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Integration of Precipitation Kinetics From an *In Vitro*, Multicompartment Transfer System and Mechanistic Oral Absorption Modeling for Pharmacokinetic Prediction of Weakly Basic Drugs

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ABSTRACT

Solubility, dissolution, and precipitation in the gastrointestinal tract can be critical for the oral bioavailability of weakly basic drugs. To understand the dissolution and precipitation during the transfer out of the stomach into the intestine, a multicompartment transfer system was developed by modifying a conventional dissolution system. This transfer system included gastric, intestinal, sink and supersaturation, and reservoir compartments. Simulated gastric fluid and fasted state simulated intestinal fluid were used in the gastric and intestinal compartment, respectively, to mimic fasted condition. The new transfer system was evaluated based on 2 model weak bases, dipyridamole and ketoconazole. Traditional 2-stage dissolution using 250 mL of simulated gastric fluid media, followed by 250 mL of fasted state simulated intestinal fluid, was used as a reference methodology to compare dissolution and precipitation results. An *in silico* model was built using R software suite to simulate the *in vitro* time-dependent dissolution and precipitation process when formulations were tested using the transfer system. The precipitation rate estimated from the *in vitro* data was then used as the input for absorption and pharmacokinetic predictions using GastroPlus. The resultant simulated plasma concentration profiles were generally in good agreement with the observed clinical data, supporting the translatability of the transfer system *in vitro* precipitation kinetics to *in vivo*.

Introduction

Understanding the *in vitro* dissolution behavior of solid oral dosage forms provides vital information about the *in vivo* performance of the drug. Typically, for immediate-release products, the apparent dissolution rate depends on tablet disintegration, followed by granules disintegration, and finally by active pharmaceutical ingredient (API) physicochemical properties that govern the dissolution of the compound. For Biopharmaceutics Classification System[1,2] (BCS) class I and III compounds, because the solubility of these compounds is very high, tablet and granule disintegration ends up being the rate-limiting step to *in vivo* dissolution. For BCS II and IV compounds, solubility and dissolution rates mainly dictate the *in vivo* performance; this is especially true for BCS II compounds because of their high permeability. For BCS class II weak bases, under normal fasted conditions, dissolution primarily takes place in the low pH gastric fluid because of higher solubility. However, on entering the small intestine where typically pH is in the 5.5-6.5 range, the significantly lower solubility can result in precipitation. In recent years, physiologically based pharmacokinetic (PBPK) models have been widely used to predict drug absorption. However, a lack of knowledge of drug precipitation kinetics remains a significant hurdle for modelers to prospectively establish, with high degree of confidence, a PBPK model for weakly basic compounds. Hence, understanding dissolution and precipitation kinetics can be considered a prerequisite to establish a predictive PBPK model for BCS II weak bases.

Abbreviations used: ACN, acetonitrile; API, active pharmaceutical ingredient; BCS, Biopharmaceutics Classification System; FaSSIF, fasted state simulated intestine fluid; GI, gastrointestinal; PBPK, physiologically based pharmacokinetic; SGF, simulated gastric fluid; TFA, trifluoro acetic acid.

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Conventional dissolution test using United States Pharmacopeia (USP) apparatus I and II at constant pH may not provide vital information about precipitation. Two-stage dissolution using simulated gastric fluid (SGF) and fasted state simulated intestine fluid (FaSSIF) as biorelevant media[3,4] is commonly used to mimic gastrointestinal (GI) pH shift.[5] In this traditional setup, after a specific time period (typically 30 min), the media pH is changed by addition of higher pH FaSSIF media to result in the final intended FaSSIF composition. This rapid shift in pH oftentimes leads to rapid drug precipitation. However, owing to the rapid pH shift and the lack of drug removal from the system to simulated absorption, the precipitation rate may be overpredicted relative to the *in vivo* situation. To try to address the shortcomings of the traditional media shift methods, several dissolution systems have been developed, such as a biphasic system using USP IV flow through system,[6] the biorelevant gastrointestinal transfer system,[7] the artificial stomach-duodenum,[8] transfer models from Kostewicz et al.[9] and Gu et al.,[10] and the GI simulator.[11] All the transfer systems consist of a stomach and a duodenum compartment and utilize volume and pH to which more closely mimic GI environment which influence the dissolution of the drug. Most of these systems have been custom made and use a variable flow rate to more accurately simulate gastric emptying and intestinal transit kinetics.

The goal of this study was to assess the ability to obtain dissolution and precipitation rate kinetics for 2 model weak bases, dipyridamole and ketoconazole, using a multicompartment transfer system with a somewhat less complex setup that may be easier to implement with available standard dissolution equipment. The proposed transfer system was developed using conventional USP I/II dissolution baths with a simple single flow rate mechanism and using a mini paddle setup in a standard 900-mL dissolution vessel. The media volumes used for the transfer system were similar to 2-stage dissolution, but instead of employing a sudden pH change, the media was pumped at a constant rate, and in addition, the dissolved drug was removed to help maintain supersaturation in the presence of absorption. The system has total gastric transfer time of 50 min which was comparable to the physiological gastric emptying time.[12] Two hundred fifty milliliters of intestinal volume was used to enable ease of setup in a conventional bath and kept some alignment with historic 2-stage dissolution. The time course of drug amounts in the different compartments of the setup was modeled using a R program to compute the drug precipitation rate. These precipitation rates were used as the input for GastroPlus simulations to compare the simulated plasma concentration profiles with available clinical data.

Materials and Methods

Materials

Fifty milligram of dipyridamole tablets and 200 mg of ketoconazole tablets were obtained from Zydus Pharmaceuticals and Taro Pharmaceuticals, respectively. Sodium taurocholate was purchased from Spectrum Chemicals (New Brunswick, NJ), lecithin was purchased from Alfa Aesar (Tewksbury, MA), and trifluoroacetic acid was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN), potassium phosphate monobasic, sodium phosphate monobasic, sodium phosphate dibasic, trifluoro acetic acid (TFA), and o-phosphoric acid (85%) were purchased from Fisher Scientific (Waltham, MA) and were used as received.

Solubility

The apparent saturation solubility of dipyridamole and ketoconazole in SGF, pH 1.8 and FaSSIF, pH 6.5 media was determined by equilibrating a known excess of API (~150 mg for SGF and ~30 mg in FaSSIF) in 15 mL of media. One milliliter of samples were taken at 4-h and 24-h intervals and were centrifuged for 15 min at 14,000 rpm in a centrifuge tube containing a 0.45-μm polyvinylidene fluoride filter (Millipore, Burlington, MA). Subsequently, 1:1 dilutions in ACN were made for FaSSIF sample solutions. Owing to the higher solubility of dipyridamole and ketoconazole at lower pH, 1:100 dilution in water with 0.1% TFA:ACN (80:20) were made for ketoconazole SGF sample solutions and 0.2:100 dilution in water with 0.1% TFA:ACN (80:20) were made for dipyridamole SGF sample solutions. Solubilized amounts of dipyridamole and ketoconazole were determined by high-performance liquid chromatography (HPLC) analysis using the methods mentioned in the following section. Every sample was analyzed in duplicate, and the mean values are reported.

