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A Modified Physiological BCS for Prediction of Intestinal Absorption in Drug Discovery

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Abstract: In this study, the influence of physiologically relevant media on the compound position in a biopharmaceutical classification system (BCS) which resembled the intestinal absorption was investigated. Both solubility and permeability limited compounds ($n = 22$) were included to analyze the importance of each of these on the final absorption. Solubility was determined in three different dissolution media, phosphate buffer pH 6.5 (PhB 6.5), fasted state simulated intestinal fluid (FaSSIF), and fed state simulated intestinal fluid (FeSSIF) at 37 °C, and permeability values were determined using the 2/4/A1 cell line. The solubility data and membrane permeability values were used for sorting the compounds into a BCS modified to reflect the fasted and fed state. Three of the seven compounds sorted as BCS II in PhB 6.5 (high permeability, low solubility) changed their position to BCS I when dissolved in FaSSIF and/or FeSSIF (high permeability, high solubility). These were low dosed (20 mg or less) lipophilic molecules displaying solvation limited solubility. In contrast, compounds having solid-state limited solubility had a minor increase in solubility when dissolved in FaSSIF and/or FeSSIF. Although further studies are needed to enable general cutoff values, our study indicates that low dosed BCS Class II compounds which have solubility normally restricted by poor solvation may behave as BCS Class I compounds in vivo. The large series of compounds investigated herein reveals the importance of investigating solubility and dissolution under physiologically relevant conditions in all stages of the drug discovery process to push suitable compounds forward, to select proper formulations, and to reduce the risk of food effects.

Keywords: Solubility; permeability; fasted state; fed state; intestinal fluid; biorelevant dissolution medium; BCS; intestinal absorption

Introduction

Lately, a considerable amount of attention has been paid to the poor solubility of new highly lipophilic druglike molecules,[1,2] because a poor solubility may lead to an inadequate absorption from the gastrointestinal (GI) tract. Indeed, new techniques for the solubility assessment of these problematic compounds have been developed to address this issue.[1,3−5] Furthermore, formulation strategies have been developed that allow more of these compounds to be dissolved or delivered to the intestinal tract (see e.g, refs

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6−8). However, the solubility in the GI tract can be significantly higher than expected from standard in vitro solubility tests in buffer due to the solubilization in the lipid phase formed by natural lipids and bile acids present in the intestinal fluid.[9−11] In solution, the final barrier for absorption is the intestinal epithelium, and experimental in vitro models are, therefore, standard methodology today for the measurement of intestinal permeability.[12,13] The interaction of solubility and permeability, and the influence of their interaction on the intestinal absorption, have been explored in different ways,[14−16] with the most widely accepted and most commonly used at present being the biopharmaceutics classification system (BCS).[17,18] However, many compounds suffer from the BCS definition of high solubility which states that the maximum dose given orally needs to be soluble in 250 mL of fluid in the pH-interval of 1−7.5, otherwise the compound will be classified as low solubility. The use of the complete pH-interval in the definition of solubility is not feasible in drug discovery. In addition, it is a drawback for many weakly acidic drugs, as they often exhibit low solubility in the stomach but a significantly higher solubility in the small intestine where most of the absorption takes place. Furthermore, simple phosphate buffers are traditionally used in the solubility assessment, limiting the solubility of lipophilic compounds which generally gain solubility in vivo where the solubilization capacity increases through the presence of naturally occurring lipids and bile acids.[11,19−21]

In this study, we investigated to which extent physiologically relevant dissolution media may impact on the BCS classification of marketed drug molecules. In addition, we explored how much solubility and permeability limit the fraction absorbed (FA) for such a data set, and we determined whether the completely absorbed compounds are correctly predicted by the available in vitro methods. Thus, we assessed drug solubility and permeability for 22 drugs by applying more physiologically relevant in vitro dissolution models than are traditionally used. The solubility experiments were designed to measure the apparent solubility in a buffer system, in fasted state simulated intestinal fluid and in fed state simulated intestinal fluid (FaSSIF and FeSSIF, respectively),[9] with the aim being to investigate the influence of lipids included in the physiologically relevant media resembling the intestinal fluid on the solubility of the compounds. The membrane permeability was assessed with the 2/4/A1 cell line, which has a paracellular permeability comparable to that of the human small intestinal epithelium in vivo.[13,22,23] This cell line has previously shown a better quantitative relationship with the human effective permeability data generated with the Loc-I-Gut perfusion technique than did the Caco-2 cell line,[24] and it was, therefore, selected for this study to improve the biorelevance of the permeability model. The data obtained were used to sort the compounds into a modified BCS designed to mimic the small intestine. We

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Table 1. Physicochemical Properties and Experimental Results for the Compounds Studied[a]

