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Research paper

Mechanistic analysis and experimental verification of bicarbonate-controlled enteric coat dissolution: Potential *in vivo* implications

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ABSTRACT

Enteric coatings have shown *in vivo* dissolution rates that are poorly predicted by traditional *in vitro* tests, with the *in vivo* dissolution being considerably slower than *in vitro*. To provide a more mechanistic understanding of this, the dependence of the release properties of various enteric-coated (EC) products on bulk pH and bicarbonate molarity was investigated. It was found that, at presumably *in vivo*-relevant values, the bicarbonate molarity is a more significant determinant of the dissolution profile than the bulk pH. The findings also indicate that this steep relationship between the dissolution of enteric coatings and bicarbonate molarity limits those coatings' performance *in vivo*. This is attributed to the relatively low bicarbonate molarities in human intestinal fluids. Further, the hydration and dehydrations kinetics of carbonic acid and carbon dioxide are not sufficiently rapid to reach equilibrium in the diffusion layer surrounding a dissolving ionizable solid. This results in the effective pKa of bicarbonate in the diffusion layer being lower than that determined potentiometrically at equilibrium in the bulk surrounding fluid. These results demonstrate the importance of thoroughly investigating the intestinal bicarbonate concentrations and using bicarbonate buffers or properly designed surrogates (if possible) when evaluating enteric drug products during product development and quality control.

1. Introduction

Enteric coatings have been used since the late 19th century to delay the onset of drug release until after the dosage form has passed the pylorus in order to: (i) Protect acid-labile active pharmaceutical ingredients (API)'s against degradation by gastric acid, (ii) To protect the gastric mucosa against the irritating effects of certain API's, and (iii) To target the release of some API's to particular intestinal segments [1]. The typical strategy employed for this purpose is through the use of a weakly acidic film-former that is unionized and thus water-insoluble at the acidic pH of the gastric juice but negatively charged and water-soluble at the higher pH values found in the intestine [1].

Polymers have been designed with various dissolution pH thresholds in order to target different sections of intestine; (i) polymers with dissolution pH thresholds of ~5–5.5 have been considered to target API release to the duodenum, (ii) those with dissolution pH thresholds of ~6 have been targeted to release in the jejunum, and (iii) those with dissolution pH thresholds of ~7 to target the ileum and colon [4]. This gave rise to the generally accepted view that an enteric polymer will quickly dissolve upon reaching an intestinal segment where the pH exceeds that of its dissolution pH threshold. This also has been implemented in physiologically-based pharmacokinetics software used to model drug absorption like GastroPlus® and Simcyp®. For example, with GastroPlus® the usual procedure assumes the dosage form to start behaving as an immediate-release product once it passes the pylorus and Simcyp® makes a similar assumption once the dosage form reaches an intestinal segment with a pH equal to the enteric polymer's dissolution pH threshold.

However, in reality, the time interval between gastric emptying and onset of API release *in vivo* can be considerably longer, reaching values

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exceeding one hour even for polymers considered to target the duodenum [9,25][9]. This contributes to the unpredictable *in vivo* behavior of enteric-coated (EC) dosage forms [4], and, in some cases, gives rise to questions concerning the dosage forms' clinical efficacy. For example, enteric-coated iron preparations suffer from low efficacy owing to low iron bioavailability from these dosage forms [40]. This has been attributed to insufficient release of the API taking place before the dosage form has passed iron's preferential absorption site in the duodenum and proximal jejunum [40]. Another example is the case with EC acetylsalicylic acid (ASA) with its low efficacy in anti-platelet therapy compared to plain ASA that was theorized to be a consequence of slow release and/or poor absorption, though conclusive investigations regarding the exact mechanism are yet to be performed [16,17,7].

This issue can be explained by the fact that the pH value directly controlling the dissolution rate of such polymers is the pH at the polymer-water interface (surface pH) rather than the bulk pH of the medium [37]. This pH is expected to be lower than the bulk pH because of the reaction between the polymer's carboxyl groups and the basic components of the buffer system. As the buffer capacity of the medium increases, the gap between the two pH values would become narrower.

In the USA and Europe, the typically used buffer in the *in vitro* compendial (according to the United States Pharmacopoeia (USP) and European Pharmacopoeia (Ph Eur)) dissolution test for EC dosage forms is a 50 mM pH 6.8 phosphate buffer in which the dosage forms are placed following a two-hour period in 0.1 M HCl. The relatively high (relative to *in vivo*) buffer capacity of this buffer seems to result in the aforementioned gap between the bulk medium pH and the surface polymer pH being less than encountered *in vivo* [4]. This results in pronounced discrepancies between the *in vitro* and *in vivo* performance of EC dosage forms [4]. For instance, the onset of release from HPMC paracetamol capsules coated with Eudragit L 30D-55 (a polymer with a dissolution pH threshold of 5.5) prepared by Cole et al was around 15 min following the start of the buffer stage of the compendial dissolution test. However, the scintigraphically observed onset of capsule disintegration *in vivo* was around 66 min post-gastric emptying [9].

Intestinal fluid is buffered by bicarbonate at molarities that are apparently not high [33,18]. Therefore, one could expect a considerable gap between the bulk pH of the medium and the surface pH of the enteric polymer. The significance of this discrepancy between the typical *in vitro* and the *in vivo* buffers with regard to the *in vivo* performance of EC dosage forms has been recognized [2,4,13,15,20,27,30,43,46], though the compendial test noted above is still the standard for design of EC dosage forms.

However to date, there has not been a systematic investigation on the effect of varying bicarbonate concentrations on the release performance of EC dosage forms though there are indications of bicarbonate molarity being a major influence [2,20]. This is because the gap between the bulk medium pH and the surface enteric polymer pH is expected to be strongly affected by buffer molarity in non-concentrated buffers [37]. Therefore, the aim of this study is to explore the role of the bicarbonate molarity in the dissolution performance for different EC products and polymers.

2. Materials and methods

2.1. Materials

Bayer Safety Coated Aspirin Regimen® 325 mg EC ASA tablets (Bayer HealthCare LLC, USA), Dulcolax® 5 mg EC bisacodyl tablets (Boehringer Ingelheim Consumer Healthcare Products, Italy) and Delzicol® mesalamine delayed release capsules (Allergan, USA) were obtained from the American market. Both Dulcolax® and Bayer Safety Coated Aspirin Regimen® tablets are coated with enteric coatings based on methacrylic acid copolymer type C-NF (with a dissolution pH threshold of 5.5). As for Delzicol®, each capsule is filled with 4 small tablets film-coated with a coating based on methacrylic acid copolymer type B-NF (Eudragit S, with a dissolution pH threshold of 7). The detailed qualitative composition for each of the products is available in the public domain [34–36].

The paracetamol was obtained from Caesar & Loretz GmbH (Hilden, Germany), size 0 hydroxypropyl methylcellulose (HPMC) transparent capsule shells (ACG Nature Caps Plus) were received as gift from ACG Associated Capsules Pvt Ltd (Ashagadh, Maharashtra, India), talc was obtained from Euro OTC Pharma GmbH (Bönen, Germany), triethyl citrate (TEC) from Merck Schuchardt OHG (Hohenbrunn, Germany), methacrylic acid copolymer type C-NF (Eudragit L100-55) was received as a free sample from Evonik Röhm GmbH (Darmstadt, Germany), and hypromellose phthalate (HP-55) and hypromellose acetate succinate (HPMCAS-LF) were received as gifts from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Carbon dioxide was obtained from Metro Welding (MI, USA). All other materials used were of analytical or HPLC grade.

2.2. Preparation of EC paracetamol HPMC hard shell capsules

Size 0 transparent HPMC hard shell capsules were filled with around 235 mg paracetamol powder per capsule manually. Then they were coated using a Labotech Capsule Coater® (Labotech, Netherlands). This apparatus is described in detail by Catteau et al. [8].

The coating liquid consisted of 3.6 g enteric polymer, 1.8 g talc, 0.72 g TEC made up to a total weight of 150 g with acetone. The film formers used were Eudragit L100-55, HP-55, and HPMCAS-LF. The capsules were coated to a level of ~5.55 mg of polymer per squared cm.

