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# A pH-Dilution Method for Estimation of Biorelevant Drug Solubility along the Gastrointestinal Tract: Application to Physiologically Based Pharmacokinetic Modeling

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**Abstract:** Physiologically based pharmacokinetic (PBPK) modeling tools have become an integral part of the modern drug discovery−development process. However, accurate PK prediction of enabling formulations of poorly soluble compounds by applying PBPK modeling has been very limited. This is because current PBPK models rely only on thermodynamic drug solubility inputs (e.g., pH−solubility profile) and give little consideration to the dynamic changes in apparent drug solubility (e.g., supersaturation) that occur during gastrointestinal (GI) transit of an enabling formulation of a water insoluble drug. Moreover, biorepresentative and predictive *in vitro* tools to measure formulation dependent solubility changes during GI transit remain underdeveloped. In this work, we have developed an *in vitro* dual pH-dilution method based on rat physiology to estimate the apparent drug concentration in solution along the GI tract during release from solubility enabling formulations. This simple dual pH-dilution method was evaluated using various solubility enabling formulations (i.e., cosolvent solution, amorphous solid dispersions) made using a model early development drug candidate with poor aqueous solubility. The *in vitro* drug concentration−time profiles from the enabling formulations were used as solubility inputs for PBPK modeling using GastroPlus software. This resulted in excellent predictions of the *in vivo* oral plasma concentration−time profiles, as compared to using the traditional inputs of thermodynamic pH−solubility profiles. In summary, this work describes a novel *in vitro* method for facile estimation of formulation dependent GI drug concentration−time profiles and demonstrates the utility of PBPK modeling for oral PK prediction of enabling formulations of poorly soluble drugs.

**Keywords:** Physiologically based pharmacokinetic (PBPK) modeling; GastroPlus; solubility enabling formulations; biorelevant dissolution; amorphous solid dispersions

## Introduction

Over the past decade, physiologically based pharmacokinetic (PBPK) modeling tools have emerged to play an increasingly important role in the discovery and development of new drugs.[1−12] PBPK modeling software packages such as GastroPlus have been introduced and broadly applied across the entire drug discovery−development process to

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address a variety of biopharmaceutical challenges related to oral absorption (e.g., solubility, dissolution, permeability) as well as drug disposition issues (e.g., metabolism, distribution, and elimination).[13−29]

Although PBPK models have rapidly evolved to become an integral part of modern biopharmaceutics, the application of these tools to solve drug solubility/dissolution problems has been limited to those related to bulk API properties, such as thermodynamic pH−solubility profiles and particle size distributions.[30−41] This is because current PBPK models rely primarily on thermodynamic equilibrium assumptions to


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model drug solubility/dissolution and give little consideration to the dynamic changes in apparent drug concentration (e.g., supersaturation) that may occur during gastrointestinal (GI) transit. As a result, the application of PBPK modeling to oral PK prediction of solubility enabling formulations, such as cosolvents, lipid solutions, and amorphous solid dispersions, has been very limited.

An additional reason for the limited application of PBPK models to solubility enabling formulations of poorly soluble drugs is the lack of biorepresentative and predictive *in vitro* tools to measure biorelevant drug solubility/dissolution. A number of *in vitro* dilution or precipitation evaluation methods have been reported.[42−44] One of the most straightforward methods is the serial dilution method in which lipid or solvent based formulations are diluted into buffer, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Rank-ordering of the formulations is then decided by visual observation of the rate and extent of precipitation within a defined time period (e.g., after 8 h).[43] A similar approach was taken by Schamp et al. Various lipid formulations were added to physiologically relevant media (SGF+, FaSSIF and FedSSIF) separately, and the concentration of the drug released into the media was measured.[44] The maximum concentration reached in these media was found to correlate with *in vivo* dog data. For lipid formulations containing long chain or medium chain triglycerides, *in vitro* dynamic lipolysis has been shown to be a useful tool to qualitatively predict *in vivo* performance.[45−47] All of these methods, while valuable on their own applications, do not adequately address some important changes (e.g., pH change) that an oral formulation undergoes *in vivo*. In many instances, it is important to mimic the physiologically relevant pH changes, fluid volumes/dilution, and residence times that the dosage form experiences during GI transit. A more physiologically relevant *in vitro* solubility measurement is especially important for water insoluble drugs which are being formulated into solubility enabling delivery systems. In these cases, the drug may undergo precipitation and/or solution-mediated phase transformation which results in supersaturation.[48−51] Supersaturation and solution-mediated phase transformation of the drug may result from pH-induced precipitation (e.g., free-base from salt/ionized form), precipitation from solution formulations (e.g., cosolvents, lipids), or amorphous/metastable to stable polymorph/hydrate phase transformations (e.g., crystallization of an amorphous solid dispersion).[48−51] Therefore, for more accurate PBPK modeling of these solubility enabling formulations, it is desired to develop more biopredictive *in vitro* drug release models that can better represent *in vivo* conditions, both statically and kinetically.

More biorelevant *in vitro* dissolution methods have been pursued by some researchers.[51,52] Gu et al. modified the conventional six-vessel USP dissolution system to a multicompartment dissolution system to include a "gastric"

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***Table 1.*** Compositions of Solubility Enabling Formulations of the Abbott Model Compound

| formulation | composition | dose (mg/kg) | method of manufacture | lot information |
|---|---|---|---|---|
| solution | 4.3 mg/mL 90:10 PEG-400:DMSO | 4.3 | na | preclinical |
| suspension A | 25:20:55 API:surfactant:polymer | 100 | rotary evaporation | pre-GLP Tox |
| suspension B | 25:20:55 API:surfactant:polymer | 100 | rotary evaporation | GLP Tox |
| suspension C | 25:20:55 API:surfactant:polymer | 100 | spray-drying | pre-GMP |
| suspension D | crystalline hydrate suspension | 100 | na | preclinical |

compartment, an "intestinal" compartment, an "absorption" compartment, and a reservoir to simulate the dissolution and absorption in the gastrointestinal tract.[52] Carino et al. developed an automated artificial stomach−duodenum (ASD) model to simulate dog physiology in the fasted state.[51] By using this model, they obtained an excellent estimation of the relative bioavailability of carbamazepine crystal forms.[51] These dissolution models provided skillful approaches to the problem described above, but the drawback is the complexity in the experimental setup or the need of large amount of drug that limits their use in the fast paced toxicology and first in human (FIH) formulation development stage.