HPLC Analysis

Chromatograms were obtained from an Agilent 1200 HPLC equipped with a UV detector (254 nm for dipyridamole and ketoconazole), autosampler, and a thermostat (Agilent Technologies, Santa Clara, CA). Samples (10 μL) were eluted onto a YMC Pro Pack C18 reverse-phase column (4.6 × 50 mm, particle size, 5 μm; YMC America, Allentown, PA) at a temperature of 40°C using a gradient HPLC method. The mobile phase A consisted of water with 0.1% trifluoroacetic acid and mobile phase B consisted of ACN with 0.1% trifluoroacetic acid. The gradient was changed from 80% mobile phase A and 20% mobile phase B to 50% mobile phase A and 50% mobile phase B linearly in 5 min. The chromatographic data were collected using Empower 3 chromatography software (Waters Corporation, Milford, MA).

Two-Stage Dissolution

Dissolution testing was performed using the USP apparatus II (Distek 2100C; Distek Inc., New Brunswick, NJ) with a paddle speed of 100 rpm. A media volume of 250 mL SGF (SGF), pH 1.8 was used at a temperature of 37 ± 0.5°C for the first stage. The dissolution study was performed in SGF for 30 min and immediately after the 30-min time point, 250 mL of 2× concentrated FaSSIF, pH 6.9 was added to achieve a final volume of 500 mL of FaSSIF, pH 6.5. The diagram of 2-stage dissolution is shown in Figure 1. Samples were withdrawn at predetermined intervals and were filtered through a 1-μm glass membrane filter (Waters Corporation, Acrodisc, Part# WAT200818). Subsequent 1:1 dilutions in ACN were made for FaSSIF sample solutions immediately after sample collection, and drug concentration was determined by HPLC analysis.

Transfer System

The diagram of the transfer system is shown in Figure 2. The transfer system includes a gastric compartment, an intestinal compartment, a sink and supersaturation compartment, and a reservoir compartment. The system was developed using a conventional dissolution system (Distek 2100C; Distek Inc.). The gastric compartment consisted of a USP paddle with a paddle speed of 100 rpm to mimic stomach forces[13] and contained 250 mL of SGF, pH 1.8 as media to mimic a human stomach under fasted conditions. The intestinal compartment contained 250 mL of FaSSIF, pH 6.5 as media to mimic fasted conditions, which gradually increased to 500 mL during the run. The intestinal compartment consisted of a mini paddle with a paddle speed of 50 rpm to mimic less force in the intestine.[14] The sink and supersaturation compartment started empty. A 1-μm glass membrane filter was used to ensure removal of only dissolved drug from the intestinal compartment to the sink and supersaturation compartment. The sink and supersaturation compartment consisted of a mini paddle with a paddle speed of 50 rpm. The reservoir compartment contained FaSSIF, pH 7 media;

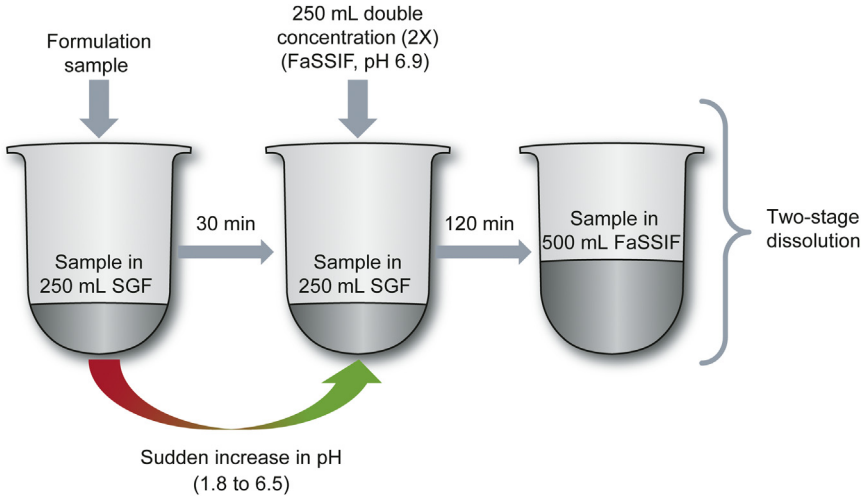

Figure 1. Diagram of 2-stage dissolution.

therefore, on addition to the intestinal compartment, it neutralized the acid from the SGF and maintained the intestinal compartment pH greater than 6. A 5 mL/min flow rate was used for all compartment transfers and was controlled by a Distek peristaltic pump. Because the aim of the study was to primarily understand dissolution and precipitation, flow was started after 15 min after addition of dosage form to allow for dissolution in SGF to be complete. Samples were withdrawn at predetermined intervals and were filtered through a 1-μm glass membrane filter. Subsequent 1:1 dilutions in ACN were made for FaSSIF sample solutions immediately after sample collection, and drug concentration was determined by HPLC analysis.

In Silico *Model to Calculate Precipitation Rate*

An *in silico* model using R software suite loaded with "deSolve"[15] and "FME"[16] program packages was developed to simulate the time-dependent dissolution and precipitation process when formulations are tested using the transfer system. The system schema is summarized in Figure 3.

The *in silico* model included the 4 compartments illustrated in Figure 3 with the volume of gastric, intestinal, and sink compartments represented by Vg, Vi, and Vs, respectively. The model further assumed first-order precipitation kinetics with a precipitation rate constant (Kp) in the intestinal compartment.[17] The liquid transfer rates (mL/min) among compartments were applied to the model based on the experimental settings, where Kgi, Kri, and Kis represented the liquid transfer rates from the gastric to the intestinal compartment, from the reservoir to the intestinal compartment, and from the intestinal to sink compartments, respectively. The change of fluid volume and drug amount in each compartment was then described by 2 sets of differential equations.

The change of fluid volume within each compartment was described using the following differential equations:

$$\frac{dVg}{dt} = -Kgi \tag{1}$$

$$\frac{dVi}{dt} = -Kis + Kri + \mathrm{Kgi} \quad (\mathrm{Kgi} = 0 \text{ if } \mathrm{Vg} = 0) \tag{2}$$

$$\frac{dVs}{dt} = Kis \tag{3}$$

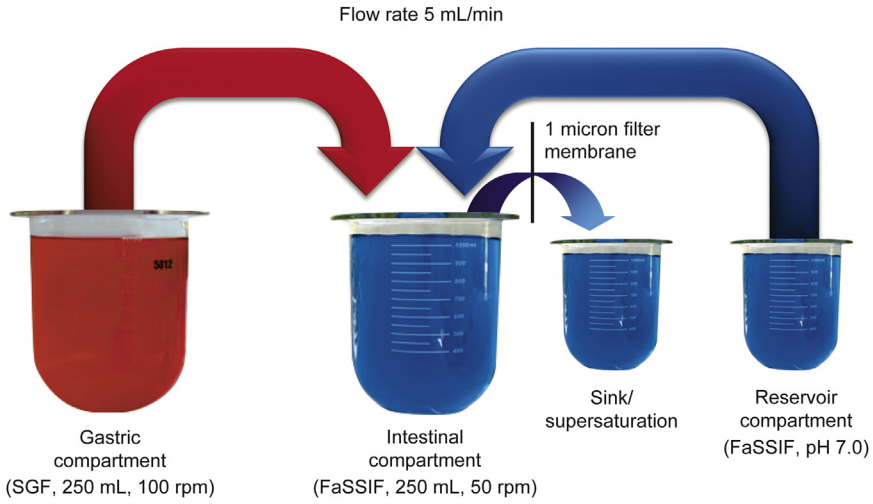

Figure 2. Diagram of the transfer system.

