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The Composite Solubility Versus pH Profile and Its Role in Intestinal Absorption Prediction

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ABSTRACT

The purpose of this study was to examine absorption of basic drugs as a function of the composite solubility curve and intestinally relevant pH by using a gastrointestinal tract (GIT) absorption simulation based on the advanced compartmental absorption and transit model. Absorption simulations were carried out for virtual monobasic drugs having a range of pKa, log D, and dose values as a function of presumed solubility and permeability. Results were normally expressed as the combination that resulted in 25% absorption. Absorption of basic drugs was found to be a function of the whole solubility/pH relationship rather than a single solubility value at pH 7. In addition, the parameter spaces of greatest sensitivity were identified. We compared 3 theoretical scenarios: the GIT pH range overlapping (1) only the salt solubility curve, (2) the salt and base solubility curves, or (3) only the base curve. Experimental solubilities of 32 compounds were determined at pHs of 2.2 and 7.4, and they nearly all fitted into 2 of the postulated scenarios. Typically, base solubilities can be simulated in silico, but salt solubilities at low pH can only be measured. We concluded that quality absorption simulations of candidate drugs in most cases require experimental solubility determination at 2 pHs, to permit calculation of the whole solubility/pH profile.

KEYWORDS: GIT, absorption simulation, pH solubility curve, BCS, solid-state properties, solubility screening

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INTRODUCTION

Drug solubility is widely accepted as important in the candidate selection process. Solubility is essential if an orally administered drug is to be absorbed across the intestinal walls and enter the portal vein. Absorption is to be distinguished from bioavailability: the latter is less because first pass metabolism, protein binding, hydrolysis, and other degradative pathways reduce the systemic concentration from that observed in the portal vein.

Absorption may occur throughout the gastrointestinal tract (GIT), the specific region of which depends on the relative ratio of transit and absorption rates. Passive absorption is also a diffusion-controlled process, and the permeability of the drug varies as a function of surface area to volume ratio and regional pH effects on ionization. The driving force for diffusion across the apical and basolateral membranes of the enterocyte is the soluble drug concentration gradient, and for ionizable drugs this varies with the pKa and the pH profile between the intestinal compartments. Only passive absorption will be considered here: although carrier-mediated absorption is sometimes seen, its existence and extent are harder to predict and beyond the scope of the present article, as are enzymatic and chemical degradative pathways.

The advanced compartmental absorption and transit (ACAT) model has been implemented in the software program GastroPlus (Simulations Plus, Inc, Lancaster, CA).[1] The current version of the ACAT model accounts for dissolution rate, the pH dependence of solubility of drugs, controlled release, absorption in the stomach or colon, metabolism in the gut or liver, degradation in the lumen, or changes in such factors as surface area, transporter densities, efflux protein densities, and other regional factors within the intestinal tract. A basic assumption of the ACAT model is that drug passing through the small intestine (SI) will have equal transit time in each of 7 compartments. Since the volume of

fluid entering the SI (8-9 L/day) in the duodenum and jejunum is more than exits the SI (0.5-1 L/day), then to satisfy the transit time constraint, volumes and transit rates of the upper SI compartments are considered to be larger than those for the lower compartments. The form of the ACAT model implemented in GastroPlus is modeled by a system of coupled linear and nonlinear rate equations. The equations include the consideration of 6 states (unreleased, undissolved, dissolved, degraded, metabolized, and absorbed), 18 compartments (9 GI [1 SI, 1 colon, and 7 stomach] and 9 enterocyte), 3 states of excreted material (unreleased, undissolved, and dissolved), and the amount of drug in up to 3 pharmacokinetic compartments (when pharmacokinetic parameters are available). The total amount of absorbed material is summed over the integrated amounts being absorbed/exsorbed from each absorption/transit compartment.

In general, the rate of change of dissolved drug concentration in a luminal GIT compartment depends on 6 different processes: (1) transit of drug into a compartment, (2) transit of drug out of a compartment, (3) release of drug from the formulation in the compartment, (4) dissolution of the drug particles, (5) luminal degradation of the drug (if any), and (6) absorption/exsorption of the drug. The time scale associated with luminal transit is set by a transfer rate constant, k_t, that is determined from the mean transit time within each compartment. The time scale of the dissolution process is set by a rate constant, k_d, that can be computed from a drug's solubility (as a function of pH), its effective particle size, its molecular density, its lumen concentration, its diffusion coefficient, and the diffusion layer thickness. The time scale associated with the absorption process is set by a rate constant, k_a, that depends on the effective permeability of the drug multiplied by an absorption scale factor (ASF) with units of cm^{-1}. The ASF corrects for changes in permeability due to changing physiology along the GIT (eg, absorption surface area, pH, transport/efflux protein densities). The rates of absorption and exsorption depend on the concentration gradients across the apical and basolateral enterocyte membranes. The time scale for luminal degradation can be set by a rate constant, k_{Degrad}, that is determined by interpolation from an input table of degradation rate (or half-life) versus pH, and the pH in the compartment.

The system of differential equations is integrated using a fourth/fifth-order Runge-Kutta numerical integration package with adaptive step size.[2] The fraction of dose absorbed is calculated as the sum of all drug amounts disappearing from the GIT as a function of time, divided by the dose, or by the sum of all doses if multiple dosing is used. Bioavailability, which is the fraction of dose reaching the systemic circulation, is distinguished from absorption, which is the fraction of dose entering the portal vein in the absence of intestinal metabolism. For this study, metabolism, transport, efflux, concentration gradient-dependent absorption, and controlled release characteristics were not considered.

The size and shape of a drug molecule, its acid and base dissociation constants, and the pH of the GIT all influence the absorption rate constant for specific regions of the GIT. The ACAT model for GIT simulation has been parameterized to account for the extent to which the paracellular and transcellular routes are used in passive absorption. The parameterization relies on experimental data using excised segments from 3 regions of rat intestine: jejunum, ileum, and colon. Ungell et al[3] determined the regional permeability coefficients of 19 drugs with different physicochemical properties. They observed a significant decrease in permeability for hydrophilic drugs and a significant increase in permeability for hydrophobic drugs aborally to the SI ($P = 0.0001$). For hydrophilic drugs (low permeability and low log D) the ratio of colon:jejunal permeability was less than 1, while for hydrophobic drugs (higher permeability and higher log D) the ratio of colon:jejunal permeability was observed to be greater than one. At certain pH values the permeability of small hydrophilic drugs may have a large paracellular component,[4] and it is well known that the transepithelial electrical resistance (TEER) of the colon is much higher than that of the SI. TEER increases as the width of tight junctions decrease, and the tight junction width has been determined to be 0.75 to 0.8 nm in the jejunum, 0.3 to 0.35 nm in the ileum, and 0.2 to 0.25 nm in the colon. The narrower tight junctions in the colon suggest that paracellular transport will be much less significant in the colon, which helps to explain the lower ratio of colon:jejunal permeability for hydrophilic drugs. Using experimental biopharmaceutical properties for a series of hydrophilic and hydrophobic drug molecules, a "log D model" has been incorporated into the ACAT model as implemented in GastroPlus.

