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Ethanol Effects on Apparent Solubility of Poorly Soluble Drugs in Simulated Intestinal Fluid

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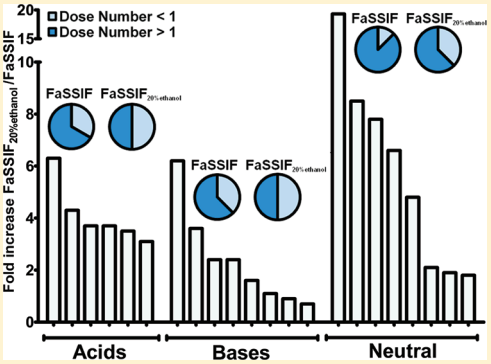
Supporting Information

ABSTRACT: Ethanol intake can lead to an unexpected and possibly problematic increase in the bioavailability of druglike compounds. In this work we investigated the effect of ethanol on the apparent solubility and dissolution rate of poorly soluble compounds in simulated intestinal fluid representing a preprandial state. A series of 22 structurally diverse, poorly soluble compounds were measured for apparent solubility and intrinsic dissolution rate (37 °C) in phosphate buffer pH 6.5 ($PhB_{6.5}$) and fasted state simulated intestinal fluid (FaSSIF, pH 6.5) with and without ethanol at 5% v/v or 20% v/v. The obtained data were used to understand for which molecules ethanol results in an increased apparent solubility and, therefore, may increase the amount of drug absorbed. In $FaSSIF_{20\%ethanol}$ 59% of the compounds displayed >3-fold higher apparent solubility than in pure FaSSIF, whereas the effects of 5% ethanol on solubility, in most cases, were negligible. Acidic and neutral compounds were more solubilized by the addition of ethanol than by lecithin/taurocholate aggregates, whereas bases showed a more substance-specific response to the additives in the buffer. The stronger solubilizing capacity of ethanol as compared to the mixed lipid aggregates in FaSSIF was further identified through Spearman rank analyses, which showed a stronger relationship between $FaSSIF_{20\%ethanol}$ and $PhB_{6.5,20\%ethanol}$ (r_S of 0.97) than $FaSSIF_{20\%ethanol}$ and FaSSIF (r_S of 0.86). No relationships were found between solubility changes in media containing ethanol and single physicochemical properties, but multivariate data analysis showed that inclusion of ethanol significantly reduced the negative effect of compound lipophilicity on solubility. For this data set the higher concentration of ethanol gave a dose number (Do) <1 for 30% of the compounds that showed incomplete dissolution in FaSSIF. Significant differences were shown in the melting point, lipophilicity, and dose profiles between the compounds having a Do < 1 and Do > 1, with the latter having higher absolute values in all three parameters. In conclusion, this study showed that significant effects of ethanol on apparent solubility in the preprandial state can be expected for lipophilic compounds. The results herein indicate that acidic and neutral compounds are more sensitive to the addition of ethanol than to the mixed lipid aggregates present in the fasted intestine.



KEYWORDS: *apparent solubility, dissolution rate, ethanol, biorelevant dissolution medium, poorly soluble compounds, molecular properties, dose number*

INTRODUCTION

Molecular properties likely to result in poor absorption as a result of low solubility and/or insufficient permeability have been acknowledged since the mid-1990s.[1] Even so, up to 90% of drugs currently in development have been estimated to be poorly soluble compounds according to the Biopharmaceutics Classification System (BCS).[2] The BCS criterion for solubility sorts all compounds that do not display a dose number (Do) <1 (i.e., completely dissolved dose) for the maximum oral dose over the pH interval 1.0−7.5 as poorly soluble.[3] The criterion has been questioned as being too strict, e.g., for acids, which display a low solubility at the acidic conditions of the stomach but up to 1000-fold higher solubility at the pH of the small intestine where most of the absorption takes place.[4,5] Furthermore, compounds that are lipophilic may gain largely in solubility through solubilization in lipid aggregates that are naturally present in the intestinal fluids.[6−10] Several different biorelevant dissolution media (BDM) that simulate the milieu of the gastrointestinal (GI) tract have been proposed. They range from simple buffer systems with low concentrations of synthetic amphiphilic additives[11−13] to more complex mixtures

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of bile salts, phospholipids, and fatty acids.[14−16] The solubilizing constituents of the fasted state simulated intestinal fluid (FaSSIF), sodium taurocholate and lecithin, self-aggregate. At the concentrations used in FaSSIF these components form larger mixed aggregates such as vesicles and disklike micelles with a mean diameter of 45 nm. Fed state simulated intestinal fluid (FeSSIF) contains a five-times higher concentration of the lipids, and at these concentrations the lipids form small and uniform micelles with a diameter of 6.4 nm.[17] Lipophilic compounds with poor aqueous solubility are solubilized by these aggregates, the extent of which is determined by molecular properties such as lipophilicity, size, shape, and charge. The solubilizing effect induced by the aggregates and the possible ionization effects caused by the pH of the buffer used result in apparent solubility in BDM sometimes several orders of magnitude higher than the compound's intrinsic aqueous solubility. There are several reports in the literature that indicate that the effects of the mixed lipid aggregates in the intestinal fluid are significant for lipophilic compounds with a partition coefficient between octanol and water (log *P*) greater than 3.[9,18−20] The drug discovery and development process currently produces a large number of such compounds. For example, 45% of all small molecule new chemical entities (NCEs) approved for human use in Sweden since 1998 (when the concept of simulated intestinal fluids first was introduced[16]) have a calculated log *P* value of 3 or above (Figure 1). It is likely

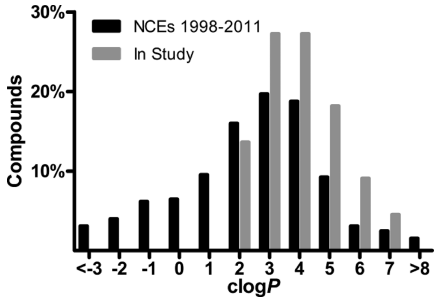

Figure 1. Lipophilicity of compounds in this study and NCEs approved for human use in Sweden between 1998 and June 2011.

that these compounds will display significantly higher solubility in media reflecting the intestinal lipid content, and that the resulting increase in concentration in the intestine may lead to an increased rate and/or extent of absorption. The risk for food related effects on absorption, through changes in the apparent solubility of the drug after food intake, is also higher for these compounds.[21]

A food related effect that may impact the absorption of lipophilic compounds is the likelihood of "dose dumping" if the medication is taken with ethanol. The term dose dumping is used for describing the premature disintegration of delayed release formulations.[22−24] The rapid dissolution of the larger dose used in modified release (MR) formulation may for highly permeable compounds result in an increased rate and/or extent of absorption and, hence, lead to higher plasma concentrations and changed pharmacokinetic profile. For drugs with a narrow therapeutic window this is a severe safety concern and has resulted in, e.g., the withdrawal of a MR product of hydromorphone from the U.S. market.[25] Concomitant intake of ethanol and MR hydromorphone resulted in significantly higher C_{max} of hydromorphone. This finding prompted dissolution studies of ethanol effects on MR formulations using simulated gastric fluid or acidic conditions, water, conditions mimicking the small intestinal fluid (phosphate buffer pH 6.5 or pH 6.8), and 0.1% SLS.[25−28] In addition the Food and Drug Administration (FDA) has given guidelines evaluating ethanol vulnerability of MR products under acidic conditions simulating the stomach.[29] If high levels of ethanol appear in the small intestine, the ethanol may also impact on drug solubilization in the intestinal fluid. This effect is likely to be transient as compared to stomach due to the dilution and rapid absorption of ethanol from the small intestine,[22] but to date neither solubilization in the small intestine after ethanol intake nor its effect on drug absorption has been studied.

In this work we therefore investigated to what extent high ethanol concentrations may impact drug solubilization. The main objectives of this study were to investigate the contributed effect of ethanol and/or mixed lipid aggregates on apparent solubility of poorly soluble compounds in small intestinal fluid and to link the extent of observed solubility increase to compound properties. For this purpose, we focused on highly lipophilic compounds with poor aqueous solubility, since such molecules are likely to be solubilized to a larger extent when ethanol is present. Through an experimental design, in which we included studies in the pure buffer and FaSSIF, and ethanol-containing mixtures of these at 5 and 20% v/v, we enabled analysis of the ethanol and mixed lipid aggregate effects separately. Finally we used the obtained experimental results to reveal for which molecules a significant increase in dissolution rate and apparent solubility can be expected when ethanol is present in the intestinal fluid.

