pH-dependent ileocolonic drug delivery, part II

Broesder, Annemarie; Kosta, Anne-Marijke M A C; Woerdenbag, Herman J; Nguyen, Duong N; Frijlink, Henderik W; Hinrichs, Wouter L J

Published in:
Drug Discovery Today

DOI:
10.1016/j.drudis.2020.06.012

Teaser Novel drugs and novel excipients in pH-dependent ileocolonic drug delivery systems have to be tested in animals. Which animal species are suitable and what in vivo methods are used to verify ileocolonic drug delivery?

pH-dependent ileocolonic drug delivery, part II: preclinical evaluation of novel drugs and novel excipients

Annemarie Broesder[1], Anne-Marijke M.A.C. Kosta[2], Herman J. Woerdenbag[1], Duong N. Nguyen[1], Henderik W. Frijlink[1] and Wouter L.J. Hinrichs[1]

[1] University of Groningen, Groningen Research Institute of Pharmacy, Department of Pharmaceutical Technology and Biopharmacy, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands
[2] University of Groningen, University Medical Center Groningen, Department of Biomedical Sciences of Cells and Systems, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Introduction

In part I of this review series we described *in vitro* and clinical methods to investigate and verify colonic drug delivery of novel pH-dependent systems [1]. These systems utilize the sharp but short pH peak of 7.4 (range 7.2–7.7) in the ileum for ileocolonic drug targeting. When evaluating the ileocolonic targeting ability of a system or the therapeutic efficacy of an ileocolonic delivered drug, it is preferable to test it directly in humans [1]. However, a novel drug and/or a novel excipient cannot be tested in humans if no safety data from animal studies are available for the drug or excipient used for the ileocolonic drug delivery systems [2]. Guidelines concerning safety testing have been provided by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [3]. According to these guidelines, safety pharmacology studies have to be conducted with the final formulation in laboratory animals if the formulation substantially alters the pharmacokinetics and/or pharmacodynamics of the drug compared with previously tested formulations [4]. Given that ileocolonic drug delivery systems target the drug to the lower parts of the gastrointestinal (GI) tract, both the pharmacokinetics and pharmacodynamics of the drug will be altered, thus necessitating safety testing with the final drug delivery system in laboratory animals. To achieve pH-dependent ileocolonic drug delivery in animals, the animal species chosen should have a sharp distinct pH peak in its terminal ileum. Ideally, the intestinal pH values of the animal should be comparable with those found in humans, including the pH peak above pH 7.2 in the terminal ileum. This would allow the use of established pH-dependent ileocolonic targeted drug delivery systems to test novel drugs. To test novel pH-dependent excipients, the pH of the GI tract of the chosen animal species has to be similar to that of humans to obtain ileocolonic targeting in humans. Various species have been used for preclinical testing of new drugs or novel excipients, including rats, mice, dogs, and rabbits. However, little emphasis has been put on the translation from animal species to humans regarding the pH values of the GI tract.

In this review, we provide an overview of the pH values of various parts of the GI tract of frequently used laboratory animal species and of humans. We aim to determine which animal species, if any, could best be used to test novel drugs or novel excipients in pH-dependent

Corresponding author: Hinrichs, Wouter L.J. (w.l.j.hinrichs@rug.nl)

Annemarie Broesder received her MSc in pharmacy from the University of Groningen (The Netherlands). Currently, she is pursuing her PhD in the Department of Pharmaceutical Technology and Biopharmacy at the University of Groningen under the supervision of Prof. Dr H.W. Frijlink and Dr. W.L.J. Hinrichs. Her research focuses on oral pH-dependent ileocolonic drug delivery and the suitability of these systems for different dosage forms.

Wouter L.J. Hinrichs studied chemistry at the University of Groningen and received his PhD from the University of Twente (The Netherlands) in 1993. Thereafter, he worked as a postdoc in the University of Twente and Utrecht University (The Netherlands) until 1998. Since then, he has been an assistant professor at the Department of Pharmaceutical Technology and Biopharmacy at Groningen Research Institute of Pharmacy (The Netherlands). One of his research interests is oral dosage forms. He has (co-)authored more than 140 original articles in peer-reviewed journals and book chapters.

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.

https://doi.org/10.1016/j.drudis.2020.06.012

ileocolonic drug delivery systems. Subsequently, *in vivo* methods used in preclinical evaluations to investigate and verify ileocolonic drug delivery are discussed.

Luminal pH in the gastrointestinal tract of animals compared with humans

The pH values of the GI tract largely determine in which part of the GI tract a drug is released from a pH-sensitive drug delivery system. For ileocolonic drug delivery, it is important to select an animal species that has a sharp and distinct pH peak in the terminal ileum, as is found in the human GI tract [1]. In addition to pH, other factors can have a role in the performance of pH-dependent ileocolonic drug delivery systems, including intestinal length, buffer capacity, fluid volume, motility, and transit time. These factors have been extensively described by Hatton *et al*. and Sjögren *et al*. [5,6], and are not further discussed in this review.

Methods to determine the pH in the gastrointestinal tract in laboratory animals

Similar to human studies, pH values in the GI tract of animals have been determined with aspiration via the oral route, tethered pH electrodes, and with pH-sensitive radio telemetry capsules. In humans, aspiration via colonoscopy has also been used to measure the pH of the lower GI tract, but to our knowledge this method has never been used in animals [1]. Aspiration via the oral route is generally limited to the upper GI tract and can be used to determine the mean pH of the collected stomach or duodenal fluid *ex vivo* [7,8]. If the pH of the entire GI tract during transit is to be measured, radio telemetry capsules can be used [9–11]. A drawback of these capsules is their large size (usually around 10 × 20 mm), which limits their use to larger animals, such as dogs and pigs (Table 1) [12–15]. With laboratory animals, *ex vivo* pH measurements of the intestinal contents after surgical collection of samples from different segments of the GI tract is possible [16–23]. A drawback of this method is that only the mean pH of the collected fluid is determined. In addition, the pH should be measured immediately after collection to prevent possible postsampling pH changes. This is particularly relevant for the content of the colon, because bacterial fermentation of polysaccharides results in the formation of acidic products which can lower the pH after collection [24]. Another option to measure the pH in animals is to open the GI tract by surgery and to measure the pH *in situ* with a pH electrode [25–32]. With *in situ* pH measurements and with a pH-sensitive radio telemetry capsule possible pH alterations after sampling are excluded, because the pH values are measured immediately.

pH values in the gastrointestinal tract of laboratory animals

The mean or median pH values found in various studies for different segments of the GI tract (stomach, duodenum, jejunum, ileum, cecum, and colon) of rabbits, pigs, dogs, rats, mice, guinea pigs, and monkeys are shown as dots in Fig. 1, together with the values for humans as reviewed in part I of this series [1]. From publications in which both the median and mean pH values were given, the mean values were used in this review. When multiple pH values were reported for a certain GI tract region, for instance the fundus and antrum of the stomach, the mean value was calculated and used. In Fig. 1, the minimum and maximum pH values are indicated by bars and the mean pH values, calculated from all different studies combined, by a vertical line. The study size was not taken into account for the calculation of the mean. In this overview, no differentiation is made between the fasted and fed states, because of a lack of sufficient data on this point. To enable comparison, the previously reported values for humans in the fasted and fed states were also combined. Table 1 provides an overview of the studies used, with information about the pH measurement method and the state (fasted or fed) under which the experiments were carried out.

Rabbits and pigs have pH values in the stomach that are within the range of the human values (Fig. 1A,B, respectively) [16–18,25,33]. The mean pH values of the duodenum and jejunum in these animal species are below the minimum pH of 7.2 that is found in the human ileum, whereas the pH values in the ileum were higher [16–18,25,33].

A broader variation was found for the pH in the stomach of dogs (pH 1.1–6.8; Fig. 1C) and rats (pH 3.2–6.7; Fig. 1D) [7–11,16,18,21,23,26,28–32]. In these animals, the minimum pH of 7.2 of the human ileum is already surpassed in the duodenum and jejunum in some of the studies. Additionally, the mean pH in the ileum of both dogs and rats was below this minimum pH of 7.2 [7,16,18–21,23,26,28–30].

Mice have a higher pH in the stomach but a lower pH in the small and large intestine compared with humans (Fig. 1E) [16,22,23,28]. The minimum pH of 7.2 found in the ileum of humans was not reached in any part of the murine GI tract.

In guinea pigs, the pH values of the GI tract are higher than those of humans (Fig. 1F). The minimum pH of 7.2 was reached in the duodenum in some of the studies and was above pH 7.2 in the jejunum in all studies until the cecum, where the pH drops until 6.7 [16,25].

In monkeys, the mean pH in the stomach was higher than in humans (Fig. 1G) [16,34]. The pH increases to pH 6.0 in the ileum and then drops to 5.0 in the cecum and colon [16]. However, care must be exercised to draw definite conclusions from these data because the pH values of the small intestine and colon are based on only one study.