In this work, we have developed a fast, easy to use and material sparing *in vitro* dual pH-dilution method aimed at mimicking the physiologically relevant pH, dilution volumes, and residence times experienced by a drug formulation during gastrointestinal transit in the rat. This dynamic process provides a better representation of the transit of the drug formulation through the GI tract, which more closely captures the kinetic aspects of the actual *in vivo* drug release process. The pH-dilution method developed here was used to estimate the regional changes in drug concentration along the GI tract from various solubility enabling formulations (i.e., cosolvent solution, amorphous solid dispersions) for a model early development drug candidate with poor aqueous solubility. The *in vitro* drug concentration−time profiles from the enabling formulations were used as solubility inputs for PBPK modeling using GastroPlus software.

## Experimental Section

**Materials.** Polyethylene glycol 400 (PEG-400) was obtained from Spectrum Chemicals & Laboratory Products (Gardena, CA). DMSO was obtained from Sigma-Aldrich (St. Louis, MO). Simulated intestinal fluid (SIF) powder was obtained from Phares AG (Muttenz, Switzerland). 0.1 N HCl was obtained from J. T. Baker (Phillipsburg, NJ) and diluted to 0.0001 N using purified water. The Model Compound was obtained from the Abbott compound library and was used as received. All other chemicals were of the highest available quality and were used as received.

**Preparation of Solubility Enabling Formulations.** The compositions of the various solubility enabling formulations of the Abbott Model Compound are summarized in Table 1. The amorphous solid dispersion formulations for pre-GLP and GLP toxicology studies were prepared by rotary evaporation. All formulation components were dissolved in 200 proof ethanol in a round-bottom flask at a total solids load of 20% (weight to volume). These ethanol solutions were then evaporated at 50 °C under vacuum using a Buchi (Flawil, Switzerland) Rotavapor R-210 until no visual solvent existed. The solid was then removed from the flask and further dried under vacuum at 40 °C. The dried solid was milled, and particles under 420 $\mu$m were collected for further studies.

The amorphous solid dispersion formulation for pre-GMP evaluation was prepared by spray-drying. All formulation components were dissolved in 200 proof ethanol at a total solids load of 8% (weight to volume). This solution was then spray-dried using a Buchi (Flawil, Switzerland) mini-spray dryer B-290 at an inlet temperature of 105 °C, outlet temperature of approximately 54 °C, and feed solution infusion rate of approximately 5 mL per minute. The size range for the resulting spray-dried particles was approximately 20−30 $\mu$m.

**Determination of Equilibrium pH−Solubility Profile.** The Abbott Model Compound was added (in triplicate) to 3 mL of liquid buffer at a range of pH conditions so that excess solid remained undissolved. The mixtures were equilibrated for 24−48 h with gentle agitation (end over end tumbling) in a constant temperature water bath held at 37 °C. After equilibration, the mixtures were inspected to ensure that excess solid was present. If not, additional solid was added and the sample was returned to the water bath for equilibration. The pH of aqueous suspensions was determined after equilibration. The solid phase was then separated from the liquid phase by filtration through a 4 mm syringe filter with 0.45 $\mu$m PTFE membrane. The filtrate was diluted with sample diluent to achieve a final concentration within the range of the standard curve and assayed by HPLC.

***In Vitro* pH-Dilution Experiments.** The rat dilution model used in the present study is designed to mimic the two compartments of the GI tract of a fasted rat: the stomach and the intestine. This is a dual pH-dilution scheme constructed by taking into consideration the rat GI physiological parameters important to formulation dilution/dissolution and supersaturation. The rat physiological parameters were obtained from the literature, and include pH change, rate of fluid secretion in the GI lumen responsible for dilution, and GI transit times.

The dilution factors were calculated based on various fluid secretion rates and transit times. In the stomach compartment, the primary dilution came from basal acid output (BAO) − the gastric secretion, since saliva secretion in rats is negli-

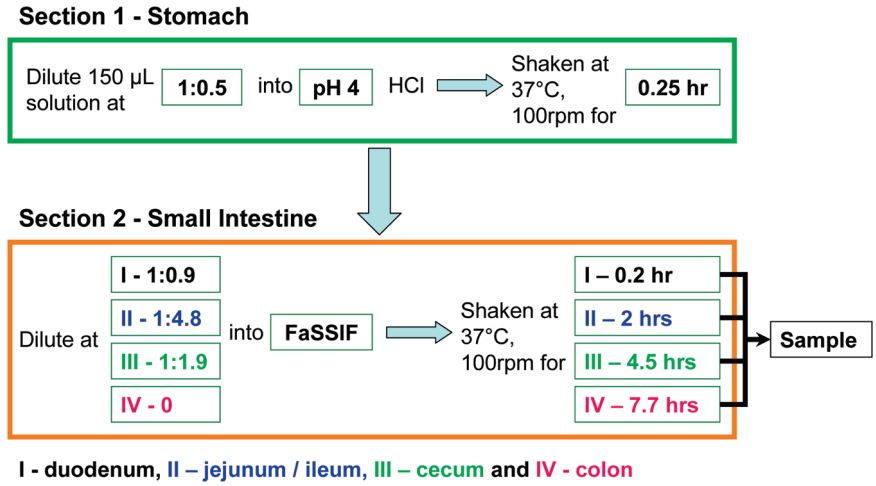


***Figure 1.*** Schematic of *in vitro* pH-dilution method to simulate rat gastrointestinal conditions for solution formulations.

