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RESEARCH ARTICLE

Dissolution of a Biopharmaceutics Classification System Class II Free Acid from Immediate Release Tablets Containing a Microenvironmental pH Modulator: Comparison of a Biorelevant Bicarbonate Buffering System with Phosphate Buffers

Dorota Haznar-Garbacz[1] · Dagmara Hoc[2] · Grzegorz Garbacz[3] · Marek Lachman[4] · Daria Słomińska[5] · Michał Romański[6]

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Abstract
Poor water dissolution of active pharmaceutical ingredients (API) limits the rate of absorption from the gastrointestinal tract. Increasing the pH of a solid form microenvironment can enhance the dissolution of weakly acidic drugs, but data on this phenomenon in a physiologically relevant bicarbonate media are lacking. In this paper, we examined the effect of a microenvironmental pH modulator (Na_2HPO_4) on the dissolution of a Biopharmaceutics Classification System (BCS) class II free weak acid (ibuprofen) at biorelevant conditions, including an automatic bicarbonate buffering system, as well as in compendial (50 mM) and low-concentration (10 mM) phosphate buffers with no external pH control. The tablets of 200 mg ibuprofen with either Na_2HPO_4 (phosphate formulation, PF) or NaCl (reference formulation, RF) were manufactured using a compression method. In a pH 2 simulated gastric fluid, only PF produced a transient supersaturation of ibuprofen, dissolving a fourfold higher drug amount than RF. In a bicarbonate-buffered simulated intestinal fluid with a dynamically controlled pH (5.7, 7.2, and 5.8 to 7.7 gradient), PF dissolved more drug within 30 min than RF ($p \leq 0.019$). Of note, the use of a 50 mM phosphate buffer pH 7.2 provided opposite results—RF dissolved the API much faster than PF. Moreover, 10 mM phosphate buffers of pH 5.6 and 7.2 could neither maintain a constant pH nor mimic the bicarbonate buffer performance. In conclusion, the use of a bicarbonate-buffered intestinal fluid, instead of phosphate buffers, may be essential in dissolution tests of BCS class II drugs combined with pH modulators.

KEY WORDS dissolution model · microenvironmental pH modulators · supersaturation · weak acid

INTRODUCTION

Dissolution of active pharmaceutical ingredients (API) from solid oral forms in the gastrointestinal tract (GIT) is a prerequisite for drug absorption into blood and exerting of systemic effects (1–3). For API belonging to the Biopharmaceutics Classification System (BSC) class II, the rate of the

Dorota Haznar-Garbacz and Michał Romański equally contributed to the paper.

✉ Michał Romański
michalroman@ump.edu.pl

[1] Department of Drug Form Technology, Wroclaw Medical University, 211a Borowska St., 50-556 Wrocław, Poland

[2] Physiolution Polska, 74 Piłsudskiego St., 50-020 Wrocław, Poland

[3] Physiolution GmbH, 49a Walther-Rathenau-Straße, 17489 Greifswald, Germany

[4] Budenheim KG, 27 Rheinstraße, 55257 Budenheim, Germany

[5] Department of Pharmacology, Poznan University of Medical Sciences, 3 Rokietnicka St., 60-806 Poznań, Poland

[6] Department of Physical Pharmacy and Pharmacokinetics, Poznan University of Medical Sciences, 3 Rokietnicka St., 60-806 Poznań, Poland

in vivo dissolution limits the rate of absorption and, sometimes, the absorbed dose fraction (bioavailability) as well (3, 4). Therefore, various formulation technologies have been developed to improve water solubility and thus dissolution rate of BCS class II members. These include micronization, nano-formulations, solid dispersions, addition of solubilizers, creation and stabilization of amorphous solids, and *in situ* micelle formation (5–8). When BCS class II drugs have the character of weak acids and bases, the use of salt forms instead of free neutral species is a common strategy of solubility enhancement. A weakness of this approach lies in the pH-dependent disproportionation of ionized species to neutral molecules that occurs either in the stomach—in case of acidic drugs or small intestine—in case of basic drugs (9, 10). The above process is usually accompanied by precipitation of neutral API in the GIT, which in turn may lead to irregular and variable drug concentrations in blood (10–13). For instance, multiple peaks of diclofenac (weak acid) in plasma observed after oral administration of its salts have been attributed to drug precipitation and agglomeration in the stomach resulting in irregular gastric emptying (13).

From a mechanistic point of view, the rate of diffusion-controlled dissolution from solid forms depends on the drug solubility in the microenvironment (diffusion layer) of the solid particles (14–17). Therefore, the modulation of the pH of the microenvironment by addition of relevant excipients has emerged as a strategy to accelerate the dissolution of BCS class II API from solid dosage oral forms (18–25). To our best knowledge, no studies examined how the microenvironmental pH modulation affects the *in vitro* dissolution of BCS class II weak acids in the small intestine-relevant bicarbonate buffer, the medium from which the drug absorption occurs *in vivo*; instead, the phosphate solutions were used. Meanwhile, it is well known that in the absence of microenvironmental pH modulators, such APIs dissolve with different kinetics in bicarbonate and phosphate buffers (14–17). A compendial 50 mM phosphate buffer or simulated intestinal fluids (SIF) buffered with phosphates have several times higher buffer capacity in the solution bulk than the physiological bicarbonate buffer, thus overestimating the dissolution rate of BCS class II weak acids and bases. Efforts of different research groups showed that it is possible to find the smaller concentration of phosphates that provide the same rate of the dissolution as *in vivo* bicarbonates; however, this is not straightforward for at least two reasons (14–17). First, a bicarbonate buffer specifically decreases its pK_a and buffer capacity in the diffusion layer compared with the fluid bulk, because the hydration of CO_2 to form carbonic acid occurs much slower in the former region than in the latter (16, 17). Second, the physicochemical properties of the API itself, like pK_a, solubility, and diffusivity, affect the drug-buffer interaction and thus contribute to the pH of the diffusion layer. Therefore, the concentration of the surrogate phosphate buffer is drug-specific (15–17). According to the model created by Krieg *et al.* (16), it depends on the target pH of the bulk solution as well as the pK_a and intrinsic solubility of API free weak acid. One can foresee that the dissolution of weak acids from solid dosage forms containing a microenvironmental pH modulator is more complex, as the pH of the diffusion layer is governed by the interplay of three contributors: the buffer, API, and pH modulator. Also, the finding of the phosphate buffer providing equivalent dissolution to the bicarbonate medium becomes more sophisticated.