Selection of an appropriate animal species

For the preclinical evaluation of novel drugs or novel excipients applied in pH-dependent systems, we found that no particular animal species is commonly used (Table 2). It is remarkable that in most studies no information is given about the rationale behind the chosen animal species. In view of the working principle of pH-sensitive ileocolonic targeted drug delivery systems, a pH peak in the terminal ileum of the animal should be considered as the most important factor. Furthermore, pH values similar to the human GI tract would be ideal, because in that case an existing and well-validated pH-dependent system can be used to obtain ileocolonic drug delivery in the chosen animal species or novel pH-dependent excipients can be evaluated.

The pH profile of the GI tract of monkeys, mice, and guinea pigs differs from that in humans and no distinctive pH peak has been found in the terminal ileum. Therefore, these laboratory animal species should be considered unsuitable for testing novel drugs in pH-dependent ileocolonic drug delivery systems (Fig. 1). Dogs and rats might be suitable because the mean pH values in the various

TABLE 1

Overview of studies investigating the pH values of the GI tract in laboratory animals and in healthy human individuals

Animal		Segment gastrointestinal tract						Method		Refs
Species	**Breed**	**Stomach**	**Duodenum**	**Jejunum**	**Ileum**	**Cecum**	**Colon**	**pH measurement**	**Fasted/fed**	
Monkey	Cynomolgus	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
		X						Bravo^{TM} capsule[b]	Fasted and fed	[34]
Pig	From farms	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
	Crossbreed of large white and Landrace	X	X	X	X	X	X	*In situ*[a]	Fed *ad libitum*	[25]
		X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[18]
	Large white, Landrace, and Essex	X	X	X	X	X	X	*Ex vivo*[a]	Fasted for 1 hour	[17]
Mouse	Balb/c	X	X	X	X	X	X	*Ex vivo*[a]	Fasted and fed	[23]
	House	X	X	X	X	X		*In situ*[a]	N/A[e]	[27]
		X				X		*Ex vivo*[a]	Fed ad libitum; high and low fiber diet	[22]
	White	X						*Ex vivo*[a]	Fed ad libitum	[16]
Rat	Albino Norwegian	X	X	X	X	X		*In situ*[a]	Fasted and fed *ad libitum*	[28]
	Porton-Wistar	X	X			X	X	*In situ*[a]	Fasted, fed *ad libitum* and fed standardized	[29]
		X	X			X	X	*In situ*[a]	Fed	[26]
	White	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
	Wistar	X	X	X	X	X	X	*Ex vivo*[a]	Fasted and fed	[23]
		X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[18]
		X	X	X	X			*In situ*[a]	Fasted	[30]
Rabbit	New Zealand Whites	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
		X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[18]
		X	X	X	X	X	X	*In situ*[a]	Fed *ad libitum*	[25]
						X	X	N/A[e]	Fed *ad libitum*	[33]
Guinea pig	N/A[e]	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
	Dunkin-Hartley White	X	X	X	X	X	X	*In situ*[a]	Fed *ad libitum*	[25]
Dog	Beagle	X						Heidelberg capsule	Fasted	[9]
		X						Aspiration	Fasted	[8]
		X						*In situ*[a]	Fasted	[31]
		X						Bravo^{TM} capsule	Fed big or small meal before capsule ingestion	[10]
		X						Bravo^{TM} capsule	Fasted, fed standard meal and fed slurry meal	[11]
		X						*In situ*[a]	Fed	[32]
	From domestic households	X	X	X	X	X	X	*Ex vivo*[a]	Fed *ad libitum*	[16]
	Labrador	X		X				Aspiration	Fasted	[7]
				X				*Ex vivo*[a]	Fasted and fed	[20]
	Mixed		X					*Ex vivo*[a]	Fasted	[19]
	N/A[e]	X	X	X	X	X	X	*Ex vivo*[c]	Fasted	[21]
Human		X	X	X	X		X	Telemetry capsule	Fasted; food when capsule left stomach	[105]
		X	X	X	X		X	Bravo^{TM} capsule	Fasted; food 30 min or 4 h after ingestion	[106]

TABLE 1 (*Continued*)

Animal		Segment gastrointestinal tract						Method		Refs
Species	**Breed**	**Stomach**	**Duodenum**	**Jejunum**	**Ileum**	**Cecum**	**Colon**	**pH measurement**	**Fasted/fed**	
		X	X	X				*In situ*[a]	Fasted	[38]
		X	X		X	X	X	Telemetry capsule	Fasted; food allowed when capsule left stomach	[40]
		X	X		X		X	Intellicap® capsule	Fasted; food allowed 4 h after ingestion	[107]
		X	X					Heidelberg capsule	Fasted and fed	[108]
		X	X					Aspiration	Fasted	[109]
		X	X					Aspiration	Fasted	[110]
			X		X		X	SmartPill	Fed; FDA standard breakfast	[111,112]
			X		X		X	SmartPill	Fasted; after 4.5 h standardized lunch	[111]
		X		X	X	X	X	Telemetry capsule	Fed; food allowed after ingestion of capsule	[36]
		X				X	X	IntelliCap®	Fasted; food 3, 6 and 10 h after ingestion	[92]
		X					X	SmartPill	Fed; gastric-emptying meal	[113]
		X						Heidelberg capsule	Fasted; food 3 h after capsule left stomach	[9]
		X						*In situ*[a]	Fasted and fed standard meal	[114]
		X						Aspiration	Fasted	[115]
		X						*In situ*[a]	Fasted	[116]
		X						Aspiration	Fasted	[117]
		X						*In situ*[a]	Fasted and fed	[118]
		X						Heidelberg capsule	Fed; standardized breakfast	[119]
		X						*In situ*[a]	Fasted and fed	[120]
		X						*In situ*[a]	Fasted and fed	[121]
			X	X	X	X	X	Telemetry capsule	Fasted; food allowed when capsule left stomach	[122]
			X	X	X		X	Telemetry capsule	Fed; normal diet	[123]
			X	X	X		X	Telemetry capsule	Fasted; food allowed when capsule left stomach	[37]
			X	X				Aspiration	Fasted	[124]
			X		X			Heidelberg capsule	Fed	[125]
			X					Aspiration	Fasted, fed and fed fat-enriched meal	[126]
			X					Bravo™ capsule[d]	Fed; standardized meal twice daily	[127]
			X					Aspiration	Fasted	[128]
			X					Aspiration	Fasted	[129]
			X					Aspiration	Fasted and fed	[130]
				X	X		X	Telemetry capsule	Fasted; food allowed when capsule left stomach	[131]
				X	X		X	SmartPill	Fasted; food allowed 6 h after ingestion	[132]
					X	X		SmartPill	Fasted; standardized meal when capsule left stomach	[133]
					X	X		SmartPill	Fed; test meal, after 6 h normal diet	[39]

[a] Measured with a pH electrode.
[b] Capsule attached to stomach.
[c] Colorimetric and with a pH electrode.
[d] Capsule attached to duodenal bulb.
[e] Not available.