METHODS

Data Set. A series of 22 compounds were selected for this study (Table 1, Figure 2). The data set was selected to include compounds displaying solvation and/or solid-state limited solubility. Primarily poorly water-soluble compounds with a calculated log *P* >3 were selected for the study (Figure 1). For such compounds an increase in dissolution rate and solubility, as the result of addition of ethanol, could significantly impact the absorption kinetics. To allow general conclusions to be drawn, efforts were made to include as structurally diverse compounds as possible, but still remain within the chemical space of poorly soluble drugs. Poor solubility in water was assessed based on expected solubility at pH 6.5. Compounds for which the expected solubility was less than 100 μM were defined as poorly soluble, and so were compounds for which the complete dose given was predicted to be insoluble in 250 mL of water. In addition, the selection of compounds was limited to those available in their free form and with sufficient UV-chromophore to allow detection by fiber optic UV probes. The final data set was composed of six anionic, eight cationic, and eight neutral drugs at the pH studied (pH 6.5) and had the following physicochemical profile: molecular weight (M_w) ranged from 230.3 to 531.4, log *P* from 1.9 to 5.7, melting point from 95 to 225 °C, and polar surface area (PSA) from 6 to 145 Å^2 (Table 1). All compounds were purchased from Sigma Aldrich (St. Louis, MO) except felodipine, which was received as a kind gift from AstraZeneca (Mölndal, Sweden), and ketoconazole, which was purchased from Toronto Research Chemicals (North York, ON, Canada).

pK_a Measurement. For ionizable compounds pK_a was measured at 37 ± 1 °C in 150 mM KCl solution, with and without 20% ethanol, using GLpKa (Sirius Analytical Instruments, U.K.). Prior to the pK_a determinations the instrument

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Table 1. Physicochemical Properties of the Investigated Compounds[a]

compound	M_W (Da)	Tm (°C)	clog $D_{pH6.5}$	PSA (Å^2)	NPSA (Å^2)	rotatable bonds (counts)
albendazole	265.4	209	3.2	68	255	5
astemizole	458.6	173	4.4	41	512	8
carvedilol	406.5	114	2.4	84	405	10
cinnarizine	368.6	120	4.3	9	465	6
corticosterone	346.5	181	2.2	69	315	2
danazol	337.5	225	3.6	48	330	0
dipyridamole	504.7	163	1.7	116	472	12
disopyramide	339.5	95	−0.3	56	354	8
felodipine	384.3	145	4.8	59	362	6
glibenclamide	494.1	170	3.9	110	442	8
griseofulvin	352.8	220	2.5	80	295	3
haloperidol	375.9	149	2.0	40	397	6
indomethacin	357.8	155	1.5	78	324	4
indoprofen	281.3	214	0.7	61	272	3
ketoconazole	531.5	146	3.8	73	481	7
naproxen	230.3	153	1.3	54	230	3
omeprazole	345.5	156	2.0	80	315	5
progesterone	314.5	121	3.8	38	332	1
tamoxifen	371.6	97	4.8	18	469	8
terfenadine	471.7	147	3.5	39	556	9
tolfenamic acid	261.7	207	2.9	47	248	3
warfarin	308.4	161	2.0	58	293	4
min	230.3	95	−0.3	9	230	0
max	531.5	225	4.8	116	556	12
median	355.3	155.5	2.7	58.5	343	5.5

[a]Molecular weight (M_W), melting temperature (T_m),[39,40] calculated octanol−water partitioning coefficient at pH 6.5 (clog $D_{pH6.5}$) from ADMET predictor, polar and nonpolar surface area (PSA, NPSA) calculated using MAREA, and number of rotatable bonds counted using DragonX.

was standardized with a blank titration allowing correction for the electrode's Four Plus parameters. At least three titrations were performed for each compound. The titrations were performed with 0.5 M HCl and 0.5 M KOH under a flow of argon to minimize the uptake of ambient carbon dioxide. The results of the titrations were monitored with potentiometric and spectrophotometric detection, and interpreted with Refinement Pro software (Sirius Analytical Instruments, U.K.).

Preparation of Media. Phosphate buffer pH 6.5 ($PhB_{6.5}$) and FaSSIF were prepared according to the protocol of Galia and co-workers.[16] The blank buffer was prepared by dissolving NaOH, $NaH_2PO_4{\cdot}H_2O$ and NaCl in MQ-water. The buffer was adjusted to pH 6.5, sterile filtered, and stored in a refrigerator. On the day of the experiment the FaSSIF was prepared from the blank buffer by addition of lecithin (0.75 mM) and sodium taurocholate (3 mM) powder (ePhares, Switzerland). A clear solution was obtained after stirring. The ethanol-containing blank buffers ($PhB_{6.5,5\%ethanol}$ and $PhB_{6.5,20\%ethanol}$) were prepared by dissolving 1.05 times and 1.25 times the amount NaOH, $NaH_2PO_4{-}H_2O$ and NaCl in MQ-water used to prepare $PhB_{6.5,}$ resulting in more concentrated blank buffers compared to $PhB_{6.5}$. The pH values of the buffers were adjusted to 6.5, and ethanol equal to 5% and 20% (v/v) respectively was added, diluting the buffers to the concentration of $PhB_{6.5}$. The buffers were sterile filtered and stored in a refrigerator. FaSSIFs containing ethanol ($FaSSIF_{5\%ethanol}$ and $FaSSIF_{20\%ethanol}$) were prepared on the day of the experiment as described above. Clear solutions were produced.

Dynamic light scattering was used to measure the size of mixed lipid aggregates present in the FaSSIF and $FaSSIF_{20\%ethanol}$. The measurements were performed at 37 °C, using a vertically polarized Uniphase He−Ne laser. Scattering at 90° was detected with a PerkinElmer (Quebec, Canada) diode detector and a multiple digital autocorrelator, ALV-5000 (ALV-laser Vertriegesellschaft mbH, Germany).

Solubility and Intrinsic Dissolution Rate Measurement. Apparent solubility and intrinsic dissolution rate in the six different media ($PhB_{6.5}$, $PhB_{6.5,5\%ethanol}$, $PhB_{6.5,20\%ethanol}$, FaSSIF, $FaSSIF_{5\%ethanol}$, $FaSSIF_{20\%ethanol}$) were measured using the μDISS Profiler (pION INC, Woburn, MA), utilizing in situ UV probes as described in a previous publication.[6] All measurements were performed in at least triplicate, and 2−20 mm UV probe path lengths were used based on chromophore strength and solubility. For compounds showing poor apparent solubility and/or weak chromophore, longer path length was used. The instrument channels were individually calibrated by collection of standard curves based on the absorbance of up to ten standard solutions. Aliquots of DMSO-stock solutions were added to preheated media and stirred at around 900 rpm for 1 min before the absorbance of the resulting standard solution was recorded.

After calibration of the UV channels, amounts equivalent to at least two times the expected solubility were weighed to each vial. The vials were transferred to the μDISS Profiler, the cross-bar magnetic stirrers were inserted, and 15 mL of preheated medium (37 °C) was added as the experiment was started. The dissolution experiments were performed at 37 ± 0.5 °C under stirring (100 rpm), and the vials were sealed to avoid evaporation. The in situ UV probes scanned the samples at predefined time intervals, commencing with scans every 5 s at the beginning of the experiment, to allow accurate determination of the dissolution rate, to one scan every 30 min at the end of the experiment, to confirm the apparent solubility. Solid material was present during the course of the experiment. The concentrations were determined from the area under the curve (AUC) in second derivative spectra, evaluated over a range of wavelengths. Interference arising from background turbidity caused by the powder was minimized by applying this spectral method.[30−32] Data refinement of the dissolution experiments was performed using the μDISS profiler software. Measurement of pH, after completion of each study, confirmed that the pH was stable in all media used and for all compounds investigated.