The use of surrogate low concentration-phosphate buffers in dissolution test is often justified by the inconvenience of continuous supply of gaseous CO_2 into a physiologically relevant bicarbonate buffer. In case of weakly acidic APIs, the practical advantage of phosphate media holds true when the concentration of the dissolved API is low and/or its pK_a is high enough to avoid shifting the bulk solution pH. Otherwise, the external control of pH by titration with alkali is necessary, which compromises the convenience of the use of phosphate media (14–16, 31). This aspect needs consideration also when studying the dissolution from solid dosage forms containing microenvironmental pH modulators.

The present paper was aimed at comparing the dissolution of a BCS class II weak acid from immediate release (IR) tablets in a biorelevant bicarbonate-buffered SIF and simple phosphate buffers. In particular, we verified if phosphate buffers with no external pH control can reflect the same acceleration effect of a diffusion layer pH modulator (Na_2HPO_4) on the dissolution as the biorelevant bicarbonate solution with automatic dynamic pH adjustment (26). We chose ibuprofen as a representative of BCS class II weak acids because of a common use in the form of IR tablets.

MATERIALS AND METHODS

Materials

A certified reference standard of racemic ibuprofen was purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile for high-performance liquid chromatography (HPLC), gradient grade, was supplied by Merck KGaA (Darmstadt, Germany). Analytical grade sodium chloride, potassium chloride, calcium chloride 2-hydrate, potassium dihydrogen phosphate, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate 2-hydrate, magnesium sulfate 7-hydrate, and sodium bicarbonate were all obtained from commercial suppliers. Deionized water with a conductivity of 5.5 μS/m, used for HPLC, was prepared in a deionizer Thermo Scientific GenPure Pro equipped with a carbon filter and UV lamp.

Preparation of Tablets

A designed tablet formulation contained 200 mg of ibuprofen, 206 mg of Na_2HPO_4 (molar ratio of API to phosphate was 1:1.5), and other excipients shown in Table I; this formulation is further referred as a phosphate formulation (PF). A reference tablet formulation (RF) had the same composition as the PF except Na_2HPO_4, which was replaced with 206 mg of NaCl. Tablets of both series were prepared with a direct compression method in a rotary tablet press Fette F100 (Fette Compacting GmbH, Schwarzenbek, Germany) using three different compression forces of 10, 15 and 20 kN.

Preparation of Dissolution Media

A simulated gastric fluid (SGF) concentrate was composed of 342 mM NaCl and 304 mM HCl in distilled water from a house system. The pH 2 and 4 SGF were prepared by adjusting commercial Volvic® mineral water (Danone Waters Deutschland GmbH, Frankfurt, Germany) to the target pH using 45.3 and 4.26 mL of the SGF concentrate per 1 L of the water, respectively. The obtained solutions were to reflect the stomach environment after the intake of a tablet with a glass of water in a fasted (pH 2) and fed state (pH 4).

A Hank's buffer concentrate contained 1369 mM NaCl, 53.6 mM KCl, 8.13 mM $MgSO_4$, 2.72 mM $CaCl_2$, 4.41 mM KH_2PO_4, and 2.72 mM NaH_2PO_4. A Hank's buffer with sodium bicarbonate was always prepared *ex tempore* by tenfold diluting of the Hank's buffer concentrate and dissolving the amount of $NaHCO_3$ that provided a concentration of 7 or 10 mM in the final solution.

A 50 mM phosphate buffer pH 7.2, for dissolution testing under compendial conditions, and 10 mM phosphate buffers pH 5.6 and 7.2 were prepared from 0.2 M KH_2PO_4 and 0.2 M NaOH.

Table I Composition of the Tablet Formulations Used in the Study

Substance	Amount in the formulation [mg]	
	PR	RF
Ibuprofen (free acid)	200	200
Na_2HPO_4	206	none
NaCl	none	206
Microcrystalline cellulose	70	70
Lactose	153	153
Croscarmellose sodium	10	10
$Ca_3(PO_4)_2$	6.5	6.5
Magnesium stearate	5.0	5.0

PF phosphate formulation (containing Na_2HPO_4), *RF* reference formulation (containing NaCl)

HPLC Method for the Quantification of Ibuprofen

An HPLC method was applied to quantify the low concentrations of ibuprofen in the acidic media. The LaChrom Elite HPLC system (Merck, Darmstadt, Germany) was equipped with a degasser, quaternary pump, autosampler, column compartment, and UV detector set at a wavelength of 220 nm. A chromatographic resolution was accomplished at 30°C on a Zorbax SB-CN C18 column (250 × 4.6 mm, 5 µm particles) from Agilent Technologies (Santa Clara, CA, USA), guarded by an on-line filter, using isocratic elution. A mobile phase was composed of 20 mM phosphate buffer (pH 7.0) and acetonitrile (74:26, v/v). The mobile phase flow rate was 1 mL/min and the injection volume was 10 µL.

The HPLC method was validated according to the ICH guidelines (27). A calibration curve of ibuprofen in a 20 mM phosphate buffer (pH 7.0) was linear from 1 to 750 mg/L. The back calculated concentrations were 99.1–105.0% of the nominal values. Accuracy and intermediate precision of the method was examined on 3 different days at 3 concentration levels (3, 400, and 750 mg/L) and ranged from 100.4 to 102.8% and 0.5 to 1.2% (coefficient of variation, CV), respectively. Repeatability was tested by analyzing 6 replicates at a concentration of 400 mg/L in the same analytical run, and the CV amounted to 0.3%. A lack of signals interfering with the ibuprofen peak confirmed the specificity of the method. In addition, the change of a matrix from the pH 7 phosphate buffer to the pH 2 SGF, pH 4 SGF, and pH 7.2 Hank's buffer did not significantly affect the ibuprofen peak area as the accuracy of the drug determination in those fluids at a concentration of 400 mg/L ($n = 3$) fell within 96.9–99.5%.

