molecular pharmaceutics

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Dissolution Rate and Apparent Solubility of Poorly Soluble Drugs in Biorelevant Dissolution Media

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Received February 25, 2010; Revised Manuscript Received May 19, 2010; Accepted May 27, 2010

Abstract: A series of poorly soluble BCS class II compounds with "grease ball" characteristics were assessed for solubility and dissolution rate in biorelevant dissolution media (BDM) with the purpose of investigating which molecular structures gain most in solubility when dissolved under physiologically relevant conditions. The compounds were studied in four media (simulated intestinal fluid in fasted (FaSSIF pH 6.5) and fed state (FeSSIF pH 5.0), and their corresponding blank buffers ($FaSSIF_{blk}$ and $FeSSIF_{blk}$)) at a temperature of 37 °C. The experimental results were used to analyze which molecular characteristics are of importance for the solubility in BDM and for in silico modeling using multivariate data analysis. It was revealed that a majority of the compounds exhibited a higher dissolution rate and higher solubility in the FaSSIF and FeSSIF than in their corresponding blank buffers. Compounds which were neutral or carried a positive charge were more soluble in FeSSIF than FaSSIF. The acidic compounds displayed clear pH dependency, although the higher concentration of solubilizing agents in FeSSIF than FaSSIF also improved the solubility. Five of the ten compounds were upgraded to BCS class I when dissolved in FaSSIF or FeSSIF, i.e., the maximum dose of these compounds given orally was soluble in 250 mL of these BDMs. Lipophilicity as described by the log D_{oct} value was identified as a good predictor of the solubilization ratio ($R^2 = 0.74$), and computed molecular descriptors were also shown to successfully predict the solubilities in BDM for this data set. To conclude, the physiological solubility of "grease ball" molecules may be largely underestimated in in vitro solubility assays unless BDM is used. Moreover, the results herein indicate that the improvement obtained in BDM may be possible to predict from chemical features alone.

Keywords: Poor solubility; dissolution rate; biorelevant dissolution media; FaSSIF; FeSSIF; apparent BCS; physicochemical properties; molecular features; solvation limited

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Introduction

Biorelevant dissolution media (BDM) include different additives which model the intestinal fluid in the fasted (FaSSIF) and fed (FeSSIF) state, and they range from being quite simple to more complex.[1,2] BDM often have a substantial impact on the apparent solubility of molecules with solvation limited solubility, i.e., molecules with poor interaction with water, through improved wetting and solubilization by additives such as surfactants and/or lipids. We have recently investigated a series of poorly soluble com-

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

10.1021/mp100049m © **2010 American Chemical Society**
Published on Web 05/27/2010

pounds, all of which displayed an intrinsic solubility of 1 μM or less in pure water.[3] When studying the relationship between physicochemical properties and solubility for this data set, it became evident that the solubility of these poorly soluble marketed drugs was mainly limited by the compounds' being poorly solvated in water. Unfortunately, only a few of the compounds investigated in our previous study have been assessed for solubility in BDM; but for the investigated compounds, the solubility in, e.g., FeSSIF was much higher than in water (40- to 10000-fold). This indicates that, to make a reasonable estimation of the in vivo solubility of poorly soluble drugs, the solubility should be assessed in BDM. Based on the findings in our previous studies, we have recommended this to be performed for compounds with a lipophilicity (log P_{oct}) > 3.[3]

The short transit time in the small intestine may lead to incomplete dissolution and subsequent inadequate absorption of poorly soluble compounds. The dissolution rate in the intestinal fluid can, therefore, be more biorelevant for some compounds than the maximum apparent solubility. Furthermore, the sink condition, which allows the dissolution to be unaffected by previously dissolved material, may be maintained in the intestinal lumen for highly permeable compounds. Studying the dissolution rate in the different BDM and using the experimental results to select compounds to advance to further development is one way to speed up the assessment of in vivo performance. Further, regulatory authorities demand to be provided with the dissolution rate and characterization, in terms of the Biopharmaceutics Classification System (BCS), to determine the bioequivalence of immediate release (IR) solid dosage forms.[4] The BCS sorts drug molecules into four different classes on the basis of their permeability and solubility.[5] A solubility is defined as high if the maximum oral dose given is soluble in 250 mL of water at 37 °C over the pH interval 1−7.5; otherwise it is considered to be low. Permeability is defined as high if 90% of the oral dose given is absorbed. The compound is considered as rapidly dissolving if $\geq$85% of the dose given is dissolved within 30 min in $\leq$900 mL of three different buffers, representing the pH interval of the gastrointestinal tract. Whereas several different methodologies are suggested for the solubility and permeability assessments, only the classical dissolution testing equipment, USP Apparatus (I and II), is recommended for the dissolution testing.[4] Such technological platforms, which require a large amount of sample for a single measurement, cannot be used if BCS profiling is to become feasible earlier in the drug discovery process. One appealing approach would be to assess apparent BCS profiles in the drug discovery setting, i.e., the BCS class obtained under specific physiological conditions, with the purpose being to include such properties in the forthcoming selection of compounds to pursue as candidate drugs. As previously shown, both solubility and permeability can be assessed adequately from small quantities of material.[6,7] Recently, we also introduced a miniaturized disk intrinsic dissolution rate (IDR) apparatus, which we have shown to produce IDR values comparable to those obtained by the traditional Wood's apparatus.[8]

In this study we wanted to investigate if the recently introduced miniaturized equipment for solubility and dissolution rate studies could be used to study the behavior of poorly soluble compounds in BDMs. If so, it would be possible to address the question of the apparent BCS class earlier in the discovery process. With this aim, we investigated the dissolution rate and the apparent solubility of ten poorly soluble molecules with "grease ball" characteristics in FaSSIF, FeSSIF, and their corresponding blanks, using the miniaturized dissolution apparatus previously described.[8] The results obtained were analyzed to determine which molecular features will gain most in solubility when dissolved in FaSSIF and FeSSIF. Finally, a glimpse of the in vivo situation for poorly soluble compounds was obtained by sorting the studied compounds according to an apparent BCS.

Experimental Section

Compounds and Their Characteristics. Ten compounds (three compounds with no net charge in the pH interval studied, three acids and four bases; Table 1) were selected from previous studies performed in our laboratories.[3,9,10] All compounds were purchased from Sigma Aldrich (St. Louis, MO) with the exception of carvedilol, which was a kind gift from Roche Diagnostics GmbH (Mannheim, Germany).

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(2) 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.

(3) 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.

(4) 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 May 182010).

(5) Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution an *in vivo* bioavailability. *Pharm. Res.* **1995**, *12* (3), 413–420.

(6) Bergström, C. A. S.; Norinder, U.; Luthman, K.; Artursson, P. Experimental and computational screening models for prediction of aqueous drug solubility. *Pharm. Res.* **2002**, *19* (2), 182–188.

(7) Alsenz, J.; Haenel, E. Development of a 7-day, 96-well Caco-2 permeability assay with high-throughput direct UV compound analysis. *Pharm. Res.* **2003**, *20* (12), 1961–1969.

(8) 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.

(9) Bergström, C. A. S.; Luthman, K.; Artursson, P. Accuracy of calculated pH-dependent aqueous drug solubility. *Eur. J. Pharm. Sci.* **2004**, *22* (5), 387–398.

(10) Wassvik, C. M.; Holmen, A. G.; Bergström, C. A. S.; Zamora, I.; Artursson, P. Contribution of solid-state properties to the aqueous solubility of drugs. *Eur. J. Pharm. Sci.* **2006**, *29* (3−4), 294–305.

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

		pK_a, 25 °C			pK_a, 37 °C			
compound	MW	acidic	basic	ref	acidic	basic	$\log P_{oct}$	*T*m (°C)[b]
albendazole	265.3	10.43	4.21	37	nd	nd	3.14[37]	178.1
astemizole	458.6	8.20	5.61	38	8.29	5.34	4.10[39]	174.4
carvedilol	406.5	7.97		40	8.20		4.14[40]	114.1
cinnarizine	365.5	7.69	2.55	*c*	7.45	nd	5.71[40]	120.2
danazol	337.5						4.53[21]	228.6
felodipine	384.3						5.58[38]	139.1
glibenclamide	494.0	5.75		38	5.88		3.08[41]	
indomethacin	357.8	4.42		40	4.27		3.51[40]	159.8
tamoxifen	371.5	8.48		40	8.36		5.26[40]	97.8
tolfenamic acid	261.7	4.20		42	4.82		5.70[42]	212.1

[a] pK_a at 37°C were determined within this work except for glibenclamide which value was taken from ref 8. Danazol and felodipine are nonprotolytic compounds in the interval 2−12 and were therefore not assessed for pK_a. Albendazole was not determined (nd) at 37 °C due to the fact that a temperature effect on the acidic pK_a (10.43) and basic pK_a (4.21) would play a minor role in the solubility and dissolution at pH 5.0 and 6.5. Further, the lower pK_a of cinnarizine of 2.55 was not measured at 37°C since a change in this basic pK_a would have no significant impact on the solubility and dissolution at pH 5.0 and 6.5 used in the study. [b] Melting points determined with DSC previously reported.[3] [c] Unpublished work (Kansy, M., Bendels, S., Wagner, B.; Hoffmann-La Roche, 2006).