gible.[53] The BAO value of rats was found to be 0.8657 mL/4 h/100 g.[54] The rat stomach transit time was found to be about 0.25 h.[55] The calculation of dilution factor in the small intestine took into account two additional dilution sources, the pancreatic juice secretion rate (0.033 mL/min/kg)[56] and bile flow (70 mL/day/kg)[57] in addition to the BAO which continues to flow from the stomach down to the small intestine. The intestine compartment was further divided into four segments, corresponding to duodenum, jejunum/ileum, cecum, and colon with transit times of approximately 0.2, 2.0, 4.5, and 8 h, respectively.[58] In the colon, the transit time is relatively long (8 h), but the dilution factor was presumed to be zero (1:0) since most fluid has been absorbed and the drug is present in a semisolid or solidlike matrix.[59] The pH in rat stomach was reported as approximately pH 4, therefore, $1.0 \times 10^{-4}$ M HCl was used to simulate the stomach pH.[60] The pH in small intestine depends on fed or fasted status. The widely used FaSSIF (fasted simulated small intestinal fluid)[1] with a pH of about 6.5 was selected as dilution buffer for the intestine segment, because the PK studies were conducted on fasted rats.[60] It should be noted that this FaSSIF was originally designed to mimic the human intestinal fluid composition, whereas the studies reported here were designed to mimic the rat. In the absence of any known and well established rat FaSSIF compositions, human FaSSIF was used as the best available surrogate. Moreover, the bile salt and phospholipid compositions of human and rat bile fluid have been reported to be similar.[57]

For *solution formulations*, the dilution factor in the stomach was calculated to be 1.5 based on the dosing volume of 1 mL/kg, the average weight of a rat (~300 g) and the BAO and stomach transit time described above. In duodenum, jejunum and ileum, the dilution factors calculated were 1.9, 5.8, and 2.9 with an overall dilution factor of ca. 32, using the pancreatic juice secretion rate, bile flow rate and BAO described above.

For *suspension formulations*, the dosing volume was increased significantly to 10 mL/kg. As a result, the dilution factors are smaller compared to dosing solution formulations. The dilution factor in the stomach was calculated as 1.1, and those in small intestine (duodenum, jejunum and ileum) were 1.1, 2.2 and 2.2, respectively. In this case, the overall dilution factor is much lower, ca. 6.

The steps of dilution are illustrated in Figure 1 (for solution formulations) and Figure 2 (for suspension formulations). The dilution volumes, pH, and sampling times are summarized in Table 2. For solution formulations, 150 $\mu$L of sample was first diluted into $1.0 \times 10^{-4}$ M HCl (pH 4 solution) at a dilution factor of 1:1.5. The sample vial was agitated at 100 rpm in an VWR orbital shaker at 37 °C for 0.25 h. The vial was withdrawn from the shaker, and further diluted at a dilution factor of 1:0.9 with FaSSIF, and returned to the water bath for another 0.2 h, at which time a 50 $\mu$L sample was taken, ultracentrifuged at ~150000*g*, diluted with HPLC diluent and injected into HPLC for determination of drug concentration. The cycle of dilution/agitation/sampling was repeated using the dilution factors and agitation time listed in the Small Intestine section of Figure 1. The same procedure using the parameters listed in Figure 2 was followed to test suspension formulations.

***In Vivo* Rat PK Studies.** All *in vivo* study procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with local

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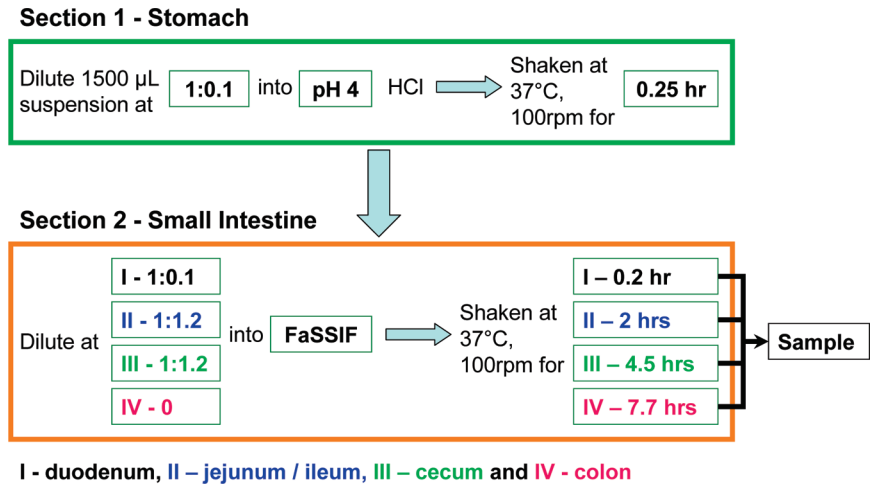


***Figure 2.*** Schematic of *in vitro* pH-dilution method to simulate rat gastrointestinal conditions for suspension formulations.