Determination of the Equilibrium Solubility of Ibuprofen in the pH 2 SGF at 37°C

The amount of 3 mg of the ibuprofen analytical standard was vortexed with 1 mL of the pH 2 SGF in 1.5 mL-polypropylene reaction tubes ($n = 6$). The tubes were stirred for 24 h ($n = 3$) or 48 h ($n = 3$) in a water bath maintained at 37°C and then centrifuged for 2 min at 37°C with 14,000 g. Thereafter, 0.5 mL of the clear supernatant was immediately transferred into the HPLC vials containing 0.5 mL of the pH 7 phosphate buffer (20 mM), using the pipette tips pre-warmed at 37°C. The concentration of ibuprofen in the final samples was determined with the HPLC method, and the solubility of the drug was calculated using the dilution factor of 2. A lack of the statistically significant difference in the solubility results obtained after 24 and 48 h of incubation ($p = 0.988$ in the analysis of variance (ANOVA) test) confirmed reaching the solubility equilibrium.

Dissolution of Ibuprofen from the Tablets

Biorelevant dissolution of the tablets were carried out in SGF (pH 2 and 4) and bicarbonate-buffered SIF (pH 5.7, 7.2, and intestinal gradient from 5.8 to 7.7). Also, standard dissolution tests were performed in 50 mM phosphate buffer pH 7.2 and 10 mM phosphate buffers pH 5.6 and 7.2 to verify the importance of using biorelevant conditions. All the dissolution tests were carried out in a USP 2 apparatus at 37 ± 0.5°C with a 75 rpm paddle stirring speed. Three replicates of the PR and RF tablets produced with different compression force (10, 15, 20 kN) were used in each experiment.

Dissolution in SGF

The dissolution of ibuprofen from the PF and RF tablets was tested in 250 mL of the pH 2 or pH 4 SGF ($n = 3$) within 35 min. Samples for the HPLC analysis (2 mL) were taken at 0 (pre-dose), 5, 10, 15, and 30 min with 5 mL-syringes coupled via a luer lock to stainless steel tubing protected at the end with a 1 µm porous filter (ProSense B.V., Oosterhout, Netherlands). The solution was transferred into HPLC glass vials. At the sampling time points, the pH of the solution was measured using FiveEasy FE 20 pH meter (Mettler Toledo AG, Schwerzenbach, Switzerland).

Dissolution in a Bicarbonate-Buffered SIF

The dissolution of ibuprofen in SIF was studied at the constant pH of 5.7 and 7.2 and also at the intestinal pH gradient. The acceptor medium was 1 L of a Hank's buffer containing $NaHCO_3$ at a concentration of 10 mM, except the dissolution test at constant pH 5.7, when a lower concentration of $NaHCO_3$ (7 mM) was necessary to reach the target pH. The pH of the medium was automatically regulated with the pHysio-grad® device (Physiolution GmbH, Greifswald, Germany) by dispensing gaseous carbon dioxide and the air into the solution. The applied dynamic HCO_3^-/CO_2 system simulated real intestinal buffering conditions, including constant ionic strength (26). Electrodes and gas diffusers were introduced into dissolution vessels through fitted holes in plastic lids to prevent solution evaporation. Prior to starting the dissolution test, the initial pH of the Hank's buffer (37°C) was adjusted from its original value (about 7.40) to a target value of either 5.67, 7.20, or 5.81 (the latter was the initial value in the pH gradient test) by automatic dispensing of carbon dioxide. After placing the tablets in the dissolution medium, the pH was maintained at a desired constant value of 5.67 or pH 7.20 (± 0.03) or changed according to the programmed small intestine pH gradient: 5.81 for 1 min, 6,01 for 9 min; 6.05, 6.17, 6.30, 6.34, 6.54, 6.71, 6.95, 7.10, 7.23, 7.36, 7.40, 7.48, 7.58, 7.57, each for 10 min; then 7.66 for 20 min; and 7.68 for 10 min. To determine the concentration of dissolved ibuprofen, the absorbance of the solution was measured online at a wavelength of 235 nm using an 8-multicell spectrophotometer Agilent 8453 equipped with an automatic sipper system and 1-cm-wide quartz flow cells (all from Agilent Technologies, Santa Clara, CA, USA). The absorbance measurements were performed every 2 min for the first 30 min of the dissolution test, and then every 5 min until 3 h.

Calibration standards of ibuprofen at a concentration ranging from 10 to 250 mg/L were prepared in the pH 5.67 Hanks's buffer containing 7 mM $NaHCO_3$ and pH 7.20 Hanks's buffer with 10 mM $NaHCO_3$. The slope of the mean calibration curve ($n = 3$) in the form of $Absorbance = Slope \times Ibuprofen\ Concentration$ was 0.006295 and 0.006337, respectively. The average slope of 0.006316 was used to calculate the concentration of ibuprofen in the pH gradient dissolution test.

Dissolution in Simple Phosphate Buffers

The dissolution test was conducted using 900 mL of three different phosphate buffers as an acceptor medium: 50 mM phosphate buffer pH 7.2; 10 mM phosphate buffer pH 7.2; and 10 mM phosphate buffer pH 5.6. As previously, sampling was performed every 2 min for the first 30 min and then every 5 min up to 3 h, and the ibuprofen concentration was determined online spectrophotometrically.