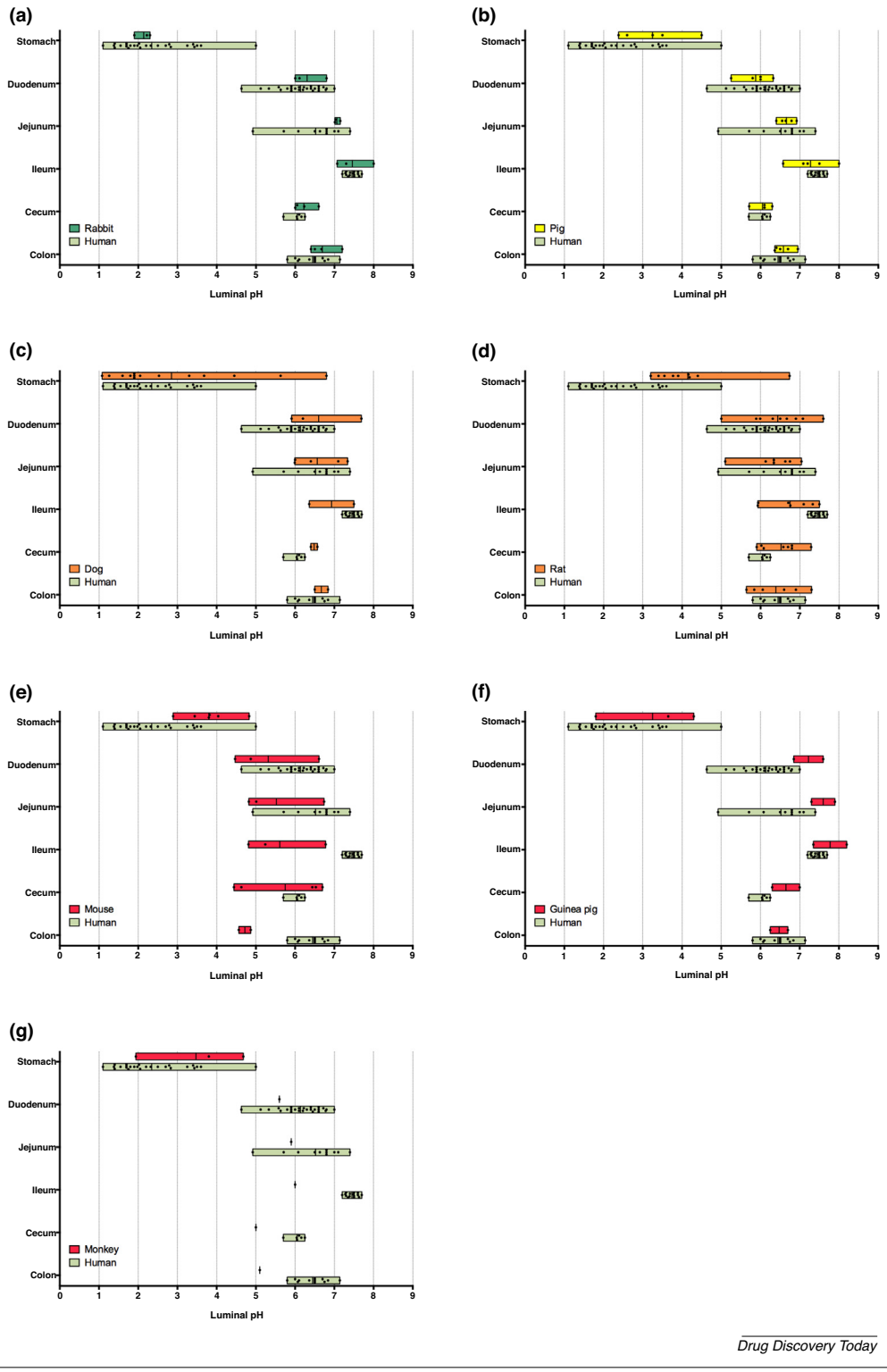

FIGURE 1

Luminal gastrointestinal (GI) pH values of laboratory animals (dark-green, orange, or red bars) and healthy human individuals (light-green bars). The different pH values of the GI tract of the **(a)** rabbit [16,18,25,33], **(b)** pig [16–18,25], **(c)** dog [7–11,16,19–21,31,32], **(d)** rat [16,18,23,26,28–30], **(e)** mouse [16,22,23,27], **(f)** guinea pig [16,25], and **(g)** monkey [16,34] are given against the values in healthy human individuals [9,36–40,92,105–133]. The GI pH ranges are indicated by bars, the vertical line in the bars indicates the mean pH. The mean or median pH values of the different segments of the GI tract found in the different studies are indicated by the black dots. Only one study was found for pH values for the duodenum, ileum, cecum, and colon in the monkey and, therefore, only this value is given as a dot and a vertical line. Table 1 in the main text details the studies that were used to obtain the minimum, maximum, and mean pH values for the different segments of the GI tract.

segments of their GI tract are generally comparable to those in humans, including a pH peak in the terminal ileum (Fig. 1). However, their mean pH value in the ileum is below the minimum value in humans. Furthermore, the pH values in the GI tract substantially varied among the different studies. Therefore, it is advised to check the inter- and intraindividual variation in pH values of the GI tract of the dogs or rats used in the experiments. Given the relatively broad range of gastric pH values in dogs, it is recommended to pretreat the animals with a 0.1 M HCl-KCl solution, via an orogastric tube, to lower the pH of the stomach [35]. Rabbits and pigs have pH values comparable to those in humans, including the pH peak in the ileum above pH 7.2 (Fig. 1). The pH values in the different segments of the GI tract showed relatively little variation between the different studies. For rabbits, the mean pH in the jejunum was slightly higher (7.1) than in humans, which might result in premature drug release. However, the rabbit is the only animal species having a distinct pH peak in the ileum, with the minimum pH above 7.0. For pigs, the minimum pH found was 6.6, which is considerably lower. In four out of five pig studies, a pH in the ileum above 7.0 was found, whereas this was the case for all three rabbit studies. Thus, when using pigs, it is advised to check the pH values of the GI tract of the individual animals to verify whether they are comparable to those of humans and constant over time.

Overall, the rabbit appears to be the most reliable species for testing novel drugs with established pH-dependent ileocolonic drug delivery systems because of the low pH variability and the distinct pH peak above 7.0 in the ileum. When the inter- and intraindividual variations in pH values of the different regions of the GI tract are checked for rabbits, ileocolonic drug targeting might be proved with even more certainty. In cases where the pH values of the GI tract for individual pigs or dogs are comparable to those in humans, are constant over time, and if the pH threshold of the system is reached, these animal species might be good alternatives to the rabbit, because larger drug delivery systems can be administered. When multiple animal species are found to have appropriate pH values in the GI tract, pilot studies could be performed to determine which species is most optimal to obtain ileocolonic drug delivery with the chosen drug delivery system. To test the therapeutic effect of a novel drug in preclinical studies (Table 2), diseased rather than healthy animals should be used. However, a diseased state could change the pH values in the GI tract in animals, even though it was found that colonic diseases did not negatively affect the pH values in humans [1,36–40]. However, despite these results, it is still recommended to check the pH values of the GI tract of diseased animals, because the effects of a colonic disease on the intestinal pH have not yet been described for animals.

It should also be possible to mimic a specific disease in the chosen animal species to enable measurement of the therapeutic effect of a novel drug. In the literature, several colonic disease models have been described for the rabbit (Table 3), which, according to our review, is the most reliable animal species to test novel drugs in a pH-dependent ileocolonic drug delivery system. Another important aspect to be taken into consideration is that the pharmacokinetics and/or pharmacodynamics of a drug might be different in an animal compared with humans. Thus, an animal species should be selected with care, to avoid false negative or false positive results.

In vivo methods to investigate or verify ileocolonic targeting in laboratory animals

Before performing *in vivo* studies, the pH dependency of a chosen delivery system should be verified in a challenging *in vitro* dissolution test. The dissolution test should mimic the pH profile and preferably the buffer capacity, buffer type, and ionic strength of the human GI tract, as described in part I of this series [1]. To draw conclusions from the *in vivo* efficacy data of a novel drug or the targeting ability of a drug delivery system containing a novel pH-dependent excipient, it is important to verify ileocolonic drug delivery in the chosen animal species. Different methods have been used to verify ileocolonic drug delivery in animals. These methods include investigation of tissue samples and utilization of imaging techniques, such as X-ray imaging (radiography and fluoroscopy), γ-scintigraphy, fluorescence microscopy, and near-infrared (NIR) fluoroscopy. Furthermore, drug plasma concentrations and therapeutic effects have been used. These methods are discussed in more detail in the following sections.

Harvesting tissue samples

After sacrificing an animal, tissue samples can be collected in which the drug concentration is determined or in which the drug delivery system and/or the drug is detected with micro- or macroscopic techniques. Different sections of the GI tract can be removed from the animal, such as the stomach, small intestine, cecum, and large intestine. Four different methods have been developed to analyze harvested tissue samples (Table 4). All of these methods are applicable for drugs that are absorbed into the systemic circulation, but, under certain conditions, three of them can be used for drugs that are not absorbed from the GI tract.

In the first method (i) the intestinal content is immediately washed away. Subsequently, the drug, if present, is extracted from the tissue by an appropriate method and quantified [41–45]. With this method, drug release is indicated by the presence of drug in the extract because only released drug can be absorbed by the intestinal tissue. In the second method (ii), the washing step is omitted and the luminal fluid is included, next to the intestinal tissue, in the extraction procedure [46,47]. To draw conclusions from the drug content data, it is important to validate the extraction procedure and to determine whether the drug is completely extracted from the drug delivery system or not at all. If the drug is indeed completely extracted (iia) from the dosage form, drug release is indicated by incomplete recovery because, in that case, part of the drug has been absorbed into the systemic circulation [47]. If the drug is not extracted from the dosage form (iib), then presence of the drug in the extract is indicative of drug release [46]. In the third method (iii), only the drug content in the luminal content is measured while the tissue itself is not used [48]. For this method, it is also important to know whether the drug is completely extracted from the drug delivery system or not at all. If the drug is completely extracted (iiia), incomplete recovery indicates drug release. If the drug is not extracted (iiib), the presence of the drug in the extract indicates drug release. In the fourth method (iv), the drug delivery system is retrieved from the luminal content and only the drug content in the delivery system itself is determined [49]. In this method incomplete recovery indicates drug release.