Reagents for preparation of FaSSIF and FeSSIF were purchased from Sigma Aldrich and ePhares.com (ePhares Drug Delivery AG, Switzerland). Molecular descriptors were calculated with DragonX (Talete, Italy) and ADMET Predictor (SimulationsPlus, CA).

pK_a Measurement. The Gemini Profiler (*p*ION INC.,MA) was used to determine the pK_a values of the ionizable drugs studied here. For each compound, at least three replicate titrations were performed at 37 ± 0.5 °C in 1 mL of 0.15 M KCl medium. The titrated solutions were bathed with argon, to minimize the ingress of ambient carbon dioxide. The double-junction pH electrode was calibrated in situ during the pK_a data collection[8,11] using the Avdeef−Bucher 4-parameter procedure,[12] thus eliminating the need for the traditional separate "blank" titration. As typical procedures, acids were titrated from pH 12.2 to 1.8 with 0.5 M HCl, while bases were titrated from pH 1.8 to 12.2 with 0.5 M KOH. Owing to the low solubility of the compounds, the 1-propanol cosolvent procedure was used,[8,11] where the apparent pK_a values at various ratios of cosolvent (12−47 wt %) were extrapolated to zero wt % cosolvent to estimate the aqueous value. The cosolvent approach was also used when determining the pK_a of cinnarizine at 37 ± 1 °C; however the GLpKa apparatus (Sirius Analytical Instruments, U.K.) was used in this assay.

Preparation of FaSSIF, FeSSIF and Corresponding Blank Buffers. The FaSSIF, FeSSIF and their corresponding blank buffers were prepared in accordance to the protocol of Galia and co-workers.[1] Briefly FaSSIF (pH 6.5) and FeSSIF (pH 5.0) blank buffers were prepared as stock solutions and short-term stored in a refrigerator. On the day of the experiment, fresh FaSSIF and FeSSIF were prepared by addition of lecithin and sodium taurocholate. The resulting FaSSIF contained 3 mM sodium taurocholate, 0.75 mM egg lecithin and had an ionic strength of 0.16 M whereas FeSSIF contained 15 mM sodium taurocholate, 3.75 mM lecithin and had an ionic strength of 0.32 M. Both FaSSIF and FeSSIF were clear solutions.

Dissolution Rate and Solubility Measurements. Dissolution rate and apparent solubility in each media were measured with the μDISS Profiler (*p*ION INC, Woburn, MA).[8] In each experiment ($n \geq 3$), dissolution rate and solubility were determined in 10−20 mL of BDM at 37 ± 1 °C using a stirring rate of 100 rpm. Dependent on the expected solubility of each compound in the BDM under investigation the path length of the in situ UV probe was varied between 2 and 20 mm; when a high solubility was anticipated, the shorter probe length was used. Each channel was calibrated with its own standard curve prior to the experiment. To calibrate, an aliquot of a DMSO stock solution of the compound under study was dispensed to 4 mL of BDM followed by one minute of stirring. Thereafter the UV absorbance was read and the concentration determined. The addition of an aliquot of DMSO stock solution was repeated 4−9 times to enable a large concentration interval to be covered by the standard curve but remaining within the linear range of the absorbance. After determination of the standard curve, the experiment was started, with data collected in triplicate. The experiment was performed in new vials to which at least 2−3 times more powder than the expected solubility value was added. Through this approach, excess material was present throughout the experiment, which allowed not only the dissolution rate but also the apparent solubility to be determined. The vials were transferred to the μDISS Profiler, the cross-bar magnetic stirrers were inserted and 10−20 mL of preheated medium (37°C) was added as the run was started. The in situ UV probes scanned the samples at predefined time intervals, commencing with scans every 5 s in the beginning of the run to allow

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(11) Avdeef, A.; Tsinman, K.; Tsinman, O.; Sun, N.; Voloboy, D. Miniaturization of powder dissolution measurement and estimation of particle size. *Chem. Biodivers.* **2009**, *6* (11), 1796–1811.

(12) Avdeef, A.; Bucher, J. J. Accurate measurements of the concentration of hydrogen ions with glass electrode: calibrations using the Prideaux and other universal buffer solutions and a computer-controlled automatic titrator. *Anal. Chem.* **1978**, *50*, 2137–2142.

accurate determination of the dissolution rate to one scan every 30 min at the end of the measurement to confirm the apparent solubility. The concentrations were determined by considering 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.[8,13,14] Measurement of pH after completion of each study confirmed that the pH was stable in all four media used and for all the compounds investigated. The obtained solubility values were combined with permeability data taken from literature[15−17] to allow an apparent BCS to be performed. Exceptions were tolfenamic acid and cinnarizine, for which we could not find literature data and therefore predicted these compounds with our in-house in silico Caco-2 model.[18,19] Both compounds were predicted to have high permeability across Caco-2. The apparent BCS classification performed in this study was based on the solubility described as dose number in the different media at the specific investigated pHs (5.0 and 6.5). This should not be regarded as a BCS for regulatory purposes, since that would require investigations of the complete pH interval of 1−7.5.[4] The dose number (Do) for the compounds was calculated from

$$\mathrm{Do} = \frac{M_0/V_0}{C_\mathrm{S}} \tag{1}$$

where M_0 is dose, V_0 is available volume (250 mL) and C_S is saturated concentration or solubility in medium.[20] A Do below 1 implies that the dose is soluble in the specified volume of the medium. A higher number means that the dose is not completely soluble in 250 mL of the medium, and hence, a larger volume is needed to solve the dose.

The solubilization ratio resulting from taurocholic acid (SR) in FaSSIF and FeSSIF was calculated in accordance with the publication by Mithani and co-workers,[21]

$$\mathrm{SR} = \frac{\mathrm{SC_{bs}}}{\mathrm{SC_{aq}}} \tag{2}$$

where $\mathrm{SC_{bs}}$ is the solubilization capacity of the micelles of taurocholic acid (moles drug/mol taurocholic acid) and $\mathrm{SC_{aq}}$ is the solubilization capacity of the water (moles drug/mol water).

Statistics and Physicochemical Analysis. The coefficient of determination (R^2) from linear regression performed in Excel (Microsoft Office XP) was used to assess the quality of the fit of, e.g., standard curves for solubility and dissolution rate measurement. Data refinement was performed with the μDISS Profiler software, and the results obtained are presented as mean values ± standard deviation ($n \geq 3$). The physicochemical analysis of how molecular features are related to the apparent solubility and the solubility difference between FaSSIF, FeSSIF and their corresponding blanks was performed using multivariate data analysis applying partial least-squares projection to latent structures (PLS) in Simca-P 11.0 (Umetrics, Sweden). In the model development, the following responses were investigated: the apparent solubility in FaSSIF, FeSSIF, $\mathrm{FaSSIF_{blk}}$ and $\mathrm{FeSSIF_{blk}}$, the solubility ratio of FaSSIF/$\mathrm{FaSSIF_{blk}}$ and FeSSIF/$\mathrm{FeSSIF_{blk}}$, and solubilization capacity expressed as the ratio (SR) of $\mathrm{SC_{bs}}$ (moles drug/mole bile salt) and $\mathrm{SC_{aq}}$ (moles drug/mole water). All responses were transformed ($\log_{10}$) prior to the model development. We used calculated molecular descriptors obtained from Dragon (Talete, Italy) and ADMET Predictor (SimulationsPlus, CA), and no experimental data were included as variables. The data were mean centered and scaled to unit variance. A variable selection was applied to decrease the complexity of the models and facilitate interpretation. First, the bottom 50% of the variables exhibiting the lowest level of importance was excluded. Second, variables duplicating the information contained within other variables (residing in the same area of the PLS loading plot) were excluded to leave just a few (2−4) variables representing the key descriptors that encoded the majority of the information related 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). To avoid overtraining the models, a maximum of two principal components was allowed.