Commercial drug companies are under pressure to maintain the flow of candidates into the pipeline in order to meet the needs of society for new medicine as well as to provide shareholder value. Quite apart from bioactivity and biospecificity, candidates must be "druggable," and capable of moving from candidate selection to first

human dose reliably without needing major, time-consuming, and expensive solubility enhancement during development. Ninety percent of drugs fail during development, 40% of them due to inadequate bioavailability. Recently, Selick et al[5] have discussed the growing importance of predictive ADME simulation. One aspect of this from the physicochemical perspective is the relationship between solubility and permeability. As made clear by the biopharmaceutics drug classification,[6] some drugs are limited by either poor solubility or poor permeability (or both). High-throughput discovery screening methods and the methods of combinatorial synthesis are reputed to be producing generally less soluble drugs than those available before.[7] In addition, drug candidates must be adequately soluble to permit high-concentration toxicology formulations, as animal toxicity testing requires concentrations higher than those contemplated for final human use. The molecule makers at the front end of discovery need to know at an early stage whether their molecule has sufficient solubility to be viably druggable for human and toxicology use, and it is the purpose of this study to seek to answer this question.

Biopharmaceutics Classification System

Since the Biopharmaceutics Classification System (BCS) became available in 1995,[6] its 4 classes have become familiar in the industry, as a development tool allowing estimation of the contributions of dissolution rate, solubility, and permeability to the absorption of immediate-release dosage forms. A drug molecule may be shown to be solubility limiting, in which case the development chemist is aware that techniques aimed at improving solubility and dissolution, including particle size management, may be explored. Similarly, a drug molecule may be shown to be permeability limiting, in which case absorption cannot be enhanced by any solubility-enhancing mechanism. Some techniques available for enhancing permeability have been summarized recently.[8] The BCS has been introduced into regulatory decision making and forms the basis for waiving high-solubility, high-permeability drugs' requirements for in vivo bioavailability and bioequivalence studies.[9] Some[10] have suggested that this approach should be extended to low-solubility, high-permeability drugs. The BCS additionally proposes 3 dimensionless ratios to classify drug absorption:

- The *dose number* is the ratio of the dose to the amount of drug that will dissolve in 250 mL of test solution at the lowest solubility within the pH range 1 to 8. Ideally, this ratio should be below 1 if full dissolution is to be possible in principle. Obviously, higher doses will raise the ratio and make good absorption less likely.

- The *absorption number* is the ratio of the transit time to the absorption time (1/absorption rate constant). Ideally, this should exceed 1. Longer absorption times resulting from lower permeability will reduce this ratio.

- The *dissolution number* is the ratio of the transit time to the dissolution time (1/dissolution rate constant). Ideally, it should exceed 1. In the case of solid dosage forms, a combination of inadequate solubility or diffusivity, or excessive particle size or density can increase the time needed for full dissolution and reduce this ratio.

The importance of all these parameters is clear. The present article will, however, concentrate mainly on the role of solubility and to a lesser extent on permeability in the absorption process.

Solubility: Theoretical Considerations

Most drugs are ionizable in water, having either proton acceptor groups (bases), proton donor groups (acids), or both (amphoterics). The principles will be illustrated with reference to bases, but the behavior of acids and amphoterics will be analogous.

At high pH (at least 2 pH units above the pKa), bases will be fully un-ionized and their solubility will be a minimum, the intrinsic solubility. As the pH is gradually lowered, increasingly more base will be protonated and the solubility will begin to rise. When the pH equals the pKa, half the molecules are protonated, and the solubility rises to double the intrinsic solubility. Further reduction in the pH causes progressively more protonation, and the solubility rises steeply according to an equation that follows the degree of ionization as described by the Henderson-Hasselbalch equation:

$$C_S = C_{OB}\,(1 + 10^{\mathrm{pKa-pH}}) \qquad (1)$$

C_S is the observed solubility at the given pH, and C_{OB} is the intrinsic solubility of the base. The equation predicts that the solubility will rise indefinitely at even lower pHs, although in practice a limit is reached at the salt solubility. This salt solubility depends on the salt itself (the acid used to lower the pH in the experiment) and on the ionic strength.[11] The salt form of the base likewise varies in solubility, becoming progressively more soluble

as the pH is raised. This solubility also obeys the Henderson-Hasselbalch equation expressed in the reverse form:

$$C_S = C_{OA} (1 + 10^{pH-pKa}) \quad (2)$$

C_S remains the observed solubility of the salt form at the given pH; C_{OA} represents the intrinsic solubility of the salt form, the limiting value at the lowest pHs.[12] The 2 intersecting concentration curves, the base solubility curve and the salt solubility curve, are combined such that the minimum at any one pH is taken to be the solubility.

In practice then, the result is a composite curve for base solubility as a function of pH comprising 2 parts separated by a sharp discontinuity. It can be shown by combination of Equations 1 and 2 that the intersection occurs at a pH given by

$$pH = pKa - \log (C_{OA}/C_{OB}) \quad (3)$$

where the ratio C_{OA}/C_{OB} is referred to as the acid solubility factor. In titrating from low to high pH, a nearly constant solubility is predicted until the pH of the discontinuity (the intersection), the solubility of the salt form. Beyond the pH of the intersection, the base solubility curve, the solubility is predicted to be sensitively dependent on the pH and on the drug pKa. It is important to note that if any point on this base solubility curve is known (solubility and the pH at which it was measured) then the whole curve, base and salt solubility, can be calculated provided the pKa and the acid solubility factor are also available. GastroPlus prompts the user for these 4 parameters in order to construct the composite curve.

Solubility: Practical Considerations

In the drug discovery setting, large numbers of molecules are synthesized in small quantities for biological and physicochemical screening. Initially, physicochemical properties may be predicted from structure by proprietary software packages, giving in silico estimates of log D, pKa, solubility, permeability, and diffusivity. Indeed, these estimates do not even require that the molecule be synthesized. Recently, much effort has been put into the in silico prediction of solubility with the purpose of making the discovery process manageable and productive. Jorgensen and Duffy[13] reviewed the prediction of solubility from structure, while Parshad et al[14] studied 22 salts within a narrow structural class. Their solubilities were measured and correlated with measured parameters and molecular descriptors. Bergstrom et al[15] also combined small-scale in-house solubility measurements of 17 compounds with correlations based on lipophilicity and molecular surface areas. Gao et al[16] used 930 compounds in their Quantitative Structure Property Relationship (QSPR) approach and claimed an estimation error of just 0.39 of the log solubility value in their test set. On the experimental side, Avdeef et al[17] have described a pH-metric titration method for determining not just the intrinsic solubility but the whole solubility-pH profile and have compared the results favorably with the shake-flask method.

However, a fraction of synthesized molecules will have their in silico properties refined by in vitro experiments, including solubility. The number of candidates needing in vitro solubilities is such that traditional equilibrium "stirred beaker" experiments are impracticable. They require more drug than is available and take too long. A trade-off is appropriate between accuracy on one hand and speed and scale on the other. Various high-throughput screening methods are currently used in the industry, based upon examination of very small quantities of material and upon automation. The methods used will not be described here, but it is worth emphasizing that they are screens and are approximate. The aim is a solubility estimate at a known pH, and received wisdom suggests a pH close to that of the intestine is most appropriate, typically around 6 or 7. It was shown above that provided the pKa is known (whether by prediction or by experiment) then the whole of the solubility/pH relationship for the base solubility curve is known, and additionally if the acid solubility factor is known then the salt solubility curve is also known. In general, salt solubilities (pH sufficiently low that it is below the pH of the intersection, Equation 3) appear not to be measured, perhaps because they are considered irrelevant to the areas of the GIT where absorption is likely to take place. However, the change in solubility due to ionization is a critical factor for the accurate simulation of drug dissolution. A more extensive determination of solubility factors for a chemically diverse set of drug molecules might lead to additional in silico models that allow us to accurately represent the complete pH versus solubility profile. The question of whether low pH solubilities as defined above are relevant to absorption predictions is one significant part of the present investigation. The question of what solubility value is required of a candidate to ensure it will not fail on that account is the other.