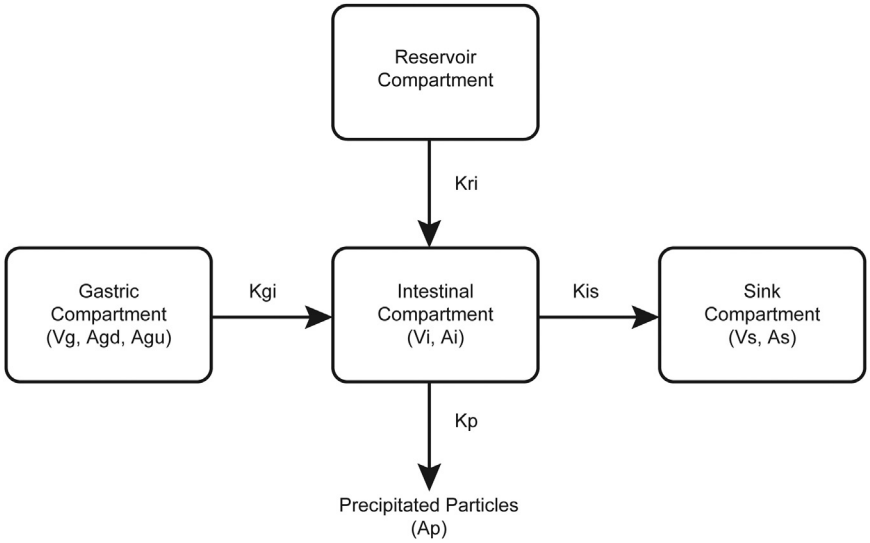

Figure 3. Schema of compartmental transfer system.

After the fluid in the gastric compartment was depleted, the volume change in the gastric compartment was set to 0, whereas the volume changes were set to 0 before the transfer valve was opened between the gastric and intestinal compartments. The dissolution rate, represented by the change of dissolved drug amount (Agd) in the gastric compartment, was calculated by a Z-factor[18] using the following equation:

$$\frac{dAgd}{dt} = z \times Dose^{1/3} \times Agu^{2/3} \times \left(SGF_{sol} - \frac{Agd}{Vg}\right) \quad (4)$$

where dose was the drug amount used in dissolution test, Agu was the undissolved drug amount in the gastric compartment, and SGF_{sol} is the drug solubility (mg/mL) in the SGF. The input rate of the dissolved drug from gastric to intestinal compartment after all valves were open in the transfer system is described in Equation 5, and the change of dissolved drug amount in the gastric compartment was described in Equation 6.

$$Input = \frac{Kgi \times Agd}{Vg} \quad (5)$$

$$\frac{dAgd}{dt} = z \times Dose^{1/3} \times Agu^{2/3} \times \left(SGF_{sol} - \frac{Agd}{Vg}\right) - \text{Input} \quad (6)$$

The differential equation to calculate the change of dissolved drug amount in intestinal compartment (Ai) depended on the difference between bulk drug concentration (equivalent to Ai divided by Vi) and solubility in FaSSIF, as described in Equations 7 and 8.

$$\frac{dAi}{dt} = -Kp \times Ai + Input - \frac{Kis \times Ai}{Vi} \ (\text{when Ai/Vi} > \text{Sol}_{\text{FaSSIF}}) \quad (7)$$

$$\frac{dAi}{dt} = Input - \frac{Kis \times Ai}{Vi} (\text{when Ai/Vi} \leq \text{Sol}_{\text{FaSSIF}}) \quad (8)$$

Equations 9 and 10 describe the mass change of drugs in the sink and in the precipitate particles, with As representing the amount of the drug in sink compartment and Ap representing the amount of the drug as precipitated particles in intestinal compartment. The current model assumed no redissolution of the precipitated particles in the intestinal compartment.

$$\frac{dAs}{dt} = \frac{Kis \times Ai}{Vi} \quad (9)$$

$$\frac{dAp}{dt} = Kp \times Ai \quad (10)$$

Numeric integration of all differential equations (Eqs. 1-10) was performed in R (version 3.4.1) using an solver for ordinary differential equations method. Estimation of precipitation rate constant (Kp) was performed by minimizing the sum of squared residuals with Nelder-Mead method. BFGS (Broyden-Fletcher-Goldfarb-Shanno) optimization methods were also explored, and the fitting results were not changed. The accuracy of model fitting was evaluated by root-mean-square error and correlation coefficient (r), which were calculated between the experimental data and model simulated results. In this work, the initial value of Vg, Vi, and Vs were 250 mL, 250 mL, and 0 mL, respectively, and all transfer rates, such as Kgi, Kri, and Kis, were set constantly at 5 mL/min. Other model-related input parameters and constants are listed in Table 1.

PBPK Modeling

GastroPlus™ v 9.6 (Simulations Plus, Lancaster, CA) was used for absorption modeling in both dipyridamole and ketoconazole case studies.

Table 1
Selected Input Parameters for Model Drugs in R and GastroPlus PBPK Model

Parameters	Dipyridamole	Ketoconazole
Dose (mg)	50	400
Formulations	IR tablet	IR tablet
Gut physiology	Fasted	Fasted
Acid/base	Base	Base
MW (g/mol)	504.6	531.4
Log D	3.95 (pH 7)	3.93 (pH 7.4)
Calculated Z-factor (mL/mg/min)	0.05	0.027
Solubility in SGF (mg/mL)	8.0	6.0
Solubility in FaSSIF (mg/mL)	0.012	0.028
Effective permeability (10^{-4} cm/s)	2.5	4.7
Diffusion coefficients (cm^2/s)	0.58	0.57
Blood-to-plasma ratio	1	0.6
Fraction unbound in plasma (f_{up})	1%	4%

Dipyridamole Physicochemical Properties

The following key compound properties were used in building the model: molecular weight 504.6, log D (pH 7) 4.0, density 1.2 g/mL, calculated human effective permeability (based on Caco-2 data) was 2.5×10^{-4} cm/s, and diffusion coefficient was calculated based on molecular weight. The solubility values were inputted as pH solubility profile, as described below: 8.0 mg/mL (pH 1.3, SGF solubility), 5 mg/mL (pH 3.0), 0.13 mg/mL (pH 5.0), 0.012 mg/mL (pH 6.0~7.0, FaSSIF data), and 0.005 mg/mL (pH 7.5). Dissolution of the formulation was described in the model using GastroPlus™ Z-factor model based on dissolution data in SGF phase in transfer system (Table 1). The precipitation time was explored in the model including the default setting of 900 s. The blood-to-plasma ratio was assumed to be 1.