The accuracy of the PLS models was judged by the R^2 and the root mean squared error (RMSE). The models were

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(13) Bijlani, V.; Yuonaye, D.; Katpally, S.; Chukwumezie, B. N.; Adeyeye, M. C. Monitoring ibuprofen release from multiparticulates: in situ fiber-optic technique versus the HPLC method. *AAPS PharmSciTech* **2007**, *8* (3), E1−E4.

(14) 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.

(15) Varma, M. V.; Sateesh, K.; Panchagnula, R. Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport. *Mol. Pharmaceutics* **2005**, *2* (1), 12–21.

(16) Custodio, J. M.; Wu, C. Y.; Benet, L. Z. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Adv. Drug Delivery Rev.* **2008**, *60* (6), 717–733.

(17) Zaki, N.; Artursson, P.; Bergström, C. A. S. A modified physiological BCS for prediction of intestinal absorption in drug discovery. Unpublished.

(18) Bergström, C. A. S.; Strafford, M.; Lazorova, L.; Avdeef, A.; Luthman, K.; Artursson, P. Absorption classification of oral drugs based on molecular surface properties. *J. Med. Chem.* **2003**, *46* (4), 558–570.

(19) Stenberg, P.; Norinder, U.; Luthman, K.; Artursson, P. Experimental and computational screening models for the prediction of intestinal drug absorption. *J. Med. Chem.* **2001**, *44* (12), 1927–1937.

(20) Oh, D. M.; Curl, R. L.; Amidon, G. L. Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model. *Pharm. Res.* **1993**, *10* (2), 264–270.

(21) 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.

validated by cross-validated R^2 (Q^2) and permutation tests (100 iterations) in which the values for the response variable were randomized and the multivariate data analysis was repeated to detect whether chance correlations had occurred.

Theoretical Methods

The literature on mechanistic modeling of powder dissolution is vast.[22] The treatments are most often framed in terms of the Noyes−Whitney[23] or the Nernst−Brünner[24,25] equation. According to the Nernst−Brünner thin film theory, a stagnant layer of solvent adhers to the surface of a solid compound immersed in an aqueous medium. The thickness of this aqueous boundary layer (ABL) is denoted as h. The concentration of the compound at the solid−liquid interface is equal to the solubility, S, of the compound. According to the classical theory, the diffusional transport of the dissolving compound across the ABL is the rate-limiting process in dissolution. The compound concentration across the ABL decreases from S at the solid surface to C in the bulk solution.

In this study, powder dissolution data were fitted with a biexponential equation (eq 3), as suggested by Tinke et al.,[26] based on the assumption that there may be up to two particle-size populations

$$C_{\mathrm{tot}}(t) = C_0^\infty[1 - \mathrm{e}^{-k_0(t-t_{\mathrm{LAG}})}] + C_1^\infty[1 - \mathrm{e}^{-k_1(t-t_{\mathrm{LAG}})}] \tag{3}$$

in which C_{tot} is the total concentration (μg mL^{-1}) of the dissolved drug as a function of time t (s); C_0^∞ and C_1^∞ are concentrations at $t = \infty$ (the sum of which is the solubility in a saturated solution), k_0 and k_1 (s^{-1}) are rate constants and t_{LAG} is the time (s) at which the dissolution starts, i.e., the t_{LAG} is a small lag arising from experimental timing delays from, e.g., poor wettability. At t_{LAG}, the derivative of eq 3 with respect to t is set equal to the limiting slope in the Nernst−Brünner equation:

$$\frac{\mathrm{d}C_{\mathrm{tot}}(t_{\mathrm{LAG}})}{\mathrm{d}t} = k_0C_0^\infty + k_1C_1^\infty = \frac{A_{\mathrm{app}}}{V}\cdot\frac{D}{h_{\mathrm{app}}}\cdot S \tag{4}$$

from which the ratio of the apparent total surface area, A_{app} (cm^2) and the apparent thickness of the ABL, h_{app} (cm), at the start of dissolution process is defined as

$$\left(\frac{A_{\mathrm{app}}}{h_{\mathrm{app}}}\right) = \frac{V}{\mathrm{DS}}(k_0C_0^\infty + k_1C_1^\infty) \tag{5}$$

in which D (cm^2 s^{-1}) is the diffusivity of the drug in the medium, V (cm^3) is the volume of the medium, and S (μg mL^{-1}) is the solubility of the drug. When excess material is present, $S = C_0^\infty + C_1^\infty$. Nonlinear weighted regression analysis in the μDISS Profiler software was used to determine the five constants associated with eq 3: C_0^∞, k_0, C_1^∞, k_1, and t_{LAG}.

Effective Diffusivity in Biorelevant Dissolution Medium. When solute molecules diffuse through a medium containing micelles, the fraction of the molecules bound to the micelles, f_{BDM}, will migrate at a slower speed than that which is unbound, owing to the larger size of the micelles. If the molecular weight (MW) of the drug−micelle complex is known, then the diffusivity would be expected to be proportional to MW$^{-1/3}$. For small druglike molecules, diffusivity is more likely to be proportional to MW$^{-1/2}$.[27] A number of investigators have proposed an "effective" diffusivity, D_{eff}, to describe the diffusion of drugs in the presence of micelles,[28,29]

$$D_{\mathrm{eff}} = f_{\mathrm{BDM}}D_{\mathrm{BDM}} + (1 - f_{\mathrm{BDM}})D_{\mathrm{aq}} \tag{6}$$

where D_{BDM} is the aqueous diffusivity of micelles (with bound drug) under the solution conditions of the BDM (37 °C, pH 5.0 and $I = 0.32$ M or pH 6.5 and $I = 0.16$ M), D_{aq} is the aqueous diffusivity under the conditions of the BDM "blank" buffer, i.e., the solution containing all the components of BDM except for the taurocholate and the lecithin, and f_{BDM} is the fraction bound by micelles. The latter can be calculated by

$$f_{\mathrm{BDM}} = \frac{S_{\mathrm{BDM}} - S_{\mathrm{BDMblank}}}{S_{\mathrm{BDM}}} \tag{7}$$

where S_{BDM} is the solubility of the compound in the solution containing micelles and $S_{\mathrm{BDM\ blank}}$ is the solubility in the corresponding buffer without micelles. Equation 7 is valid if $S_{\mathrm{BDMblank}} < S_{\mathrm{BDM}}$, otherwise $f_{\mathrm{BDM}} = 0$. The D_{aq} (cm^2 s^{-1}) at 37 °C were calculated from the empirical equation[27]

$$D_{\mathrm{aq}} = (99.15 \times 10^{-6})\mathrm{MW}^{-0.453} \tag{8}$$

The D_{BDM} (cm^2 s^{-1}) values were estimated according to the equation presented by Sugano et al.[30]

$$D_{\mathrm{BDM}} = (6.63 \times 10^{-6})d_{\mathrm{BDM}}{}^{-1} \tag{9}$$

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(22) Dokoumetzidis, A.; Macheras, P. A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system. *Int. J. Pharm.* **2006**, *321* (1−2), 1–11.

(23) Noyes, A. S.; Whitney, W. R. The rate of solution of solid substances in their own solutions. *J. Am. Chem. Soc.* **1897**, *19*, 930–934.

(24) Brünner, E. Reaktionsgescwindigkeit in heterogenen systemen. *Z. Phys. Chem.* **1904**, *57*, 56–102.

(25) Nernst, W. Theorie der reaktionsgeschwindigkeit in heterogenen systemen. *Z. Phys. Chem.* **1904**, *47*, 52–55.

(26) Tinke, A. P.; Vanhoutte, K.; De Maesschalck, R.; Verheyen, S.; De Winter, H. A new approach in the prediction of the dissolution behavior of suspended particles by means of their particle size distribution. *J. Pharm. Biomed. Anal.* **2005**, *39* (5), 900–907.

(27) Avdeef, A. Leakiness and Size Exclusion of Paracellular Channels in Cultured Epithelial Cell Monolayers-Interlaboratory Comparison. *Pharm. Res.* **2010**, *27* (3), 480–489.

(28) Higuchi, W. I. Effects of Interacting Colloids on Transport Rates. *J. Pharm. Sci.* **1964**, *53*, 532–535.

(29) Amidon, G. E.; Higuchi, W. I.; Ho, N. F. Theoretical and experimental studies of transport of micelle-solubilized solutes. *J. Pharm. Sci.* **1982**, *71* (1), 77–84.

(30) Sugano, K.; Okazaki, A.; Sugimoto, S.; Tavornvipas, S.; Omura, A.; Mano, T. Solubility and dissolution profile assessment in drug discovery. *Drug. Metab. Pharmacokinet.* **2007**, *22* (4), 225–254.