Dose number (Do) was calculated for all compounds in all media according to

$$Do = \frac{M_0}{V_0 C_S}$$

where M_0 is dose, V_0 is available volume (in this study set to 250 mL), and C_S is the apparent solubility.[33]

Statistics and In Silico Modeling. Linear regression and Spearman rank analysis were performed in Excel (Microsoft Office). The coefficient of determination (R^2) was used to assess the quality of the fit of, e.g., standard curves for solubility and dissolution rate measurement. All the experimental results are presented as mean values ± standard deviation ($n \geq 3$). Standard error (SE) for the ratios was calculated from

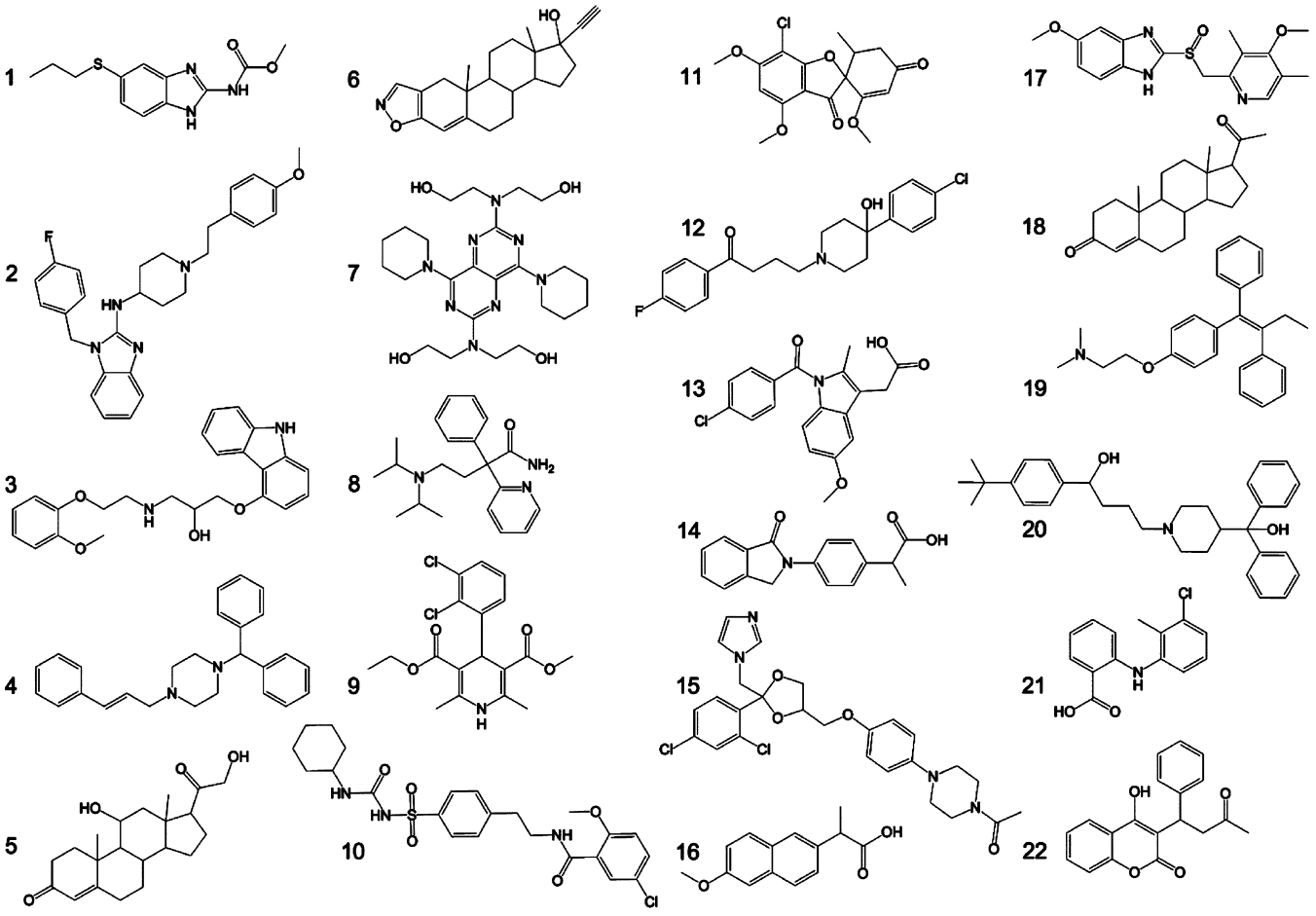

Figure 2. Chemical structures of compounds included in the study. 1: Albendazole. 2: Astemizole. 3: Carvedilol. 4: Cinnarizine. 5: Corticosterone. 6: Danazol. 7: Dipyridamole. 8: Disopyramide. 9: Felodipine. 10: Glibenclamide. 11: Griseofulvin. 12: Haloperidol. 13: Indomethacin. 14: Indoprofen. 15: Ketoconazole. 16: Naproxen. 17: Omeprazole. 18: Progesterone. 19: Tamoxifen. 20: Terfenadine. 21: Tolfenamic acid. 22: Warfarin.

$$SE_{FI} = FI \times \sqrt{\frac{S_A^2}{A^2} + \frac{S_B^2}{B^2}}$$

where FI is mean Fold Increase or ratio between apparent solubility in medium A over apparent solubility in medium B. A, B, S_A, and S_B is the mean measured apparent solubility and standard errors for media A and B, respectively. Spearman rank analysis was used to compare measured apparent solubility in the different media to allow analysis of the effect of the different components on the overall solubility, and the following comparisons were made: $PhB_{6.5,20\%ethanol}$ vs $PhB_{6.5}$, FaSSIF vs $PhB_{6.5}$, FaSSIF vs $PhB_{6.5,\ 20\%ethanol}$, $FaSSIF_{20\%ethanol}$ vs $PhB_{6.5,\ 20\%ethanol}$, and $FaSSIF_{20\%ethanol}$ vs FaSSIF.

Molecular structures were acquired as SMILES strings from chemicalize.org, and the three-dimensional structures were generated using Corina 3.0 (Molecular Networks, Erlangen, German). The resulting structures were used to calculate a large number of physicochemical properties and molecular descriptors using DragonX 1.4 (Talete, Italy), ADMETPredictor 5.0 (SimulationsPlus, CA), and MAREA 3.02 (in-house program). The descriptors were used to identify structural diversity within the data set and to analyze which molecular features that are linked to apparent solubility increase in biorelevant media with or without ethanol. To allow the latter, both linear regression between single molecular descriptors and solubility and multivariate data analysis, in the form of partial least-squares projection to latent structures (PLS) (Simca-P 11.0,Umetrics, Sweden), were performed. In the PLS model development, the following nine responses were investigated: the apparent solubility in $PhB_{6.5}$, $PhB_{6.5,20\%ethanol}$, FaSSIF, and $FaSSIF_{20\%ethanol}$; the fold increase obtained in FaSSIF, $PhB_{6.5,20\%ethanol}$, and $FaSSIF_{20\%ethanol}$ compared to that in $PhB_{6.5}$; and the solubilization ratio (SR) obtained from bile salt (SR_{bile}) or ethanol ($SR_{ethanol}$). SR was calculated as suggested by Mithani et al.,[34] in which [mol of drug/mol of bile] or [mol of drug/mol of ethanol] was divided by [mol of drug/mol of water]. All responses were transformed (log_{10}) prior to the model development. The molecular descriptor matrix mainly consisted of Dragon descriptors. To this we added log $D_{pH6.5}$ from ADMET Predictor, NPSA from MAREA, and the experimentally determined melting point. The descriptors were deidentified, mean centered, and scaled to unit variance. After removal of skewed descriptors, a matrix consisting of 1321 descriptors was submitted for PLS. A variable selection was applied to decrease the complexity of the models and facilitate interpretation. First, all descriptors except the 100 with highest importance for the response under investigation were excluded. Second, variables with low or little influence on the model, as identified by the variable of importance plot, and variables that duplicated the information contained within other variables (residing in the same area of the PLS loading plot) were excluded to leave just a few variables representing the key descriptors that encoded the majority of the information related

Table 2. Experimentally Determined Dissociation Constants and Apparent Solubility Values at 37 °C[a]