In three of the different approaches, the location of release of drugs that are not absorbed into the systemic circulation can be

TABLE 2

Overview of pH targeted ileocolonic drug delivery systems tested in laboratory animals

Animal			Drug delivery system		*In vivo* test		Refs
Species	**Rationale**	**pH values GI tract mentioned**	**pH-dependent matrix (M) and/or coating (C)**	**Drug/marker**	**Read out**	**Colon arrival determined with**	
Dog	N/A[a]	Yes	Eudragit S (C)	Mesalazine; tegafur	Plasma samples	Plasma concentrations compared with previous determined colon arrival time	[77]
	Based on practical considerations	Yes	Eudragit FS 30 D (C)	Meloxicam	Plasma samples	Plasma concentrations and colon arrival time from literature	[78]
	GI tract comparable to human	Yes	Eudragit S (C)	Mesalazine	Tissue sections harvested; plasma samples	Plasma and tissue concentrations	[42]
	Pharmacokinetics	No	Eudragit FS 30 D	Lovastatin	Plasma samples	N/A	[134]
Dog and rat	N/A	No	Eudragit S (M)	Insulin; salicylic acid	Plasma samples and tissue sections harvested	Plasma concentrations and visual examination of harvested tissue sections	[53]
	GI tract comparable to human	Yes	Eudragit P-4135 F (C); Eudragit L (C); Eudragit S (C)	Norfloxacin; fluorescein	Tissue sections harvested; plasma samples	Sulfasalazine method	[52]
Rat	N/A	No	Eudragit S (C)	Mesalazine and/or curcumin	Colitis severity	N/A	[135]
	N/A	No	*N*-succinyl chitosan/Zn^{2+} (M)	Mesalazine and/or zinc	Colitis severity	Colitis severity and *in vitro* release	[99]
	N/A	No	Acrylic acid and butyl meth-acrylate polymers (M)	Aceclofenac	Colitis severity	N/A	[136]
	N/A	No	Eudragit S (M)	Celecoxib and/or curcumin	Colitis severity	N/A	[137]
	N/A	No	Poly(starch/acrylic acid) (M)	Rutin	Colitis severity	Colitis severity and *in vitro* release	[100]
	N/A	No	Eudragit P-4135 F (M)	Tacrolimus	Colitis severity, plasma samples	N/A	[138]
	N/A	No	Eudragit P-4135 F (M)	Tacrolimus	Colitis severity	Colitis severity and *in vitro* release	[101]
	N/A	No	Eudragit S (M)	Aceclofenac	Paw edema severity	N/A	[139]
	N/A	No	Eudragit P-4135 F (M)	Calcitonin; carboxy-fluorescein	Plasma samples	Plasma concentrations and colon arrival time from literature	[79]
	N/A	No	P(LE-IA-MEG) (M)	Dexamethasone	Plasma samples	Plasma samples and *in vitro* release	[81]
	N/A	No	Eudragit S (C); Eudragit L (C); Eudragit RS100 (C)	Insulin	Plasma samples	N/A	[140]
	N/A	No	Eudragit L100-55 (M); Eudragit L (M); Eudragit S (M)	Celecoxib	Plasma samples; colitis severity	N/A	[141]

TABLE 2 (*Continued*)

Animal			Drug delivery system		*In vivo* test		Refs
Species	**Rationale**	**pH values GI tract mentioned**	**pH-dependent matrix (M) and/or coating (C)**	**Drug/marker**	**Read out**	**Colon arrival determined with**	
	N/A	No	Eudragit S (C)	Budesonide	Tissue sections harvested	Tissue concentrations	[41]
	N/A	No	Eudragit S (C); alginate (M)	Ginger extract	Tissue sections harvested; clinical effects on colon cancer	Visual observation of ileocecal junction	[50]
	N/A	No	Eudragit S/Compritol (M)	10-hydroxy-camptothecin; coumarin-6	Tissue sections harvested; microscopy; plasma samples	Plasma and tissue concentrations	[46]
	Disease model available	No	Eudragit S (M)	Carboxy-fluorescein	Tissue sections harvested; plasma samples	Plasma and colon concentrations	[44]
	Disease model available	Yes	Eudragit P-4135F (M)	Ellagic acid with and without fluorescein	Tissue sections harvested; plasma samples	Plasma concentrations and visual observation of lower part of small intestine	[51]
	Based on practical considerations	No	Eudragit S (C)	IgY	Tissue sections harvested; plasma samples	Tissue concentrations	[142]
	N/A	No	Eudragit S (M); Eudragit L (M); Eudragit S/Eudragit L (M)	Insulin	Tissue sections harvested; plasma samples	Insulin content in microspheres from harvested tissue sections	[49]
	Disease model available	No	Eudragit S/PLGA[b] (M); Eudragit S (M)	Budesonide; coumarin-6	Tissue sections harvested; plasma samples; microscopy; colitis severity	Plasma and tissue concentrations	[47]
	N/A	Yes	Eudragit S (M); Eudragit L (M); Eudragit L100-55 (M)	Prednisolone	Plasma samples	Not determined because of failure of system (pH threshold not reached in rat)	[143]
	N/A	No	Eudragit S/L and Surelease® (C)	Capecitabine	Tissue sections harvested	Tissue concentrations	[144]
	N/A	No	Eudragit S (C)	Curcumin and cyclosporine	Colitis severity	Colitis severity and *in vitro* release	[103]
	N/A	No	Eudragit S (M)	Tacrolimus	Colitis severity	*In vitro* release	[145]
Rat and mouse	N/A	No	Eudragit S (C)	Budesonide; DiR[c]; coumarin-6	Tissue sections harvested; plasma samples	Visual observation of harvested tissue sections combined with plasma and colon concentrations	[45]
	N/A	No	Pluronic/Polyacrylic acid (M)	Epirubicin; Toluidine Blue O	Tissue sections harvested; plasma samples; tumor size	Visual observation of harvested tissue sections	[54]
	N/A	No	P(CFC-MAA-MEG) (M)	Dexamethasone	Tissue sections harvested; plasma samples	Plasma and colon concentrations	[43]

TABLE 2 (*Continued*)

Animal			Drug delivery system		*In vivo* test		Refs
Species	**Rationale**	**pH values GI tract mentioned**	**pH-dependent matrix (M) and/or coating (C)**	**Drug/marker**	**Read out**	**Colon arrival determined with**	
Mouse	N/A	No	Eudragit S/Eudragit L (C)	Sulfasalazine	Paw edema severity	N/A	[146]
	N/A	No	P(CE-MAA-MEG) (M)	Mesalazine	Colitis severity	Colitis severity	[102]
	Disease model available	No	Eudragit S/PLGA (M)	Curcumin	Colitis severity	N/A	[147]
	N/A	No	Eudragit P-4135 F (M)	Tacrolimus	Colitis severity	N/A	[148]
	Disease model available	No	Eudragit S/PLGA[b] (M)	Curcumin	Tissue sections harvested, colitis severity	Visual accumulation in colon	[58]
	N/A	Yes	Eudragit FS 30 D (C); Eudragit L100-55 (C)	FITC-BSA; luciferase DNA plasmid; CpG vaccine	Tissue sections harvested	Cellular uptake in harvested tissue	[57]
	Disease model available	No	Eudragit FS 30 D/PLGA[b] (M)	Cyclosporine; DiR[c]	Tissue sections harvested; colitis severity	Visual observation of harvested tissue sections	[55]
	Disease model available	No	Eudragit S (C)	Budesonide; DiR[c]; coumarin-6	NIR spectroscopy; tissue sections harvested; disease severity	NIR spectroscopy and colon concentrations	[56]
	Disease model available	No	Eudragit S (M)	Curcumin	Colitis severity; plasma samples; fecal matter	Fecal concentrations	[48]
Mouse and rabbit	Disease model available (mouse) and N/A (rabbit)	No	Eudragit P-4135F (M)	Enoxaparin	Plasma samples	Not possible because of negligible systemic absorption of enoxaparin	[149]
Rabbit	N/A	No	Eudragit S/Ethyl cellulose (C)	Metronidazole	Plasma samples	Plasma samples and colon arrival time from literature	[80]
	GI tract comparable to human	Yes	Eudragit S/Ethyl cellulose (C)	Theophylline	Radiography, plasma samples	Radiography and colon arrival time from literature	[62]

[a] Not available.
[b] Poly(lactic-co-glycolic acid.
[c] 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide.

TABLE 3

Examples of colonic disease models in rabbits

Disease	Disease induction	Refs
Colon cancer	VX2 carcinoma transplantation	[150,151]
	Azoxymethane subcutaneously	[152,153]
Irritable bowel syndrome	Moist heat, stress, and low-dose laxatives	[154]
	Colorectal distension with induced visceral hypersensitivity	[155,156]
Inflammatory bowel disease	Diluted acetic acid intrarectally	[157]
	Hapten intrarectally (e.g., 2,4,6-trinitrobenzenesulfonic acid or dinitrochlorobenzene)	[158–161]
	Inoculation with *Eimeria magna* oocytes intragastrically	[162]
	Degraded carrageenan orally	[163]
	Lipopolysaccharide intrarectally after 1% formalin enema	[164]
	Inoculation with Crohn's tissue homogenates intraileally	[165]
	Immune complex intravenously in combination with dilute formalin intrarectally	[166]
	Inoculation with *Bacteroides vulgatus* intra-appendiceal	[167]
	Dextran sodium sulfate orally	[168]

identified (iib, iiib, and iv) (Table 4). One option is to retrieve the drug delivery system itself (iv) and to measure the drug content in the system, in which incomplete recovery indicates drug release. In the other approaches (iib and iiib), it is important that the drug is not extracted from the drug delivery system and that the luminal content is included in the assay either with or without the tissue. Drug release is then indicated by the presence of drug in the extract.

