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Simulating Fasted Human Intestinal Fluids: Understanding the Roles of Lecithin and Bile Acids

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Abstract: The purpose of this work is to evaluate the roles of lecithin and bile salts in a new generation of fasted simulated small intestinal fluid (FaSSIF-II), thus enhancing the closer mimic of simulated fluids to the real human intestinal fluids (HIF) in drug discovery and drug product development. To assess the effects of lecithin in FaSSIF-II, solubility studies were conducted at 37 °C using four media including first generation simulated intestinal fluid (FaSSIF-I), FaSSIF-II, phosphate pH 6.5 buffer, and HIF. A total of 24 model compounds representing a wide range of biopharmaceutic properties were included. The drug solubility values measured in the FaSSIF-II were compared with those in FaSSIF-I, pH 6.5 buffer and HIF. To assess the effects of bile acids, solubility was measured for 4 compounds in the FaSSIF-I containing five different bile acids of various concentrations. The lecithin concentration in the FaSSIF-II is lowered from 0.75 mM to 0.2 mM. The results suggested that the FaSSIF-II is a better medium to reflect HIF, compared with pH 6.5 phosphate buffer and FaSSIF-I. Solubility of neutral compounds including atovaquone, carbamazepine, cyclosporine, danazol, diethylstilbestrol, felodipine, griseofulvin and probucol in FaSSIF-II showed improvement in predicting the *in vivo* solubility. The relative standard deviation (SD) of solubility measurement in FaSSIF-II is comparable with FaSSIF-I. For the acidic and basic tested compounds, the FaSSIF-II performs similarly to the FaSSIF-I. Experimental results showed that the level of bile salts typically is less than 5 mM under fasted state. Among the five studied bile acids, the conjugation (glycine or taurine) has no impact on the drug solubilization, while there may be a minimal effect of the degree of hydroxylation of the steroid ring system on solubilization. The lecithin concentration of 0.2 mM in FaSSIF-II has been demonstrated to closely represent HIF, for both neutral and ionizable compounds. In the composition of simulated intestinal fluids, the structure of bile acids has minimal effect, providing the flexibility of choosing one bile salt to represent complex *in vivo* bile acids.

Keywords: Intestinal fluids; lecithin; bile acids; drug solubility

Introduction

Across the pharmaceutical community, *in vitro* testing models have been well recognized and extensively utilized, playing an eminent role through the spectrum of stages in drug development. Reliable and reproducible correlation between *in vitro* models and *in vivo* human clinical observations, however, remains challenging to development scientists due to numerous causes. Ultimately, the drug products will be investigated in humans, with greater clinical merits in patients. However, the use of human subjects is appreciably hampered by concerns in ethics, especially for seriously diseased therapeutic areas such as oncology where recruiting patients only to seek formulation development is almost

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impossible. In addition, restrictions of extensive cost and competition of marketing timeline constitute additional shortcomings. Therefore, the importance of using *in vitro* models in drug research and development is evident. The key is to ensure that the *in vitro* testing is scientifically equipped with the power of predicting *in vivo* characteristics, thus enabling meaningful extrapolation to the *in vivo* performance. This approach has drawn attention increasingly because it serves as a valuable tool to profile lead compounds in the early stage and to optimize drug products in the late stage of drug development. Vital areas most desiring *in vitro* tools to predict the *in vivo* performance transpire in forecasting the bioavailability and absorption of wide-ranging drug molecules and the corresponding dosage forms through various stages of pharmaceutical development. Specifically, this paper reports further investigations and the advanced understanding of *in vitro* simulated small intestinal fluid, thus significantly improving the estimate of drug substance solubility and drug product dissolution *in vivo*.

Solubility measurements of drug substances and dissolution testing of drug products are the most common analytical methodologies to evaluate orally administered dosage forms. Driving forces to determine the solubility of active pharmaceutical ingredient (API) and the dissolution profiles of drug product exist for diverse purposes. The ultimate goal is to ensure the efficacy and safety of marketed products in patients. Therefore, one of the important purposes of conducting *in vitro* solubility and dissolution measurement is to closely link the *in vitro* values with the solubility and dissolution of *in vivo*, namely, to develop *in vitro* systems predictive of *in vivo* solubility or dissolution. *In vivo* predictive solubility is utilized in screening and selecting lead compounds, and *in vivo* predictive dissolution is employed in establishing and optimizing drug product compositions and manufacturing processes. In addition, such knowledge can be further used as input parameters for *in silico* modeling and simulation, thus reducing the guesswork and improving the prediction accuracy to effectively support product development.

To establish *in vivo* predictive solubility or dissolution methodologies, many aspects, such as gastrointestinal (GI) hydrodynamics, transit time and GI media, deserve attention. The aspirated human intestinal fluid (HIF), when it is used as a GI medium determining *in vivo* solubility or dissolution, is the gold standard. Evidently, HIF is composed of numerous ingredients essential to solubility and dissolution, which are bile acids, enzymes, buffer capacity species and other ions. The practical expenditure of HIF, however, is constrained due to its limited availability, exceptional price and exigent storage conditions. As an alternative, simulated intestinal fluid is certainly turning into an attractive option for pharmaceutical scientists.

