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Research paper

# Precipitation in the small intestine may play a more important role in the *in vivo* performance of poorly soluble weak bases in the fasted state: Case example nelfinavir

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ABSTRACT

The aim of this study was to evaluate the utility of biorelevant dissolution tests coupled with *in silico* simulation technology to forecast *in vivo* bioperformance of poorly water-soluble bases, using nelfinavir mesylate as a model compound.

An *in silico* physiologically based pharmacokinetic (PBPK) model for poorly water-soluble, weakly basic drugs was used to generate plasma profiles of nelfinavir by coupling dissolution results and estimates of precipitation with standard gastrointestinal (GI) parameters and the disposition pharmacokinetics of nelfinavir. *In vitro* dissolution of nelfinavir mesylate film-coated tablets was measured in biorelevant and compendial media. Drug precipitation in the small intestine was estimated from crystal growth theory. GI parameters (gastric emptying rate and fluid volume) appropriate to the dosing conditions (fasting and fed states) were used in the PBPK model. The disposition parameters of nelfinavir were estimated by fitting compartmental models to the *in vivo* oral PK data. The *in vivo* performance in each prandial state was simulated with the PBPK model, and predicted values for AUC and $C_{max}$ were compared to observed values.

Dissolution results in FaSSIF-V2 and FeSSIF-V2, simulating the fasting and fed small intestinal conditions, respectively, correctly predicted that there would be a positive food effect for nelfinavir mesylate, but overestimated the food effect observed in healthy human volunteers. In order to better predict the food effect, an *in silico* PBPK simulation model using STELLA® software was evolved. Results with the model indicated that invoking drug precipitation in the small intestine is necessary to describe the *in vivo* performance of nelfinavir mesylate in the fasted state, whereas a good prediction under fed state conditions is obtained without assuming any precipitation. *In vitro–in silico–in vivo* relationships (IVISIV-R) may thus be a helpful tool in understanding the critical parameters that affect the oral absorption of poorly soluble weak bases.



## 1. Introduction

Food ingestion is known to induce various physiological changes in the GI environment. Poorly soluble compounds are especially prone to higher systemic exposure when given in the fed state, with the main effect being faster and more extensive dissolution under fed conditions, due to higher levels of native surfactant, presence of lipophilic meal components and the products of fat digestion, larger volumes of fluids available to dissolve the drug, and the longer upper GI residence time [1–3]. The *in vivo* dissolution behavior of weak bases is additionally influenced by variations in upper GI pH, making these drugs especially prone to food effects.

Different systemic exposure between the prandial states is often problematic in terms of ensuring safe and efficient medication. These problems have been addressed by the Food and Drug Administration (FDA, US), which provides guidance to pharmaceutical companies about conducting food-effect bioavailability (BA) studies for orally administered dosage forms with respect to study design, data analysis, and product labeling [4]. In some cases, oral administration of the drug with a meal may be required to attain sufficient oral bioavailability of a poorly soluble, lipophilic drug [5,6]. Nelfinavir mesylate, which is a potent and highly selective inhibitor of human immunodeficiency virus (HIV) protease, is a representative example of drugs that are recommended to be taken with food to enhance bioavailability and minimize pharmacokinetic variability. When nelfinavir mesylate 1250 mg is given to

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healthy humans with a high-fat meal, it exhibits a significant increase in AUC and $C_{max}$, with about a 5-fold advantage over fasted state administration in both parameters [7].

For the above-stated reasons, prediction of food effects for poorly soluble, lipophilic drugs would be highly advantageous during oral formulation development. The Biopharmaceutics Classification System (BCS) criteria [8] can be a trigger to initiate early assessment of food effects [2]. Animal studies, usually in dogs, are often applied to predict food effects early in oral formulation development since differences in GI physiology between dogs and humans are well characterized [9]. Although several research groups have successfully applied the dog model [10,11], it is far from a perfect surrogate to predict *in vivo* performance in humans due to limitations in terms of dosing conditions, diet, the specific GI parameters, etc. *In vitro* dissolution studies are a useful means of determining the dissolution characteristics of oral solid dosage forms and to control their quality. Biorelevant media, which can simulate the human gastric, small intestinal, and colonic fluid in each prandial state [12–15], can also be a useful tool to predict *in vivo* dissolution behavior in the GI tract for lipophilic drugs in humans [16,17]. *In vitro* biorelevant dissolution testing combined with *in silico* human PBPK modeling (IV–IS–IV) has the potential to predict food effects on the pharmacokinetics of poorly soluble compounds, as shown in our previous reports using celecoxib and aprepitant as model compounds [18,19], as well as in reports from other researchers using similar strategies [20–23].

In order to explore the IV–IS–IV approach further, nelfinavir mesylate, which is a poorly soluble weak base and exhibits a significant food effect, was chosen as a model compound. Due to its highly pH-dependent solubility [24], nelfinavir mesylate dissolves well in the stomach, whereas it is poorly soluble at the more neutral pH found in the small intestine. Nelfinavir mesylate could thus partially precipitate in the small intestinal fluid, with a negative impact on its bioperformance. Therefore, the aims of this study were to

(1) evolve the PBPK simulation model to consider the transfer of basic compounds from the stomach to the intestine, including generation of supersaturation and possible precipitation in the small intestine,
(2) forecast *in vivo* oral absorption of nelfinavir mesylate in the pre- and post-prandial states, and hence its food effect, by coupling *in vitro* biorelevant dissolution testing with PBPK modeling and
(3) determine whether biorelevant media offer an advantage over simple buffer media for predicting *in vivo* performance of weak bases.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Both nelfinavir and its methanesulfonic salt were kindly donated by F. Hoffmann-La Roche AG (Basel, Switzerland). Viracept® 250 mg film-coated tablets (lot E0028E1) were purchased commercially from German market. Long-life, heat-treated, and homogenized milk (UHT milk) containing 3.5% fat (Milfina Hochwald, Kaiserslautern, Germany) was purchased commercially. Glyceryl monooleate (GMO, Rylo M19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was kindly donated by Danisco Specialities, Brabrand, Denmark. Egg phosphatidylcholine (Lipoid E PC®, 99.1% pure, lot 108015-1/42) was kindly donated by Lipoid GmbH, Ludwigshafen, Germany. Eighty-five percentage orthophosphoric acid ($H_3PO_4$), 37% hydrochloric acid (conc. HCl), and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were obtained from Fluka Chemie AG, Buchs, Switzerland. Maleic acid (99% pure, lot 4039128) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, 97% pure, lot 2007100274) was purchased from Prodotti Chimici Alimentari SpA, Basaluzzo, Italy. Sodium hydroxide solution (0.1 N NaOH) and hydrochloric acid solution (0.1 N HCl) were purchased from VWR International GmbH (Darmstadt, Germany). Dichloromethane, acetonitrile, glacial acetic acid, sodium acetate trihydrate, sodium chloride, potassium dihydrogen phosphate triethylamine, and sodium hydroxide pellets were all of analytical grade and purchased from Merck KGaA (Darmstadt, Germany).

