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In silico prediction of drug dissolution and absorption with variation in intestinal pH for BCS class II weak acid drugs: ibuprofen and ketoprofen

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ABSTRACT: The FDA Biopharmaceutical Classification System guidance allows waivers for *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms only for BCS class I. Extensions of the *in vivo* biowaiver for a number of drugs in BCS class III and BCS class II have been proposed, in particular, BCS class II weak acids. However, a discrepancy between the *in vivo* BE results and *in vitro* dissolution results for BCS class II acids was recently observed. The objectives of this study were to determine the oral absorption of BCS class II weak acids via simulation software and to determine if the *in vitro* dissolution test with various dissolution media could be sufficient for *in vitro* bioequivalence studies of ibuprofen and ketoprofen as models of carboxylic acid drugs. The oral absorption of these BCS class II acids from the gastrointestinal tract was predicted by GastroPlus™. Ibuprofen did not satisfy the bioequivalence criteria at lower settings of intestinal pH of 6.0. Further the experimental dissolution of ibuprofen tablets in a low concentration phosphate buffer at pH 6.0 (the average buffer capacity 2.2 mmol l^{-1}/pH) was dramatically reduced compared with the dissolution in SIF (the average buffer capacity 12.6 mmol l^{-1}/pH). Thus these predictions for the oral absorption of BCS class II acids indicate that the absorption patterns depend largely on the intestinal pH and buffer strength and must be considered carefully for a bioequivalence test. Simulation software may be a very useful tool to aid the selection of dissolution media that may be useful in setting an *in vitro* bioequivalence dissolution standard. Copyright © 2012 John Wiley & Sons, Ltd.

Key words: weak acid; ibuprofen; ketoprofen; pH; simulation; GastroPlus; *in vitro* dissolution; dissolution media

Introduction

The U.S. Food and Drug Administration (FDA) released guidelines for the pharmaceutical industry based on the Biopharmaceutics Classification System (BCS) in 2000 [1]. The BCS classifies drugs according to the biopharmaceutical properties governing their absorption and the controlling factors: permeability, solubility and product dissolution. The European Medicines Agency (EMA) and FDA have released guidances for the investigation of bioequivalence (BE) and requirements of the BCS-based biowaiver for immediate release (IR) drug products [2]. The BCS classifies drugs as highly soluble when their highest IR dose

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strength is soluble at 37 °C in 250 ml or less of aqueous medium over a pH range of 1.0–7.5 [3,4]. On the other hand, the EMA has defined a drug as being highly soluble at 37 °C in 250 ml or less of buffer over a range of 1.0 to 6.8 at the highest drug dose [2,5]. The current FDA criteria for a drug to be termed highly soluble in order to be granted a biowaver are likely conservative, while both the WHO and the EMA extend biowaivers to some drugs when they meet the specific criterion for weak acids (rapid dissolution at pH 6.8) and are categorized as BCS class II [2,6].

The feasibility of granting biowavers to some BCS class II drugs has been evaluated previously; many BCS class II drugs such as flurbiprofen, naproxen, ketoprofen, rifampicin and carbamazepine exhibit good oral absorption, even though they are almost insoluble in acidic or basic pH conditions [7–10]. Some BCS class II weak acids, particularly the small molecule non-steroidal anti-inflammatory drugs (NSAIDs), are potential candidates for biowaivers [11]. These drugs can dissolve quickly and behave like BCS class I drugs at intestinal pH (6.5–7.0) in the GI tract, even though they exhibit low solubility at acidic pH [4]. Ibuprofen, an NSAID, has been considered as a BCS biowaiver candidate due to its high permeability and high solubility at gastrointestinal pH [12]. However, in a recent report, it was determined that it would be risky to biowaiver BCS class II acidic drugs using only *in vitro* dissolution tests [13]. Thus, this report raises questions of the system used for *in vitro* dissolution study and of the differences between *in vitro* and *in vivo* dissolution and, hence, *in vivo* absorption and systemic availability.

It is well known that the buffering capacity, ionic strength, pH and buffer composition of the dissolution medium can have significant effects on drug dissolution [14]. It has been suggested that the media used for *in vitro* dissolution media are not adequate for assessing *in vivo* drug dissolution [15,16]. The USP simulated intestinal fluid (SIF) and fasted-state simulated small intestine fluid (FaSSIF) have been used mainly for *in vitro* dissolution studies, even though the buffer species employed in those media are phosphate [17,18]. However, the main physiological buffer in the human intestine is predominantly bicarbonate and, therefore, bicarbonate buffers may better reflect the *in vivo* environment and provide more suitable *in vitro* dissolution media for assessing the *in vivo* dissolution of test products [18]. Yet SIF is predominantly used for *in vitro* dissolution studies and was used for *in vitro* dissolution tests of ibuprofen for the BE studies noted previously [13]. Sheng and colleagues reported that drugs whose pKa values are lower than 5.5 would exhibit a significant difference in their solubility and dissolution rates in SIF compared with bicarbonates and have suggested a lower concentration of phosphate for *in vitro* dissolution studies [18]. The adoption of inappropriate buffer media for *in vitro* BE studies could cause a failure in concluding the bioinequivalence for those noted ibuprofen tablets. Those dissolution media were constituted with either 70–80 mM phosphate buffer (pH 6.8–7.2) or 20–2270 mM acetate buffer (pH 4.5–6.0), not bicarbonate. The aim of this study is to determine that the current *in vitro* dissolution methods are suitable for concluding bioequivalence for test compounds and to predict the *in vivo* dissolution of test drug products and, hence, their absorption and systemic availability as a function of gastrointestinal variables, especially pH and buffer capacity. When the buffer species for *in vitro* dissolution does not replicate the *in vivo* buffer medium, the results of *in vitro* dissolution tests may neither reflect *in vivo* dissolution, nor correctly determine bioequivalence/bioinequivalence of test drug products.