compound	M_w	c log *P*	PSA (Å^2)	pK_a	P_{app} ($\times 10^{-6}$ cm/s)	$S_{PhB6.5}$ (μM)	S_{FaSSIF}(μM)	S_{FeSSIF}(μM)	FA (%)
5-FU	130.1	−0.6	64.5	8.0 (a)	26.5 ± 1.2	soluble	soluble	soluble	*28*
acarbose	645.6	−6.7	361.1	5.8 (b)	1.7 ± 0.1	soluble	soluble	soluble	*2*
amiloride	229.6	−0.6	159.7	8.7 (b)	26.9 ± 8.3	soluble	soluble	soluble	*50*
amitriptyline	277.4	4.9	1.2	9.5 (b)	356.0 ± 35.6	>3000	>3000	>3000	100
carbamazepine	236.3	2.0	46.8		356.0 ± 13.7	1135 ± 33	1284 ± 69	1887 ± 133	**72−100**
cyclosporine A	1202.7	14.4	177.8		9.0 ± 2.4	2 ± 1	25 ± 3	66 ± 9	***35−45***
diazepam	284.8	3.2	28.8	3.3 (b)	229.0 ± 7.8	123 ± 20	1265 ± 100	443 ± 59	90
digoxin	781.0	1.6	215.2		56.1 ± 3.0	soluble	soluble	soluble	85
dipyridamole	504.6	2.5	134.7	6.4 (b)	130.0 ± 25.0	22 ± 6	19 ± 2	295 ± 39	**27−88**
enalapril	376.5	0.9	101.9	5.4 (b); 3.0 (a)	5.0 ± 0.5	soluble	soluble	soluble	*66*
erythromycin	734.0	1.5	203.3	8.8 (b)	5.5 ± 0.4	9163 ± 485	8075 ± 701	6036 ± 1673	*35*
felodipine	384.3	5.6	68.7		175.0 ± 25.1	2 ± 1	17 ± 8	117 ± 24	100
furosemide	330.7	1.9	130.0	3.9 (a)	54.5 ± 3.6	>5000	7556 ± 761	2782 ± 202	*65*
hyoscine-*N*-butyl	361.5	1.6	67.7		21.6 ± 2.4	soluble	soluble	soluble	*6*
ketoconazole	531.4	2.6	57.8	6.5 (b)	131.0 ± 4.0	12 ± 4	40 ± 6	455 ± 12	**15−90**
loperamide	477.1	4.7	43.2	8.7 (b)	210.0 ± 25.2	92 ± 6	214 ± 79	230 ± 86	**65−100**
methyldopa	211.2	−2.7	114.5	10.6 (b); 2.2 (a)	44.6 ± 5.4	27294 ± 1516	28271 ± 3421	28937 ± 3234	*41*
metoclopramide	299.8	2.2	70.8	9.7 (b)	289.0 ± 17.7	685 ± 282	1172 ± 25	1332 ± 356	100
pravastatin	424.5	2.3	135.6	4.7 (a)	39.4 ± 2.0	soluble	soluble	soluble	*34*
spironolactone	416.6	2.3	63.6		197.0 ± 13.3	53 ± 7	62 ± 7	110 ± 6	**30−100**
tranexamic acid	157.2	−1.8	68.8	10.6 (a); 4.3 (a)	14.9 ± 4.2	soluble	soluble	soluble	*55*
verapamil	454.6	4.5	56.3	9.1 (b)	312.0 ± 24.3	soluble	soluble	soluble	98
min	130.1	−6.7	1.2	neutral	1.7	2	17	66	2
max	1202.7	14.4	361.1	10.6 (b); 2.2 (a)	356.0	soluble	soluble	soluble	100

[a] Abbreviations used: Molecular weight (M_w), calculated log *P* (c log *P*), polar surface area (PSA), acidic dissociation constant (pKa), apparent permeability (P_{app}), solubility (*S*), and fraction absorbed (FA). M_w, c log *P*, and PSA were calculated with the AstraZeneca program Selma and kindly provided by Dr. Ulf Norinder. pK_a ((a) = acid; (b) = base) were taken from literature sources found at PubMed for all compounds except for acarbose (ACD predicted value is reported) and enalapril (source: the Merck Index). The reported solubility was obtained after 4 h for all samples with the exception of methyldopa, for which the 1 h solubility was used because the compound was no longer stable at the later time points. The solubility is given qualitatively (e.g., >3000) when the maximum solubility was not reached although excess material as compared to the maximum dose given was added to the vial. Soluble is given as the end result for compounds assayed in the visual screen. The FA values of the studied compounds were taken from the literature.[43,44] FA values are stylized to highlight the parameter dominating the final absorption: italic denotes permeability, bold denotes solubility, and bold italic (cyclosporine A only) denotes a combination of poor solubility and poor permeability.

then investigated the influence of the simulated intestinal media on the classification of the compounds in the modified BCS.

Methods

Data Set Selection. A data set of 22 drugs was selected with the intention of ensuring that the drugs included would be structurally and physicochemically diverse, enabling a large volume of the druglike space to be covered, and to secure an even spread over the range of data for the fraction absorbed (Table 1). Both permeability and solubility limited compounds were considered. The diversity of the compounds was analyzed using molecular descriptors generated by the program Dragon (Talete, Italy). The structural diversity and the chemical space represented by the data set were verified through principal component analysis. The compounds were superimposed on top of the oral drug space as defined by a reference data set of 527 drugs registered for oral administration in Sweden. The 22 compounds were found to be spread throughout the chemical space of orally administered drugs and were thereby judged as good model compounds for the oral drug space.

Drugs. With the exception of felodipine, which was a gift from AstraZeneca (Sweden), all compounds used in this study were purchased from Sigma (St. Louis, MO). Amiloride, amitriptyline, loperamide, metoclopramide, and verapamil were used as their corresponding HCl salt. The maleate of enalapril and the sodium salt of pravastatin were used.