MATERIALS AND METHODS

The drugs studied in the simulation experiments are virtual and defined solely in terms of physicochemical properties and the estimated in vivo permeability. In effect, the user must define the parameters, and the rationale adopted here is outlined below. Two databases have been helpful in defining the parameters below: one based on 130 basic drugs,[18] and the other the authors' smaller database.

Ionizability

Basic drugs are considered in this study, as these are seen as dominant in the industry. A variety of pKa values are considered. The majority of drugs in both databases were in the range pKa 6 to 10, and accordingly values of 6, 8, and 10 were used for simulation purposes as representing most basic drugs.

Partitioning

Various values of log D were considered (at a stated pH). The software uses log D along with pKa to calculate the absorption rate in the various GIT compartments. The majority of drugs in both databases were in the range log D7.4 -1 to 3, and accordingly values of -1, 1, and 3 were used for simulation purposes as representing most basic drugs.

Dose

In most of the simulations in this article, the relatively high single dose of 100 mg was assumed. This ensured a challenging scenario to absorption, as the most potent drugs (lowest doses) will succeed as candidates with a relatively lower solubility than higher-dose drugs. Doses of 10 mg and 1 mg were also considered to quantify the differences.

Permeability

Again, a wide range of values were considered to test the significance to required solubility of this parameter. Throughout this article, human effective permeability will be expressed in the units 10^{-4} cm sec^{-1}. Permeability was allowed to vary over the range 0.05 to 3.0 ($*10^{-4}$ cm sec^{-1}), a 60-fold range that is thought to include the actual permeabilities of many drugs.

Solubility

Although a suggested limiting solubility is an output variable of this study, it is necessary to input values initially in order to predict absorption. As discussed above, 2 pairs of solubility data are needed in any instance to construct the solubility/pH relationship over the entire pH range. One of these is typically a solubility value at an intestinally relevant pH, and the other is the acid solubility factor, which defines the salt solubility at lower pHs. For the present purposes, the 80-fold range of solubility at pH 7 from 0.2 µg to 0.016 mg/mL was used, as this range is thought to include the observed solubilities of many drugs. The corresponding salt (low pH) solubilities, with the presently assumed acid solubility factor of 1000, would be in the range 0.2 mg/mL to 16 mg/mL.

Absorption

The difference between absorption and bioavailability has been discussed earlier. Absorption is one of the main outputs from the GastroPlus software, and values are predicted for each intestinal compartment, as well as the total into the portal vein. Several variations on the ACAT model are available, but here the log D model was used throughout for defining ASFs for the SI and large intestine, with a fixed 24-hour time period.

Critical Success Factors

The question of what absorption is "good enough" to make a candidate likely to succeed has to be answered subjectively. In this study it will be taken as 25%. A similar study could just as easily be conducted on a different basis if preferred.

Many basic compounds were screened in the context of high-throughput solubility screens and were run manually. A small quantity of drug was dissolved in a small quantity of Dimethyl Sulphoxide DMSO, and 5-mL increments of the DMSO solution were added to 90 mL of solvent, which in this study was phosphate-buffered saline with pH 7.4, or 0.01M HCl with pH 2.2. In many cases, precipitation was rapidly seen, but if not, a second increment of drug in DMSO solution was added. The vials in which the mixing took place were mechanically swirled, allowed to stand for an hour, and centrifuged. The supernatant phase was assayed by UV (with dilution if needed) and compared to standards. The concentration of drug was simply calculated. Many drugs showed no precipitation at the low pH, and the solubility could be expressed in terms of only a minimum figure.

There are several sources of error in the method as described. The presence of DMSO is unavoidable and raises the solubility to some extent. It should be noted that many of the commercially available methods are open to the same criticism. The ionic strengths of the chosen solvents differ from each other, as does the nature of the dominant counterion. High ionic strengths will tend to depress the result. Supersaturation is often encountered, leading to a falsely high result. These errors

are real and are the price to be paid for rapid screening of a large number of compounds available on only a minute scale. At a later stage in candidate selection, more satisfactory methods can be used.

Data will be presented below for 32 basic compounds for which solubility measurements at both pHs were finite, neither value being a "greater than," and for which measured pKa data were available. They will be presented in terms of the solubility ratio at the 2 pHs.

Validation of the Software

In a brief and independent validation of the overall concept of predicting absorption from supplied physical parameter values, 21 drugs were selected for which literature values for absorption were available,[19] as in **Table 1** below.

The correlation between predicted and observed absorption is shown in **Figure 1A**. The correlation is seen to be poor, with a correlation coefficient of just 0.44. However, 3 of these drugs are believed to be transported by an active mechanism, and 2 are substrates for the P-gp efflux pump. The ACAT model's ability to simulate transport, efflux, and metabolism was not used for this study. If these 5 drugs are left out of the correlation, the remaining 16 give a much better correlation coefficient of 0.80 and all have estimates with 26% of the experimental values (**Figure 1B**).

Although the number of drugs here is small, the overall result is encouraging and suggests the feasibility of large-scale screening of candidates for absorption behavior.[19]

RESULTS

Benchmarking

The BCS, as discussed above, considers the solubility and permeability of a drug as the means of classification. This article will quantify the limiting behavior of these quantities in different regions of parameter space. To do this, we should suggest a benchmark virtual molecule with consensus properties.

A basic molecule was chosen, with log $D_{7.4}$ set to 1 and the pKa set to 8. The single dose was assumed to be 100 mg, and the solubility of the ionized form was taken as 1000 times greater than the intrinsic solubility, which is 10 times the software's default value of 100 for acids and 50 for bases. The permeability was allowed to vary over the range 0.05 to 3.0 ($*10^{-4}$cm sec^{-1}) and the solubility at pH 7 over the range 0.2 µg to 0.016 mg/mL. The corresponding salt (low pH) solubilities, with the present acid solubility factor of 1000, are in the range 0.2 mg/mL to 16 mg/mL. Percentage of dose absorbed into the portal vein (F_a) was evaluated for each combination of solubility and permeability (**Figure 2**).

The 4 classifications of the BCS are clearly shown on **Figure 2**. The most relevant and helpful measure of the degree of solubility limiting (or sensitivity of absorption to solubility) is to compute the percentage increase in absorption for a 1% increase in solubility, and likewise for permeability. It may be shown that this is a dimensionless quantity equal to the following:

$$\text{Sensitivity} = (dF_a/dS_w) * (S_w/F_a) \qquad (4)$$

where F_a is the absorption (% of administered dose) and S_w is the solubility (mg/mL). The equation for permeability is analogous. The sensitivities calculated from the data of **Figure 2** appear in **Table 2**.

The low and high values of solubility and permeability were the limits mentioned above. When both parameters are high, BCS class 1, absorption is also high (96%) and sensitivities of absorption to small changes in either parameter are understandably low, as there is little scope for improvement. When both are low, BCS class 4, the absorption is also very low (1.4%), but the sensitivities to both parameters are high, as small increments in solubility or permeability make relatively large differences to the absorption. Classes 2 and 3 are intermediate in absorption and in sensitivities to absorption. It should be noted that the BCS does not propose numerical limits for low or high solubility or permeability but classifies on the basis of whether absorption is limited by these parameters.

Convenient Presentation of Results

As stated above, absorption of 25% is considered a reasonable criterion of suitability for a drug. For the parameters used in this initial benchmarking scenario, this absorption can be achieved by many combinations of solubility and permeability, and these may conveniently be presented as a 2-dimensional graph. In **Figure 3**, all such combinations yielding absorption of 25 ± 2% are shown. In effect, this is a horizontal "slice" through the 3-dimensional graph in **Figure 2**.