***Table 2.*** Dilution Volumes, pH, and Sampling Times for Rat pH-Dilution Method

| | | volume ($\mu$L) | | | |
|---|---|---|---|---|---|
| accumulated time (h) | dilution factor | starting | sample | dilution | dilution buffer |
| | | For Solutions | | | |
| 0 | 1.5 | 150 | none | 81 | $10^{-4}$ N HCl |
| 0.25 | 1.9 | 231 | none | 212 | FaSSIF |
| 0.45 | 5.8 | 393 | 50 | 1879 | FaSSIF |
| 2.45 | 2.9 | 2222 | 50 | 4135 | FaSSIF |
| 6.95 | 1.0 | 6307 | 50 | 0 | FaSSIF |
| 14.95 | | 6257 | 50 | | FaSSIF |
| | | For Suspensions | | | |
| 0 | 1.1 | 1500 | none | 81 | $10^{-4}$ N HCl |
| 0.25 | 1.1 | 1581 | none | 212 | FaSSIF |
| 0.45 | 2.2 | 1743 | 50 | 2059 | FaSSIF |
| 2.45 | 2.2 | 3752 | 50 | 4572 | FaSSIF |
| 6.95 | 1.0 | 8274 | 50 | 0 | FaSSIF |
| 23 | | 8224 | 50 | | FaSSIF |

regulations. Male Sprague−Dawley derived rats, weighing 250−400 g, were obtained from Charles Rivers Laboratories, Portage, MI. All animals were fasted overnight and allowed continuous access to water.

In the preliminary study the Abbott Model Compound was prepared at concentrations appropriate for a 1 mL/kg dose volume in a solution of DMSO:PEG-400 (10:90, by volume). Groups of three rats received either a 10 $\mu$mol/kg intravenous or oral dose. The intravenous dose was administered as a slow bolus in the jugular vein under light isoflurane anesthetic; the oral dose was administered by gavage. Serial samples were collected into heparinized blood collection tubes (0.4−0.5 mL) via the tail vein of each animal at 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after administration.

In the collection of studies evaluating the crystalline Abbott Model Compound and amorphous solid dispersion formulations, a 100 mg/kg oral dose of the Abbott Model Compound was administered by oral gavage to groups of three animals at concentrations appropriate for a 10 mL/kg dose volume. The Abbott Model Compound was prepared as a suspension in 0.2% HPMC; the amorphous solid dispersion formulations were prepared by suspension in 0.2% HPMC (for lots prepared by rotovap) or water (for lot prepared by spray drying). Serial samples were collected into heparinized blood collection tubes (0.4−0.5 mL) via the tail vein of each animal at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after administration.

In all studies, plasma was separated by centrifugation (13000 rpm × 8 min, ~4 °C). A 200 $\mu$L aliquot of plasma from each sample (nonpooled) was transferred to 96-well polypropylene plate and stored frozen (<−15 °C) until analysis. Plasma concentrations were determined using liquid−liquid extraction to separate the Abbott Model Compound compound from plasma, followed by reverse phase HPLC with MS/MS quantitation. An aliquot of plasma (200 $\mu$L, sample or spiked standard) was combined with 25 $\mu$L of internal standard (prepared in methanol:water; 1:1 by volume) in a 96-well polypropylene plate. Following a brief mixing, *tert*-butyl methyl ether (0.9 mL) was added to each well in an automated manner (Tomtec Quadra). Following vortexing, the plate was centrifuged at 2000 rpm for 10 min (4 °C). In an automated manner (Tomtec Quadra), 0.8 mL of the organic extract was transferred to a clean 96-well plate and evaporated to dryness on a Micro-Vap under a stream of dry nitrogen with low heat (~35 °C). The samples were reconstituted by vortexing for 30 s with 200 $\mu$L of mobile phase. Samples were analyzed simultaneously with spiked plasma standards.

Parent compound and the internal standard were separated from each other and coextracted contaminants on a 50 × 3 mm 5 $\mu$m Betabasic CN column (Thermo) with an acetonitrile:0.1% trifluoroacetic acid mobile phase (60:40, by volume) at a flow rate of 0.4 mL/min. Analysis was performed on a Sciex API2000 Biomolecular Mass Analyzer with a turbo-ionspray interface. Analytes were ionized in the positive ion mode with a source temperature of approximately 400 °C. Detection was in the multiple reaction monitoring (MRM) mode. Parent and internal standard peak

***Table 3.*** Measured Physicochemical Properties of the Abbott Model Compound

| | |
|---|---|
| MW | ~450 |
| p$K_a$ | 5.3 (base) |
| log *D* (pH 7.4) | 4.6 |
| intrinsic solubility | 0.11 $\mu$g/mL |
| FaSSIF solubility (pH 6.5) | 1.6 $\mu$g/mL |
| FeSSIF solubility (pH 5.8) | 10.4 $\mu$g/mL |

areas were determined using Sciex TurboQuan software. The plasma drug concentration of each sample was calculated by least-squares linear regression analysis of the peak area ratio (Abbott Model Compound/internal standard) of the spiked plasma standards versus concentration. The limit of quantitation from a 0.2 mL plasma sample was estimated to be ~1 ng/mL.

**PBPK Modeling.** Whole body PBPK modeling was carried out on a Dell OptiPlex GX620 with 2.0 Gb of RAM and a 2.8 GHz Intel processor running Microsoft Windows XP Professional and ver. 6.1.0003 of GastroPlus (Simulations Plus, Inc., Lancaster, CA). The physical−chemical parameters of the Abbott Model Compound are listed in Table 3. In rat, the blood:plasma concentration ratio was experimentally determined to be 0.5. The fraction unbound in rat plasma was determined to be 0.4%. A particle size radius of 10 $\mu$m was used for the solid and suspension formulations; precipitant was assumed to form new particles with a radius of 1 $\mu$m. Based on the molecular weight, the diffusion coefficient was estimated by GastroPlus to be $0.6 \times 10^{-5}$ cm$^2$/s.

Tissue partition coefficients ($K_p$s) were calculated using the "Rodgers-Single" method developed by Simulations Plus, Inc., based on a combination of two original Rodgers equations.[61,62] All tissues were defined as perfusion-limited. Unbound, nonionized concentrations in extracellular, intracellular, and plasma regions was assumed to be equal.