Solubility of Compounds in FaSSIF and FeSSIF. A primary solubility screen was performed in 150 mM K_2HPO_4 buffer pH 6.5 (PhB 6.5) with ionic strength (*I*) of 0.15, FaSSIF (pH 6.5, $I = 0.16$) and FeSSIF (pH 5.0, $I = 0.32$).[9]

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These buffers had the capacity to maintain their pH throughout the experiments; that is, the pH remained constant over the 24 h period investigated. The maximum oral dose given was obtained from Martindale.[25] When the dose was stated as being "elevated in rare occasions," then the Swedish Physician Desk Reference[26] was used to confirm and decide on which dose to select as the highest single oral dose. To obtain trustworthy results from this screen, we added compound in excess to that needed for the maximum *single* oral dose to be dissolved. Since we worked in a small scale format, that is, with 0.5 mL instead of the 250 mL used in the BCS system, we added at least an amount of each drug corresponding to 1/500 of the maximum *daily* dose to this volume. Compounds only administered once per day had excess material added to the maximum dose given, to avoid reaching borderline solubility and to certify that compounds having a dose number (Do)[27] of less than 1 in this qualitative screen were correctly sorted. The amount of material was weighed into glass vials (1.5 mL standard HPLC vials), and 0.5 mL preheated dissolution medium (37 °C) was added. The samples were visually inspected directly after the dissolution media was added and after 24 h on the shaker (330 rpm) at 37 °C.

The compounds which were visually inspected to be insoluble in this qualitative assay (amitriptyline, carbamazepine, cyclosporine A, diazepam, dipyridamole, erythromycin, felodipine, furosemide, ketoconazole, loperamide, methyldopa, metoclopramide, and spironolactone) were measured in PhB 6.5, FaSSIF, and FeSSIF at 37 °C with an in-house semiautomated shake flask method[28] using the Tecan Freedom EVO 200 instrument (Tecan, Switzerland). The temperature was maintained constant at 37 ± 1 °C. The same schedule as was used in the visual screening was also applied to the drug used in each of the quantitative experiments. The drug was added to a 96-deep-well propylene plate, and 500 μL of preheated dissolution medium was added to each well. At 1, 4, and 24 h, 150 μL samples were withdrawn to 1.5 mL eppendorf tubes with 500 μL glass inserts and immediately centrifuged in a preheated Eppendorf centrifuge 5403 (Eppendorf AG, Hamburg, Germany) at 10 000*g* for 15 min. After the centrifugation, 30 μL of the supernatant was drawn into the wells of a preheated 96-deep-well propylene plate and then diluted to give appropriate concentrations for analysis with LC-MS/MS. The experiment was performed in at least triplicate, and the obtained solubility values were used to calculate the percentage dissolved of the maximum oral dose and the Do, for the analysis of possible solubility limited absorption and to perform the BCS classification.

Cell Culture. The cell culture media and supplements were purchased from Gibco BRL Life Technologies AB (Täby, Sweden) unless otherwise stated. 2/4/A1 cells originating from fetal rat intestine were conditionally immortalized with a pZipSVtsa58 plasmid containing a temperature-sensitive mutant of SV40 large T antigen.[29] As previously described,[30] the cells were cultured at 33 °C in 15 mL of RPMI 1640 supplemented with 1% L-glutamine, 20 ng/mL epidermal growth factors, and 5% fetal calf serum (FCS). The cells were expanded in 75 cm^2 plastic cell culture flasks at 33 °C, 5% CO_2, and 95% humidity. The medium was changed every second day, and the cells were passaged at 80% confluence by trypsinization. Cells cultivated during passage numbers 32 through 45 were used.

The drug transport experiments were conducted using 2/4/A1 cells seeded at a density of 200 000 cells/cm^2 on polycarbonate filter inserts (Transwell Costar, Badhoevedorp, The Netherlands, 0.4 μm pore size, 12 mm in diameter) coated with ECM-gel (extracellular matrix; Sigma, St. Louis, MO). The cells were grown at 39 °C in Optimem supplemented with 1% glutamine, 0.4% bovine serum albumin, 1 nM triiodothyronine (T3), 65 ng/mL dexamethasone, 2 mM sodium pyruvate, 0.1% dimethyl sulfoxide (DMSO), 1.2 mM calcium chloride, 1% PEST (penicillin and streptomycin), and ITS premix containing 10 μg/mL insulin, 5.5 μg/mL selenic acid, and 5 ng/mL transferrin. The 2/4/A1 cells were cultivated for 5 or 6 days before they were used in drug transport experiments.

Permeability of Drugs in 2/4/A1 Cell Monolayer. All transport experiments were performed in Hank's balanced salt solution containing 25 mM HEPES (HBSS) at pH 7.4 and 37 °C as previously described.[22] Hence, we performed the permeability experiments using a simple buffer without added lipids, instead of applying more biorelevant buffers such as the FaSSIF and/or FeSSIF. This decision was made based on the following two reasons. First, and most important, the cell monolayers lose integrity when subjected to a biorelevant dissolution medium (BDM) such as FeSSIF. Second, we wanted to use the 2/4/A1 permeability values obtained to sort the compounds as being either high or low permeability compounds. This was performed by the application of our in-house 2/4/A1 permeability versus FA correlation curve, in which the previously obtained permeability values were determined in HBSS at a pH of 7.4.[24]

The drugs were dissolved in the HBSS to give a final concentration of 0.1−1 mM, with each concentration being

