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Teaser After the pH dependency of novel pH-dependent ileo-colonic drug delivery systems is confirmed *in vitro*, their performance should be evaluated in human volunteers.



# pH-dependent ileocolonic drug delivery, part I: *in vitro* and clinical evaluation of novel systems

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pH-dependent ileocolonic drug delivery systems rely on the sharp pH peak reaching 7.2–7.7 usually found in the ileum of healthy individuals and patients with colonic diseases. The pH dependency of novel drug delivery systems should first be evaluated in *in vitro* dissolution tests mimicking the human gastrointestinal pH profile and buffer composition. When proven successful *in vitro*, the clinical applicability of a novel system should be confirmed in humans. Various methods have been published to verify ileocolonic drug delivery in humans. Of those, we recommend the caffeine-sulfasalazine method and the dual stable isotope approach.

## Introduction

Drug delivery to the ileocolonic region is receiving substantial attention because it could improve the local treatment of disorders, such as ulcerative colitis (UC), Crohn's disease (CD), and colorectal cancer (CRC) [1]. In addition, the potential of ileocolonic delivery for systemic treatment is a subject of investigation. Examples are the systemic delivery of protein and peptide drugs, which are spared because of the low proteolytic activity in the colon [2], and the delayed delivery of drugs to treat diseases that follow a circadian rhythm, such as asthma, angina pectoris, and rheumatoid arthritis [3].

Several approaches have emerged to achieve ileocolonic targeted drug delivery. They include systems that are dependent on time, pressure, enzymes, pH, and combinations thereof. Comprehensive overviews on this topic have been published elsewhere [1,4,5]. Although time-, pressure-, and enzyme-based systems have also shown their potential, here we focus on pH-dependent systems for ileocolonic drug delivery. In general, these pH-dependent systems are based on pH-sensitive polymeric coatings surrounding the drug or pH-sensitive matrices in which the drug is embedded. We describe different methods to measure the pH in the various parts of the human gastrointestinal (GI) tract and compare the reported values for healthy individuals and

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**Henderik W. Frijlink** has been a professor and chairman of the Department of Pharmaceutical Technology and Biopharmacy at the Groningen Research Institute of Pharmacy since 1998. He is interested in solid oral dosage forms, their technological and biopharmaceutical aspects and gastrointestinal targeting strategy. Further interests of his current research are technical and biopharmaceutical aspects of pulmonary drug delivery. The formulation of biopharmaceuticals, such as peptides, proteins, vaccines, and gene-based products, using stabilizing sugar glasses is a further research topic in his department. He has published over 200 peer-reviewed papers in the field.



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patients with various colonic diseases. Thereafter, current methods to evaluate novel systems *in vitro* as well as in clinical trials are described and critically discussed. Finally, we suggest methods that are most suitable for evaluating the performance of novel systems.

## pH in the lumen of the human gastrointestinal tract

### Methods to determine the pH in the human gastrointestinal tract

For the development and performance of pH-dependent ileocolonic drug delivery systems, it is a prerequisite to know the pH of the content in the different segments of the GI tract of both healthy and diseased humans. Three different methods are used to measure these pH values. The first is aspiration, during which GI fluid is collected and the pH of the collected fluid is measured *ex vivo* with a pH electrode [6–16]. This can be performed via the oral route for the stomach, duodenal, and jejunal fluid [6–14] or via colonoscopy for the ileal and colonic fluid [15,16]. The disadvantage of colonoscopy is that it requires the administration of either bisacodyl or Klean-Prep® before the procedure, which alters the contents of the GI tract and, therefore, could alter the pH. In the second method, a tethered pH electrode is used to measure the pH *in situ* of the stomach, duodenum, or jejunum [17–24]. In the third method, a pH-sensitive radio telemetry capsule is used, which measures the pH during its transit through the entire GI tract [25–45]. The major advantage of a tethered pH electrode or a pH-sensitive radio telemetry capsule is that the pH changes over time of the different segments in the GI tract can be measured, whereas the aspiration and colonoscopy methods only generate mean values. Furthermore, pH-sensitive radio telemetry capsules measure the pH during its transit through the entire GI tract. Thus, this method represents a dynamic measurement. Examples of telemetry capsules are the Heidelberg capsule, SmartPill, Bravo™ pH monitoring capsule, and the IntelliCap® (Table 1) [46–49]. The SmartPill and IntelliCap® measure not only the pH in the GI tract, but also the temperature, which is helpful to determine when the capsule exits the body. Furthermore, when pyloric passage is assumed, ice-cold water can be orally administered and, when no immediate temperature decrease is measured, it can be concluded that the telemetry capsule has passed the stomach [47,49,50]. The IntelliCap® also contains a fluid reservoir that can be used to locally deliver compounds to the GI tract, for instance to enable pharmacokinetic studies [49,50]. The downside of this system is that the capsule is considerably larger than the other systems and has not been commercially available since 2017. The Bravo™ system was originally developed to be attached to the esophageal mucosa via endoscopic intervention to measure esophageal acid exposure over time [48]. Without endoscopic interven-

tion, the capsule becomes a freefall system and the pH over the entire GI tract can be measured [30]. Overall, there is no system superior to another, and the choice of the system depends on the specific research question or preference.

Besides measuring pH, temperature, and intestinal pressure, radio telemetry capsules can be used to measure the transit time through the different regions of the GI tract, without the aid of imaging techniques. The transit times, based on pH readings, have been found to be comparable to those obtained with imaging techniques [37,42,51].

### pH values in the gastrointestinal tract of healthy human individuals

In the literature, 36 studies were found in which the pH of the different segments of the GI tract of healthy humans was measured [6–14,17–23,25,28–45,52]. When compiling an overview of the mean pH values of the various segments of the GI tract, both intra- and interindividual variation has to be taken into account. For pH-dependent ileocolonic drug delivery, the pH values in the small intestine and ascending colon are most important.