Physiology

The human fasted physiological model in GastroPlus™ (Opt logD SA/v6.1) was used in these simulations.

Pharmacokinetic Parameters

Human PK parameters were estimated by fitting 20 mg intravenous bolus PK data[19] using a 3-compartment model in WinNonlin, version 5.2. The PK parameters used in these simulations were clearance (CL) = 0.104 L/h/kg, Vc = 0.0869 L/kg, $k_{12} = 2.38\ h^{-1}$, $k_{21} = 3.63\ h^{-1}$, $k_{13} = 0.50\ h^{-1}$, and $k_{31} = 0.074\ h^{-1}$. The first-pass extraction were set as 8% based on the ratio of hepatic CL and hepatic blood flow (assuming $CL_h = CL_{total}$).

Simulation

Single simulations were conducted to predict the mean PK profiles and parameters for 50 mg dose of dipyridamole IR tablets in fasted subjects.[20] Parameter sensitivity analysis was conducted to assess the impact of precipitation time on fraction dissolved or absorbed and PK. Other PBPK model-related input parameters are listed in Table 1.

Ketoconazole Physicochemical Properties

The following key compound properties were used in building the model: molecular weight 531.4, log D (pH 7.4) 3.9, density 1.2 g/mL, calculated human effective permeability (based on Caco-2 data) was 4.7×10^{-4} cm/s, and diffusion coefficient was calculated based on molecular weight. The solubility values were inputted as pH solubility profile, as described below: 6.0 mg/mL (pH 1.3, SGF solubility), 1.8 mg/mL (pH 3.0), 0.7 mg/mL (pH 3.5), 0.25 mg/mL (pH 4.5), 0.1 mg/mL (pH 5.0), and 0.028 mg/mL (pH 6.0 to 7.0, FaSSIF solubility). Dissolution of the formulation was described in the model based on dissolution data in SGF phase in the transfer system (Fig. 4). The precipitation time was explored in the model including the default setting of 900 s. The blood-to-plasma ratio was assumed to be 0.6.

Physiology

The human fasted physiological model in GastroPlus™ (Opt logD SA/v6.1) was used in these simulations.

Pharmacokinetic Parameters

Human PK parameters were estimated by simultaneous fitting of 200, 400, and 800 mg oral solution PK data[21] using a nonlinear PK model in GastroPlus PK-Plus (Simulations Plus). The nonlinear PK parameters used in these simulations were Km = 0.0585 mg/L, Vmax = 0.0194 mg/s, Vc/F = 0.33 L/kg, $k_{12} = 0.0148\ h^{-1}$, and $k_{21} = 0.0633\ h^{-1}$.

Simulation

Single simulations were conducted to predict the mean PK profiles and parameters for the 200 mg dose of ketoconazole IR tablets in fasted subjects.[22] Parameter sensitivity analysis was conducted to assess the impact of precipitation time on PK. Other PBPK model-related input parameters are listed in Table 1.

Results

Solubility

Dipyridamole and ketoconazole have pH-dependent solubility. Solubilites were determined in SGF and FaSSIF media and are summarized in Table 2.

Two-Stage Dissolution

The 2-stage dissolution data of the dipyridamole 50 mg tablets are shown in Figure 5a. Dipyridamole was able to dissolve quickly and achieved complete dissolution in the first 10 min during the acid stage. Minimal precipitation was observed after changing the pH of the media to FaSSIF, pH 6.5, and it was able to maintain supersaturation during the run time of 120 min.

Two-stage dissolution data of the ketoconazole 200 mg tablet are presented in Figure 5b. Ketoconazole was dissolved in 15 min during the acid stage of the dissolution run but a significant precipitation was observed after changing the pH of media to FaSSIF, pH 6.5 and reached a concentration similar to solubility of API in FaSSIF, pH 6.5.

Transfer System

During the transfer system run, media volume in gastric, intestinal, and sink and supersaturation compartments were changing, and volume change is shown in Figure 6. Figure 7a presents percent

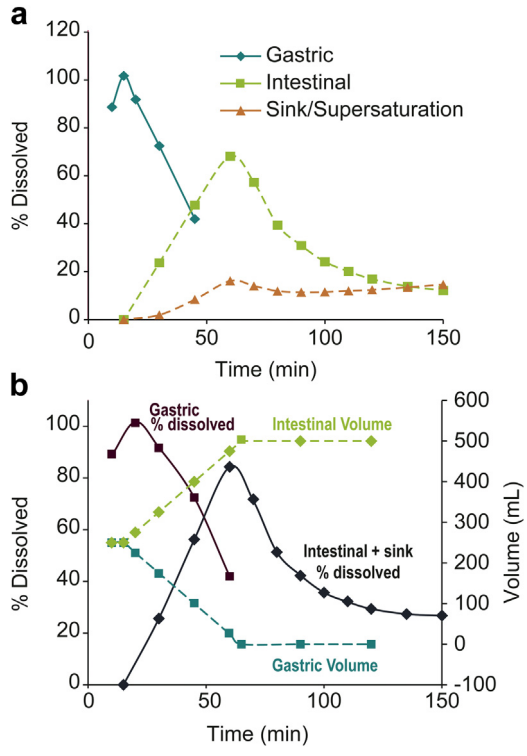

Figure 4. (a) Percent dissolved as a function of time profile of ketoconazole in a transfer system; (b) cumulative percent dissolved from intestinal and sink and supersaturation compartment as a function of time profile of ketoconazole in a transfer system.

Table 2
Solubility Summary of Dipyridamole and Ketoconazole in Dissolution Media

Dipyridamole		
Vehicle	4 h	24 h
	Solubility (mg/mL)	Solubility (mg/mL)
SGF, pH 1.8	8.0	7.8
FaSSIF, pH 6.5	0.012	0.012
Ketoconazole		
SGF, pH 1.8	6.0	6.2
FaSSIF, pH 6.5	0.028	0.025

dissolved of dipyridamole as a function of time profiles in each chamber of the transfer system experiments. Dipyridamole has very rapid onset, and within 10 min, complete dissolution was achieved in the gastric compartment before transfer started. The dissolved amount in the gastric compartment was decreased due to transfer in the intestinal compartment, and at same time, the dissolved amount in intestinal compartment was increased. At the end of the gastric transfer, the reduction in intestinal compartment amount was observed because of the removal of the dissolved drug in the sink and supersaturation compartment, which resulted in a constant increase of percent dissolved drug in the sink and supersaturation compartment. To understand total drug recovery after transfer, the dissolved amount in the intestinal and sink and supersaturation compartments were combined. A prolonged precipitation rate was observed from collective data of the intestinal and sink and supersaturation compartments. The combined data are presented in Figure 7b.