Table 1. Compounds Whose Absorptions Were Calculated With the Software, and the Corresponding Literature Values*

Name	Human P_{eff}[†]	Log D (6.8)[§]	Sol. (6.8)[§], mg/mL	Dose, mg[¶]	Diffusivity[†]	Predict. F_a%[#]	Exper. F_a%[§]	Transporter/efflux
Paromomycin	0.05[‡]	-2.0	10.00	250	0.75	9	3	
Acyclovir	0.15	-1.8	0.80	200	1.06	25	20	
Miconazole	12.00	4.5	0.0029[‖]	250	0.741	43	25	P-gp
Famotidine	0.55	-1.0	0.80	40	0.804	62	45	
Amiloride	1.60	-0.6	0.10	5	1.07	88	50	P-gp
Rifabutin	0.29	4.3	0.10	150	0.419	77	53	
Atenolol	0.30	-1.3	10.00	50	0.804	41	56	
Furosemide	0.30	-1.0	0.80	40	0.878	55	61	
Xipamide	1.25	0.5	0.80	20	0.75	96	70	
Olanzapine	2.05	2.0	0.02	10	0.793	91	75	
Fluoxetine	6.27	1.9	2.50	30	0.81	100	80	
Ibuprofen	5.36	1.6	2.50	200	0.917	100	80	
Terbinafine	12.00	5.5	0.02	250	0.722	99	80	
Zopiclone	0.93	1.3	0.10	8	0.776	85	80	
Zidovudine	0.25	-0.7	7.50	100	0.953	42	90	Nucloside
Allopurinol	0.30	0.1	0.40	300	1.63	51	90	Hypoxanthine
Aspirin	3.07	-2.0	0.80	500	1.21	99	90	
Propranolol	1.89	1.2	7.50	80	0.829	97	90	
Nizatidine	0.60	-0.3	10.00	300	0.741	66	99	Organic cation
Moxonidine	0.85	0.4	0.80	0.3	0.75	79	99	
Diclofenac	3.07	1.4	0.80	50	0.901	100	99	

*Literature values published by Balon et al[19] or estimated using QMPRPlus (version 2.3.0) or simulated using GastroPlus (version 3.1.0). Sol. = Solubility; Predict. = Predicted; Exper. = Experimental.

[†]Human jejunal effective permeability ($S + P_{eff}$) (cm sec^{-1} x 10^4) and molecular diffusion coefficient (cm^2 sec^{-1} x 10^5) were estimated using QMPRPlus (version 2.3.0).

[‡]Parshad et al.[14]

[§]Literature value for dose was from "Clinical Pharmacology Online" (Gold Standard Multimedia).[21]

[‖]Fraction absorbed was simulated using GastroPlus (version 3.1.0).

[¶]Because of the large number of H-bond donors[19] in paromomycin (more than any compound in the training set), the $S + P_{eff}$ (1.62 from QMPRPlus) was considered to be an unreliable estimate. Therefore, a value of 0.05 was assigned as being representative of the lowest values measured in humans.

[#]Because of the experimental lower limit of solubility measured in Parshad et al[14] (0.02 mg/mL), we obtained the intrinsic solubility (0.0029 mg/mL) by personal communication from Alex Avdeef, April, 2001.

Location of Absorption

The software treats each compartment of the GIT separately and estimates the absorption there, as well as summing the total absorption into the portal vein. The combinations of solubility and permeability in the above graph greatly influence the relative importance of the various equal time compartments, even with the constant total absorption of 25% in this case. Thus, at the relatively lower solubilities in the above graph, the colon contributes 94% of the total absorption, while at the higher solubilities this contribution declines to 44%.

Sensitivity to Log $D_{7.4}$

When using the GastroPlus software, users may choose among several variations on the ACAT model. Some use simplified models that are log D insensitive, but a more recent development is a log D model in which log D is

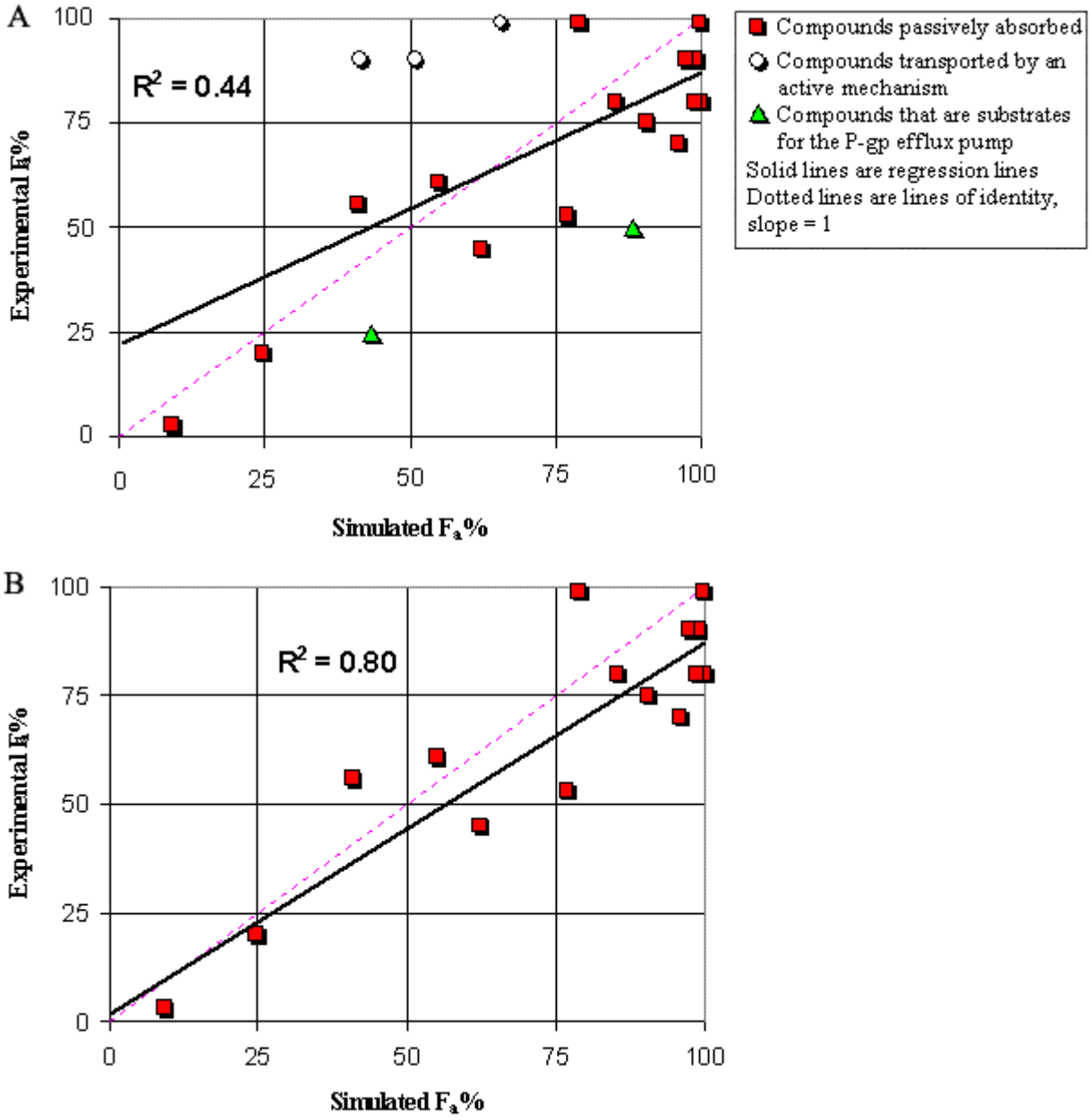

Figure 1. Correlation of calculated absorption (x-axis) with literature values (y-axis): (A) uses all 21 data points; (B) omits 5 transported or effluxed drugs.

recalculated in each compartment according to the prevailing pH in the compartment, and the prevailing ASF is adjusted accordingly. This is the model used throughout this work, other than the initial validations. Predicted absorption is significantly influenced by the log $D_{7.4}$ of the drug. **Figure 4** compares the solubility/permeability combinations giving rise to an overall absorption of 25 ± 2% for log $D_{7.4}$ values of -1, 1, and 3.