Table 2. Apparent Solubility and Intrinsic Dissolution Rate[a]

	apparent solubility (μg/mL)				IDR (μg/min/cm^2)			
	pH 6.5		pH 5.0		pH 6.5		pH 5.0	
compound	FaSSIF$_{\mathrm{blk}}$	FaSSIF	FeSSIF$_{\mathrm{blk}}$	FeSSIF	FaSSIF$_{\mathrm{blk}}$	FaSSIF	FeSSIF$_{\mathrm{blk}}$	FeSSIF
albendazole	0.85 ± 0.36	1.9 ± 0.0	1.1 ± 0.0	6.1 ± 0.1	0.09 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.30 ± 0.01
astemizole	19.2 ± 0.2	97.9 ± 2.1	248 ± 6	182 ± 1	1.80 ± 0.10	7.76 ± 0.17	22.7 ± 0.6	16.9 ± 0.1
carvedilol	46.0 ± 1.0	55.9 ± 1.0	243 ± 2	305 ± 2	4.40 ± 0.10	5.40 ± 0.09	23.2 ± 0.2	26.0 ± 0.2
cinnarizine	1.4 ± 2.5	13.4 ± 0.9	11.8 ± 0.2	112 ± 2	0.14 ± 0.25	0.33 ± 0.02	1.18 ± 0.17	4.44 ± 0.07
danazol	0.6 ± 0.0	8.4 ± 0.7	0.9 ± 0.1	28.8 ± 0.4	0.06 ± 0.01	0.16 ± 0.01	0.09 ± 0.01	0.95 ± 0.01
felodipine	1.2 ± 0.0	54.5 ± 3.7	1.1 ± 0.0	237 ± 1	0.12 ± 0.01	0.64 ± 0.04	0.11 ± 0.00	7.16 ± 0.01
glibenclamide	4.5 ± 1.1	4.7 ± 0.1	0.3 ± 0.0	2.5 ± 0.2	0.48 ± 0.11	0.41 ± 0.01	0.03 ± 0.00	0.10 ± 0.01
indomethacin	219 ± 78	443 ± 10	14.9 ± 0.2	109 ± 7	21.7 ± 7.8	33.5 ± 0.7	1.49 ± 0.02	4.63 ± 0.28
tamoxifen	5.9 ± 0.2	156 ± 2	22.6 ± 0.9	236 ± 13	0.58 ± 0.02	2.25 ± 0.03	2.21 ± 0.09	23.1 ± 1.3
tolfenamic acid	27.4 ± 0.5	63.0 ± 2.7	0.8 ± 0.0	41.0 ± 0.5	2.98 ± 0.06	4.01 ± 0.17	0.09 ± 0.01	1.31 ± 0.02
min	0.6	1.9	0.3	2.5	0.06	0.13	0.03	0.10
max	219	443	248	305	21.7	33.5	23.2	26.0

[a] Apparent solubility and intrinsic dissolution rate (IDR) in fasted state simulated intestinal fluid (FaSSIF), fed state simulated fluid (FeSSIF) and their corresponding blank buffers without taurocholic acid and lecithin (FaSSIF$_{\mathrm{blk}}$ and FeSSIF$_{\mathrm{blk}}$).

where d_{BDM} (nm units) is the micelle particle diameter, estimated as a function of the concentration of the mixed micelle, C_{BDM} which is equal to the concentration of taurocholate (mM),

$$d_{\mathrm{BDM}} = \frac{700}{37.1 - 7.90C_{\mathrm{BDM}}} \tag{10a}$$

and is valid for $C_{\mathrm{BDM}} < 4$ mM, e.g., FaSSIF, whereas the diameter of the micelles in a medium containing $C_{\mathrm{BDM}} >$ 4 mM (e.g., FeSSIF) is calculated through

$$d_{\mathrm{BDM}} = 5.31 + \frac{1}{0.143C_{\mathrm{BDM}} - 0.562} \tag{10b}$$

These calculations result in $D_{\mathrm{BDM}} = 0.13 \times 10^{-6}$ cm^2 s^{-1} for C_{BDM} of 3 mM (FaSSIF) and $D_{\mathrm{BDM}} = 1.1 \times 10^{-6}$ cm^2 s^{-1} for C_{BDM} of 15 mM (FeSSIF). These diffusion coefficients were used when the data were refined with the μDISS Profiler software.

Approximation of the Rotating Disk IDR in BDM. In BDM, the IDR based on the rotating disk thin-film model can be stated to be

$$\mathrm{IDR}_{\mathrm{disk}} = \frac{\mathrm{DR}^{\max}_{\mathrm{disk}}}{A_{\mathrm{disk}}} = \frac{1}{h_{\mathrm{disk}}}D_{\mathrm{eff}}S \tag{11}$$

in which $\mathrm{DR}^{\max}_{\mathrm{disk}}$ (μg s^{-1}) is the maximum slope in the disk dissolution curve, i.e., slope of amount dissolved ($C_{\mathrm{tot}}V$) versus time (s), evaluated at $t > t_{\mathrm{LAG}}$. The IDR based on polydisperse powders may be similarly stated as

$$\mathrm{IDR}_{\mathrm{pwd}} = \frac{\mathrm{DR}^{\max}_{\mathrm{pwd}}}{A_{\mathrm{app}}} = \frac{1}{h_{\mathrm{app}}}D_{\mathrm{eff}}S \tag{12}$$

in which $\mathrm{DR}^{\max}_{\mathrm{pwd}}$ (μg s^{-1}) is the maximum slope in the powder dissolution curve. When eq 12 is substituted into eq 11, the common $D_{\mathrm{eff}}S$ term is removed, giving

$$\mathrm{IDR}_{\mathrm{disk}} = \mathrm{DR}^{\max}_{\mathrm{pwd}}\left(\frac{h_{\mathrm{app}}}{A_{\mathrm{app}}}\right)\frac{1}{h_{\mathrm{disk}}} \tag{13}$$

in which $h_{\mathrm{app}}/A_{\mathrm{app}}$ is obtained from eq 5. The h_{disk} (cm) can be estimated from the Levich equation,[31]

$$h_{\mathrm{disk}} = 4.98\eta^{1/6}{D_{\mathrm{eff}}}^{1/3}\cdot \mathrm{RPM}^{-1/2} \tag{14}$$

where RPM (rev min^{-1}) is the rotation speed, and η is the solvent kinematic viscosity (0.00696 cm^2 s^{-1} in aqueous solution at 37 °C[32]). In this study, it was assumed that the aqueous viscosity is unchanged in the presence of 3 or 15 mM mixed micelles. Equations 5 and 14 and the aqueous kinematic viscosity value are then inserted into eq 13, to produce the following expression to approximate traditional rotating disk IDR (μg s^{-1} cm^{-2}), using data obtained from powder measurements (37 °C), similarly to the approach described earlier for aqueous solutions:[11]

$$\mathrm{IDR}_{\mathrm{disk}} = 0.460{D_{\mathrm{eff}}}^{2/3}\sqrt{\mathrm{RPM}}(C_0^{\infty} + C_1^{\infty}) \tag{15}$$

D_{eff} in eq 15 can be estimated from eqs 6−10.

Results

Solubility and Dissolution Rate Measurements. Ten poorly soluble drugs were assessed to determine their intrinsic dissolution rate and apparent solubility in FaSSIF, FeSSIF and the corresponding blank buffers using a miniaturized dissolution method (Table 2). Of the compounds studied, four were bases (Figure 1), three were acids (Figure

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(31) Levich, V. G. In *Physicochemical hydrodynamics*; Prentice-Hall: Englewood Cliffs, NJ, 1962; pp 39−72.

(32) Bull, H. In *An Introduction to Physical Biochemistry*; FA Davies Co.: Philadelphia, PA, 1964; p 233.

(33) Bergström, C. A. S.; Wassvik, C. M.; Norinder, U.; Luthman, K.; Artursson, P. Global and local computational models for prediction of aqueous solubility of drug-like molecules. *J. Chem. Inf. Comput. Sci.* **2004**, *44* (4), 1477–1488.