To closely mimic true human intestinal fluids, several aspects merit careful consideration in developing a sensible simulated fluid, namely, the pH, the species and capacity of the medium buffer, ionic strength, enzymes, phospholipids, and bile acids. Pioneering investigations have been published over the past decade, where continuous understanding of fasted human small intestinal fluids has been gained and significant progress has been made.[1−7] The initial effort to reflect an *in vivo* GI fluid was to adjust the pH values of small intestine changing from pH 7.2 to 6.8.[8] Later, the first and now the widely employed simulated intestinal fluid has been FaSSIF, which was proposed by Dressman and Galia, based on the knowledge of dog and human small intestine fluids in 1998.[2,9] Then, the comparison of simulated fluids with HIF was realized as a result of advanced techniques of aspirating human small intestinal fluids. The early attempts of characterizing HIF were conducted by Lindahl et al.,[1] and subsequently both solubility and intrinsic dissolution rate (IDR) of hydrocortisone were compared using simulated intestinal fluids (SIFs) and HIF under the fasted state.[3] Further investigations were carried out to characterize the HIF under not only the fasted state but also under the fed state.[6] Similar comparison but with the target of simulating the conditions of FDA bioavailability and bioequivalence studies was also accomplished.[10] In addition, comparison between dog intestinal fluids and HIF was addressed recently.[11]

In 2008, Jantratid et al. updated the current FaSSIF, namely, FaSSIF-V2, in which a lower concentration of lecithin, reduced osmolality and new buffer species of maleic

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acid were proposed.[12] Validation of FaSSIF-V2 with model compounds of diverse physicochemical properties is yet to be established. The focus of this work is to investigate the role of phospholipids such as lecithin and bile acids in simulated human small intestinal fluids. Specifically, this research is to enhance further understanding of the impact of the concentration levels of lecithin and bile acid structures on solubility of poorly soluble compounds and on the solubilizing capacity of simulated intestinal fluids. In addition to enhance the understanding of *in vivo* performance, the physical stability of current FaSSIF is unsatisfactory. Therefore, for practical reasons simulated fluids with improved stability is desirable.

The outcome of this investigation is applicable to the following phases of drug product development: (1) early stage development in selecting lead drug candidates by reducing early clinical failures due to undesirable exposure in human, (2) late stage development by speeding up the dosage platform and formulation ranking, and (3) the NDA stage of bioequivalence (BE) studies bridging clinical pivotal to commercial formulation for innovators and general BE studies for generic industries, and to *in vitro* dissolution testing under the concept of FDA initiative of Quality-by-Design (QbD).

Materials and Methods

Materials. Sodium taurocholate was purchased from Prodotti Chimici E Alimentari S.P.A, (Basaluzzo, Italy) and lipoid E PC S from Lipoid Gmbh (Ludwigshafen, Germany). Glycodeoxycholic acid (GDCA) and glycocholic acid (GCA) were purchased from Calbiochem (USA). Glycochenodeoxycholic acid (GCDCA) and taurodeoxycholic acid (TDCA) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Aprepitant and felodipine were supplied in house (AstraZeneca R&D, Mölndal, Sweden). Amiodarone hydrochloride, carbamazepine, cyclosporin A, danazol, dipyridamole, flufenamic acid, glipizide, ketoconazole, nimesulid, piroxicam, probenecid, rimonabant, sulfasalazine and terfenadine were all from Sigma-Aldrich. Carvedilol was purchased from BIONET Research Intermediates (Camelford, U.K.); diclofenac acid was purchased from Tyger Sci. Inc. (Ewing, NJ); diethylstilbestrol was purchased from TCI Europe (Zwijndrecht, Belgium); griseofulvin was purchased from Acros Organics (Morris Plains, NJ); irbesartan was purchased from Kemprotec Lim. (Dorset, U.K.); probucol was purchased from ICN Biomedicals (Eschwege, Germany); retinol was purchased from Fluka Chemie Gmbh (Germany); and warfarin and atovaquone were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada).

All water used was produced by a Millipore Milli-Q System (Molsheim, France). Methanol, acetonitrile and trifluoroacetic acid were of gradient grade and supplied from Merck KGaA (Darmstadt, Germany). Acetic acid (99−100%) came from J. T. Baker (Deventer, Holland).

Preparation of FaSSIF-I, FaSSIF-II and pH 6.5 Buffer. 1.65 g of sodium taurocholate and lecithin (0.59 or 0.152 g) were weighed into a 1 L volumetric flask for preparation of FaSSIF-I and FaSSIF-II, respectively. Approximately 200 mL of phosphate buffer pH 6.5 (1.7 g of NaOH, 20 g of $NaH_2PO_4 \cdot H_2O$ and 31 g of NaCl to 5 L, pH was adjusted to 6.5 with 1 M HCl or 1 M NaOH) was transferred to the flask. The mixture was stirred until a clear solution was obtained, after which the volume was adjusted to 1 L with phosphate buffer pH 6.5.

Preparation of Mixed Micelle Solutions with Various Bile Acids. The impact of bile acid molecular structure on the solubilization capacity of mixed micelle solutions was investigated using five different bile acids: glycocholic acid (GCA), taurocholic acid (TCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), and glycochenodeoxycholic acid (GCDCA). Mixed micelle stock solutions of each bile acid (20 mM) were prepared and diluted with phosphate buffer pH 7.0 to concentrations of 15, 10, 6, 3, and 1 mM. The bile acid:lecithin ratio was chosen to be 4:1.