The two major objectives of this study were: (1) to investigate how lowering the pH of the intestinal medium affects the oral absorption of BCS class II weak acids using the simulation software GastroPlus™ in order to understand the *in vivo* dissolution of those acids; and (2) to determine if *in vitro* dissolution tests with SIF would be sufficient for BE studies. For these purposes, ibuprofen (pKa 4.5) and ketoprofen (pKa 3.7), being lower than pKa 5.5, were selected as model drugs for *in silico* simulation. Their oral drug absorption, C_{max}, $AUC_{0\text{-inf}}$ and F_a %, were predicted while incorporating the effects of dissolution kinetics along with intestinal pH. For testing *in vitro* dissolution media, the *in vitro* dissolution rate of ibuprofen tablets was performed in the simulated intestinal fluid (SIF) and the low phosphate concentration of SIF (LSIF) at pH 6.0 and 6.8 in order to investigate the dissolution rate along with the pH changes of the dissolution media.

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Materials and Methods

Materials

Ibuprofen tablets were obtained from Amneal Pharmaceuticals (Hauppauge, NY). Potassium phosphate monobasic, sodium hydroxide, acetonitrile, high-performance liquid chromatography (HPLC) grade and other reagents were obtained from Fisher Scientific (St Louis, MO). All chemicals were either analytical or HPLC grade.

Computer hardware and software

GastroPlus™ version 7.0 (SimulationPlus, Inc., Lancaster, CA) was run using an IBM computer with Intel Core™ 2 Duo processors. This software allows the input of different dissolution velocities for pharmacokinetic predictions.

Simulation design

The oral drug absorption was predicted based on the physicochemical, pharmacokinetic and drug dissolution properties of two selected BCS class II drugs using a previously reported simulation method. Briefly, the default gastric emptying time (15 min) was used for this virtual trial. The virtual trials were performed with 24 h monitoring for ibuprofen and ketoprofen. In virtual trials, the variations in population physiology were defined as the mean with coefficients of variation in log space and were randomly selected within those ranges. Virtual trials were performed as references ($n = 500$) on each drug compound with an IR dosage form. Virtual trials ($n = 24$) with randomly selected physiological conditions were performed as samples with an IR dosage form at the different intestinal pHs. The results of the dissolution rates for those drug compounds with an IR dosage form were obtained by GastroPlus™ single simulation (Figure 1).

Input parameters for pharmacokinetic simulations

The physicochemical and biopharmaceutical properties of ibuprofen and ketoprofen used in the GastroPlus™ simulations, as well as the chemical, physiological and pharmacological parameters for drugs cited in the literature are presented in Table 1 [13,19–25]. The Johnson model was adopted as a dissolution model for this set of simulations [26].

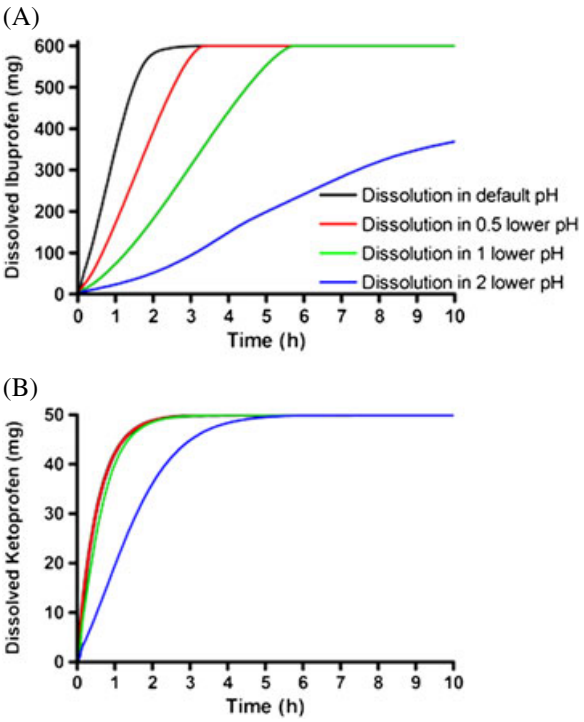

Figure 1. **The delayed dissolution rates of an IR dosage form to the lowered pH of GI tract used in the simulations**. (A) ibuprofen and (B) ketoprofen. Black line represents the drug dissolution of an IR dosage form in default pH at the small intestine, red line represents the drug dissolution of an IR dosage form in half point lower pH at the small intestine, green line represents the drug dissolution of an IR dosage form in 1 point lower pH in the small intestine, blue line the drug dissolution of an IR dosage form in 2 points lower pH in the small intestine