*In vitro* metabolism and rat *in vivo* pharmacokinetic data indicated that the Abbott Model Compound was eliminated primarily by CYP3A metabolism. Hepatic clearance was estimated by scaling the rat *in vitro* hepatocyte intrinsic clearance of 3 $\mu$L/min/10$^6$ cells, assuming the fraction unbound in hepatocytes and plasma are equal, 120 million hepatocytes per gram of liver, and a liver weight of 10.3 g. As a first estimate, unbound intrinsic clearance in the intestine was assumed to be similar to that in the liver, and the regional abundance of CYP3A is 3-fold greater than in humans (eg, duodenum contains 0.4% of hepatic abundance).

The rate and extent of oral absorption of the Abbott Model Compound formulations were simulated using the fasted rat advanced compartmental absorption and transit (ACAT) model in GastroPlus. Human effective jejunal permeability ($P_{eff}$) was estimated to be $10 \times 10^{-4}$ cm/s, based on high Caco-2 permeability, which was converted to an estimated $P_{eff}$ in rat of $3.7 \times 10^{-4}$ cm/s by the built-in GastroPlus converter. The presence of solubilizers has been shown to decrease apparent permeability as a result of increased apparent solubility.[63−65] This was accounted for by adjusting $P_{eff}$ for the solubility enabling formulations. $P_{eff}$ was reduced by a factor of 2 for the solution formulation, and by a factor of 4 for the solid dispersion formulations as compared to the crystalline suspension.

***Table 4.*** Equilibrium pH-Solubility Profile of the Abbott Model Compound

| final pH | solubility ($\mu$g/mL) |
|---|---|
| 1.0 | 3352 |
| 2.2 | 236 |
| 3.7 | 7.7 |
| 4.0 | 3.7 |
| 4.6 | 1.5 |
| 5.0 | 0.47 |
| 5.4 | 0.30 |
| 5.7 | 0.16 |
| 6.8 | 0.17 |
| 7.4 | 0.12 |
| 9.2 | 0.11 |

Simulations were performed either by utilizing physicochemical-based accounting for solubility changes throughout the intestine (methods 1 and 2), or by inputting the estimated GI concentration−time profile determined from the *in vitro* pH-dilution method (method 3). *Method 1:* Simulations were run utilizing the equilibrium solubility−pH profile and the GastroPlus p$K_a$-based solubility model (Table 4). *Method 2:* In addition to inputting the equilibrium solubility−pH profile, simulations were run after utilizing the GastroPlus function that fits biorelevant solubility (FaSSIF and FeSSIF solubility) to the *in vitro* data and estimates a solubilization ratio (159 for the Abbott Model Compound) used to calculate solubility in the presence of bile salts (Table 3). *Method 3:* The estimated GI concentration−time profile was interpolated from the concentration−time values determined by the *in vitro* pH-dilution method (Figure 3). The concentration−time profiles (Figure 3) were input into GastoPlus using the experimental pH-solubility function, where pH values were

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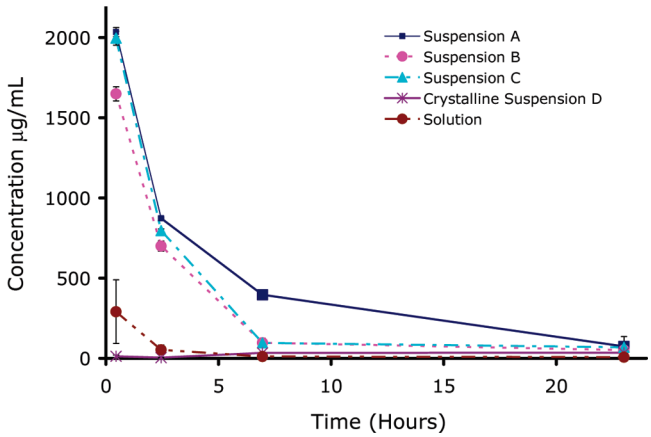


***Figure 3.*** Rat intestinal concentration−time profiles for solubility enabling formulations of the Abbott Model Compound in the *in vitro* pH-dilution model.

set to correspond to the set GI transit times for each compartment in the compartment data table.

## Results

**Physicochemical Properties of the Abbott Model Compound.** The physicochemical properties of the Abbott Model Compound are summarized in Table 3. The compound is a weak base (p$K_a$ 5.3) with molecular weight of approximately 450 Da. The compound is relatively lipophilic (log *D* 4.6) and exhibits very poor intrinsic aqueous solubility of 0.11 $\mu$g/mL.

Table 4 contains the equilibrium solubility of the Abbott Model Compound as a function of pH. As expected for a weak base, the compound shows much higher solubility at low pH and extremely poor solubility at higher pH. The p$K_a$ value estimated by fitting the pH-solubility data to well established pH-solubility relationships was found to be 5.39, which is in close agreement with the value of 5.30 measured by titration.

***In Vitro* pH-Dilution Experiments.** The concentration−time profiles ($n$ = 3) from the *in vitro* pH-dilution test of the five formulations of the Abbott Model Compound are shown in Figure 3. The rank ordering for the peak drug concentration and AUC for the formulations was A > C > B > solution > D. Figure 4 compares the AUC obtained from the *in vitro* pH-dilution concentration−time data to the AUC obtained *in vivo* in the rat plasma concentration−time data. An excellent IVIVR ($R^2$ = 0.9797) was obtained between the AUC *in vitro* and the AUC in *in vivo*.

***In Vivo* Rat PK Data and PBPK Modeling.** Figure 5 shows the simulated and observed plasma concentration−time profiles following a solution iv bolus dose (10 $\mu$mol/kg) of the Abbott Model Compound. The simulated plasma concentration−time profile after iv bolus administration of 10 $\mu$mol/kg agreed well with the observed profile (Figure 5). The mean observed clearance, CL, and steady-state volume of distribution $V_{ss}$ obtained from the iv plasma concentration−time data were 0.5 L/h/kg and 1.2 L/kg.