The coating process parameters were as follows: a coating fluid input rate of 0.6–0.8 g/min, air pressure of 2.0 bar, a batch size of 30 capsules per batch, and inserted dipping tube length of 7.1 cm. The procedures were performed at room temperature. After the pre-determined amount of coat solution was applied, the process was allowed to continue without solvent input for further 10 min. After the coating process ended, the capsules were spread on aluminum foil and left to dry overnight.

2.3. Dissolution testing

All dissolution tests were in an SR6 USP Type II dissolution tester (Hanson Research, USA) using 1.0 L of dissolution medium and 50 rpm paddle rotation speed at a temperature of 37 °C in the various dissolution media described later.

2.3.1. Preparation of bicarbonate-based media

The bicarbonate solutions were prepared by dissolving the appropriate amounts of sodium bicarbonate and sodium chloride (all the bicarbonate buffers had their ionic strength adjusted to a value equal to that of physiologic saline (0.154 M) using NaCl) in deionized water. The solutions were then poured into the dissolution vessels, and the pH was adjusted to the desired value and maintained at it throughout the run by continuously sparging the medium with a CO_2-air mixture of a certain composition depending on the bicarbonate molarity and the desired pH value. An initial estimate for the necessary $\%CO_2$ was calculated using the values of bicarbonate pKa in bulk and Henry's constant of CO_2 in water listed by Krieg et al. [23]. The pH was monitored using a pH-meter (Beckman Φ40 -Brea, California, USA) until it became constant for at least 15 min. If it was within 0.05 units of the target pH in all the three vessels, the dissolution test was started, and if it deviated slightly, the composition of the gas mixture was adjusted accordingly, and the setup was monitored again until the pH was within the desired range and constant for at least 15 min, and then was the dissolution test started. The pH was continuously monitored throughout each dissolution test.

2.3.2. Dissolution tests for EC formulations targeted to the proximal small intestine

Dissolution testing was performed in 50 mM pH 6.8 phosphate

buffer (corresponding to the USP-specified buffer) as well as bicarbonate buffers of different pH and bicarbonate molarity values. For Dulcolax® pH values of 5.8, 6.4 and 7.0, and bicarbonate molarities of 5 10 and 20 mM were used. For the in house-manufactured paracetamol EC capsules, the pH values were 6.0, 6.5 and 6.8, while the bicarbonate molarities were 5, 10 and 15 mM. As for Bayer Aspirin EC tablets, pH was maintained at 6.8 with the bicarbonate molarities ranging from 5 to 20 mM for all the experiments except one where pH was maintained at 6.0 (with a bicarbonate molarity of 15 mM). Uncoated paracetamol capsules were evaluated in 10 mM pH 6.5 buffer (since both paracetamol and HPMC are non-ionizable, this single medium was deemed sufficient).

The selected pH and bicarbonate molarity ranges were based on literature values for these quantities in the human intestinal fluid. Duodenal pH has been reported to be around 6 and to rise distally along the small intestine until the ileocecal junction is reached [22]. As for the proximal intestinal bicarbonate molarity, it was reported to be around 8 mM [6,29]with Banwell et al. reporting value of 8.2 ± 6 mM in the proximal jejunum.

Since the Dulcolax® tablets had a thick sugar coat the dissolution of which was slow, before dissolution testing, they were placed for 15 min in purified water in a disintegration tester without discs (BJ-2, Tianjin Guoming Medicinal Equipment Co. Ltd, China) at room temperature. After that, any sugar coat remnants were washed away using a stream of purified water from a wash bottle.

Spring-style capsule sinkers (Sotax, Switzerland) 31 mm long and 11 mm wide were used to keep the EC paracetamol capsules from floating. Those sinkers were also used with the Bayer Aspirin EC tablets because, in bicarbonate, those tablets exhibited a propensity to float probably owing to the generation of carbon dioxide by the reaction between bicarbonate and ASA. For the Dulcolax® tablets, no sinkers were used since no sign of buoyancy was observed.

For EC paracetamol capsules, the determination of the %released was done using a chromatographic method as follows: 5 µL samples were injected onto an Agilent 1100 high-performance liquid chromatography (HPLC) chromatograph (Agilent, USA), employing an Agilent Eclipse Plus C18 (3.5 µm, 4.6 × 150 mm) column and a mobile phase consisting of methanol and water mixed in a 1:3 v:v ratio flowing at a rate of 0.6 ml/min. Detection was photometric at a wavelength of 243 nm. The paracetamol peak was observed at 5 min.

For the Bayer Aspirin EC tablets, the determination of %released was done spectrophotometrically (Hewlett Packard 8453, Hewlett Packard, USA) using the isosbestic point method prescribed by the USP. For the Dulcolax® tablets, only the dissolution onset was estimated by monitoring the tablets for the first sign of coat rupture.

In addition to bicarbonate and USP phosphate buffers, experiments were made using a maleate buffer containing 10 mM maleate divalent anion at pH 6.4 for Dulcolax® and pH 6.8 for Bayer Aspirin. The ionic strength was also adjusted to 0.154 M using sodium chloride. The purpose behind using maleate buffer is explained later in the discussion section.

2.3.3. Dissolution tests for Delzicol® delayed release mesalamine capsules

The dissolution testing conditions were similar to those in 2.3.2 except for the phosphate buffer used to represent the USP conditions (151 mM pH 7.2 phosphate buffer) and the range of pH and bicarbonate molarity values selected for the bicarbonate buffers, which was between 6.8 and 7.7 for the pH and between 30 and 120 mM for the bicarbonate molarity. The human terminal ileum has been reported to have a pH of about 7.4 [12] and a bicarbonate molarity of around 30 mM [6]. This explains the pH range used and the use of 30 mM bicarbonate. The rationale behind using very high bicarbonate molarity values of 60 and 120 mM is clarified in the results section.

As for the phosphate buffer, Sodium phosphate was used instead of the potassium phosphate prescribed by the USP. This was because sodium is the major cation in intestinal fluid, and also to make the cation uniform across all the dissolution experiments of this work.

Each capsule was opened and the four tablets inside it were placed into the dissolution medium. In this way any possible variability from the capsule shell dissolution was eliminated. Since the tablets sank to the bottom of the vessel at the start of the experiment, sinkers were not used. In addition to the phosphate and bicarbonate buffers, a run was made employing a 31 mM pH 6.9 maleate buffer (29 mM conjugate base) with the ionic strength adjusted to 0.154 M using NaCl. Determination of the amount released was performed spectrophotometrically at a wavelength of 311 nm (Hewlett Packard 8453, Hewlett Packard, USA).

2.3.4. Statistical analysis

Student's t-tests were performed using Microsoft Excel while analysis of variance (ANOVA) was performed using Vassarstats (vassarstats.net last accessed on January 14, 2019).

3. Results and discussion

3.1. Dulcolax® tablets

The first signs of coat rupture were observed at 15 ± 2 min in the 50 mM phosphate buffer, which when compared to the values in different bicarbonate buffers shown in Fig. 1 is considerably earlier. To make the comparison of the two effects on the same scale, the bicarbonate molarity was expressed in terms of -log [HCO_3^-] which was 2.3 for 5 mM (0.005 M), 2.0 for 10 mM (0.01 M) and 1.7 for 20 mM (0.02 M). Bicarbonate molarity is clearly more significant than bulk pH in terms of effect on the coat rupture time. For instance, changing the bulk pH of a 10 mM bicarbonate buffer (-log [HCO_3^-] = 2) from 5.8 to 6.4 resulted in a considerable decrease in the initial rupture time from 42 to 34 min. However, when this + 0.6 change in bulk pH was accompanied by a +0.3 change in -log [HCO_3^-] (i.e. halving the bicarbonate molarity), the initial rupture time was significantly increased from the 42 to 50 min (p-value = 0.034 for a two-tailed two independent sample *t*-test) indicating that bicarbonate molarity effect is stronger. Increasing the pH from 6.4 to 7.0 had only a small effect, but changes in bicarbonate molarity had considerable effects also within this pH range.