Data Analysis and Statistics

Dissolution rate of ibuprofen from the tablets in SIF and phosphate-based media was examined based on the percentage drug fraction dissolved in the medium at 30 min (D30). This parameter was calculated as a percentage ratio of the ibuprofen concentration at 30 min to the drug concentration at 180 min (plateau). Statistical analysis of the data was performed in Statistica 13 (StatSoft Inc.). A two-way factorial ANOVA was used to examine the effect of the formulation type and compression force on the D30, as this statistical test is fairly robust to violations of data normality and variance homogeneity, particularly in equinumerous groups (28). If the ANOVA indicated a significant effect of the independent variable on the D30, a post-hoc analysis was carried out using the Tukey's test to examine a statistically significant difference between all the possible pairs of the six D30 subgroups (2 formulation types × 3 compression force levels).

Modeling of Ibuprofen Dissolution in the Bicarbonate-Buffered SIF

To gain some insight into the mechanism of ibuprofen dissolution in the bicarbonate-buffered SIF at strictly controlled pH, the observed dissolution data were fitted to the three models: (1) first-order kinetics, (2) Hixson-Crowell,

and (3) Korsmeyer-Peppas, of which particular cases are zero-order kinetics and Higuchi models (29, 30). The analysis included the ibuprofen concentrations up to the first concentration exceeding 75% of the value observed at 3 h (plateau). The respective linear equations used in the model fitting were as follows: (1) $\ln(C_\infty - C_t) = A - K \bullet t$; (2) $(C_\infty - C_t)^{1/3} = A - K \bullet t$; and (3) $\log(C_t / C_\infty) = A + n \bullet \log(t)$, where C_t and C_∞ denote the drug concentration in solution at a given time t and at the final time of 3 h (plateau), respectively, and A as well as K are constants (29, 30). The goodness of fit was evaluated by a coefficient of determination (R^2) and visual inspection of the best-fitted line superimposed on the observed data.

RESULTS

Dissolution of Ibuprofen in SGF

In the pH 2 SGF, the PF tablets disintegrated within 30–35 min, which was much longer than the RF tablets (2–3 min). Also, the PF increased the solution pH by 0.4–0.6, whereas the RF tablets caused negligible pH changes (Fig. 1a). Only the dissolution of the PF produced a transient supersaturation state, lasting about 10 min, after which the ibuprofen concentrations decreased approximately to the level observed for the RF tablets. This process was associated with the drug precipitation on the paddles and high variability of the ibuprofen concentration in the solution between 5 and 15 min; the CV was up to 49%. The mean maximal amount of ibuprofen dissolved from the PF and RF corresponded to 8% and 4% of the tablet strength (200 mg),

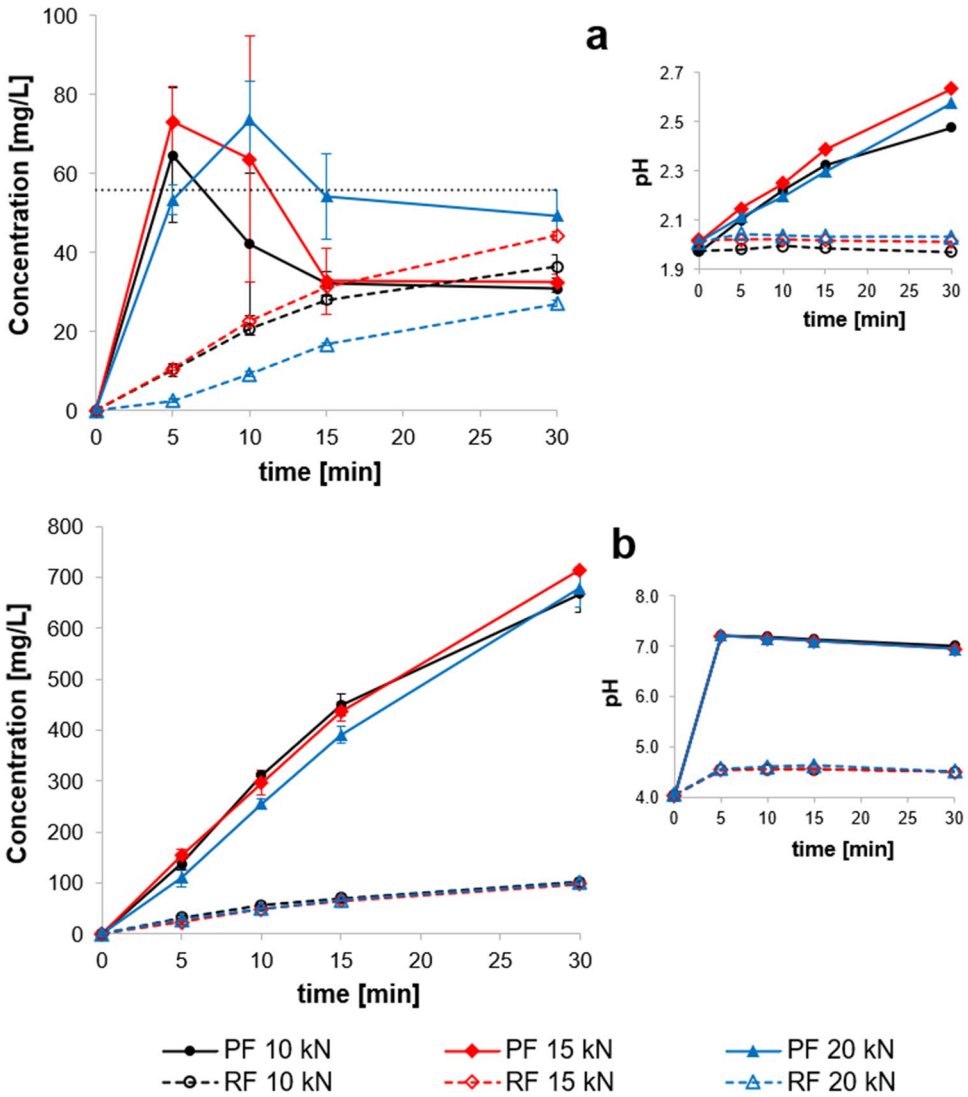

Fig. 1 Dissolution of ibuprofen (mean ± SD, $n = 3$) from the PF and RF tablets in **a** the pH 2 SGF and **b** pH 4 SGF, at 37°C. The inset graphs show the changes of the bulk pH. The dotted line in (**a**) shows the equilibrium solubility of ibuprofen in the pH 2 SGF

respectively. In an additional experiment, the equilibrium solubility of ibuprofen in the pH 2 SGF at 37°C was determined to be 55.8 ± 2.1 mg/L (mean ± SD, $n = 6$). Therefore, the degree of supersaturation produced by the PF was 1.2, as expressed by a ratio of the maximal concentration of ibuprofen in the pH 2 SGF (~ 70 mg/L) to its thermodynamic solubility.