	dissociation constant 37 °C			apparent solubility (μM)					
compound	function[b]	pK_a	p$_sK_{a,20\%ethanol}$	$PhB_{6.5}$	$PhB_{6.5,5\%ethanol}$	$PhB_{6.5,20\%ethanol}$	FaSSIF	$FaSSIF_{5\%ethanol}$	$FaSSIF_{20\%ethanol}$
albendazole[c]	b	3.86 ± 0.02	3.61 ± 0.02	3 ± 1	2 ± 0	10 ± 2	7 ± 0	6 ± 1	15 ± 2
	a	9.94 ± 0.02	10.37 ± 0.08						
astemizole[c]	b	5.34 ± 0.10	5.72 ± 0.03	42 ± 0	63 ± 8	179 ± 21	214 ± 5	174 ± 9	342 ± 1
	b	8.29 ± 0.08	8.13 ± 0.11						
carvedilol[c]	b	7.80 ± 0.07	7.41 ± 0.17	113 ± 3	246 ± 6	804 ± 13	138 ± 3	365 ± 53	850 ± 13
cinnarizine[c]	b	7.45	nd[d]	4 ± 7	1 ± 0	4 ± 1	36 ± 2	28 ± 2	25 ± 2
corticosterone	n	na[e]	na	262 ± 6	1030 ± 19	3788 ± 139	530 ± 31	1145 ± 33	4145 ± 190
danazol[c]	n	na	na	2 ± 0	4 ± 1	24 ± 3	25 ± 2	20 ± 1	46 ± 5
dipyridamole	b	6.20 ± 0.02	5.55 ± 0.25	13 ± 0	26 ± 3	297 ± 44	23 ± 0	50 ± 1	443 ± 25
disopyramide	b	9.89 ± 0.06	9.73 ± 0.20	577 ± 17	2004 ± 50	3613 ± 75	927 ± 24	2116 ± 124	3351 ± 50
felodipine[c]	n	na	na	3 ± 0	7 ± 1	112 ± 9	142 ± 10	131 ± 1	265 ± 4
glibenclamide[c]	a	5.88 ± 0.05	nd	9 ± 2	7 ± 3	41 ± 5	10 ± 0	10 ± 1	60 ± 2
griseofulvin	n	na	na	42 ± 3	62 ± 1	298 ± 42	46 ± 5	77 ± 0	395 ± 9
haloperidol	b	8.55 ± 0.10	7.71 ± 0.41	207 ± 16	237 ± 8	439 ± 16	294 ± 2	475 ± 4	700 ± 21
indomethacin[c]	a	3.91 ± 0.09	4.94 ± 0.03	612 ± 218	962 ± 93	3725 ± 536	1238 ± 28	1675 ± 144	4298 ± 97
indoprofen	a	4.02 ± 0.07	4.89 ± 0.01	1050 ± 60	3944 ± 104	6513 ± 199	2176 ± 95	4337 ± 94	8016 ± 18
ketoconazole	b	3.37 ± 0.21	2.71 ± 0.03	28 ± 2	31 ± 0	176 ± 17	314 ± 26	405 ± 0	741 ± 8
	b	6.32 ± 0.17	6.46 ± 0.16						
naproxen	a	4.23 ± 0.06	5.11 ± 0.02	1003 ± 89	5448 ± 175	8646 ± 371	2154 ± 116	4386 ± 527	9321 ± 602
omeprazole	a	4.22 ± 0.22	4.50 ± 0.06	523 ± 37	596 ± 52	3216 ± 526	803 ± 64	1095 ± 8	3814 ± 2
	b	8.21 ± 0.14	8.80 ± 0.09						
progesterone	n	na	na	36 ± 3	61 ± 3	318 ± 44	81 ± 4	125 ± 5	531 ± 3
tamoxifen[c]	b	8.37 ± 0.06	8.27 ± 0.13	16 ± 1	21 ± 0	96 ± 11	420 ± 5	292 ± 24	393 ± 16
terfenadine	b	8.76 ± 0.03	7.08 ± 0.07	29 ± 3	33 ± 2	81 ± 10	189 ± 9	184 ± 9	208 ± 7
tolfenamic acid[c]	a	4.08 ± 0.04	4.76 ± 0.13	105 ± 2	148 ± 1	794 ± 15	241 ± 10	278 ± 37	751 ± 89
warfarin	a	4.73 ± 0.07	6.08 ± 0.02	643 ± 7	1098 ± 42	3514 ± 682	1160 ± 108	1571 ± 36	4308 ± 292
min				2	1	4	7	6	15
max				1050	5448	8646	2176	4386	9321

[a]Dissociation constant in 0.15 M KCl solution (pK_a) and in the same solution containing 20% ethanol (p$_sK_{a,20\%ethanol}$). Apparent solubility in $PhB_{6.5}$ and FaSSIF and corresponding 5% and 20% ethanol-containing media. [b]a denotes acid, b denotes base, and n denotes neutral compound. [c]pK_a, apparent solubility in $PhB_{6.5}$, and FaSSIF have been published previously.[6] [d]Not determined. [e]Not applicable; no protolytic function in the pH range 2−12 is present.

to the response variable. The aim of the variable selection was to maintain predictivity and increase the robustness of the model by removing information that was not directly related to the response variable (i.e., noise). All compounds were included in the analysis, and the accuracy of the PLS model was judged by the R^2 and the root mean squared error of the estimate (RMSEE). The models were validated by cross-validated R^2 (Q^2) and permutation tests (100 iterations); in the latter the values for the response variable were randomized and the multivariate data analysis was repeated to detect whether chance correlations had occurred. The results were used to allow further analyses of molecular properties of importance for the obtained apparent solubility in the different media, rather than applying them as quantitative predictive models.

RESULTS

Ethanol Effect on pK_a. All acidic functions studied had a slightly higher disassociation constant in the presence of the cosolvent ethanol. Studied bases, on the other hand, exhibited a lower pK_a in 20% ethanol compared to aqueous media (Table 2).

Apparent Solubility in FaSSIF and after Simulation of Ethanol Intake. The higher Spearman rank correlation of $FaSSIF_{20\%ethanol}$ vs $PhB_{6.5,20\%ethanol}$ as compared to $FaSSIF_{20\%ethanol}$ vs FaSSIF shows that the solubility of the compound series studied was more affected by ethanol inclusion than by presence of mixed lipid aggregates. The solubility in FaSSIF ranged from 1-fold (glibenclamide), i.e., showing no increase in solubility after addition of lecithin and taurocholate to the buffer, to being up to 45-fold higher (felodipine) as compared to the pure buffer ($PhB_{6.5}$). After addition of 20% ethanol to the buffer, the solubility measured varied from equal to that in pure buffer (cinnarizine) to up to 35-fold higher solubility (felodipine). The synergistic effect of mixed lipid aggregates and ethanol resulted in 3-fold (haloperidol) up to 84-fold (felodipine) higher apparent solubility than in the buffer alone (Figure 3A−C and Table 2). The solubilizing capacity, after the addition of 20% ethanol to FaSSIF, ranged from being less than that in FaSSIF (cinnarizine) to 19-fold higher (dipyridamole) (Figure 3D).

All studied anionic compounds (n = 6) exhibited similar solubility increase profiles due to solubilization from mixed lipid aggregates and the cosolvent effect from ethanol. Presence of mixed lipid aggregates increased the apparent solubility of the acids in general 2-fold. In the buffer spiked with 20% ethanol, the apparent solubility was significantly higher, resulting in on average 6-fold higher apparent solubility as compared to the $PhB_{6.5}$. The combined effect from mixed lipid aggregates and ethanol amounts to a 7-fold solubility increase as compared to what is achieved in a pure buffer (Figure 3A).

The studied cationic compounds (n = 8) were solubilized to a higher degree by the constituents of FaSSIF and slightly less

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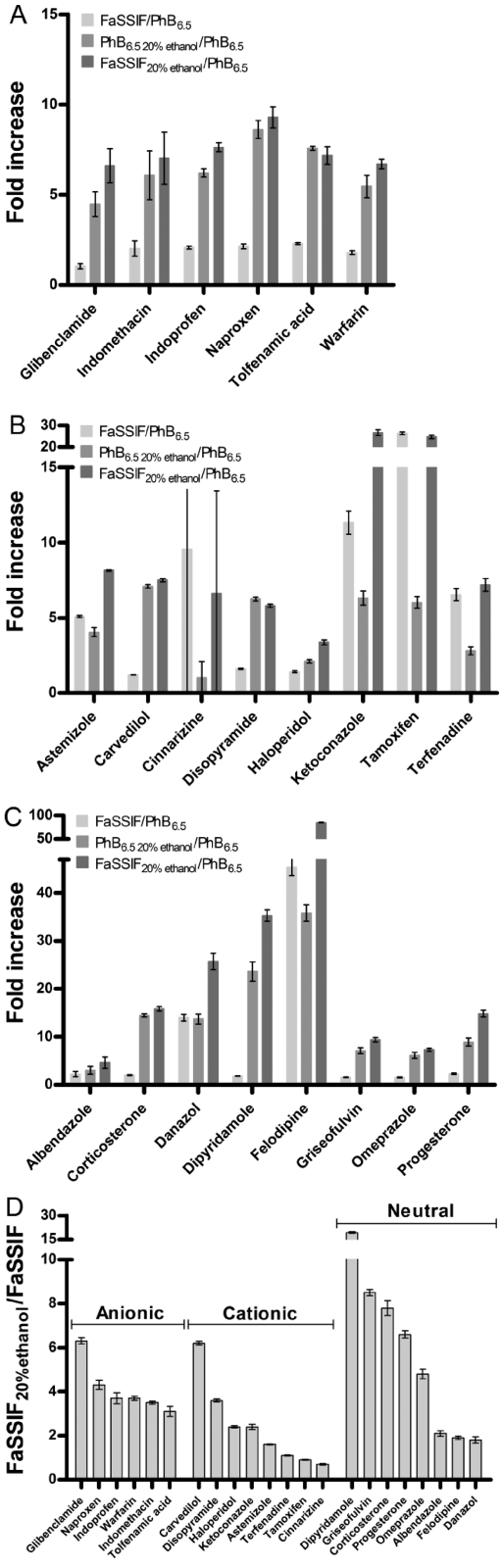