3.2. In house-manufactured paracetamol capsules

Release in 50 mM phosphate was faster than in all the bicarbonate buffers tested (Fig. 2). It is clear from Fig. 2 that bicarbonate molarity had a stronger effect on dissolution performance than bulk pH, with the dissolution curves in the 5 mM buffers bundled separately from those in 15 mM. The 10 mM pH 6.5 bicarbonate buffer gave dissolution profiles that were positioned quite close to those of the 15 mM buffers for

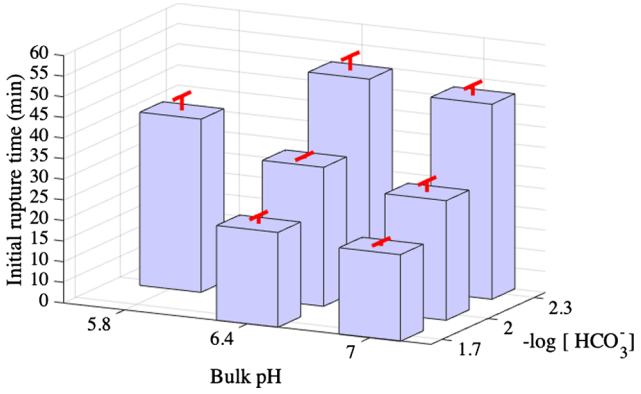

Fig. 1. Dependence of the mean initial coating rupture for Dulcolax® EC tablets (n = 3) on bulk pH and bicarbonate molarity.

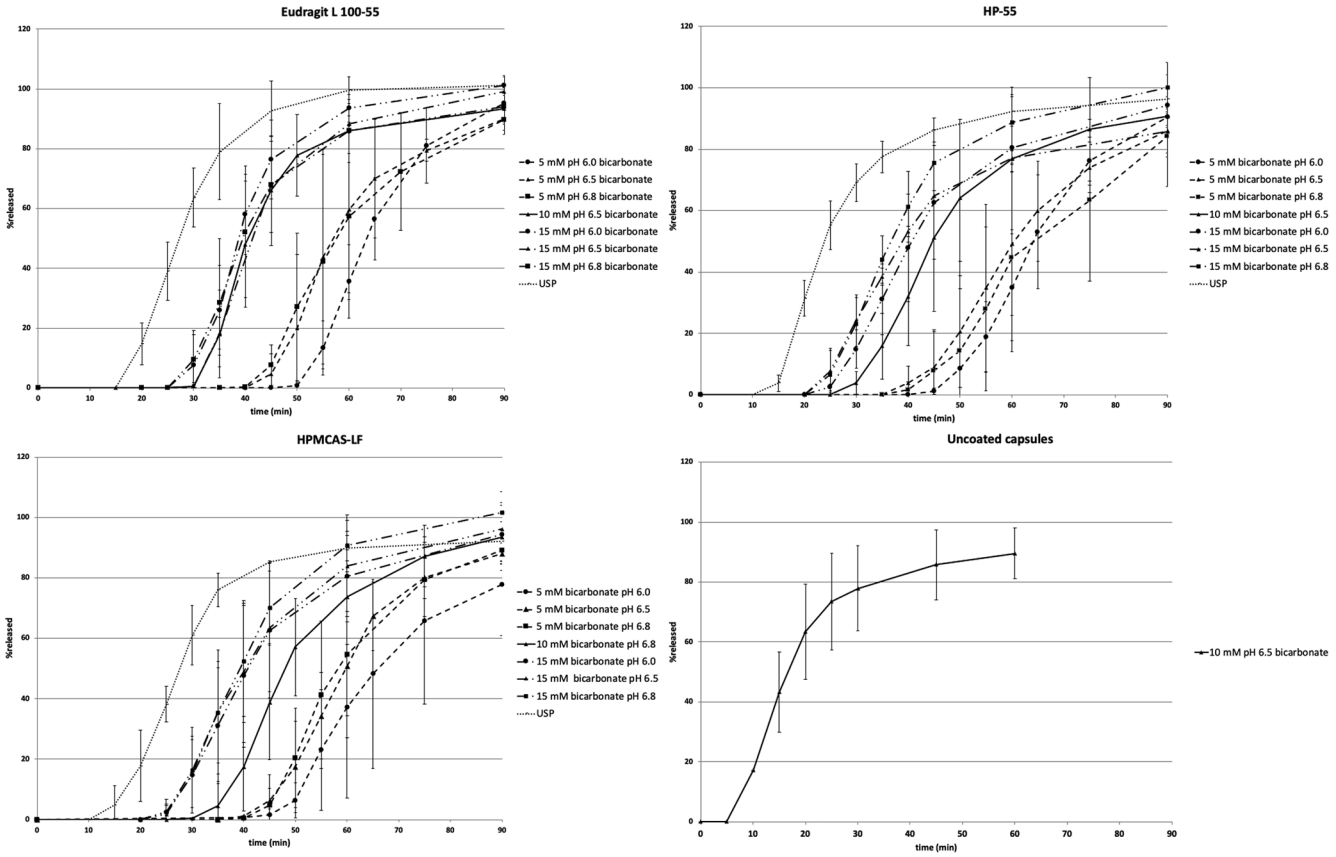

Fig. 2. Dissolution profiles of uncoated and enteric-coated paracetamol-filled HPMC hard shell capsules (mean ± SD; n = 3).

Eudragit L100-55, and less so for HP-55. But for HPMCAS-L, it gave a dissolution profile positioned right in the middle between those of the 5 and 15 mM buffers. Whether this indicates a different bicarbonate molarity-dependence for HPMCAS-LF in the range 10–15 mM remains to be ascertained because of the dissolution studies being performed only in triplicate.

The release can be divided into three stages: first stage where the capsule is closed and no measurable paracetamol concentration is obtained, a second stage of early release where a rupture develops (typically at the joint between the capsule body and cap) and paracetamol powder starts coming out and dissolving, and a third stage where the rupture becomes large and powder gets quickly emptied from the capsule forming a cone that dissolves relatively slowly. In this third stage, the coat effect on dissolution rate is limited, and it is likely due to this coning that the uncoated capsule, though releasing 64% at 20 min, took 60 min to achieve 90% release. Therefore, to provide the most accurate assessment of the dissolution medium effect on the polymers, the evaluation was focused on the early portion of the dissolution profiles.

Fig. 3 shows the time required to achieve 20% release for the different coated capsules in different buffers. As for the uncoated capsules they gave $t_{20\%}$ values with a mean of 10.67 min and a standard deviation of 2.19 min (not shown on the figure), which when compared to the results in Fig. 3 shows that the enteric coat was the primary release-delaying component in, at least , all the bicarbonate buffer experiments.

All formulations showed $t_{20\%}$ values that were clearly more dependent on bicarbonate molarity than on bulk pH values. Actually, with the possible exception of 5 mM bicarbonate buffer in the pH range 6.0–6.5, no indication of bulk pH-dependence was observed. When the 5 and 15 mM buffers were compared against each other across the three tested bulk pH values statistically using two way ANOVA, the buffer molarity effect gave a p-value < 0.0001 for each of the three polymers, while the bulk pH effect gave p-values between 0.41 and 0.61, as shown in Table 1. This strongly indicates that bicarbonate molarity has a stronger influence on the surface pH and so on enteric polymer performance than bulk pH within the studied range of values. As for the 10 mM pH 6.5 buffer, its $t_{20\%}$ was significantly different from that of the 15 mM pH 6.5 buffer only for HPMCAS-LF (p-value = 0.045 for a two-tailed two independent sample *t*-test). For HP-55 this p-value was 0.081 while for Eudragit L100-55 it was 0.89. However, more studies with larger number of replicates need to be performed before making any inferences out of this.

Since the capsule shell material (HPMC) is a readily soluble neutral material with no expected acidifying/alkalinizing effect on the enteric coat, and paracetamol is a highly soluble non-ionizable drug within the normal gastrointestinal pH range, with a solubility of 23.7 mg/ml [19] the release properties of the in house-manufactured EC capsules (particularly the early portion of the release profile) can be considered fairly reflective of the intrinsic polymer performance. Therefore, it could be stated that, over a bulk pH range of 6.0–6.8 and bicarbonate molarity range of 5–15 mM, the bicarbonate molarity is seemingly the more important and the dominant factor in the dissolution of the studied enteric polymers.