It is clear that higher values of log $D_{7.4}$ favor good absorption in that the same overall absorption is achieved either with less solubility or with lower permeability.

Sensitivity to Dose

Qualitatively, there are several reasons for progressively larger doses becoming increasingly more of a challenge to oral absorption. A given solubility requires a progressively larger volume to dissolve the higher

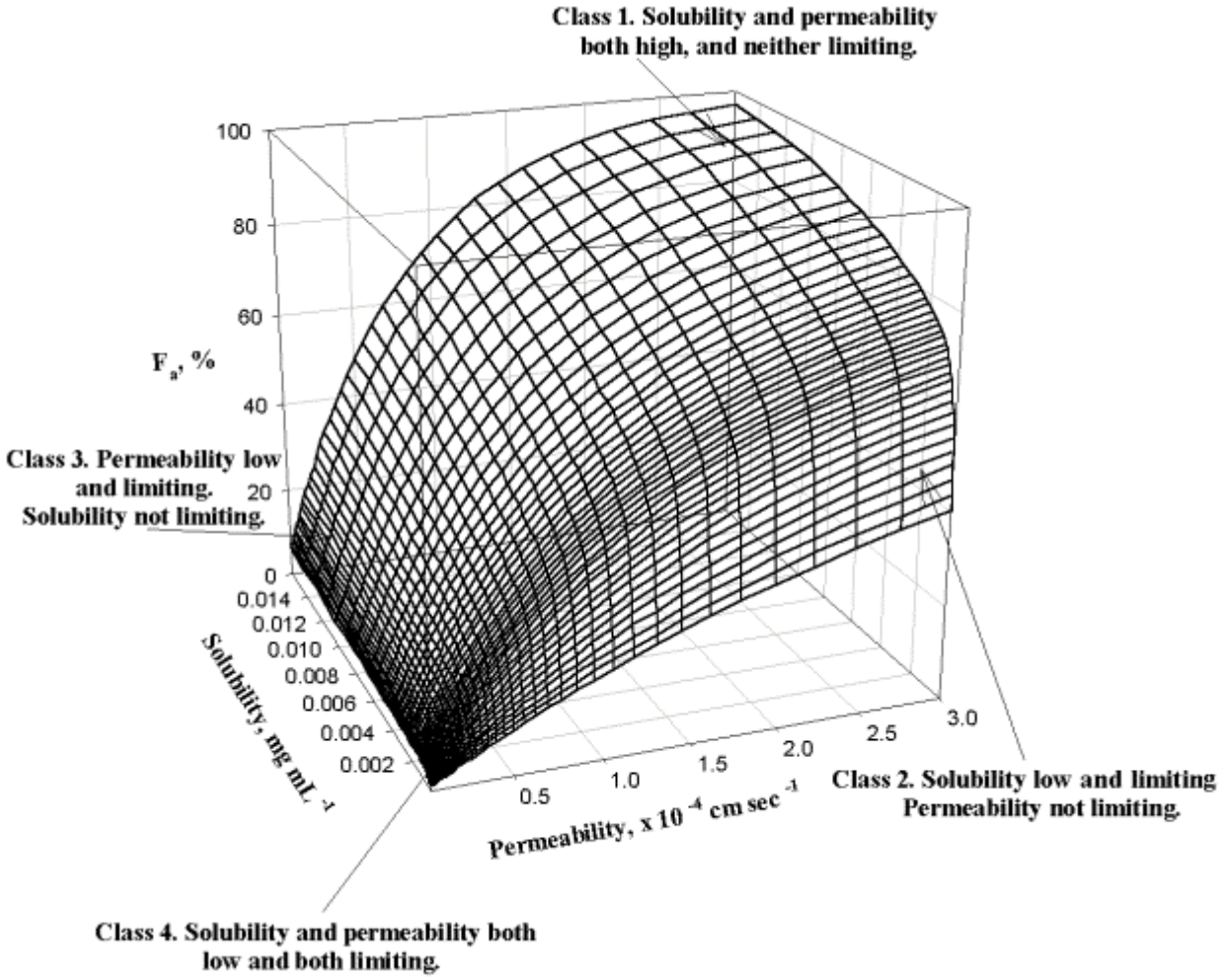

Figure 2. Absorption (fraction absorbed, F_a) as a function of solubility and of permeability.

dose—that is, a higher dose number. A given permeability leads to a rate of absorption that progressively becomes more inadequate because transit time is always finite—that is, a higher absorption number. Although dissolution rates are not primarily in view in this study, higher doses will also slightly reduce dissolution rates as the dissolution process moves further from sink conditions.

The combinations of dose and solubility studied here are such that the dose number is always in excess of 1; that is, the required dose is never able to dissolve in the assumed 250 mL of gastric contents. This accounts for the relatively large importance of colonic absorption in the benchmark drug, as transit times in the colon are so much longer.

Figure 5 indicates clearly the advantage that low-dose, high-potency drugs can have, in that lower solubilities or permeabilities can be tolerated within the limit of 25 ± 2% overall absorbance.

Theoretical Significance of Solubility Curves

As discussed in the introduction, the pH/solubility curve comprises 2 parts, representing the salt and base solubilities. These intersect at the pH given by Equation 3. Their potential significance for GIT absorbance is shown schematically in **Figure 6** for 3 drug scenarios. The shaded areas, the bands of pH significance, represent the low and high limits of the pH values used in the simulations, here taken as 5 to 7.5. The graphs are considered generically, with salt solubilities normalized at an arbitrary solubility of 1 unit, purely for illustrative purposes. For the present, the important point is the overall juxtaposition of the band of pH significance upon the composite curve.

Table 2. Sensitivities of Absorption to Permeability and to Solubility as a Function of Their Class Within the BCS*

Class in BCS	Solubility	Permeability	Sensitivities of absorption (0 = low, 1 = high)		
			To permeability	To solubility	Absorption (%)
1	High	High	0.10	0.07	96
2	Low	High	0.59	0.66	44
3	High	Low	0.96	0.36	7
4	Low	Low	0.99	0.92	1.4

*The table refers to the fixed and variable parameters detailed in the benchmarking section of the text. BCS indicates Biopharmaceutics Classification System.

Three Scenarios

In scenario 1, the band of pH significance lies entirely on the base solubility part of the curve, where solubility is a relatively sensitive function of pH. The intersection point must by the definition employed here be below pH 5. In this scenario, the simulation is not influenced by the salt solubility (except for stomach dissolution rate at pH = 1), and from the simulation standpoint a solubility measurement on the salt curve is not necessary to simulate small bowel dissolution. Equation 3 indicates that the intersection point is a function of both the pKa and the acid solubility factor (effectively, the acid solubility factor embodies the salt solubility). Before a measurement, one cannot confidently predict the location of the intersection point, but as a generalization it is likely that drugs of pKa up to about 7 will usually fit this scenario.

