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Toward an *In Vivo* Dissolution Methodology: A Comparison of Phosphate and Bicarbonate Buffers

Jennifer J. Sheng,[†,‡] Daniel P. McNamara,[§] and Gordon L. Amidon*[,†]

College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065, and Penwest Pharmaceuticals Co., Patterson, New York 12563

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Abstract: The purpose of this research was to evaluate the difference between the pharmaceutical phosphate buffers and the gastrointestinal bicarbonates in dissolution of ketoprofen and indomethacin, to illustrate the dependence of buffer differential on biopharmaceutical properties of BCS II weak acids, and to recommend phosphate buffers equivalent to bicarbonates. The intrinsic dissolution rates of ketoprofen and indomethacin were experimentally measured using a rotating disk method at 37 °C in USP SIF/FaSSIF and various concentrations of bicarbonates. Theoretical models including an improved reaction plane model and a film model were applied to estimate the surrogate phosphate buffers equivalent to the bicarbonates. Experimental results show that the intrinsic dissolution rates of ketoprofen and indomethacin in USP and FaSSIF phosphate buffers are 1.5−3.0 times that in the 15 mM bicarbonates. Theoretical analysis demonstrates that the buffer differential is largely dependent on the drug pK_a and second on solubility, and weakly dependent on the drug diffusivity. Further, in accordance with the drug pK_a, solubility and diffusivity, a simple phosphate surrogate was proposed to match an average bicarbonate value (15 mM) of the upper gastrointestinal region. Specifically, phosphate buffers of 13−15 mM and 3−4 mM were recommended for ketoprofen and indomethacin, respectively. For both ketoprofen and indomethacin, the intrinsic dissolution using the phosphate surrogate buffers closely approximated the 15 mM bicarbonate buffer. This work demonstrates the substantial difference between pharmaceutical phosphates and physiological bicarbonates in determining the drug intrinsic dissolution rates of BCS II weak acids, such as ketoprofen and indomethacin. Surrogate phosphates were recommended in order to closely reflect the *in vivo* dissolution of ketoprofen and indomethacin in gastrointestinal bicarbonates, which has significant implications for defining buffer systems for BCS II weak acids in developing *in vitro* bioequivalence dissolution methodology.

Keywords: BCS; weak acid; bicarbonate; buffer species; in vivo dissolution media

Introduction

Drug dissolution is the prerequisite to drug absorption and the subsequent clinical response for almost all drugs administered orally. For Biopharmaceutics Classification System (BCS) class II drugs, those with high permeability and low solubility, drug absorption is often rate limited by *in vivo* drug dissolution. Thus, a correlation between an *in vitro* dissolution and *in vivo* performance is expected if the *in vitro* dissolution rate is similar to the *in vivo* dissolution rate.[1] In order to develop the *in vitro*−*in vivo* correlation, the *in vitro*

* To whom correspondence should be addressed: Gordon L. Amidon, College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109-1065. Tel: 734-764-2440. Fax: 734-763-6423. E-mail: glamidon@umich.edu.

† University of Michigan.

‡ Current address: PAR&D, AstraZeneca LLP, 1800 Concord Pike, Wilmington, DE 19850.

§ Penwest Pharmaceuticals Co.

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dissolution testing method should be reflective of the *in vivo* situation. However, the inherent physiological complexity and variability of the GI tract, including such variables as transit time, unsteady hydrodynamics and changing fluid contents, presents a truly complex system to attempt to mimic.[2−7] It is important that the *in vitro* dissolution media, whose contents should mimic GI fluids, are matched closely for pH, buffer species and concentration, bile salts, electrolytes, enzymes and a wide range of lipids. Development of a suitable biorelevant *in vitro* dissolution medium has attracted intense interest.[4,8−12] Several dissolution media have been proposed as biorelevant, and some have been widely applied as *in vitro* dissolution media. For example, the most dominant dissolution media include the USP simulated intestinal fluids (SIFs)[13] and fasted-state simulated small intestine fluids (FaSSIF),[4,8,14] as shown in Table 1. However, these media were developed primarily to simulate the GI pH and bile salts concentration. Further, the buffer species employed in these two media is phosphate, which is not the primary constituent buffer of GI fluids. One seemingly very obvious choice as a buffer for a biorelevant dissolution media would be to include the GI buffer species, namely, the bicarbonates.

Table 1. Commonly Used Pharmaceutical Dissolution Media/Buffers for Simulating Upper Small Intestine

dissolution media	pH	buffer components
USP SIF	6.8	phosphate: 50 mM
		1% pancreatin
FaSSIF	6.5	phosphate buffer: 29 mM
		sodium taurocholate: 3 mM
		lecithin: 0.75 mM
		NaOH: adjust pH to 6.5
		NaCl: adjust to isotonicity

The principal physiological buffer in the human GI tract, and all mammalian species, is not phosphate, but rather bicarbonate. Gastroduodenal bicarbonate has long been shown as the main buffer system maintaining a pH gradient along the gastrointestinal lumen.[15] Bicarbonate is a ubiquitous component in human secreted fluids and is actively secreted by the pancreas to neutralize gastric secretion in the GI lumen. It has also been shown that epithelial cells of the duodenum copiously secrete bicarbonate, as an important mechanism to protect the duodenal epithelium from damage in the face of ongoing acid discharged from the stomach.[16−21] Bicarbonate concentrations in human GI fluids have been reported to be within a dynamic range, depending on the fasted and fed states as well as local regions along the GI

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tract.[17,19,22,23] For example, as early as in 1935, the bicarbonate concentration was measured directly from the fasted human duodenum using a titration method, and the values were reported in the range of 4−21 mM,[24,25] with an average of 15 mM.[23] In 2001, Repishti et al. measured the pH and the partial pressure of CO_2 (g) (P_{CO_2}) in human duodenum, and used the Henderson−Hasselbalch equation to calculate the bicarbonate HCO_3^- in fasted-state duodenum with a mean value of 6.7 ± 0.34 mM at pH 7.22.[26] Persson et al. [5] showed that the mean value of the buffer capacity of human jejunum under fasted conditions is 2.4−2.8 mmol L^{-1} pH^{-1}, which corresponds to 18.1 mM of bicarbonate concentration at pH 7.5, assuming that the buffer capacity is primarily determined by the buffer species and not by proteins. Recently, Kalantizi et al.[6] reported the range of buffer capacity for distal duodenum of fasted human, which is equivalent to 4.35−21.6 mM of HCO_3^- at pH 6.2.

