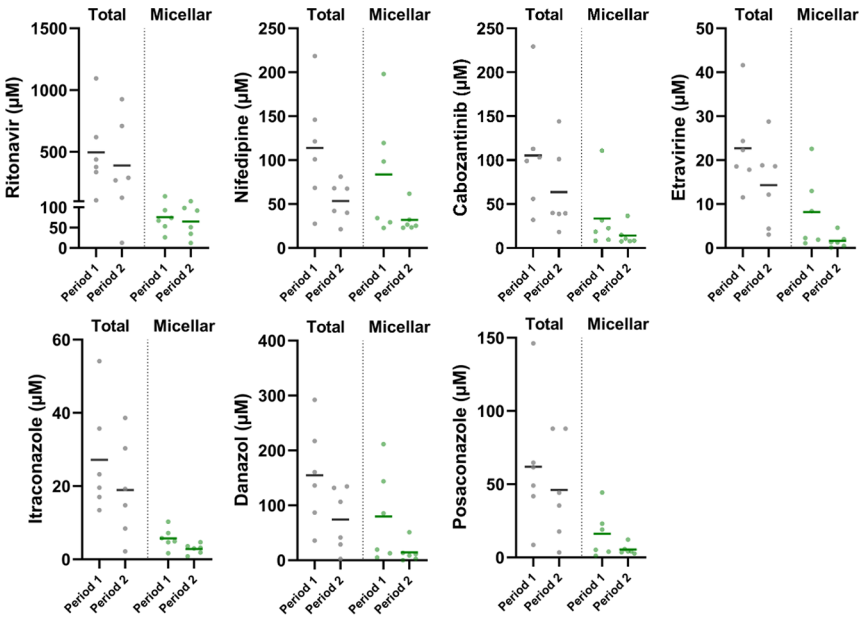


Figure 7. Equilibrium solubility of seven poorly water-soluble model drugs in SM-HIF pools representing period 1 (0−90 min after water administration) versus period 2 (90−180 min). Data points represent the mean of an experiment in triplicate, with the solid lines depicting the mean of the solubility values in the different HIF pools per period (n = 6 for SM-HIF). Total samples (gray dots) and micellar samples (green dots) are displayed separately.

between LM-HIF and SM-HIF pools representing **period 1**. Figure 7 analyses possible differences in the solubilizing capacity of SM-HIF pools representing **period 1 and 2**. Solubility was measured in both the micellar and the total samples.

As shown in Figure 6, most compounds exhibited higher average solubility in the total sample of LM-HIF pools compared to SM-HIF pools collected during **period 1** (representing the early and late fed state 1). The most pronounced difference was observed for etravirine, which had a 72% higher solubility in LM-HIF pools. This difference is caused by the late fed state 1 pool, where significant higher solubility is present in the LM-HIF pools (Figure S1). For other compounds, including ritonavir, nifedipine, cabozantinib, itraconazole, and danazol, average solubility values were 25−60% higher in LM-HIF pools. Only posaconazole deviated from the general trend, with a 29% lower solubility in LM-HIF compared to SM-HIF pools.

In contrast, the average solubilizing capacity of the micellar fraction did not differ between LM- and SM-HIF pools for four out of seven compounds (i.e., ritonavir, etravirine, danazol, and posaconazole), with differences not exceeding 7% (Figure 6). For itraconazole, nifedipine and cabozantinib, more pronounced solubility differences were seen, ranging from 35 to 57% in either direction.

It seems reasonable that the lower solubilizing capacity of SM-HIF versus LM-HIF pools, observed for most compounds in the total samples, is primarily due to the reduced lipid concentrations in these pools (see Figure 4). Lower lipid levels directly reduce the volume of the lipid fraction, and thus its contribution to the solubilizing capacity of the total samples. In contrast, the difference in solubilizing capacity of the micellar fraction between LM-HIF and SM-HIF was less pronounced. Although micellar lipid concentrations (key contributors to solubilizing capacity) were lower, the average concentrations of bile salts and phospholipids were similar between LM-HIF and SM-HIF micellar samples during **period 1**. We hypothesize that the maintained solubilizing capacity for certain compounds, despite the reduced lipid content, may be attributed to the comparable bile salt levels, given their well-established role in promoting micellar solubilization.