Since the size of drug particles affects the drug dissolution rate, a default radius of 25 µm was set as the mean with 10% log-normal distribution as its coefficient of variation [27]. Virtual trials were performed using the GastroPlus™ standard physiological conditions: the averaged human intestinal pH at each intestinal segment was used as the default setting. The pH at each intestinal compartment except the caecum and colon, was lowered from the default setting and was used as the lower pH setting (Table 2). In those virtual trials, the dose, dose volume, molecular weight, log P, pKa, particle density and diffusion coefficient of the drug compounds were fixed. All other parameters for virtual trials such as effective human permeability, intestinal transit time and pharmacokinetic clearance were defined as

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Table 1. Chemical/physiological/pharmacological parameters of BCS class II drugs for GastroPlus™ simulation

		Ibuprofen	Ketoprofen
MW		206.3	254.3
Dose	mg	600[a]	50[f]
Dose number		63[#]	197[#]
Dose volume	ml	250	250
Solubility (pH 1.2)	mg/ml	2.1×10^{-2}[b]	5.1×10^{-2}[b]
logP		4.0[c]	2.8[g]
pKa		4.5[d]	3.7[b]
Mean precipitation time	s	900	900
Human P_{eff}	$\times 10^{-4}$ cm^2/s	4.10*	8.70[h]
Body weight	kg	70	70
V_c	l/kg	0.2[e]	0.4[f]
Total clearance	l/h/kg	0.10[a]	0.06[f]

V_c, volume of central compartment; [#]calculated by GastroPlus™ 7.0; *calculated by ADMET predictor; [a]Ref [13]; [b]Ref [23]; [c]Ref [20]; [d]Ref [21]; [e]Ref [19]; [f]Ref [22]; [g]Ref [25]; [h]Ref [24].

Table 2. Absorption scale factor (ASF), fluid volume (ml) and pH settings at different intestinal sites for simulation

	pH condition for the simulation					
Intestinal	Default pH	0.5 lower pH	1.0 lower pH	2.0 lower pH[a]	Fluid volume	ASF
Duodenum	6.0	5.5	5.0	4.0	41.6	7.0
Jejunum 1	6.2	5.7	5.2	4.2	154.2	10.0
Jejunum 2	6.4	5.9	5.4	4.4	122.3	7.0
Ileum 1	6.6	6.1	5.6	4.6	94.3	2.7
Ileum 2	6.9	6.4	5.9	4.9	70.5	2.6
Ileum 3	7.4	6.9	6.4	5.4	49.8	2.5
Caecum	6.4	6.4	6.4	6.4	47.5	0.1
Asc colon	6.8	6.8	6.8	6.8	50.3	0.3

[a]Simulations with lowered pH numbers were strictly hypothetical to determine the effect of intestinal pH for drug absorption.

variables, which were randomly created within a 5–20% log-normal distribution based on their mean values.

In vitro dissolution of ibuprofen immediate release (IR) tablets and pH of dissolution media

The dissolution profile of an ibuprofen IR tablet was determined using a Hanson SR6 Dissolution Test Station (Chatsworth, CA) equipped with a USP apparatus II (paddles). The paddle speed was set to 100 rpm.

Dissolution tests were conducted on a single dosage unit (400 mg ibuprofen tablet) at $37 \pm 0.5\,^{\circ}C$ in 500 ml of either simulated intestinal fluid (SIF) (50 mM phosphate buffer) at pH 6.0 or pH 6.8 or a low phosphate concentration of SIF (LSIF) (10 mM phosphate buffer; buffer capacity 1.6–2.8 mmol l^{-1} /pH) at pH 6.0 or pH 6.8. Tablets were introduced to the vessel by adding them directly to the medium without sinkers. Sampling was performed over 3 h and was filtered to remove any undissolved solid particles. Drug release profiles were determined on an Agilent HPLC system (Agilent Technologies, Santa Clara, CA). The HPLC system consisted of Agilent pumps (1100 series), an Agilent autosampler (1200 series) and an Agilent UV-vis detector (1100 series) controlled by Chemstation® 32 software (version B.01.03). Samples were resolved in an Agilent Eclipse Plus C_{18} reverse-phase column (3.5 µm, 4.6 × 75 mm) equipped with a guard column. The mobile phase consisted of 0.1% TFA/water (solvent A) and 0.1% TFA/acetonitrile (solvent B) with the solvent B gradient changing over 0–56% at a rate of 2%/min during a 20 min run for ibuprofen. A standard curve generated for ibuprofen was utilized for quantitation of the integrated area under peaks. The detection wavelength was 254 nm and spectra were acquired in the 220–380 nm range.

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The pH was monitored at each time point by SevenEasy Mettler Toledo (Columbus, OH) equipped with InLab 413 SG/2m probe. All experiments were performed in triplicate and average values were calculated.