Even though bicarbonate is the prevailing buffer in human gastrointestinal (GI) tract, it has rarely been used in dissolution studies[27] mainly owing to the perceived inconvenience of having to maintain a constant pH and HCO_3^- concentration in the dissolution medium through continuous CO_2 gas sparging. Almost all pharmaceutical dissolution studies apply phosphate or acetate buffers, subsequently creating a true inconsistency or disconnect with the chosen buffer species between the *in vitro* method and the physiological GI *in vivo* system. This disconnect should be closely scrutinized, because it has been previously shown that not only buffer concentration but also buffer species in dissolution media can significantly impact dissolution rates of ionizable drugs, even if the pH of buffers between media is held constant.[28−30] It was shown that the intrinsic dissolution rates of naproxen increase with the escalation of buffer concentration, which was demonstrated consistently in three buffers including phosphate, citrate and acetate.[30] More interestingly, this work also showed that naproxen demonstrated a decreasing dissolution rate in the buffer species phosphate > citrate > acetate, despite all the buffers being maintained at a comparable molar buffer concentration and same pH value.[30] The buffer differential, namely, the difference in intrinsic dissolution rates for a given compound in various buffer systems, is thus defined. Therefore, we propose that it is important to compare dissolution in a bicarbonate buffer with the commonly used USP SIF/FaSSIF phosphate buffers, thus investigating the impact of buffer species on dissolution rates of BCS II acidic drugs. Using model BCS II weak acids, such as ketoprofen and indomethacin, we not only demonstrated the dissolution difference between phosphates and bicarbonates experimentally but also provided a mechanistic understanding of the buffer differential theoretically, i.e., the dependence of buffer differential on biopharmaceutical properties of drugs. The work proposed simple phosphate buffer surrogates to be equivalent to *in vivo* bicarbonates, which is essential in establishing the *in vitro* meaningful bioequivalence dissolution medium.

Theoretical Section

Reaction Plane Model. The reaction plane model was initially developed in chemical engineering for describing the effect of rapid irreversible chemical reactions on mass transport phenomena.[31] The model was extended to pharmaceutical dissolution applications by Amidon et al.[30,32] In the current paper, the reaction plane model was further revised to better predict the effect of buffer concentration acidic drug flux.

The general continuity equation describing one-dimensional mass transport in a fluid[33] is composed of diffusive, convective, and reactive terms as:

$$\partial c_i / \partial t = D_i \mathrm{d}^2 c_i / \mathrm{d}z^2 - v_z \mathrm{d}c_i / \mathrm{d}z + R_i \qquad (1)$$

where D_i, c_i and R_i are the diffusion coefficient, the molar concentration, and the rate of reaction per volume of species i (mol/cm^3•s), v_z is the fluid velocity (cm/s), and t is the time (s). The overall mass transport model includes the acid−base reactions and convective diffusion, which are fast and slow process, respectively. The continuity equation can then be further simplified by recognizing that slow processes control the overall mass transport for a solid dissolving acidic drug.

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Assuming instantaneous reactions at the solid−liquid surface, mass transport in a rotating disk system at steady state can be simplified as

$$\partial c_i/\partial t = D_i \mathrm{d}^2 c_i/\mathrm{d}z^2 - v_z \mathrm{d}c_i/\mathrm{d}z = 0 \quad (2)$$

where v_z is the axial velocity of the fluid toward the disk. Litt and Serad[31] have shown that eq 2 can be scaled by introducing the dimensionless distance variable, n, for the axial distance z, as

$$n = (\Omega/\nu)^{1/2} z = 1.61\left(\frac{D}{\nu}\right)^{1/3}\frac{z}{h} = 1.61(Sc)^{-1/3}\frac{z}{h}$$
$$C_{in}(n) = (c_{in} - c_{ib})/(c_{i0} - c_{ib}) V(n) = v_z/(\nu\Omega)^{1/2}$$
$$Sc_i = \nu/D_i \quad (3)$$

where Ω is the angular velocity of the disk (rad/s), ν is the kinematic viscosity of the fluids (cm^2/s), c_{in} is the molar concentration of species at distance n, C_{in} is the dimensionless concentration of species i at position n, v_z is the axial velocity of the fluid as reported by Riddiford[34] [$-(v\Omega)^{1/2}(0.510n^2 - 0.333n^3 + ...)$], $V(n)$ is the dimensionless velocity of the liquid, and Sc_i is the dimensionless Schmidt number of species i.

$$\mathrm{d}^2 C_i/\mathrm{d}n^2 - VSc_i \mathrm{d}C_i/\mathrm{d}n = 0 \quad (4)$$

Levich has shown that the molar flux of acid from the disk surface is

$$N_{HA} = D_{HA}(\Omega/\nu)^{1/2} C_{HA_0} (Sc_{HA})^{1/3}/1.613$$
$$\mathrm{d}C_i/\mathrm{d}n = -(Sc_i)^{1/3}/1.613 \quad (5)$$

At the solid surface, the following reactions exist:

$$H_2O \rightleftarrows H^+ + OH^-$$
$$HA \rightleftarrows H^+ + A^-$$
$$HB \rightleftarrows H^+ + B^-$$
$$HA + B^- \rightleftarrows HB + A^-$$
$$HA + OH^- \rightleftarrows H_2O + A^-$$
$$HB + OH^- \rightleftarrows H_2O + B^- \quad (6)$$

After identifying various chemical reactions at the reaction plane, a set of mass balance equations accounting for all reacting species were established. When the flux of one species changes due to a chemical reaction at the reaction plane, it must be reflected by the flux of the corresponding reactants or products as defined in the aforementioned reactions (eq 6). Therefore at steady state, the following flux condition must hold at the solid−liquid interface:

$$\vec{N}_{H^+} + \vec{N}_{HA} + \overleftarrow{N}_{HB} = \overleftarrow{N}_{OH^-} + \vec{N}_{A^-} + \overleftarrow{N}_{B^-} \quad (7)$$

where $\vec{N}_{H^+}$, $\vec{N}_{HA}$, $\overleftarrow{N}_{HB}$, $\overleftarrow{N}_{OH^-}$, $\vec{N}_{A^-}$ and $\overleftarrow{N}_{B^-}$ individually denote the molar flux of H^+, HA, HB, OH^-, A^- and B^-. It should be noticed that the fluxes of species of A^- and B^- have different signs because they are supplied from opposite directions, i.e., from the solid surface or from the medium bulk to the reaction plane, respectively. Fluxes are vector quantities and as such they have magnitude and direction. Thus, electrical neutrality is maintained at the solid surface in eq 7.