When comparing the SM-HIF pools in **period 1** versus **period 2** (Figure 7), a reduction in average solubilizing capacity was observed for both the total and micellar samples. For the total samples, solubility was between 21 and 53% higher in the early 90 min of aspiration; for the micellar samples, this was between 15 and 82%. Etravirine and danazol were the most affected compounds. Here, the solubility in the micellar fraction of samples from late-stage pools decreased by 80 and 82%, respectively. This reduction in solubilizing capacity can be attributed to the declining concentrations of both bile salts and lipids in **period 2** (Figure 5).

In summary, the lower average solubility values in the total sample of SM-HIF reflect the downstream effects of the delayed gastric emptying. The reduced intestinal lipid concentrations after the solid meal ingestion resulted in a decreased volume of the lipid fraction, thereby limiting its ability to dissolve poorly soluble compounds. However, just like the differences in composition, the solubilizing capacity of the samples also varied a lot, so the results should be interpreted with caution. Due to this variability, most of the observed trends were not statistically significant. In fact, a significant difference was only detected for itraconazole when comparing solubility values between **period 1 and 2** of SM-HIF aspiration.

To better understand the variability in solubility, maximum-to-minimum ratios (MMR) were calculated. During **period 1** of SM-HIF collection, the micellar fraction showed notably high variability, with an average MMR of 19 across all compounds, compared to 8.3 in the total fraction. A similar trend was observed in LM-HIF, where the micellar fraction showed substantial variability with an average MMR of 43 across all compounds, compared to 10 in the total fraction. Notably, during **period 2** of SM-HIF aspiration, the average MMR increased markedly to 27 for the total samples, while it remained relatively stable for the micellar samples (21). The observed variability in solubilizing capacity reflects the fluctuations

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

observed in lipid concentrations, suggesting a direct link between lipid content and the solubilizing potential of intestinal fluids. This was further tested by evaluating the correlations between the solubilizing capacity and the composition in the micellar fraction of the HIF pools (LM- and SM-HIF combined). Significant correlations between lipid concentrations and solubility in micellar samples were observed across all model compounds, with moderate to strong positive Spearman coefficients (r = 0.51−0.88) (complete table with Spearman coefficients are presented in Table S1). Positive correlations were also observed for other components, though these were generally weaker and not consistent across all compounds. These findings highlight the critical role of accurately simulating the lipid fraction in biorelevant media when predicting the in vivo performance of poorly water-soluble drugs.

3.3. Implications for Drug Development. Understanding the complex and dynamic nature of HIF under fed-state conditions is crucial for accurately predicting gastrointestinal drug behavior in the framework of drug candidate selection and drug product development. While substantial progress has been made in characterizing HIF, a gap exists in comprehending the variability in intestinal fluid composition due to factors such as inter- and intrapersonal differences, age, disease states, and meal types. Notably, the impact of meal consistency on gastrointestinal physiology and its subsequent effects on drug solubility has received limited attention.

Despite notable intersample variability in composition and solubilizing capacity, the results of this study demonstrate a consistent trend: compared to a high-fat liquid meal, the ingestion of a solid meal results in a slower appearance of exogenous compounds, reflecting a slower gastrointestinal transit, leading to a reduced concentration of exogenous components (i.e., lipids, cholesterol, and protein) in HIF, without substantially altering the levels of endogenous components (i.e., bile salts and phospholipids). The decrease in intestinal lipid levels limits the volume of the lipid fraction, thereby diminishing the solubilizing capacity of total HIF for lipophilic, poorly soluble compounds. In contrast, the solubilizing capacity of the micellar fraction of HIF is less affected, as it is primarily governed by the still-abundant endogenous surfactants. Furthermore, the delayed gastric emptying observed with solid meals prolongs the fed-state condition, with higher concentrations of exogenous compounds compared to the fasted state, persisting up to 210 min postmeal ingestion. The lower concentrations of lipids in SM-HIF, despite the solid meal containing more lipids, highlight that it is not just the nutritional content, but also the physical consistency of a meal that shapes the composition and solubilizing capacity of HIF over time. Considering the importance of solubilization for the absorption of lipophilic drugs, the observed effects of meal consistency on fluid composition should be considered when simulating postprandial intestinal drug behavior using either advanced or simple in vitro tools.