Collection of HIF. The sampling of the human intestinal fluid was performed at the Clinical Research Department of the University Hospital in Uppsala, Sweden, and was approved by the Ethics Committee of the Medical Faculty at Uppsala University. All subjects had been given informed consent to their participation in this study. Jejunal fluids were aspirated from healthy, fasted volunteers via an oral intubation tube (Loc-I-Gut).[13,14] The tube is 175 cm long with an external diameter of 5.3 mm. It is a multichannel polyvinyl tube with two inflatable balloons 10 cm apart and a tungsten weight at the tip. The position of the tube was checked fluoroscopically, and once the desired location was reached, only the lower balloon was inflated with 25−30 mL of air to prevent fluid from passing down the gastrointestinal tract, thus achieving complete sampling of jejunal fluids. Fluids were collected from the jejunum by continuous vacuum drainage without prior administration of liquid. A separate tube was positioned in the stomach to drain gastric fluid to prevent nausea. The intestinal fluid from each subject was collected on ice, pooled with fluids from the other subjects

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and stored at −70 °C. Each pooled HIF batch was subsequently characterized with respect to pH, buffer capacity, osmolality, and content of bile acids, phospholipids, and neutral lipids. All the properties of the HIF batches used here were inside the ranges normally found for fasted state HIF.[15]

Solubility Measurements. The solubility of the model substances was determined in phosphate buffer pH 6.5, simulated intestinal media and HIF. An excess amount of substance was weighed into a vial (approximately 1 mg/mL, $n = 3$), and 1 mL of medium was added. The vials were placed in a Thermomixer comfort (Eppendorf, Hamburg, Germany) for 24 h at 37 °C and 1000 rpm (shaking of retinol was done in darkness and under anaerobic conditions). A volume of 200 μL of each sample was withdrawn after 24 h, transferred to Eppendorf tubes, and centrifuged (Hettich mikro 200R, Tuttlingen, Germany) for 15 min at 37 °C and 10000 rpm. 100 μL of the supernatant was transferred to HPLC vials and diluted with the mobile phase. For HIF samples, the centrifugation was repeated after the dilution to remove precipitated protein. The supernatant produced after the second centrifugation was transferred to HPLC vials.

Analytical Methods - HPLC. The concentration of all model substances was analyzed by HPLC with UV detection (Waters Alliance 2695 separation module connected to a Waters 2487 dual band UV detector, Waters, Milford, MA, USA). Samples or standards of 80 μL were injected. HPLC conditions were set up in accordance with Table 1. Substance for standards were dissolved in a small volume of methanol and further diluted ($n = 2$ for each substance) in mobile phase. The standard concentrations were within 50% of the concentrations for the samples. All samples were over LOQ (definition as 5 times of the baseline noise). RSD for the areas from at least 6 standard injections in all analysis was under 5%. Empower 2 (Waters) was used to collect and process all chromatographic data.

Calculation of Solubilization Capacity. The ability of each of the tested simulated media to correctly predict the solubility in the human small intestine was assessed by calculating the following ratio:

$$\frac{S_{\text{simulated medium}}}{S_{\text{HIF}}}$$

where $S_{\text{simulated medium}}$ is the solubility in each of the simulated media and S_{HIF} the solubility in HIF. The obtained ratio indicates the degree of discrepancy in solubility between a simulated medium and the real human intestinal fluid, and the closer the ratio to the value of 1 the more *in vivo* predictive a simulated medium is.

Light Scattering Measurements. The physical stability of FaSSIF-I and FaSSIF-II during storage in room temperature was monitored over a period of 7 days by light scattering measurements using a Perkin-Elmer LS55 Fluoresence Spectrometer. Excitation and emission wavelengths were both set to 700 nm, and the intensity of scattered light was determined at a scattering angle of 90°. Measurements were made on the preparation day (day 0), day 1, day 2 and day 7 post preparation.

Results

Effect of Phospholipid Concentration on Drug Solubility: Comparing FaSSIF-I and FaSSIF-II. The solubilities in phosphate buffer pH 6.5, FaSSIF-I, FaSSIF-II and HIF of 8 neutral, 10 acidic and 6 basic compounds are listed in Table 2. For acidic and basic compounds the pH at equilibrium is one of the important factors determining the solubility. Hence, the supernatant pH was determined, but not adjusted, for the ionizable compounds. Figure 1 shows the supernatant pHs at equilibrium for those compounds. A comparison of the pH at equilibrium with the pK_a values listed in Table 2 indicates that all ionizable compounds were completely, or at least to a large extent, charged in all media at equilibrium. The results also indicate that the dissolution of the ionizable compounds had only a minor impact on the pH in the simulated media as expected for poorly soluble acids and bases. The only exception was the acid probenecid, for which the equilibrium pH decreased from the initial pH 6.5 to pH <6 as a result of its relatively high solubility. Several different HIF batches with pHs ranging from 6.5 to 7.5 were used, which explains the scattering of equilibrium pH observations. However, the dissolution of acids and bases

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Table 1. Analytical HPLC Conditions for the Solubility Measurement of Investigated Compounds in FaSSIF-I, FaSSIF-II, pH 6.5 Buffer and HIF