Results

The oral absorption of BCS class II drugs was predicted using GastroPlus™ virtual trials. Those predicted numbers, mean C_{max}, $AUC_{0\text{-inf}}$ and Fa % ± standard deviation (SD), were obtained with 500 virtual trials using an IR dosage form as a reference and with 24 virtual trials as samples using the same dosage at different intestinal pHs.

The impact of release rate and gastrointestinal pHs on oral drug absorption

Ibuprofen. Ibuprofen exhibited good oral absorption (81.6–99.9%) throughout all tested release rates. The dissolved drug amounts of an IR dosage form at different intestinal pHs were calculated by a GastroPlus™ single simulation and were plotted as a function of time for the first 10 h (Figure 1A). Figure 1A exhibits the delayed dissolution rates of ibuprofen due to the lowering of the intestinal pH. Ibuprofen would dissolve 100% in 5 h after oral administration, except in the condition of lowering the intestinal pH by 2.0 points. The IR dosage form of ibuprofen exhibited a similar $AUC_{0\text{-inf}}$ but did not maintain a similar C_{max} (Figure 2), as expected. The ibuprofen tablets did not demonstrate bioequivalence in any simulation in the lower intestinal pH conditions, even though those simulations were performed with the same formulation. As expected, the gastrointestinal pH would have an enormous impact on the dissolution and, hence, the oral absorption of ibuprofen due to its pH-dependent solubility in the physiological range. With the lowest tested gastrointestinal pHs in the GI tract, the value of C_{max} was reduced by 58.1% compared with that in the average physiological intestinal pH (pH range of 6.0–7.4) in human, while the $AUC_{0\text{-inf}}$ of ibuprofen exhibited a BE with a similar predicted F_a as expected (Figure 2, Table 5).

Ketoprofen. Ketoprofen exhibited complete oral absorption (99.9–100.0%) throughout all tested release rates (Table 5). Ketoprofen exhibited similar $AUC_{0\text{-inf}}$ values throughout all the different conditions (the difference; $AUC_{0\text{-inf}}$ 0.0–4.3%). The dissolved drug amounts of an IR dosage form at different intestinal pHs were calculated by a GastroPlus™ single simulation and were plotted as a function of time for the first 10 h (Figure 1B). Figure 1B exhibits delayed dissolution rates of ketoprofen due to the setting of lower intestinal pHs. However, the difference in dissolution rates was minimal until the pH was lowered by 2.0 points. The dissolved amount of ketoprofen under each intestinal pH condition reached 100% at 5 h after oral administration. Unlike ibuprofen, an IR dosage form of ketoprofen at each lowered pH condition in human intestine exhibited BE when

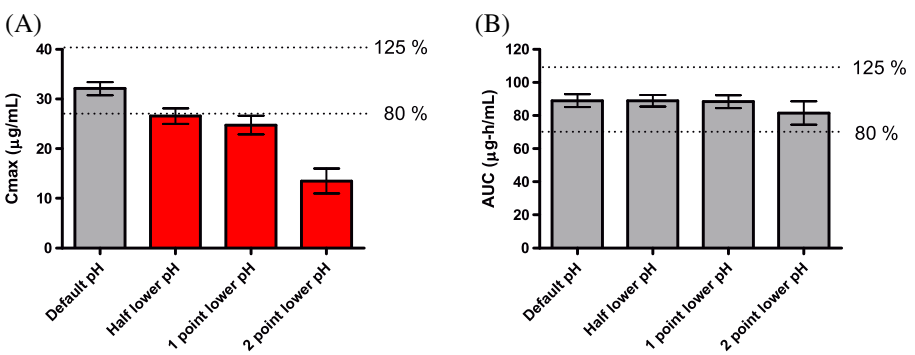

Figure 2. C_{max} (A) and $AUC_{0\text{-inf}}$ (B) of ibuprofen predicted by computer simulations. Data reported as mean ± SD, 90% confidence interval (CI) of C_{max} (A) and $AUC_{0\text{-inf}}$ (B), the simulation being taken with lowered intestinal pHs corresponding to an IR dosage form in default intestinal pHs as comparator. Black bars represent bioequivalence, and red bars represent outside of bioequivalence criteria (outside of 80% and 125% of the comparator)

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the result was compared with the reference intestinal pH (Figure 3). Oral absorption of ketoprofen was not affected as much as that of ibuprofen by lowering the intestinal pH due to its lower pKa. With the lowest intestinal pH setting in this simulation (pH range of 4.0–5.4), the value of C_{max} was only reduced 8.4% compared with the reference result. $AUC_{0\text{-inf}}$ and predicted F_a of ketoprofen in all tested conditions satisfied BE and exhibited similar results (Figure 3, Table 5).