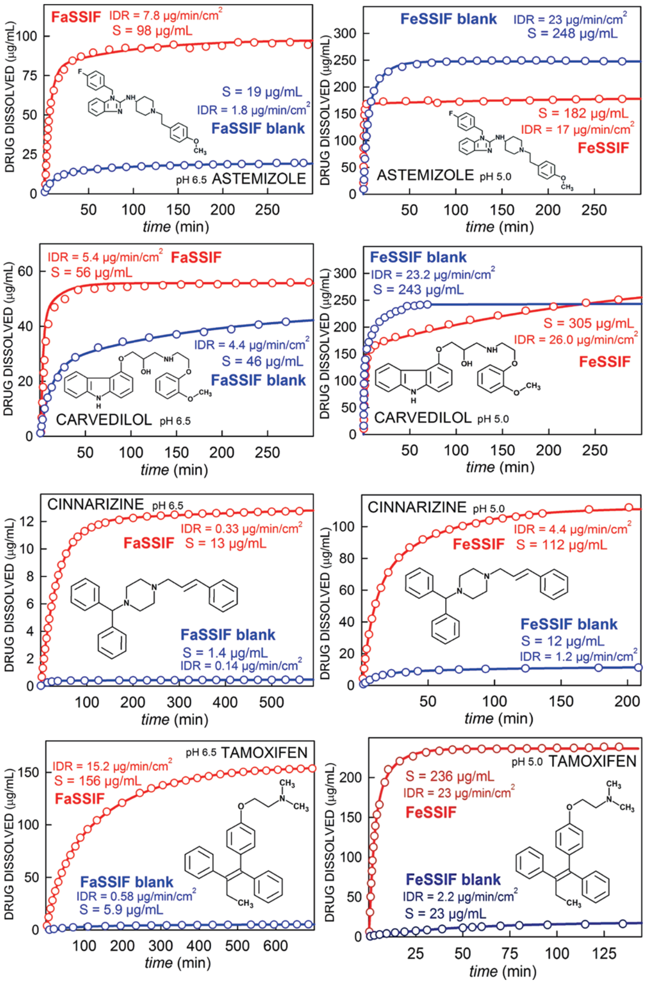


Figure 1. Dissolution rate curves of the four bases investigated. The four dissolution curves obtained in FaSSIF and FeSSIF and their corresponding blank buffers are shown. S_x/S_0 is the ratio between the apparent solubility obtained in the modified media (FaSSIF or FeSSIF) and the solubility obtained in the corresponding blank buffer. All bases are more ionized at pH 5 (FeSSIF and FeSSIF$_{blk}$) than pH 6.5 (FaSSIF and FaSSIF$_{blk}$).

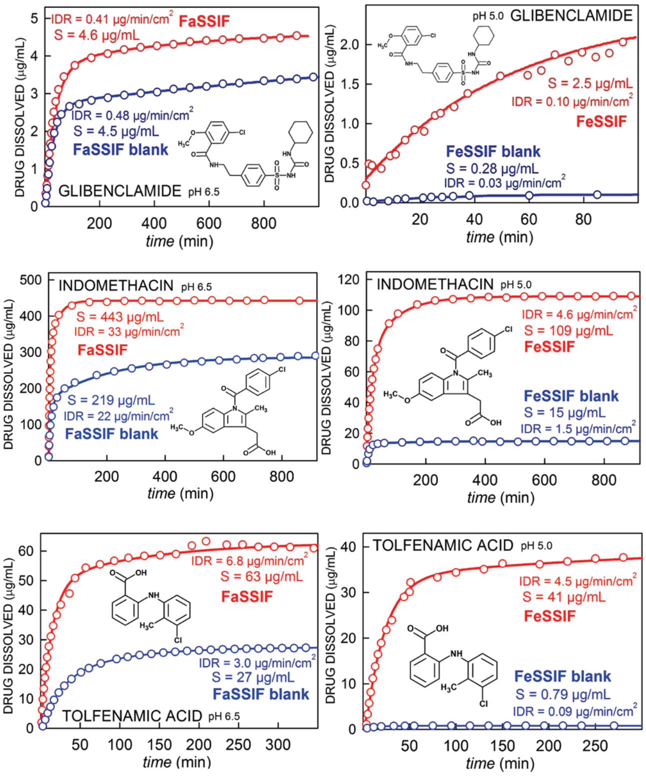


Figure 2. Dissolution rate curves of the three acids investigated. The dissolution curves obtained in FaSSIF and FeSSIF and their corresponding blank buffers are shown. S_x/S_0 is the ratio between the apparent solubility obtained in the modified media (FaSSIF or FeSSIF) and the solubility obtained in the corresponding blank buffer. All acids are more ionized at pH 6.5 (FaSSIF and FaSSIF$_{blk}$) than pH 5.0 (FeSSIF and FeSSIF$_{blk}$).

2) and three were essentially neutral at the pHs studied (Figure 3). The method used collects in situ dissolution profiles in 1−20 mL vessels filled with the aforementioned media without having to filter the solutions. The powder dissolution experiments required samples in the lower milligram scale and used only 10−20 mL of dissolution media. Excess material was added to the dissolution medium to allow determination of the apparent solubility of the compounds, which in this study ranged from 0.3 μg/mL (glibenclamide in FeSSIF$_{blk}$) to 443 μg/mL (indomethcin in FaSSIF) (Table 2). Dose number (Do) for the examined substances was calculated as the ratio between oral dose and amount possible to dissolve in 250 mL of medium[20] (Table 3). The dose number for the studied compounds ranged from 0.1 (low dose of carvedilol and felodipine in FeSSIF) to 1882 (albendazole FaSSIF blank buffer). The intrinsic dissolution rate ranged from 0.03 μg/min/cm^2 (glibenclamide in FeSSIF$_{blk}$) to 33.5 μg/min/cm^2 (indomethacin in FaSSIF).

A large increase in solubility in the modified media containing the lecithin and taurocholate was seen for a majority of the compounds (Figure 4). The fold difference ($S_{BDM}/S_{BDMblank}$) ranged from 1.0 (glibenclamide) to 45 (felodipine) in FaSSIF and from 0.7 (astemizole) to 215 (felodipine) in FeSSIF in comparison with the blank buffers. The fold difference in dissolution rate ranged from 0.9 (glibenclamide) to 5.3 (felodipine) in FaSSIF and from 0.7 (astemizole) to 65.1 (felodipine) in FeSSIF.

Inspired by the work performed by Mithani et al.,[21] the relationship between lipophilicity and solubilization ratio (SR) was investigated for the ten compounds (Figure 5). In their work Mithani and co-workers investigated neutral compounds and a good correlation between log P_{oct} and log SR was obtained. When log P_{oct} was correlated with the logarithm of the SR of our compounds, a weak relationship was found ($R^2 = 0.32$, Figure 5A). This was somewhat expected as a majority of the compounds carry a net charge at the pHs investigated. Thus the extent of ionization was considered through the exchange of log P_{oct} to log $D_{oct,pH6.5}$ (SR in FaSSIF) and log $D_{oct,pH5.0}$ (SR in FeSSIF). The resulting correlation showed an R^2 of 0.74 (Figure 5B).

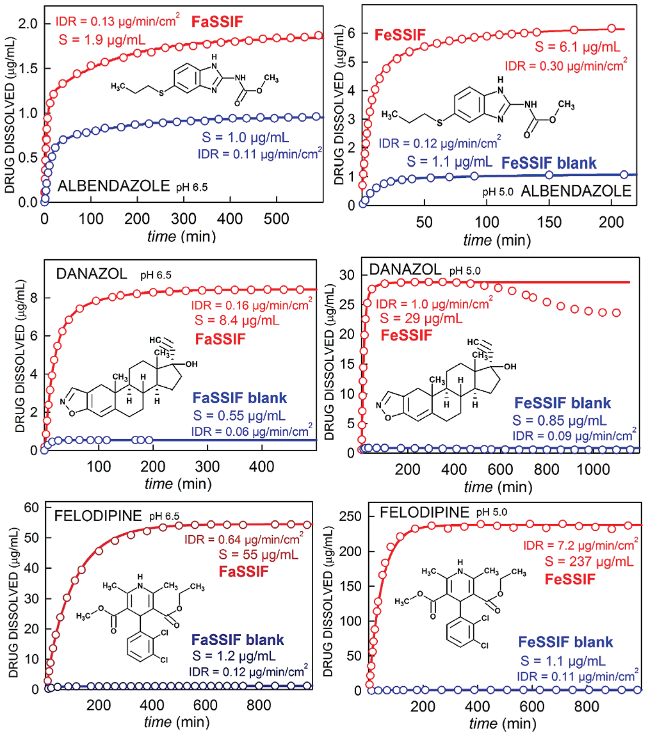


Figure 3. Dissolution rate curves of the three compounds being neutral at the pH interval studied. The dissolution curves obtained in FaSSIF and FeSSIF and their corresponding blank buffers are shown. S_x/S_0 is the ratio between the apparent solubility obtained in the modified media (FaSSIF or FeSSIF) and the solubility obtained in the corresponding blank buffer. The three compounds were essentially neutral at pH 5.0 and 6.5. The apparent decline of solubility with danazol FeSSIF above 400 min is likely due to photodecomposition of the compound.[36] Only the early time points were therefore used to characterize the solubility reported.