Combined with eqs 5 and 7, boundary conditions at the surface ($n = 0$),

$$C_{HA} = 1$$
$$C_{A^-} = 1$$
$$C_{H^+} = 1$$
$$C_{HB} = 1$$

and boundary conditions in the bulk solution ($n = \infty$),

$$C_{HA} = 0$$
$$C_{A^-} = 0$$
$$C_{H^+} = 0$$
$$C_{HB} = 0$$

the flux condition was rewritten explicitly in the following system equation (eq): where CT_{HB} is the total molar buffer

$$\begin{Bmatrix} C_{H^+,0} \times C_{B^-,0} = K_a^{HB}(CT_{HB} - C_{B^-,0}) \\ D_{A^-}[HA]_0 K_a^{HA}(Sc_{A^-})^{1/3}/C_{H^+,0} + D_{B^-}(C_{B^-,0} - C_{B^-,b})(Sc_{B^-})^{1/3} + D_{OH^-}\left(\frac{K_a^w}{C_{H^+,0}} - \frac{K_a^w}{C_{H^+,b}}\right)(Sc_{OH^-})^{1/3} \\ = D_{H^+}(C_{H^+,0} - C_{H^+,b})(Sc_{H^-})^{1/3} + D_{HB}(C_{HB,b} - C_{B^-,0})(Sc_{HB})^{1/3} + D_{HA}[HA]_0(Sc_{HA})^{1/3} \end{Bmatrix} \quad (8)$$

concentration, $C_{B^-,0}$ is the molar concentration of the basic component at the solid−liquid interface, and K_a^{HA} and K_a^{HB} are the ionization constant of the drug and buffer, respectively. The only unknown variables in system eq are $C_{H^+,0}$ and $C_{B^-,0}$, which is readily solvable if the bulk pH, ionization constants of the acidic drug and the buffer, the total buffer concentration, the intrinsic solubility of the drug, and the diffusion coefficients of all species are available. Once $C_{H^+,0}$ is solved, the relative flux increase $(N_{total})/(N_0)$ can be calculated with eq 9:

$$\frac{N_{total}}{N_0} = 1 + \frac{K_a^{HA}}{C_{H^+,0}} \quad (9)$$

where N_{total} is the total drug flux at a specific pH and rotating speed, which includes the flux from both species HA and A^-; N_0 is the drug flux at the same rotating speed, but dominantly from species HA when the ionization or reaction of HA is negligible at low pH ($pH \ll pK^{HA}_a$). Using the relative flux increase $(N_{total})/(N_0)$, where N_0 is used to scale the effect of buffers, rather than the absolute total drug flux at specific condition makes the buffer comparisons more straightforward.

Film Model. The film model was derived originally in 1920's and further developed in 1960s,[35] and applied to drug

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Table 2. Parameters Used in Theoretical Analysis

species	pK_a	D ($\times 10^6$), cm^2/s	MW	solubility (M)
ketoprofen	4.76[a]	9.3[b] for HA form 9.2[c] for A^-	254.3	9.95×10^{-4}[a]
indomethacin	4.17[d]	8.0[b] for HA form 7.9[c] for A^-	253.3	9.58×10^{-6}
$H_2PO_4^-$ /HPO_4^{2-}	7.21[e] 7.18[f]	11.5[g]	98	
H_2CO_3	6.37[e] 6.31[f]	19.2[h]	44.0	
HCO_3^-		12.3[g]		
H^+		104.9[i]		
OH^-		63.0[i]		

[a] From ref 9. [b] Calculated using ADMET Predictor based on Einstein−Stokes equation for 37 °C. [c] Calculated using harmonic average of HA and H^+ forms. [d] From ref 27. [e] At 25 °C, from Physical pharmacy, fourth edition, by A. Martin and Lange's handbook of chemistry, fifth edition, by J. A. Dean, McGraw-Hill, Inc., 1999. [f] Calculated using Gibbs equation ($\ln K_a = \Delta G^\circ/RT$) with the consideration of temperature. [g] Limiting ionic mobility for $H_2PO_4^-$, HPO_4^{2-} and HCO_3^- at 37 °C is 41.6 and 44.5 cm^2/Ω/equiv, being converted to D using the unit conversion factor from $D = 2.769 \times 10^{-6}\lambda_i/Z_i$ (E. L. Cussler, Diffusion). [h] At 25 °C, from E. L. Cussler, second ed., 1997, p 112; [i] At 37 °C, from Lange's handbook of chemistry, fifth edition, by J. A. Dean, McGraw-Hill, Inc., 1999, p 8.168.

dissolution by Higuchi et al.[36] and further modernized by Stella et al.[28,37] Assumptions inherent in film models include an adherent stagnant layer of fluid through which it is assumed the dissolved drug (HA) must diffuse into the aqueous media.[28,29,37] This stagnant or diffusion layer can be calculated using a simplification to the Levich's rotating-disk model. The length of the stagnant layer is based entirely on the diffusivity of the drug (D_{HA}) irrespective of the presence of other species. Within this boundary layer, all concentration gradients of the reactants and products exist as a result of diffusion and instantaneous reactions between the dissolving drug, buffers, protons, hydroxyl ions and water. Unlike the reaction plane model, all reactions are reversible and homogeneous, and they are concurrent with diffusion throughout the boundary layer. The final working equation was derived and is detailed by Mooney et al.[28] The only unknown variable in film model is $C_{H^+,0}$, i.e., the H^+ concentration at the solid−liquid interface, which is solvable by the Newton iterative method using Mathematica 5.1 (Wolfarm Research, Inc., Champaign, IL). Once $C_{H^+,0}$ is solved, the relative flux increase can be calculated using eq 9.

Table 2 lists the physical parameters that were used in the reaction plane and film model predictions, including the pK_a values of model drugs and buffers, diffusivity values of all species, and the solubility of the model drugs. In both models, only the predominant ionization of phosphate or bicarbonate, under the working pH range of 6.5−6.8, was considered because other ionizations are negligible. Specifically, for the phosphate buffer, only the p$K_{a,2}$ of $H_2PO_4^-$ was utilized; and for the bicarbonate, the p$K_{a,1}$ of H_2CO_3 was considered.