All methods described above measure drug content. Next to this approach, it is possible to detect the drug delivery system (e.g., microspheres or tablets) in harvested tissue samples visually or with a light microscope. In case of dissolving or eroding systems, these methods allow for the conformation of drug release from the disappearance of the drug delivery system [50–54]. When the drug delivery system is still present, drug release cannot be ruled out. More information is obtained when release can be visualized by fluorescence microscopy or NIR fluorescence microscopy, when a marker is included in the delivery system. For fluorescence microscopy, fluorescein or coumarin-6 have been used as markers and for NIR fluorescence imaging, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR), has been used [45,51,55,56]. Furthermore, cellular uptake of the marker/drug can be used to verify release [45,46,57,58].

When tissue sections are harvested, multiple animals are required. The consequence of this is that individual animals with variable GI transit times are compared, which makes interpretation of the data less reliable when the data are pooled. Moreover, the exact location of drug release cannot easily be determined, especially for multiparticulate formulations, such as microspheres, because they spread throughout the GI tract. Additionally, many animals have to be sacrificed to obtain data at sufficient time points. Furthermore, possible degradation or metabolization and/or transfer of the drug to the plasma have to be taken into account. The advantage of harvesting tissue samples is that it is possible to assess whether the drug has reached the colon. If the drug does not reach the colon, no drug would be measured or visually detected in the colonic tissue and/or luminal content. However, if no drug is measured or visually detected in the colonic tissue, it cannot be excluded with certainty that the drug has not reached the colon. More reliable conclusions can be drawn when plasma samples are taken at the same time points as the tissue samples, on the condition that the drug is immediately absorbed into the systemic circulation after release. The presence of drug in plasma indicates drug release and the presence in tissue sections might indicate the site of release.

Non-invasive imaging techniques

An attractive alternative to harvesting tissue samples, also in the light of the 3Rs (reduction, refinement, and replacement) for animal experiments [59], is the use of non-invasive imaging techniques. Most frequently used are radiography and fluoroscopy, followed by γ-scintigraphy and NIR fluorescence imaging [60–71].

Ionizing radiation, X-rays, are used in radiography and fluoroscopy to capture the images while in γ-scintigraphy the marker in the dosage form emits ionizing γ-radiation [72–74]. To visualize the dosage form in radiography or fluoroscopy, a contrast agent (e.g., barium sulfate) has to be integrated in the drug delivery system [73,74]. For radiography, fluoroscopy, and γ-scintigraphy, the cumulative ionizing radiation exposure has to be considered in the study design to ensure humane treatment of the animals. This is especially the case when animals are not sacrificed after the study, because radiation can cause long-term effects [75]. With NIR fluorescence imaging, no ionizing radiation is used and instead a fluorescent agent (e.g., DiR) is used as a marker compound [60,74]. The downside of NIR fluorescence imaging is that it suffers from a low resolution because of attenuation, scattering, and dispersion of the emitted light when it passes through tissues [60,74]. With all imaging techniques, the animals have to be restrained or brought under anesthesia to prevent blurred images, which causes

TABLE 4

Overview of methods used to analyze harvested tissue samples

Method	Drug extracted			Drug release indicated by	Applicable for drugs that are not absorbed
	Tissue	Luminal content	Delivery system		
i	X			Presence of drug	
iia	X	X	X	Incomplete recovery[a]	
iib	X	X		Presence of drug	X
iiia		X	X	Incomplete recovery	
iiib		X		Presence of drug	X
iv			X	Incomplete recovery	X

[a] The total drug content is not retrieved.

discomfort to the animal [75]. Radiography, fluoroscopy, and γ-scintigraphy are also used in clinical studies. The advantages and disadvantages of these methods were described in more detail in part I of this series [1].

Based on the evaluation of published animal studies in which non-invasive imaging techniques are applied, we suggest aspects that could be improved. Generally, only images were taken from one angle, generating a 2D image of the abdomen [60–71,73]. Reasons for taking only one image angle could be, for instance, reduction of radiation exposure, animal welfare, or practical considerations. Images from only one angle might lead to misinterpretation of disintegration and/or erosion of the drug delivery system or of the exact location of the drug delivery system in the GI tract because the depth cannot be determined. When the location of the different segments of the GI tract is determined with, for instance, a barium sulfate meal study in radiography or fluoroscopy studies, interpretation of the images is more straightforward [61,62,66,67,73,76]. Interpretation of images is more problematic in animal studies than in clinical trials because, in humans, the location of the large intestine is fixed in the body and the outline of the large intestine becomes visible when the marker is released [1]. In addition, the projection of the 2D images (e.g., dorsoventral) is often not given and the printed image quality and size is sometimes not optimal [60–62,64–70], which complicates interpretation of these images by the readers. Furthermore, image exposure and animal positioning could be improved, which would simplify interpretation of the images [60–64,66–69,71]. When these factors are not optimal and when only images from one angle are used, one should be reluctant with statements about colon targeting, because structures in the abdomen overlap and the exact 3D position cannot be determined with certainty.

Imaging techniques, when performed correctly, provide useful information about the position of a drug delivery system in the GI tract. However, these techniques do not provide information about the drug release from a system and, therefore, do not automatically give information about the ileocolonic targeting ability. This shortcoming can be overcome by combining imaging techniques with measuring plasma concentrations, especially when the drug and an imaging marker are combined in the same drug delivery system [60,62,67,71]. However, this is only valid for drugs that are absorbed over the entire GI tract.

Plasma samples

Drug and/or drug metabolite concentrations in plasma samples have been frequently used as a proof for ileocolonic drug delivery in animal studies (Table 2) [52,77–81]. However, plasma concentrations generally do not provide enough information to confirm ileocolonic drug delivery. An option is to compare the pharmacokinetic data obtained from the plasma curve to the colon arrival time, to determine whether the observed lag time of the system is sufficiently long to warrant targeting to the colon [62,77–80]. Kennedy *et al*. developed a method to measure the colonic arrival time, also called mouth-to-cecal transit time, in humans, using sulfasalazine [82], which has been validated by others [83]. This method is based on the fact that sulfasalazine is poorly absorbed by the GI tract but is converted by bacteria in the colon into sulfapyridine, which is subsequently absorbed [84,85]. Several researchers have used this method to circumvent interindividual variation in colon arrival times [52,86]. It is also possible to circumvent the intraindividual variation in colon arrival time by combining sulfasalazine with a release marker (e.g., theophylline) in the drug delivery system [1,87]. In this method, theophylline is used as a marker for drug release, since it is absorbed over the entire GI tract. If there is no difference in plasma arrival time between theophylline and sulfapyridine, it indicates that the formulation released its contents into the colon. A downside of this method is that it only gives an answer to the question whether the drug delivery system releases its contents into the colon, but does not provide information as to the exact location in the colon. To determine the exact location of drug release, imaging techniques can be used in combination with the theophylline-sulfasalazine method. Furthermore, the pharmacokinetics and/or pharmacodynamics of the investigated drug might be influenced by either sulfasalazine or the release marker (in case the drug itself cannot be used as a release marker). In addition, sulfasalazine is degraded into not only sulfapyridine, but also mesalazine, which is a pharmacologically active compound used in the treatment of inflammatory bowel disease [82,88]. This should be taken into account if the compounds are combined into one drug delivery system.

Breath and urine samples

In addition to using plasma samples to determine the orocecal transit time, it is possible to use breath samples [89,90]. This method utilizes ^{13}C-urea (a stable isotope), which is metabolized by bacteria into $^{13}CO_2$ that is subsequently exhaled. In human volunteers, this method in combination with measuring ^{13}C-urea and ^{15}N-urea (an internal standard) in urine has been used to verify colonic drug delivery [1,91–93]. This principle could also be used in laboratory animals. Collection of urine in animals is possible by using a metabolic cage or by catheterization [94]. However, the use of metabolic cages generates a stressful environment for animals because of individual housing and the wire mesh floors, which can influence the therapeutic effect of a drug or exacerbate disease symptoms [94,95]. The latter stressor can be prevented by using hydrophobic sand for urine collection [96], but individual housing would remain an issue. In addition, catherization is problematic because, among other issues, the catheter can be removed by the animal, and inserting the catheter is a stressor on its own [97]. The collection of breath samples is also problematic because of stress caused by handling or individual housing in a breath-test system [94,98]. The non-invasive character of the method makes it an ideal method to verify colonic drug delivery in humans [1]; however, because of the implications of urine and breath collection in laboratory animals, we do not recommend this method for animal studies.