In the pH 4 SGF, the disintegration of the PF and RF took the same time as in the pH 2 SGF (30–35 min and 2–3 min, respectively), but the bulk pH changes and ibuprofen dissolution behavior were different (Fig. 1b). During the first 5 min, a rapid increase in the bulk pH occurred, followed by a plateau at the pH level of 7.1–7.2 for the PF and 4.5–4.6 for the RF until the end of the test (35 min). Under such conditions, the dissolution profiles had an exponential-like plot with the maximal ibuprofen concentration of 670–710 mg/L for the PF and approximately 100 mg/L for the RF, which corresponded to 85% and 12% of the tablet strength, respectively.

No clear trend in the influence of the tablet compression force on the drug dissolution profile in the SGF was observed.

Dissolution of Ibuprofen in the Bicarbonate-Buffered SIF

At all the tested SIF conditions—constant pH 5.7, constant pH 7.2, and a pH gradient from 5.8 to 7.7—the PF and RF tablets disintegrated within about 30 and 2 min, respectively, regardless of the compression force. The dissolution of ibuprofen from the tablets was always complete, but its rate, expressed as D30, depended on the tablet formulation, compression force, and the pH of the medium (Fig. 2, Table II). A two-way ANOVA showed that at all pH conditions, both the formulation and compression force had a statistically significant effect on the D30 ($p < 0.0001$ and $p < 0.0003$, respectively). It meant that at least at one level of the compression force (10, 15 or 20 kN), the D30 differed significantly between the formulation type (PF and RF), or vice versa. Post hoc multiple comparisons, done with the Tukey's test, revealed that for each compression force level, the PF dissolved significantly faster than RF (Table 2). In opposition, the effect of the compression force depended on the formulation type and presented a diverse pattern among the pH conditions. A general tendency was the highest D30 at 10 kN of the compression force, and the lowest one at 20 kN, but a statistical significance of the means difference was present only in few cases (Tables S1–S3 in Supporting Information).

The mean concentrations of ibuprofen dissolved from the PR tablets were best fitted to the Hixson-Crowell model (R^2 0.978–0.999), whereas those from the RF tablets—to the first-order model (R^2 0.989 – 0.999). Figure 3 presents the

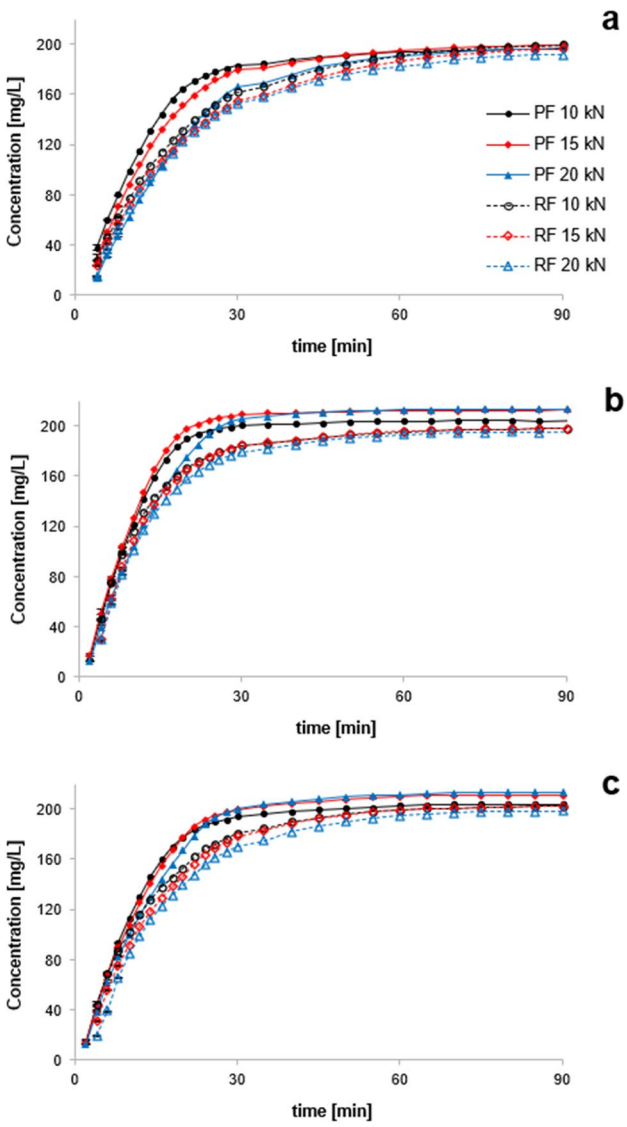

Fig. 2 Dissolution of ibuprofen (mean concentration, $n = 3$) from the PF and RF tablets in the bicarbonate-buffered SIF at **a** constant pH 5.7, **b** constant pH 7.2, and **c** intestinal pH gradient from 5.8 to 7.7, at 37°C. The drug concentrations from 90 min to the end of the test (3 h) were all at the plateau level and therefore are not shown

observed drug concentrations with the superimposed best-fit lines for the tablets produced with the 10 kN compression force; the data for the 15 and 20 kN forces are shown in Fig. S1 in Supporting Information.