### 2.2. Media preparation

The compositions and the preparation procedures of the media used for dissolution tests and solubility determination have been described previously [12–14]. Fasted State Simulated Gastric Fluid (FaSSGF), Fed State Simulated Gastric Fluid (FeSSGF), and compendial Simulated Gastric Fluid without pepsin ($SGF_{sp}$) (USP 33) were used for the gastric media. For the upper small intestine, updated versions of Fasted State Simulated Intestinal Fluid (FaSSIF-V2), Fed State Simulated Intestinal Fluid (FeSSIF-V2), and compendial Simulated Intestinal Fluid without pancreatin ($SIF_{sp}$) (USP 33) were used in this study.

### 2.3. Analytical methods

#### 2.3.1. The high-performance liquid chromatography (HPLC) system

The samples obtained from solubility and dissolution testing were quantitatively analyzed for nelfinavir concentration using an isocratic HPLC system, modified and validated based on a literature method [24]. The HPLC system consisted of a pump (Merck Hitachi L7100), an autosampler (Merck Hitachi L-7200), and a UV detector (Merck Hitachi L-7400). The chromatograms were evaluated with EZChrom Elite™ Version 2.8 Software (Biochrom Ltd., Cambridge, UK). The analytical column used was Capcell Pak C18 MGII (4.6 mm-i.d. × 50 mm, 5 μm, Shiseido Co., Ltd., Japan). The mobile phase was a mixture of acetonitrile and 1% triethylamine (40:60) adjusted pH to 3 with phosphoric acid. The injection volume was 10 μL. The detection wavelength was set at UV 240 nm. The analysis was performed under ambient conditions.

### 2.4. Solubility measurements

The shake-flask method was used to determine the solubility of nelfinavir mesylate in each medium. Measurements were performed by adding an excess amount of the drug substance to a medium in a glass vial. The vial was incubated in a water bath at 37 °C and shaken vigorously at appropriate intervals. Samples were taken after at least 6 h and filtered through PVDF membrane filters having a pore size of 0.45 μm (25 mm GD/X, Whatman GmbH, Dassel, Germany). The filtrate was diluted immediately with the mobile phase and then analyzed by HPLC. For FeSSGF, with milk as a major component, the procedure described in a previous report was applied [18].

### 2.5. Dissolution testing of nelfinavir mesylate film-coated tablets

#### 2.5.1. Dissolution testing

A scaled-down apparatus (the mini-paddle assembly produced by Erweka GmbH, Heusenstamm, Germany) was used to measure the dissolution profile as well as the initial dissolution rate. This apparatus is based upon the USP paddle setup but scaled down geometrically with respect to all dimensions, so that hydrodynamics remain essentially similar at a given paddle rotation speed [25]. The dissolution conditions consisted of a medium volume of

250 mL per vessel with a paddle revolution speed of 50 rpm and a temperature of 37 °C ± 0.5 °C.

Because of its high solubility under acidic conditions, dissolution studies of nelfinavir under simulated fasted gastric state conditions were conducted in two different ways: in one case, one 250 mg tablet was used per vessel, while in the other case, five tablets were run in each vessel (since the dose in the clinical study was 5 × 250 mg tablets = 1250 mg). In the media simulating the fed state in the stomach or the fasted and fed states in the intestine, the solubility of nelfinavir is much lower and results were obtained with one tablet per vessel.

Experiments were conducted in triplicate. The volume withdrawn was approximately 5 mL for each sampling time point. The samples were filtered immediately through a 0.45-μm PVDF filter (25 mm GD/X, Whatman GmbH, Dassel, Germany), and the filtrate was assayed by HPLC. FeSSGF sample analysis was performed with the same procedure as for the solubility measurements.

### 2.6. Available pharmacokinetic data

The plasma drug concentration–time profiles after oral administration of 1250 mg nelfinavir (five 250 mg tablets) were taken from the literature [7]. In this clinical study, healthy male subjects, whose mean age was 26.0 years (range 18–41 years) and mean weight was 72.1 kg (range 55–96 kg), were administered 1250 mg nelfinavir under fasting conditions ($n$ = 50 completed this phase) or after ingestion of a standard breakfast (approximately 820 kcal, protein: 110 kcal, carbohydrates: 310 kcal, fat: 400 kcal) within 10 min prior to dosing ($n$ = 52 completed this phase), in a single dose randomized crossover study design. Each dose was administered with 200 mL of water. Plasma drug concentrations were estimated directly from the profiles. Pharmacokinetic analysis was performed using WinNonlin® Professional Edition 4.1 software (Pharsight Corporation, Mountain View, CA, USA). Since no appropriate intravenous administration data were available, post-absorptive disposition parameters needed for the simulations were estimated by fitting *in vivo* mean plasma concentration–time curves after oral administration in the fed state, based on an oral one-compartment model (WinNonlin® Model 4): These included the volume of distribution corrected for the fraction absorbed, V/F (211.9 L), and the elimination rate constant $K_{10}$ (0.163 $h^{-1}$). These parameters were used for the simulations in both the fed and fasted states as there is no reason to suspect that the disposition kinetics are altered by meal intake. The amount of drug entering the plasma as nelfinavir was translated into the plasma concentration using V/F. Although nelfinavir is known to be metabolized in liver by cytochrome P450 enzymes (e.g., 3A4, 2C9, 2C19, and 2D6), the V/F parameter is based on the levels of nelfinavir and not its metabolites, circumventing the need to have a separate parameter for the first-pass metabolism in these simulations. Both observed and simulated plasma nelfinavir concentration vs. time profiles were evaluated to determine the following pharmacokinetic parameters based on non-compartmental analysis: area under the plasma concentration–time curve (AUC), the peak plasma concentration ($C_{max}$), and the time to reach $C_{max}$ ($T_{max}$).

### 2.7. PBPK-based simulation of plasma nelfinavir profiles

The plasma nelfinavir concentration–time profiles were simulated using the STELLA® 9.0 software (Cognitus Ltd., North Yorkshire, UK). The basic setup is the same as the one previously used for celecoxib and aprepitant [18,19], the solubilities of which are independent of pH. The model was modified only to provide the possibility of supersaturation with subsequent precipitation in the small intestine. For poorly soluble weak bases, drug precipitation is more likely to occur due to the solubility gap between the stomach and the small intestine. Fig. 1 shows the structure of the modified model. As stated previously, the model assumes negligible absorption from the stomach and simultaneous solid and liquid emptying from the stomach.