Applying the resulting equation displayed in Figure 5B resulted in an RMSE of 0.28 log unit. When separate correlations were produced for FaSSIF and FeSSIF, the R^2 obtained was 0.58 and 0.89, respectively. We also calculated the SR when including the moles of lecithin in the FaSSIF and FeSSIF (data not shown), which resulted in SR that were equal to those obtained from the more simplified calculations based on taurocholic acid alone.

Apparent BCS Classification and Impact of Dissolution Rate. All compounds were shown to be class II compounds in FaSSIF blank buffer when combining the experimentally determined solubility with permeability data taken from the literature.[15,16] The large increase in solubility resulted in a shift from BCS class II to class I in the apparent classification of indomethacin (acid) and tamoxifen (base) in FaSSIF and of four compounds in FeSSIF (Figure 6). According to FDA requirements, 85% or more of the dose needs to be dissolved in 900 mL of the medium within 30 min for a substance to be considered to be rapidly dissolving.[4] The amount of drug that had been dissolved after 30 min was calculated based on IDR obtained from μDISS and compared to the normal and maximum dose given for the studied compounds (Table 3). As anticipated, an increase in the dissolution rate was observed for a majority of the compounds in media containing wetting and solubilizing agents. The dissolution of the maximum dose of astemizol (10 mg) in FeSSIF was sufficiently rapid for the substance to be classified as fast dissolving in accordance with the regulations provided by the FDA, i.e., the dissolution rate in this medium allows 85% of the maximum dose to be dissolved within 30 min. Also carvedilol would be sorted as a rapidly dissolving compound in FeSSIF, but only at the low dose region (6.25 mg up to 12.4 mg).

Investigation of Chemical Features of Importance for Solubility in BDMs. With the purpose to investigate the applicability and usefulness of in silico modeling for prediction of solubility in BDM, we continued our study by analyzing whether calculated molecular descriptors and multivariate analysis can be used to estimate BDM solubility. The models were established with the purpose of indicating if it is likely that in silico modeling can also be used for the prediction of charged compounds in rather complex in vitro media, in the same manner as we have previously shown for the prediction of intrinsic solubility in pure water and simple buffers.[6,18,33] The general applicability of models based on small data sets is difficult to evaluate, but trends can be identified and the obtained results can be used to increase the understanding of the solubility behavior of compounds similar to the ones included in this work. The statistics of the models developed are shown in Table 4. Good to excellent multivariate models were obtained for the small and chemically diverse data set, and descriptors related to rigidity, aromaticity, log *D*, shape, hydrophilicity and polarity were predictive for the compounds studied herein. The models were evaluated through leave-one-out cross-validated R^2 (Q^2) and permutation tests, but so far the models have not been evaluated with a test set. Therefore, to investigate the general applicability of the models on a larger chemical space, further studies are needed.

Discussion

The novel miniaturized powder dissolution approach is applicable to measurements of very poorly soluble compounds. Both intrinsic dissolution rate and apparent solubility could successfully be determined applying this methodology, as shown by the 40 different combinations of poorly soluble drugs and BDM tested. The lowest apparent solubility determined in this data set was 0.3 μg/mL (603 nM), and the lowest intrinsic dissolution rate measured was 0.03 μg/min/cm². The method was shown to be able to handle poorly soluble compounds properly as long as the compound under study had a strong enough chromophore, allowing UV to be used for detection. The solutions were extensively stirred, and a reasonably good wetting of the hydrophobic powders was obtained. We believe that the experimental setting used, i.e., the rigorous stirring, the elevated temperature, the pH of the buffers allowing many of the compounds to be ionized

Table 3. Low Dose and Max Dose and Dose Number (Do) in Examined Media

compound	dose (mg): low; max	Do FaSSIF$_{blk}$: low; max dose	FeSSIF$_{blk}$: low; max dose	FaSSIF: low; max dose	FeSSIF: low; max dose
albendazole	400[a]	1882.4	1454.5	864.9	262.3
astemizole	10[a]	2.1	**0.2**[b]	**0.4**	**0.2**
carvedilol	6.25; 50	**0.5**; 4.3	**0.1**; **0.8**	**0.45**; 3.6	**0.1**; **0.7**
cinnarizine	15; 75	42.9; 214.3	5.0; 25.4	4.5; 22.4	**0.5**; **2.7**
danazol	50; 200	333.3; 1333.3	222.2; 888.8	23.8; 95.2	6.9; 27.8
felodipine	2.5; 20	8.3; 66.7	9.1; 72.7	**0.2**; 1.5	**0.1**; **0.3**
glibenclamide	2.5; 10	2.2; 8.9	33.3; 133.3	2.2; 8.6	4.0; 16.0
indometacin	25; 100	**0.5**; 1.8	6.7; 26.8	**0.2**; **0.9**	**0.9**; 3.7
tamoxifen	10; 20	6.8; 13.6	1.8; 3.5	**0.3**; **0.5**	**0.2**; **0.3**
tolfenamic	100; 200	14.6; 29.2	500.0; 1000.0	6.4; 12.7	9.8; 19.5

[a] Substance is not given in a dose interval: only one dose is suggested. [b] Bold figures denotes Do lower than 1 resulting in a BCS shift from class II to class I. All doses were taken from Martindale.[43]

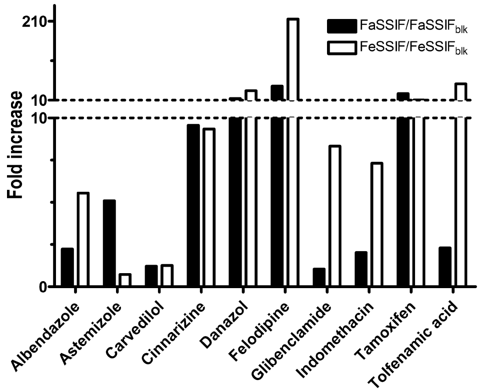


Figure 4. Difference in solubility obtained in FaSSIF and FeSSIF as compared to the corresponding blank buffers. The compounds displayed an apparent solubility ratio (the solubility in the modified media over the solubility in the blank buffer) from 0.7 (astemizole in FeSSIF/FeSSIF$_{blk}$) to 215 (felodipine in FeSSIF/FeSSIF$_{blk}$).

and the inclusion of taurocholic acid and lecithin in the FaSSIF and FeSSIF, allowed the powder material to be wetted.

In this study we wanted to investigate the extent to which it is possible to increase the apparent solubility of poorly soluble compounds and to improve the intrinsic dissolution rate in media reflecting the fasted and fed state in humans. Furthermore, we wanted to determine the reasons for the improved dissolution and solubility. With this aim, we assessed these properties both in simulated media and in the blank buffer system. Through this approach, it was possible to investigate whether the solubility and dissolution changes were related to the extent of ionization of the compound or to improvements brought about by the additives in the simulated media. The FeSSIF used in this study was a homogeneous medium containing small micelles with a diameter in the lower nanometer scale as identified through Cryo-TEM (data not shown). These micelles have the capacity to solubilize lipophilic compounds, and thereby improve their apparent solubility in the medium. When a detailed analysis of the compounds was done, it became