In vitro dissolution test of ibuprofen

The dissolution of ibuprofen tablets in SIF and LSIF at pH 6.8 exhibited a similar profile with LSIF exhibiting a slower dissolution rate of ibuprofen (Figure 4A). Ibuprofen tablets in SIF at pH 6.8 were completely dissolved at 30 min while those in LSIF at pH 6.8 exhibited complete dissolution at 60 min. Although the pH of the SIF medium did not change, the pH of LSIF was decreases from 6.8 to 6.1 during the experiment over 3 h (Figure 4A). Figure 4B demonstrated that the dissolution (average 22%) of ibuprofen in LSIF was dramatically reduced at pH 6.0 compared with that (average 74%) in SIF over 3 h. The pH in SIF was slightly lowered from 6.0 to 5.7 over 3 h, while the pH in LSIF was decreased from 6.0 to 5.0 in 45 min in accordance with the dissolution of ibuprofen (Figure 4B). At pH 6.0, the dissolved ibuprofen in LSIF was sufficient to lower the pH of the dissolution medium and, as a result, only 22% (88 mg of 400 mg dose) of ibuprofen dissolved in LSIF, due to the lower buffering capacity compared with SIF (the average buffering capacity; LSIF 2.2 mmol l^{-1}/pH vs 12.6 mmol l^{-1}/pH).

Discussion

The FDA guidelines currently allow biowaivers only for BCS class I drugs, while the EMA has discussed allowing biowaivers for some BCS Class II and III drug products [2,28,29]. The possibility of extending biowaivers to BCS class II has been discussed due to the conservative FDA requirement for drugs to be highly soluble [30]. It has been argued that biowaivers should be extended to some BCS class II compounds that have specific biopharmaceutical characteristics, such as weak acids, particularly the small molecule nonsteroidal anti-inflammatory drugs (NSAIDs) [8,9,31]. The proposed rationale is that poorly soluble weak acids with pKa values of ≤ 5.0, such as ibuprofen, ketoprofen and naproxen, would have solubilities of ≥ 1 mg/ml at around pH 6.5, which is the average pH value found in the fasted state in the jejunum, resulting in rapid (less than 0.5 h) and reliable dissolution of these drugs [32]. Weak acids will exhibit high solubility and act like BCS class I drugs in the small intestine, even though these drugs are classified as having low solubility because of *in vitro* dissolution tests performed in a wide pH range (FDA; pH 1.0–7.5, EMA; 1.0–6.8). pH-regulating excipients and surfactants are often

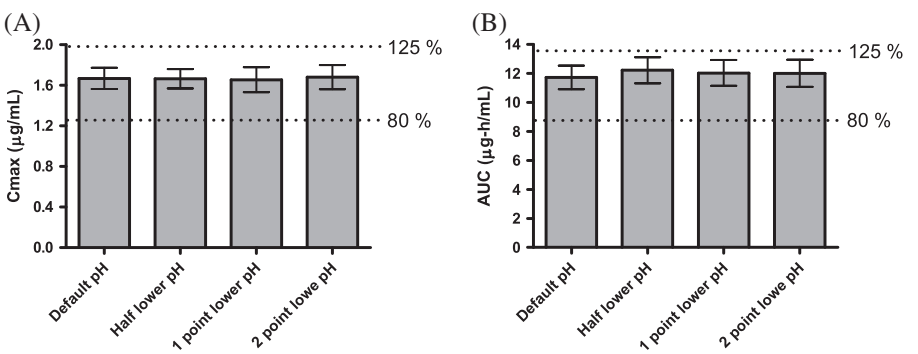

Figure 3. C_{max} (A) and $AUC_{0\text{-inf}}$ (B) of ketoprofen predicted by computer simulations. Data reported as mean ± SD, 90% confidence interval (CI) of C_{max} (A) and $AUC_{0\text{-inf}}$ (B), the simulation being taken with lowered intestinal pHs corresponding to an IR dosage form in default intestinal pHs as comparator. Black bars represent bioequivalence, and red bars represent outside of bioequivalence criteria (outside of 80% and 125% of the comparator)

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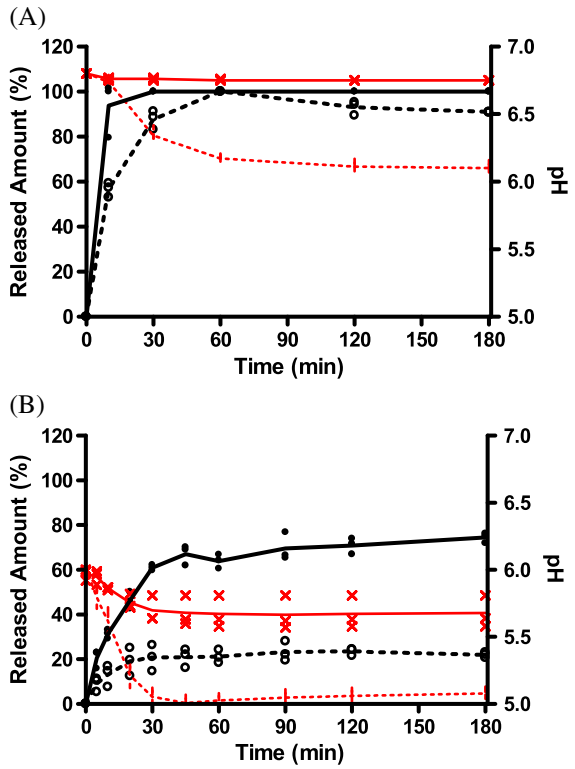