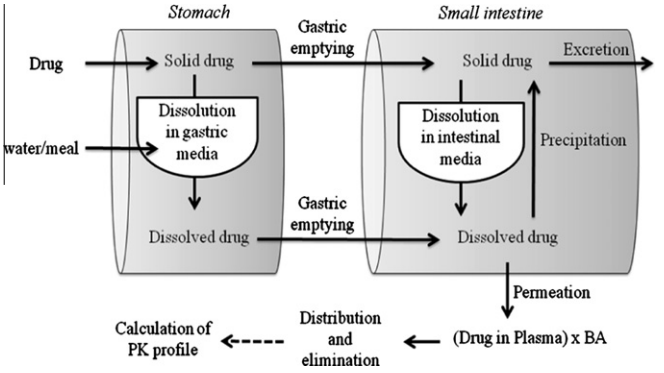


**Fig. 1.** Model structure used to simulate the pharmacokinetic profile of nelfinavir.

#### 2.7.1. Standard gastrointestinal parameters

First-order gastric emptying rate in the fasted state at 2.8 $h^{-1}$ and calorie-controlled zero-order gastric emptying rate in the fed state at 4 kcal/min were used in accordance with the average population values [26]. Basal intestinal fluid volume in the fasted and fed states was assumed to be 100 mL and 250 mL, respectively. Co-administered water (200 mL) and/or meal volume (assuming 500 mL) was modeled to leave the stomach and enter the small intestinal compartment in accordance with gastric emptying. The intestinal transit time is known to be similar in both prandial states, approximately 3–5 h [27]. Hence, the dissolution process in the small intestine was terminated at 4 h to coincide with the average time at which nelfinavir would reach the end of the small intestine after administration in the fasted state. In the fed state, due to the longer gastric residence, the dissolution process in the small intestine was terminated at 6 h after administration. These values assume that dissolution and absorption in the colon are negligible for nelfinavir.

#### 2.7.2. In vivo dissolution process

To estimate *in vivo* dissolution in the PBPK model, *in vitro* dissolution kinetics based on the modification of Noyes–Whitney theory [28] were incorporated. The Noyes–Whitney equation is based on the assumption of dissolution of isometric, similarly sized particles, occurring under continuously decreasing surface area conditions. The ratio $D/\delta$, where $D$ is the diffusion coefficient and $\delta$ is the diffusion layer thickness, is assumed to remain constant during the dissolution process. When these conditions apply, the dissolution rate is given by the following equation:

$$\frac{dW_t}{dt} = \frac{D\Gamma N^{1/3}}{V\delta\rho^{2/3}} W^{2/3}(X_s - W_t) = zW^{2/3}(C_s - C) \quad (1)$$

where $W_t$ is the amount dissolved at time $t$, $W$ is the amount of drug remaining to be dissolved, $X_s$ is the amount of drug that saturates the volume $V$ of the dissolution medium, $C_s$ is the solubility of drug, $C$ is the concentration of the dissolved drug at time $t$, $\rho$ is the particle density, $\Gamma$ is the shape factor related area to volume of the particles, $N$ is the number of particles to be dissolved, and $z$ is the dissolution parameter, which is a constant equal to $D\Gamma N^{1/3}/\delta\rho^{2/3}$. Where the administered dose *in vivo* (1250 mg) differed from that tested *in vitro* (250 mg), $z_{vitro}$ differed from $z_{vivo}$ since $z$ depends on $N$ (Eq. (1)). In these cases, $z_{vivo}$ was estimated according to the following equation:

$$\frac{z_{vitro}}{z_{vivo}} = \frac{N_{vitro}^{1/3}}{N_{vivo}^{1/3}} = \frac{N_{vitro}^{1/3}}{(aN)_{vivo}^{1/3}} = \frac{1}{a^{1/3}} \quad (2)$$

where $a$ is the ratio of $Amount_{vivo}$ to $Amount_{vitro}$.

For FeSSGF, a $z$ value could not be obtained from *in vitro* dissolution profile because the dissolution profile followed first-order kinetics rather than the Noyes–Whitney model. In this case, the cumulative amount dissolved is given by the following equation:

$$W_t = W_{\max}(1 - e^{-kt}) \quad (3)$$

where $W_t$ and $W_{\max}$ are the amount dissolved at time $t$ and the maximum amount dissolved, respectively. The first-order rate constant $k$ was estimated by fitting with SigmaPlot® for windows version 10.0.

#### 2.7.3. Precipitation process in the small intestine

Crystal growth theory was used to estimate the kinetics of drug precipitation in the small intestine [29]. The rate of drug precipitation is given by the following equation:

$$\frac{dP_t}{dt} = k_{cg}(C - C_s) \cdot 4\pi r_t^2 \cdot N \quad (4)$$

where $P_t$ is the amount of drug crystallized at time $t$, $k_{cg}$ is the rate constant of crystal growth, $r_t$ is the mean radius of the particles at time $t$, and $N$ is the number of particles remaining to be dissolved. This equation assumes neither agglomeration nor fracture of the particles during crystal growth. The increase in the particle size with time is given by the following equation:

$$\frac{dr_t}{dt} = \frac{k_{cg}}{\rho}(C - C_s) \quad (5)$$

#### 2.7.4. Intestinal absorption process

In our previous work [19], the permeation rate was estimated from a comparison of Caco-2 permeability with substances of known fraction absorbed and no solubility restrictions to absorption, i.e., metoprolol (high permeability reference) and mannitol (low permeability reference). In this case, although Caco-2 data for nelfinavir mesylate ($P_{app}$ value of $3.4 \times 10^{-6}$ cm/s [30]) were available in the literature, no accompanying permeability data were given for reference compounds. As an alternative, the equation for diffusion through the unstirred water layer (UWL) that Takano et al. successfully applied to predict intestinal absorption of diverse lipophilic compounds [21,31] was used. According to Takano, intestinal permeation rate is given by the following equation:

$$\frac{dA_t}{dt} = P_{UWL} \times S \times \frac{dX_{d_t}}{V_{available}} \quad (6)$$

where $A_t$ is the amount of drug absorbed at time $t$, $P_{UWL}$ is the permeability through the UWL, $S$ is the effective surface area of the small intestine, $V_{available}$ is the available fluid volume (fluid volume arriving from the stomach to the small intestine at time plus volume of basal small intestinal fluid), and $X_d$ is the dissolved amount of the drug in the small intestine at time $t$. $P_{UWL}$ was estimated by the equation proposed by Takano et al. based on the molecular weight of the drug [21]. $S$ is estimated from the equation:

$$S = \frac{S_{whole}}{V_{whole}} \times V_{available} \quad (7)$$

where $S_{whole}$ and $V_{whole}$ are the whole effective surface area and whole volume of the small intestine, respectively, using 2.25 for the ratio of $S_{whole}/V_{whole}$ calculated in previous work [19].