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nontoxic to the cells as judged by the [^{14}C]-mannitol permeability. Whenever necessary, DMSO at a final concentration of 0.5%, which has been proven nontoxic to the cells, was used to dissolve the poorly soluble compounds. Transport experiments were initiated by incubating the monolayers in HBSS at 37 °C for 10 min in a humidified atmosphere. Studies were performed in the apical to basolateral direction only owing to the lack of functional transporters in the cell line used.[22] The experiment was started with the addition of 0.4 mL of drug solution in HBSS to the apical chamber, and the filter inserts were stirred at 500 rpm using a plate shaker (IKA-Schuttler MTS4) in order to obtain permeability values that are unbiased by the presence of an unstirred water layer adjacent to the cells. Samples of 0.6 mL were withdrawn from the receiver side at regular time intervals and were replaced with fresh preheated HBSS. For lipophilic drugs (with c log $P \geq 2$), the experimental protocol was modified such that the filter inserts were transferred every 5 min to fresh preheated HBSS receiver medium and the complete volume of HBSS in the receiver chamber (1.2 mL) was sampled at the end of experiment. This was followed by washing the plastic receiver chamber with 0.6 mL of acetonitrile to recover any drug that had adsorbed to the plastic. All samples analyzed with LC-MS/MS were kept at −20 °C pending analysis.

All of the experiments were performed in quadruplicate, and the integrity of the cell monolayer was determined for each filter batch by measuring the transepithelial resistance (TEER) as well as the membrane permeability to [^{14}C]-mannitol. The mass balance was assessed by sampling the donor and receiver chamber after completing the transport experiment.

Analytical Methods. [^{14}C]-Mannitol and [^{3}H]-cyclosporine were analyzed using a liquid scintillation counter (Packard Instruments 1900CA TRICARB; Canberra Packard Instruments, Downers Grove, IL). Samples of amiloride, amitriptyline, furosemide, metoclopramide, pravastatin, and verapamil were analyzed directly by UV spectrophotometry (Saphire^2, Tecan, Switzerland). All other samples were analyzed with LC-MS/MS, which consisted of a ThermoFinnigan TSQ Quantum Discovery triple-quadrupole mass spectrometer using electrospray ionization (ESI), coupled to a ThermoFinnigan Surveyor autosampler and Surveyor HPLC-MS pump (Thermo Electron Corp., Waltham, MA). For separation, an XTerra MSC18 column (3.5 μm, 2.1 × 20 mm) (Waters, Milford, MA) and a flow rate of 200 μL/min were used. A total of 10 μL of the samples was injected and run with a gradient using water and acetonitrile with or without formic acid, and electrospray ionization was used.

Calculations. The dose number (Do) was calculated as suggested by Amidon and colleagues[27]

$$\mathrm{Do} = \frac{\mathrm{dose}}{S \times V} \tag{1}$$

in which dose is given in mg, solubility (S) in mg/mL, and the volume (V) in mL. The volume was set to 250 mL in the calculations, which corresponds to the volume used in the BCS classification.[18] Compounds for which the dose was not soluble in this volume will obtain a dose number greater than 1.

In general, the apparent permeability coefficients (P_{app}, cm/s) were calculated from the following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t}\frac{1}{AC_0} \tag{2}$$

where $\Delta Q/\Delta t$ is the steady-state flux (mol/s), C_0 is the initial concentration in the donor chamber at each time interval (mol/mL), and A is the surface area of the filter (cm^2).

For rapidly transported compounds, for which sink conditions could not be maintained for the duration of the experiments, P_{app} was calculated, as described previously,[31] from

$$C_R(t) = \frac{M}{V_D + V_R} + \left(C_{R,O} - \frac{M}{V_D + V_R}\right) e^{-P_{app}A(1/V_D + 1/V_R)t} \tag{3}$$

in which $C_R(t)$ is the time-dependent drug concentration in the receiver compartment, M is the amount of drug in the system, V_D and V_R are the volumes of the donor and receiver compartment, respectively, and t is the time from the start of the interval. P_{app} was obtained from nonlinear regression, minimizing the sum of squared residuals ($\Sigma(C_{R,i,obs} - C_{R,i,calc})^2$), where $C_{R,i,obs}$ is the observed receiver concentration at the end of the interval and $C_{R,i,calc}$ is the corresponding concentration calculated according to eq 2. The mass balance was calculated as the amount of drug recovered in receiver samples after each interval plus that in the donor chamber at the end of the experiment, divided by the amount of drug in the donor chamber at the beginning of the experiment. Permeability coefficients are presented as the mean ± standard deviation ($n \geq 3$).

The positioning in the BCS system was obtained from the permeability values in the 2/4/A1 cell line and the solubility values obtained after 4 h in the three media investigated. A high solubility corresponds to complete dissolution of the maximum oral dose given per administration in 250 mL of the investigated medium, otherwise it is low. High permeability is defined by the Food and Drug Administration (FDA) as being 90% FA, otherwise it is low.[18] In the 2/4/A1 cell line, 90% FA has been shown to correspond to a permeability value of 55 × 10^{-6} cm/s.[24]

Results and Discussion

In this study, we investigated a data set of chemically diverse oral drugs covering a range of FA from 2 to 100% (Table 1). From the solubility and permeability experiments, it was shown that all compounds exhibiting complete absorption (FA > 90%; $n = 5$) were predicted as having complete absorption. Moreover, it proved to be possible to

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analyze why the remaining compounds were incompletely absorbed, and for some compounds clear effects of the fed state was observed. The experimental methods applied herein and the explanations on a molecular level to the solubility and, thereby, BCS class changes are applicable at several levels of the drug discovery process, ranging from predictions of potential food effects in early drug discovery to guidance on how to design food effect investigations in the drug development setting.[32]

Solubility in Buffer and Physiologically Relevant Media. The apparent solubility values obtained in PhB 6.5, FaSSIF, and FeSSIF at 37 °C are shown in Table 1. The apparent solubility in the different solvents was studied at 1, 4, and 24 h to allow assessment of time dependent solubility. Of the 13 compounds investigated quantitatively, only two showed more than 2-fold increase in solubility between the 4 and 24 h time point (these being felodipine in all three media and ketoconazole in FaSSIF and FeSSIF). However, based on the fact that a window of approximately 3−7 h is available in vivo for absorption from the small intestine,[33,34] we selected the 4 h apparent solubility value for subsequent analyses.