Figure 3. Fold increase in apparent solubility after addition of lipids and/or ethanol compared to buffer. All graphs are presented as mean fold difference ± standard error bars. (A) Apparent solubility of anionic compounds. (B) Apparent solubility of cationic compounds. The large SE obtained for cinnarizine is an effect of the poor wetting of the powder in $PhB_{6.5}$ resulting in large variability in the solubility value obtained in pure buffer. However, since this study investigated the wetting and solubilizing effects of ethanol and lecithin/taurocholate aggregates, it was decided to perform the solubility measurement without adding other wetting agents to the buffer. (C) Apparent solubility of neutral compounds. Note that the base dipyridamole is presented among the neutral compounds since the ethanol effect on the pKa results in that the compound mainly exists in its neutral form in the solution. (D) Solubility of all compounds in FaSSIF spiked with 20% ethanol compared to in FaSSIF. Light gray bars in A–C denote fold increase in $FaSSIF/PhB_{6.5}$. Gray and dark gray bars denote solubility ratios between $PhB_{6.5,20\%ethanol}/PhB_{6.5}$ and $FaSSIF_{20\%ethanol}/PhB_{6.5}$, respectively. Light gray bars with black borders in D denote fold increase in $FaSSIF_{20\%ethanol}/FaSSIF$.

by the cosolvent effect of ethanol (Figure 3B). Ketoconazole and tamoxifen showed >10-fold higher solubility in FaSSIF as compared to the pure buffer, whereas carvedilol, disopyramide, and haloperidol all showed equal solubility in both these media. All bases, except cinnarizine which was unaffected, gained in solubility after addition of ethanol, the apparent solubility being up to a maximum of 7-fold (carvedilol) higher after addition of ethanol compared to the buffer solubility. The solubility increase in media containing both lipid aggregates and ethanol was largest for tamoxifen and ketoconazole, which showed 25- and 27-fold higher solubility, respectively, in $FaSSIF_{20\%ethanol}$ than in $PhB_{6.5}$. The overall effect of the addition of ethanol to FaSSIF was negative for cinnarizine, resulting in a lesser amount dissolved after inclusion of ethanol as compared to FaSSIF alone. Ketoconazole, haloperidol, disopyramide, and carvedilol showed a 2- to 6-fold increase in $FaSSIF_{20\%ethanol}$. The remaining cationic compounds (astemizole, tamoxifen, and terfenadine) were negligibly affected by the addition of ethanol to FaSSIF.

For the neutral compounds (n = 8) it was revealed that, in general, they responded relatively weakly to the addition of the lecithin and taurocholate at the concentration used in FaSSIF, with six of the compounds displaying <2.3-fold higher solubility in FaSSIF than in buffer. In comparison, all compounds responded relatively strongly to the addition of ethanol, with only one compound showing <6-fold higher solubility in this media (Figure 3C). Of the eight compounds studied, seven showed equal or higher solubility in $PhB_{6.5,20\%ethanol}$ as compared to FaSSIF. The apparent solubility of the lipophilic compound felodipine was improved immensely by both mixed lipid aggregates (45-fold) and ethanol (36-fold), and the synergistic effect of mixed lipid aggregates and ethanol resulted in 85-fold higher solubility as compared to $PhB_{6.5}$. Albendazole and omeprazole, which are ionizable ampholytic compounds that are essentially neutral at the pH of this study (pH 6.5), were found to display only subtle solubilization by mixed lipid aggregates and ethanol.

The effects of media composition on dissolution rate were in close agreement with the effects on apparent solubility. Examples of dissolution profiles are given in Figure 4. Typically two different profiles could be seen, here exemplified with griseofulvin and felodipine.The dissolution of griseofulvin was unaffected by the solubilizing constituents in FaSSIF, but increased to a large extent by the addition of ethanol in $FaSSIF_{20\%ethanol}$. Felodipine dissolution rate in $PhB_{6.5}$ was slow, but considerably more rapid in FaSSIF. The addition of ethanol increased the dissolution rate of this neutral compound even further. Of the 22 compounds only cinnarizine showed a reduced dissolution rate in $FaSSIF_{20\%ethanol}$ compared to that in FaSSIF.

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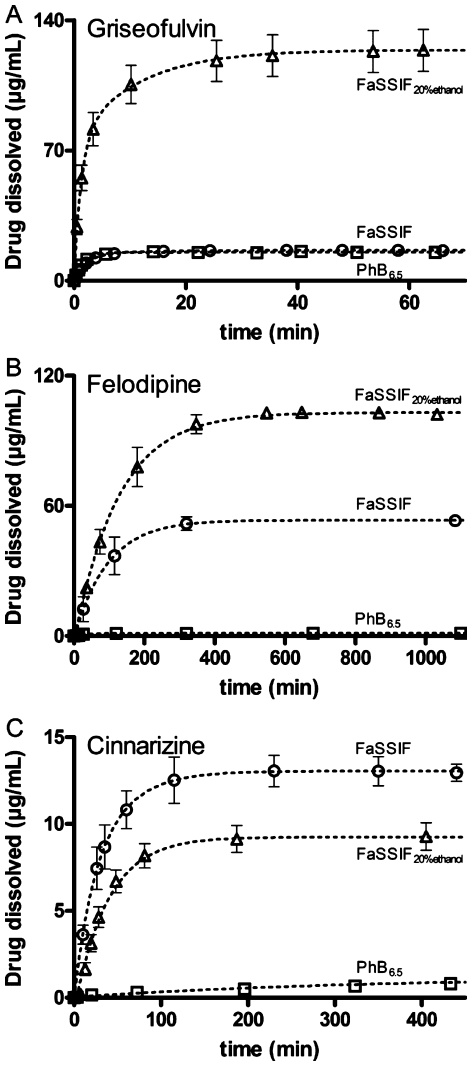

Figure 4. Dissolution profiles of three model compounds. Dissolution of (A) griseofulvin, (B) felodipine, and (C) cinnarizine in $PhB_{6.5}$ (□), FaSSIF (○), and $FaSSIF_{20\%ethanol}$ (△) reported as mean concentrations over time with standard deviations for triplicates. For clarity, only a fraction (1−5%) of measured values are shown.

Physicochemical Properties Resulting in Increased Solubility in Media Containing Ethanol. We developed nine PLS models with the purpose of analyzing which molecular properties are most important in terms of affecting apparent solubility in mixed lipid aggregates and in ethanol, and after the addition of both these components. All solubility data generated in this study was used for training the models to allow as much structural information as possible to influence the outcome. Hence, cross-validation and permutation tests were used for validation rather than a test set. The resulting models used 1−2 principal components and 5−8 variables, which produced R^2 of 0.72−0.88 and Q^2 of 0.64−0.80 (see Supporting Information). In general, the responses that reflected the fold increase after addition of ethanol only (i.e., $PhB_{6.5,20\%ethanol}/PhB_{6.5}$ and $SR_{ethanol}$) were more problematic to model and required 7−8 variables to produce models with R^2 of 0.72 and Q^2 of 0.67−0.69. These were the only models that required the PSA of the molecule as a descriptor. In agreement with our previous study, lipophilicity (log $D_{6.5}$) was needed to explain fold increase in FaSSIF (i.e., $FaSSIF/PhB_{6.5}$ and SR_{bile}), but not for the model of FaSSIF solubility.[6] In addition to these easily interpreted descriptors, less interpretable descriptors weighted for Sanderson electronegativity, polarizability, or atomic mass, and related to, e.g., Getaway, WHIM index, MoRSE signal, and Geary and Moran autocorrelation, remained after the variable selection (see Supporting Information).

Apparent Solubility and Dose Number. The obtained solubility values in each respective medium were used to calculate the *D*o (Table 3). The normal dose given for 77% of the compounds was not able to be dissolved in $PhB_{6.5}$. This number decreased to 59% in FaSSIF and 41% in $FaSSIF_{20\%ethanol}$. No simple relationships between, e.g., dose given, lipophilicity, charge, hydrophobicity, and solid state characteristics were revealed as common attributes to the group which had a reduction to *D*o < 1 after ethanol addition. When dividing the compounds into two groups based on the *D*o, using 1 as a cutoff value, it was shown that the groups had significant differences in the melting point, lipophilicity, and dose profiles (Figure 5). The compounds still having a *D*o > 1 after addition of both mixed lipid aggregates and ethanol had a higher melting point (average of 173 compared to 151), a higher log *D* (average of 3.3 compared to 2.4), and a higher dose (average of 192 compared to 76 mg, and a median of 200 and 10 mg, respectively) than the group *D*o < 1.