To assess the impact of the permeability parameter on the simulation, a sensitivity analysis was run on this parameter.

## 3. Results and discussion

### 3.1. Solubility of nelfinavir mesylate in biorelevant and compendial media

Solubility of nelfinavir mesylate, as well as data for dissolution of its film-coated tablets, in biorelevant and compendial media at 37 °C is shown in Table 1. Nelfinavir mesylate is not only poorly soluble but also exhibits strongly pH-dependent solubility. With a basic p*K*a at 6.0 [24], the solubility of nelfinavir mesylate increases dramatically under acidic conditions. Solubility of nelfinavir mesylate in the fasted gastric simulating media, FaSSGF (pH 1.6) and also $SGF_{sp}$ (pH 1.2), was much higher than that in media of about neutral pH, even in the presence of solubilizers such as bile salts. Solubility in FeSSGF was equivalent to FeSSIF-V2, indicating a possible incorporation of the drug into casein micelles in the milk. Both FaSSIF-V2 and FeSSIF-V2 enhanced drug solubility, depending on the level of mixed micelle in the medium.

### 3.2. Dissolution of nelfinavir mesylate in biorelevant and compendial media

As shown in Fig. 2a, drug release from five tablets, representing the 1250 mg clinical dose, approached a plateau level within 30 min, achieving an average dissolution of 68.4% and 57.6% in FaSSGF and $SGF_{sp}$, respectively. Thus, nelfinavir mesylate is expected to dissolve quickly, if not completely, in the fasting stomach. Note that the dissolution from five tablets generated a profile with quicker, but less extensive dissolution than the dissolution from one tablet. As shown in Fig. 2b, dissolution of nelfinavir under fasted-state small intestinal conditions was well under 5%, reflecting its poor solubility in this medium. Fed-state dissolution curves (in FeSSGF and FeSSIF-V2) lay between the profiles obtained under fasting conditions in the stomach and in the small intestine and were similar to each other.

In formulation development, dissolution profiles are often compared between FaSSIF and FeSSIF to obtain a rough idea of the expected food effect. In the case of nelfinavir, dissolution profiles in FeSSIF-V2 were significantly higher than in FaSSIF-V2, anticipating a positive food effect *in vivo*. However, the ratio of the extent of *in vitro* dissolution (FeSSIF-V2/FaSSIF-V2) is about 11-fold, which is an overestimate of the observed *in vivo* food effect (about a 5-fold increase in the systemic exposure after a high-fat meal ingestion, compared to the fasted state). A plausible reason for this apparent discrepancy lies in the dissolution characteristics under gastric conditions. For nelfinavir (and other weak bases), the dissolution in the fasting stomach is much higher than that in the fasting intestinal media. As a result of its high solubility in the fasted stomach, supersaturation followed by drug precipitation could be a further important event with respect to predicting nelfinavir bioavailability. As shown in Table 1, the solubility difference between

**Table 1**
Summary of solubility and $z$ values of nelfinavir mesylate in biorelevant and compendial media at 37 °C.

| | Medium (pH) | Solubility (mg/mL) | $z$ Value[a] (mL/mg$^{2/3}$/h) |
|---|---|---|---|
| $SGF_{sp}$ | (pH 1.2) | 2.163 ± 0.084 | 0.1015 ± 0.0067 |
| FaSSGF | (pH 1.6) | 3.070 ± 0.062 | 0.0732 ± 0.0050 |
| FeSSGF | (pH 5.0) | 0.353 ± 0.006 | 1.564 ± 0.036[b] |
| $SIF_{sp}$ | (pH 6.8) | 0.003 ± 0.006 | 0.2877 ± 0.1009 |
| FaSSIF-V2 | (pH 6.5) | 0.022 ± 0.002 | 0.1661 ± 0.0273 |
| FeSSIF-V2 | (pH 5.8) | 0.243 ± 0.012 | 0.1397 ± 0.0013 |

[a] Value corrected for *in vivo* dose (1250 mg) according to Eq. (2).
[b] This is a first-order rate constant, not a $z$ value, since the first-order model was more appropriate for dissolution in FeSSGF.

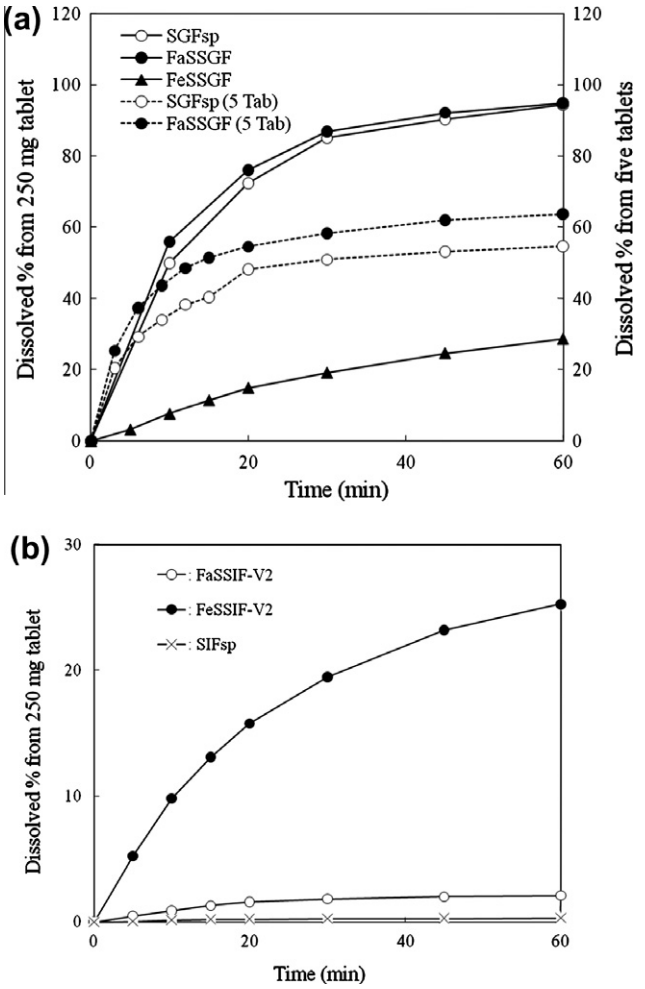


**Fig. 2.** Dissolution of nelfinavir mesylate tablets in the gastric and small intestinal media: (a) the gastric media using either one or five tablets and (b) the small intestinal media using one tablet per vessel.

fasted-state conditions in the stomach and small intestine is about two orders of magnitude; these large differences are anticipated to predispose nelfinavir to precipitation in the fasted small intestine. On the other hand, neither supersaturation nor drug precipitation is expected to occur in the small intestine in the fed state since dissolution in FeSSGF and FeSSIF-V2 is comparable. In summary, from the dissolution and solubility results alone, it is difficult to weigh the contributions from events in the stomach and small intestine accurately; this is the rationale for coupling the results with a PBPK model.