Regarding intraindividual variation, Mikolajczyk et al. showed that, over 24 h, pH fluctuations in a single subject were only minor, with pH variations in the colon being slightly higher ( $\Delta\text{pH}$ : 0.45) than in the proximal small intestine ( $\Delta\text{pH}$ : 0.14) and distal small intestine ( $\Delta\text{pH}$ : 0.22) [43]. According to Ibekwe et al. the intraindividual variability in pH is partially the result of differences in transit times of the telemetry capsules [30]. With telemetry capsules, the transit time determines the number and location of pH measurements taken in each region of the GI tract. Therefore, differences in transit time, including stasis and retro-pulsion, could influence the mean pH value. With aspiration, the intestinal fluid is homogenized and the mean pH is based on the aspirated sample taken; therefore, the location of aspiration could influence the mean pH. Koziolok et al. investigated the interindividual variability and showed that the pH was highly variable in the stomach and colon, but only small differences were seen in the proximal small intestine and even smaller differences occurred in the distal small intestine [31]. This is in line with other studies, which showed that gastric and colonic pH showed larger interindividual variations [25,41].

The mean or median pH found in the individual studies, for both the fasted and fed state in healthy adults, are displayed in Fig. 1 as dots. When multiple pH values were given for a GI tract segment, for instance the fundus and antrum of the stomach, the mean of these values is shown. The range of found pH values is shown as bars, in which a vertical line shows the mean pH of all studies with healthy adults. To calculate this mean value, the

TABLE 1

### Overview of pH-measuring radio telemetry capsules

Brand radio telemetry capsule	Dimensions (mm)	Telemetry			Fluid reservoir	Commercially available	Refs
		pH	Temperature	Intestinal pressure			
Heidelberg	8 × 18	X				X	[46]
IntelliCap®	11 × 27	X	X		X		[49,50]
Bravo™	6 × 5.5 × 25	X				X	[48]
SmartPill	11.7 × 22	X	X	X		X	[47]

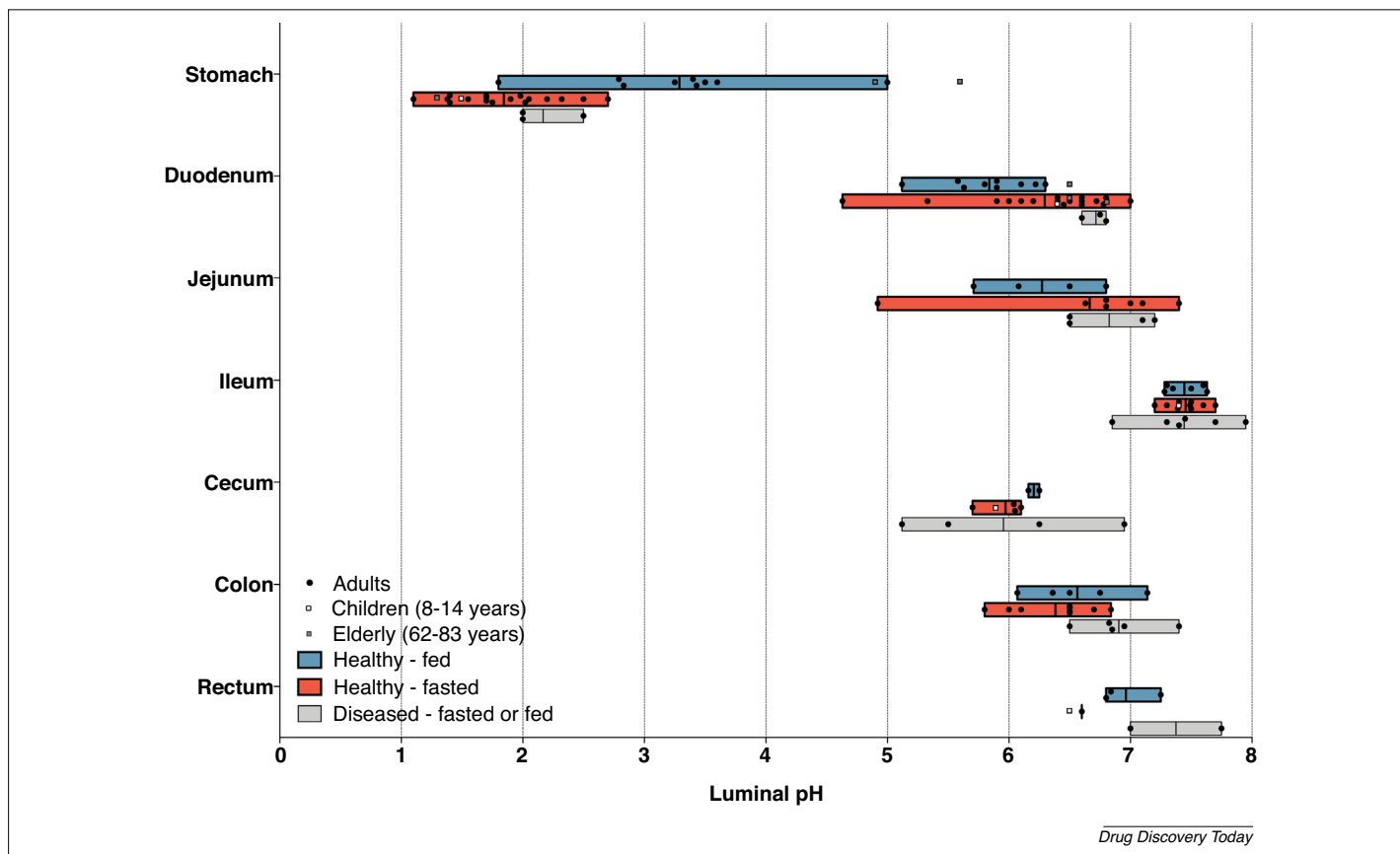


FIGURE 1

pH values of various parts of the gastrointestinal (GI) tract of healthy and diseased humans. The maximum, minimum, and mean pH of the stomach [9–12,17–19,21–23,28–33,36,40,42,52], duodenum [6–9,12–14,18–20,25,30–35,39,41,45], jejunum [8,18,30,32,34–36,38,43,45], ileum [25,30–39,41,43–45], cecum [28,33,34,36,37,44], colon [25,28,30–36,38,41–43,45], and rectum [34,36,43,45] are given for healthy individuals in the fed (blue) and fasted (orange) state. The pH values of diseased human individuals (gray) are grouped for the fasted and fed state; the mean pH value is not given [33,35,36,44]. The maximum and minimum pH values are indicated by the bars, in which a vertical line indicates the mean pH of healthy individuals, and the dots the mean or median pH values of the individual studies. The white squares, in the fasted state, indicate the pH values of the GI tract of healthy children aged 8–14 years [26]. The gray squares, in the fasted and fed state, indicate the pH values of the GI tract of older subjects, aged 62–83 years [7,24,40]. Given the limited amount of studies in the fasted state for the pH in the rectum (one study), the bar and vertical line overlap.