The solubility in the two biorelevant dissolution media as compared to the solubility determined in the buffer is shown in Figure 1. The majority of compounds exhibited a higher solubility in FaSSIF and FeSSIF than in the buffer, although erythromycin, furosemide, and methyldopa all revealed a rather large decrease in solubility between the value obtained in buffer and the values obtained in FaSSIF and/or FeSSIF. Four compounds, cyclosporine A, dipyridamole, felodipine, and ketoconazole, showed more than 10-fold increase in solubility in FeSSIF when compared to the value in the buffer. These compounds had a moderate to high lipophilicity (Table 1) and were either neutral or positively charged when dissolved in FeSSIF. In addition to the pH-dependent increase in solubility resulting from the net positive charge of the bases, the solubilization capacity of the mixed micelles in the FeSSIF will further increase the apparent solubility of the compounds.[19] Other studies conducted in our laboratory show that also negatively charged lipophilic compounds gain in solubility when dissolved in BDM in comparison to a pure buffer system of the same pH, although not to the same extent as the cationic or neutral compounds.[35] Thus, the mixed micelles seem to have a larger capacity to solubilize neutral or positively charged compounds than acids carrying a negative charge at the pH under investigation; this is because the net negative charge of the lecithin/taurocholate micelles.

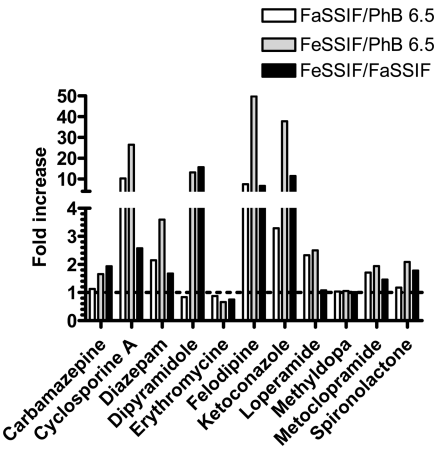

Figure 1. Fold increase in solubility in the fasted (white bars) and fed state (gray bars) in comparison to the phosphate buffer, and in the fed state as compared to the fasted (black) of the compounds studied quantitatively. Amitriptyline and furosemide are not shown because the solubility was determined to be greater than 3000 μM in all three media for amitriptyline and greater than 5000 μM for furosemide in PhB 6.5.

For diazepam, felodipine, and loperamid, the maximum dose becomes soluble when moving from buffer to FaSSIF and/or FeSSIF (Table 2, Figure 2). According to the Physician Desk Reference, the only food-related recommendation for the compounds listed in Table 2 concerns felodipine for which it is stated that fatty foods should be avoided since these can increase the rate of absorption of felodipine.[26] The observed solubility increase in the FeSSIF, and the resulting higher dissolution rate,[35] of felodipine supports this recommendation. Furthermore, a fatty meal impacts on several other physiological factors, such as the gastric emptying rate (decreases)[34] and the bile secretion (increases), with the latter resulting in an increase in the mixed micelles present which can be absorbed by the lymphatic system.[36] All these factors will contribute to the observed increased rate of absorption of felodipine.

For the other substances for which the solubility was determined quantitatively, the solubility improved considerably for three of the compounds although the dose numbers[14] still remained greater than one (Table 2). Ketoconazole showed a 38-fold increase, cyclosporine A a 33-fold increase,

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Table 2. Dose, Proportion of the Maximum Dose That Was Dissolved, and the Dose Number (Do) in the Three Different Media

compound	dose (mg)	$dissolved_{PhB6.5}$ (% of dose)	$dissolved_{FaSSIF}$ (% of dose)	$dissolved_{FeSSIF}$ (% of dose)	$Do_{PhB6.5}$	Do_{FaSSIF}	Do_{FeSSIF}
5-FU	1000	100	100	100	<1	<1	<1
acarbose	100	100	100	100	<1	<1	<1
amiloride	20	100	100	100	<1	<1	<1
amitriptyline	150	100	100	100	<1	<1	<1
carbamazepine	1000	6.7	7.6	11.1	14.9	13.2	9.0
cyclosporine A	500	0.1	1.5	3.9	672.5	65.4	25.4
diazepam	10	87.6	100	100	1.1	0.5	0.3
digoxin	1.5	100	100	100	<1	<1	<1
dipyridamole	200	1.4	1.2	18.6	70.7	84.4	5.4
enalapril	20	100	100	100	<1	<1	<1
erythromycin	500	100	100	100	0.3	0.3	0.5
felodipine	10	2.3	16.8	100	44.3	6.0	0.9
furosemide	250[a]	100	100	92.0	<1	0.4	1.1
hyoscine-*N*-butyl	20[b]	100	100	100	<1	<1	<1
ketoconazole	400	0.4	1.3	15.1	249.9	75.9	6.6
loperamide	16	68.5	100	100	1.5	0.6	0.6
methyldopa	1500	100	100	100	1.0	1.0	1.0
metoclopramide	20	100	100	100	0.4	0.2	0.2
pravastatin	80	100	100	100	<1	<1	<1
spironolactone	400	1.4	1.6	2.9	72.8	62.0	34.8
tranexamic acid	1500	100	100	100	<1	<1	<1
verapamil	120	100	100	100	<1	<1	<1