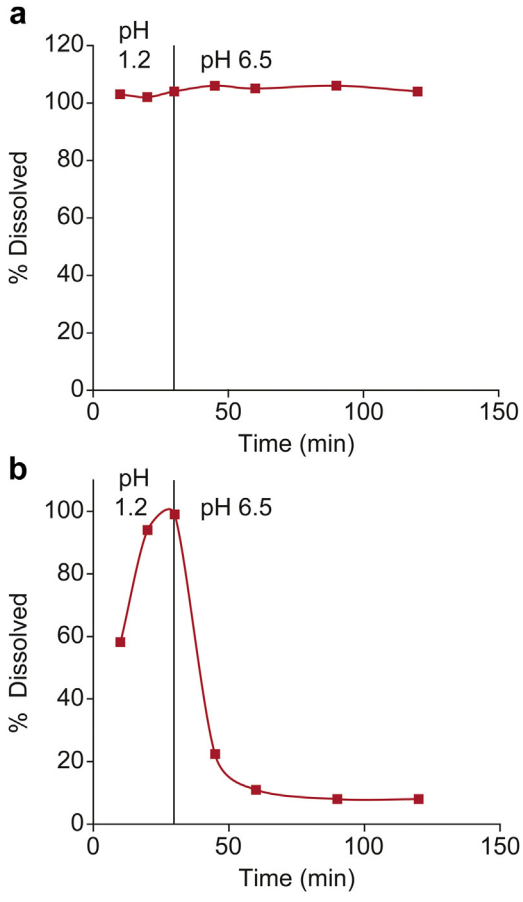

Figure 5. Two-stage dissolution profile of a (a) dipyridamole tablet and (b) ketoconazole tablet.

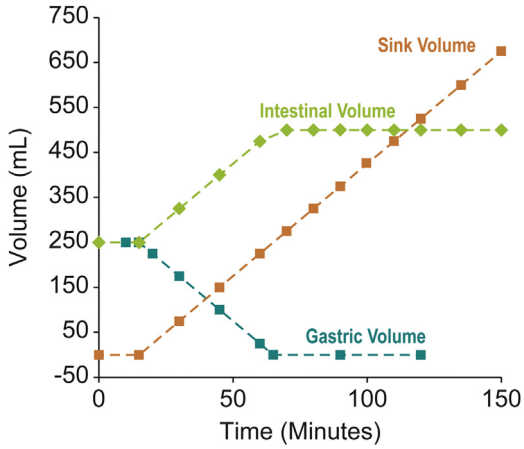

Figure 6. Change in media volume in each compartment as a function of time in transfer system.

Figure 4a presents the percent dissolved of ketoconazole as a function of time profiles in each chamber of the transfer system experiments. In the gastric compartment, ketoconazole achieved complete dissolution within 15 min before transfer. The dissolved amount in the gastric compartment was decreased due to transfer in the intestinal compartment, but at same time, the dissolved amount in the intestinal compartment was increased. At the end of gastric transfer, a reduction in the intestinal compartment was observed due to the removal of the dissolved drug from the sink and supersaturation compartment. Only dissolved drug gets transferred between the intestinal and sink and supersaturation compartments because of the filter, but after a while, visual

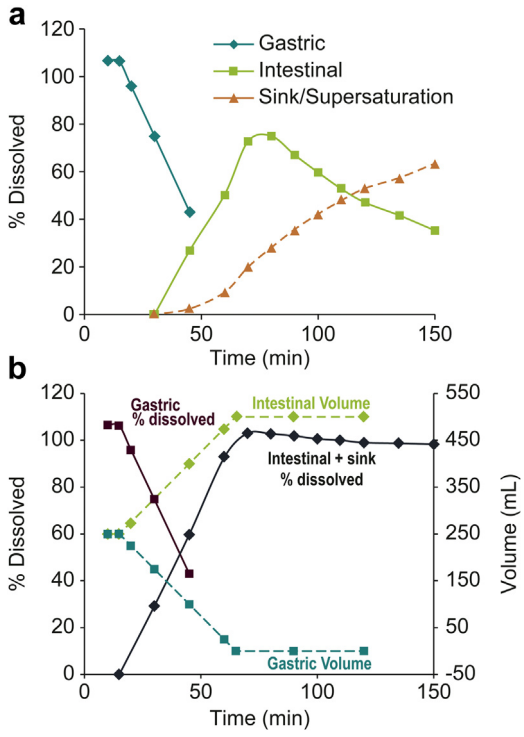

Figure 7. (a) Percent dissolved as a function of time profile of dipyridamole in transfer system (b) Cumulative percent dissolved from intestinal and sink and supersaturation compartment as a function of time profile of dipyridamole in transfer system.

precipitation was observed in the sink and supersaturation compartment, which led to a minimal increase of percent dissolved drug in the sink and supersaturation compartment. To understand total drug recovery after transfer, the dissolved amount in the intestinal and sink and supersaturation compartments was combined. About 85% of the drug was recovered collectively from the intestinal and sink and supersaturation compartments, and a slight terminal slope was observed due to precipitation in the sink and supersaturation compartment, but overall a much slower precipitation rate was observed compared with 2-stage dissolution; cumulative data are presented in Figure 4b.

In Silico *Model to Calculate Precipitation Rate*

Estimation of the precipitation rate constant using the R program was performed on the compounds. The R program accurately simulated change of fluid volume within each compartment (Fig. 6), including deletion of gastric compartment fluid and final volume of intestinal compartment (500 mL).

For dipyridamole, 50 mg API was quickly dissolved after 10 min, and the Z-factor was initially calculated as 0.05 mL/mg/min based on the dissolution data of dipyridamole (from 0 to 15 min) in the gastric compartment. Good fit was obtained for the time versus dissolved drug amount curve (Fig. 8a) in each compartment, and the correlation between experimental data and simulated results was greater than 0.9. The model predicted a low Kp value of 0.0040 h^{-1}. The Kp value can be used to calculate the mean drug precipitation time as the parameter used in GastroPlus model; we defined the mean precipitation time as the reciprocal value of Kp. Thus, the calculated precipitation time is approximately 34 h, suggesting that drug precipitation phenomenon did not occur in the small intestine.

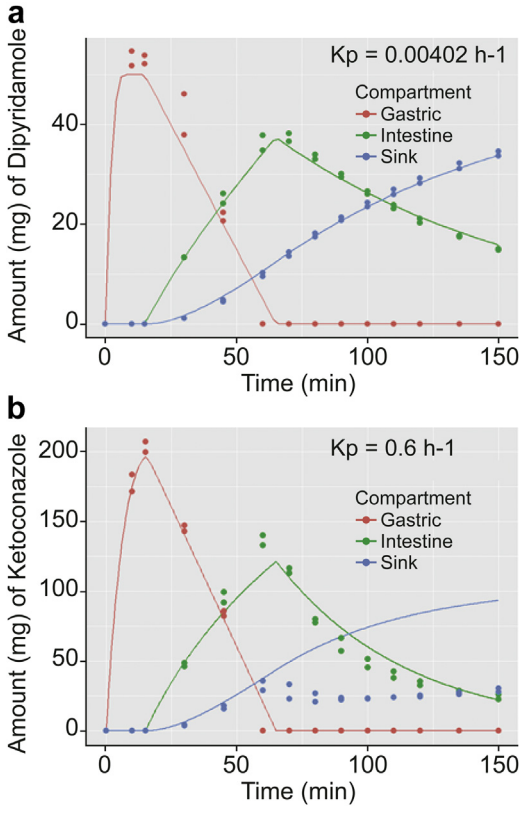

Figure 8. (a) Observed versus simulated data for dipyridamole; (b) observed versus simulated data for ketoconazole.