mean pH values were used if they were mentioned in the individual studies. Mean values were preferred to prevent data loss of individuals with outlying pH values. If no mean value was given, the median value was used. The number of subjects in the separate studies was not used in the calculations. Studies only reporting pH ranges were excluded from the overview and from our calculations. We also excluded studies that used colonoscopy to access the pH value of the lower GI tract, because of the use of Bisacodyl of Klean-Prep®. We did not find marked differences in the pH values obtained with aspiration, tethered pH-electrodes, or telemetry capsules.

The pH rises during transit from the stomach to the ileum, after which it drops in the cecum and rises again slightly in the colon (Fig. 1). The precise location of the post ileum pH drop of 1.5 and 1.2 units in the fasted and fed state, respectively, was found to be in the proximal colon. The drop can occur either in the cecum, the ascending colon, or during the transit from the cecum to the ascending colon [37]. This pH drop can be explained by the bacterial fermentation of polysaccharides to short-chain fatty acids [53]. The food status of the subjects only appeared to influence the pH of the stomach; that is, the pH is substantially higher

after food intake. However, fewer data were available on the fed state than on the fasted state. Furthermore, in the postprandial state the stomach has regions of different pH values, namely a proximal acid layer (pH 2.9), a buffered layer (pH 5.0), and a distal acid layer (pH 2.3) [23].

Fallingborg et al. studied the pH in the GI tract of fasted healthy children, aged 8–14 years [26]. The mean pH values, indicated by white squares in Fig. 1, only slightly differed from the values of fasted healthy adults. For healthy older subjects, aged 62–83 years, three studies were found in which the gastric and/or duodenal pH was studied. These are indicated by gray squares in Fig. 1 [7,24,40]. Contradictory results were found on the influence of age on the gastric and duodenal pH. Comparative studies from the same group indicated that the gastric fasted and peak-fed pH were significantly lower for older (65–83 years) [24] than for young individuals (21–35 years) [19]. By contrast, Mojaverian et al. found that the postprandial pH values in the stomach of the older subjects (65–79 years) were significantly higher than those of young individuals (2–34 years) [40]. In the fasted state, no significant differences in gastric pH between three different age groups (20–39, 40–59, and 60–70 years) was found [10]. The duodenal pH

in the fasted and fed state was significantly higher in older (65–83 years) [24] than young (21–35 years) individuals [19]. By contrast, Annaert et al. found no significant differences between duodenal pH values for the older (62–72 years) and younger individuals (18–25 years) in the fasted state [7].

Next to age, gender differences in GI pH values have been investigated. No significant differences were found for the fasted and fed states for gastric [18,19,24,40], duodenal [18,19,24,35], and jejunal [18,35] pH. For the ileum and colon, only fasted state data were available, that showed no significant gender effect [35]. To the best of our knowledge, no influence of ethnicity on the GI pH has been reported in literature. The influence of age-related diseases on GI pH has been sparsely addressed and, therefore, was not included in this overview [54,55].

The sharp, local pH peak in the terminal ileum to values >7.2 (Fig. 1) has been used for the development of colon targeted drug delivery systems. When a system uses this pH value as a trigger to initiate drug release, release will start in the terminal ileum which is in close proximity of the cecum (i.e., the beginning of the large intestine) [1]. Thus, as stated by Ibekwe et al., the term ‘ileocolonic drug targeting’ is more appropriate for these systems than the term ‘colonic drug targeting’ [56]. When continuation of the release depends on the presence of the high pH value in the terminal ileum, the full release should occur quickly because the pH drops again in the cecum (pH 6.1). This will slow down or halt further disintegration and/or dissolution of the pH-dependent component in the system. Although the mean and minimum pH in the jejunum were found to be 6.5 and 4.9, respectively, a maximum pH of 7.4 was found in this GI segment. Therefore, there is a risk of premature drug release.

### *pH values in the gastrointestinal tract of humans with colonic diseases*

For a successful therapeutic application of an ileocolonic targeted drug delivery systems, it is important to have insight into the pH of the GI tract of patients with GI tract diseases, such as CD, UC, irritable bowel syndrome (IBS), and CRC. Therefore, here, we compare the pH of various segments of the GI tract of healthy subjects (Fig. 1) with those of the aforementioned patient groups. To visualize the overall differences more clearly, pH values of diseased individuals are also given in Fig. 1, in which the different diseases are grouped together.

The pH values of the GI tract of individuals with CD did not differ from those of healthy individuals, including the median pH peak of 7.4 in the terminal ileum [36]. For patients with UC, contradictory pH values have been reported. Press et al. found a median pH of 7.95 in the ileum and 6.95 in the cecum of patients with UC, which was higher than the maximum values of 7.70 and 6.25, respectively, found in healthy individuals [33]. By contrast, in a study by Ewe et al., a median pH of 6.8 in the ileum and 5.5 in the cecum of patients with UC was found [36], which was lower than the minimum values of 7.2 and 5.7, respectively in healthy individuals. However, the median pH increased to 7.3 in the ascending colon, which is higher than the maximum value of 7.1 found in the colon of healthy individuals, making pH-dependent colonic targeted drug delivery possible. In patients with IBS, the mean pH in the ileum was 7.7 [44], which falls within the pH range (7.2–7.7) of the ileum of healthy individuals. The mean pH

in the cecum in patients with IBS was 5.1, which is lower than the minimum value of 5.7 found in the ileum of healthy individuals. This lower pH could be attributed to excessive bacterial fermentation of polysaccharides leading to the production of short-chain fatty acids. Lastly, patients with colorectal adenoma or carcinoma were found to have pH values comparable to those in healthy volunteers in the various segments of the GI tract [35].