In advanced, dynamic, in vitro models such as the tiny-TIM system, used to assess food effects at later stages of drug development, intestinal fluids in different prandial states are generated in situ through a complex interplay between the added meal and preset physiological parameters, including bile secretion and gastrointestinal transit. Our findings emphasize the necessity of adjusting these physiological parameters when introducing meals of varying consistency, as meal texture clearly influences gastrointestinal dynamics. This observation is supported by a previous study from our group, which employed the tiny-TIM system to investigate the impact of meal consistency.[43] When physiological parameters such as gastric emptying kinetics were held constant, the resulting intestinal fluid composition remained largely unchanged, irrespective of whether a solid or liquid meal was administered. A comparison of those findings with the current study highlights that altering meal composition alone is insufficient to produce a biorelevant intestinal fluid profile. Therefore, rather than solely administering meals with different consistencies, we underscore the importance of adapting in vitro system parameters to reflect the physiological changes in gastrointestinal dynamics induced by these different meal consistencies.

For early stage formulation development using simple, static in vitro models, we support the approach proposed by Pyper et al. and Silva et al.[44,45] These works advocate for a multiple SIF-system that moves away from relying on a single ″average″ SIF, such as commercially available fed state SIF, to represent average fed conditions. In the context of developing robust formulations, the wide variability in HIF composition observed in vivo, and its impact on solubility outcomes, is more important than the differences between average fasted and fed states alone. Compositions deviating from the average may cause relevant variations in drug solubilization, making it essential to assess how new APIs respond to a full range of gastrointestinal conditions. Instead of a one-size-fits-all approach, generating multiple SIFs that reflect realistic variations, especially in bile salt and lipid levels, offers a more accurate prediction of in vivo behavior. Therefore, although the average composition of HIF differs between solid and liquid meals, relying on a SIF solely based on the average SM-HIF composition remains questionable. Rather, the compositional range of SM-HIF should be considered in the development of multiple SIF. Considering that SM-HIF generally shows lower variability than LM-HIF and falls within its range, designing SIFs based on LM-HIF variability should also capture most of the relevant conditions seen with solid meals.

3.4. Limitations of the Study. This study employed a solid meal which corresponded to half a portion of the standard meal as recommended in the respective FDA guidance. We decided not to administer the full portion in order to avoid technical difficulties. The full portion with its large volume and high caloric load can cause substantial gastric distension and thus, catheter displacement. In addition, the half-portion meal (478 kcal) more closely matched the caloric content of the liquid meal used in prior studies (600 kcal), enabling better comparison to previously generated data. Consequently, the study was not designed to simulate the worst-case scenario for food effect testing in regulatory settings. As compared to the full portion, reducing the meal size to half a portion most likely had an impact on various physiological parameters such as gastric emptying, bile secretion, as well as volume and composition of luminal contents. Hence, it may have also influenced the solubilization of the poorly water-soluble drugs tested in this work. Future investigations using the full FDA standard meal are warranted to delineate these effects and extend the reference data to the worst-case scenario.

Another limitation of the present work is the sample size. Whereas 21 fluid collections were successful in the liquid meal study, only eight collections were obtained in the solid meal study. This discrepancy was primarily due to the technical challenges of aspirating in the presence of solid contents, which frequently caused catheter displacement and rendered several aspirations unusable. The results should therefore be interpreted

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

in this context. Following this exploratory study, future investigations with larger sample sizes may help to confirm and expand our findings.

4. CONCLUSIONS

The data of this work show that meal consistency has a strong impact on the average HIF composition, and thus, affects the solubility of poorly water-soluble drug in HIF. Interestingly, the micellar solubilizing capacity of the micellar fraction remained largely unaffected. Despite the lower variability in SM-HIF as compared to LM-HIF, the broad substantial range in composition highlights the need to move beyond average-based SIF formulations and instead capture a broader spectrum of physiological compositions in order to assess the solubility extremes. The findings of this work underscore the importance of incorporating realistic variability into biorelevant in vitro models for the prediction of food effects.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.5c01002.

Comparison of the composition and solubilizing capacity of the LM- and SM-HIF pools; period 1 and period 2 further divided into early and late stage 1, and late stage 2 and stage 3, respectively; and R- and P-values from Spearman correlation analyses examining the relationship between the solubility of model compounds in fed-state HIF pools and the characteristics of these pools provided (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Patrick Augustijns − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium;* orcid.org/0000-0003-2595-388X; Email: patrick.augustijns@kuleuven.be

Authors

Brecht Goovaerts − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium*

Mare Oja − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium*

Sepanta Ehtemam − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium*

Joachim Brouwers − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium*

Álvaro López Mármol − *Synthetic Molecules CMC R&D, AbbVie Deutschland GmbH & Co. KG, Ludwigshafen am Rhein 67061, Germany;* orcid.org/0009-0008-5838-4840