Experimental Section

Materials and Dissolution Media Preparation. Ketoprofen, indomethacin (>99% purity), carbonic anhydrase, and all other chemicals were of analytical grade and were purchased from Sigma (St. Louis, MO). Distilled, deionized and filtered water was prepared in house and used for all experiments. Dried and compressed 100% CO_2 was purchased from LifeGas (Ann Arbor, MI). All 3-D plots were generated using Sigmaplot 10.0 (SPSS Inc., Chicago, IL).

The USP SIF pH 6.8, 50 mM phosphate buffer without pancreatin[13] and fasted-state simulated intestinal fluid (FaSSIF, 29 mM pH 6.5 phosphate buffer) without bile salts[4] were prepared following standard procedures. Sodium phosphate was used instead of potassium phosphate because the principle cationic species in fasted small intestine is sodium.[2,38,39] Previous study conducted by Reppas et al.[4] has demonstrated that substituting sodium for potassium in standard USP and International Pharmacopeia buffer systems has no practical effects on the dissolution process of weak acids. The 15 mM bicarbonate buffer was established by initially preparing a 15 mM of sodium carbonate solution, and then the solution was continuously sparged with CO_2 (g) until pH was reduced to 6.8 or 6.5. To ensure the bicarbonate buffer was equilibrating, both the pH and the total H_2CO_3/HCO_3^- (aq) content in the bicarbonate buffer were monitored. The pH was continuously monitored with a standard pH meter and electrode (PHI40, Beckman Coulter, Inc., Fullerton, CA), and the H_2CO_3/HCO_3^- (aq) content was checked periodically using methods described previously[40−42] with a standard CO_2 assay kit purchased from Sigma. After the CO_2 (aq) and the pH of the bicarbonate buffer reach an equilibrium, which takes 30−40 min at 37 °C, only pH is followed through the intrinsic dissolution testing. To maintain a steady CO_2 (aq) concentration and pH in the bicarbonate buffer at 37 °C, a continuous CO_2 (g) purge with a flow rate ~50 mL/min was generally required. Prior to dissolution testing, all buffers were adjusted to be isotonic to normal saline with NaCl.

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Table 3. Relative Intrinsic Flux of Ketoprofen and Indomethacin in Phosphate and Bicarbonate Buffer Systems Using Rotating Disk Method

ketoprofen			indomethacin		
buffer components	pH	exptl[a] (SD_{total})	buffer components	pH	exptl[a] (SD_{total})
USP SIF, w/o pancreatin, 50 mM phosphate buffer	6.8	35.6 (1.7)	USP SIF, 50 mM phosphate buffer: 5% CO_2, 6.4 mM bicarbonate	6.8	2.4 (0.3)
FaSSIF w/o bile salts, 29 mM phosphate buffer	6.5	17.6 (0.9)	USP SIF, 50 mM phosphate buffer: 10% CO_2, 12.9 mM bicarbonate	6.8	1.7 (0.3)
5.0 mM bicarbonate buffer	6.5	6.1 (0.3)	USP SIF, 50 mM phosphate buffer: 15% CO_2, 19.3 mM bicarbonate	6.8	1.5 (0.3)
15 mM bicarbonate buffer	6.5	10.5 (0.5)	USP SIF, 50 mM phosphate buffer: 20% CO_2, 25.8 mM bicarbonate	6.8	1.4 (0.3)
20 mM bicarbonate buffer	6.5	14.2 (0.8)	USP SIF, 50 mM phosphate buffer: 15 mM bicarbonate	6.8:6.5	2.4 (0.5)

[a] Relative flux (N_{total}/N_0), where N_{total} is the total intrinsic drug flux including the ionized and un-ionized forms under buffered conditions, and N_0 is the intrinsic drug flux for un-ionized form only occurring under acidic conditions.

Intrinsic Dissolution Measurement. The intrinsic dissolution rates of ketoprofen and indomethacin in various buffers were measured using a rotating disk system. Some 150 mg of ketoprofen and indomethacin powder were compressed under 2000 and 5000 lb, respectively, for 60 s to form a circular compact with a radius of 0.45 cm using a hydraulic laboratory press (Fred Carver, Inc., Summit, NJ). The die containing the compact was mounted onto a Plexiglass shaft attached to an overhead synchronous motor (Cole-Parmer Scientific, Niles, IL). The die was rotated at 100 rpm, which was calibrated with a digital tachometer (Cole-Parmer Scientific, Niles, IL). The single face of the compact was exposed to 150 mL of the dissolution media in a jacketed beaker maintained at 37 ± 1 °C via circulating water heated with a water bath circulator (Isotemp Constant Temperature Circulator Model 8000, Fisher Scientific, Pittsburgh, PA). At predetermined time points, 1.0 mL of dissolution sample was withdrawn and same amount of blank dissolution medium was replaced. The ketoprofen and indomethacin concentration in the dissolution media samples were measured using UV absorption at 258 and 265 nm, respectively, using a UV spectrophotometer (Beckman Coulter DU 650, Fullerton, CA). To ensure sink conditions, the concentrations of ketoprofen or indomethacin in the dissolution media were maintained less than 10% of their solubility for the entire experiment. Dissolution flux (mass/time/area) of ketoprofen and indomethacin were calculated using the linear slope of the concentration vs time plot, volume of dissolution medium (150 mL), and area of the exposed disk (0.636 cm^2). Individual dissolution experiments typically were carried out for 10 min for ketoprofen and for up to several hours for indomethacin.

For reference and comparison, dissolution flux of ketoprofen and indomethacin in the following isotonic media were determined: pH 6.8 USP SIF 50 mM phosphate buffer without pancreatin, pH 6.5 FaSSIF containing 29 mM phosphate buffer without sodium taurocholate or Lecithin, and pH 6.5 various concentrations of bicarbonate buffers that covers a normal range of bicarbonate concentrations along the fasted human duodenum. Enzymes and bile salts were not included in the dissolution media in these studies as the purpose of these investigations was specifically to reveal the effects of buffer species. The final pH values of the dissolution media using phosphates were checked at the end of the dissolution experiments to ensure that a constant pH has maintained throughout the dissolution testing.