Now, as stated before in the materials and methods section, the pH of the fasted duodenum is around 6 and rises in the aboral direction, while the proximal intestinal bicarbonate molarity was reported to be around 8 mM, with a standard deviation of around 6 mM. Thus the results in Fig. 3 imply that it is the bicarbonate molarity rather than the bulk pH of the intestinal fluid that limits the ability of the studied enteric polymers to target the proximal small intestine *in vivo*.

These findings strongly suggest that the notion of an enteric polymer being able to target the most proximal intestinal segment it encounters where the bulk pH exceeds its dissolution pH threshold is questionable. Actually, with the mean transit time through the

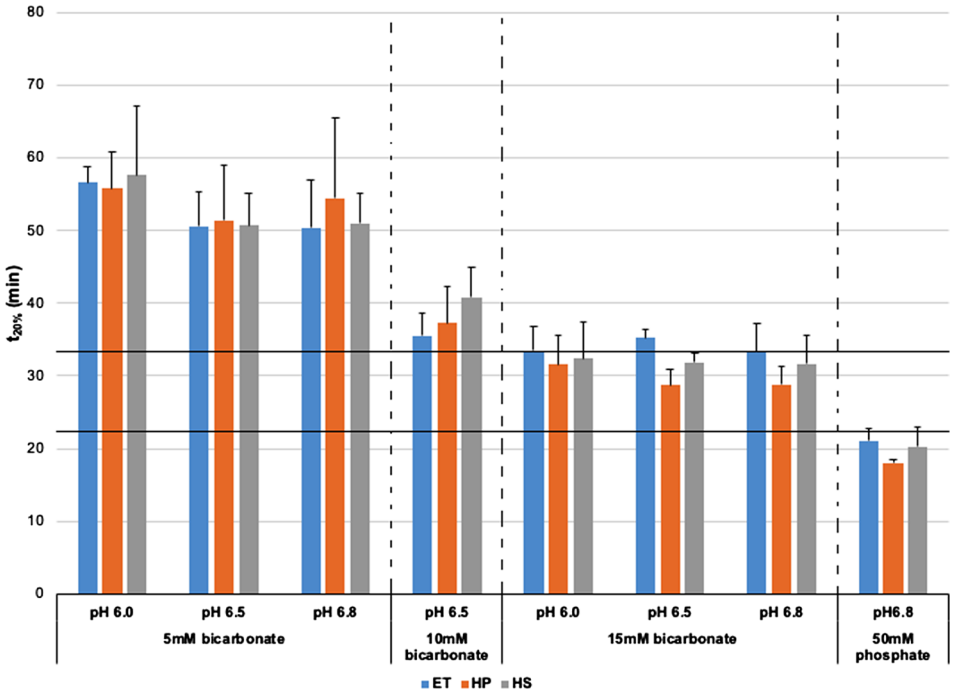

Fig. 3. Dependence of the time required to achieve 20% release on bulk pH and bicarbonate molarity for in house-manufactured paracetamol HPMC hard shell capsules (mean ± SD; n = 3). The blackened horizontal lines represent estimates of the transit time through the duodenum and proximal jejunum with the upper one representing the arithmetic mean (33.4 min) and the lower representing the median (22.5 min) of the mean transit times of phenol red as found by Paixão et al. in the fasted state [39]. Though these data are for the transit of a liquid, they most probably provide a good estimate for the transit of solids since the small bowel transit time has been found to be independent of the physical state of the dosage form (solution, pellets or single unit) [10]. ET: Eudragit L100-55; HP: HP-55; HS: HPMCAS-LF.

Table 1
The results of comparing the $t_{20\%}$ results for in house manufactured paracetamol EC capsules for the different polymers using two-way ANOVA with interaction. Comparison was done between the results in 5 and 15 mM bicarbonate buffers across the three pH bulk values of 6.0, 6.5 and 6.8. 10 mM buffer was not included since it was evaluated at one pH value only.

Polymer	p-value		
	Bicarbonate molarity effect	Bulk pH effect	Bicarbonate molarity and bulk pH interaction
Eudragit L100-55	< 0.0001	0.41	0.2519
HP-55	< 0.0001	0.44	0.9236
HPMCAS-L	< 0.0001	0.61	0.5395

duodenum and proximal jejunum being reported at around half an hour (mean = 33.6 min; median = 22.5 min) [39], and taking into account that (at least for Eudragit L100-55) the coating levels in terms of polymer mass applied per cm^2 are near the lower end of the range recommended by the manufacturer, it can be inferred from Fig. 3 that the currently used enteric polymers have difficulty targeting the drug release to duodenum and proximal jejunum owing to insufficient sensitivity to bicarbonate. This is of importance in the case of API's with a preferential absorption site as in the case of the aforementioned oral iron delivery.

3.3. Bayer EC ASA tablets

As shown in Fig. 4, release in 50 mM phosphate buffer was significantly faster than in all the tested bicarbonate buffers. Compared to the previously discussed products, the release of the API is further delayed. This is most likely due to the acidifying effect that the acidic ASA in the core exerts on the surrounding enteric-coat [38].

The release shows strong dependence on bicarbonate molarity at pH 6.8, particularly in the 5 to 10 mM range where the change in release rate is quite dramatic, with 80% release at 2.5 h in 10 mM pH 6.8 bicarbonate buffer in contrast to less than 5% release in at 4 h in 5 mM pH 6.8 bicarbonate buffer. This might provide new insights concerning the clinical evidence regarding poor efficacy of EC ASA in anti-platelet therapy, which has been theorized, by a few authors, to be a consequence of slow release and/or poor absorption of ASA from EC formulations [16,17,7] though the exact mechanism has not been thoroughly investigated as of yet.

A surprising finding is that the release rate in 15 mM bicarbonate buffer at pH 6.0 was faster than that in the corresponding pH 6.8 bicarbonate buffer. When evaluated using a two-tailed *t*-test for two independent samples, the time required to achieve 80% release was found to be significantly different between the two pH values with a p-value of 0.0013. A similar trend was also observed in the study by Karkossa and Klein [20]. A probable explanation is related to gas generation.

Gas generation inside the EC ASA tablet, though not directly observable, can be implied from the tumbling and floating behavior that the tablets exhibit when tested in bicarbonate (Fig. 5) but not in phosphate. This can be expected for a drug like ASA, with its relatively high intrinsic solubility of 26.1 mM and low pKa of 3.5 [11]. Actually gas formation in bicarbonate has been observed by Krieg et al. even at the surface of a rotating disc composed of benzoic acid, a compound with lower intrinsic solubility and higher pKa compared to ASA [24], which further supports the gas generation inside the EC ASA tablet hypothesis.

The gas generation could provide and additional disintegrating force (pressure) promoting ASA release [20], and it is expected to be more intense in a 15 mM bicarbonate buffer at pH 6.0 than in a 15 mM bicarbonate buffer at pH 6.8. This is because CO_2 bubble formation at rough solid liquid interfaces occurs primarily through heterogenous nucleation [47], with dissolved CO_2 diffusing from the liquid into air pockets entrapped within pits at the rough solid surface to generate bubbles. At pH 6.0, a bicarbonate buffer will be richer with dissolved carbon dioxide ($CO_{2(aq)}$) than at pH 6.8. The higher dissolved CO_2 concentration will result in a concentration gradient that is more favorable for CO_2 diffusing from the liquid into air pockets entrapped within the tablet. And this will facilitate the heterogenous nucleation of carbon dioxide bubbles. This issue of gas generation complicates the process of developing a biopredictive non-bicarbonate surrogate buffer through the resulting alteration in disintegrating force and interfacial contact area between the solid drug and the dissolution medium, and it needs further investigation.