■ DISCUSSION

In this work we investigated the likelihood of ethanol to result in food-related solubilization effects that might have an impact on absorption and bioavailability of lipophilic poorly soluble drugs. We performed these studies in fluids simulating the fasted state of the small intestine before and after intake of ethanol. At the low concentration, i.e., 5% ethanol v/v, the effect was negligible for most compounds, whereas significant effects were obtained at the high concentration of 20% ethanol v/v. The 20% ethanol v/v may be regarded as a "worst case" scenario of ethanol levels in the small intestine. The solubilization effects in the intestine is likely temporary since the ethanol rapidly will be diluted and absorbed from the intestinal compartment. Nevertheless, changed absorption kinetics may result if significantly higher dissolution rate and apparent solubility are obtained in the presence of ethanol. In particular, compounds that under normal physiological conditions show poor solubility and high permeability (BCS class 2) are at risk, since these show dissolution and/or solubility limited absorption.

Ethanol may impact the solubilization capacity of BDM at both the water and the micelle level. The ethanol molecules are completely miscible with the water molecules, resulting in a decrease in the dielectric constant. For example, a 20% ethanol in water mixture has a dielectric constant of 62, whereas the dielectric constant of pure water is 73 at physiological temperature.[35] The lower dielectricity of the ethanol mixtures results in increased apparent solubility for nonpolar and/or lipophilic compounds compared to what is obtained in pure water. In addition, the ethanol molecules are also likely to affect the morphology and solubilizing effect of the lecithin and taurocholate aggregates present in the fasted and fed state. Ethanol has been shown to decrease the critical micelle concentration (cmc) of surfactants in aqueous media at low concentrations and increase the cmc at higher concentrations. The effect that ethanol has on the cmc of surfactants is related to its impact on the monomer solubility, the aggregate size, and the polarity of the aggregate core.[36−38] Indeed, measurements

Table 3. Dose Number (*Do*) Obtained in the Different Media[a]

		Do			
compound	dose (mg) low; max	$PhB_{6.5}$	$PhB_{6.5,20\%ethanol}$	FaSSIF	$FaSSIF_{20\%ethanol}$
albendazole	400	1882	619	842	407
astemizole	10	2	**0.5**	**0.4**	**0.3**
carvedilol	6.25; 50	**0.5**; 4	**0.1**; **0.6**	**0.4**; 4	**0.1**; ***0.6***
cinnarizine	15; 75	43; 214	42; 209	5; 22	7; 32
corticosterone	100	4	**0.3**	2	***0.3***
danazol	50; 200	333; 1333	24; 97	24; 95	13; 52
dipyridamole	200; 300	126; 189	5; 8	69; 103	4; 5
disopyramide	200; 400	4; 8	**0.7**; 1	3; 5	***0.7***; 1.4
felodipine	2.5; 20	8; 67	**0.2**; 1.9	**0.2**; 1.5	**0.1**; ***0.8***
glibenclamide	2.5; 10	2; 9	**0.5**; 2	2; 9	***0.3***; 1
griseofulvin	500; 1000	135; 270	19; 38	85; 171	14; 29
haloperidol	2; 5	**0.1**; **0.3**	**0.1**; **0.1**	**0.1**; **0.2**	**0.0**; **0.1**
indomethacin	25; 100	**0.5**; 2	**0.1**; **0.3**	**0.2**; **0.9**	**0.1**; **0.3**
indoprofen	100; 200	1.4; 3	**0.2**; **0.4**	**0.7**; 1.3	**0.2**; ***0.4***
ketoconazole	200; 400	54; 109	9; 17	5; 10	2; 4
naproxen	500; 1000	9; 17	**1**; 2	4; 8	***0.9***; 1.9
omeprazole	20; 60	**0.4**; 1	**0.1**; **0.2**	**0.3**; **0.9**	**0.1**; **0.2**
progesterone	200; 400	71; 142	8; 16	31; 63	5; 10
tamoxifen	10; 20	7; 14	1; 2	**0.3**; **0.5**	**0.3**; **0.6**
terfenadine	60; 120	18; 35	6; 13	3; 5	2; 5
tolfenamic acid	100; 200	15; 29	2; 3	6; 13	2; 4
warfarin	5; 10	**0.1**; **0.2**	**0.0**; **0.0**	**0.1**; **0.1**	**0.0**; **0.0**

[a]*Do* < 1 shows that the dose given is soluble. These numbers are given in bold. Physiologically relevant *Do* < 1 as a consequence of ethanol intake, assessed by addition of ethanol to the lipid containing FaSSIF, is shown in bold italics.

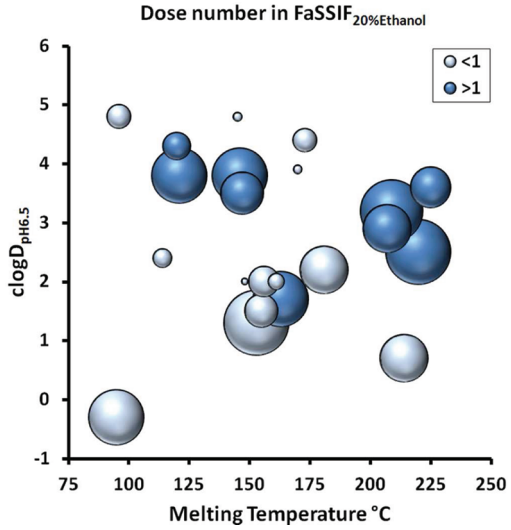

Figure 5. Compounds sorted according to dose number (*Do*). The light blue bubbles denote the 13 compounds which have a *Do* < 1 in FaSSIF spiked with 20% ethanol. The dark blue bubbles denote the nine compounds which have *Do* > 1 in this media. The area of each bubble corresponds to the size of its dose; the larger the bubble, the larger the dose. The minimum and maximum doses displayed in this graph are 2 and 500 mg, respectively.

of the aggregate size before and after addition of 20% v/v ethanol to FaSSIF showed that aggregates in FaSSIF containing ethanol are considerably larger and more variable in size (⌀ 94 ± 35 nm) compared to aggregates in FaSSIF alone (⌀ 59 ± 5 nm).

To analyze if ethanol or mixed lipid aggregates had the highest solubilization capacity for poorly soluble lipophilic drugs, we performed Spearman rank analyses. The r_S of 0.97 obtained for $FaSSIF_{20\%ethanol}$ vs $PhB_{6.5,20\%ethanol}$ in comparison to the r_S of 0.86 for $FaSSIF_{20\%ethanol}$ vs FaSSIF suggests that ethanol was more important for the solubility of this compound series than the mixed lipid aggregates. Hence, for compounds which are lipophilic and poorly water-soluble, simple buffers with ethanol may be sufficient to use as a screen for identifying compounds that are sensitive to ethanol in the preprandial state. From the obtained results, the compounds whose solubility increases largely after addition of ethanol can be selected and further studied for apparent solubility in BDM in the presence of ethanol.

The addition of ethanol results in changes in the compounds' protolytic function. Typically a downward shift is obtained in the pK_a for bases, whereas the pK_a goes in the opposite direction for acids in the presence of ethanol. In particular, this leads to a significant lower degree of ionization for the compounds with a pK_a in the vicinity of the pH of the buffer, i.e., pK_a of 6.5. In addition to decreased polarization and possibly lower aqueous solubility, this decrease in charge results in a higher apparent lipophilicity at pH 6.5 (log $D_{6.5}$) than in the media not containing this cosolvent. Hence, such molecules will respond to the addition of ethanol not only as a result of molecular properties, such as molecular size, flexibility, and aromaticity, but also due to their physicochemical profile in terms of charge and lipophilicity. For our data series, this effect will be greatest for dipyridamole, ketoconazole, and warfarin, which show significant changes in their pK_a in the close vicinity to the buffer pH of 6.5 (Table 2).