For ketoconazole, 200 mg of API was fully dissolved after 15 min, and the Z-factor was initially calculated as 0.027 mL/mg/min based on the dissolution data of dipyridamole (from 0 to 15 min) in the gastric compartment. Good fit was obtained for the time versus dissolved drug amount curve (Fig. 8b) in gastric and intestinal compartments. However, precipitation was visually observed in the sink compartment during the transfer system experiment at ~60 min after initiation of the experiment. Because the *in silico* R model assumed no precipitation, the dissolved drug amount was significantly overpredicted in the sink compartment. However, the precipitation of the drug in the sink compartment was not the focus of this work, and therefore, it was not necessary to adjust the model. The correlation between experimental data and simulated results was greater than 0.95. The model predicted a low Kp value of 0.60 h^{-1}, and its converted mean precipitation time was 1.66 h, suggesting that drug precipitation occurs in the small intestine, but the process is relatively slow.

GastroPlus

For dipyridamole, a prolonged precipitation time (Tprep = 120,000 s) was required to match the observed plasma concentration-time profile (Fig. 9a). The predicted Fa ~100% indicated complete absorption of the drug under this situation. It should be acknowledged that this does not represent a unique solution to the simulation, as even longer precipitation times resulted in practically similar estimation of C_{max}. Simulations with shorter precipitation times of 3600s and 900 s resulted in a decreased C_{max} of ~30% and ~60%, respectively (Fig. 9a). The time-dependent total dissolution and absorption profiles of the drug are shown in Figure 9b. Assuming a faster *in vivo* precipitation of 3600 s or 900 s in the absorption model resulted in an impaired absorption of the drug as expected in the model compared with the scenario when *in vivo* precipitation of 120,000s was assumed. Taken together, the redissolution of the drug particles in intestinal compartment partially compensated the difference on estimated area under curve (AUC) under different *in vivo* precipitation rates (based on total %absorbed of the drug in Fig. 9b) but not the C_{max}.

For ketoconazole, simulations with a Tprep = 120,000, 6000, or 900 s are shown in Figure 10. Although a simulation assuming that a prolonged *in vivo* precipitation (120,000 s) appeared to provide a slightly better fit of the AUC against observed PK data, a simulation assuming 6000 s *in vivo* precipitation time showed a somewhat better alignment with the onset of the PK profile (absorption phase) and the observed C_{max} (Fig. 10 and Table 3). It should be noted that according to the transfer system dissolution data, visual precipitation was observed in the sink and supersaturation compartment, which led to a minimal increase of percent dissolved drug in the sink and supersaturation compartment. Therefore, for PBPK modeling purposes, the particle radius for newly formed particle due to precipitation was set as 1000 μm, which allows minimal redissolution of the APIs in the gut once precipitated.

Discussion

Dipyridamole and ketoconazole solubility in acidic media SGF, pH 1.8, was measured at 8.0 mg/mL and 6.0 mg/mL, respectively, whereas solubility in neutral pH media with bile salts FaSSIF were observed to be 0.012 mg/mL and 0.028 mg/mL, respectively. The solubility study results were in good agreement with the physicochemical properties of both compounds and also well in agreement with previously published solubility data. Dipyridamole was able to maintain supersaturation in FaSSIF media after a pH change in 2-stage dissolution. A similar phenomenon was observed during the transfer system experiment and can be observed cumulatively

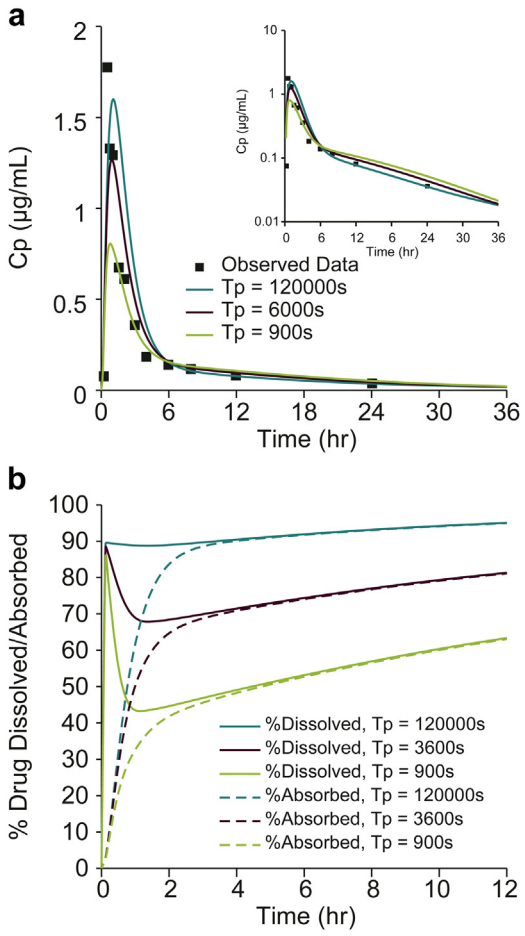

Figure 9. (a) Observed versus simulated PK for dipyridamole (50 mg fasted) with *in vivo* precipitation at 120,000, 3600, and 900 s. (b) Simulated percentage of dissolved/absorbed drug for dipyridamole (50 mg fasted) with *in vivo* precipitation at 120,000, 3600, and 900 s.

in the intestinal and sink and supersaturation compartment profiles. Dipyridamole has a concentration-dependent precipitation mechanism, and the results were well aligned with the study by Psachoulias et al[23] performed in fasted adults. In the ketoconazole case study, rapid precipitation was observed in 2-stage dissolution,

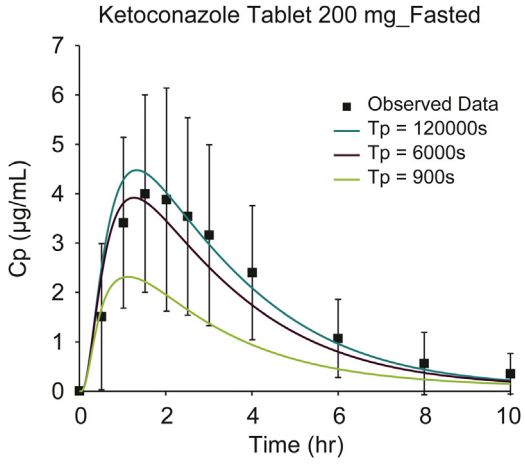

Figure 10. Observed versus simulated PK for ketoconazole (200 mg fasted) with *in vivo* precipitation assumed at 120,000, 6000, or 900 s.