substance	flow rate (mL/min)	mobile phase (v/v)	isocratic or gradient	wavelength (nm)	column
amiodarone	1.0	methanol/phosphate buffer pH = 3, i = 0.05 (83:17)	isocratic	242	Waters XTerra RP8 (3.9 × 100 mm, 3.5 μm)
aprepitant	1.0	acetonitrile/water (60:40)	isocratic	210	Waters XTerra C18 (4.6 × 150 mm, 5 μm)
atovaquone	0.8	(A) acetonitrile/water (20:80) + 0.1% TFA (B) acetonitrile + 0.1% TFA	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	254	Waters Symmetry C18 (3.0 × 50 mm, 3.5 μm)
carbamazepine	1.0	acetonitrile/water (30:70)	isocratic	220 (3.9 × 100 mm, 3.5 μm)	Waters XTerra RP8
carvedilol	0.8	(A) acetonitrile/water (20:80) + 0.1% TFA (B) acetonitrile + 0.1% TFA	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	240	Waters Atlantis T3 (4.6 × 150 mm, 3 μm)
cyclosporin a	1.0	acetonitrile/water (71:29)	isocratic	212	Agilent Zorbax Eclipse C18 (4.6 × 150 mm, 5 μm)
danazol	1.0	acetonitrile/50 mM acetic acid (60:40)	isocratic	270	Agilent Zorbax Eclipse C18 (4.6 × 150 mm, 5 μm)
diclofenac	1.0	(A) acetonitrile/water (10:90) (B) acetonitrile	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	280	Waters Atlantis dC18 (4.6 × 150 mm, 5 μm)
diethylstilbestrol	1.0	(A) acetonitrile/water (20:80) (B) acetonitrile	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	254	Waters Atlantis dC18 (4.6 × 150 mm, 5 μm)
dipyridamole	1.0	(A) acetonitrile/water (10:90) + 0.1% TFA (B) acetonitrile + 0.1% TFA	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	280	Waters Atlantis dC18 (4.6 × 150 mm, 5 μm)
felodipine	1.0	acetonitrile/methanol/phosphate buffer pH = 3, i = 0.05 (40:20:40)	isocratic	220	Waters XTerra C18 (4.6 × 150 mm, 5 μm)
flufenamic acid	1.0	(A) acetonitrile/water (10:90) (B) acetonitrile	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	280	Waters Atlantis dC18 (4.6 × 150 mm, 5 μm)
glipizide	1.0	(A) acetonitrile/water (10:90) (B) acetonitrile	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	230	Waters Atlantis dC18 (4.6 × 150 mm, 5 μm)
griseofulvin	1.0	acetonitrile/50 mM acetic acid (37:63)	isocratic	254	Waters XTerra C18 (4.6 × 150 mm, 5 μm)
irbesartan	0.8	(A) acetonitrile/water (10:90) (B) acetonitrile	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	280	Waters Xbridge C18 (4.6 × 100 mm, 3.5 μm)
ketoconazole	1.0	methanol/acetonitrile (75:25)	isocratic	254	Agilent Zorbax Eclipse C18 (4.6 × 150 mm, 5 μm)
nimesulid	1.0	methanol/phosphate buffer pH = 3, i = 0.05 (55:45)	isocratic	258	Waters XTerra RP8 (3.9 × 100 mm, 3.5 μm)
piroxicam	0.8	(A) acetonitrile/water (20:80) + 0.1% TFA (B) acetonitrile + 0.1% TFA	0 min: 100% A 10 min: 10% A 12 min: 10% A 14 min: 100% A	330	Waters Atlantis T3 (4.6 × 150 mm, 3 μm)
probucol	1.0	acetonitrile/water (85:15)	isocratic	254	Waters XTerra RP8 (3.9 × 100 mm, 3.5 μm)

Table 1. Continued

substance	flow rate (mL/min)	mobile phase (v/v)	isocratic or gradient	wavelength (nm)	column
probenecid	1.0	(A) acetonitrile/water (10:90) + 0.1% TFA	0 min: 100% A	242	Waters Atlantis dC18
		(B) acetonitrile + 0.1% TFA	10 min: 10% A		(4.6 × 150 mm, 5 μm)
			12 min: 10% A		
			14 min: 100% A		
retinol	1.0	(A) acetonitrile/water (10:90) + 0.1% TFA	0 min: 100% A	285	Waters Atlantis dC18
		(B) acetonitrile + 0.1% TFA	10 min: 10% A		(4.6 × 150 mm, 5 μm)
			12 min: 10% A		
			14 min: 100% A		
rimonabant	1.0	acetonitrile/water (70:30)	isocratic	210	Waters XTerra RP8 (3.9 × 100 mm, 3.5 μm)
sulfasalazine	0.8	(A) acetonitrile/water (20:80) + 0.1% TFA	0 min: 100% A	228	Waters Atlantis T3
		(B) acetonitrile + 0.1% TFA	10 min: 10% A		(4.6 × 150 mm, 3 μm)
			12 min: 10% A		
			14 min: 100% A		
terfenadine	1.0	acetonitrile/50 mM acetic acid (75:25)	isocratic	230	Waters XTerra RP8 (3.9 × 100 mm, 3.5 μm)
warfarin	1.0	(A) acetonitrile/water (10:90)	0 min: 100% A	280	Waters Atlantis dC18
		(B) Acetonitrile	10 min: 10% A		(4.6 × 150 mm, 5 μm)
			12 min: 10% A		
			14 min: 100% A		