Dissolution in the Phosphate Buffers

The time of disintegration of the PF and RF tablets in the three examined phosphate buffers (50 mM and pH 7.2, 10 mM and pH 7.2, and 10 mM and pH 5.6) increased with the compression force and ranged from 7 to 10 min and 1 to 3 min, respectively. Only the 50 mM phosphate

Table II Comparison of the D30 Results Obtained for the PF and RF Tablets in the Bicarbonate-Buffered SIF and Phosphate Buffers at Different pH Conditions at 37°C

Buffer pH and concentration	COMP [kN]	D30 [%]		*p* (ANOVA)[a]	*p* (Tukey's test)
		PF	RF		
Bicarbonate-buffered SIF					
pH 5.7	10	93.0 ± 0.3	80.1 ± 0.6	< 0.00001 FORM	0.00016
7 mM $NaHCO_3$	15	88.8 ± 3.4	77.3 ± 0.5	0.00009 COMP	0.00016
	20	83.1 ± 0.9	78.2 ± 1.0		0.019
pH 7.2	10	98.1 ± 0.2	92.1 ± 0.3	< 0.00001 FORM	0.00016
10 mM $NaHCO_3$	15	98.4 ± 0.1	92.4 ± 0.5	0.00024 COMP	0.00016
	20	96.7 ± 0.9	91.0 ± 0.3		0.00016
pH gradient	10	95.4 ± 0.7	88.8 ± 0.1	< 0.00001 FORM	0.00016
(5.8–7.7)	15	94.5 ± 0.6	87.7 ± 1.2	0.00006 COMP	0.00016
10 mM $NaHCO_3$	20	94.1 ± 0.5	84.7 ± 0.4		0.00016
Phosphate buffers					
pH 5.6	10	88.0 ± 1.0[b]	51.8 ± 4.3[b]	< 0.00001 FORM	0.00013
10 mM	15	84.5 ± 1.9[b]	52.0 ± 3.0[b]	0.0344 COMP	0.00013
	20	83.3 ± 4.5[b]	48.4 ± 5.4[b]		0.00013
pH 7.2	10	97.8 ± 2.0	97.3 ± 1.0[b]	0.882 FORM	0.995
10 mM	15	96.9 ± 2.3	97.1 ± 0.6[b]	0.690 COMP	1.00
	20	97.3 ± 1.9	97.3 ± 0.4[b]		1.00
pH 7.2	10	98.3 ± 1.8	99.4 ± 1.3	0.0344 FORM	0.983
50 mM	15	98.3 ± 4.6	99.7 ± 0.1	0.434 COMP	0.943
	20	95.9 ± 4.2	99.4 ± 0.8		0.243

[a]The *p* values in the column indicate the general effect of the formulation type (FORM) and compression force (COMP) on the ibuprofen dissolution

[b]Experiments in which the bulk solution pH was not maintained

D30 percentage fraction of the drug dissolved at 30 min, *COMP* compression force, *FORM* formulation type, *PF* phosphate formulation (containing Na_2HPO_4), *RF* reference formulation (containing NaCl)

buffer pH 7.2 maintained the nominal bulk pH during a 3 h dissolution test of both the PR and RF tablets, as shown in details in Table S4 in the Supporting Information. In the 10 mM phosphate buffers, the pH was unstable either in case of the two tested formulations or RF only. The major observations on the pH monitoring and dissolution of ibuprofen in these media are gathered in Table III, and the detailed dissolution results are depicted in Fig. 4 and Table II. According to the two-way ANOVA test, the formulation type generally had a statistically significant effect on the D30 for the 10 mM phosphate buffer pH 5.6 and 50 mM phosphate buffer pH 7.2. However, the compression force-based pairwise comparisons indicated that only the former medium revealed the acceleration effect of the microenvironment pH modulator. It is worth noting that the dissolution of ibuprofen in the compendial 50 mM phosphate buffer pH 7.2 was complete within 30 min, but in the earlier phase, the PF tablets clearly demonstrated lower dissolution rates than the RF (Fig. 4c). This result was opposite to that obtained in the pH 7.2 SIF buffered with a bicarbonate/carbon dioxide system (Fig. 2b). On the other hand, the use of the 10 mM phosphate buffer pH 5.6 exaggerated the dissolution-acceleration effect of Na_2HPO_4 in the PF tablets.

DISCUSSION

Modulation of the pH of a diffusion layer (microenvironment) in solid oral dosage forms is a promising strategy for enhancing the dissolution of weakly acidic and basic BCS class II API in the GIT (18–25). To the best of our knowledge, this concept was experimentally tested using non-bicarbonate biorelevant media only, which basically alter the dissolution characteristics compared with the bicarbonate-based intestinal fluid (15–25). The major aim of our work was to verify the effect of a microenvironmental pH modulator (Na_2HPO_4) on the dissolution rate of free BCS class II weak acid (ibuprofen) in a physiologically-relevant bicarbonate-buffered SIF *versus* compendial (50 mM) and low-concentration (10 mM) phosphate buffers, and also in SGF. We used Na_2HPO_4 as a microenvironmental pH modulator due to its good water solubility, biocompatibility, cheapness, and beneficial pK_a of the conjugate acid ($H_2PO_4^-$) at physiological ionic strength and temperature (~6.8) in relation to that of ibuprofen (pK_a 4.4) (16). The co-existence of neutral ibuprofen/deprotonated ibuprofen and $H_2PO_4^-/HPO_4^{2-}$ as conjugate acid/base pairs in the microenvironment was supposed to produce the pH close