[a] Furosemide shows a large dose interval dependent on disease status. In rare occasions doses up to 500 mg can be given; these are also completely soluble in PhB 6.5 and FaSSIF. [b] Oral dose found at http://sigmatausudan.com/product%20PDF/Tablets/Nospasmin.pdf accessed March 24, 2010.

and dipyridamole a 13-fold increase in FeSSIF when compared to PhB 6.5. Cyclosporine A is a neutral compound, and hence, the increase in its solubility can be attributed to the increased solubilization capacity of the BDM when compared to the buffer. Ketoconazole and dipyridamole are bases, so the main contribution to the increased solubility for these compounds is likely to be a pH effect obtained when changing the solvent. For dipyridamole, this was emphasized through the study of solubility in FaSSIF, which resulted in a solubility value equal to that obtained in the buffer of the same pH. In comparison, ketoconazole showed a 3.3-fold increase in solubility in FaSSIF compared to that in PhB 6.5. The substantial impact of the pH on the absorption of these two compounds is also reflected in the administration recommendations of them. Hypochlorhydria, which can occur either due to aging or through the administration of H2-blockers, results in lower amounts of dipyridamole and ketoconazole being absorbed.[20,37,38]

Permeability in 2/4/A1. The permeability values of the 22 compounds studied are shown in Table 1. The apparent permeability ranged from 1.7 to 356 $\times$ 10^{-6} cm/s, and hence, the data set included in the study displayed a 209-fold range in membrane permeability in the 2/4/A1 cell line. We had determined the cutoff value for high permeability compounds previously, that is, for those compounds that have a FA of >90%, to 55 $\times$ 10^{-6} cm/s in this cell line.[24] By applying this cutoff value, 11 of the compounds were sorted as low permeability compounds, and the remaining 11 compounds were sorted as high permeability compounds. However, two compounds were on the borderline: furosemide with a permeability of 54.5 $\times$ 10^{-6} cm/s and digoxin with a permeability of 56.1 $\times$ 10^{-6} cm/s. According to our cutoff value, furosemide is determined to be a low permeability compound, which is in agreement with the permeability class given in the BCS guidelines.[18] The correct permeability classification of these two compounds was further supported by the study performed by Wu and Benet,[39] in which digoxin was sorted as a BCS class II compound (i.e., high permeability) and furosemide as a BCS class III or IV compound (i.e., low permeability).

Modified Physiological BCS. On the basis of the experimentally determined solubility and permeability values, and values for the FA collected from the literature, it is possible to analyze the extent to which the FA is restricted by the

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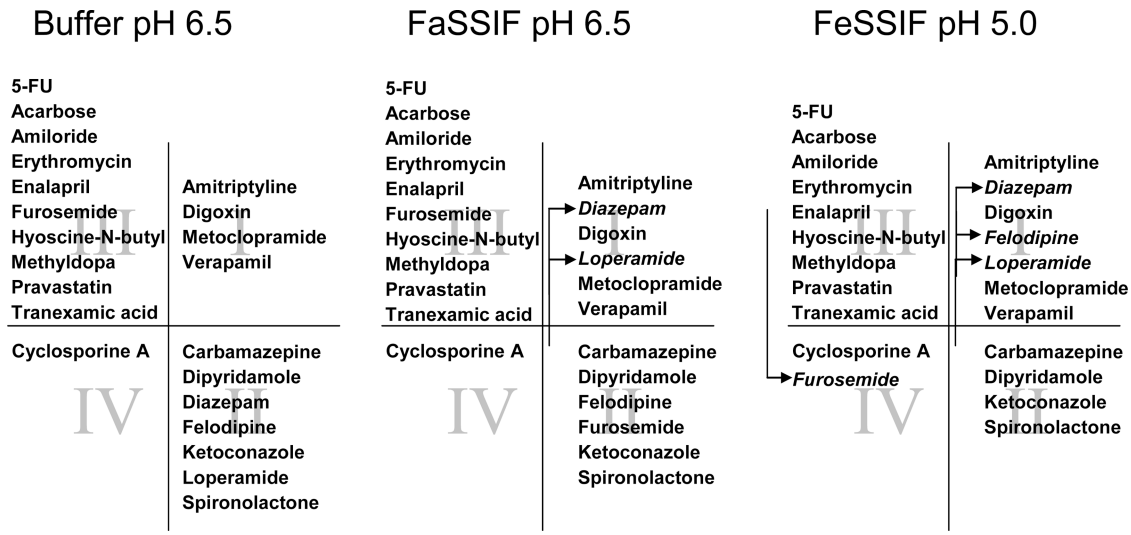

Figure 2. Modified BCS classification in PhB 6.5, FaSSIF, and FeSSIF. Three of the compounds behaved as BCS Class I compounds in FeSSIF but as BCS Class II in PhB 6.5. These three compounds were low dosed molecules of "grease ball" character.

permeability and/or the solubility (Table 1). Of the 22 compounds included in this study, only cyclosporine A was found to be limited by both parameters. In addition, furosemide exhibited limited permeability in the cell model used and limited solubility when solubility was measured in FeSSIF. This shows the negative effect of the more acidic pH of the FeSSIF, in comparison to FaSSIF and PhB 6.5, on the solubility of a weak acid. Ten compounds showed incomplete absorption, with one of the reasons for this likely to be poor membrane permeability. Moreover, solubility limited absorption was indicated for five of the compounds irrespective of which solvent that was used for the solubility measurement.