Based on the data from the studies described above, it is expected that pH-dependent ileocolonic drug delivery systems can successfully be applied in patients with the above-mentioned chronic GI diseases. Therefore, it is not surprising that various products are available on the market that utilize pH-dependent excipients for ileocolonic drug targeting [e.g., Budenofalk® (Budesonide; Dr. Falk Pharma), Lialda® (Mesalazine; Shire), and Asacol® (Mesalazine; Allergan)].

### ***In vitro* methods to simulate the human gastrointestinal tract**

Before clinical evaluation, novel pH-dependent systems are generally first tested in *in vitro* dissolution studies. Obviously, an optimal *in vitro* dissolution test mimics the physiological pH of the various segments of the GI tract as close as possible (Fig. 1). In addition, the type of buffer and exposure times to the different pH values should be carefully chosen.

Table 2 presents an overview of clinical studies together with information about the drug delivery system and details about the *in vitro* dissolution test, if applicable. As expected, the pH of the dissolution medium is often adjusted during the dissolution test to mimic the *in vivo* conditions. This can be achieved by either transferring the drug delivery system from one vessel to another vessel containing medium with a different pH or by changing the pH of the dissolution medium by adding another solution to the original vessel. The latter option is preferred because the drug delivery system will then only experience the pH change.

The GI tract is particularly buffered by bicarbonate in the fasted state, whereas in the fed state different buffer species are present [57]. Systems that use bicarbonate buffers with buffer capacities reflecting the *in vivo* situation have been found to better represent the *in vivo* situation compared with the more commonly used phosphate buffers (Table 2) [56,58–61]. According to Amaral Silva et al., a major difference is that bicarbonate has a lower buffering capacity in the diffusion layer near the surface of the drug delivery system compared with phosphate, resulting in a better *in vivo* predictability of bicarbonate systems [62]. A complicating factor of bicarbonate buffers in *in vitro* studies is their pH instability because of the loss of CO<sub>2</sub> as a gas from the system, which alters the pH. Garbacz et al. utilized this in their pHydro-grad® device in which the pH during the dissolution test can be dynamically altered by purging N<sub>2</sub> or CO<sub>2</sub> gas into the system to increase or decrease the pH, respectively, thereby eliminating the major drawback of bicarbonate systems [63]. Purging of gasses and loss of CO<sub>2</sub> gas in the system introduces bubbles into the dissolution medium, which could influence the drug release [62]. To avoid effects of CO<sub>2</sub> bubbles on the drug release when bicarbonate buffers are used, alteration of the buffer capacity of phosphate buffers has been proposed. However, not only the buffer capacity influences the release of pH-dependent systems, but also the buffer type and the ionic strength [61,64]. An investigation into the



TABLE 2

## Overview of pH-dependent ileocolonic drug delivery systems tested in clinical trials

Drug delivery system		In vitro dissolution test			In vivo test		Refs
pH-dependent matrix (M) and/or coating (C)	Drug/marker	pH (t in h) including shifts	Fixed	Buffer type	Readout	Colon arrival determined with	
Cellulose acetophtalate and diethylphtalate (C)	Riboflavin monophosphate, <sup>13</sup> C-urea, and <sup>15</sup> N-urea		1.2; 5.0, 6.0, and 7.0	Phosphate	Breath and urine samples	Breath and urine samples and confirmed with inulin, inulin- <sup>14</sup> C-carboxylic acid, and lactose- <sup>13</sup> C-ureide breath tests	[106]
Enteric acrylic resin (C)	Mesalazine and <sup>153</sup> Sm	1.2 (1); 2.5 (2); 4.5 (1.5); 7 (1.5); 7.2 (2)		N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[78,111]
Eudragit E and L (C) <sup>e</sup>	<sup>111</sup> In	1.2 (4); 6.8 (4); 5 (1)		Citrate-phosphate and phosphate	γ-Scintigraphy	γ-Scintigraphy	[82]
Eudragit E and S (C) <sup>e</sup>	<sup>153</sup> Sm	1.2 (2); 7.4 (2); 6.4 among others <sup>c</sup>		Citrate-phosphate and phosphate	γ-Scintigraphy	γ-Scintigraphy	[94]
Eudragit FS 30 D (C)	Caffeine	1.2 (2); 6.5, 7.0, 7.5 (12)		Phosphate	Plasma and breath samples	Lactose- <sup>13</sup> C-ureide breath test	[98,112]
Eudragit FS 30 D (C)	Paracetamol; <sup>153</sup> Sm	1.2 (2); 6.8 (1); 7.4		Phosphate	γ-Scintigraphy	γ-Scintigraphy	[80]
	Diclofenac sodium; barium sulfate	1.2 (2); 6.5 (1); 6.8 (2); 7.2		Phosphate	Radiography	Radiography	[113]
Eudragit L (C)	Mesalazine and <sup>153</sup> Sm	1.2 (2); 6.8 (1 and 5 <sup>d</sup> )		N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[85]
Eudragit L 100-55 (C)	Mesalazine and <sup>111</sup> In	N/A <sup>b</sup>	N/A	N/A	γ-Scintigraphy; plasma samples	γ-Scintigraphy	[92]
	<sup>2</sup> H <sub>8</sub> -budesonide; budesonide and <sup>111</sup> In	N/A	N/A	N/A	γ-Scintigraphy; plasma, urine, and fecal samples	γ-Scintigraphy	[91,114]
Eudragit L 30 D-55 (C)	<sup>2</sup> H <sub>8</sub> -budesonide; budesonide and <sup>111</sup> In	N/A	N/A	N/A	γ-Scintigraphy; plasma, urine, and fecal samples	γ-Scintigraphy	[89,114]
	Paracetamol; <sup>153</sup> Sm	1.2 (2); 6.8		Phosphate	γ-Scintigraphy	γ-Scintigraphy	[80]
Eudragit L or Eudragit L/S (C)	Barium sulfate	1.2 (2); 6.8, 7.5 <sup>c</sup>		Phosphate	Fluoroscopy	Fluoroscopy	[76]
Eudragit L/S (C)	Mesalazine and <sup>153</sup> Sm	N/A	N/A	N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[79]
Eudragit L (C)	Mesalazine and <sup>111</sup> In	N/A	N/A	N/A	γ-Scintigraphy; plasma samples	γ-Scintigraphy	[88]
Eudragit L 100-55 (C)	Beclomethasone dipropionate and <sup>153</sup> Sm	N/A	N/A	N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[83]
Eudragit S (C)	Salicylic acid; DTPA <sup>a</sup> labeled with <sup>99m</sup> Tc	1.1 (2); 6.1 (1); 7.0 (2); 6.5 (2), 1.1 (2); 7.2 (1); 7.8 (2)	7.5	Phosphate	γ-Scintigraphy	γ-Scintigraphy	[115,116]
	Sulfapyridine and barium sulfate	N/A	N/A	N/A	Radiography; plasma samples	Radiography	[75]
	<sup>99m</sup> Tc-DTPA	1.2 (0.5 or 2); 6.8, 7.0, 7.2, 7.4 (6)		Phosphate and bicarbonate	γ-Scintigraphy	γ-Scintigraphy	[30,56]
	Mesalazine	N/A	N/A	N/A	Fecal dialysate; urine samples and samples	N/A	[117]
	Theophylline	1.2 (2); 6.8 (2); 7.4 (2)		Phosphate	γ-Scintigraphy; plasma samples	γ-Scintigraphy	[95]
<sup>3</sup> H-Prednisolone	Prednisolone and <sup>99m</sup> Tc-DTPA or <sup>111</sup> In-DTPA	N/A	N/A	N/A	Radiography; urine samples	Radiography	[118]
		1.2 (2); 7.4; 1.2 (2); 6.0 (1); 7.2		Phosphate and bicarbonate	γ-Scintigraphy	γ-Scintigraphy	[84]