Figure 4. Release profile of ibuprofen tablets in different buffer concentration of SIF at pH 6.8 (A) and pH 6.0 (B) with a fixed rotation speed of 100 rpm as well as monitoring pH change. Data reported ($n = 3$). (●) Ibuprofen in SIF (50 mM phosphate buffer), (○) ibuprofen in LSIF (10 mM phosphate buffer). (—) Average of released ibuprofen in SIF, (·····) Average of released ibuprofen in LSIF (10 mM phosphate buffer). (X) pH of SIF (50 mM phosphate buffer), (|) pH of LSIF (10 mM phosphate buffer). (—) Average pH of SIF (50 mM phosphate buffer), (·····) Average pH of diluted SIF (10 mM phosphate buffer)

used in the formulation of low soluble BCS class II drugs, in order to increase the rate of dissolution and, hence, absorption [6,12,30,33]. Indeed, it is not evident that all ibuprofen IR dosage forms with a marketing authorization achieve bioequivalence [12,34]. However, the bioinequivalence between those formulations could be detected by the right *in vitro* dissolution studies. The BCS class currently categorizes high and low solubility on active pharmaceutical ingredient (API) itself and does not consider solubility changes caused by its formulation. Extending biowaivers to BCS class II drug products must be evaluated carefully to ensure the safety of drug products and to understand the formulation effects on drug dissolution and absorption.

The demonstration of bioequivalence by *in vitro* dissolution studies seems feasible for a biowaiver based approval. However, it has been reported that *in vitro* dissolution studies failed to detect the bioinequivalence between the comparator and its test compound of BCS class II [13]. This discrepancy between *in vitro* BE dissolution studies and *in vivo* BE studies for a BCS class II drug raises questions regarding the current *in vitro* BE dissolution tests. In order to understand the difference between *in vivo* and *in vitro* behaviour of BCS class II weak acids, the *in vivo* dissolution and absorption of ibuprofen and ketoprofen were predicted by the simulation software GastroPlus™. Additionally, *in vitro* dissolution studies were performed in two different dissolution media to understand the importance of the selection of dissolution media for BE studies.

The small-intestinal transit time, approximately 3–4 h in the fasted state, is longer than the gastric residence time of approximately 15–60 min [35–37]. It has been reported that the residence times in the caecum and colon are around 3–7 h and 12–24 h, respectively, which are much longer than the 3 h residence time reported for the entire small intestine [38,39]. In our simulations, the residence times used for the stomach, small intestine (duodenum, jejunum and ileum), caecum and ascending colon were 0.2 h, 3.2 h, 4.2 h and 12.6 h within a 10% log-normal distribution based on those mean values, respectively.

The measured pH of human intestine in the fasted state reportedly ranged from 5.5 to 7.5 in the duodenum, from 6.2 to 6.7 in the proximal small intestine, from 6.3 to 7.3 in the middle small intestine, from 6.7 to 7.7 in the distal small intestine, and from 5.5 to 7.6 in the ascending colon [38,40,41]. The pH values of the duodenum, jejunum and ileum were set to 6.0, 6.2–6.4, and 6.6–7.4, respectively. Those starting pH values were gradually lowered in this set of simulations within a 6–10% log-normal distribution based on those mean values (Table 2). At those starting pHs, a weakly acidic drug product such as ibuprofen (pKa 4.5) and ketoprofen (pKa 3.7) would exhibit high solubility in the duodenum, jejunum, ileum, caecum and colon and have a longer residence time in the small intestine, caecum and colon for a total of 18–35 h. As a result, it is highly likely that ibuprofen and ketoprofen may behave similarly to

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a BCS class I drug in the small and large intestines, even though they are still classified as low solubility drugs due to their poor solubility at the gastric pH. Therefore, weak acids of BCS class II drugs may be completely absorbed because of their high solubility in small and large intestines and have sufficient residence time throughout the whole intestine. The BCS class II acidic drugs, ibuprofen and ketoprofen, exhibited complete absorption (99.9–100.0%) in this prediction study with an IR dosage form. Indeed, it has been reported that weakly acidic BCS class II drug products have been shown to be completely orally absorbed due to their high solubility at the pH range (pH 6.0–6.4) of the small intestine [31,32]. Thus, drugs with pKa values ≤ 5.0 of IR oral dosage forms would have sufficient time for complete dissolution and absorption in the small intestine due to their high permeability.