In this study we found that neutral and acidic compounds were more soluble in media containing ethanol than in media containing aggregate forming lipids at the concentrations found in the preprandial state. In contrast, cationic compounds were more substance specific and, for the eight compounds studied, some molecules gained more in solubility by mixed lipid

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aggregates than by ethanol, whereas others showed the opposite tendency. Molecular properties of importance for the obtained solubility in each of the investigated media were not able to be revealed through analysis of single molecular properties. Therefore, we performed multivariate data analysis to reveal which molecular functions are most influential for solubility in each of the media. The models developed herein have not yet been tested for their general predictability of ethanol effects; rather they were developed to analyze which molecular structures are likely to show significant changes in apparent solubility when ethanol is present. It became evident that lipophilicity (as described by the log $D_{6.5}$) was the most important factor for improving the solubility in ethanol-containing media. This descriptor was not one of the most important for solubility in FaSSIF, a finding in agreement with our previous study.[6] However, it was found to be important for the apparent solubility in $PhB_{6.5}$, $PhB_{6.5,\ 20\%ethanol}$, and $FaSSIF_{20\%ethanol}$. In the resulting models, the coefficients for log $D_{6.5}$ decreased from −0.40 ($PhB_{6.5}$) to −0.28 ($PhB_{6.5,20\%ethanol}$) and −0.23 in $FaSSIF_{20\%ethanol.}$ The multivariate analysis further revealed that polarizability, electronegativity, and size are important descriptors for solubility in the studied media. Electronegativity was related positively to solubility in all media, and it may be a property that reflects the capacity of a compound to form hydrogen bonds with the water. Further, the WHIM descriptor E2p, which is a descriptor depicting polarizability of the molecule, proved to be negatively related to solubility in $PhB_{6.5}$, FaSSIF, and $FaSSIF_{20\%ethanol}$ (coefficient of −0.30, −0.30, and −0.16, respectively). In contrast, it was not found to be important for solubility in $PhB_{6.5,20\%ethanol}$. The lower relative contribution of this parameter in the FaSSIF containing ethanol, and the fact it is not an important descriptor for solubility in $PhB_{6.5,20\%ethanol}$, indicate that this property is of less importance after inclusion of ethanol. The negative effect of polarizability on solubility in media containing mixed lipid aggregates was further supported by the 3D descriptors MOR13p.

In addition to the multivariate data analyses on absolute solubility obtained in each of the media, we also investigated the fold increase in solubility after addition of lecithin and taurocholate, ethanol, or both mixed lipid aggregates and ethanol, as compared to solubility in the pure buffer. Through this we could reveal that the compounds whose solubility increased the most in the mixed lipid aggregate-containing media were lipophilic, whereas the compounds that gained most in solubility after addition of ethanol to the buffer were the ones with lower PSA values. We interpret this to reflect that compounds that were well solubilized in pure water, through the use of hydrogen bonds with water molecules, were affected negatively by the decrease in polarity of the medium after the addition of ethanol. A number of more complex descriptors were also included in these models, many of which reflected two or more properties (e.g., size, branching, symmetry, electronegativity, polarizability). The interpretation of these is not as straightforward as for the properties log $D_{6.5}$ and PSA. Interestingly, the combination of both mixed lipid aggregates and ethanol resulted in the fact that neither log $D_{6.5}$ nor PSA was the most important descriptor for the fold increase in solubility, leaving seven other more complex descriptors. Hence, for lipophilic compounds, for which an improved solubility would be expected in BDM, the solubilization when ethanol is present will also be dependent on, among other things, charge and polar surface area of the molecule.

To summarize the different analyses of molecular properties, the solubility increase obtained for neutral compounds in the media containing mixed lipid aggregates and/or ethanol was on average higher than for the acids and bases. It was also revealed that neutral compounds and compounds carrying a net negative charge at the investigated pH were solubilized to a larger extent by the addition of 20% ethanol than by mixed lipid aggregates at the concentrations used in FaSSIF. For the investigated negatively charged and neutral compounds the presence of lipid aggregates and ethanol resulted in a synergistic positive effect on solubility. This effect was not seen for bases, which showed negative, positive, or no effects on solubility after inclusion of all additives. These compounds, which carry a net positive charge at pH 6.5, were the ones for which the resulting solubility, after inclusion of mixed lipid aggregates and/or ethanol, was most substance specific. The multivariate data analysis showed that the lipophilicity of a compound was less restrictive for solubility after the addition of ethanol. Indeed, the largest influence of high compound lipophilicity was seen in $PhB_{6.5}$, and this solvation limitation was decreased in both $PhB_{6.5,20\%ethanol}$ and $FaSSIF_{20\%ethanol}$. However, other molecular properties are also of importance for this to occur. For example, charge, PSA, size, polarizability, and electronegativity will influence the resulting concentration obtained.

For some of the compounds the effect of ethanol and the synergistic effect of ethanol and mixed lipid aggregates resulted in large increase in apparent solubility, and for some this led to a $Do < 1$, i.e., the complete dose was dissolved. Indeed, for 31% of the compounds that were sorted as poorly soluble in FaSSIF a Do of < 1 was obtained in $FaSSIF_{20\%ethanol}$. The compounds that were able to dissolve in FaSSIF containing ethanol were corticosterone, disopyramide, glibenclamide, and naproxen. These represent neutral, acidic, and basic compounds which are given in doses ranging from 2.5 mg (glibenclamide) to 500 mg (naproxen). The compounds are hydrophilic (disopyramide, log $D_{pH6.5}$ of −0.3) to lipophilic (glibenclamide, log $D_{pH6.5}$ of 3.9), and display intermediate melting points of 153−181 °C. The analysis of physicochemical difference between completely soluble compounds ($Do < 1$) and compounds for which undissolved drug material would be present after oral administration ($Do > 1$) showed that the latter were more lipophilic, had higher melting points, and were administered at higher doses (Figure 5). Hence, even though dose is not always known in early drug development, the analysis shows that high melting compounds which are also highly lipophilic will be poorly soluble regardless of the solvent used. Indeed, the addition of five times more lipids did not change the grouping of the compounds, as all compounds with a $Do > 1$ remained within this group even when measured in FeSSIF (data not shown). However, among the compounds for which the dose was not completely soluble in the simulation experiments of ethanol intake, many still showed significantly higher apparent solubility and dissolution rate after the addition of ethanol.

The results from this study suggest that the dissolution rate and solubility in the intestine and, hence, the rate and/or extent of absorption may be significantly different for highly lipophilic drugs if they are concomitantly taken with ethanol. This effect can be disastrous for lipophilic compounds with a narrow therapeutic window. Therefore, possible ethanol effects on intestinal solubility should be evaluated for such molecules during early drug development.

CONCLUSION

In this work we investigated the likelihood of increased intestinal concentration of poorly soluble compounds to appear after ethanol intake. To simulate this condition in vitro we performed dissolution studies using FaSSIF with and without ethanol. At low concentrations of ethanol, FaSSIF itself reflected the apparent solubility in the media. However, 59% of the compounds displayed 3-fold or higher apparent solubility in FaSSIF containing 20% ethanol. Acidic and neutral compounds were more solubilized by the addition of ethanol than by the mixed lipid aggregates, whereas bases showed a more substance-specific response to the additives in the buffer. No strong relationships were found between solubility changes in media simulating ethanol intake and single physicochemical properties. Multivariate data analysis revealed that log $D_{6.5}$, PSA, polarizability, electronegativity, and size are important properties affecting solubility in the studied media. Further, it was revealed that at the higher concentration of ethanol the *D*o decreased to < 1 for 31% of the compounds which showed incomplete dissolution in FaSSIF. Significant differences were shown in the melting point, lipophilicity, and dose profiles between the compounds having a *D*o < 1 and those having a *D*o > 1, with the latter having higher absolute values in all three parameters. The results of this study clearly indicate that dissolution rate and total concentration of poorly soluble, lipophilic compounds in the intestinal fluid may change tremendously after intake of ethanol. It is important to bear in mind that even for lipophilic compounds with a *D*o > 1 the ethanol intake may result in dramatic effects on the pharmacokinetic profile due to a more rapid dissolution process and higher concentration available for absorption to the systemic circulation.

ASSOCIATED CONTENT

Supporting Information

The results of the PLS models. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23* (1−3), 3−25.

(2) Benet, L. Z.; Wu, C. Y.; Custodio, J. M. Predicting drug absorption and the effects of food on oral bioavailability. *Bull. Tech. Gattefosse* **2006**, No. 99, 9−16.

(3) FDA. Guidance for Industry. *Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system*. For further information: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070246.pdf (accessed June 10, 2011), 2000.

(4) Yazdanian, M.; Briggs, K.; Jankovsky, C.; Hawi, A. The "High Solubility" Definition of the Current FDA Guidance on Biopharmaceutical Classification System May Be Too Strict for Acidic Drugs. *Pharm. Res.* **2004**, *21* (2), 293−299.