Figures 6−10 compare the simulated plasma concentration−time profiles using methods 1−3 to the observed

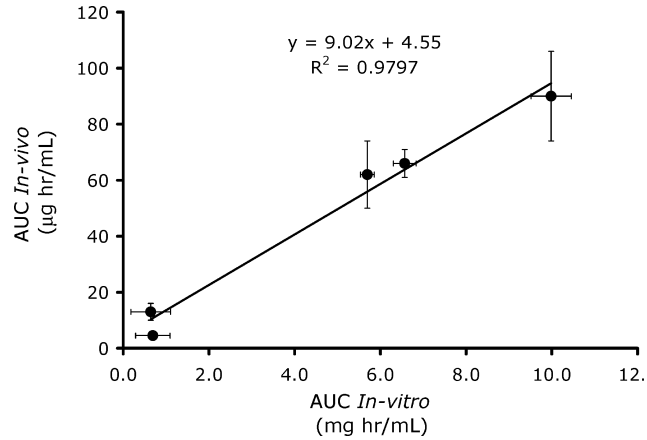


***Figure 4.*** IVIVR of AUC obtained from the *in vitro* pH-dilution concentration−time data to AUC obtained *in vivo* in the rat plasma concentration−time data.

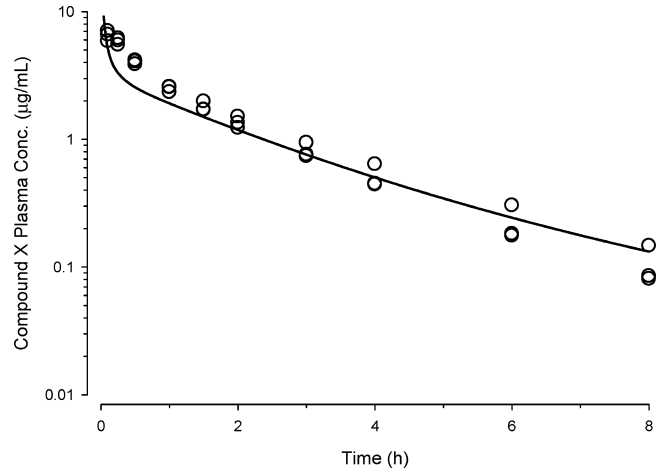


***Figure 5.*** Simulated (line) and observed (symbols, $N$ = 3) plasma concentration−time profiles following a 10 $\mu$mol/kg dose of the Abbott Model Compound administered as a solution by iv bolus.

profiles. Figure 11 shows the predicted total percent of the dose dissolved for each formulation using simulation method 2 vs method 3. Table 5 compares the fraction dose absorbed ($F_a$) and oral bioavailability ($F$) predicted using simulation methods 1−3 to the observed values. In all cases, the simulations using the *in vitro* pH-dilution data (method 3) were superior for predicting the observed plasma concentration−time profiles, % released vs time profiles, and $F_a$ as compared to the simulations using thermodynamic pH-solubility profiles (method 1) and FaSSIF/FeSSIF thermodynamic solubility (method 2).

## Discussion

The Abbott Model Compound was an early discovery-development candidate received in our laboratories for preclinical efficacy and safety evaluation. Like many drug candidates in the current pipeline of many pharmaceutical companies, the compound was relatively high molecular weight (~450 Da) and lipophilic (log *D* 4.6). As a result, the compound suffered from poor intrinsic aqueous solubility

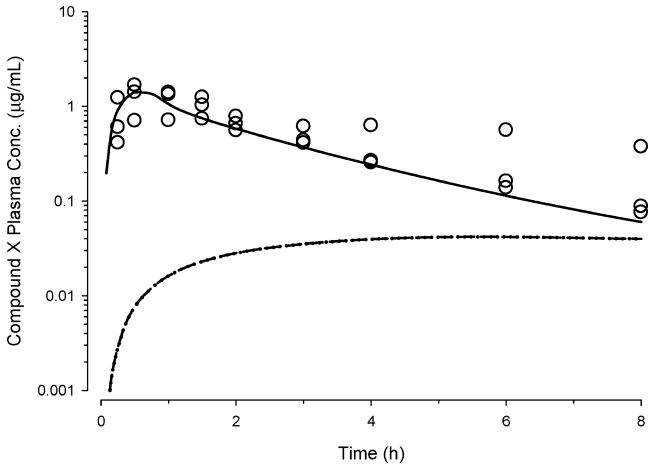


***Figure 6.*** Simulated (method 1 = dotted line; method 2 = dashed line; method 3 = solid line) and observed (symbols, $N = 3$) plasma concentration−time profiles following a 4.3 mg/kg dose of the Abbott Model Compound solution formulation administered po.

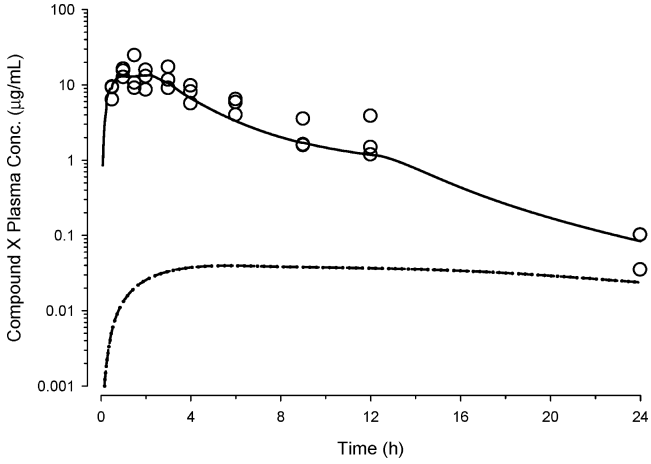


***Figure 7.*** Simulated (method 1 = dotted line; method 2 = dashed line; method 3 = solid line) and observed (symbols, $N = 3$) plasma concentration−time profiles following a 100 mg/kg dose of the Abbott Model Compound solid dispersion suspension formulation A administered po.