### 3.3. Simulation of the plasma profiles using STELLA® software

In order to predict the food effect for nelfinavir mesylate more quantitatively, *in silico* PBPK simulation technology was invoked and combined with results of *in vitro* dissolution in both biorelevant gastric and small intestinal media. The *z* value, a dissolution parameter necessary to run the simulation model, was estimated by fitting the curve based upon the Noyes–Whitney theory to *in vitro* dissolution profile; the values for nelfinavir are shown in Table 1. For FeSSGF, since a good fit could not be obtained from the Noyes–Whitney theory, the first-order rate constant instead of a *z* value was used for the PK profile simulation.

As a first step, the previously published model [19] was applied, and the results are shown in Fig. 3.

Simulated nelfinavir profiles in the fed state predicted the *in vivo*-observed data well. Point estimate ratios of pharmacokinetic parameters generated from the simulated profile compared to the observed values met the preliminary bioequivalence criteria of 0.8–1.25 for both AUC and $C_{max}$ as summarized pharmacokinetic parameters in Table 2. Therefore, there appears to be no reason to consider precipitation processes for the drug transferring from the stomach into the small intestine in the fed state, as was also anticipated directly from the *in vitro* biorelevant dissolution results.

"Best" and "worst" case simulations were performed for the fasted state. In the best case, drug dissolution in the stomach was taken into consideration, but no drug precipitation was assumed to occur in the small intestine, with the result that any drug dissolving in the stomach contributes to intestinal absorption. The worst case simulation, by contrast, assumes that no drug dissolution occurs in the stomach, an assumption that corresponds to complete precipitation of drug upon reaching the small intestinal compartment. Since neither simulated profile (see Fig. 3) was close to the *in vivo* nelfinavir plasma profile, it was hypothesized that both transient supersaturation and ensuing precipitation need to be considered in order to simulate the *in vivo* performance.

In general, crystallization proceeds first by triggering nucleation, which is then followed by crystal growth. The rate of crystallization is known to be affected by many factors, such as temperature, solvent, co-existent impurities, agitation speed, presence of seed crystals, particle size of the crystal, crystal lattice, and aggregation. Although it is obviously difficult to estimate the precipitation rate *in vivo* because of the heterogeneous conditions in the GI tract, the precipitation process may be crucial to predict *in vivo* performance of poorly soluble bases. In their work on the precipitation behavior in biorelevant media of three diverse weakly bases using the transfer model, Kostewicz et al. found that a faster transfer rate led to a faster precipitation rate, but the maximum concentration did not increase proportionally [32]. As the drugs in those studies were fully dissolved in the gastric compartment and thus no seed crystals were present, the authors deemed it inappropriate to apply crystal growth theory. As an alternative, Sugano showed that classical nucleation theory could explain the precipitation the Kostewicz results [33]. In case of nelfinavir mesylate, by contrast, undissolved drug ingredient would be present and provide seed crystals in the proximal small intestine (drug dissolution in the fasting stomach is predicted to be incomplete at the 1250 mg dose, see Fig. 2). Therefore, it appears to be appropriate to

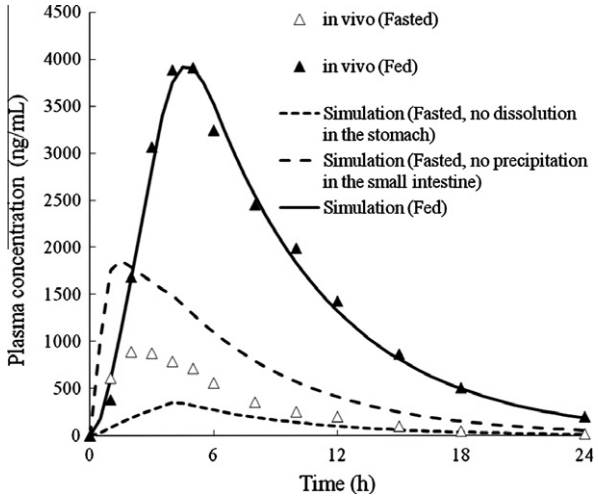


**Fig. 3.** Simulated profiles of orally administered nelfinavir 1250 mg in the fasted and fed states using the previously published model structure (which did not address precipitation in small intestine).

**Table 2**
Pharmacokinetic parameters estimated from simulated plasma profiles vs. mean observed data.

| | $T_{max}$ (h) | $C_{max}$ (ng/mL) | $AUC_{0-inf}$ (h ng/mL) | Predicted/observed | | | Fed/fasted | |
|---|---|---|---|---|---|---|---|---|
| | | | | $T_{max}$ | $C_{max}$ | $AUC_{0-inf}$ | $C_{max}$ | $AUC_{0-inf}$ |
| *Fasted* | | | | | | | | |
| Observed[a] | 2.0 | 895 | 7076 | | | | 4.4 | 5.1 |
| Predicted[b] | 2.0 | 837 | 7914 | 1.00 | 0.94 | 1.12 | 4.7 | 4.4 |
| (a) "Worst" case[c] | 4.0 | 351 | 2929 | 2.00 | 0.39 | 0.41 | 11.2 | 12.0 |
| (b) "Best" case[d] | 1.5 | 1857 | 14,942 | 0.75 | 2.07 | 2.11 | 2.1 | 2.3 |
| *Fed* | | | | | | | | |
| Observed[a] | 5.0 | 3912 | 35,808 | | | | | |
| Predicted[b] | 4.5 | 3919 | 35,012 | 0.90 | 1.00 | 0.98 | | |

[a] Each value estimated from *in vivo* data taken from the literature (using WinNonlin).
[b] These parameters were predicted from the model which took precipitation in the fasted state, but not in the fed state, in the small intestine into account.
[c] "Worst" case predictions with the previous model assumed that there is no drug dissolution in the stomach.
[d] "Best" case predictions with the previous model assumed that there is no drug precipitation in the small intestine.

apply crystal growth theory as the driving force for precipitation of nelfinavir in the small intestine.