In scenario 2, the band of pH significance straddles both parts of the composite curve, and the intersection point must by definition be within this band. The expectation is that both base and salt solubilities will influence the small bowel absorption simulation, and in practical terms 2 solubility measurements will be needed to define the whole solubility curve, one on the salt curve and one on the base curve. As suggested above, only a generalization can be made, but drugs with pKa in the range 7 to 11 will probably fit this scenario.

In scenario 3, the band of pH significance overlies the salt solubility curve only; by the present definition the intersection point is at a pH in excess of 7.5. This contrasts with scenario 1; here, the simulation is not influenced by the base solubility, and from the simulation standpoint a solubility measurement on the base curve is not necessary. It seems likely that drugs of very high pKa, above 11, may fit this scenario.

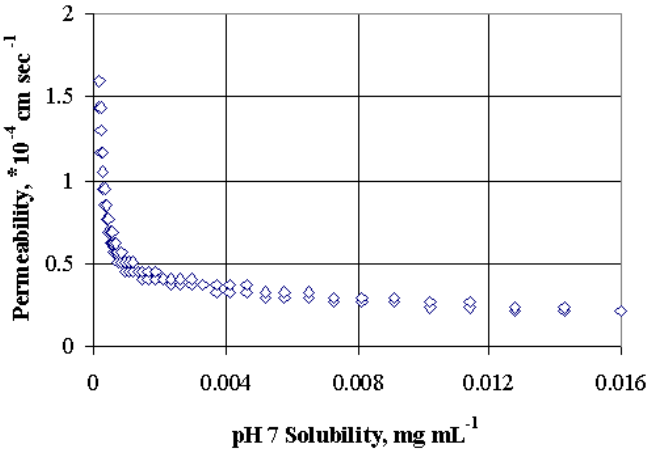

Figure 3. Combinations of permeability and of pH 7 solubility that yield 25 ± 2% absorption for fixed parameters. There are often multiple estimates of absorption for each simulated solubility, because of the width of the absorption range studied (as also in Figures 4 and 5).

pH of Solubility Measurements

The above discussion refers to experimentally determined solubilities on the salt (low pH) and base (high pH) parts of the solubility relationship. This needs further explanation. Low and high pH are relative terms dependent on their context, but here the distinction is based on the need to define these 2 parts of the solubility

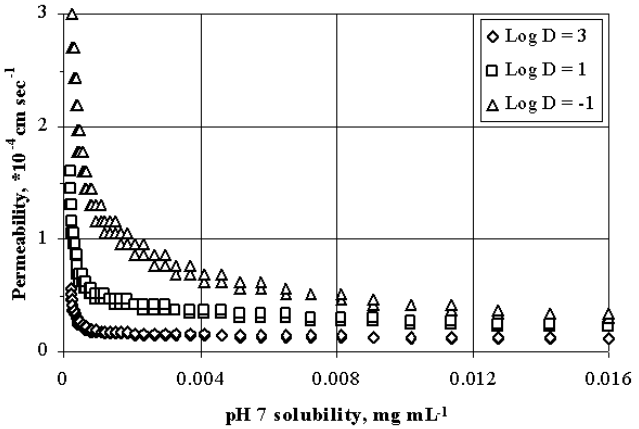

Figure 4. Effect of log D on the solubility/permeability relationship, all at constant 25 ± 2% absorption and for fixed parameters.

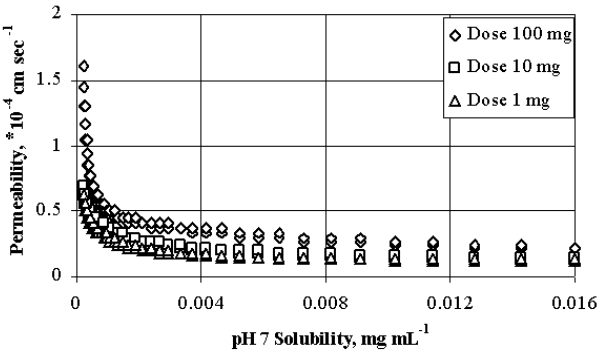

Figure 5. Effect of dose on the solubility/permeability relationship, all at constant 25 ± 2% absorption and for fixed parameters.

curve. Solubilities are clearly needed on either side of the intersection point, but this intersection point cannot be known without the solubility values, so practical guidelines are helpful in overcoming this interdependence.

For the salt solubility curve, it is generally convenient and simple to use 0.01M HCl as the solvent, with a pH of about 2.2. The HCl's pH has the virtue of being similar to the pH of gastric juices, which oral drugs will encounter in use. For drugs or combinatorial libraries of drugs known to be acid labile, a somewhat higher pH could be used, but the pH should be at least 1 and preferably 2 units less than the pKa to ensure that the intersection point pH is not exceeded.

For the base solubility curve, experimenters have traditionally used intestinal pHs of around 6 or 7. Such pHs do have the virtue of being intestinally relevant. For drugs of high pKa (above 10-11), a higher pH of solubility measurement would be needed to ensure that it was really the base solubility curve that was being measured, but pHs above 9 would not be helpful for several reasons. First, they are not intestinally relevant. Second, they are likely to cause an in vitro degradation not matched by an in vivo degradation. Third, as such drugs are likely to be in scenario 3, the base part of the curve, being above pH 7.5, is not relevant to the absorption simulation. In practice there is an advantage in deciding upon a fixed pH near 7 for all "base" measurements and accepting that for some high-pKa compounds this produces merely a second estimate of the salt solubility.

Much of the time, experimenters will use high-throughput strategies that do not easily lend themselves to individual adjustment of solvent pHs. In such cases, there is less scope for error if 2 fixed-solvent pHs are employed. Where individual adjustments are practicable, these may be advantageous.

Acid Solubility Factors

Assuming 2 experimental solubilities are determined at low and high pH as defined above, the acid solubility factor required by the software for the simulation may be calculated from a combination of Equations 1 and 2, which gives

$$\text{Acid solubility factor} = (S_L/S_H) * (1 + 10^{\text{pKa-H}}) \qquad (5)$$

where S_L and S_H are the measured solubilities at low and high pH respectively, and H is the high pH value. Note that the low pH value L does not enter this equation, as salt solubility is modeled to be effectively independent of pH. The combination of this calculated acid solubility factor and the known pKa defines the intersection point and thus which of the 3 scenarios the drug fits into. It should also be noted that Equation 5 would not be applicable to scenario 3, in which S_L and S_H are duplicate estimates of the salt solubility, but this will be obvious, as they will be very close to each other.

Importance of pKa and Acid Solubility

The 3 drug scenarios described above arise on account of the interplay of 3 factors. The pKa of the drug is the major determinant. Without a doubt, pKa is one of the most fundamentally important parameters of a drug[20] and has given rise to both predictive software (eg, from Compudrug, or ACD Laboratories) and, on an experimental level, high-throughput automated titrators (eg, from Sirius Analytical Instruments). In addition to

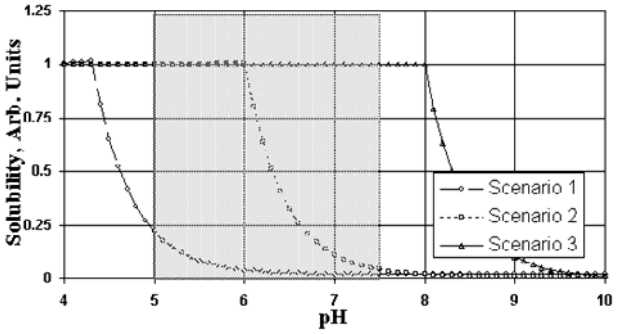

Figure 6. The 3 scenarios that may exist for the juxtaposition of the solubility curve upon the range of pH relevant for the absorption model (shaded).

the pKa, the acid solubility as expressed in the acid solubility factor (Equation 5) is also a major determinant. Between them, these parameters determine the intersection point of the pH/solubility curve through Equation 3 and thus determine which of the 3 scenarios will prevail in any given case. Finally, it will be appreciated from the discussion above that the pHs at which solubilities are measured could, in some cases, be adjusted to fit their properties.