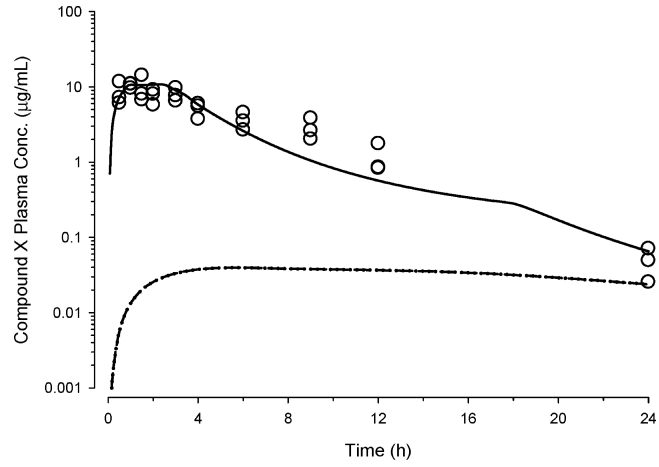


***Figure 8.*** Simulated (method 1 = dotted line; method 2 = dashed line; method 3 = solid line) and observed (symbols, $N = 3$) plasma concentration−time profiles following a 100 mg/kg dose of the Abbott Model Compound solid dispersion suspension formulation B administered po.

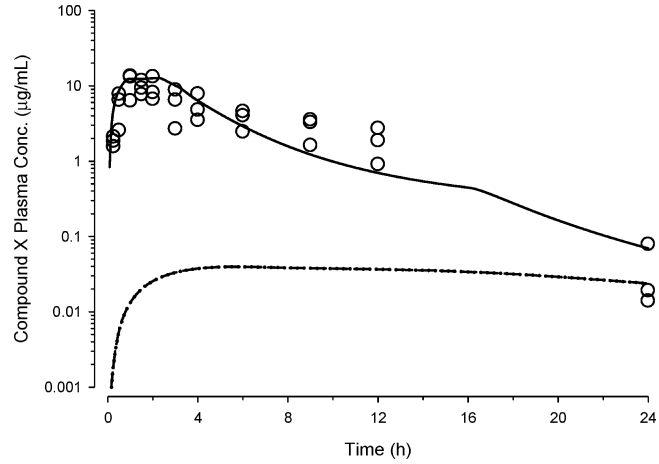


***Figure 9.*** Simulated (method 1 = dotted line; method 2 = dashed line; method 3 = solid line) and observed (symbols, $N = 3$) plasma concentration−time profiles following a 100 mg/kg dose of the Abbott Model Compound solid dispersion suspension formulation C administered po.

and oral absorption (Table 3). The compound was only weakly basic ($pK_a$ 5.3), which left limited chance for successful salt formation and/or pH-adjusted cosolvent solutions to improve aqueous solubility. Therefore, amorphous solid dispersions (Table 1) were prepared in an effort to improve the apparent solubility and *in vivo* absorption of the Abbott Model Compound.

The pH-dilution method developed here was used to estimate the regional changes in drug concentration along the GI tract from the various solubility enabling formulations (PEG-400 solution, amorphous solid dispersions) of the Abbott Model Compound. Figure 3 contains the concentration−time profiles in the pH-dilution rat model for the crystalline suspension, PEG-400 solution, and amorphous solid dispersion formulations of the Model Compound. For all of the formulations, peak drug concentration was observed at the first data point taken at 0.2 h, after the formulation went through the simulated stomach region and started to enter the jejunum region. Afterward, the drug concentration declined steadily following further dilution, pH change, and longer incubation period, albeit at different rates among the five formulations. As expected, the crystalline drug suspension D released very little drug, consistent with the low intrinsic aqueous solubility (0.11 $\mu$g/mL) and thermodynamic solubility in FaSSIF (1.6 $\mu$g/mL). The PEG-400 based oral solution formulation yielded a much lower concentration than any of the enabling amorphous solid dispersions, primarily because of the much lower dose. It is well-known that concentration in aqueous solution decreases exponentially upon dilution of a solvent based formulation into aqueous medium.[42] Most of the drug must have precipitated from

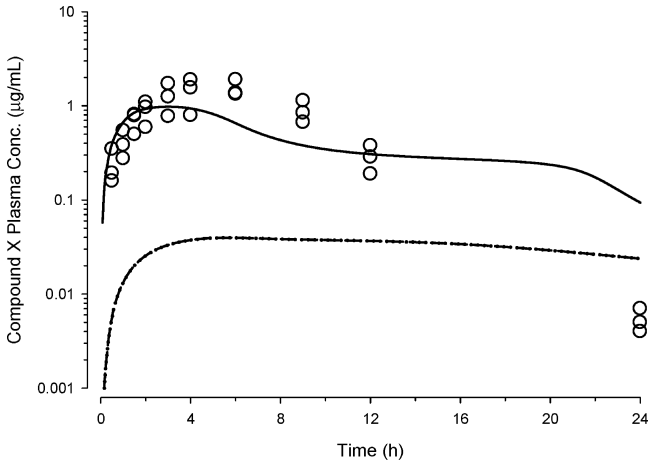


***Figure 10.*** Simulated (method 1 = dotted line; method 2 = dashed line; method 3 = solid line) and observed (symbols, $N = 3$) plasma concentration−time profiles following a 100 mg/kg dose of the Abbott Model Compound crystalline suspension formulation D administered po.