Therapeutic effect

The last method, abundantly used to evaluate ileocolonic drug delivery systems in laboratory animals, is to determine a therapeutic effect (Table 2) [99–103]. However, the therapeutic effect as such does not directly answer the question whether the drug is in fact targeted to, and released into, the ileocolonic region. Therapeutic proteins or peptides given orally in an ileocolonic drug delivery system are an exception because they will be degraded in the upper GI tract. Therefore, they can only elicit a therapeutic effect if they are released in the colon [104]. In other cases, a

therapeutic effect should not be used as such to verify ileocolonic drug targeting, but should be used in combination with imaging techniques and plasma sampling to verify successful targeting. When the theophylline–sulfasalazine method is used or urine is collected with a metabolic cage, the influence on therapeutic effects of the drug under investigation should first be taken into account before conducting the experiments.

Overall, the determination of the therapeutic effect is valuable, because it can answer the questions whether the drug delivery system can improve clinical symptoms and whether it is superior to nontargeted drug delivery systems.

Concluding remarks

For testing novel drugs for ileocolonic delivery and/or for pH-dependent ileocolonic drug delivery systems containing novel excipients, animals have to be used. To obtain ileocolonic drug delivery, a sharp distinct pH peak in the terminal ileum is crucial, thus a similar pH to humans is not essential. However, if the pH profile of the GI tract in animals is similar to that of humans, established ileocolonic drug delivery systems can be used to test novel drugs. When novel pH-dependent excipients have to be tested, a pH profile similar to that in humans is a prerequisite to obtain ileocolonic drug delivery in humans. In this respect, the rabbit is the most appropriate animal species compared with other frequently used laboratory animals, because their GI pH values are most similar to those of the human GI tract. However, not only the pH values of the GI tract, but also the desired disease model and the size of the delivery system have to be taken into account. If the rabbit cannot be used, then the pig, rat, and dog might be suitable alternatives, on the condition that the pH values of individual animals are verified first. To properly draw conclusions from the obtained efficacy data, ileocolonic drug delivery must be verified. The different methods used for this verification all have specific advantages and limitations, thus the optimal method should be determined for each study. Non-invasive imaging techniques in combination with plasma sampling can be used if the therapeutic effect of a novel drug is investigated. When a novel excipient in the drug delivery system itself is subject of investigation, the theophylline-sulfasalazine method is an elegant way to verify colonic drug delivery.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 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 reported in this paper. The literature search was partially funded by Janssen Pharmaceutica. Janssen Pharmaceutica had neither role in study design, in the collection, analysis, and interpretation of data, nor in the writing of the report and in the decision to submit the paper for publication.

Acknowledgments

This literature search was partially funded by Janssen Pharmaceutica. Janssen Pharmaceutica had neither a role in the study design, in the collection, analysis, and interpretation of data, nor in the writing of the report and in the decision to submit the paper for publication.

References

1 Broesder, A. *et al.* (2020) pH-dependent ileocolonic drug delivery, part I: in vitro and clinical evaluation of novel systems. *Drug Discov. Today* . http://dx.doi.org/10.1016/j.drudis.2020.06.011 Published online June XX, 2020

2 Bespalov, A. *et al.* eds (2020) *Good Research Practice in Non-Clinical Pharmacology and Biomedicine*, Springer

3 ICH (2020) *Safety Guidelines*. ICH

4 ICH (2000) *Guideline S7A Safety Pharmacology Studies for Human Pharmaceuticals*. ICH

5 Hatton, G.B. *et al.* (2015) Animal farm: considerations in animal gastrointestinal physiology and relevance to drug delivery in humans. *J. Pharm. Sci.* 104, 2747–2776

6 Sjögren, E. *et al.* (2014) *In vivo* methods for drug absorption – comparative physiologies, model selection, correlations with *in vitro* methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *Eur. J. Pharm. Sci.* 57, 99–151

7 Arndt, M. *et al.* (2013) Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state. *Eur. J. Pharm. Biopharm.* 84, 633–641

8 Akimoto, M. *et al.* (2000) Gastric pH profiles of beagle dogs and their use as an alternative to human testing. *Eur. J. Pharm. Biopharm.* 49, 99–102

9 Lui, C.Y. *et al.* (1986) Comparison of gastrointestinal pH in dogs and humans: implications on the use of the Beagle dog as a model for oral absorption in humans. *J. Pharm. Sci.* 75, 271–274

10 Sagawa, K. *et al.* (2009) Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. *J. Pharm. Sci.* 98, 2494–2500

11 Mahar, K.M. *et al.* (2012) Gastric pH and gastric residence time in fasted and fed conscious Beagle dogs using the Bravo® pH system. *J. Pharm. Sci.* 101, 2439–2448

12 Steinberg, W.H. *et al.* (1965) Heidelberg capsule I. *In vitro* evaluation of a new instrument for measuring intragastric pH. *J. Pharm. Sci.* 54, 772–1726

13 Dickman, R. and Fass, R. (2006) Ambulatory esophageal pH monitoring: new directions. *Dig. Dis.* 24, 313–318

14 Chotiprashidi, P. *et al.* (2005) ASGE Technology Status Evaluation Report: wireless esophageal pH monitoring system. *Gastrointest. Endosc.* 62, 485–487

15 Becker, D. *et al.* (2014) Novel orally swallowable IntelliCap® device to quantify regional drug absorption in human GI tract using diltiazem as model drug. *AAPS PharmSciTech* 15, 1490–1497

16 Smith, H.W. (1965) Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J. Pathol. Bacteriol.* 89, 95–122

17 Smith, H.W. and Jones, J.E.T. (1963) Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. *J. Pathol. Bacteriol.* 86, 387–412

18 Merchant, H.A. *et al.* (2015) Gastrointestinal characterisation and drug solubility determination in animals. *J. Pharm. Pharmacol.* 67, 630–639

19 Gupta, P.K. and Robinson, J.R. (1988) Gastric emptying of liquids in the fasted dog. *Int. J. Pharm.* 43, 45–52

20 Kalantzi, L. *et al.* (2006) Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. *Pharm. Res.* 23, 1373–1381

21 Graysel, D.M. and Miller, E.G. (1928) The pH of the contents of the gastrointestinal tract in dogs, in relation to diet and rickets. *J. Biol. Chem.* 76, 423–436

22 Kohl, K.D. *et al.* (2013) Effects of anatomy and diet on gastrointestinal pH in rodents. *J. Exp. Zool. A Ecol. Genet. Physiol.* 319, 225–229

23 McConnell, E.L. *et al.* (2008) Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *J. Pharm. Pharmacol.* 60, 63–70

24 Cummings, J.H. and Macfarlane, G.T. (1991) The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* 70, 443–459

25 Merchant, H.A. *et al.* (2011) Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development. *Eur. J. Pharm. Sci.* 42, 3–10

26 Ward, F.W. *et al.* (1986) Nitrate reduction, gastro-intestinal pH and N-nitrosation in gnotobiotic and conventional rats. *Food Chem. Toxicol.* 24, 17–22

27 Haiba, M.H. (1954) The pH of the alimentary tract in normal and Giardia -infected culture mice. *Parasitology* 44, 387–391

28 Haiba, M.H. and Williamson, J. (1952) The pH of the small intestine of normal, starved and Giardia-Infected norway rats. *Trans. R. Soc. Trop. Med. Hyg.* 46, 85–93