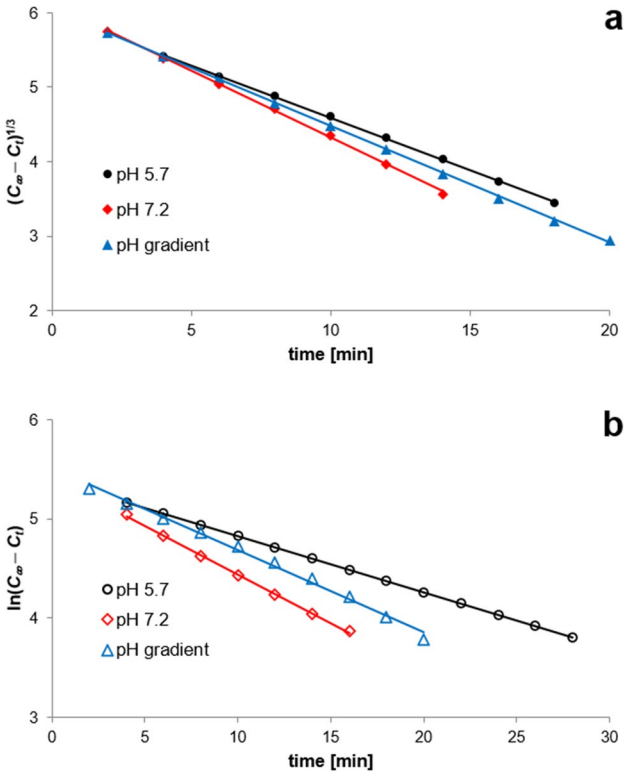

Fig. 3 Fitting of the mean concentrations of ibuprofen ($n=3$) dissolved from the 10 kN compression force-tablets to dissolution models: **a** Hixson-Crowell model for the PF tablets and **b** first-order kinetics model for the RF tablets. The analysis included the drug concentrations up to the first concentration exceeding 75% of the plateau value

to the average of the two pK_a values (~5.6). This in turn could provide a relatively high buffer capacity of the two buffering systems and increased ionization of ibuprofen in the diffusion layer and thus also accelerated dissolution of the API.

In the pH 2.0 SGF, simulating the stomach fasted state, the PF tablets produced a 10 min-lasting supersaturation of ibuprofen in the solution bulk, associated with the dissolution of 8% of the tablet strength. The pH of the medium during the supersaturation state (2.1–2.4) was far from the pK_a of ibuprofen (4.4) (16), indicating that dissolved ibuprofen was present in a unionized form in the medium bulk. Of note, the enhanced drug dissolution in the pH 2.0 SGF together with fast emptying of the stomach at fasted conditions offers the benefit of a fast onset of the absorption *in vivo*. Also, the occurrence of the supersaturation state for the PF, but not RF, proves the role of Na_2HPO_4 in this phenomenon.

In the pH 4 SGF, reflecting a fed state of the stomach, the PF rose the pH of the medium bulk to 7.0, which considerably exceeded the pK_a of ibuprofen. At these conditions, ibuprofen was supposed to exist predominantly in an ionized form, which well explained the high amount of the dissolved drug (85% of the tablet strength). Due to the significant changes in the bulk solution pH, we could not unequivocally attribute the increased dissolution of ibuprofen to the Na_2HPO_4-dependent pH increase of the diffusion layer.

The role of Na_2HPO_4 as a microenvironmental pH modulator was clearly proved by the enhanced ibuprofen dissolution in the bicarbonate-buffered SIF of which pH was strictly controlled with an automatic dynamic system (26). At these conditions, the PF provided a statistically higher D30 than RF, which might stem only from the altered microenvironment of dissolving particles (Table II). The gain in the D30 obtained with the PF was most pronounced at pH 5.7. A weaker effect of Na_2HPO_4 observed for the pH 7.2 SIF and intestinal pH gradient program (pH change from 5.8 to 6.2) might be caused by a greater difference between the bulk pH and pK_a of ibuprofen, thus enhancing drug solubility in the medium bulk (14–18).

In accordance with the previous reports (14–17, 31), replacement of the pH 7.2 bicarbonate-buffered SIF with the compendial 50 mM phosphate buffer pH 7.2 significantly overestimated the rate of ibuprofen dissolution from RF (D30 99% instead of 92%, dissolution half-life about

Table III Main Observations on the Ibuprofen Dissolution from PF and RF in the Phosphate Buffers *versus* Biorelevant Bicarbonate Buffer

Phosphate buffer	Observation	
	PF	RF
pH = 5.6 C = 10 mM β = 1.3 mM/ΔpH	Despite the released Na_2HPO_4 increases the bulk pH by *ca.* 0.4, the API dissolves at the rate similar to the bicarbonate-buffered SIF	The released API decreases the bulk pH by *ca.* 0.8; both the bulk solubility and dissolution rate of API are strikingly reduced compared with the bicarbonate-buffered SIF
pH = 7.2 C = 10 mM β = 4.7 mM/ΔpH	The bulk pH is maintained; the API dissolves at the rate similar to the bicarbonate-buffered SIF	The released API decreases the bulk pH by *ca.* 0.2, but the dissolution is faster than in the bicarbonate-buffered SIF
pH = 7.2 C = 50 mM β = 23.4 mM/ΔpH	The bulk pH is maintained; the API dissolves at the rate similar to the bicarbonate-buffered SIF	The bulk pH is maintained, but the API dissolution is strikingly faster than in the bicarbonate-buffered SIF

C total buffer concentration, β buffer capacity, *PF* phosphate formulation (containing Na_2HPO_4), *RF* reference formulation (containing NaCl)