Results

Dissolution of Ketoprofen and Indomethacin in Phosphate and Bicarbonate Buffers. The purpose of these studies was to evaluate the dissolution difference of ketoprofen and indomethacin determined in bicarbonate buffers as compared to the commonly used phosphate buffers. Table 3 lists the intrinsic dissolution rates of ketoprofen in two phosphates (USP and FaSSIF), and three bicarbonate buffers with concentrations of 5, 15 and 20 mM, and it also lists the flux ratio of indomethacin in phosphates versus in bicarbonates with concentrations of ranging 6.4−25.8 mM. As expected, the higher the concentration of bicarbonate, the faster the drug flux of indomethacin or ketoprofen. The increase is because a greater driving force for the forward reaction between the weakly acidic drug and basic species HCO_3^-, as shown in eq 10, exists with the presence of higher bicarbonate concentrations.

$$HA + HCO_3^- \rightleftharpoons A^- + H_2CO_3\,(aq) \qquad (10)$$

More importantly, the intrinsic dissolution rates in the USP SIF buffer and in the FaSSIF are higher than in all bicarbonate buffers. For example, the respective dissolution rate of ketoprofen in the USP SIF and FaSSIF is at least 200% and 50% faster than that in the 15 mM bicarbonate, an average value in fasted duodenum. Again, the intrinsic dissolution rate of indomethacin in phosphate buffer is higher, showing about 30−150% of flux increase compared with that in various concentrations of bicarbonate buffers.

Comparison of the results in Table 3 between the phosphates and the bicarbonates is confounded by main differences in pH (6.8 versus 6.5), buffer concentration and buffer species. All of these could contribute to the observed dissolution rate differences. To simplify the comparison, the intrinsic dissolution rates of ketoprofen were measured in phosphate and bicarbonate media with the same pH, i.e., 6.8, and the same buffer concentration, i.e., 50 mM. Table 4 lists the results of the simplified comparison and show that the

Table 4. Intrinsic Dissolution Rates of Ketoprofen in 50 mM pH 6.8 Phosphate and Bicarbonate Buffers

buffer components	pH	mean flux (mg/cm^2/min) (n = 3, SD)	exptl (SD_{total})
USP SIF, phosphate	6.8	0.783 (0.010)	35.6 (1.7)
bicarbonate	6.8	0.352 (0.003)	16.1 (0.7)
SGF, 0.1 N HCl	1.2	0.022 (0.001)	1.0

ketoprofen flux in phosphate was still 2-fold of that in the bicarbonate in spite of the comparable pH and buffer concentration. These experimental results support the hypothesis that even if at the same pH and the same buffer concentration is used, the dissolution of ketoprofen in phosphate and bicarbonate buffers are inherently distinctive and different from each other. The distinction lies in the natural chemical and physical differences between phosphate and bicarbonate, namely, the pK_a and the diffusion coefficient.

First of all, the pK_a of the buffer plays an important role in the driving force for the reaction between the acidic drug and the basic buffer species. At the solid−liquid interface, the primary chemical reaction driving the drug dissolution is the following:

$$HA + B^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} HB + A^-, K = \frac{k_f}{k_r} = \frac{K_a^{HA}}{K_a^{HB}} \qquad (11)$$

Equation 11 clearly indicates that a basic buffer with a higher pK_a value translates into a smaller K_a^{HB}, which yields a larger overall equilibrium constant K for the reaction. Consistent with the experimental results, at 37 °C phosphate buffer with an effective pK_a of 7.19 provides greater driving force for the acidic drug−buffer reaction than that of bicarbonate with a pK_a of 6.31 (shown in Table 2), thus leading to faster dissolution rate for ketoprofen and indomethacin.

Second, the pK_a difference in these two buffer species also results in different buffer capacity at the solid−liquid interface. For a system the same pH and the same buffer concentration, phosphate buffer has about 23% higher buffer capacity relative to the bicarbonate, according to eq 12.

$$\beta = 2.303C\frac{K_a^{HB}[H^+]}{(K_a^{HB} + [H^+])^2} \qquad (12)$$

where β is the buffer capacity. In general, the $C_{H^+,0}$ concentration at the solid−liquid interface is lower than that in the bulk due to the ionization of acidic drug molecule. Within the boundary layer or at the solid−liquid interface, the buffer capacity is controlled not only by the extent of acidic drug disassociation but also the buffer capacity. The higher buffer capacity of phosphate maintains the pH at the solid−liquid interface lower than, but closer, to the basic environment of the bulk, in relative to the bicarbonate system. Thus, a greater extent ionization of acidic drugs and the subsequent increase of drug dissolution in the phosphates are present.

Third, the diffusion coefficient of buffer species may also have an indirect impact on drug dissolution rates through diffusional layer thickness. The Levich equation[43] indicates that the diffusional layer thickness $h = 1.612D^{1/3}\upsilon^{1/6}\varpi^{-1/2}$ is accounted for one species, generally the drug molecule. In reality, there are a number of species involved in drug dissolution using the rotating disk. Therefore, it may be logical to consider the boundary layer thickness is an average value composed of all of the species involved in the dissolution. Thus, the species include not only the drug molecules but also the conjugate buffers HB and B^-, and OH^- and H^+. It is apparent that a smaller diffusion coefficient D would produce a thinner boundary layer thickness, which implies less resistant during mass transport. Specifically, the diffusivity for $H_2PO_4^-/HPO_4^{2-}$ is 11.5×10^{-6} cm^2/s, which implies a thinner diffusional layer thickness and a subsequent faster drug flux, relative to H_2CO_3 and HCO_3^- with respective diffusivity values of 12.3×10^{-6}cm^2/s and 19.5×10^{-6} cm^2/s.

Dependence of Buffer Differential on Biopharmaceutical Properties of Drugs. Experimentally, this work has shown that ketoprofen and indomethacin demonstrated up to 200% dissolution rate increase in USP SIF and FaSSIF phosphate than in the 15 mM bicarbonate buffer. Theoretically, the buffer differential can be forecasted reasonably well using reaction plane model and film model. The question is: for a given new drug entity, what is the magnitude of the buffer differential? To answer this question, three key parameters including drug pK_a, solubility and diffusion coefficient, which are used in the theoretical analysis, are investigated to determine their importance on the buffer species effects.