TABLE 2 (Continued)

Drug delivery system		In vitro dissolution test			In vivo test		Refs
pH-dependent matrix (M) and/or coating (C)	Drug/marker	pH (t in h) including shifts	Fixed	Buffer type	Readout	Colon arrival determined with	
Eudragit S (M) with Aqoat AS-HF (C)	Ibuprofen		5.8; 6.8, 7.4	Phosphate	Plasma samples	N/A	[119]
Eudragit S or Eudragit FS (C)	Prednisolone; <sup>99m</sup> Tc-DTPA; <sup>111</sup> In-DTPA	1.2 (0.5 or 2); 6.8, 7.0, 7.2 and 7.4 (6)		Phosphate and bicarbonate	γ-Scintigraphy	γ-Scintigraphy	[56,60]
Eudragit S or Eudragit L (C)	Prednisolone	N/A	N/A	N/A	Plasma samples and samples; clinical symptoms	N/A	[120]
	metasulfabenzozate; prednisolone acetate	N/A	N/A	N/A	Radiography; plasma and urine samples	Radiography	[121]
	Mesalazine and barium sulfate	N/A	N/A	N/A	Radiography; plasma and urine samples	Radiography	[121]
Eudragit S, Eudragit L, or Aqoat AS-HF (M) with Aquateric or Aqoat AS-HF (C)	Ibuprofen; furosemide		5.0; 6.8; and 7.4	Phosphate	Plasma samples	Literature	[122]
Eudragit S/NE 30 D for granules (C) and Eudragit L for capsules (C)	Mesalazine	1.2 (2); 6.4 (1); 7.2 (1)		N/A	Plasma and urine samples	Sulfasalazine method	[123]
Eudragit S (C)	<sup>13</sup> C-urea	1.2 (2); 6.8 (2); 7.5 (0.5); 6.0 (1.5)		Phosphate	Breath and urine samples	Breath and urine samples, confirmed with IntelliCap®	[28,124]
	<sup>13</sup> C-urea	1.2 (2); 6.8 (2); 7.5 (0.5); 6.0 (1.5)		Phosphate	Breath and urine samples	Breath and urine samples	[108,124]
	Mesalazine; <sup>13</sup> C <sub>6</sub> -glucose	1.2 (2); 6.8 (2); 7.5 (0.5); 6.0 (1.5)		Phosphate	Breath samples	Literature (based on intake of a 'subsequent meal')	[105,124]
	Bee venom peptide; labeled bee venom with <sup>99m</sup> Tc-MIBI	1.2 (2); 6.8 (3); 7.4 (3)		Phosphate	γ-Scintigraphy	γ-Scintigraphy	[90]
Methacrylic acid copolymer soluble > pH 7.0 (C)	Insulin; barium sulfate	N/A	N/A	N/A	Plasma samples; radiography	Radiography	[125]
Polymethacrylate (C)	Mesalazine and <sup>153</sup> Sm	N/A	N/A	N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[77]
	Budesonide and <sup>153</sup> Sm	N/A	N/A	N/A	γ-Scintigraphy; plasma and urine samples	γ-Scintigraphy	[81]

<sup>a</sup> Diethylenetriamine penta-acetic acid.<sup>b</sup> Not available.<sup>c</sup> In vitro disintegration test.<sup>d</sup> 1 h for tablets and 5 h for pellets.<sup>e</sup> Inner coating Eudragit E.

nfluence of pH, electrolyte composition, and ionic strength showed that the drug release from an enteric-coated aspirin formulation increased with an increasing ionic strength [64]. Next to this, it was shown that a phosphate and a bicarbonate buffer with an equal pH and a similar buffer capacity did not result in a similar dissolution profile. Similarly, Fadda et al. found that a phosphate buffer with a buffer capacity and ionic strength comparable to Hanks buffer, a bicarbonate buffer, did not give a similar dissolution profile of Asacol tablets [61]. Therefore, one should be reluctant to substitute the buffer type, even if the buffer capacity and ionic strength is kept the same, because this could result in a poor *in vitro*–*in vivo* correlation.