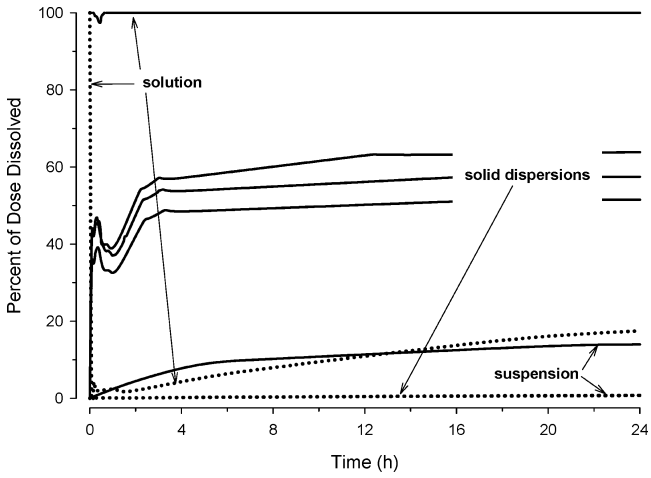


***Figure 11.*** Simulated total percent of the dose dissolved for each orally administered formulation, comparing method 2 (dotted lines) to method 3 (solid lines).

the solution formulation upon dilution in the FaSSIF in the 0.2 h incubation time simulating duodenum region. In contrast, the amorphous solid dispersion formulations were capable of reaching substantially higher concentrations than the other formulations and also far exceeded the thermodynamic solubility of the Model Compound in FaSSIF. More importantly, the high supersaturation was maintained for several hours, which results in much higher amounts of drug in solution available for absorption.

The pH-dilution test was also capable of discriminating the solubility/dissolution performance of different lots of the amorphous solid dispersion formulation that were prepared at various stages of development (Figure 4). For example, at about 7 h, amorphous solid dispersion suspension A generated a supersaturation ratio of 247, while the amorphous solid dispersion suspension B only produced a supersaturation ratio of 60. Microscopic examination of these two solid dispersion materials revealed phase separation between excipients of polymer and surfactant in the suspension B solid dispersion lot (data not shown), which may have caused a greater tendency of drug crystallization and precipitation.

The *in vitro* drug concentration−time profiles from the enabling formulations were used as solubility inputs for PBPK modeling using GastroPlus software (i.e., method 3). This method was compared to traditional methods using pH−solubility data (i.e., method 1) and FeSSIF/FaSSIF solubility data (method 2). Oral absorption was poorly simulated when equilibrium pH−solubility data (method 1) were used for the Abbott Model Compound solubility inputs (Figures 6−10). Accounting for estimated increased solubility in the presence of bile salts (method 2) resulted in negligible improvement in the predictions (Figures 6−10). Using these methods, absorption from the solubility-enabling formulations was greatly underpredicted, and there was little predicted difference in the absorption across formulations (Table 5). Alternatively, excellent correlations between the simulated and observed plasma concentration−time profiles were obtained when the *in vitro* pH-dilution data (method 3) were used as solubility inputs in GastroPlus (Figures 6−10). Moreover, the predicted values of $F_a$ and $F$ for the various formulations correlated well with the observed values (Table 5).

Compared to methods 1 and 2, the superior predictability of the method 3 simulations to the observed concentration−time profiles and $F$ can be attributed to better representation of the simulated *in vivo* concentration−time profiles of the Model Compound in the intestinal lumen (Figure 11). Thus, the dynamic aspects of the pH-dilution test provides a better representation of the transit of the drug formulation through the GI tract, which more closely captures the kinetic aspects (e.g., supersaturation) of the actual *in vivo* drug release process.

***Table 5.*** Summary of Estimated Fraction of Dose Absorbed ($F_a$) and Oral Bioavailability ($F$) *vs* Observed $F$ Following Oral Doses of the Abbott Model Compound to Rat

| | method 1 | | method 2 | | method 3 | | |
|---|---|---|---|---|---|---|---|
| formulation and dose | $F_a$ % | $F$ % | $F_a$ % | $F$ % | $F_a$ % | $F$ % | mean obsd $F$ % |
| solution 4.3 mg/kg | 17 | 9 | 18 | 10 | 99 | 39 | 48 |
| suspension A SD rotovap small scale 100 mg/kg | 1 | <1 | 1 | <1 | 63 | 34 | 41 |
| suspension B SD rotovap GLP scale 100 mg/kg | 1 | <1 | 1 | <1 | 51 | 27 | 28 |
| suspension C SD spray dried small scale 100 mg/kg | 1 | <1 | 1 | <1 | 57 | 31 | 30 |
| suspension D 100 mg/kg | 1 | <1 | 1 | <1 | 14 | 5 | 7 |

It is worth noting that similar *in vitro* pH-dilution methods can be developed to mimic other species (e.g., mouse, monkey, dog, human) and these data could be used for PBPK modeling and prediction in other species. Good agreement between predicted versus observed plasma concentration−time profiles across several preclinical species would increase confidence in the predicted human PK. This is particularly advantageous for low-solubility compounds dosed in solubility-enabling formulations, where there is often considerable uncertainty in the estimated human oral absorption and bioavailability. Additionally, the IVIVR established with these methods could be used to guide clinical formulation development, and also in setting formulation quality control specifications.

## Conclusions

This work describes an *in vitro* dual pH-dilution method to estimate the formulation dependent drug concentration in solution along the GI tract of the fasted rat. The utility of this simple dual pH-dilution method was evaluated using various solubility enabling formulations (i.e., cosolvent solution, amorphous solid dispersions) of a model early development drug candidate with poor aqueous solubility. The *in vitro* drug concentration−time profiles from the enabling formulations were used as solubility inputs for PBPK modeling using GastroPlus software. This resulted in excellent predictions of the *in vivo* oral plasma concentration−time profiles, as compared to using the traditional inputs of thermodynamic pH−solubility profiles and FaSSIF/FeSSIF solubility. In summary, this work describes a novel *in vitro* method for facile estimation of formulation dependent GI drug concentration−time profiles and demonstrates the utility of PBPK modeling for oral PK prediction of enabling formulations of poorly soluble drugs.

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