It is worth noting that GastroPlus works out whether this high-pH solubility measurement is on the salt or neutral side of the intersection and treats it accordingly. If it is on the neutral curve, then the neutral curve is constructed from this data point, as indicated earlier, and the salt curve on the basis that C_{OA} is acid solubility factor * C_{OB}. If it is on the salt curve, then the salt curve is constructed from this data point, and the neutral curve on the basis that C_{OB} is C_{OA}/acid solubility factor. This is a "best possible" reconstruction without additional measurements at higher pH. Polyprotics with n pKa's would need n + 1 solubility measurements to define n acid solubility factors, but from experience, it has been found that in the absence of extensive experimental data, a default value of 100 for acids and 50 for bases is a good start.

Consequently, it is essential that these important factors be considered together. As an example, consider a basic drug of pKa = 8, having a solubility at pH 7 of 3.26 µg/mL and a permeability of $1*10^{-4}$ cm sec^{-1}. If the low-pH solubility (expressed as the acid solubility factor) is allowed to vary over wide limits, the absorption is as shown in **Figure 7**.

From points A to B, we have scenario 3. The absorption appears to be insensitive to the acid solubility factor (or, alternatively, to the salt solubility) in this region. The present simulation assumed a fixed solubility at pH 7. According to Equation 3, this is at or below the intersection point, meaning that the pH 7 solubility is in effect the salt solubility. In terms of the simulation, the software detects this and produces (and uses) a base solubility curve. The neutral solubility curve is also produced, and its values do depend upon the acid solubility factor value entered, but the information is not used in the simulation. This accounts for the observed insensitivity of the calculated result to the acid solubility factor, consistent with the earlier discussion.

Between points B and C, scenario 2, the absorption is seen to be very sensitive to the acid solubility factor. The dimensionless sensitivities are 0.91 (acid solubility factor just over 10), declining to 0.54 (acid solubility factor = 100) and declining further to 0 at acid solubility factor = 1000. This underlines the considerable value of a quality estimate of the acid solubility factor. This should be achieved through an experimental solubility measurement on the salt part of the solubility curve.

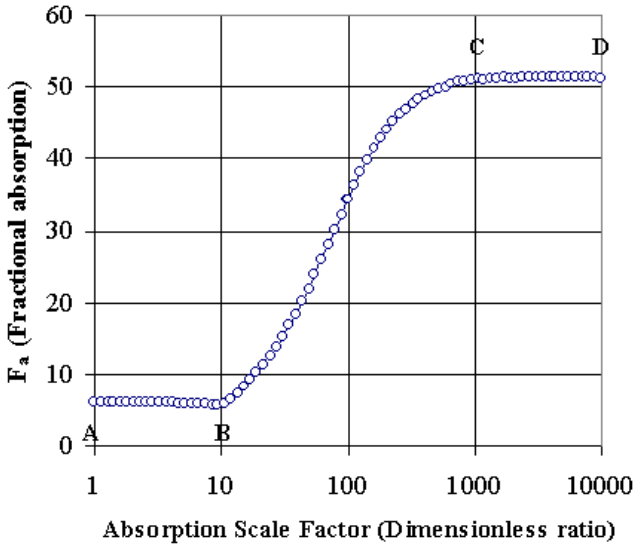

Figure 7. The 3 scenarios that may exist for the juxtaposition of the solubility curve upon the range of pH relevant for the absorption model (shaded).

Point C corresponds to the benchmarking scenario described earlier. From point C to point D we have scenario 1, where again the result is seen not to depend upon the assumed value of acid solubility factor, as this influences only estimated solubilities below pH 5, which are not relevant to the simulation. Thus, the benchmarking exercise assumed an acid solubility factor

Table 3. Measured Values for 32 Drugs*

Sample no.	pKa	Experimental solubility ratio, $S_{2.2}/S_{7.4}$	Absorption Scale Factor	Intersection pH of solubility curves	Scenario
1	4.11	291.8	292.0	1.65	1
2	4.34	60.5	60.5	2.56	1
3	4.77	194.9	195.4	2.48	1
4	5.00	280.3	281.4	2.55	1
5	5.20	94.2	94.9	3.23	1
6	5.32	94.2	95.0	3.34	1
7	5.47	1299.4	1314.8	2.36	1
8	6.41	556.7	613.5	3.62	1
9	6.51	80.2	90.5	4.55	1
10	7.01	37.2	52.5	5.29	2
11	7.21	11.6	19.0	5.93	2
12	7.24	98.1	166.8	5.02	2
13	7.34	28.1	52.7	5.62	2
14	7.40	16.0	32.1	5.90	2
15	7.61	130.2	343.1	5.08	2
16	7.63	17.3	46.4	5.96	2
17	7.66	16.0	45.2	6.01	2
18	7.74	9.4	29.9	6.26	2
19	7.81	19.5	69.8	5.97	2
20	8.20	159.3	1172.9	5.13	2
21	8.77	25.9	631.8	5.97	2
22	9.03	9.1	393.4	6.43	2
23	9.03	6.8	296.6	6.56	2
24	9.15	9.4	540.6	6.42	2
25	9.21	68.2	4490.3	5.56	2
26	9.25	18.8	1341.7	6.12	2
27	9.57	3.4	510.3	6.86	2
28	9.60	27.0	4349.8	5.97	2
29	9.80	4.6	1155.7	6.74	2
30	9.88	1.9	563.2	7.12	2 or 3
31	10.15	6.8	3813.7	6.56	2
32	10.38	4.8	4537.3	6.72	2

*The GIT pH range overlapping only the salt solubility curve is scenario 3, overlapping only the base curve is scenario 1, and overlapping both is scenario 2.

of 1000 to ensure that there was no contribution to the result by any assumption about low-pH solubility.

What Scenarios Are Common Among Drugs?

Solubilities at pH 2.2 and 7.4 were determined for 32 basic drugs, and their experimental ratios are given in **Table 3** below, along with their measured pKa values. From the simple solubility ratios, the acid solubility

factor was calculated from Equation 5, and the crossover pH for the solubility curves from Equation 3. Finally, the "scenario" as described above was calculated.

It will be seen that in this admittedly rather small database, the great majority of drugs (22 out of 32) fall into scenario 2, where considerable sensitivity to acid solubility is anticipated. One drug, number 30, has a very low experimental ratio and a calculated pH of crossover of 7.12. As stated above, the calculations of the crossover pHs using Equation 5 assume that the 2 individually measured solubilities are on either side of the crossover point. Drug 30 illustrates the uncertainty that arises when the measured solubilities are close to each other. Bearing in mind the potential errors in the solubility values, ratios as low as this could well indicate that the crossover point is above the higher of the 2 pH values employed and that this drug could be in scenario 3.

The database has its limitations. The solubility measurements were part of high-throughput screens, accuracy was sacrificed for speed and throughput, and, as with any sample, a degree of uncertainty remains. However, taking this database as a whole, one can conclude that most of these basic drugs fall into scenario 2 and thus have absorption that is sensitively dependent upon the solubility at low pH, and that experimentally such measurements are essential to good simulations.