Table 2. Solubility Values [Relative SD] of Investigated Compounds in Phosphate Buffer pH 6.5, FaSSIF-I, FaSSIF-II, and HIF

	solubility (μg/mL)			
compound	phosphate buffer pH 6.5	FaSSIF-I	FaSSIF-II	HIF
Neutral				
atovaquone	0.43[24]	1.9 [0.8%]	0.40 [7.7%]	0.32 [5.3%]
carbamazepine	127 [2.6%]	236 [0.5%]	132 [0.3%]	283 [2.0%]
cyclosporine	1.9 [5.8%]	5.7 [8.7%]	2.2 [7.8%]	3.5 [11%]
danazol	0.50 [7.9%]	8.7 [11%]	3.5 [42%]	4.9 [22%]
diethylstilbestrol	13 [2.0%]	40 [3.3%]	22 [2.1%]	38 [31%]
felodipine	1.0 [9.1%]	53 [3.1%]	12 [1.8%]	14 [0.2%]
griseofulvin	15 [0.3%]	20 [4.4%]	17 [1.4%]	17 [7.8%]
probucol	0.006	3.4 [7.9%]	1.6 [43%]	0.92 [49%]
Acidic/pK_a				
diclofenac/4.1[25]	486 [2.6%]	762 [1.6%]	678 [15%]	892 [1.3%]
flufenamic acid/4.0[25]	502 [1.2%]	931 [8.7%]	874 [1.9%]	428 [0.2%]
glipizide/5.1[25]	29 [3.9%]	58 [5.7%]	65 [17%]	42 [2.2%]
irbesartan/3.7; 4.4[26]	102 [3.9%]	112 [3.0%]	106 [1.0%]	123 [3.0%]
nimesulide/6.5[27]	23 [30%]	36 [11%]	26 [9.9%]	85 [27%]
piroxicam/1.9; 5.3[25]	176 [1.8%]	339 [2.0%]	309 [14%]	397 [1.0%]
probenecid/3.4[28]	1291 [8.0%]	1632 [9.4%]	1695 [5.2%]	738 [4.8%]
rimonabant	0.19 [8.6%]	11 [5.8%]	3.3 [18%]	5.4 [5.2%]
sulfasalazine/2.5; 8.1[25]	588 [4.5%]	761 [23%]	567 [16%]	544 [36%]
warfarin/4.9[25]	318 [3.9%]	443 [1.5%]	437 [3.7%]	313 [3.2%]
Basic/pK_a				
amiodarone/8.7[29]	0.032[29]	351 [10%]	6 [35%]	376 [27%]
aprepitant/4.0; 8.1[30]	0.37 [4.1%]	23 [2.0%]	5.2 [19%]	13 [22%]
carvedilol/8.0[29]	78 [4.1%]	136 [2.6%]	89 [1.1%]	36 [1.4%]
dipyridamole/6.4[29]	5.4 [4.8%]	19 [2.2%]	18 [1.1%]	29 [1.4%]
ketoconazole/2.9; 6.5[31]	6.5 [23%]	26 [20%]	11 [9.4%]	56 [7.6%]
terfenadine/9.2[32]	13 [12%]	104 [11%]	43 [16%]	not measured

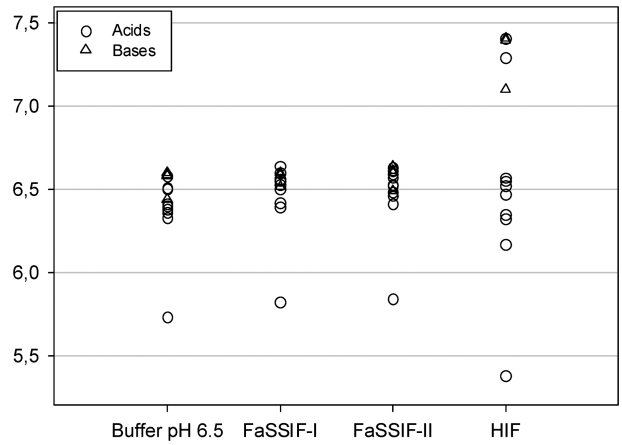

Figure 1. The pH values at solubility equilibrium in buffer pH 6.5, FaSSIF-I, FaSSIF-II, and HIF for the investigated acids and bases.

influenced the pH only marginally also in HIF, again with the exception of probenecid.

The major determinant of the solubility of neutral compounds in HIF is the content of solubilizing agents, i.e. primarily bile acids. The bile acid concentration in the HIF batches used in the present study was very consistent, approximately 2−3 mM. In Figure 2 the solubility ratio, $S_{\text{simulated medium}}/S_{\text{HIF}}$, is shown for all investigated compounds in all simulated media (phosphate buffer pH 6.5, FaSSIF-I and FaSSIF-II). For neutral compounds, FaSSIF-I tends to over-predict the solubility in HIF while phosphate buffer pH 6.5 clearly under-predicts the solubility in HIF. FaSSIF-II is the simulated medium that appears to provide the best agreement with HIF. For ionizable compounds the overall predictive capability appeared to be very similar for all tested simulated media (Figure 2b). With the exception of one outlier, the weak base amiodarone, FaSSIF-I and FaSSIF-II provided essentially the same *in vivo* solubility predictions