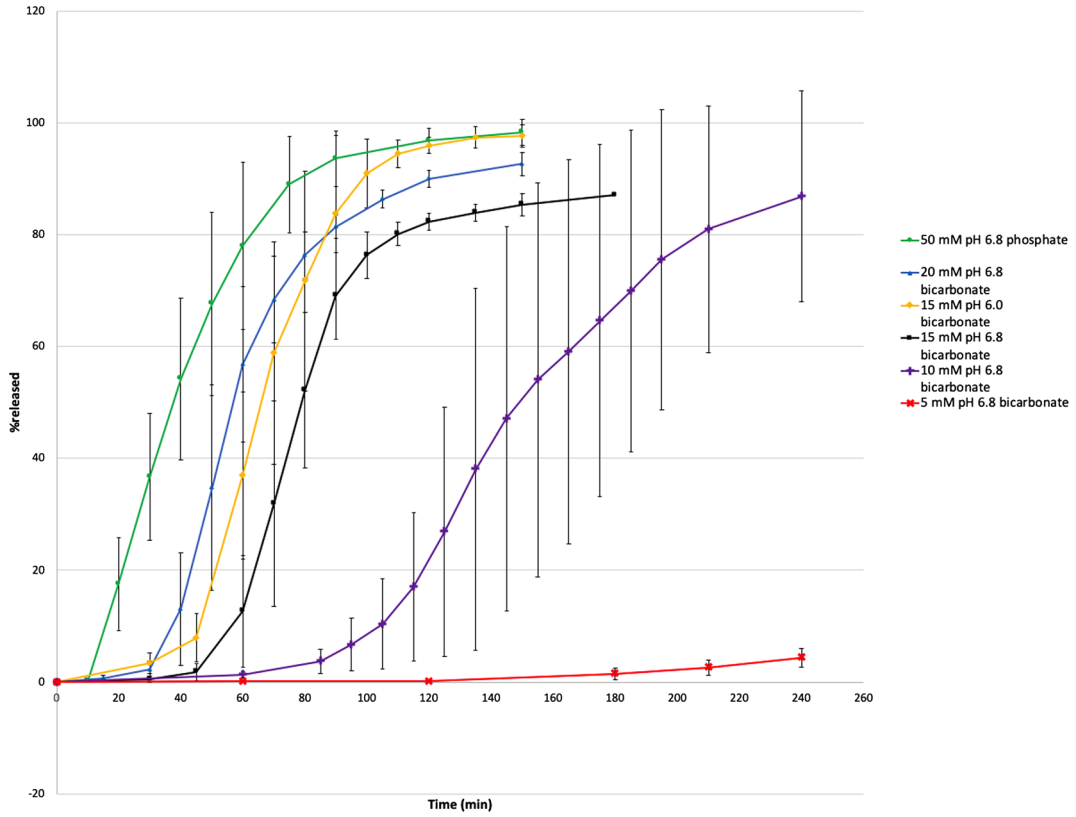

Fig. 4. Dissolution of Bayer Aspirin EC tablets in different media (mean ± SD; n = 6).

3.4. *Delzicol®*

What sets Delzicol® apart from the other formulations investigated is that it is a formulation intended to target the ileum and the colon by virtue of the dissolution pH threshold of the coating film-former (Eudragit S) being stated to have a dissolution pH threshold of 7 [5].

As shown in Fig. 6, even though bulk pH had considerable influence on API release, the bicarbonate molarity was the more important factor, with doubling it consistently appearing to have a stronger influence than halving the hydrogen ion activity (represented by an increase in pH value equal to 0.3). Fig. 7 shows that the release in the USP phosphate buffer is appreciably faster than that in all the bicarbonate buffers even the 120 mM bicarbonate buffer despite having lower conjugate base molarity (113 mM HPO_4^{2-}) and pH (7.2 vs 7.4), showing that phosphate is inherently more capable of dissolving Eudragit S-based coatings than bicarbonate, most probably because of its stronger buffering action at the relevant pH values. Slower release in bicarbonate-based buffers compared to phosphate-based ones was also observed by Karkossa and Klein [21].

At physiological conditions representing the terminal ileum (pH 7.4 and 30-mM bicarbonate), dissolution is too slow for significant release of mesalamine to be expected in the terminal ileum, with less than 50% release at 6 h and less than 5% release at 4 h. Actually, taking into account that the total small intestinal transit time is ~4 h[22], it is questionable if even a very high bicarbonate molarity of 60 mM would achieve considerable release in the terminal ileum (less than 5% released in 4 h). This indicates that the low responsiveness of the Eudragit S-based coat to bicarbonate could be a factor limiting the *in vivo* performance of this product.

This might actually be one of the factors contributing to what was observed in literature reports concerning observed disintegration failure and incomplete therapeutic response of pH-responsive mesalamine formulations *in vivo* [41,41,45]. Also it might be a factor behind the modest benefit of mesalamine in the treatment of Crohn's disease [26], which often involves the ileum. And also is in line with mesalamine being more effective when Crohn's disease involves the colon than when it involves the ileum only [31]. Actually, the prescribing information for Delzicol® states that the intact dosage form could occasionally be observed in the stool following the bowel movement [5]. However, further investigations are required before a role could be confirmed for this apparently poor bicarbonate-responsivity of Eudragit S regarding those issues.

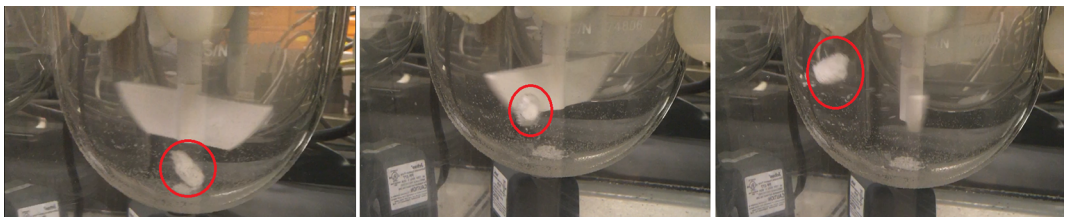

Fig. 5. Snapshots showing the floating behavior of a Bayer EC ASA tablet in 15 mM pH 6.8 bicarbonate buffer.