The average volumes of human intestinal fluid from the duodenum and jejunum in a fasted state are reportedly 184 ml and 63 ml, respectively [41]. With those volumes in a fasted state at the duodenum and jejunum, more than 50 mg of ibuprofen and ketoprofen would be dissolved at those small intestinal segments at pH 6.0 and 6.2, respectively, indicating that about 10% and 100% of orally dosed ibuprofen (600 mg dose) and ketoprofen (50 mg dose) were dissolved without any pH change. Therefore, the ranges of dissolved ibuprofen and ketoprofen at the duodenum and jejunum would be 2–4 mmol/l and 1–3 mmol/l, respectively. The buffer capacity values obtained for fasted-state human intestinal fluid in several reports were in the range 2.4–5.6 mmol l^{-1}/pH [42,43]. This suggests that the buffer capacity at the duodenum and proximal jejunum is relatively lower than *in vitro* dissolution media [44,45]. As a result, the dissolved amount of weak acids *in vivo* could significantly lower the intestinal pH and, hence, slow the dissolution rate. Indeed, Lee and colleagues have reported a significant decrease of duodenal pH by the presence of acid in humans [40]. In our simulations and *in vitro* dissolution studies, drastically slowed dissolution rates of an IR dosage form of ibuprofen and ketoprofen were observed when the intestinal pH and buffer concentration were reduced (the range of 0.5–2 pH at each segment of the small intestine and 10–50 mM buffer concentration of SIF at pH 6.0 and 6.8) (Figures 1 and 4). The slowed dissolution rates of ibuprofen and ketoprofen reduced the rate of absorption and, therefore, the values of C_{max} (Tables 3 and 4). However, the changes of $AUC_{0\text{-inf}}$ for ibuprofen and ketoprofen in the lowered intestinal pH condition were minimal, 0.8–8.4% for ibuprofen and 0.9–4.3% for ketoprofen (Tables 3 and 4). The predicted F_a values of ibuprofen and ketoprofen were unchanged and exhibited ranges of 81.6–99.9% and 99.9–100.0%, respectively (Table 5). These results indicate that BCS class II acidic drugs have a long enough transit time in the small intestine to be completely absorbed because of their high permeability and high solubility at physiological pH. The lowered pH in the intestinal segments slowed the dissolution rate and, hence, the absorptive rate for acidic drugs such as ibuprofen and ketoprofen.

The results of the *in vitro* dissolution study for ibuprofen tablets in different pH and buffer strength supported the simulation outcomes. The lowered pH of the dissolution media attributed the slower dissolution rate for ibuprofen in LSIF (Figure 4B), suggesting that the dissolution rate of acidic drugs would be affected more at the proximal small intestine such as duodenum and proximal jejunum (pH 5.5–6.2) than one at the distal small intestine such as ileum (pH 6.7–7.7). At pH 6.8, the starting pH was high enough not to affect the dissolution rate of acids, even though the pH in LSIF was reduced to pH 6.1. The difference of the dissolution rates in LSIF at pH 6.0 and one in SIF at pH 6.0 was significant and as the pH of the dissolution media decreases, the solubility of ibuprofen would be largely affected at pH 5.0, which is closer to the pKa of ibuprofen. Based on this result, ketoprofen, which possesses

Table 3. Simulated C_{max} and $AUC_{0\text{-inf}}$ of IR ibuprofen at different intestinal pHs

Release rate	An IR dose			
	Default pH	0.5 lower pH	1.0 lower pH	2.0 lower pH
C_{max} (μg/ml)	32.2 ± 1.3	26.6 ± 1.6	24.8 ± 1.9	13.5 ± 2.5
$AUC_{0\text{-inf}}$ (μg-h/ml)	89.1 ± 3.9	90.0 ± 3.5	88.4 ± 3.9	81.6 ± 5.8

Data reported as mean ± SD.

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Table 4. Simulated C_{max} and $AUC_{0\text{-inf}}$ of IR ketoprofen at different intestinal pHs

	An IR dose			
Release rate	Default pH	0.5 lower pH	1.0 lower pH	2.0 lower pH
C_{max} ($\times 10^{-1}$ μg/ml)	16.7 ± 1.1	16.6 ± 1.0	16.5 ± 1.2	15.3 ± 1.5
$AUC_{0\text{-inf}}$ (μg-h/ml)	11.7 ± 0.8	12.2 ± 0.9	12.0 ± 1.0	11.7 ± 1.0

Data reported as mean ± SD.

Table 5. Predicted F_a of ibuprofen and ketoprofen at different intestinal pH

	An IR dose			
Release rate	Default pH	0.5 lower pH	1.0 lower pH	2.0 lower pH
Ibuprofen (%)	99.9 ± 0.0	99.7 ± 0.2	98.9 ± 0.4	81.6 ± 7.1
Ketoprofen (%)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	99.9 ± 0.0

Data reported as mean ± SD.

similar physiological characteristics to ibuprofen, would exhibit a similar result of *in vitro* dissolution in a similar condition. However, the pKa of ibuprofen is higher than that of ketoprofen (ibuprofen; pKa 4.5 vs ketoprofen; pKa 3.7). Therefore, the dissolution rate of ibuprofen would be more sensitive to pH changes in the human intestine. Indeed, our simulation results indicated that ibuprofen would be more sensitive to a change of intestinal pH than ketoprofen (Figures 2 and 3).

The *in vivo* dissolution rates of acidic drugs would be altered by the lowered pHs of buffer in the human intestine. As shown in Figures 4A and B, the pH of dissolution media would shift especially when the buffering capacity of the media is low. The shift of pH in *in vivo* dissolution media would occur gradually in accordance with the dissolved amount of acidic drugs. The limitation of this phenomenon in *in silico* simulation is that the gradually decreasing pH of the dissolution medium in each intestinal compartment due to low buffer capacity cannot be accomplished. GastroPlus™ has a function to create a gradually decreasing/increasing pH profile at any given time in the GI tract. However, this function cannot be used for this series of simulations with an IR dosage form without knowing the *in vivo* dissolution rate, specific intestinal pH and the buffer capacity. Therefore, in these simulations, the physiological pHs in gastrointestinal segments were adjusted as an initial setting and those pHs were altered by the dissolved amount of acidic or basic drugs. The assumption in the simulation software is that the buffer capacity of *in vivo* media is high enough to maintain the pH independently of how much of the acidic or basic drugs dissolved. The initial pH settings in those simulations were assumed to be the final pH of *in vivo* media when the drug is completely dissolved at a given transit time in specific intestinal segments. Therefore, those simulations give a progressive prediction by setting the lowest pHs in the GI segments as the initial settings.