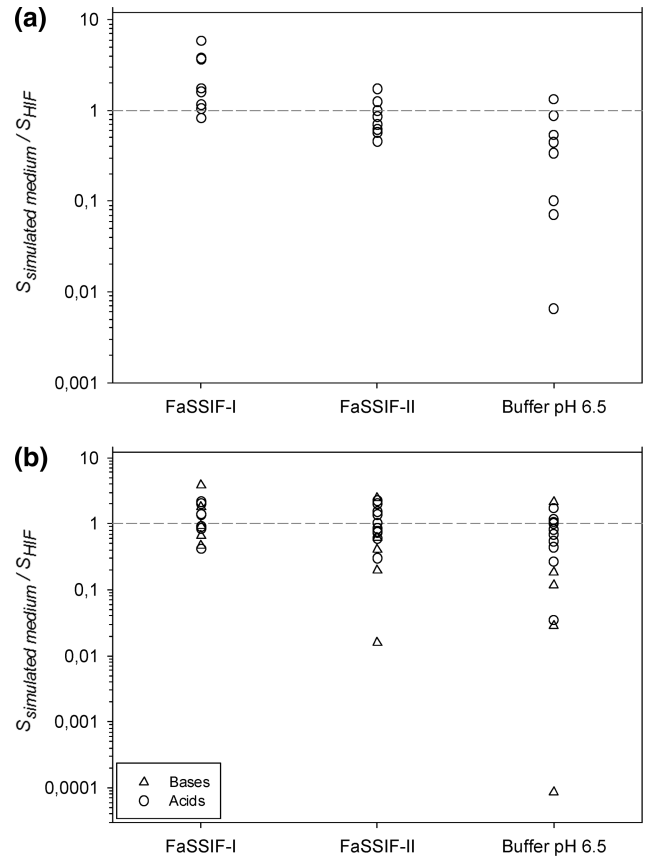

Figure 2. Solubility ratios of investigated poorly solubles in FaSSIF-I, FaSSIF-II and buffer pH 6.5 compared to HIF: (a) neutral compounds and (b) acids and bases.

while the plain buffer pH 6.5 was slightly less predictive providing more variable results. The relationship between the solubility in FaSSIF-II and FaSSIF-I is shown in Figure 3. Both for neutral and ionizable compounds the solubility in FaSSIF-II appears to be correlated in a linear fashion with the solubility in FaSSIF-I, although data for neutral compounds are limited. However, for neutral compounds the slope of the regression line (Figure 3a), $k = 0.56$, indicates that the solubility in FaSSIF-II was approximately half the solubility in FaSSIF-I. For ionizable compounds the solubilities in FaSSIF-II and FaSSIF-I were approximately the same, as indicated by the slope (Figure 3b), $k = 0.99$.

Physical Stability of Simulated Media. The physical stability of simulated media was assessed by light scattering

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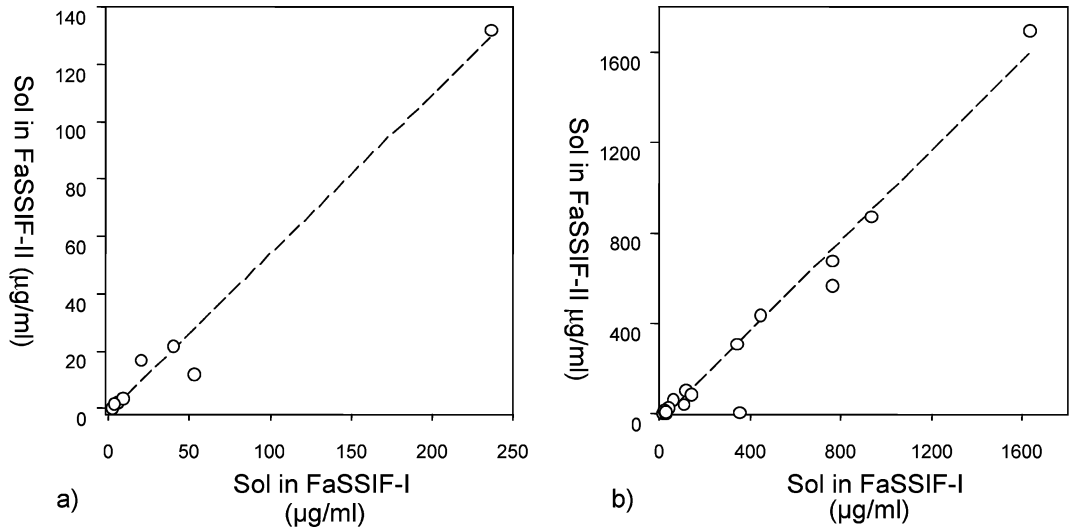

Figure 3. Solubility of investigated compounds in FaSSIF-II vs FaSSIF-I of (a) neutral compounds and (b) acids and bases.

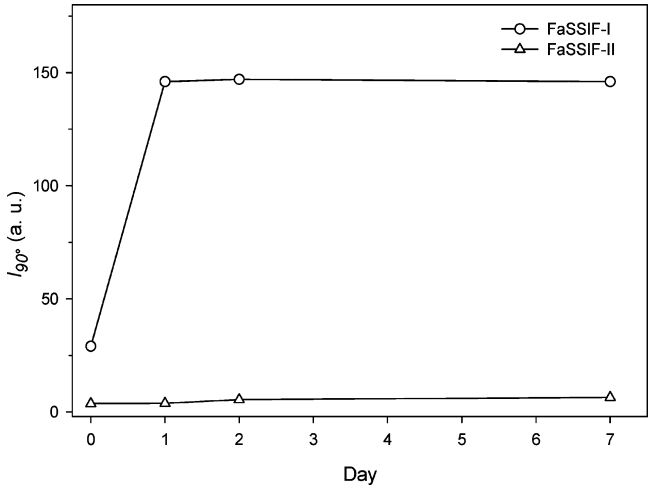

Figure 4. Scattered light intensity in arbitrary units at a scattering angle of 90° for FaSSIF-I and FaSSIF-II during storage at room temperature ($n = 1$).

measurements indicating the presence and growth of lipid aggregates. The intensity of scattered light in arbitrary units at a scattering angle of 90° from FaSSIF-I and FaSSIF-II during storage in room temperature during 1 week post preparation is shown in Figure 4. Although both simulated media appeared visually clear on preparation day (day 0), FaSSIF-I actually scattered light slightly more than FaSSIF-II. Then, from day 1 onward, FaSSIF-I appeared turbid to the eye, indicating a phase separation, while FaSSIF-II remained clear. Consistent with the visual observations, from day 1 the light scattering from FaSSIF-I was significantly larger than from FaSSIF-II. For FaSSIF-II the light scattering intensity remained at close to background level during the entire experiment period. Furthermore, FaSSIF-II remained visually clear upon storage at room temperature for several weeks after preparation.