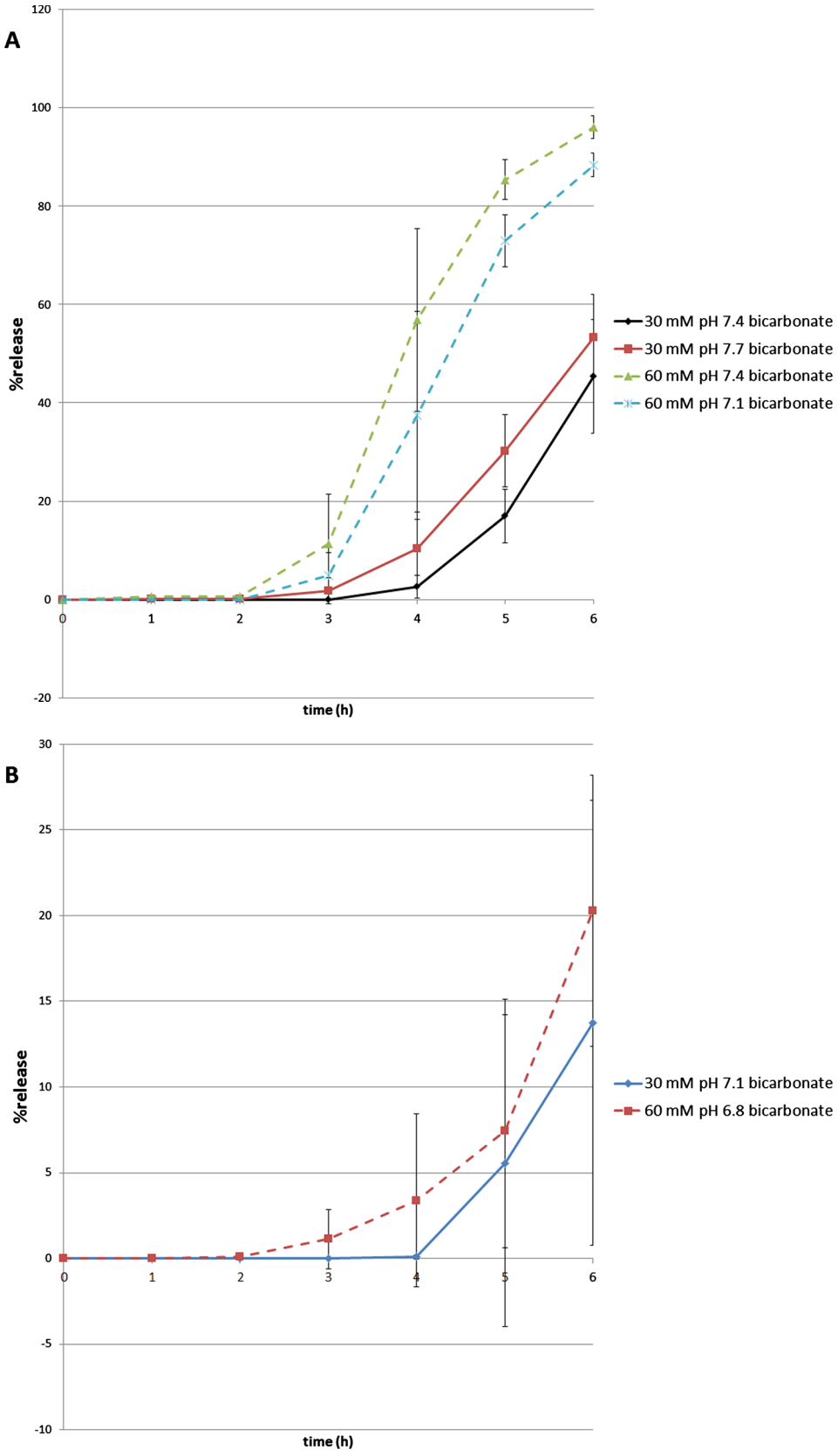

Fig. 6. Dissolution of Delzicol® in different bicarbonate buffers (mean ± SD; n = 3). Graph A shows the main contrast being between the different bicarbonate molarities (solid line for 30 mM and dashed line for 60 mM) regardless of bulk pH. Graph B shows that even dropping the pH below the polymer's reported dissolution pH threshold of 7 did not make the bulk pH effect stronger than the bicarbonate molarity effect.

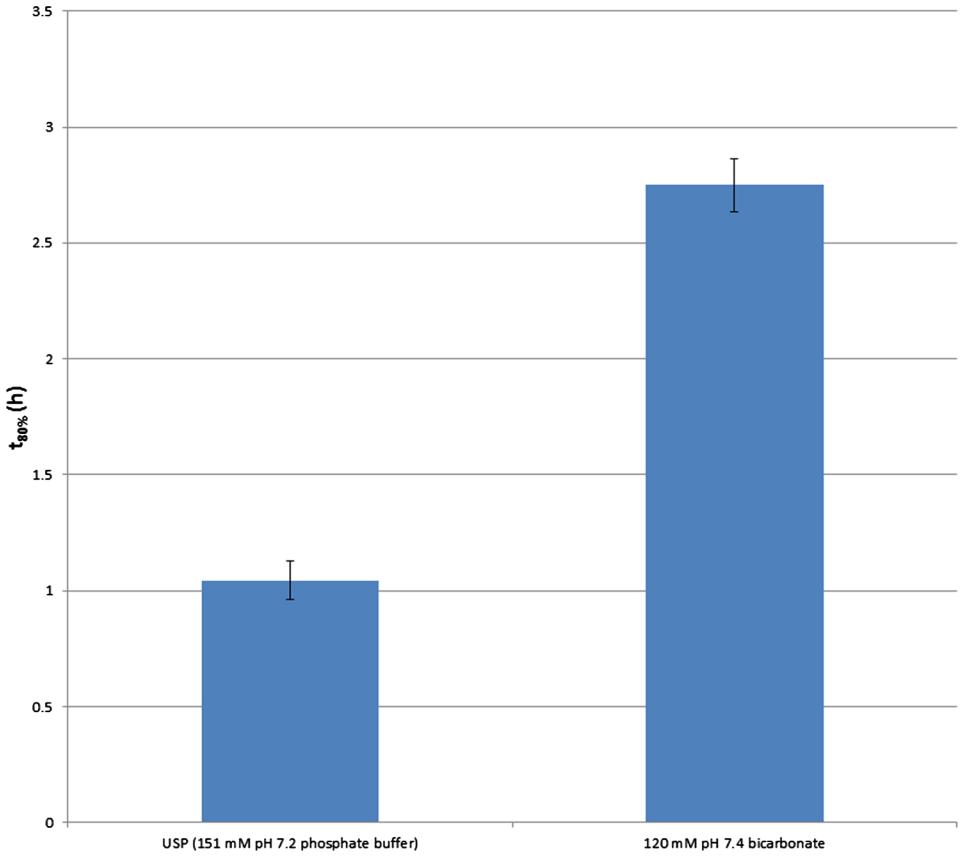

Fig. 7. Dissolution Delzicol® in USP phosphate buffer and 120 mM pH 7.4 bicarbonate buffer expressed by the time required to reach 80% release (mean ± SD; n = 3).

Table 2
Comparison of the performance of different products under bicarbonate and maleate buffers of comparable bulk pH and conjugate base molarity values. The molarities of both buffers represent the conjugate base molarities. The p-values are calculated by comparing the bicarbonate and maleate results using a two-tailed *t*-test for two independent samples.

Product	Enteric Polymer	Buffer	Van Slyke's Buffer capacity (mM/ΔpH)*	Initial rupture time or $t_{1\%}$ (h)#	p-value
Dulcolax®	Methacrylic acid copolymer type C NF	10 mM pH 6.4 bicarbonate (n = 3) 10 mM pH 6.4 maleate (n = 6)	7.00 4.80	0.56 ± 0.01 0.40 ± 0.06	0.00017
Bayer Aspirin	Methacrylic acid copolymer type C NF	10 mM pH 6.8 bicarbonate (n = 6) 10 mM pH 6.8 maleate (n = 3)	3.41 2.18	1.84 ± 0.32 0.51 ± 0.20	0.00080
Delzicol®	Eudragit S	30 mM pH 7.1 bicarbonate (n = 3) 29 mM pH 6.9 maleate (n = 3	5.54 5.13	4.79 ± 0.64 3.06 ± 0.79	0.044827

* i.e. estimated using the van Slyke equation [44].
# Initial rupture times are shown for Dulcolax® and Bayer Aspirin, while time required to achieve 1% release ($t_{1\%}$) is shown for Delzicol®. Due to the long time involved, continuous observation of Delzicol® was not possible, so $t_{1\%}$ values were used as surrogates. They were found to be in good agreement with observations for rupture made at half-hour intervals (the first such time points at which a rupture was observed were 2.5, 3.5 and 4 h for maleate, and 4.5, 5 and 5.5 h for bicarbonate).

3.5. Maleate buffer

The results of the experiments performed in maleate buffer are all summarized in Table 2. Maleate buffers gave consistently faster coat rupture than comparable bicarbonate buffers. Fig. 8 also shows the dissolution profile of Delzicol® in maleate buffer being sandwiched between those obtained in comparable phosphate and bicarbonate buffers. The significance of these findings is discussed in Section 3.6.2.

3.6. General discussion

It has been shown that bicarbonate concentration has a stronger effect on the dissolution performance of enteric coatings than bulk pH, and that insufficient sensitivity of those coatings to bicarbonate ion is the main factor limiting their performance *in vivo*. Even at pH values considerably higher than their known dissolution thresholds of pH at high buffer capacity, the enteric coatings do not dissolve sufficiently fast at bicarbonate molarity values that correspond to the actual *in vivo* bicarbonate concentrations reported in literature.