29 Ward, F.W. and Coates, M.E. (1987) Gastrointestinal pH measurement in rats: influence of the microbial flora, diet and fasting. *Lab. Anim.* 21, 216–222
30 Eaimtrakarn, S. *et al.* (2001) Retention and transit of intestinal mucoadhesive films in rat small intestine. *Int. J. Pharm.* 224, 61–67
31 Mori, C. and Kondo, H. (2006) Effect of gastric acidity regulation on the gastrointestinal transit time and secretion of gastric fluids in beagle dogs. *J. Drug Deliv. Sci. Technol.* 16, 467–472
32 Yamada, I. and Haga, K. (1990) Measurement of gastric pH during digestion of a solid meal in dogs. *Chem. Pharm. Bull. (Tokyo)* 38, 1755–1756
33 Gidenne, T. and Lebas, F. (2006) Feeding behaviour in rabbits. In *Feeding in Domestic Vertebrates: From Structure to Behaviour* (Bels, V., ed.), pp. 179–194, CABI
34 Chen, E.P. *et al.* (2008) Gastric pH and gastric residence time in fasted and fed conscious cynomolgus monkeys using the Bravo® pH system. *Pharm. Res.* 25, 123–134
35 Polentarutti, B. *et al.* (2010) Modification of gastric pH in the fasted dog. *J. Pharm. Pharmacol.* 62, 462–469
36 Ewe, K. *et al.* (1999) Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. *Dig. Dis. Sci.* 44, 1434–1439
37 Pye, G. *et al.* (1990) Gastrointestinal intraluminal pH in normal subjects and those with colorectal adenoma or carcinoma. *Gut* 31, 1355–1357
38 Ovesen, L. *et al.* (1986) Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. *Gastroenterology* 90, 958–962
39 Farmer, A.D. *et al.* (2014) Cecal pH is a biomarker of excessive colonic fermentation. *World J. Gastroenterol.* 20, 5000–5007
40 Press, A.G. *et al.* (1998) Gastrointestinal pH profiles in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 12, 673–678
41 Xu, Q. *et al.* (2013) Preparation, *in vitro* and *in vivo* evaluation of budesonide loaded core/shell nanofibers as oral colonic drug delivery system. *J. Nanosci. Nanotechnol.* 13, 149–156
42 Hirayama, M. *et al.* (2011) Concentration dependence of 5-aminosalicylic acid pharmacological actions in intestinal mucosa after oral administration of a pH-dependent formulation. *Mol. Pharm.* 8, 1083–1089
43 Dong, K. *et al.* (2014) Assessment of the safety, targeting, and distribution characteristics of a novel pH-sensitive hydrogel. *Colloids Surf. B Biointerfaces.* 123, 965–973
44 Kietzmann, D. *et al.* (2010) Colonic delivery of carboxyfluorescein by pH-sensitive microspheres in experimental colitis. *Eur. J. Pharm. Biopharm.* 76, 290–295
45 Kim, H.Y. *et al.* (2020) Ternary nanocomposite carriers based on organic clay-lipid vesicles as an effective colon-targeted drug delivery system: preparation and *in vitro*/*in vivo* characterization. *J. Nanobiotechnol.* 18, 17
46 Gan, L. *et al.* (2013) Novel pH-sensitive lipid-polymer composite microspheres of 10-hydroxycamptothecin exhibiting colon-specific biodistribution and reduced systemic absorption. *J. Pharm. Sci.* 102, 1752–1759
47 Makhlof, A. *et al.* (2009) pH-sensitive nanospheres for colon-specific drug delivery in experimentally induced colitis rat model. *Eur. J. Pharm. Biopharm.* 72, 1–8
48 Kesharwani, S.S. *et al.* (2018) Site-directed non-covalent polymer-drug complexes for inflammatory bowel disease (IBD): formulation development, characterization and pharmacological evaluation. *J. Control. Release* 290, 165–179
49 Morishita, I. *et al.* (1993) Enteral insulin delivery by microspheres in 3 different formulations using Eudragit L100 and S100. *Int. J. Pharm.* 91, 29–37
50 Deol, P.K. and Kaur, I.P. (2013) Improving the therapeutic efficiency of ginger extract for treatment of colon cancer using a suitably designed multiparticulate system. *J. Drug Target.* 21, 855–865
51 Jeong, Y.-I. *et al.* (2001) Application of Eudragit P-4135F for the delivery of ellagic acid to the rat lower small intestine. *J Pharm Pharmacol.* 53, 1079–1085
52 Hu, Z. *et al.* (1999) Characterization of norfloxacine release from tablet coated with a new pH-sensitive polymer, P-4135F. *J. Drug Target.* 7, 223–232
53 Geary, R.S. and Schlameus, W.H. (1993) Vancomycin and insulin used as models for oral delivery of peptides. *J. Control. Release* 23, 65–74
54 Lo, Y.-L. *et al.* (2013) pH-and thermo-sensitive pluronic/poly(acrylic acid) in situ hydrogels for sustained release of an anticancer drug. *J. Drug Target.* 21, 54–66
55 Naeem, M. *et al.* (2018) Colon-targeted delivery of cyclosporine A using dual-functional Eudragit® FS30D/PLGA nanoparticles ameliorates murine experimental colitis. *Int. J. Nanomed.* 13, 1225–1240
56 Naeem, M. *et al.* (2018) pH-triggered surface charge-reversal nanoparticles alleviate experimental murine colitis via selective accumulation in inflamed colon regions. *Nanomedicine* 14, 823–834
57 Zhu, Q. *et al.* (2012) Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. *Nat. Med.* 18, 1291–1296
58 Beloqui, A. *et al.* (2014) pH-sensitive nanoparticles for colonic delivery of curcumin in inflammatory bowel disease. *Int. J. Pharm.* 473, 203–212
59 Russell, W.M.S. and Burch, R.L. (1959) *The Principles of Humane Experimental Technique*. Methuen & Co. Ltd
60 Hou, L. *et al.* (2016) Smart nanocomposite hydrogels based on azo crosslinked graphene oxide for oral colon-specific drug delivery. *Nanotechnology* 27, 315105
61 Sharma, P. and Pathak, K. (2013) Inulin-based tablet in capsule device for variable multipulse delivery of aceclofenac: optimization and *in vivo* roentgenography. *AAPS PharmSciTech* 14, 736–747
62 Patel, M.M. and Amin, A.F. (2011) Design and optimization of colon-targeted system of theophylline for chronotherapy of nocturnal asthma. *J. Pharm. Sci.* 100, 1760–1772
63 Hong, S. *et al.* (2012) Colon-targeted cell-permeable NFκB inhibitory peptide is orally active against experimental colitis. *Mol. Pharm.* 9, 1310–1319
64 Omar, S. *et al.* (2007) Colon-specific drug delivery for mebeverine hydrochloride. *J. Drug Target.* 15, 691–700
65 Avachat, A.M. and Shinde, A.S. (2016) Feasibility studies of concomitant administration of optimized formulation of probiotic-loaded Vancomycin hydrochloride pellets for colon delivery. *Drug Dev. Ind. Pharm.* 42, 80–90
66 Yassin, A.E.B. *et al.* (2010) New targeted-colon delivery system: *in vitro* and *in vivo* evaluation using X-ray imaging. *J. Drug Target.* 18, 59–66
67 Patel, M.M. and Amin, A.F. (2011) Formulation and development of release modulated colon targeted system of meloxicam for potential application in the prophylaxis of colorectal cancer. *Drug Deliv.* 18, 281–293
68 Moghimipour, E. *et al.* (2018) *In vivo* evaluation of pH and time-dependent polymers as coating agent for colonic delivery using central composite design. *J. Drug Deliv. Sci. Technol.* 43, 50–56
69 Amrutkar, J.R. and Gattani, S.G. (2012) A novel hydrogel plug of *Sterculia urens* for pulsatile delivery: *in vitro* and *in vivo* evaluation. *J. Microencapsul.* 29, 72–82
70 Mastiholimath, V.S. *et al.* (2007) Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma. *Int. J. Pharm.* 328, 49–56
71 Patel, M.M. (2017) Formulation and development of di-dependent microparticulate system for colon-specific drug delivery. *Drug Deliv. Transl. Res.* 7, 312–324
72 Perkins, A.C. and Frier, M. (2004) Radionuclide imaging in drug development. *Curr. Pharm. Des.* 10, 2907–2921
73 Owens, J.M. and Biery, D.N. (1998) *Radiographic Interpretation for the Small Animal Clinician* (2nd edn), Williams & Wilkins
74 Kiessling, F. *et al.* eds (2017) *Small Animal Imaging*, Springer
75 University of Britisch Columbia Animal Care Committee (2014) *UBC ACC Guideline on Imaging of Rodents*. UBC
76 Hardy, J.G. *et al.* (1987) Evaluation of an enteric-coated delayed-release 5-aminosalicylic acid tablet in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 1, 273–280
77 Takaya, T. *et al.* (1998) Importance of dissolution process on systemic availability of drugs delivered by colon delivery system. *J. Control. Release* 50, 111–122
78 Gao, C. *et al.* (2006) *In vitro* release and *in vivo* absorption in beagle dogs of meloxicam from Eudragit® FS 30 D-coated pellets. *Int. J. Pharm.* 322, 104–112
79 Lamprecht, A. *et al.* (2004) pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. *J. Control. Release* 98, 1–9
80 Shah, N. *et al.* (2016) Design of experiment approach for formulating multi-unit colon-targeted drug delivery system: *in vitro* and *in vivo* studies. *Drug Dev. Ind. Pharm.* 42, 825–835
81 Dong, K. *et al.* (2016) Assessment of the drug loading, *in vitro* and *in vivo* release behavior of novel pH-sensitive hydrogel. *Drug Deliv.* 23, 174–184
82 Kennedy, M. *et al.* (1979) A pharmacological method of measuring mouth cecal transit time in man. *Br. J. Clin. Pharmacol.* 8, 372–373
83 Kellow, J.E. *et al.* (1986) Sulfapyridine appearance in plasma after salicylazosulfapyridine. *Gastroenterology* 91, 396–400
84 Dahan, A. and Amidon, G.L. (2009) Small intestinal efflux mediated by MRP2 and BCRP shifts sulfasalazine intestinal permeability from high to low, enabling its colonic targeting. *Am. J. Physiol. Liver Physiol.* 297, G371–G377
85 Schröder, H. *et al.* (1973) Metabolism of salicylazosulfapyridine in healthy subjects and in patients with ulcerative colitis; Effects of colectomy and of phenobarbital. *Clin. Pharmacol. Ther.* 14, 802–809
86 Takaya, T. *et al.* (1995) Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in Beagle dogs. *J. Pharm. Pharmacol.* 47, 474–478
87 Ishibashi, T. *et al.* (1999) Evaluation of colonic absorbability of drugs in dogs using a novel colon-targeted delivery capsule (CTDC). *J. Control. Release* 59, 361–376
88 Khan, A.K.A. *et al.* (1977) An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* 310, 892–895
89 Uchida, M. and Yoshida, K. (2009) Non-invasive method for evaluation of gastrocecal transit time by using a breath test in conscious rats. *J. Pharmacol. Sci.* 110, 227–230