Marlies Braeckmans − *Drug Delivery and Disposition, KU Leuven, Leuven 3000, Belgium*

Tim Vanuytsel − *Translational Research Centre for Gastrointestinal Disorders, TARGID, KU Leuven, Leuven 3000, Belgium*

Zahari Vinarov − *Department of Chemical and Pharmaceutical Engineering, Sofia University, Sofia 1164, Bulgaria*

Thomas B. Borchardt − *Product Development Science and Technology, AbbVie Inc., North Chicago, Illinois 60064, United States*

Mirko Koziolek − *Synthetic Molecules CMC R&D, AbbVie Deutschland GmbH & Co. KG, Ludwigshafen am Rhein 67061, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.molpharmaceut.5c01002

Author Contributions

#B.G. and M.O. contributed equally to this work.

Funding

This work was cofunded by the European Union (grant agreement: 101108993) and AbbVie.

Notes

ALM, TBB, and MK are employees of AbbVie and may own AbbVie stock.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank all volunteers for their participation in this study. We also thank Raf Mols for his assistance with the bile salt analysis and the staff of the Department of Gastroenterology at University Hospitals Leuven (Belgium) for their valuable support during the aspirations. M.O. is supported by the European Union (grant agreement: 101108993). Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them. T.V. is supported by a senior clinical research fellowship (1830522N) from the Flanders Research Foundation (FWO Vlaanderen). Z.V. gratefully acknowledges the support of the Bulgarian national science fund, under the National Research Program "VIHREN-2021", project 3D-GUT (No KP-06-DV-3/15.12.2021).

■ REFERENCES

(1) Dressman, J. B.; Vertzoni, M.; Goumas, K.; Reppas, C. Estimating drug solubility in the gastrointestinal tract. *Adv. Drug Deliv Rev.* **2007**, *59* (7), 591−602.

(2) Mudie, D. M.; Amidon, G. L.; Amidon, G. E. Physiological parameters for oral delivery and in vitro testing. *Mol. Pharm.* **2010**, *7* (5), 1388−1405.

(3) Clarysse, S.; Tack, J.; Lammert, F.; Duchateau, G.; Reppas, C.; Augustijns, P. Postprandial Evolution in Composition and Characteristics of Human Duodenal Fluids in Different Nutritional States. *J. Pharm. Sci.* **2009**, *98* (3), 1177−1192.

(4) Dressman, J. B.; Amidon, G. L.; Reppas, C.; Shah, V. P. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm. Res.* **1998**, *15* (1), 11−22.

(5) Fadda, H. M.; Sousa, T.; Carlsson, A. S.; Abrahamsson, B.; Williams, J. G.; Kumar, D.; et al. Drug solubility in luminal fluids from different regions of the small and large intestine of humans. *Mol. Pharm.* **2010**, *7* (5), 1527−1532.

(6) Galia, E.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res.* **1998**, *15* (5), 698−705.

(7) Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. B. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharm. Res.* **2008**, *25* (7), 1663−1676.

(8) Kalantzi, L.; Goumas, K.; Kalioras, V.; Abrahamsson, B.; Dressman, J. B.; Reppas, C. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm. Res.* **2006**, *23* (1), 165−176.

(9) Pentafragka, C.; Vertzoni, M.; Dressman, J.; Symillides, M.; Goumas, K.; Reppas, C. Characteristics of contents in the upper