The buffer differential effects were indicated by the drug flux ratio in the USP SIF to that in the 15 mM bicarbonate. Theoretical analysis indicated that drug pK_a has a profound impact on buffer differential, as shown in Figure 1. For weak acids with pK_a values of 7 or higher, the drug flux ratios in these two tested buffers were close to 1. This result is reasonable because the ionization difference of these drugs in pH 6.8 USP SIF and pH 6.5 bicarbonate is limited. The observed drug flux is essentially contributed from the un-ionized form and thus determined by the intrinsic solubility of the un-ionized form, which should not differ appreciably in various buffers. In comparison, for weakly acidic drugs with pK_a values of 6.5 or less, their intrinsic dissolution rates is about 50−200% higher in the USP SIF than that in the 15 mM bicarbonate buffer (Figure 1). Thus, the magnitude of buffer differential depends strongly on drug pK_a whose value is in the regions of <6.5, and very weakly in regions of drug pK_a > 7.0. It should be noticed that this theoretical calculation underestimates the flux ratio in these two tested buffers. For example, the film model and reaction model predicted that the flux ratio of ketoprofen in these two buffers was 1.77 and 2.12, respectively, which was lower than the experimental ratio of 3.33. A similar result was seen with

(43) Levich, V. G. *Physicochemical hydrodynamics*; Prentice-Hall: Englewood Cliffs, N.J., 1962.

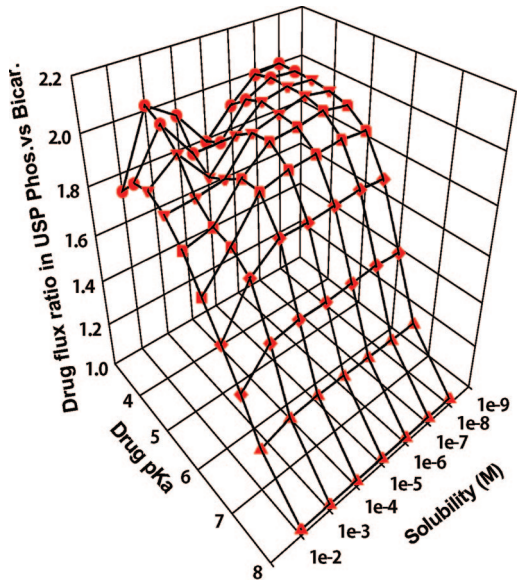

Figure 1. Dependence of drug flux ratio in the USP 50 mM phosphate buffer versus 15 mM bicarbonate buffer on drug pK_a and solubility. The drug diffusion coefficient is assumed to be 5×10^{-5} cm^2/s.

indomethacin, i.e., the flux ratio forecasted theoretically was less than the experimental value. It is also evident from Figure 1 that the buffer differential is more sensitive to the changes of drug pK_a but much less to the drug solubility. The following hypothesizes the high dependence of drug flux ratio on drug pK_a. It is the drug pK_a, rather than the drug solubility, predominantly controls the extent of ionization of drug molecules at the solid−liquid interface. As a result, the concentration of ionized drug species varies mainly with the drug pK_a, and subsequently controls the overall reaction with the buffer components from the dissolution medium and the buffer differential.

In addition to drug pK_a, drug solubility also plays an important role in considering buffer effects in drug dissolution due to its self-buffering capability. Therefore, based on eq 9, $N_{total}/N_0 = 1 + (K_a^{HA}/C_{H^+,0})$, acidic high-solubility drug increases the $C_{H^+,0}$ at the solid−liquid interface, leading to an increase of absolute drug flux N_{total} but a decrease of the relative drug flux N_{total}/N_0. Further, the ratio of the relative drug flux in two buffers, i.e., the buffer differential, is much weakly dependent on drug solubility, due to a similar magnitude change of N_{total}/N_0 in two buffers. Theoretical analysis showed that the buffer differential varies less significantly with drug solubility variation (data not shown). In addition to drug pK_a and solubility, drug diffusivity has a negligible impact on the buffer differential (data not shown). In summary, the importance of drug properties on the magnitude of buffer differential decreases in the following order: drug pK_a > drug solubility ≈ drug diffusivity.

Surrogate Buffer Equivalent to the Bicarbonate. As demonstrated evidently in this work, ketoprofen and indomethacin show higher intrinsic dissolution rates in the commonly accepted USP SIF and FaSSIF phosphate buffer than in the physiological 15 mM bicarbonate buffer. Even though bicarbonate is the prevailing human physiological buffer, it is rarely used as an *in vitro* dissolution media. The decision to not use bicarbonate is mainly due to its inconvenience because maintaining a stable bicarbonate buffer system requires an equilibrium between P_{CO_2} (g) and the dissolution media at a given pH, which is generally achieved by continuous purging CO_2 (g) into the media to compensate the fast loss of CO_2 to the atmosphere at 37 °C.[44] Practical considerations lead to the alternative using a simple buffer such as phosphate buffer, as a surrogate for the bicarbonate. The ideal surrogate buffer should behave similarly to the physiological bicarbonates, exhibit almost the same dissolution rates for the same drug, and preferably be easily prepared and maintained. In this work, phosphate buffer surrogates for ketoprofen and indomethacin were initially forecasted using both the reaction plane model and film model, and then were confirmed using intrinsic dissolution measurement. For ketoprofen, the reaction plane model and film model predicted that 12−14 mM phosphate buffer at pH 6.5 is equivalent to the 15 mM bicarbonate, and experimentally 13.0 mM phosphate was selected, as shown in Table 5. The intrinsic dissolution rate of ketoprofen in 13.0 mM phosphate buffer is 0.198 mg/cm^2/min, which is 85% of 0.231 mg/cm^2/min, the dissolution rate in the 15 mM bicarbonate. Similarly, for indomethacin, theoretical analysis predicted that ∼3−4 mM phosphate buffer is suitable as the bicarbonate surrogate. Then, a 3.5 mM phosphate buffer was used, in which the indomethacin intrinsic dissolution rate was 26.0 μg/cm^2/min. This drug flux is equivalent to 108% of the intrinsic dissolution rate in the 15 mM bicarbonate. Here, theoretical analysis is useful to recommend a suitable phosphate concentration that is replaceable for the bicarbonate. It is also apparent in Table 5 that the surrogate phosphate buffer is not one single universal medium for all drug

Table 5. Phosphate Buffer as an Equivalent Substitute for 15 mM Bicarbonate Buffer

	exptl drug flux (SD)	
buffer components (pH 6.5)	ketoprofen (mg/cm^2/min)	indomethacin (μg/cm^2/min)
29 mM phosphate	0.386 (0.010)	40.0 (3.5)
15 mM bicarbonate	0.231 (0.002)	24.0 (4.8)

	theor phosphate concn (mM)	
theor analysis	ketoprofen	indomethacin
reaction plane model	12.0	∼3.0
film model	13.7	∼4.0

	ketoprofen	indomethacin
exptl phosphate	13.0	3.5
exptl drug flux (SD) using the bicarbonate substitute	0.198 (0.004)	26.0 (0.3)
proficiency to bicarbonate	86%	108%

(44) Boni, J. E.; Brickl, R. S.; Dressman, J. Is bicarbonate buffer suitable as a dissolution medium? *J. Pharm. Pharmacol.* **2007**, *59*, 1375–1382.

molecules, but rather it should be individualized according to the unique properties of drug molecules including pK_a, solubility and diffusivity.