(5) Yu, L. X.; Amidon, G. L.; Polli, J. E.; Zhao, H.; Mehta, M. U.; Conner, D. P.; Shah, V. P.; Lesko, L. J.; Chen, M. L.; Lee, V. H. L.; Hussain, A. S. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *19* (7), 921−925.

(6) Fagerberg, J. H.; Tsinman, O.; Sun, N.; Tsinman, K.; Avdeef, A.; Bergström, C. A. S. Dissolution rate and apparent solubility of poorly soluble drugs in biorelevant dissolution media. *Mol. Pharmaceutics* **2010**, *7* (5), 1419−1430.

(7) Dressman, J. B.; Reppas, C. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl. 2), S73−S80.

(8) Perez De La Cruz Moreno, M.; Oth, M.; Deferme, S.; Lammert, F.; Tack, J.; Dressman, J.; Augustijns, P. Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. *J. Pharm. Pharmacol.* **2006**, *58* (8), 1079−1089.

(9) Persson, E. M.; Gustafsson, A. S.; Carlsson, A. S.; Nilsson, R. G.; Knutson, L.; Forsell, P.; Hanisch, G.; Lennernäs, H.; Abrahamsson, B. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharm. Res.* **2005**, *22* (12), 2141−2151.

(10) Sunesen, V. H.; Pedersen, B. L.; Kristensen, H. G.; Müllertz, A. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *Eur. J. Pharm. Sci.* **2005**, *24* (4), 305−313.

(11) Lehto, P.; Kortejärvi, H.; Liimatainen, A.; Ojala, K.; Kangas, H.; Hirvonen, J.; Tanninen, V. P.; Peltonen, L. Use of conventional surfactant media as surrogates for FaSSIF in simulating in vivo dissolution of BCS class II drugs. *Eur. J. Pharm. Biopharm.* **2011**, *78* (3), 531−538.

(12) Taupitz, T.; Klein, S. Can biorelevant media be simplified by using SLS and Tween 80 to replace bile compounds? *Open Drug Delivery J.* **2010**, *4* (SPEC. ISSUE 1), 30−37.

(13) Zoeller, T.; Klein, S. Simplified biorelevant media for screening dissolution performance of poorly soluble drugs. *Dissolution Technol.* **2007**, *14* (4), 8−13.

(14) 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.

(15) Ilardia-Arana, D.; Kristensen, H. G.; Müllertz, A. Biorelevant dissolution media: Aggregation of amphiphiles and solubility of estradiol. *J. Pharm. Sci.* **2006**, *95* (2), 248−255.

(16) 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*, 698−705.

(17) Boni, J. E.; Brickl, R. S.; Dressman, J.; Pfefferle, M. L. Instant FaSSIF and FeSSIF-Biorelevance meets practicality. *Dissolution Technol.* **2009**, *16* (3), 41−45.

(18) Ottaviani, G.; Gosling, D. J.; Patissier, C.; Rodde, S.; Zhou, L.; Faller, B. What is modulating solubility in simulated intestinal fluids? *Eur. J. Pharm. Sci.* **2010**, *41* (3−4), 452−457.
(19) Söderlind, E.; Karlsson, E.; Carlsson, A.; Kong, R.; Lenz, A.; Lindborg, S.; Sheng, J. J. Simulating fasted human intestinal fluids: Understanding the roles of lecithin and bile acids. *Mol. Pharmaceutics* **2010**, *7* (5), 1498−1507.
(20) Zaki, N. M.; Artursson, P.; Bergström, C. A. S. A modified physiological BCS for prediction of intestinal absorption in drug discovery. *Mol. Pharmaceutics* **2010**, *7* (5), 1478−1487.
(21) Kawai, Y.; Fujii, Y.; Tabata, F.; Ito, J.; Yukiko, M.; Kameda, A.; Akimoto, K.; Takahashi, M. Profiling and trend analysis of food effects on oral drug absorption considering micelle interaction and solubilization by bile micelles. *Drug Metab. Pharmacokinet.* **2011**, *26* (2), 180−191.
(22) Lennernäs, H. Ethanol-drug absorption interaction: Potential for a significant effect on the plasma pharmacokinetics of ethanol vulnerable formulations. *Mol. Pharmaceutics* **2009**, *6* (5), 1429−1440.
(23) Hendeles, L.; Iafrate, R. P.; Weinberger, M. A clinical and pharamacokinetic basis for the selection and use of slow release theophylline products. *Clin. Pharmacokinet.* **1984**, *9* (2), 95−135.
(24) Yeh, K. C. Indomethacin and indomethacin sustained release--A comparison of their pharmacokinetic profiles. *Semin. Arthritis Rheum.* **1982**, *12* (2, Suppl. 1), 136−141.
(25) Walden, M.; Nicholls, F. A.; Smith, K. J.; Tucker, G. T. The effect of ethanol on the release of opioids from oral prolonged-release preparations. *Drug Dev. Ind. Pharm.* **2007**, *33* (10), 1101−1111.
(26) Fadda, H. M.; Mohamed, M. A. M.; Basit, A. W. Impairment of the in vitro drug release behaviour of oral modified release preparations in the presence of alcohol. *Int. J. Pharm.* **2008**, *360* (1−2), 171−176.
(27) Levina, M.; Vuong, H.; Rajabi-Siahboomi, A. R. The influence of hydro-alcoholic media on hypromellose matrix systems. *Drug Dev. Ind. Pharm.* **2007**, *33* (10), 1125−1134.
(28) Traynor, M. J.; Brown, M. B.; Pannala, A.; Beck, P.; Martin, G. P. Influence of alcohol on the release of tramadol from 24-h controlled-release formulations during in vitro dissolution experiments. *Drug Dev. Ind. Pharm.* **2008**, *34* (8), 885−889.
(29) FDA. Suggests Dissolution tests of MR products in acidic media containing 5%, 20% and 40% ethanol in Draft Guidances on Bupropion Hydrochloride, Desvenlafaxine Succinate, Lamotrigine, Morphine Sulfate, Quetiapine Fumarate, Ropinirole Hydrochloride, Trazodone Hydrochloride, *Trospium Chloride and Venlafaxine Hydrochloride*. For further information: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/...UCM082973.pdf, UCM179177.pdf, UCM209231.pdf, UCM234971.pdf, UCM089520.pdf, UCM194667.pdf, UCM261531.pdf, UCM090751.pdf and UCM091259.pdf (accessed June 10, 2011).
(30) Avdeef, A.; Tsinman, O. Miniaturized rotating disk intrinsic dissolution rate measurement: Effects of buffer capacity in comparisons to traditional wood's apparatus. *Pharm. Res.* **2008**, *25* (11), 2613−2627.
(31) Bijlani, V.; Yuonayel, D.; Katpally, S.; Chukwumezie, B. N.; Adeyeye, M. C. Monitoring ibuprofen release from multiparticulates: In situ fiber-optic technique versus the HPLC method: A technical note. *AAPS PharmSciTech* **2007**, *8* (3).
(32) Tsinman, K.; Avdeef, A.; Tsinman, O.; Voloboy, D. Powder dissolution method for estimating rotating disk intrinsic dissolution rates of low solubility drugs. *Pharm. Res.* **2009**, *26* (9), 2093−2100.
(33) Amidon, G. L.; Lennernaes, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12* (3), 413−420.
(34) Mithani, S. D.; Bakatselou, V.; TenHoor, C. N.; Dressman, J. B. Estimation of the increase in solubility of drugs as a function of bile salt concentration. *Pharm. Res.* **1996**, *13* (1), 163−167.
(35) Åkerlöf, G. Dielectric constants of some organic solvent-water mixtures at various temperatures. *J. Am. Chem. Soc.* **1932**, *54* (11), 4125−4139.
(36) Kawakami, K.; Oda, N.; Miyoshi, K.; Funaki, T.; Ida, Y. Solubilization behavior of a poorly soluble drug under combined use of surfactants and cosolvents. *Eur. J. Pharm. Sci.* **2006**, *28* (1−2), 7−14.
(37) Suzuki, H. Studies of the Effect of Ethanol and Sodium Chloride on the Micellization of Sodium Dodecyl Sulfate by Gel Filtration. *Bull. Chem. Soc. Jpn.* **1976**, *49* (6), 1470−1474.
(38) Zana, R. Aqueous surfactant-alcohol systems: A review. *Adv. Colloid Interface Sci.* **1995**, *57* (C), 1−64.
(39) O'Neil, M. J., Ed. *The Merck Index*, 13th ed.; Merck: 2001
(40) Bergström, C. A. S.; Wassvik, C. M.; Johansson, K.; Hubatsch, I. Poorly soluble marketed drugs display solvation limited solubility. *J. Med. Chem.* **2007**, *50* (23), 5858−5862.