gastrointestinal lumen after a standard high-calorie high-fat meal and implications for the in vitro drug product performance testing conditions. *Eur. J. Pharm. Sci.* **2020**, *155*, No. 105535.
(10) 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.
(11) Butler, J.; Hens, B.; Vertzoni, M.; Brouwers, J.; Berben, P.; Dressman, J.; et al. In vitro models for the prediction of in vivo performance of oral dosage forms: Recent progress from partnership through the IMI OrBiTo collaboration. *Eur. J. Pharm. Biopharm.* **2019**, *136*, 70−83.
(12) Vinarov, Z.; Abdallah, M.; Agundez, J. A. G.; Allegaert, K.; Basit, A. W.; Braeckmans, M.; et al. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. *Eur. J. Pharm. Sci.* **2021**, *162*, No. 105812.
(13) Hens, B.; Brouwers, J.; Corsetti, M.; Augustijns, P. Gastrointestinal behavior of nano- and microsized fenofibrate: In vivo evaluation in man and in vitro simulation by assessment of the permeation potential. *Eur. J. Pharm. Sci.* **2015**, *77*, 40−47.
(14) Koziolek, M.; Alcaro, S.; Augustijns, P.; Basit, A. W.; Grimm, M.; Hens, B.; et al. The mechanisms of pharmacokinetic food-drug interactions - A perspective from the UNGAP group. *Eur. J. Pharm. Sci. Off J. Eur. Fed Pharm. Sci.* **2019**, *134*, 31−59.
(15) Schulze, K. Imaging and modelling of digestion in the stomach and the duodenum. *Neurogastroenterol. Motil.* **2006**, *18* (3), 172−183.
(16) Meyer, J. H.; Ohashi, H.; Jehn, D.; Thomson, J. B. Size of liver particles emptied from the human stomach. *Gastroenterology* **1981**, *80* (6), 1489−1496.
(17) Malagelada, J. R.; Longstreth, G. F.; Summerskill, W. H.; Go, V. L. Measurement of gastric functions during digestion of ordinary solid meals in man. *Gastroenterology* **1976**, *70* (2), 203−210.
(18) Achour, L.; Méance, S.; Briend, A. Comparison of gastric emptying of a solid and a liquid nutritional rehabilitation food. *Eur. J. Clin. Nutr.* **2001**, *55* (9), 769−772.
(19) Van Den Abeele, J.; Rubbens, J.; Brouwers, J.; Augustijns, P. The dynamic gastric environment and its impact on drug and formulation behaviour. *Eur. J. Pharm. Sci. Off J. Eur. Fed. Pharm. Sci.* **2017**, *96*, 207−231.
(20) Camilleri, M.; Malagelada, J. R.; Brown, M. L.; Becker, G.; Zinsmeister, A. R. Relation between antral motility and gastric emptying of solids and liquids in humans. *Am. J. Physiol.* **1985**, *249*, G580−G585.
(21) Kelly, K. A. Gastric emptying of liquids and solids: roles of proximal and distal stomach. *Am. J. Physiol.* **1980**, *239* (2), G71−G76.
(22) Abuhelwa, A. Y.; Williams, D. B.; Upton, R. N.; Foster, D. J.R. Food, gastrointestinal pH, and models of oral drug absorption. *Eur. J. Pharm. Biopharm* **2017**, *112*, 234−248.
(23) Hörter, D.; Dressman, J. B. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv. Drug Deliv. Rev.* **1997**, *25* (1), 3−14.
(24) FDA. *Assessing the Effects of Food on Drugs in INDs and NDAs − Clinical Pharmacology Considerations [Internet]*; FDA; 2022 [cited 2025 May 20]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/assessing-effects-food-drugs-inds-and-ndas-clinical-pharmacology-considerations.
(25) Rubbens, J.; Brouwers, J.; Tack, J.; Augustijns, P. Gastric and Duodenal Diclofenac Concentrations in Healthy Volunteers after Intake of the FDA Standard Meal: In Vivo Observations and in Vitro Explorations. *Mol. Pharm.* **2019**, *16* (2), 573−582.
(26) Goovaerts, B.; Brouwers, J.; Vinarov, Z.; Braeckmans, M.; Indulkar, A. S.; Lopez Marmol, A.; et al. Understanding the Impact of Lipids on the Solubilizing Capacity of Human Intestinal Fluids. *Mol. Pharm.* **2024**, *21*, 6398−6410.
(27) Hristova, K.; Wimley, W. C. Determining the statistical significance of the difference between arbitrary curves: A spreadsheet method. *PLoS One* **2023**, *18* (10), No. e0289619.