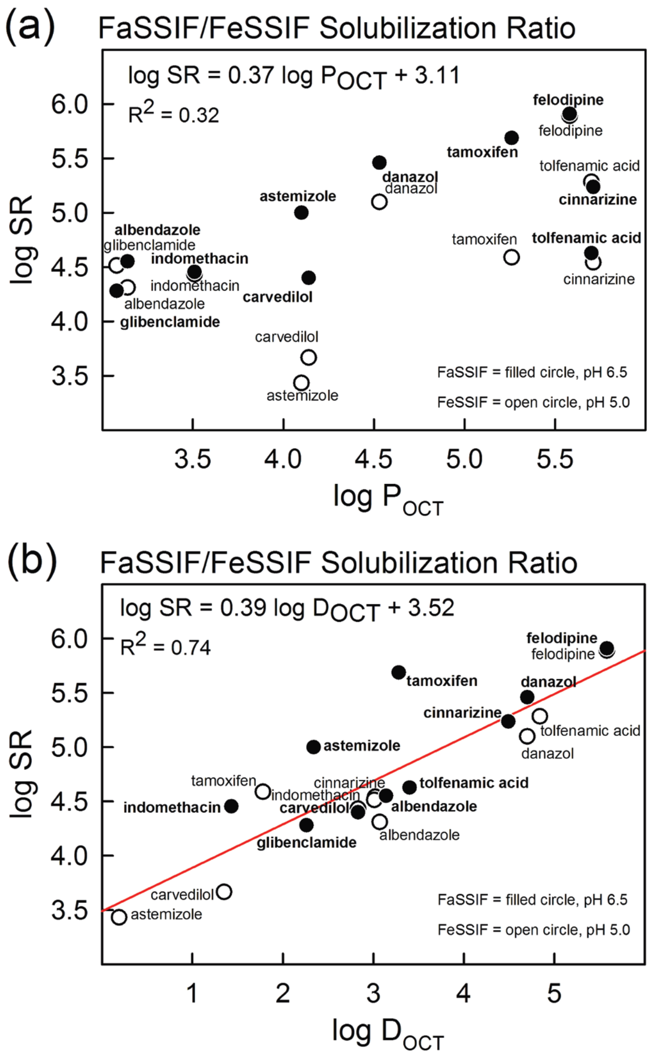
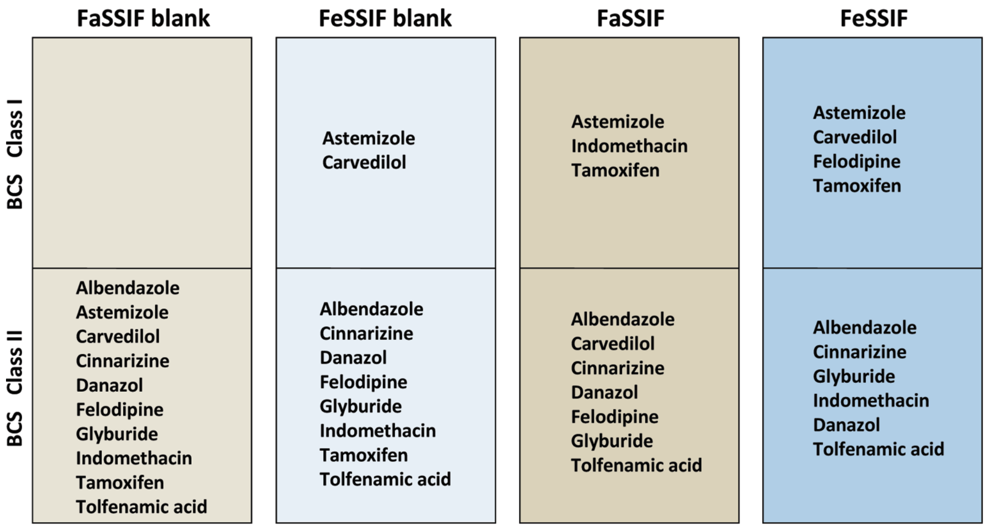


Figure 5. The solubilization capacity of FaSSIF and FeSSIF for the data set studied. (A) The log SR versus the log P_{oct}; an R^2 of 0.32 was obtained. (B) To include the effect of charge on lipophilicity and SR, log P_{oct} was exchanged to log D_{oct}. A better correlation was obtained and R^2 increased from 0.32 to 0.74.



	FaSSIF blank	FeSSIF blank	FaSSIF	FeSSIF
BCS Class I		Astemizole Carvedilol	Astemizole Indomethacin Tamoxifen	Astemizole Carvedilol Felodipine Tamoxifen
BCS Class II	Albendazole Astemizole Carvedilol Cinnarizine Danazol Felodipine Glyburide Indomethacin Tamoxifen Tolfenamic acid	Albendazole Cinnarizine Danazol Felodipine Glyburide Indomethacin Tamoxifen Tolfenamic acid	Albendazole Carvedilol Cinnarizine Danazol Felodipine Glyburide Tolfenamic acid	Albendazole Cinnarizine Glyburide Indomethacin Danazol Tolfenamic acid

Figure 6. Apparent BCS classification for the fasted and fed state. Shift in apparent BCS class when compounds are dissolved in FaSSIF and FeSSIF in comparison to the corresponding blank buffers. Permeability values for the BCS classification were taken from the literature.[15−17]

Table 4. Results of PLS Models[a]

	log S_{app}						log SR	
	$FaSSIF_{blk}$	FaSSIF	$FeSSIF_{blk}$	FeSSIF	log FaSSIF/$FaSSIF_{blk}$	log FeSSIF/$FeSSIF_{blk}$	pH 6.5	pH 5.0
R^2	0.82	0.86	0.85	0.79	0.91	0.95	0.88	0.92
Q^2	0.66	0.71	0.72	0.66	0.84	0.92	0.83	0.87
no. of PCs	1	1	1	1	1	1	1	1
no. of descr	4	4	2	4	4	4	3	2
response interval ($\log_{10}$)	2.54	2.24	2.99	2.17	1.62	2.47	1.64	2.47
RMSE ($\log_{10}$)	0.38	0.33	0.53	0.34	0.18	0.22	0.22	0.23
Ui	•							
$SssCH_2$	•							
SdssC	•				•		•	
SHsOH	•							
Mv		•						
nBM		•						
nN		•		•				
MSD		•			•		•	
HOMT			•	•				
SdO			•					
PW5				•				
PSA				•				
log *D*					•		•	
Hy					•			
nAB						•		•
J						•		
HOMA						•		
AROM						•		•

[a] The models were developed to investigate properties of importance for the solubility of this data set, and were not used for prediction purposes. The findings herein indicate that solubility ratio and solubilization ratio are easier to calculate based on molecular descriptors than the separate solubility values. However, the data set must be expanded to prove the accuracy of predictions by such models. The following descriptors were used in the final models. Dragon descriptors: Unsaturation index (Ui); mean atomic van der Waals volume (scaled on carbon atom) (Mv); number of multiple bonds, nitrogens and aromatic bonds (nBM, nN and nAB, respectively); Balaban mean square distance index (MSD); Harmonic oscillator model of aromaticity index (HOMA); total HOMA (HOMT); Path walk 5 − randic index (PW5); polar surface area (PSA); hydrophilic factor (Hy); Balaban distance connectivity index (J); aromaticity (AROM). ADMETPredictor: Electrotopological state indices of single and double bonded carbons and oxygens (SssCH2, SdssC, SHsOH, SdO, respectively); pH-dependent log P_{oct} (log *D*).

evident that the more solubilizing additives that were introduced the more the neutral compounds (albendazole, danazol and felodipine) dissolved and the higher the apparent solubility became (Table 2 and Figure 4). As expected for nonionizable compounds, no major effect was observed on the apparent solubility or IDR when changing the buffer system from the FaSSIF$_{blk}$ to the FeSSIF$_{blk}$ for these compounds. The trends are more difficult to reveal for the acids and bases. The micelles can bind both ionized and nonionized forms of the compound, but with different binding constants, and therefore the extent of ionization affects not only the pH dependent solubility but also the ability to be solubilized by the mixed micelles. Due to the net negative charge of the micelles formed, it is reasonable to assume that the bases gain more in solubility from the introduction of micelles than do the acids. Among the seven charged compounds measured for solubility and IDR we found one base, astemizole, for which the apparent solubility and IDR were lower in FeSSIF compared to FeSSIF$_{blk}$. However, in FaSSIF, astemizole displays a higher apparent solubility and IDR than in the corresponding blank buffer. Astemizole is a compound with two ionizable amines, and displays two positive charges in the FeSSIF. We also found that carvedilol, a base with pK_a of 8.2 at 37 °C, showed only 1.2-fold and 1.3-fold higher solubility in FaSSIF and FeSSIF, respectively, in comparison to the corresponding blank buffers. When we refined the experimental data with the aggregation module in the μDISS Profiler software, these two compounds, astemizole and carvedilol, were the only ones for which we could identify aggregation effects. When the compounds aggregate with negatively charged counterions, fewer molecules are available for partitioning into micelles than in a monomer solution of the compound. Therefore, the solubility shift does not become as large as would be expected from the log D and net charge profile. If the aggregations occur in the solid state, i.e., at the surface of the powder or by precipitations formed, possibly in situ Raman spectroscopy as described by Lehto and co-workers[34] could be used to identify such transformations. With regard to the acidic compounds, the solubilities of the three acids included in our data set were more strongly affected by changes in pH than by inclusion of the additives, although the latter further improved the apparent solubility. Hence, the negatively charged micelles do not repulse the negatively charged compounds electrostatically, but rather, they solubilize them, although to a lesser extent than they solubilize positively charged compounds.