To accurately test the pH dependency of a system, the pH profile of an *in vitro* dissolution test should mimic the *in vivo* situation and simulate challenging conditions. The exposure time and pH (which we refer to as pH profile in the remainder of this review), but preferably also a representative buffer type, buffer capacity, and ionic strength, have to be taken into account [57,61].

In the stomach, acid can penetrate the pH-dependent system and the amount of acid is dependent on the residence time [30,56]. This acid penetration can influence the performance of the system because it is based on neutralizing its acidic components. Substantially different values for the mean gastric residence time in the fasted state are given in two different systematic literature reviews [65,66]. Pilar et al. showed that, when a meal was administered before the dosage form had exited the stomach, gastric residence time was longer than when a meal was administered after the dosage form exited the stomach [65]. This indicates that food administration while the dosage form is in the stomach does not truly reflect a fasted state transit time. Therefore, to obtain the gastric residence time in the fasted state, they only included studies in which a meal was administered after 4 h, to ensure that the dosage form had passed into the small intestine. Based on these studies, a fasted gastric residence time of 48 min was found. In their review, Abuhelwa et al. did not consider whether the dosage form exited the stomach at the time a meal was administered [66]. Therefore, the higher gastric residence time of 1.37 h that they found for the fasted state could be explained by the fact that studies were included where a meal was administered while the dosage form was still in the stomach. Size of the dosage form did not affect gastric residence time in the fasted state [66,67]. In the fed state, the gastric residence time was found to be 2.5 and 3.5 h for a light (300 kcal) and heavy (700 kcal) breakfast, respectively [66,67]. Larger dosage forms (generally single unit) remain in the stomach longer than do smaller dosage forms (generally multi unit) in the fed state [66,67]. Based on these data and the pH data shown in Fig. 1, we recommend a residence time of 50 min at a pH of 1.85 or a residence time of 2.5 h at a pH of 3.30 for the fasted or fed state, respectively, for simulation of the stomach in an *in vitro* dissolution test.

A proper simulation of the small intestine requires that both the proximal and distal intestine should be taken into account, in other words pH values just below and at the pH peak in the terminal ileum should be simulated. Exposure of a drug delivery system to a pH just below the pH peak might result in drug release because of slow dissolution, disintegration, and/or swelling of the pH-dependent matrix or coating. An optimal drug delivery system should have the capacity to withstand exposure to this pH for a

biologically relevant amount of time. The total small intestinal transit time is unaffected by food status and is around 3–4 h [65–68]. There is no difference in small intestinal transit time for larger dosage forms (generally single unit) and smaller dosage forms (generally multi unit) [66–68]. The transit time can be decreased by 50% when a meal is given at the time the drug delivery system reaches the proximal small intestine [69]. Therefore, exposure of the drug delivery system for 3 h at a pH of 6.8 would be a challenging condition to simulate the proximal small intestine. For the ileocecal junction, a range of residence times has been found, from almost instantaneous to >10 h [30,65]. The drug delivery system is supposed to start releasing its drug content in the terminal ileum. When the peak pH value determines drug release, a short exposure time and a relatively low pH value within the range found in the terminal ileum (7.2–7.7) are challenging conditions, which means, for example, 30 min at a pH of 7.3.

Simulation of the large intestine requires the introduction of the pH drop that occurs *in vivo*, with exposure time being less relevant. As a result, a pH of 6.50 until the end of the test is recommended. Table 3 details the pH profiles recommended for simulating the dissolution test in a fasted and fed state.

Different pH profiles have been used in the various studies (Table 2). In our opinion, these conditions are often not challenging enough. Simple and, thus, easily implementable dissolution models only mimic the GI transit time and pH, but lack simulation of, for example, motility, intestinal pressure, and viscosity, and, thus, are limiting. However, when a challenging pH profile is chosen, valuable information is obtained. The recommended pH profile presented in Table 3 does not take into account the intra- and interindividual variations [30,65–71]. If more information about the robustness of the drug delivery system is desired, individual pH profiles obtained *in vivo* could be simulated with, for example, the pPhysio-grad® system [72]. Other physiological parameters, such as motility, intestinal pressure, the presence of enzymes and bile, fluid volumes, and viscosity, are usually not simulated. The complicated and not easily implementable TIM-I and TIM-II system [73] are exceptions. Even these systems are a simplification of the *in vivo* situation and their full *in vivo* predictability has yet to be established. Therefore, novel ileocolonic delivery systems always have to be tested in humans as well.

### ***In vivo* methods to investigate or verify ileocolonic targeting in humans**

Various methods have been used to investigate the ileocolonic drug delivery in humans, as listed in Table 2. They include imaging techniques, such as  $\gamma$ -scintigraphy, radiography, and fluoroscopy (continuous X-ray imaging), and indirect methods, such as determination of plasma drug concentrations or stable isotope concentrations in breath and urine samples.

**TABLE 3**  
**pH profile dissolution test<sup>a</sup>**

Segment GI tract	Fasted (pH; time)	Fed (pH; time)
Stomach	1.85; 50 min	3.30; 150 min
Proximal small intestine	6.80; 180 min	6.80; 180 min
Distal small intestine	7.30; 30 min	7.30; 30 min
Colon	6.50; end of test	6.50; end of test

<sup>a</sup> pH values might vary  $\pm$  0.10 pH unit.