Effect of Bile Acid Structures on Solubilization Capacity of Simulated Intestinal Fluids. Figure 5 shows the solubilization curves for four neutral model compounds in solutions containing varying types and concentrations of bile acid (BA)/lecithin mixed micelles, where the ratio of bile acid to the lecithin remained constant, namely, 4:1.

Discussions

Role of Lecithin in the Simulated Intestinal Fluids. The lecithin product in the present study primarily consists of phosphatidylcholine, with only very minor amounts of other phospholipids or neutral lipids. Phospholipid is the most important feature in mimicking *in vivo* GI fluids, where it affects both the solubilization of poorly soluble drugs and the stability of simulated fluids. Attributed to the inherent amphipathic properties of phospholipids, lecithin interacts with surfactants such as bile salts to form lipid aggregates that enhance the apparent drug solubility. Meanwhile, due to the low solubility of 4.6×10^{-10} mol/L in water,[16] lecithin forms liposomes, bilayer sheets, or lamellar structures, in aqueous solution, which is the major cause of physical instability manifested as visual cloudiness of FaSSIF after storage at room temperature beyond 1 day.

In the current modified simulated small intestine fluid, the concentration of lecithin is reduced from 0.75 mM to 0.2 mM, based on the recent findings of *ex vivo* small intestinal fluids.[11,12] In terms of comparable solubility enhancement, current evidence suggests that lecithin concentration of 0.2 mM provides closer or comparable reflection of HIF, the gold standard of *in vivo* GI fluids, in comparison to the conventional FaSSIF-I. First for neutral compounds (Figure 2a), an improvement of *in vivo* reflection to HIF was achieved by decreasing the overestimated solubility values resulted from the FaSSIF-I that contains a higher lecithin content of 0.75 mM than the aspirated HIF. This observation contrasts with results from a recent comparison of solubilities of BCS class II drugs in simulated and true intestinal fluid,