In previous work on predictions and/or estimations of solubility in media containing bile salts, log P_{oct} has been used as a predictor.[21] Indeed, a good correlation was established between log P_{oct} and the solubilization capacity, expressed as the logarithm of the ratio (log SR) of SC$_{bs}$ (moles of drug/mol of bile salt) over SC$_{aq}$ (moles of drug/mol of water) for a small series of steroids. The studied steroids were neutral, hence the effect of simultaneously changing the lipophilicity and the net charge of the molecule was not addressed. Therefore, we exchanged log P_{oct} to log D_{oct} to include the effect of the charge on the solubilization ratio (Figure 5B), which resulted in $R^2 = 0.74$ for this data set. We note that the correlation between log SR calculated for FaSSIF and log D_{oct} is weaker ($R^2 = 0.58$) compared to log SR FeSSIF versus log D_{oct} ($R^2 = 0.89$). The correlation found between log D_{oct} and log SR obtained in FeSSIF is surprisingly high as it is known that not only the lipophilicity of compounds will influence the partitioning to mixed bilesalt/lecithin micells. Also factors related to solid state properties affect this, and an excellent overview of the effects of the solid state and the lipophilicity on the solubility in BDM has been published by Stella and Nti-Addae.[35] However, from the results obtained in this study we propose that log D_{oct} can be used as a guide to the extent the solubility of "grease ball" molecules will improve in biorelevant media in comparison to aqueous media, in particular for BDMs of high ionic strength containing also micellar systems.

We further investigated the possibility to predict and understand physiologically relevant solubility by using multivariate analysis tools. Well aware of that the data set is small and only consists of marketed compounds, which are supposed to have good pharmaceutical profiles, we still wanted to investigate if it is likely that apparent solubility in biorelevant media will be predictable from chemical structure alone. In our modeling attempts we developed computational models for the prediction of apparent solubility in FaSSIF, FeSSIF, their corresponding blank buffers, the solubility ratio (FaSSIF/FaSSIF$_{blk}$, FeSSIF/FeSSIF$_{blk}$), and the solubilization ratio log SR (SC$_{bs}$/SC$_{aq}$ pH 6.5 and 5.0, respectively). All information related to the computational models is gathered in Table 4. The models obtained showed impressive statistics with R^2 of 0.77−0.92, Q^2 of 0.61−0.89 and valid permutation tests. We have not been able to validate our models with a test set due to the sparse data available

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(34) Lehto, P.; Aaltonen, J.; Tenho, M.; Rantanen, J.; Hirvonen, J.; Tanninen, V. P.; Peltonen, L. Solvent-mediated solid phase transformations of carbamazepine: Effects of simulated intestinal fluid and fasted state simulated intestinal fluid. *J. Pharm. Sci.* **2009**, *98* (3), 985–996.

(35) Stella, V. J.; Nti-Addae, K. W. Prodrug strategies to overcome poor water solubility. *Adv. Drug Delivery Rev.* **2007**, *59* (7), 677–694.

(36) Kariem, E. A.; Abounassif, M. A.; Hagga, M. E.; Al-Khamees, H. A. Photodegradation kinetic study and stability-indicating assay of danazol using high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* **2000**, *23* (2−3), 413–420.

(37) Escher, B. I.; Berger, C.; Bramaz, N.; Kwon, J. H.; Richter, M.; Tsinman, O.; Avdeef, A. Membrane-water partitioning, membrane permeability, and baseline toxicity of the parasiticides ivermectin, albendazole, and morantel. *Environ. Toxicol. Chem.* **2008**, *27* (4), 909–918.

(38) Bendels, S.; Tsinman, O.; Wagner, B.; Lipp, D.; Parrilla, I.; Kansy, M.; Avdeef, A. PAMPA - excipient classification gradient map. *Pharm. Res.* **2006**, *23* (11), 2525–2535.

(39) Hansch, C.; Leo, A. *Pomona College Medicinal Project*, Claremont, CA, 1987.

(40) Avdeef, A. In *Absorption and Drug Development*; Hoboken, NJ, 2003.

(41) Macdonald, F. In *Dictionary of pharmacological agents*; Chapman and Hall: London, U.K., 1997; Vol. 1.

(42) Österberg, T.; Svensson, M.; Lundahl, P. Chromatographic retention of drug molecules on immobilised liposomes prepared from egg phospholipids and from chemically pure phospholipids. *Eur. J. Pharm. Sci.* **2001**, *12* (4), 427–439.

(43) Sweetman, S. C., Ed. *Martindale: The complete drug reference [online]*; London: Pharmaceutical Press(accessed on Jan 29, 2010).

on poorly soluble compounds with "grease ball" characteristics in FaSSIF and FeSSIF in the literature; most of the compounds for which published data are available are already included in our training set, leaving too few compounds to allow a fair evaluation. From the analysis performed herein, the following could be concluded: apparent solubility in BDMs, the solubility ratio and solubilization ratio were related to descriptors of rigidity, aromaticity, log *D*, shape, hydrophilicity/polarity and electrotopological descriptors. For our data set, the extent of ionization was only included once, in the form of a log *D* parameter instead of log *P*. Although the models obtained in this work reveal that it appears possible to derive properties of importance for the solubilization to a certain extent, more studies are needed to develop truly predictive and easily interpretable computational approaches. We will continue our modeling efforts by expanding our data set, and further, we will investigate whether rapidly calculated molecular descriptors will enable the in vivo relevant solubility to be predicted.

The apparent BCS classification showed that five compounds are sorted as BCS class I in media reflecting the small intestinal fluid at the fasted and fed state. From these results it becomes evident that compounds with "grease ball" characteristics, i.e., lipophilic and quite large compounds, often with a high molecular flexibility, will severely suffer as a result of the solubility classification based on the complete pH interval 1−7.5.[4] Although a majority of the compounds show a higher apparent solubility in the full BDM than in their corresponding buffers, this did not result in the same compounds being sorted as rapidly dissolved. One explanation is the lag time for the dissolution to start in the in vitro setting which arises due to less efficient wetting and stirring than what is expected in vivo. Although only a few compounds were rapidly dissolved, we found that, in the BDM, the majority of the compounds had equilibrated to their apparent solubility within the four hour transit time of the drug in the intestinal fluid. This, together with the fact that all compounds display high membrane permeability, which should make them easily absorbed from the intestine once they are in solution, supports the assumption that the complete dose will be dissolved for the compounds upgraded to class I in FaSSIF and FeSSIF. For the compounds studied in this work, food is often discussed in the explanations given to the patients on how to take their medicine. For some drugs, it is clearly stated that they should be taken with food to increase the absorption (e.g., albendazole), but for others it is said that they should not be taken at the same time as food (e.g., astemizole), the latter most likely to ensure a more controlled absorption process. Irrespective of the administration recommendation, this study has clearly identified that the physiological dissolution rate and solubility may differ largely for "grease ball" compounds administered in the fasted and fed state. It is of utmost importance to consider these effects on the final absorption, particularly for "grease ball" molecules displaying a narrow therapeutic window, for which it is likely that a change in the intestinal concentration may lead to a lack of effect or to side effects.

Conclusions

The solubility and dissolution rate of poorly soluble compounds with "grease ball" characteristics are likely to be greatly improved in physiologically relevant dissolution media. The results presented herein show that, in such media, a large proportion of compounds belonging to the BCS class II will be sorted as class I compounds and that greatly enhanced apparent solubility and dissolution rates in the fed state are to be expected. How this will influence the absorption needs to be investigated in greater detail. In the analysis of the molecular characteristics found to improve solubility, we found that neutral and positively charged compounds gain most in solubility in BDM, as long as the compound does not form aggregates with components of the simulated media, whereas for negatively charged compounds ionization has the most substantial effect on solubility. It was revealed that log D_{oct} can be used as a guide to the extent that the solubility of solvation limited compounds will be improved when aqueous buffers are exchanged to biorelevant media containing micellar systems. The results from the computational modeling indicate that it will be possible to estimate the solubility in BDM from the chemical structure alone. Currently, we are expanding our data set on poorly soluble compounds with "grease ball" characteristics to evaluate the accuracy and general applicability of such in silico models.

Acknowledgment. Financial support from the Swedish Medical Products Agency, the Swedish Research Council (Grant 621-2008-3777), the Swedish Fund for Research without Animal Experiments and the Knut and Alice Wallenberg Foundation is gratefully acknowledged. We are grateful to Professor Katarina Edwards and Mr Göran Karlsson at Department of Physical Chemistry, Uppsala University, for the Cryo-TEM analysis of the FeSSIF solution, Dr. Kiyohiko Sugano (Pfizer, U.K.) for sharing preprints of several of his publications and for discussions concerning dissolution technology, and Dr. Manfred Kansy (Roche, Basel) for sharing the unpublished cinnarizine pK_a values at 25 °C.

Supporting Information Available: Tabulation of the $\mathrm{DR}_{\mathrm{pwd,max}}$, k_0, k_1, C_0, C_{inf} and T_{lag} values. This material is available free of charge via the Internet at http://pubs.acs.org.

MP100049M