Table 3
Simulated and Observed Pharmacokinetic Parameters for Ketoconazole (200 mg Fasted)

Precipitation Time (s)	PK Parameters		
	$AUC_{0\text{-}10hr}$ (µg/mL*h)	C_{max} (µg/mL)	T_{max} (h)
120,000	17.5	4.5	1.3
6000	15.0	4.0	1.3
900	8.7	2.3	1.1
PK parameters calculated by GastroPlus from the data presented by Chin et al.[22]	17.7	4.0	1.5

which can overpredict precipitation risk and project compromised *in vivo* performance. However, in the transfer system experiment, a much higher drug amount was observed in the cumulative intestinal and sink and supersaturation compartment profiles, which resulted in a maximum of 85% recovery in higher pH compartments. The overall precipitation trend is much slower compared with 2-stage dissolution, and data were very well aligned with human *in vivo* performance. The precipitation in the sink and supersaturation compartment caused a slower decline in the amount but the sink and supersaturation compartment data were not used in R program for precipitation rate calculation; hence, it did not cause any challenges. In the future work, the system can be improved by maintaining the drug in solution in the sink and supersaturation compartment. Both the results were in good agreement with those of Psachoulias et al[23] performed in fasted adults. The transfer system with similar volume to 2-stage dissolution but with gradual volume transfer in the presence of dissolved drug removal helped predict *in vivo* dissolution and precipitation. With standard media volumes, commercially available mini paddles in the intestinal and sink and supersaturation compartments and simple flow rate made a multicompartment transfer system consisting solely of standard component, which can be easily constructed in standard pharmaceutical laboratories.

To apply the transfer system for the prediction of drug precipitation process of weakly basic compounds, we have developed a novel computation model using R program to simulate the drug dissolution and precipitation kinetics for the model compounds tested in the dissolution transfer system. This model has the potential to provide a more reasonable estimation of key parameter input of precipitation time (or rate) required in physiologically based absorption models (e.g., GastroPlus). First-order kinetics were assumed in this model to quantitatively describe the precipitation in the intestinal compartment, similar to the precipitation model commonly used in the commercially available PBPK software and the Dissolution and Precipitation Model reported previously.[17] Estimation of precipitation rates from *in vitro* "transfer" system (where there is a flow between compartments) represented the most common approach for PBPK modeling (where compartments are "static") as shown with both GastroPlus and Simcyp simulations.[24-27] The model currently assumes no redissolution of precipitates and no precipitation in the sink compartment for the purpose of simplicity. For the 2 model compounds described in the article, redissolution of precipitates can be negligible for dipyridamole because no precipitation was observed in the transfer system. For ketoconazole, the precipitates were observed as visible particles in the FaSSIF medium, and it can be safely assumed that redissolution of those "large particles" will be a very slow process. The computational model also overpredicted the concentration in the sink compartment (blue curve vs. circles, Fig. 8b). In fact, visible precipitated particles were also observed in the sink compartment in the ketoconazole study, leading to decreased soluble drug concentration, which the computational model attempted to predict. It is most likely due to the supersaturated solution being initially

transferred from the intestinal compartment to the empty sink compartment and which then started to precipitate from its supersaturated status until the soluble drug concentration reached its solubility limits in the FaSSIF medium. Although prediction of drug concentration is unsuccessful for ketoconazole in the sink compartment, the system does not need to be further optimized due to its irrelevancy on the prediction precipitation time in intestinal lumen (or compartment).

To demonstrate the clinical relevance of the calculated precipitation kinetics in the transfer system, physiologically based absorption models (i.e., Advanced Compartmental and Transit Model) were developed for dipyridamole and ketoconazole in GastroPlus. The objective of this study was to describe how to use the data from the transfer system (assuming a first-order precipitation kinetics) within the most commonly used framework of oral absorption modeling (i.e., GastroPlus). Recently, an alternative model has been reported to imply the drug absorption and precipitation process as a discrete GI emptying process involving fluid pockets interrupted by variable time periods.[28] However, to the best of our knowledge, no mathematic model has been established to quantitate this proposed discretively-emptying process. Thus, our approach is currently considered as a "fit-for-purpose" and robust methodology to assess biorelevant drug precipitation behavior during formulation development in pharmaceutical industry.

For dipyridamole, an absorption model assuming a prolonged *in vivo* precipitation time (120,000 s) from the *in silico* precipitation model was able to predict the PK profile with reasonable accuracy. The sensitivity analysis against *in vivo* precipitation setting showed that a decreased C_{max} when shorter *in vivo* precipitation times of 3600 and 900 s, respectively, were assumed (Fig. 9a). The overall conclusion from the simulation of the clinical data was that dipyridamole had little tendency to precipitate *in vivo*. This is consistent with the calculated slow precipitation rate in the *in silico* model (Kp = 0.00402 h-1) as well as the observations from the study by Psachoulias et al.,[23] in which a minimal precipitation fraction ($\leq$7%) was observed in the clinic. To further illustrate the dissolution and absorption process of dipyridamole, time-dependent dissolution and absorption profiles of the drug are shown in Figure 9b under these scenarios with different *in vivo* precipitation settings. A 3600 or 900 s *in vivo* precipitation setting resulted in impaired absorption of the drug compared with the scenario when *in vivo* precipitation of 120,000 s was assumed. According to the total percentage of the drug absorbed as shown in Figure 9b, although the decrease of C_{max} reflected the evidence of *in vivo* precipitation in these models with shorter *in vivo* precipitation, the slow redissolution of the drug particles in the intestinal compartment likely compensated partly for the AUC differences in the absorption model with different precipitation settings. The slow redissolution of the precipitated particles is not unusual, as was suggested previously for other BCS IIb compounds, such as posaconazole[29] and LY2157299.[30]

For ketoconazole, an interesting observation in the transfer system was that the precipitates were observed as visible particles, which led to a minimal increase of percent dissolved drug in the sink and supersaturation compartment. Therefore, the absorption model assumed those "large particles" would undergo a very slow redissolution process once precipitated. The absorption model assuming 6000 s *in vivo* precipitation time provided better alignment with the observed onset of the PK profile and C_{max} at a 200-mg dose in fasted subjects (Fig. 10 and Table 3). A 6000 s *in vivo* precipitation is consistent with the calculated precipitation rate in the *in silico* model (K_p = 0.60 h-1). Simulations assuming a prolonged *in vivo* precipitation (120,000 s) showed a slightly better fit of AUC but somewhat overpredicted the absorption phase and C_{max}. On the other hand, the simulation with a shorter *in vivo* precipitation (900 s, GastroPlus default setting) was expected to underestimate the rate and extent of the absorption. Taken together, the exercise of transfer system–based PBPK modeling on ketoconazole is in line with prior observations made by Psachoulias et al.,[23] in which a minimal precipitation fraction ($\leq$16%) was observed in the clinic. Taken together, it appears that the absorption models using the precipitation rate inputs suggested by the transfer system were able to predict the plasma concentration profiles of both dipyridamole and ketoconazole with reasonable accuracy.