It seems that only at bulk pH values close to the polymer's dissolution pH threshold that bulk pH starts exerting a significant effect on the enteric polymer dissolution. This is probably because the pH-solubility profile of an enteric polymer reaches a point where even small changes in pH result in large solubility changes, and so even small changes in surface pH result in appreciable changes in the dissolution rate (an example of a pH-solubility profile of an enteric polymer,

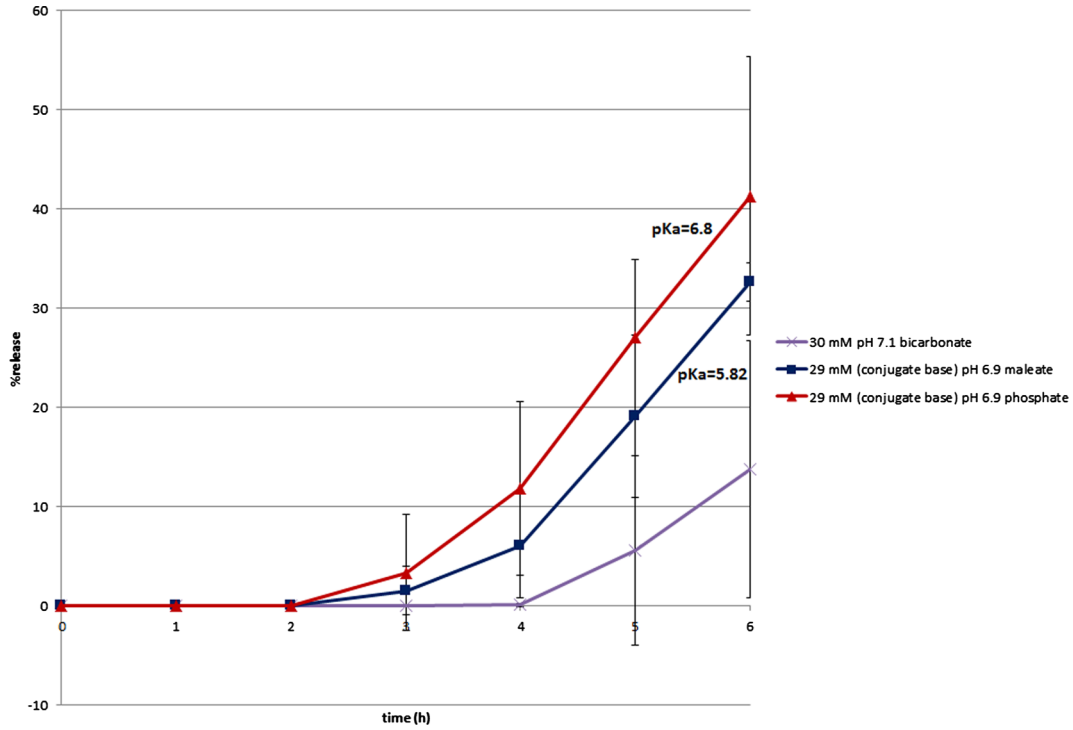

Fig. 8. Dissolution of Delzicol® in different buffers containing similar conjugate base concentrations at comparable pH values (mean ± SD; n = 3).

exhibiting such a region, is shown in the paper of Nguyen and Fogler [37]. This is also perhaps the reason why for the in house-manufactured paracetamol capsules, an indication of a possible bulk-pH effect appeared only at 5 mM bicarbonate molarity. With it being possible that, at this molarity, the surface pH was in this range with strong pH-dependence of the polymer solubility.

However, as seen in Figs. 1 and 6, even at values close to the dissolution pH threshold of the polymer, the bulk pH effect still does not seem to get stronger than the bicarbonate molarity effect (at least within the investigated range of values). This is particularly clear in the case of the Delzicol® (coated with a polymer with a dissolution pH threshold of 7). The dissolution of this product in 30 mM bicarbonate pH 7.1 was not faster than that in 60 mM pH 6.8 (double the hydrogen ion activity and double the bicarbonate molarity) bicarbonate buffer. For the mechanistic reasons behind our findings, a closer look on the mechanism of enteric polymer dissolution and chemistry of bicarbonate is needed.

3.6.1. Dissolution of enteric polymers

Enteric polymers dissolve when they are penetrated by water, and their carboxyl groups react with hydroxide ions and the buffer's conjugate base, producing the ionized polymer, which relaxes (owing to electrostatic repulsion) and undergoes disentanglement, swelling and ultimately dissolution. This eventually results in the formation of a gel layer at the surface of which the polymer chains are disentangled to a degree where they leave the polymer gel phase and dissolve into the surrounding medium [37].

The dissolution rate at the surface of the polymer will be determined by the pH maintained at that surface [37], which in turn will be determined among others by a combination of the buffer's bulk pH and buffering capacity. With a pKa of 6.04 [23], bicarbonate would not be expected to have much difficulty in buffering the polymer surface at values higher than pH = 6 which would be conducive to prompt enteric polymer dissolution. However, as explained below, the kinetics of the hydration and dehydration reactions of carbon dioxide and carbonic acid result in a different situation.

3.6.2. Chemistry of bicarbonate

When a bicarbonate ion reacts with a proton it forms carbonic acid that in turn readily dehydrates into carbon dioxide as shown below:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

The protonation of bicarbonate to form carbonic acid is associated with a pKa of around 3.55 [23], we refer this as the intrinsic pKa. However, in water, an equilibrium between proton, bicarbonate, carbonic acid and carbon dioxide will lead to the apparent pKa of bicarbonate being equal to about 6.04 (apparent pKa in water) at physiological temperature and ionic strength when determined potentiometrically [23], which is the value of bicarbonate pKa typically encountered in literature, and it will govern the buffering capacity in situations like titrating a bicarbonate solution with HCl [3].

However, in the diffusion layer around a typical dissolving weak acid with typical agitation (convection), the diffusional processes are too fast for the interconversion between the generated carbonic acid and carbon dioxide to be at equilibrium. This results in the effective pKa of bicarbonate in the boundary layer being lower than the aforementioned value of 6.04 [23]. Therefore, the buffering capacity of bicarbonate in the boundary layer, and accordingly, the flux of a dissolving ionizable solute across it will not be governed by the potentiometrically determined pKa value of bicarbonate.

The fact that the effective pKa of bicarbonate in the diffusion layer is lower than the 6.04 value is clearly illustrated by Fig. 8. Here despite its pKa of 5.82[2] [14], a pH 6.9 maleate buffer containing 29 mM of the conjugate base promotes faster Delzicol® dissolution than a 30 mM pH 7.1 bicarbonate buffer. This means that maleate is more efficient in maintaining a near-neutral surface pH than bicarbonate, indicating that the effective pKa of bicarbonate is actually lower than that of maleate.

This is in line with the observations by Krieg et al with intrinsic dissolution rates for small molecule weak acids in bicarbonate buffers. These rates were higher than what would be expected for a pKa of 3.55 buffer due to the presence of some net carbonic acid dehydration that

[2] At temperature of 37 °C and ionic strength of 0.15 M.

would limit the acidity of the diffusion layer. Nevertheless, they were still considerably lower than what would be expected for a pKa 6.04 buffer [23].

To further validate this hypothesis, Dulcolax® and Bayer Aspirin EC tablets were also evaluated in maleate buffer, thus evaluating the effect across two different enteric polymers and three different tablet cores. The results are shown in Table 2. It is clear that maleate consistently results in the enteric coat starting to rupture earlier than in bicarbonate buffers of similar pH and conjugate base molarity. This is despite the supposed buffering capacity of bicarbonate (assuming the pKa of 6.04 were applicable for buffering the boundary layer) being consistently a bit higher than that of maleate owing to the pKa of maleate being 5.82, i.e. a bit lower than the potentiometrically determined pKa of bicarbonate, and so a bit farther away from the bulk pH values used.

This supports our hypothesis that, in the boundary layer around the dissolving polymer, the effective pKa of bicarbonate is considerably lower than the value of 5.82 of maleate. This causes the buffering capacity of bicarbonate in the boundary layer (in contrast to that in the bulk) to be lower than that of maleate in the reported experiments. The implication of this is that the effective pKa of bicarbonate is lower than the potentiometrically determined value of 6.04. And as mentioned before, the reason behind this is the incomplete equilibration between carbon dioxide and carbonic acid in the boundary layer.