The physiological BCS is shown in Figure 2. Our BCS gives a glimpse of in vivo conditions likely to occur in the small intestinal fluid and is suitable for a less demanding BCS classification in the discovery setting. The simplest physiologically relevant screen for solubility included in our study was a pure buffer (PhB 6.5) of equal osmotic pressure and pH as the intestinal fluid in the fasted state. The more complex media FaSSIF and FeSSIF enable the in vitro measurements to resemble the in vivo situation through the inclusion of lipids, which may increase the wetting of the drug particles and the solubilization of the compounds. Our data show that the three buffers can help to clarify poor in vitro−in vivo correlations and large variability in FA values reported, since the solubility in one of these solvents can be 50-fold higher than that in another (Table 1, Figure 1). Hence, for such compounds, the change in lipid composition of the intestinal fluid following the intake of food can be expected to significantly influence the FA. As can be seen in Figure 1, dipyridamole, felodipine, and ketoconazole increased their solubility more than 6-fold in the fed state as compared to the fasted, and hence, food effects would be anticipated for these compounds based on the in vitro results. We have already discussed the food effect observed for felodipine and the pH effect observed for dipyridamole and ketoconazole

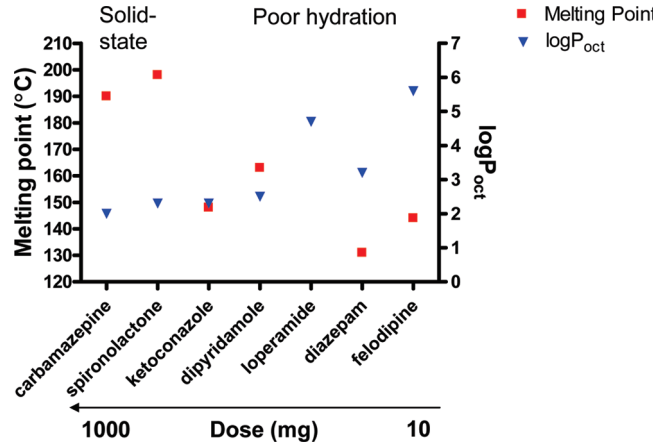

Figure 3. Analysis of the BCS class II compounds from a "grease ball" and "brick dust" viewpoint. The general solubility equation established by Yalkowsky and co-worker (see, e.g., ref 42) estimates the aqueous solubility from log P_{oct} and melting point. In this plot, it is seen that two compounds have higher impact of melting point than lipophilicity and therefore are regarded as being solid-state-limited in their solubility; that is, they are of "brick dust" character. To the right, compounds with a higher impact of lipophilicity on the solubility are found; these compounds are poorly hydrated in the media investigated and are of "grease ball" character. No melting point was included for loperamide in this analysis, since we were not able to find information with regard to the free base.

above. However, we note that the latter two are the two compounds displaying highest variability in FA, ranging from 27−88% and 15−90% for dipyridamole and ketoconazole, respectively (Table 1).

Of the seven compounds defined as Class II using PhB 6.5 (Figure 3), diazepam, felodipine, and loperamide improved their positioning in the BCS when the solubility was assessed in FeSSIF. Notably, these three compounds are the