Discussion

Significance of Physiologic Bicarbonate Buffer. To constitute an ideal *in vitro* dissolution medium, buffer species and concentration, pH, bile salts and viscosity of the GI fluids should all be considered. This work was to seek the significance of physiological buffer species and concentration, without inclusion of any bile salts, in dissolution of BCS II acidic drugs. The rationale is the following. First, studying a buffer system without including any other variables such as bile salts would distinctively reveal the buffer effects. Second, bile salts at fasting stage may not be important, due to its low concentration rage of 3−5 mM, with an average value of 4.3 mM.[8] A more recent study reported that the bile salt level in fasted human intestinal fluids was 2 ± 0.2 mM.[5] Third, even though bile salts contribute significantly on solubilization and dissolution of low-solubility drugs, their effects on ionizable drugs become minimal if pH change is present. This is particularly true for drugs with pK_a values within the pH range of proximal small intestine. Here, the two model compounds ketoprofen and indomethacin have pK_a values of 4.76 and 4.18, respectively. If the pH in proximal small intestine is around 6.5, their solubility would be increased approximately 100-fold. In comparison, their solubility enhancement from a low concentration of 2−5 mM bile salt would be negligible. Our previous studies have demonstrated that buffer/pH effects appear to be more important than surfactant effects in the case of BCS II acids such as piroxicam[11] and ketoprofen.[45]

Evidently even with FaSSIF, a phosphate buffer with much lower concentration of 29 mM at pH 6.5, the dissolution of ketoprofen and indomethacin still demonstrated a higher rate in the FaSSIF than in the bicarbonate. Thus, an *in vitro* dissolution testing in either USP SIF or FaSSIF is generally overestimating the true dissolution rates of ketoprofen and indomethacin *in vivo*. This overestimation, suggested by theoretical analysis, is extrapolated to other BCS II weak acids particularly for those with pK_a values less than 6.5. Therefore, although the pH value of USP SIF buffer and FaSSIF may mimic small intestine fluids, the buffer composition and concentration also have significant impacts on BCS II weakly acidic drugs. It is concluded here that not only the pH but also, equally important, the buffer species and concentrations should be considered in composing the *in vitro* dissolution media to closely reflect the *in vivo* dissolution fluids. This is key to poorly and ionizable drugs in selecting the appropriate buffer systems in developing the *in vitro* BE dissolution medium.

Similar to weak acids, BCS II weakly basic drugs may demonstrate the buffer differential between the phosphate and bicarbonate highly dependent on the drug pK_a. For a weak base with pK_a values close to or higher than the pH range in the small intestine, its significant ionization is expected. As a result, the ionized form and the free base form react with the buffer components, and the buffer differential effects will be observed. In comparison, for weak bases with pK_a lower than the pH range and very low solubility, which subsequently exhibit negligible disassociation in upper small intestine, a difference between the phosphate and bicarbonate is trivial. Dipyridamole is an example of such drug, i.e., a weak base with pK_a value of 6.05−6.10 and low solubility of 5.8 μg/mL at 25 °C. At pH 6.8, dipyridamole intrinsic flux is independent of the buffer species or concentration, where USP SIF, FaSSIF and bicarbonate buffer were employed.[27]

Table 6. pK_a Values, Maximum Dose, and Salt Forms of Some BCS II Weak Acids

compound	pK_a values	maximum dose (mg)	acid/salt form	provisional BCS classification
acetyl-salicylic acid	3.5[47]	975	free acid	BCS I or III
atorvastatin	4.46[47]	80	calcium salt	BCS II[48]
diacerein	4.74[a]	50	free acid	non-US marketed drug
diclofenac	4.2[49]	50	K and Na salts	BCS II
diflunisal	3.0[47]	500	free acid	BCS II
etodolac	4.7[47]	400	free acid	BCS II
epalrestat	3.2[a]	50	free acid	non-US drug
fenoprofen	4.5[49]	600	calcium salt	BCS II
flurbiprofen	4.3[49]	100	free acid	BCS II
fluvastatin	4.76[a]	40	Na salt	BCS II
furosemide	3.88, 9.37[a]	80	free acid	BCS II
ibuprofen	4.4[49]	800	free acid	BCS II
indomethacin	4.5[49]	50	free acid	BCS II
ketoprofen	4.76[49]	75	free acid	BCS II
ketorolac	3.5[49]	20	tromethamine salt	salt form: BCS I
mefenamic acid	4.2[47]	250	free acid	BCS II
meloxicam	1.1, 4.2[50]	15	free acid	BCS II
naproxen	4.2[49]	500	free acid	Acid: BCS II[51]
		500	Na salt	Na salt: BCS I[52]
oxaprozin	4.3[50]	600	free acid	BCS II
piroxicam	1.8, 5.1[50]	20	free acid	BCS II
salicylic acid	3.0[47]	750	free acid	BCS I
sulindac	4.5[49]	200	free acid	BCS II
triflusal	4.15[a]	300	free acid	non-US drug
tolmetin[53]	3.5[49]	600	Na salt	BCS II
zaltoprofen	4.44[a]	80	free acid	non-US drug
warfarin	5.35[a]	10	Na salt[54]	BCS I

[a] Calculated using Program ADMET version 1.2.3.