(28) Yu, J.; Yu, D.; Lane, S.; McConnachie, L.; Ho, R. J.Y. Controlled Solvent Removal from Antiviral Drugs and Excipients in Solution Enables the Formation of Novel Combination Multi-Drug-Motifs in Pharmaceutical Powders Composed of Lopinavir, Ritonavir and Tenofovir. *J. Pharm. Sci.* **2020**, *109* (11), 3480−3489.
(29) van der Lee, R.; Pfaffendorf, M.; Koopmans, R. P.; van Lieshout, J. J.; van Montfrans, G. A.; van Zwieten, P. A. Comparison of the time courses and potencies of the vasodilator effects of nifedipine and felodipine in the human forearm. *Blood Press.* **2001**, *10* (4), 217−222.
(30) Williams, H. D.; Ford, L.; Han, S.; Tangso, K. J.; Lim, S.; Shackleford, D. M.; et al. Enhancing the Oral Absorption of Kinase Inhibitors Using Lipophilic Salts and Lipid-Based Formulations. *Mol. Pharm.* **2018**, *15* (12), 5678−5696.
(31) Schöller-Gyüre, M.; Kakuda, T. N.; Raoof, A.; De Smedt, G.; Hoetelmans, R. M.W. Clinical Pharmacokinetics and Pharmacodynamics of Etravirine. *Clin Pharmacokinet* **2009**, *48* (9), 561−574.
(32) Bhardwaj, S. P.; Arora, K. K.; Kwong, E.; Templeton, A.; Clas, S. D.; Suryanarayanan, R. Correlation between molecular mobility and physical stability of amorphous itraconazole. *Mol. Pharm.* **2013**, *10* (2), 694−700.
(33) Kumar, S.; Jog, R.; Shen, J.; Zolnik, B.; Sadrieh, N.; Burgess, D. J. Formulation and performance of danazol nano-crystalline suspensions and spray dried powders. *Pharm. Res.* **2015**, *32* (5), 1694−1703.
(34) Steuer, C.; Huber, A. R.; Bernasconi, L. Where clinical chemistry meets medicinal chemistry. Systematic analysis of physico-chemical properties predicts stability of common used drugs in gel separator serum tubes. *Clin. Chim. Acta Int. J. Clin. Chem.* **2016**, *462*, 23−27.
(35) Vertzoni, M.; Alsenz, J.; Augustijns, P.; Bauer-Brandl, A.; Bergström, C.; Brouwers, J.; et al. UNGAP best practice for improving solubility data quality of orally administered drugs. *Eur. J. Pharm. Sci.* **2022**, *168*, No. 106043.
(36) Kong, F.; Singh, R. Disintegration of Solid Foods in Human Stomach. *J. Food Sci.* **2008**, *73* (5), R67−R80.
(37) Mehdikhani, E.; Walter, M. Chapter 13 - Gastroparesis. In *GI/Liver Secrets (Fourth Edition)*, McNally, P. R., ed.; Mosby: Philadelphia, 2010; pp. 86−93.
(38) Grundy, S. M.; Mok, H. Y. Determination of cholesterol absorption in man by intestinal perfusion. *J. Lipid Res.* **1977**, *18* (2), 263−271.
(39) Turley, S. D.; Dietschy, J. M. The intestinal absorption of biliary and dietary cholesterol as a drug target for lowering the plasma cholesterol level. *Prev. Cardiol.* **2003**, *6* (1), 29−64.
(40) Tan, Y.; Zhang, Z.; Liu, J.; Xiao, H.; McClements, D. J. Factors impacting lipid digestion and nutraceutical bioaccessibility assessed by standardized gastrointestinal model (INFOGEST): oil. *Food Funct.* **2020**, *11* (11), 9936−9946.
(41) Kaminski, D. L.; Nahrwold, D. L. Neurohormonal control of biliary secretion and gallbladder function. *World J. Surg.* **1979**, *3* (4), 449−456.
(42) Mutirangura, P.; Siwawetkul, W. Gallbladder contraction capacity in response to liquid fatty meal: a real time ultrasonographic study. *J. Med. Assoc Thail. Chotmaihet. Thangphaet.* **1996**, *79* (10), 640−647.
(43) Goovaerts, B.; Mármol, Á. L.; Brouwers, J.; Vinarov, Z.; Indulkar, A. S.; Borchardt, T. B.; et al. Tiny-TIM intestinal fluids versus human intestinal fluids: A comparison of their composition and solubilizing capacity for poorly soluble drugs. *Eur. J. Pharm. Sci.* **2025**, *211*, No. 107150.
(44) Pyper, K.; Brouwers, J.; Augustijns, P.; Khadra, I.; Dunn, C.; Wilson, C. G.; et al. Multidimensional analysis of human intestinal fluid composition. *Eur. J. Pharm. Biopharm.* **2020**, *153*, 226−240.
(45) Silva, M. I.; Khadra, I.; Pyper, K.; Halbert, G. W. Structured solubility behaviour in fed simulated intestinal fluids. *Eur. J. Pharm. Biopharm.* **2023**, *193*, 58−73.

https://doi.org/10.1021/acs.molpharmaceut.5c01002
Mol. Pharmaceutics XXXX, XXX, XXX−XXX