### Imaging techniques

An advantage of imaging techniques for the visualization of drug delivery systems in the GI tract is that they are non-invasive and direct. The most frequently used imaging technique is  $\gamma$ -scintigraphy followed by X-ray imaging (radiography and fluoroscopy) (Table 2). For *in vivo* visualization by  $\gamma$ -scintigraphy a radionuclide (e.g.,  $^{99m}\text{Tc}$  or  $^{111}\text{In}$ ) is incorporated into the formulation, whereas, for radiography or fluoroscopy, a contrast agent (e.g., barium sulfate) is needed [74–76]. Such nonabsorbable markers can either be formulated alone in the drug delivery system or together with the drug, depending on the research question. Various studies have confirmed that the markers do not influence the *in vitro* release profile of the drug [30,60,77–88]. Although it is generally assumed that marker and drug would show the same release behavior, and the site of marker release indicates the site of drug release, this is not necessarily the case [77]. Therefore, imaging techniques by themselves cannot truly verify ileocolonic drug delivery, but they provide a generally useful indication.

As with all drug administrations and medical procedures, possible drug interactions, adverse effects (including allergic reactions), contra-indications, and complications because of the procedure have to be considered. Barium sulfate is used as a diagnostic contrast agent for radiographic visualization of the GI tract, in which a suspension of 150–180 g of barium sulfate is administered. At these high amounts, barium sulfate might be responsible for disturbed absorption of drugs, allergic reactions, and gastrointestinal adverse effects, such as diarrhea, nausea, abdominal pain or distention, and constipation [87]. The amounts used in the pH-dependent drug delivery systems are small; for example, Aimone et al. only used 2.1 g [76], thus no adverse effects are expected. Both X-ray imaging and  $\gamma$ -scintigraphy make use of radiation to visualize the dosage form and, thus, radiation exposure should be taken into consideration.

An advantage of  $\gamma$ -scintigraphy is that the radiation exposure does not increase with an increasing number of images, because the radionuclide is the radiation source. In fluoroscopy and radiography, an external ionizing radiation source, X-rays, is used for visualization. Therefore, the exposure to radiation increases with each additional image taken, which results in substantially higher doses of radiation than with  $\gamma$ -scintigraphy [74]. Other advantages of  $\gamma$ -scintigraphy include a more accurate quantitative analysis and an increased sensitivity [74]. The higher sensitivity simplifies the identification of the site of release, even though the location of organs is not visible with  $\gamma$ -scintigraphy (in contrast to radiography and fluoroscopy) [74]. However, visualization of organs is not strictly necessary when evaluating ileocolonic drug delivery systems, because the human large intestine has an inverted U shape [77,79,81,82,85,88]. This inverted U shape becomes prominently visible when the radionuclide is released in the large intestine from a single unit drug delivery system, because the marker spreads over the colon when released from the system [82,85,89]. For multi unit drug delivery systems, this is more complicated because of the inevitable spread of the individual particles before drug release occurs [90]. If anatomical information is desired with  $\gamma$ -scintigraphy, an aqueous solution of  $^{99m}\text{Tc}$ -labeled diethylenetriamine penta-acetic acid (DTPA) or  $^{99m}\text{Tc}$ -labeled colloid can be taken together with  $^{111}\text{In}$  incorporated in a drug delivery system, such as a multi unit formulation [88,89,91,92]. The delivery system can

then be visualized together with the different segments of the GI tract outlined by  $^{99m}\text{Tc}$ . A disadvantage of  $\gamma$ -scintigraphy is that the shelf-life of the formulation is limited depending on the half-life of the radionuclide. Furthermore, for safe manufacturing of radiopharmaceutical dosage forms, specific conditions are required and often the production process must be downscaled [93]. This can be circumvented, for example, by using stable  $^{152}\text{Sm}$ -oxide during the production process, which can be neutron activated to the radionuclide  $^{153}\text{Sm}$  before administration [77–81,85,93,94]. A major drawback remains that, for isotope techniques, specialized equipment is required to produce, measure, or visualize the isotopes, which complicates broad applicability. Furthermore, simultaneous release of the marker and drug must be verified to draw definite conclusions about the ileocolonic targeting ability of the formulation.

### Plasma samples

Whereas imaging techniques generate information about the location of the drug delivery system, plasma drug concentrations will render valuable information about the drug release, on the condition that the drug can be absorbed over the entire length of the GI tract [77,79–81,85,88,89,91,95]. Combining imaging techniques and blood sampling allows for verification of ileocolonic drug delivery. Plasma samples as such are generally insufficient to verify ileocolonic drug delivery, because the appearance of drug in the plasma does not give information about the location of drug release. An exception to this is an adjusted method of Kennedy et al., in which sulfasalazine is used in combination with a compound with good solubility and permeability across the entire intestinal membrane (e.g., theophylline) [96–98]. Sulfasalazine is poorly absorbed from the GI tract but is metabolized by bacteria in the colon into sulfapyridine, which is subsequently rapidly absorbed. Therefore, the occurrence of sulfapyridine in plasma points to colonic arrival [99,100]. By contrast, theophylline is well absorbed over the entire GI tract and, therefore, its appearance in plasma indicates drug release from the drug delivery system [97]. Based on comparison of the plasma curves of theophylline and sulfapyridine, it can be determined whether the formulation has released its content solely in the colon. When drug release occurs in the colon, theophylline and sulfapyridine occur simultaneously in plasma. When drug release occurs before the ileocolonic region, theophylline will appear in the plasma previous to sulfapyridine. The method was successfully used in beagle dogs [97], but never in humans. Theoretically, it can be applied in humans, because the sulfasalazine–sulfapyridine method was developed and validated for humans [96,101]. Replacing theophylline with, for example, caffeine (which is also well absorbed over the entire GI tract) is advised to circumvent possible toxicity issues related to theophylline [98,102]. Furthermore, to avoid the invasive character of blood sampling, saliva samples could possibly be used [103,104].