Conclusions

In conclusion, an *in silico* dissolution and precipitation model in combination with PBPK modeling based on transfer system data was successful in simulating the drug dissolution and precipitation kinetics and predict bioperformance of ketoconazole and dipyridamole. The transfer system utilizing simple flow rates, mini paddles in the intestinal and sink and supersaturation compartments, demonstrated the flexibility to use traditional dissolution bath. The dissolution data generated from the transfer system predicted *in vivo* relevant precipitation rate using R program. The precipitation rates suggested by the *in silico* dissolution and precipitation model were introduced in PBPK model (GastroPlus), and the PK profiles of dipyridamole and ketoconazole, respectively, simulated by GastroPlus model generally agreed with the observed clinical data. The data showed promising results to support this integrated *in vitro* transfer system with *in silico* models as an alternative approach to estimate *in vivo* precipitation in the intestinal compartment, which is one of the critical attributes for prediction of clinical bioperformance for weakly basic compounds. This transfer system can be further applied to supersaturating formulations of poor solubility compounds to understand their behavior during GI transit.

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Supporting information: This material is available free of charge via the Internet.

References

1. Mudie D, Amidon GL, Lennernäs 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):413-420.
2. Tsume Y, Mudie DM, Langguth P, Amidon GE, Amidon GL. The Bio-Pharmaceutics Classification system: subclasses for *in vivo* predictive dissolution (IPD) methodology and IVIVC. *Eur J Pharm Sci*. 2014;57:152-163.
3. Dressman JB, Amidon GL, Reppas C, Shah VP. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm Res*. 1998;15(1):11-22.
4. Galia E, Nicolaides E, Hörter D, Löbenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs. *Pharm Res*. 1998;15(5):698-705.
5. Mann J, Dressman J, Rosenblatt K, et al. Butler J validation of dissolution testing with biorelevant media: an OrBiTo study. *Mol Pharm*. 2017;14(12):4192-4201.
6. Xu H, Vela S, Shi Y, Marroum P, Gao P. *In vitro* characterization of ritonavir drug products and correlation to human *in vivo* performance. *Mol Pharm*. 2017;14(11):3801-3814.
7. Kourentas A, Vertzoni M, Stavrinoudakis N, et al. An *in vitro* biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: design, implementation, and evaluation. *Eur J Pharm Sci*. 2016;82:106-114.
8. Carino SR, Sperry DC, Hawley M. Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model. *J Pharm Sci*. 2006;95(1):116-125.

9. Kostewicz ES, Wunderlich M, Brauns U, Becker R, Bock T, Dressman JB. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. *J Pharm Pharmacol*. 2004;56(1):43-51.
10. Gu CH, Rao D, Gandhi RB, Jon H, Raghavan K. Using a novel multicompartment dissolution system to predict the effect of gastric pH on the oral absorption of weak bases with poor intrinsic solubility. *J Pharm Sci*. 2005;94(1):199-208.
11. Matsui K, Tsume Y, Takeuchi S, Searls A, Amidon GL. Utilization of gastrointestinal simulator, an in vivo predictive dissolution methodology, coupled with computational approach to forecast oral absorption of dipyridamole. *Mol Pharm*. 2017;14(4):1181-1189.
12. Mudie DM, Murray K, Hoad CL, et al. Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Mol Pharm*. 2014;11(9):3039-3047.
13. Kamba M, Seta Y, Kusai A, Ikeda M, Nishimura K. A unique dosage form to evaluate the mechanical destructive force in the gastrointestinal tract. *Int J Pharm*. 2000;208(1):61-70.
14. Kamba M, Seta Y, Kusai A, Nishimura K. Comparison of the mechanical destructive force in the small intestine of dog and human. *Int J Pharm*. 2002;237(1):139-149.
15. Soetaert K, Petzoldt T, Setzer RW. Solving differential equations in R: package deSolve. *J Stat Softw*. 2010;33(9):1-25.
16. Soetaert K, Petzoldt T. Inverse modelling, sensitivity and Monte Carlo analysis in R using package FME. *J Stat Softw*. 2010;33(3):28.
17. Jakubiak P, Wagner B, Grimm HP, Petrig-Schaffland J, Schuler F, Alvarez-Sánchez R. Development of a unified dissolution and precipitation model and its use for the prediction of oral drug absorption. *Mol Pharm*. 2016;13(2):586-598.
18. Takano R, Sugano K, Higashida A, et al. Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. *Pharm Res*. 2006;23(6):1144-1156.
19. Bjornsson TD, Mahony C. Clinical pharmacokinetics of dipyridamole. *Thromb Res*. 1983;29:93-104.
20. Russell TL, Berardi RR, Barnett JL, O'Sullivan TL, Wagner JG, Dressman JB. pH-related changes in the absorption of dipyridamole in the elderly. *Pharm Res*. 1994;11(1):136-143.
21. Huang YC, Colaizzi JL, Bierman RH, Woestenborghs R, Heykants J. Pharmacokinetics and dose proportionality of ketoconazole in normal volunteers. *Antimicrob Agents Chemother*. 1986;30(2):206-210.
22. Chin TW, Loeb M, Fong IW. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob Agents Chemother*. 1995;39(8):1671-1675.
23. Psachoulias D, Vertzoni M, Goumas K, et al. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. *Pharm Res*. 2011;28(12):3145-3158.
24. Hens B, Pathak SM, Mitra A, et al. In silico modeling approach for the evaluation of gastrointestinal dissolution, supersaturation, and precipitation of posaconazole. *Mol Pharm*. 2017;14(12):4321-4333.
25. Pathak SM, Ruff A, Kostewicz ES, Patel N, Turner DB, Jamei M. Model-based analysis of biopharmaceutic experiments to improve mechanistic oral absorption modeling: an integrated in vitro in vivo extrapolation perspective using ketoconazole as a model drug. *Mol Pharm*. 2017;14(12):4305-4320.
26. Kesisoglou F, Vertzoni M, Reppas C. Physiologically based absorption modeling of salts of weak bases based on data in hypochlorhydric and achlorhydric biorelevant media. *AAPS PharmSciTech*. 2018;19(7):2851-2858.
27. Kou D, Zhang C, Yiu H, et al. In vitro, in silico, and in vivo assessments of intestinal precipitation and its impact on bioavailability of a BCS class 2 basic compound. *Mol Pharm*. 2018;15(4):1607-1617.
28. Talattof A, Amidon GL. Pulse packet stochastic model for gastric emptying in the fasted state: a physiological approach. *Mol Pharm*. 2018;15(6):2107-2115.
29. Cristofoletti R, Patel N, Dressman JB. Differences in food effects for 2 weak bases with similar BCS drug-related properties: what is happening in the intestinal lumen? *J Pharm Sci*. 2016;105(9):2712-2722.
30. Bhattachar SN, Perkins EJ, Tan JS, Burns LJ. Effect of gastric pH on the pharmacokinetics of a bcs class II compound in dogs: utilization of an artificial stomach and duodenum dissolution model and gastroplus,™ simulations to predict absorption. *J Pharm Sci*. 2011;100(11):4756-4765.