Marketed BCS II Weak Acids. Evidently, the magnitude of the buffer effects depends on the biopharmaceutical properties of the drug molecule. Table 6 lists almost all BCS II weak acidic drugs currently in US market and foreign countries. All of the listed drugs have pK_a values less than 5.5. As demonstrated in this work, significant buffer differential between USP SIF and FaSSIF, and the bicarbonates, should be expected for any BCS II weak acids with a pK_a less than 5.5. Specifically, only considering the effect of

(45) Sheng, J. J.; Kasim, N. A.; Chandrasekharan, R.; Amidon, G. L. Solubilization and dissolution of insoluble weak acid, ketoprofen: effects of pH combined with surfactant. *Eur. J. Pharm. Sci.* **2006**, *29*, 306–314.

buffer species, the dissolution rates of these weak acids in the USP SIF and FaSSIF would have been overestimated 50−200% fold of the true values *in vivo*.

In Table 6 several BCS II weak acids are marketed as sodium, potassium and calcium salts. In general, the salt forms would have a faster dissolution rate than the corresponding acid in the upper small intestine. We hypothesized that the salt forms of poorly soluble acids should also demonstrate differential dissolution rates in USP SIF and FaSSIF relative to the bicarbonate buffers. This is because at upper small intestine the salt form of a weak acid, at a greater extent but behave similarly to the corresponding acids, disassociates to the ionized acidic species that will react with the buffer systems. A very low solubility salt form such as atorvastatin calcium, which is insoluble in aqueous solution at pH equal or below 4.0, and is very slightly soluble in distilled water at 37 °C,[46] the impacts of buffer differential may be similar to the case of indomethacin. The quantitative impact of buffer differential, however, on the salt forms of weak acids requires further research.

Buffer Systems for *In Vitro* Bioequivalence Dissolution: Phosphate Surrogates. Dissolution tests are used to achieve two major objectives during drug product development: (1) to serve as quality control (QC) specification checking the reproducibility of manufacturing processes and products; and (2) to forecast the *in vivo* performance of drug products. The experimental test conditions for QC are designed to detect manufacturing variables and stability changes on storage, whereas test conditions for BE should discriminate adequately among products/batches with different *in vivo* release behavior. For the QC testing of BCS II weak acids, the USP[13] suggests a wide range of dissolution media. For example, the media used in the USP monograph for indomethacin is 20% pH 7.2 phosphate and 80% water, for etodolac ($pK_a = 4.7$) is pH 6.8 phosphate, and for sulindac ($pK_a = 4.5$) is 0.1 M pH 7.2 phosphate. However, none of these pharmacopeial monograph phosphate buffers is physiologically relevant. They are primarily useful in checking reproducibility of products during manufacturing procedures to meet regulatory requirements. However, they poorly predict the *in vivo* performance of a drug product. Therefore, an *in vitro* bioequivalence (BE) dissolution testing media, with which physiologically relevant conditions of the GI fluids are closely reflected, is essential to improve the assessment of *in vivo* drug product dissolution.

Across the industry, a lack of success has been observed in developing IVIVC for numerous BCS II immediate-release oral dosage forms, which is attributed to the dissolution discrepancy between the *in vitro* testing and the *in vivo* situation. Among many factors designed into the *in vitro* dissolution bioequivalence testing, evidently the buffer system is very important and yet to be fully considered in order to mimic the *in vivo* bicarbonates. For example, as demonstrated in this work, the dissolution profiles for BCS II drugs such as ketoprofen and indomethacin are showed to exhibit 200% and 50% differences in the monograph phosphate buffer from the average physiological bicarbonate. As a result, when *in vivo* dissolution is the rate-limiting step to the overall absorption of a BCS II weak acid, an expected IVIVC may not be observed, if the pharmacopeial phosphate buffer is used.

In establishing *in vitro* BE dissolution medium, it is evident that the bicarbonates are the best buffer system representing the *in vivo* GI situation. Practical considerations, however, lead to the use of easily prepared buffer systems, such as phosphate buffers to surrogate the bicarbonates. The concentrations of surrogate phosphate vary most significantly with the drug pK_a and second with the drug solubility. For drugs with high pK_a values such as above pH 7.0, the drug dissolution rate is only weakly influenced by buffer species and concentration. In this scenario, the commonly used USP SIF or FaSSIF behaves similarly to the bicarbonates. In contrast, BCS II weak acids with pK_a values lower than 5.5, which prevails the current market, would show around 50−200% difference in USP SIF from the bicarbonates (Figure 1). To minimize this discrepancy, a lower concentration of phosphate is required to match the drug flux in the bicarbonates at pH 6.5, as suggested in our work. In addition, for drugs with similar pK_a, a low-solubility drug may require a lower phosphate buffer to mimic the bicarbonates than a relative high-solubility drug. For example, ketoprofen with an intrinsic solubility of 9.95×10^{-4} M requires 13−15 mM of phosphate to mimic the bicarbonates, whereas 3−4 mM of phosphate appears to be sufficient for a lower solubility drug such as indomethacin with solubility of 9.58×10^{-6} M. The effect of drug solubility on the surrogate phosphate concentration may result from the self-buffering effect of drug molecules at the solid−liquid interface. As the drug molecules dissolve and then disassociate into the ionized acid−base pairs, which maintains the microenvironmental

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pH within the boundary layer and functions as self-buffering species in contact with the incoming bulk buffers. A weak acid with high solubility has a higher concentration of the acid−base pair within the boundary layer, which leads to a higher self-buffering capacity and less susceptibility to the changes of bulk buffer.

It should be noted that, if a dissolution medium is designed in order to reach maximum or even 100% of release within the duration of the test, it may satisfy the dissolution specification of a product and serves the QC purpose. However, it generally does not represent the *in vivo* condition and therefore may not be suitable for BE dissolution purposes. In a typical QC dissolution testing, more than 75−80% of the drug release has dissolved at the final evaluation time point. In contrast, a BE or biorelevant dissolution medium for BCS II drugs should be aimed to reflect the true GI fluids as closely as possible, whereas mirroring the reality of the *in vivo* dissolution is essential rather than simply to aim the 100% drug release.

Conclusions

This work underscores the importance of using physiological buffers when determining the drug intrinsic dissolution rates, particularly for BCS II weakly acidic drugs. Based on drug pK_a, solubility and diffusivity, and buffer characteristics, theoretical analysis was successfully applied to semiquantitatively forecast the drug flux in various phosphates and bicarbonates. Practical considerations and convenience may lead to the use of surrogate buffers such as phosphate to mimic physiological bicarbonates. It is expected that the bicarbonate surrogate should better reflect the *in vivo* dissolution fluid, thus further mimicing the *in vivo* performance and further improving the *in vitro* dissolution medium for BE purposes.

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