The results of *in silico* and *in vitro* studies clearly demonstrate that the possibility of slow dissolution rates of acidic drugs *in vivo* can cause bioinequivalence, even though test products show a similar *in vitro* USP type dissolution. Our *in silico* results demonstrated that ibuprofen dosage forms could fail the bioequivalence test when the oral absorption, C_{max} and $AUC_{0\text{-inf}}$, of ibuprofen with the default intestinal pH setting was compared with that with a lowered intestinal pH setting. The simulation and *in vitro* dissolution results suggest that dissolved ibuprofen and ketoprofen could lower the regional intestinal pH by up to 1–3 units and, hence, the *in vivo* dissolution rate of ibuprofen and ketoprofen might be slower due

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to this lowered solubility. Ketoprofen (pKa 3.7), which has similar chemical and physiological characteristics to ibuprofen, exhibited a slower *in vivo* dissolution rate than the *in vitro* dissolution rate, which is attributed to a lower pH and poorer buffering capacity *in vivo* [44]. *In vitro* dissolution medium, fasted USP SIF, FaSSIF, FaSSIFm and FaSSIF-V2, retain strong buffering capacities, 18.4, 12, 12 and 10 mmol l^{-1}/pH, which are 1.8–7.7-fold higher than the reported buffering capacity of *in vivo* fluid, 2.4–5.6 mmol l^{-1}/pH [16,44–47]. The buffering capacity and the pH of the dissolution medium clearly has a significant effect on the dissolution rate of these NISAID compounds. These differences between *in vivo* and *in vitro* would produce different dissolution results. Thus, in order to perform *in vitro* BE studies, the rational selection of a buffer medium with appropriate buffering capacity and pH is extremely important. Real human intestinal fluid is the most relevant medium for *in vitro* dissolution studies. However, the supply of human intestinal fluid is very limited. Therefore, simulated intestinal fluid (SIF) as an alternative to human intestinal fluid mainly has been utilized for *in vitro* dissolution. While the compendial simulated intestinal fluid (50 mM phosphate buffer) has been accepted as an *in vitro* dissolution medium, the main physiological buffer in the human intestine is bicarbonate. Therefore, *in vitro* dissolution studies in bicarbonate buffer would better predict *in vivo* dissolution rates. Further the concentration of phosphate buffer (50 mM) is very likely too high for BE studies reflecting *in vivo* dissolution media compared with bicarbonate [18]. The bicarbonate concentration was reported to be in the range 4–21 mM with an average of 15 mM [18,48]. In our calculation, 5–15 mM bicarbonate buffers at pH 6.0 and pH 6.8 are equivalent to 8–45 mM phosphate buffers. The *in vitro* dissolution studies with 50 mM phosphate buffer may not be suitable for an *in vitro* BE dissolution medium, especially for BCS class II acidic drugs. The importance of the selection of the dissolution medium for BE studies was demonstrated by *in silico* studies and *in vitro* dissolution studies with the same dosage form. Indeed, the importance of selecting the right *in vitro* dissolution medium and methods for test compounds has been indicated for the development of solid oral dosage forms [49,50]. It is hypothesized that the slower drug dissolution of ibuprofen *in vivo* caused bioinequivalence due to the lower buffering capacity, the different pKa of the *in vivo* buffer and the pH shift of the intestinal fluid *in vivo*, which would not be represented by *in vitro* dissolution studies with SIF due to its high buffering capacity. It is expected that if the dissolution studies were performed with media reflecting human intestinal fluids and bicarbonate buffers, the bioinequivalence of ibuprofen products could be detected by the *in vitro* dissolution studies. Therefore, biorelevant dissolution media such as bicarbonate buffers and pHs should be considered for use as *in vitro* dissolution media for *in vitro* BE dissolution studies. The selection of dissolution media for *in vitro* BE studies is key for predicting *in vivo* dissolution of test compounds and would depend on the physiochemical property of test compounds. With our simulation results, the simulation software can be a valuable tool for predicting the *in vivo* dissolution of test compounds and can aid in the selection of suitable *in vitro* dissolution media for BE studies.

Conclusions

The *in vitro* dissolution rate is clearly dependent on the pH, buffer species and buffer capacity of the medium for NSAID drug products. The USP test media clearly do not reflect the human intestinal *in vivo* environment and may not be suitable for *in vitro* BE dissolution studies. The results of this principally computational study could provide a guide for selecting a dissolution method and medium that can be used for establishing an *in vitro* bioequivalence standard.

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Conflict of Interest

The authors declare no conflict of interest.

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