### Breath and urine samples

Formulations containing stable isotopes have been used to assess ileocolonic drug delivery in a non-invasive manner with breath and urine samples. Schellekens et al. used  $^{13}\text{C}_6$ -glucose, which is absorbed rapidly from the GI tract and then partly metabolized into  $^{13}\text{CO}_2$  in the systemic circulation [105]. Given that glucose metabolism does not depend on bacteria, the appearance of  $^{13}\text{CO}_2$

in breath is an indicator of drug release and not a marker for colonic drug release. In the study, the motility of the GI tract was activated by giving the test subjects a subsequent meal, which caused the dosage form to pass the ileocecal junction. In this way, the authors obtained an indication of the location of drug release. In another study,  $^{13}\text{C}$ -urea and  $^{15}\text{N}$ -urea were incorporated into an ileocolonic drug delivery system [106]. The authors investigated the appearance of  $^{13}\text{CO}_2$  (from  $^{13}\text{C}$ -urea) and  $^{15}\text{NH}_3$  (from  $^{15}\text{N}$ -urea) in the breath and urine, respectively. Released  $^{13}\text{C}$ -urea in the colon will be partially fermented by bacteria generating  $^{13}\text{CO}_2$ , which is exhaled, whereas the remainder of the  $^{13}\text{C}$ -urea is excreted into the urine. When the formulation releases its content before it reaches the ileocolonic region, no  $^{13}\text{CO}_2$  will be formed and all  $^{13}\text{C}$ -urea is excreted into the urine. In case of failed release, the complete  $^{13}\text{C}$ -urea dose will be excreted into the feces. When no  $^{13}\text{CO}_2$  is detected in the breath, the presence or absence of  $^{15}\text{NH}_3$  in urine indicates premature release in the small intestine or no release at all, respectively. However, this method lacks an internal standard for variations in  $^{13}\text{C}$ -urea metabolism. Maurer et al. optimized the dual stable isotope approach [28,107,108]. In this method, an ileocolonic targeted drug delivery system with  $^{13}\text{C}$ -urea incorporated and an immediate release formulation with  $^{15}\text{N}$ -urea incorporated were administered simultaneously. The immediate release formulation with  $^{15}\text{N}$ -urea was used as an internal standard. It reflects variation in urea metabolism and, therefore, gives a reference value for 100% absorption of  $^{13}\text{C}$ -urea. Thus, by comparing the  $^{13}\text{CO}_2$  amount in breath and the  $^{13}\text{C}$ -urea and  $^{15}\text{N}_2$ -urea in urine, it is possible to verify whether or not ileocolonic delivery was successful.

A major advantage of utilizing stable isotopes is that volunteers or patients are not exposed to irradiation (in contrast to  $\gamma$ -scintigraphy, radiography, and fluoroscopy), and the method is non-invasive (in contrast to plasma sampling) [109].

### Performance of pH-dependent ileocolonic drug delivery systems

pH-dependent ileocolonic drug delivery systems often fail in humans, even though *in vitro* release studies showed promising results, as described in a review by Maroni et al. [110]. This discrepancy could be explained by the fact that the systems were tested with phosphate buffers and not bicarbonate buffers, thus failing to identify suboptimal systems. Another explanation could be that the pH profile used in the *in vitro* dissolution test does not adequately reflect the pH profile in humans (Table 2).

However, there are four systems, given in Table 2, that show good performance in humans (Table 2). The first system contains a double coating layer. The inner coating layer comprises a pH-sensitive polymer, Eudragit S, which was neutralized to pH 8.0 with phosphate salts [84]. The outer layer comprises a non-neutralized coating of Eudragit S. The authors hypothesized the following mechanism for rapid drug release once the pH threshold is passed. When the outer coating starts to dissolve in the terminal ileum, water can pass through this layer and dissolve the inner coating with the buffer salts. This results in a high local pH with a high buffer capacity, which enhances the dissolution rate of the inner layer. As a result of diffusion, the pH in the outer layer increases, which facilitates dissolution of this layer. The authors showed that the double coating layer yielded superior targeting

capabilities in humans compared with a single coating layer system. They also showed that an *in vitro* dissolution test with phosphate buffers was incapable of distinguishing the release profiles of the single and double coated formulation, whereas a bicarbonate buffered system reflected the situation in humans more accurately. The second system showing the required behavior in humans is the ColoPulse system in which a disintegrant, such as sodium starch glycolate or croscarmellose sodium, is incorporated into a pH-sensitive polymeric coating layer in a nonpercolating manner [105]. Once the pH-sensitive polymer, Eudragit S, starts to dissolve, the protective layer around the disintegrant is removed and the disintegrant swells and quickly ruptures the coating layer resulting in a fast release of the drug. The third system is based on a combination of Eudragit L and Eudragit S, which results in a pH threshold between 6 and 7 depending on the ratio of the two polymers [76,79]. By combining the two polymers, the disadvantages of the individual polymers are circumvented, that is, the pass-through of intact tablets with Eudragit S and premature release with Eudragit L. The fourth system is based on an outer Eudragit S coating layer and an inner Eudragit E coating layer (acid-soluble layer) [94]. The outer layer of Eudragit S dissolves in the terminal ileum (pH > 7.0) and the inner layer in the more acidic pH of the cecum. If the outer coating opens prematurely, such as in the jejunum, the inner coating will prevent premature release.

The first three systems are designed to result in fast disruption of the usually slow dissolving Eudragit S coating [56,60,105], thus decreasing the risks of pass-through of intact dosage forms or not fully opened systems, whereas the fourth system prevents premature release.

### Concluding remarks

Based on the temporary pH increase to values between 7.2 and 7.7 in either the terminal ileum or at the start of the ascending colon in both healthy volunteers and in patients with CD, UC, IBS, or CRC, site-specific drug release from pH-dependent ileocolonic targeted drug delivery systems can be attained. To investigate the performance of novel pH-dependent drug delivery systems *in vitro*, the pH profile in the dissolution medium should mimic the pH profile of the human GI tract, with a short pH peak of pH 7.3 being crucial. The use of dissolution media based on bicarbonate buffers, such as the pHysio-grad® system, is recommended instead of the frequently used phosphate buffers. For studies in humans, the caffeine-sulfasalazine method is an easy implementable method to verify ileocolonic drug delivery. However, a non-invasive method, such as the dual stable isotope approach with an internal standard, which requires only breath and urine samples to verify ileocolonic drug delivery, might be an attractive alternative.

### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Potential conflict of interest: HWF is one of the inventors of a patent (WO 2007/013794) describing a method for colon targeting, which is held by his employer. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work

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