3.6.3. Impact of bicarbonate chemistry on dissolution of enteric coatings

The low effective pKa of bicarbonate in the diffusion layer means that the ability of a bicarbonate-based buffer to buffer the pH of the dissolving polymer's surface at a value conducive to prompt dissolution is limited. The higher the surface pH value required for prompt polymer dissolution, the more difficult it is for bicarbonate buffers to achieve this pH, and the higher the bicarbonate molarity needed for dissolution. In other words, due to the effective pKa of bicarbonate in the boundary layer being seemingly much lower than the surface pH values conducive to prompt polymer dissolution, its effective buffering action in this boundary layer is poorly capable of maintaining such surface pH values unless high molarities are used.

This, for example, can be illustrated by using an analogous situation involving a simple small molecule-carboxylic acid. The well-known Mooney film model for the dissolution of carboxylic acids in buffered media [32] which has been validated through experiments involving the intrinsic dissolution (rotating disc) of 2-naphthoic acid in different buffers at 450 rpm, can be used to more clearly illustrate the dependence of a buffer's ability to maintain the surface pH above a certain threshold on its pKa. The physico-chemical data for naphthoic acid and one of the buffers in which it was tested (acetate buffer) were taken from the Mooney paper and the model was applied with varying buffer pKa values to a MATLAB software code (MathWorks, MA, USA). The model is based on calculating the surface H^+ molarity using a cubic equation the coefficients of which are functions of the different physico-chemical properties of the dissolving substance and of the buffer. For more information, the reader is referred to the original publication cited here.

Fig. 9 shows how a lower buffer pKa necessitates higher buffer conjugate base molarity values to get the same surface pH values. Fig. 9 also illustrates how a lower buffer pKa results in the surface pH being less dependent on bulk pH. An explanation for that is as follows:

The buffer capacity (β) is given by $\beta = 2.303CKa[H]^+/(Ka + [H^+])^2$

where C is the total buffer concentration [44].

A rearrangement of the Henderson-Hasselbalch equation gives us the conjugate base concentration ($C_{conj.base}$) $= CKa/(Ka + [H^+])$

So, when $[H^+] \ll$ Ka (i.e. pH is much above the pKa)

The van Slyke equation reduces to $\beta \approx 2.303C_{conj.base}[H]^+/Ka$

This means that a 10-fold decrease in hydrogen ion activity (corresponding to increasing the pH by one unit) will give rise to a 10-fold-drop in buffer capacity. This means that even when the diffusional layer separating the hydrogen ion activity in the bulk from a value at the surface increases by one $\log_{10}$-cycle, the ease with which this barrier can be overcome drops by one $\log_{10}$-cycle.

This enables a dissolving weak acid to bring the surface pH to similar values in buffers with different bulk pH values at low buffer capacity. Therefore, the increase in surface pH decreases as the bulk pH becomes higher and higher above the buffer pKa. As a result, the surface pH becomes less dependent on the bulk pH.

3.6.4. Implications for pH-dependent drug delivery

pH-dependent oral drug delivery has so far been based on the concept of a polymer targeting drug release to the intestinal segment with a pH exceeding the polymer's dissolution pH threshold. However, this concept faces two challenges: lack of a precise standardized definition of dissolution pH, and the fact that the dissolution rate of an enteric polymer is directly determined by its surface pH rather than the bulk pH. This surface pH is a function of the polymer's properties as well as the buffer's bulk pH and buffering capacity, which (i.e. buffering capacity) is a function of the buffer's molarity and pKa, as well as of the bulk pH. Since surface pH determination is not straightforward, there is a need for the identification of which intestinal fluid parameter has the strongest influence on it and, accordingly, on polymer dissolution, to be able to classify different polymers as to which intestinal segment they can target.

The main buffering species in the human intestine is bicarbonate, and both the bulk pH and the molarity of this buffer seem to increase along the small intestine. In this work, when the effects of those two factors on the release properties of different EC formulations were investigated, it was found that, within biorelevant ranges, the effect of the bicarbonate molarity tended overall to be considerably stronger than that of the bulk pH. Therefore, if a certain threshold should be used as a parameter to classify enteric polymers as to which small intestinal segment they would target effectively, it might, from a practical point of view, be more appropriate for it to be a bicarbonate molarity threshold rather than a bulk pH threshold. As for the large intestine, further investigations should be performed in this regard to account for presence of significant concentrations of short-chain fatty acids and their buffering effects [28].

Furthermore, in addition to bicarbonate, protein molecules could also contribute to the buffering of the intestinal fluid. Their large molecular size and accordingly low diffusivity might limit their impact on drug and excipient dissolution relative to bicarbonate. Nevertheless, investigating their effects could be worthwhile.

4. Conclusion

API release from pH-dependent EC products depends on the surface pH of the dissolving polymer, which, *in vivo*, is often more dependent on bicarbonate molarity than on bulk pH. It is insufficient responsiveness to bicarbonate that seems to limit the ability of at least some of the currently available enteric coatings to target API release to the intended intestinal segments. Therefore, taking into account that data on intestinal bicarbonate concentrations are relatively scarce, a rigorous and thorough investigation of the bicarbonate molarity profiles along the human intestine is needed to facilitate appropriate design of *in vitro* dissolution media.

Overall, these results indicate that the use of appropriately designed *in vitro* testing buffers will be of great utility in designing pH-dependent drug delivery systems. For this purpose, the specifics of the physical chemistry of bicarbonate must be taken into account, and either a physiological bicarbonate buffer or a properly designed surrogate buffer (if possible) should be employed in order to avoid test results that would misdirect the formulation development process.

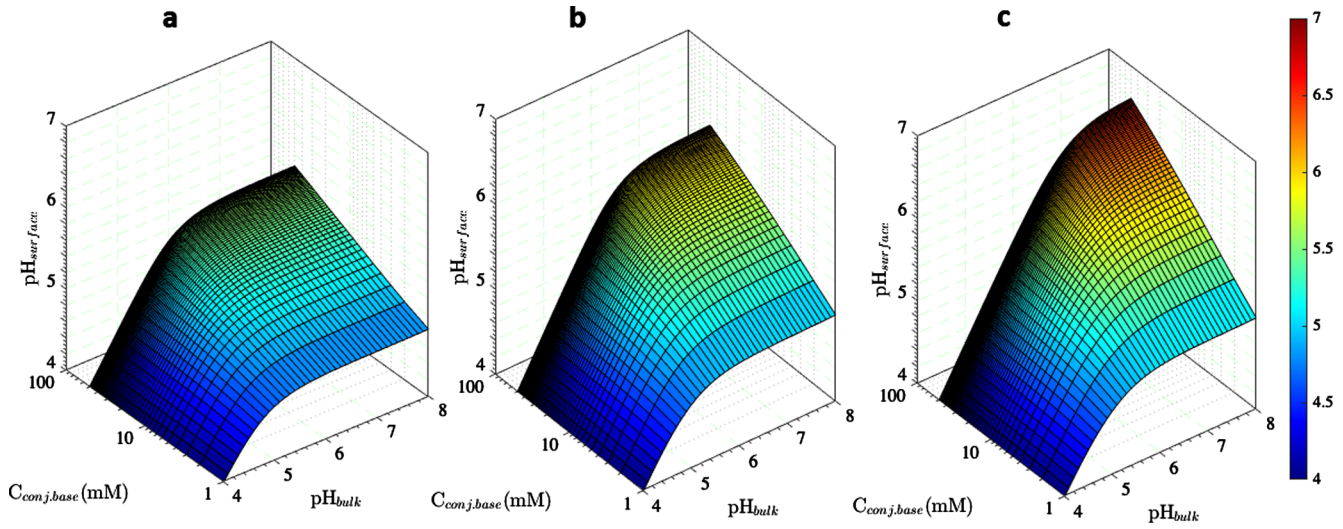

Fig. 9. Surface pH dependence of 2-naphthoic acid on bulk pH and conjugate base molarity of the buffer as a function of buffer pKa. a: buffer pKa = 4.6; b: buffer pKa = 5.5; c: buffer pKa = 7. It shows that higher buffer pKa facilitates buffering the surface pH to higher values. It also shows that at bulk pH values exceeding the buffer pKa, the dependence of the surface pH on the bulk pH is weaker.

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