Only 1 sample in this database could have been in scenario 3. It seems likely that only rather high-pKa drugs (with pKa around 10 or more) will fall into this classification. The consequence is that all solubility measurements below pH 7.5 will be on the salt curve and will be about the same. As the simulation does not make use of solubilities below pH 7.5, the result is insensitive to the base part of the solubility curve.

What Solubility Values Are Required?

The scientist producing drug candidates has to consider many factors in developing an Structural Activity Relationship (SAR), including solubility. Compromised solubility at candidate selection can cause excessive expenditure at the development stage to make the candidate commercially druggable, and wondering what solubility is needed is perfectly justified. Emphasis has been given in this article to the need for 2 solubility values (rather than 1) in most cases. This point will be amplified here. The absorption prediction, even in the simplest possible case of a monoprotic base administered as a single immediately available dose, needs at least the following experimental (or in silico predicted) parameters:

- Solubility value of the base form at a given pH
- Acid solubility factor (but see discussion below)
- Log D
- P_{eff}
- pKa

It is not practical to represent such multidimensional predicted absorption data in any convenient 2-dimensional format. Even the relatively complex 3-dimensional representation of **Figure 3** represented only 2 of these 5 variables, with the acid solubility factor, log D, and pKa fixed. Accordingly, the only satisfactory expedient is to run the simulation with the relevant parameters and determine whether the predicted absorption exceeds the predetermined target value (25% in this article). It is a simple matter to write files that will simulate thousands of parameter combinations per day.

In practice, log D and pKa are commonly predicted in silico or are experimentally measured. Peff is readily predicted in silico. Solubilities of the base form may be predicted with various products. ACD predictive software[22] leads to the solubility of the un-ionized form, which may be converted with Equation 1 to the pH 7.4 solubility, while QMPRPlus software (Simulations Plus)[1] leads to the "native" solubility, meaning the solubility at the pH assumed by a saturated solution of base in pure water. This represents a spot value on the base solubility curve, which can also be converted to the pH 7.4 solubility.

Predictive solubility software products (such as QMPRPlus, Simulations Plus; and ACD-Solubility and pKa DB, ACD Labs) cannot as yet predict salt solubilities. Consequently, the acid solubility factor cannot be predicted but must be measured by determining 2 solubility values (and thus the solubility ratio) as already discussed, using Equation 5. The acid solubility factor influences the absorption prediction for all drugs in scenario 2, as illustrated by **Figure 7**. It would be helpful, therefore, to be able to predict simply which scenario a drug would fall into, which reduces to determining the pH of the intersection point. Intersection pHs below 5 are scenario 1, while above 7.5 they are scenario 3. Most drugs in the database presented above were in scenario 2. The intersection point in turn is determined (Equation 3) by the pKa and the acid solubility factor,so rigorously that one requires knowledge of the acid solubility factor in order to establish whether the acid solubility factor needs to be

measured! In practice, experimenters will measure the acid solubility factor for all drugs as part of a high-throughput screen, without attempting to make individual exceptions, or else the approximation will be accepted that scenario is determined by pKa alone. In this approximation, drugs with pKa between 6.5 and 10 would be scenario 2, and it is only these drugs that require the acid solubility factor to be measured, and consequently only these that require a low pH (acid) solubility to be determined.

CONCLUSION

Absorption simulations with GastroPlus require physicochemical data that are potentially always available as in silico estimates, even in the absence of synthesized material. In many cases, they are subsequently refined postsynthesis by experimental determination in automated processes. There is, however, a weakness in this approach. Solubility values are usually needed at low and at high pH for good simulations of absorption, and these need to be measured. Estimations of high pH solubility for bases are now available, but low pH solubilities cannot be estimated; they can only be measured. It has been common practice in the industry to make a single high-pH measurement, but it is shown here that the result is often greatly influenced by the low pH solubility too. Accordingly, it is suggested that within discovery settings, 2 solubility measurements should be determined. In general, these could conveniently be at approximately pH 2.2 and 7, but it is appreciated that some libraries of compounds could benefit from the use of pHs different from these when high pKa or acid lability is known to be an issue. More meaningful absorption predictions, and thus more meaningful candidate screens, will be the result.

REFERENCES

1. Simulations Plus, Inc. Available at: http://www.simulations-plus.com.

2. Press WH. Numerical recipes. In: Teukolsky SA, Vetterling WT, Flannery BP, eds. C: The Art of Scientific Computing. New York, NY: Cambridge University Press; 1992: 566-580.

3. Ungell A-L, Nylander S, Bergstrand S, Sjöberg Å, Lennernäs H. Membrane transport of drugs in different regions of the intestinal tract of the rat. J Pharm Sci. 1998;87(3):360-366.

4. Adson A, Burton PS, Raub TJ, Barsuhn CL, Audus KL, Ho NF. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers. J Pharm Sci. 1995;84(10):1197-1204.

5. Selick HE, Beresford AP, Tarbit MH. The emerging importance of predictive ADME simulation in drug discovery. Drug Discov Today. 2002;7(2):109-116.

6. Amidon GL, Lennemas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995;12(3):423-420.

7. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997;23(1-3):3-25.

8. Devane J. Oral drug delivery technology: addressing the solubility/permeability paradigm. Pharm Technol. 1998; Nov:68-80.

9. Hussein AS, Lesko LJ, Lo KY, Shah VP, Volpe D, Williams RL. The biopharmaceutics classification system: highlights of the FDA's draft guidance. Dissolution technologies. 1999; May, article 1. Available at: http://www.dissolutiontech.com/DTresour/599articles/Biopharm_Class2_copy.html.

10. Blume HH, Schug BS. The Biopharmaceutics Classification System (BCS): class III drugs—better candidates for BA/BE waiver? Eur J Pharm Sci. 1999;9:117-121.

11. Streng WH, His SK, Helms PE, Tan HGH. General treatment of pH-solubility profiles of weak acids and bases and the effects of different acids on the solubility of a weak base. J Pharm Sci. 1984;73(12):1679-1684.

12. Kramer SF, Flynn GL. Solubility of organic hydrochlorides. J Pharm Sci. 1972;61(12):1896-1904.

13. Jorgensen WL, Duffy EM. Prediction of drug solubility from structure. Adv Drug Deliv Rev. 2002;54:355-366.

14. Parshad H, Frydenvang K, Liljefors T, Larsen CS. Correlation of aqueous solubility of salts of benzylamine with experimentally and theoretically derived parameters: a multivariate data analysis approach. Int J Pharm. 2002;237:193-207.

15. Bergstrom CAS, Norinder U, Luthman K, Artursson P. Experimental and computational screening models for prediction of aqueous drug solubility. Pharm Res. 2002;19(2):182-188.

16. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Controlled Rel. 2000;65:271-284.

17. Gao H, Shanmugasundaram V, Lee P. Estimation of aqueous solubility of organic compounds with QSPR approach. Pharm Res. 2002;19(2):497-503.

18. Yoshida F, Topliss JG. QSAR model for drug human oral bioavailability. J Med Chem. 2000;43:2575-2585.

19. Balon K, Riebesehl BU, Muller BW. Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. Pharm Res. 1999;16(6):882-888.

20. Wells JI. Pharmaceutical Preformulation. Chichester, England: Ellis Horwell Ltd; 1988:14.

21. "Clinical Pharmacology," online at Gold Standard Multimedia. Available at: http://www.gsm.com.

22. ACD Labs, Toronto, Canada.