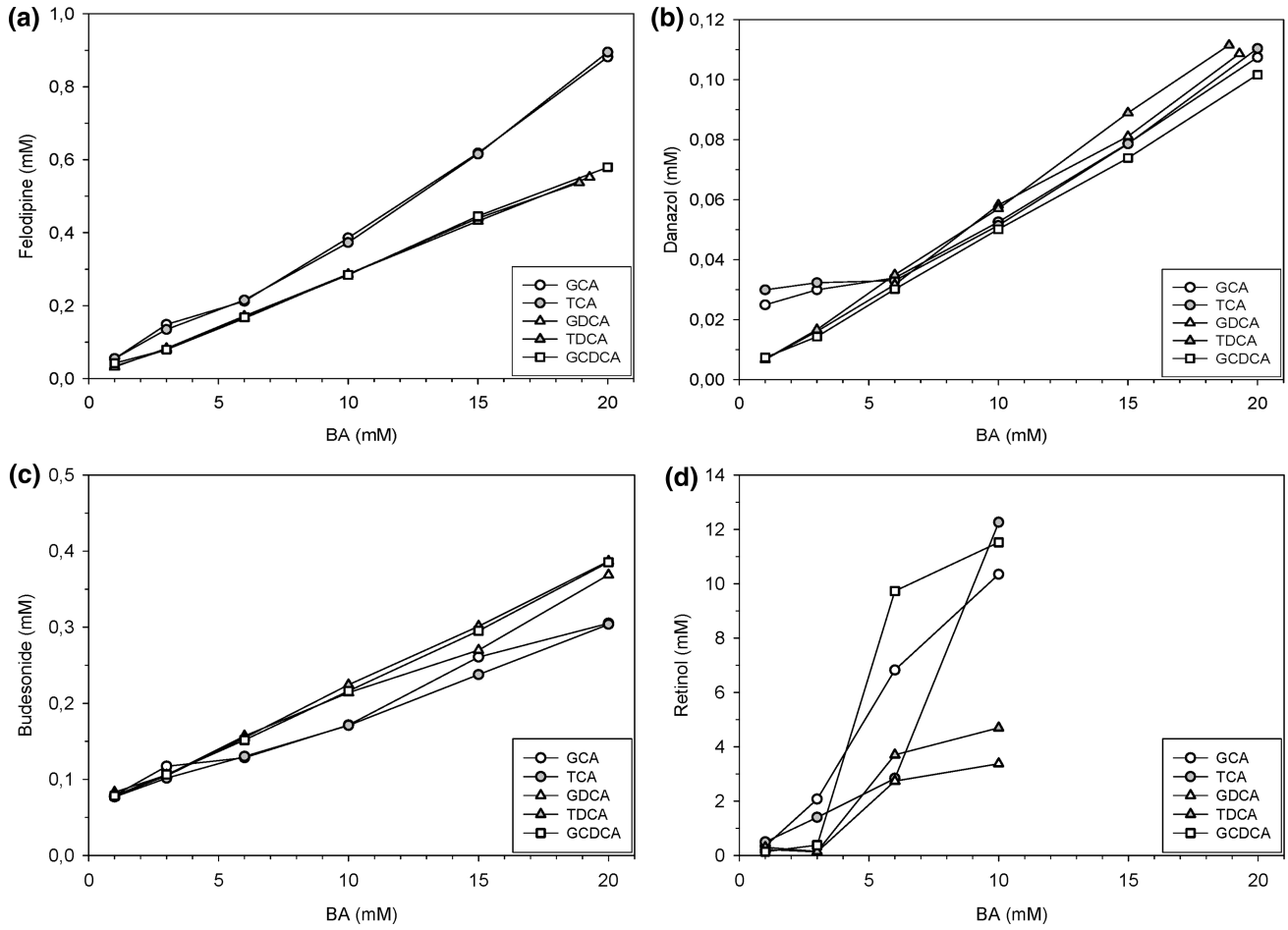

Figure 5. Solubilization of (a) felodipine, (b) budesonide, (c) danazol, and (d) retinol in aqueous solutions with varying concentrations of lecithin and five different bile acids (BA) in the proportions of BA:lecithin = 4:1.

where the solubility of two neutral drugs was found to be higher in duodenal HIF than in conventional FaSSIF.[22] The discrepancy may be due to differences in HIF collection procedures, for instance the position of the sampling tube (duodenum vs jejunum), and composition of the intestinal fluid.[23] However, for a large number of poorly soluble neutral drug compounds in pharmaceutical development at AstraZeneca R&D, FaSSIF-I has consistently overpredicted the solubility in several jejunal HIF batches collected by the same procedure as employed in the present study (data not shown). These observations suggest that 0.2 mM lecithin is the appropriate concentration for estimating *in vivo* solubility of neutral compounds. Second for ionizable compounds, the total drug solubility is determined not only by the content of lecithin and bile salt, but also the pH of the media, of which the later factor plays a more significant role because of logarithmic effects of pH on drug solubility. Evidently, the obtained solubility values in FaSSIF-II are comparable with those in HIF and FaSSIF-I. In fact, the solubility values of investigated model compounds in pH 6.5 phosphate buffer are similar to those in FaSSIF-I, FaSSIF-II and HIF, further suggesting that the solubility of ionizable compounds largely depends on the medium pH whereas it is weakly dependent on the lecithin concentration. Therefore, in estimating the *in vivo* solubility of ionizable compounds, it is adequate to use a lower level of lecithin. Further, practical considerations, such as physical stability under normal experimental conditions, also led to the recommendation of using 0.2 mM lecithin in FaSSIF-II. The improved stability of FaSSIF-II correlated well with the ratio of lecithin to bile salts. Specifically, for the higher ratio, a marked increase of the aggregate size was indicated by light-scattering measurements suggesting formation of liposomes, i.e., a phase separation into an aqueous phase and liquid crystalline phase. This observation is consistent with an independent research report on the phase behavior of the lecithin/sodium taurocholate/water system.[17]

It should be noted here that the solubility of neutral compounds in FaSSIF-II is almost half of that in FaSSIF-I, which is more physiologically relevant than the overestimation of FaSSIF-I. The implication of applying the appropriate solubility values in modeling and simulation, when assessing the absorption of lead compound potential or drug product ranking, merits careful considerations. In particular, the meaning of the apparent solubility, the dissolution rate of the compound and the associated micelles, and the underlying assumptions of the computational methodology are important aspects.

In the investigation, the work was focused on the solubility measurement. When considering the dynamic absorption process *in vivo*, the kinetic release rate of drug is essential. For example, intrinsic rotating disk has been explored in previous investigations to compare the simulated fluids with HIF.[4,18] It should be also noted that, in general, the amplification of dissolution rate is at a reduced extent comparing with the augmentation of solubility, because of the lower diffusivity of micelles that have resulted from larger size.[19−21] Therefore, it is logical to hypothesize that the solubility discrepancy between the FaSSIF-II and HIF would be further reduced in a dissolution testing.

Role of Structures of Bile Acids on the Solubilization of Neutral Compounds. As illustrated in Figure 5, the solubilization increases with the increase of concentration of bile acids, as expected, for all four tested neutral compounds. In addition, the major endogenous bile acids in the small intestine are the GCA and TCA, with small percentage of GDCA, TDCA and GCDCA.[2,10,12,22] Here, the results indicate that the impact of the bile acid molecular structure on the solubilization of neutral compounds is relatively small, at least in bile acid concentration range corresponding to the fasted state (2−6 mM). At higher bile acid concentrations, i.e. more than 6 mM, different bile acids may show slightly different ability to solubilize poorly soluble compounds. Clearly, the type of conjugating amino acid (glycine or taurine) has no impact on the solubilization, while the degree of hydroxylation of the steroid ring system may influence the solubilization capacity of the mixed micellar system. The amount of data is too limited to allow for extrapolations of the effect of degree of hydroxylation since aspects such as localization of the solubilized compounds in the mixed micelles and dielectric constants of the micellar interior are unknown. However, for the investigated compounds, the steroid structure has a negligible impact on the solubilization at *in vivo* relevant lecithin and bile acid concentrations. In preparation of simulated small intestinal fluids, one type of bile acid appears to be sufficient to represent an *in vivo* scenario rather than choosing multiple bile salts.

Conclusion

The new concentration of lecithin in FaSSIF-II, based on the solubility evaluation of 24 compounds, was recommended to be 0.2 mM, which provides improved prediction of *in vivo* solubility. This medium was found to be stable for up to at least 1 week under room temperature, enhancing its practical use in pharmaceutical development. The structure of bile salts has minimal impact on solubilization of poorly soluble compounds, suggesting the flexibility of bile acid selection in preparation of simulated small intestinal fluids.

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