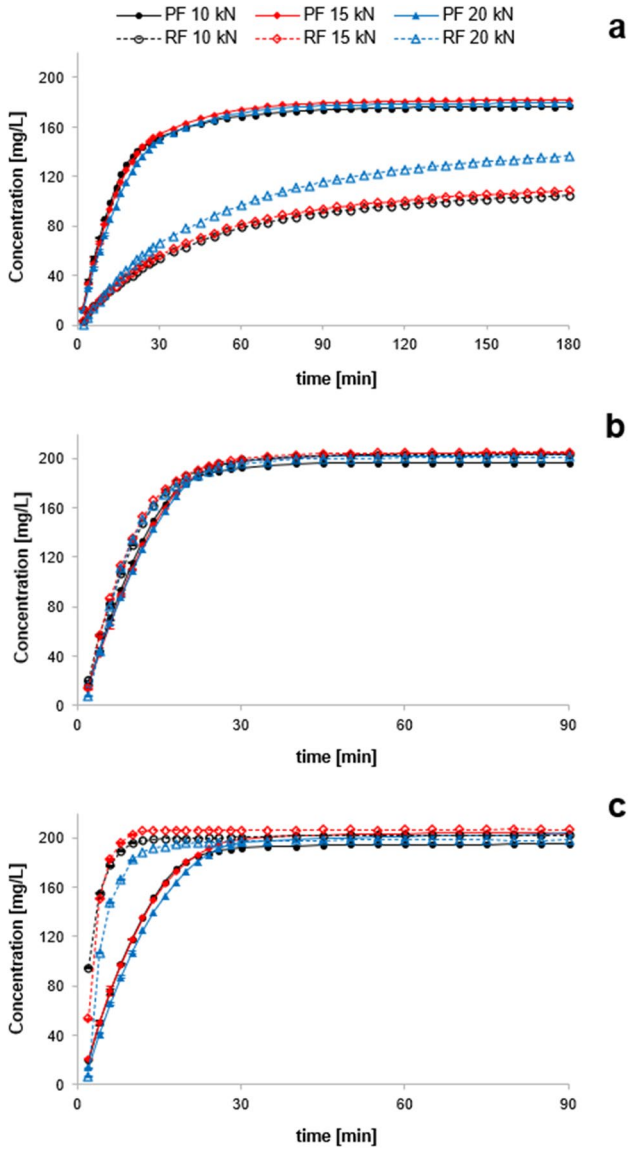

Fig. 4 Dissolution of ibuprofen (mean concentration, $n=3$) from the PF and RF tablets in the phosphate buffer of **a** 10 mM concentration and pH 5.6, **b** 10 mM concentration and pH 7.2, and **c** 50 mM concentration and pH 7.2, at 37°C. In (**b**) and (**c**), the drug concentrations from 90 min to the end of the test (3 h) were all at the plateau level and therefore are not shown. The nominal pH values in (**a**) and (**b**) were not maintained during the dissolution test (details in the text)

3 min instead of 10 min). This phenomenon can be attributed to the high buffer capacity as well as high pH in the diffusion layer of the dissolving particles of the RF tablets. Such rapid dissolution was not observed for PF, which dissolved ibuprofen at the same rate as in the SIF (D30 about 97%, dissolution half-life 8–10 min). We hypothesize that the above discrepancy stems from the presence of Na_2HPO_4 in PF: The excipient slows down the tablet disintegration compared with RF (containing NaCl) and thus makes the disintegration process a factor limiting the dissolution rate at pH 7.2. A support for this hypothesis is provided by the similar rate of ibuprofen dissolution from the PF in the 10 mM phosphate buffer pH 7.2 (D30 about 97%, dissolution half-life 8–10 min). Interestingly, the API dissolution from RF in this buffer was not diminished compared with PF, despite the bulk pH shifting to approximately 7.0 and, most likely, even lower pH in the RF tablet microenvironment caused by the saturated solution of ibuprofen. The maintenance of the dissolution rate under such conditions indicates that the process was more governed by the type of buffer species (phosphates instead of bicarbonate) than by the surface pH. This accords with the recognized overestimation of dissolution rates of BCS class II weak acids, particularly those with the pK_a below 6.5, like ibuprofen, in phosphate and maleate media compared with a biorelevant bicarbonate buffer (14–17, 31). As expected from the low buffer capacity (1.3 mM/ΔpH), the 10 mM phosphate buffer pH 5.6 provided the worst performance with regard to a bulk pH control. The RF tablets caused the pH shift to 4.8 on average, which resulted in the very slow dissolution of ibuprofen as well as smaller equilibrium solubility of the drug, expressed by the lowered dissolution profile plateau level. On the other hand, the Na_2HPO_4 excipient released from PF increased the bulk pH to approximately 6.0; however, the microenvironmental pH was probably lower due to the interplay with ibuprofen, as indicated by dissolution rate similar to that in the pH 5.7 bicarbonate-buffered SIF (Table II, Figs. 2 and 4). Altogether, the dissolution experiments in the phosphate buffers could not reflect the same effect of the microenvironmental pH modulator Na_2HPO_4 as was observed in the biorelevant bicarbonate-buffered SIF.

The addition of Na_2HPO_4 to the tablet not only did improve the dissolution rate of ibuprofen in SIF, but also changed the dissolution model from the first-order kinetics to the Hixon-Crowell model (Figs. 2, 3, and S1). Interpretation of this result needs referring to the derivation of the two models. The first-order kinetics model comes from the classical Noyes-Whitney model when the solubility of a drug in the unstirred diffusion layer (C_s) is replaced with the C_∞ in the solution bulk. This model assumes that the difference between C_s and the actual drug concentration in the well-stirred solution bulk (C_s–C) changes over time of dissolution. In opposition, the Hixon-Crowell model (cube-root law) is derived considering the C_s–C difference remains constant during dissolution (29, 30). Therefore, the fitting results obtained in our study indicate that the C_s of ibuprofen in the boundary layer of PR in a bicarbonate-buffered SIF was significantly higher than in RF, providing an indirect mechanistic support for the role of Na_2HPO_4 in accelerated dissolution.

CONCLUSION

This work shows for the first time that addition of a micro-environmental pH modulator (Na_2HPO_4) to an IR tablet enhances the dissolution of BCS class II free weak acid (ibuprofen) at biorelevant conditions including SGF and bicarbonate-buffered SIF, but not a compendial 50 mM phosphate buffer. Low-concentration phosphate media (10 mM) with no external pH adjustment do not mimic the bicarbonate buffer performance. Moreover, the bulk pH shift observed when using these surrogate phosphate buffers hinders the soundness of the obtained data.

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DECLARATIONS

CONFLICT OF INTEREST The authors declare no competing interests.

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