Fig. 4a compares the simulated plasma profiles with the amount of dissolved drug in the small intestinal compartment at time *t*, under application of different precipitation rates in the model. The maximum dissolved amount existing in the small intestine ($C_{max(SI)}$), shown in Fig. 4b, decreases proportionally with precipitation rate. The mean precipitation time ($T_{max(SI)}$) was within the range from 900 to 1260 s, in agreement with values reported by Kostewicz et al. [32]. The simulations suggest that supersaturation followed by drug precipitation may play an important role in *in vivo* bioperformance of nelfinavir in the fasted state and that slight variations in the crystal growth rate could have a big impact on the plasma profile. Additionally, the

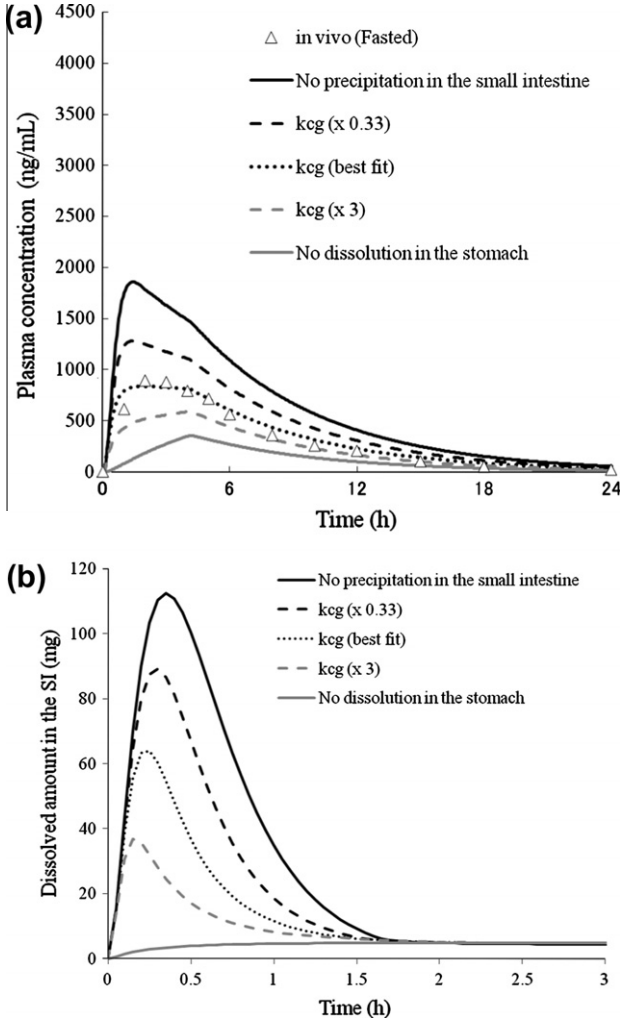


**Fig. 4.** Simulated plasma profiles and dissolved amount in the small intestine vs. time profiles generated from various precipitation rates: (a) simulated plasma profiles and (b) corresponding amounts dissolved in the small intestine as a function of time. $k_{cg}$ is the rate constant for crystal growth and was varied by a factor of three from the value giving the best fit.

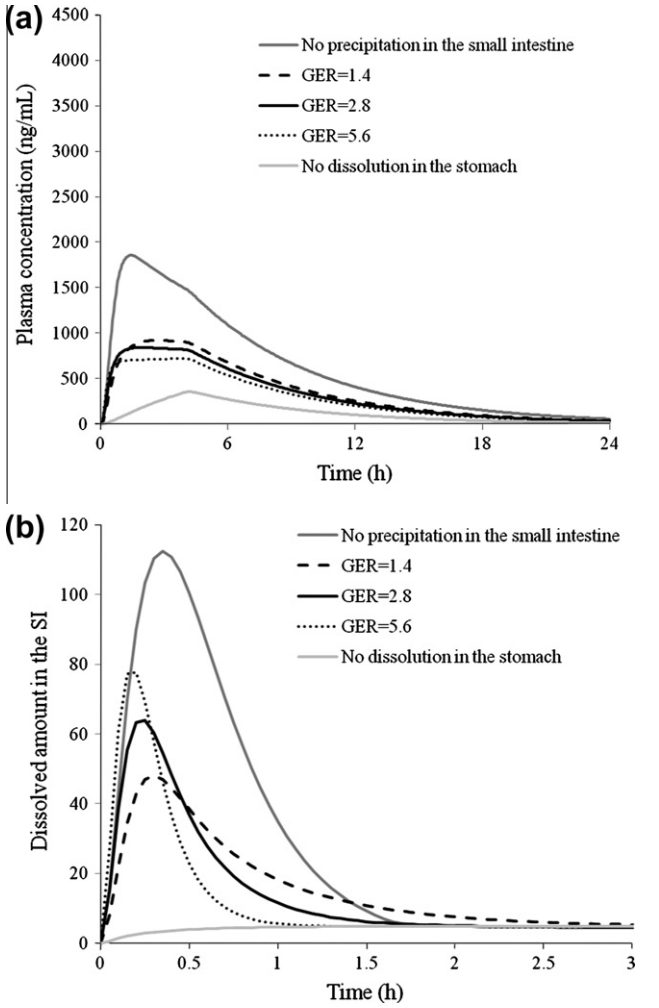


**Fig. 5.** Simulated plasma profiles generated with various gastric emptying rates: (a) simulated plasma profiles and (b) corresponding profiles of amount dissolved in the small intestine. The best and worst case profiles (no precipitation upon entry into the small intestine and no dissolution in the stomach) are also depicted.

**Table 3**
Summary of sensitivity analysis for the variable parameters used in the Model.

| Parameter | Fasted state | | Fed state | |
|---|---|---|---|---|
| | Range of values tested | Impact[a] | Range of values tested | Impact[a] |
| Gastric emptying rate | 1.4–5.6 $h^{-1}$ | None | 3–5 kcal/min | None |
| Solubility in the gastric fluid | $F$[b] = 0.5–2 | Significant | $F$ = 0.5–2 | None |
| Solubility in the intestinal fluid | $F$ = 0.5–2 | Marginal | $F$ = 0.5–2 | Marginal |
| Basal intestinal fluid volume | 50–150 mL | Marginal | 150–350 mL | None |
| Intestinal surface area/volume ratio | 1.33–4.16 | Significant | 1.33–4.16 | None |
| Intestinal permeability | $F$ = 0.1–10 | Significant | $F$ = 0.1–10 | Significant[c] |

[a] The impact of a change in the parameter was defined as follows according to the indices AUC and $C_{max}$ ratio between the simulations and the observed profiles: *None*: within 0.9–1.1, *Marginal*: within 0.8–1.2, *Moderate*: within 0.7–1.3, *Significant*: outside 0.7–1.3.
[b] $F$: Factor applied to the input value used in the simulations.
[c] Only significant when $F$ was decreased.

particle size of the drug was found to significantly affect the precipitation rate within the range explored (factor: from 0.33 to 3), with results similar in magnitude to those in Fig. 4 (data not shown).