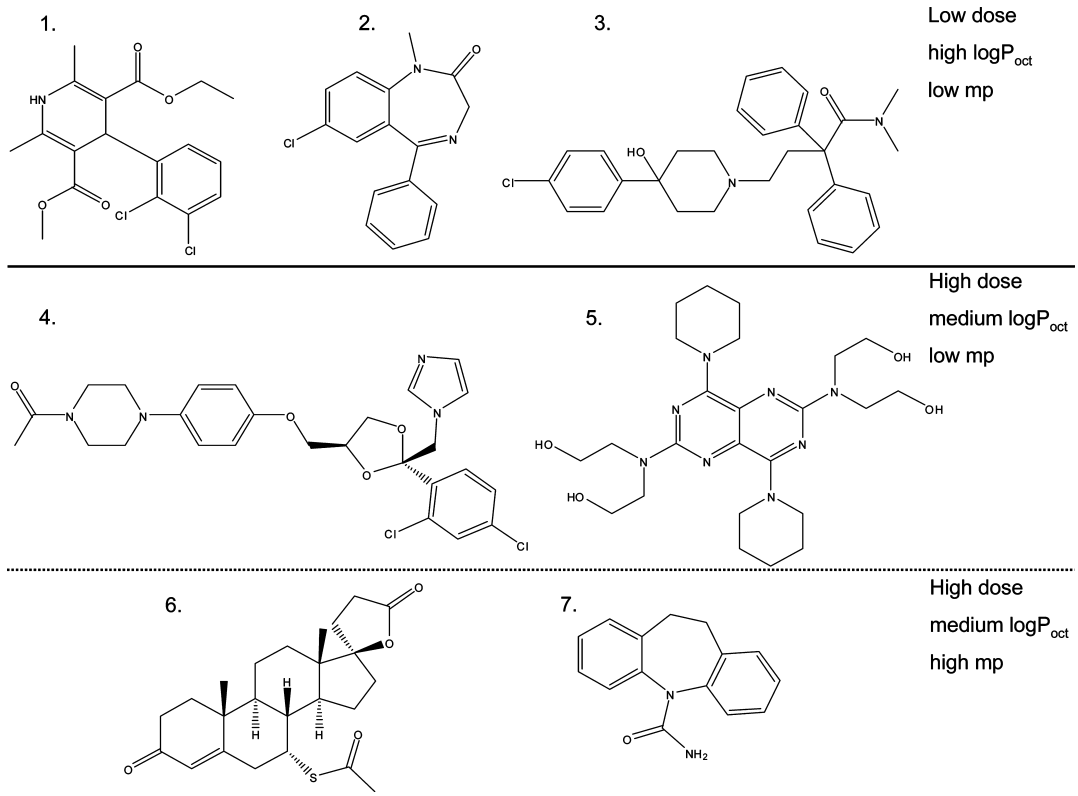

Figure 4. The three upper compounds improved their solubility in FaSSIF and/or FeSSIF. The two compounds in the middle did not change BCS class when changing from the PhB 6.5 to the two modified media, although they showed more than 10-fold increase in solubility in these media. The bottom two compounds did only increase their solubility in the modified media with 1.7- to 2.1-fold. The compounds shown are as follows: (1) felodipine; (2) loperamide; (3) diazepam; (4) ketoconazole; (5) dipyridamole; (6) spironolactone; (7) carbamazepine.

only ones of the poorly soluble compounds that are administered in a low dose: diazepam and felodipine at a maximum dose of 10 mg and loperamide of 16 mg, whereas the other compounds are given at doses ≥ 200 mg. Another interesting fact is that felodipine and loperamide are quite large and flexible molecules with a high lipophilicity and typically would be sorted as "grease ball" molecules.[4,40] These characteristics are not as pronounced for diazepam, although it, too, is lipophilic in nature (Figure 4). Lipophilic compounds often have a higher apparent solubility in modified media owing to the increased solubilization capacity of these media;[19] therefore, we recently performed a detailed study of the dissolution and the apparent solubility of a series of "grease ball" molecules.[35] In that study, it was found that a majority of these compounds showed a higher intrinsic dissolution rate and apparent solubility when dissolved in a medium containing lipids at physiologically relevant concentrations than in their corresponding blank buffer. In the present study, the improvement in the solubility was insufficient for four of the seven compounds to enable a shift to Class I. However, two of them improved their solubility more than 13-fold in FeSSIF in comparison to their solubility value in PhB 6.5. Interestingly, the solubility of two neutral compounds, carbamazepine and spironolactone, was essentially unaffected by the shift from PhB 6.5 to FeSSIF (carbamazepine exhibited a 1.7-fold increase and spironolactone a 2.1-fold increase in solubility in FeSSIF in comparison to that in PhB 6.5). These compounds are rather rigid and flat molecules, show a moderate lipophilicity, and are compounds with high melting points, of 190 °C or more (Figure 4). Therefore, these are molecules of "brick dust" character,[40] for which the solubility is mainly restricted through the inability of the compound to dissociate from the crystal lattice.[41] Hence, it is expected that a disruption of the stable crystal lattice through, for example, salt formation, amorphization, or chemical modification is needed to further improve the solubility of such compounds.

Although further studies are needed to establish general cutoff values for when a shift in class in the modified BCS is to be expected, our study indicates that low dosed (20 mg or less) BCS Class II compounds of "grease ball" character may be most influenced by the naturally occurring lipids in

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the intestinal fluids and behave as Class I in vivo. For such compounds, the guidelines for bioequivalence studies can therefore be regarded as being too rigid.[18] Although the FDA guidelines take the pH-gradient in the gastrointestinal tract into consideration, the solubilization capacity of the intestinal fluid is neglected. However, a larger series of BCS Class II compounds needs to be assessed in BDM, and proof of principle needs to be shown in suitable animal models as well as in humans to elucidate which BCS Class II compounds that will be completely dissolved in the small intestine in vivo.

Conclusion

In total, 22 compounds had their solubility determined in phosphate buffer pH 6.5 and simulated intestinal fluids and their permeability assessed in 2/4/A1 cells, and a modified physiological BCS was constructed. It was revealed that the compounds which displayed significantly increased solubility in modified media were moderately to highly lipophilic and were either neutral or positively charged compounds. Of these, only those that were low dosed drugs of 20 mg or less improved their positioning in the modified BCS. Finally, it was shown that the compounds that will be least affected by solubility assessment in different physiologically relevant media are those that are highly dosed and/or of "brick dust" character, that is, drugs for which the solubility mainly is solid-state limited. Through the analysis performed in this paper, we hope to have shed some light on the reasons why compounds are sorted into a certain BCS class and which compounds are likely to be assigned to another class when a more physiological setting is used. We believe it will be useful to include a similar "grease ball" and "brick dust" analysis early on in the drug discovery process to flag potential improvements or hazards that can be expected in the drug development setting. Suitable computational tools for such analysis have been proposed previously;[4,41] it could be useful to apply these tools in drug discovery to examine the likely solubility behavior in vivo.

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