Fig. 5a shows profiles simulated with the crystal growth rate that gave the best fit (from Fig. 4), combined with various gastric emptying rates within the usual physiological range. The corresponding graphs for the amount of nelfinavir in solution in the small intestine with time can be found in Fig. 5b. As shown in Fig. 5, however, the gastric emptying rate had only a modest effect on the simulated plasma profiles (within the physiologically relevant range explored), even though the amount dissolved in the small intestine vs. time profiles varied with the gastric emptying rate.

According to additional sensitivity analyses (similar to those conducted for crystal growth and gastric emptying rates, shown in Figs. 4 and 5), solubility in the gastric media, intestinal surface area/volume ratio, and intestinal permeability can also significantly affect fasted-state simulated profiles (see Table 3). The other variable parameters in the simulation model are the drug solubility in the small intestine and the intestinal fluid volume, both of which had only a marginal influence on the profiles in the fasted state.

As reported by Longer et al. [24], the solubility of nelfinavir mesylate decreases drastically at pH > 3. In general, the gastric pH in young, healthy, fasting humans who have ingested a glass of water varies within the range of pH 1.5–5 [34]. The achlorhydric population may have even higher gastric pH (pH > 5) [35]. With this range of gastric pH, systemic exposure of nelfinavir in the fasted state could be highly dependent on gastric pH. The worst case simulation, which assumes no dissolution in the stomach and is probably closest to a prediction for performance in achlorhydrics, predicts an AUC of only approximately 37% of the AUC predicted from dissolution results at pH 1.6 in FaSSGF.

In contrast to the fasted state, sensitivity analyses for simulations in the fed state revealed that in the fed state, more robust behavior can be expected. Simulations were affected most clearly by changing the intestinal permeability and only marginally by varying the solubility in the small intestine: other parameters played little or no role. The results of Table 3 are consistent with the prescribing information for Viracept®, according to which administration with food increases nelfinavir exposure and decreases nelfinavir pharmacokinetic variability relative to the fasted state and which indicates that systemic exposure in the fed state depends on the calories or fat content of a meal ingested before taking nelfinavir mesylate [6].

### 3.3.1. Biorelevant or compendial media?

Fig. 6a compares simulation profiles generated from the compendial and biorelevant dissolution results in the fasted state. Use of biorelevant media for both the stomach and small intestine resulted in the closest simulation of the plasma profiles observed in the pharmacokinetic study. With the compendial media combination ($SGF_{sp}/SIF_{sp}$), the plasma profiles were underestimated.

Fed-state simulations are shown in Fig. 6b. The profile generated with a combination of $SGF_{sp}$/FeSSIF-V2 showed slightly faster absorption, reflecting the faster dissolution in $SGF_{sp}$ than FeSSGF. Use of dissolution results in a simple buffer solution, such as the intestinal medium, $SIF_{sp}$, does not result in accurate simulation of

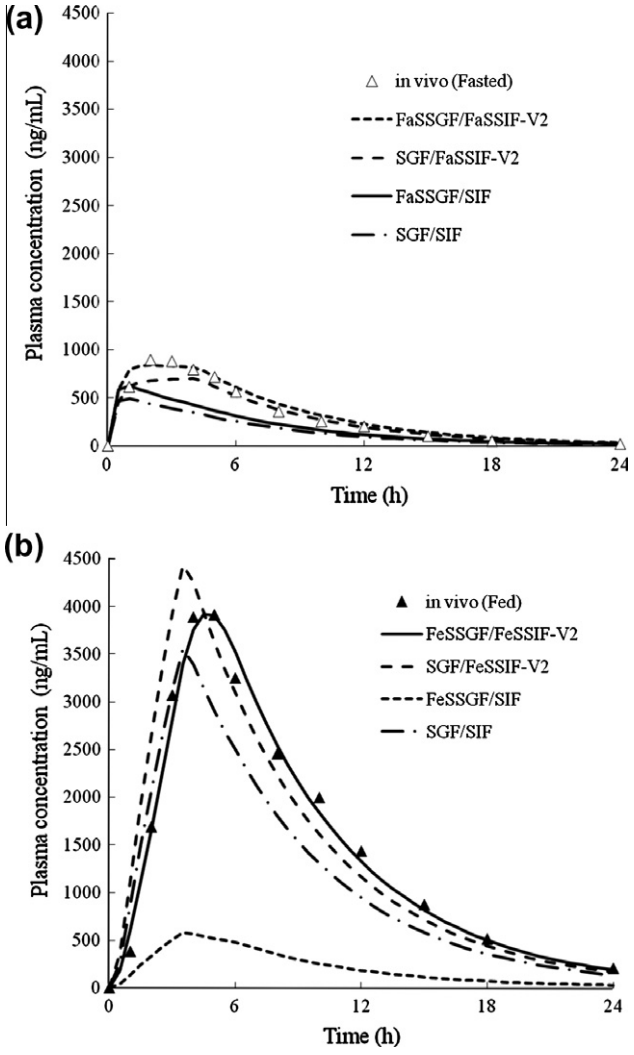


**Fig. 6.** Comparison of simulated profiles using biorelevant or compendial dissolution data in (a) the fasted state and (b) the fed state.

the PK profile, consistent with results from the sensitivity analysis. As expected, results in the compendial media combination ($SGF_{sp}$/$SIF_{sp}$) did not simulate the *in vivo* performance accurately.

Taking the results together, *in vivo* profiles of nelfinavir could only be simulated appropriately when the dissolution results in biorelevant media were coupled with the PBPK model. These results support the conclusion from previous reports that composition of dissolution media is an essential consideration in the prediction of the *in vivo* performance of poorly soluble drugs.

## 4. Conclusions

An extension of a previous PBPK model, incorporating drug precipitation kinetics, was successfully applied to predict the *in vivo* performance of the poorly soluble base, nelfinavir, in the fasted state. It was also demonstrated from both *in vitro* results and the simulations that precipitation is unlikely to limit nelfinavir absorption in the fed state. Plasma profiles generated from biorelevant media were better able to predict the *in vivo* performance of nelfinavir than those generated from compendial media. Based on these results, it appears that the *in vitro–in silico–in vivo* relationships (IVISIV-R) approach can be a powerful tool to explore the *in vivo* performance of poorly soluble, weakly basic drugs, and hence streamline the pharmaceutical development of these compounds.

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## References

[1] J.B. Dressman, G.L. Amidon, C. Reppas, V.P. Shah, Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms, Pharm. Res. 15 (1998) 11–22.
[2] D. Fleisher, C. Li, Y. Zhou, L.H. Pao, A. Karim, Drug, meal and formulation interactions influencing drug absorption after oral administration, clinical implications, Clin. Pharmacokinet. 36 (1999) 233–254.
[3] B.N. Singh, Effects of food on clinical pharmacokinetics, Clin. Pharmacokinet. 37 (1999) 213–255.
[4] US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), Food-Effect Bioavailability and Fed Bioequivalence Studies, Guidance for Industry, 2002.
[5] F. Hoffmann-La Roche Ltd., INVIRASE® Capsules (Saquinavir Mesylate), Complete Product Information, December, 2003.
[6] Pfizer Inc. Canada, Product Monograph, Viracept®, www.pfizer.ca (Date of Revision: 10.09.06).
[7] B. Kaeser, J.E. Charoin, M. Gerber, P. Oxley, H. Birnboeck, N. Saiedabadi, L. Banken, Assessment of the bioequivalence of two nelfinavir tablet formulations under fed and fasted conditions in healthy subjects, Int. J. Clin. Pharmacol. Ther. 43 (2005) 154–162.
[8] US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, Guidance for Industry, 2000.
[9] T.T. Kararli, Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, Biopharm. Drug Dispos. 16 (1995) 351–380.
[10] Y. Wu, A. Loper, E. Landis, L. Hettrick, L. Novak, K. Lynn, C. Chen, K. Thompson, R. Higgins, U. Batra, S. Shelukar, G. Kwei, D. Storey, The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human, Int. J. Pharm. 285 (2004) 135–146.
[11] N. Parrott, V. Lukacova, G. Fraczkiewicz, M.B. Bolger, Predicting pharmacokinetics of drugs using physiologically based modeling-application to food effects, AAPS J. 29 (2009).
[12] E. Jantratid, N. Janssen, C. Reppas, J.B. Dressman, Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update, Pharm. Res. 25 (2008) 1663–1676.
[13] M. Vertzoni, J. Dressman, J. Butler, J. Hempenstall, C. Reppas, Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds, Eur. J. Pharm. Biopharm. 60 (2005) 413–417.
[14] E. Galia, E. Nicolaides, D. Horter, R. Lobenberg, C. Reppas, J.B. Dressman, Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, Pharm. Res. 15 (1998) 698–705.
[15] M. Vertzoni, A. Diakidou, M. Chatzilias, E. Soderlind, B. Abrahamsson, J.B. Dressman, C. Reppas, Biorelevant media to simulate fluids in the ascending colon of humans and their usefulness in predicting intracolonic drug solubility, Pharm. Res. 27 (2010) 2187–2196.
[16] J.B. Dressman, C. Reppas, In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs, Eur. J. Pharm. Sci. 11 (2) (2000) S73–S80.
[17] E. Nicolaides, E. Galia, C. Efthymiopoulos, J.B. Dressman, C. Reppas, Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data, Pharm. Res. 16 (1999) 1876–1882.
[18] Y. Shono, E. Jantratid, N. Janssen, F. Kesisoglou, Y. Mao, M. Vertzoni, C. Reppas, J.B. Dressman, Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling, Eur. J. Pharm. Biopharm. 73 (2009) 107–114.
[19] Y. Shono, E. Jantratid, F. Kesisoglou, C. Reppas, J.B. Dressman, Forecasting in vivo oral absorption and food effect of micronized and nanosized aprepitant formulations in humans, Eur. J. Pharm. Biopharm. 76 (2010) 95–104.
[20] K. Sugano, Introduction to computational oral absorption simulation, Expert Opin. Drug Metab. Toxicol. 5 (2009) 259–293.
[21] R. Takano, K. Sugano, A. Higashida, Y. Hayashi, M. Machida, Y. Aso, S. Yamashita, Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test, Pharm. Res. 23 (2006) 1144–1156.
[22] K. Thelen, E. Jantratid, J.B. Dressman, J. Lippert, S. Willmann, Analysis of nifedipine absorption from soft gelatin capsules using PBPK modeling and biorelevant dissolution testing, J. Pharm. Sci. 99 (2010) 2899–2904.
[23] V.H. Sunesen, B.L. Pedersen, H.G. Kristensen, A. Mullertz, In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media, Eur. J. Pharm. Sci. 24 (2005) 305–313.
[24] M. Longer, B. Shetty, I. Zamansky, P. Tyle, Preformulation studies of a novel HIV protease inhibitor, AG1343, J. Pharm. Sci. 84 (1995) 1090–1093.
[25] S. Klein, The mini paddle apparatus? A useful tool in the early developmental stage? Experiences with immediate-release dosage forms, Dissolution Technol. 13 (4) (2006) 6–11.
[26] P. Macheras, C. Reppas, J.B. Dressman, Biopharmaceutics of Orally Administered Drugs, Ellis Horwood Ltd., London, 1995.
[27] V.H. Sunesen, R. Vedelsdal, H.G. Kristensen, L. Christrup, A. Mullertz, Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug, Eur. J. Pharm. Sci. 24 (2005) 297–303.
[28] E. Nicolaides, M. Symillides, J.B. Dressman, C. Reppas, Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration, Pharm. Res. 18 (2001) 380–388.
[29] K. Yamaguchi, Application of probability and statistics in chemical engineering, Kogyo Chosakai Publishing Co Inc., Tokyo, 1972.
[30] B.J. Aungst, N.H. Nguyen, J.P. Bulgarelli, K. Oates-Lenz, The influence of donor and reservoir additives on Caco-2 permeability and secretory transport of HIV protease inhibitors and other lipophilic compounds, Pharm. Res. 17 (2000) 1175–1180.
[31] R. Takano, K. Furumoto, K. Shiraki, N. Takata, Y. Hayashi, Y. Aso, S. Yamashita, Rate-limiting steps of oral absorption for poorly water-soluble drugs in dogs; prediction from a miniscale dissolution test and a physiologically-based computer simulation, Pharm. Res. 17 (2008).
[32] E.S. Kostewicz, M. Wunderlich, U. Brauns, R. Becker, T. Bock, J.B. Dressman, Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine, J. Pharm. Pharmacol. 56 (2004) 43–51.
[33] K. Sugano, A simulation of oral absorption using classical nucleation theory, Int. J. Pharm. 378 (2009) 142–145.
[34] L. Kalantzi, K. Goumas, V. Kalioras, B. Abrahamsson, J.B. Dressman, C. Reppas, Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies, Pharm. Res. 23 (2006) 165–176.
[35] M. Morihara, N. Aoyagi, N. Kaniwa, S. Kojima, H. Ogata, Assessment of gastric acidity of Japanese subjects over the last 15 years, Biol. Pharm. Bull. 24 (2001) 313–315.