90 Uchida, M. and Yoshida-Iwasawa, K. (2012) Simultaneous measurement of gastric emptying and gastrocecal transit times in conscious rats using a breath test after ingestion of acetic acid and lactose- ureide. *J. Smooth Muscle Res.* 48, 105–114

91 Maurer, M.J.M. *et al.* (2013) Isotope-labelled urea to test colon drug delivery devices *in vivo*: principles, calculations and interpretations. *Isotopes Environ. Health Stud.* 49, 473–491

92 Maurer, J.M. *et al.* (2015) Gastrointestinal pH and transit time profiling in healthy volunteers using the IntelliCap system confirms ileocolonic release of ColoPulse tablets. *PLoS One* 10, e0129076

93 Maurer, J.M. *et al.* (2013) ColoPulse tablets perform comparably in healthy volunteers and Crohn's patients and show no influence of food and time of food intake on bioavailability. *J. Control. Release* 172, 618–624

94 van Zutphen, L.F. *et al.* eds (2001) *Principles of Laboratory Animal Science*, Elsevier

95 Whittaker, A.L. *et al.* (2016) Effects of metabolic cage housing on rat behavior and performance in the social interaction test. *J. Appl. Anim. Welf. Sci.* 19, 363–374

96 Hoffman, J.F. *et al.* (2018) Hydrophobic sand versus metabolic cages: a comparison of urine collection methods for rats (Rattus norvegicus). *J. Am. Assoc. Lab. Anim. Sci.* 57, 51–57

97 Horst, P.-J. *et al.* (1988) A new method for collecting urine directly from the ureter in conscious unrestrained rats. *Kidney Blood Press. Res.* 11, 325–331

98 Uchida, M. *et al.* (2005) Simple and noninvasive breath test using 13C-acetic acid to evaluate gastric emptying in conscious rats and its validation by metoclopramide. *J. Pharmacol. Sci.* 98, 388–395

99 Duan, H. *et al.* (2017) Co-delivery of zinc and 5-aminosalicylic acid from alginate/N-succinyl-chitosan blend microspheres for synergistic therapy of colitis. *Int. J. Pharm.* 516, 214–224

100 Abdel Ghaffar, A.M. *et al.* (2016) Radiation synthesis of poly(starch/acrylic acid) pH sensitive hydrogel for rutin controlled release. *Int. J. Biol. Macromol.* 92, 957–964

101 Lamprecht, A. *et al.* (2005) FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression. *Pharm. Res.* 22, 193–199

102 Bai, X.Y. *et al.* (2016) Novel pH-sensitive hydrogels for 5-aminosalicylic acid colon targeting delivery: *in vivo* study with ulcerative colitis targeting therapy in mice. *Drug Deliv.* 23, 1926–1932

103 Desai, N. and Momin, M. (2020) Colon targeted bioadhesive pellets of curcumin and cyclosporine for improved management of inflammatory bowel disease. *Drug Deliv. Transl. Res.* 34, 893–900

104 Aguirre, T.A.S. *et al.* (2016) Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials. *Adv. Drug Deliv. Rev.* 106, 223–241

105 Fallingborg, J. *et al.* (1998) Small intestinal transit time and intraluminal pH in ileocecal resected patients with Crohn's disease. *Dig. Dis. Sci.* 43, 702–705

106 Ibekwe, V.C. *et al.* (2008) Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive ileocolonic release systems. *Pharm. Res.* 25, 1828–1835

107 Koziolek, M. *et al.* (2015) Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the Intellicap® system. *J. Pharm. Sci.* 104, 2855–2863

108 Dressman, J.B. *et al.* (1990) Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm. Res.* 7, 756–761

109 Kalantzi, L. *et al.* (2006) Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm. Res.* 23, 165–176

110 Psachoulias, D. *et al.* (2011) Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. *Pharm. Res.* 28, 3145–3158

111 Schneider, F. *et al.* (2016) Resolving the physiological conditions in bioavailability and bioequivalence studies: Comparison of fasted and fed state. *Eur. J. Pharm. Biopharm.* 108, 214–219

112 Koziolek, M. *et al.* (2015) Intragastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. *J. Control. Release* 220, 71–78

113 Maqbool, S. *et al.* (2009) Wireless capsule motility: Comparison of the SmartPill® GI monitoring system with scintigraphy for measuring whole gut transit. *Dig. Dis. Sci.* 54, 2167–2174

114 Hila, A. *et al.* (2006) Postprandial stomach contents have multiple acid layers. *J. Clin. Gastroenterol.* 40, 612–617

115 Pedersen, P.B. *et al.* (2013) Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities. *Eur. J. Pharm. Biopharm.* 85, 958–965

116 Savarino, V. *et al.* (1988) 24-hour study of intragastric acidity in duodenal ulcer patients and normal subjects using continuous intraluminal pH-metry. *Dig. Dis. Sci.* 33, 1077–1080

117 Pounder, R.E. *et al.* (1976) Effect of cimetidine on 24-hour intragastric acidity in normal subjects. *Gut* 17, 133–138

118 Fimmel, C.J. *et al.* (1985) Long-term ambulatory gastric pH monitoring: Validation of a new method and effect of H2-antagonists. *Gastroenterology* 88, 1842–1851

119 Mojaverian, P. *et al.* (1988) Effects of gender, posture, and age on gastric residence time of an indigestible solid: pharmaceutical considerations. *Pharm. Res.* 5, 639–644

120 Simonian, H.P. *et al.* (2005) Regional postprandial differences in pH within the stomach and gastroesophageal junction. *Dig. Dis. Sci.* 50, 2276–2285

121 Vo, L. *et al.* (2005) The effect of rabeprazole on regional gastric acidity and the postprandial cardia/gastro-oesophageal junction acid layer in normal subjects: a randomized, double-blind, placebo-controlled study. *Aliment. Pharmacol. Ther.* 21 1321–1230

122 Fallingborg, J. *et al.* (1989) pH-Profile and regional transit times of the normal gut measured by a radiotelemetry device. *Aliment. Pharmacol. Ther.* 3, 605–614

123 Bown, R.L. *et al.* (1974) Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. *Gut* 15, 999–1004

124 Perez de la Cruz Moreno, M. *et al.* (2006) Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. *J. Pharm. Pharmacol.* 58, 1079–1089

125 Mojaverian, P. *et al.* (1989) Gastrointestinal transit of a solid indigestible capsule as measured by radiotelemetry and dual gamma scintigraphy. *Pharm. Res.* 6, 719–724

126 Clarysse, S. *et al.* (2009) Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. *J. Pharm. Sci.* 98, 1177–1192

127 Bratten, J. and Jones, M.P. (2009) Prolonged recording of duodenal acid exposure in patients with functional dyspepsia and controls using a radiotelemetry pH monitoring system. *J. Clin. Gastroenterol.* 43, 527–533

128 Annaert, P. *et al.* (2010) Ex vivo permeability experiments in excised rat intestinal tissue and *in vitro* solubility measurements in aspirated human intestinal fluids support age-dependent oral drug absorption. *Eur. J. Pharm. Sci.* 39, 15–22

129 Brouwers, J. *et al.* (2006) Intraluminal drug and formulation behavior and integration in *in vitro* permeability estimation: A case study with amprenavir. *J. Pharm. Sci.* 95, 372–383

130 Riethorst, D. *et al.* (2016) Characterization of human duodenal fluids in fasted and fed state conditions. *J. Pharm. Sci.* 105, 673–681

131 Evans, D.F. *et al.* (1988) Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* 29, 1035–1041

132 Mikolajczyk, A.E. *et al.* (2015) Assessment of tandem measurements of pH and total gut transit time in healthy volunteers. *Clin. Transl. Gastroenterol.* 6, e100

133 Zarate, N. *et al.* (2010) Accurate localization of a fall in pH within the ileocecal region: validation using a dual-scintigraphic technique. *Am. J. Physiol. Liver Physiol.* 299, G1276–G1286

134 Hubert, S. *et al.* (2018) Development of a modified-release formulation of lovastatin targeted to intestinal methanogens implicated in irritable bowel syndrome with constipation. *J. Pharm. Sci.* 107, 662–671

135 Duan, H. *et al.* (2016) Mucoadhesive microparticulates based on polysaccharide for target dual drug delivery of 5-aminosalicylic acid and curcumin to inflamed colon. *Colloids Surf. B Biointerfaces* 145, 510–519

136 Arya, A. *et al.* (2017) Design and evaluation of acrylate polymeric carriers for fabrication of pH-sensitive microparticles. *Drug. Dev. Ind. Pharm.* 43, 305–318

137 Gugulothu, D. *et al.* (2014) pH-sensitive nanoparticles of curcumin–celecoxib combination: Evaluating drug synergy in ulcerative colitis model. *J. Pharm. Sci.* 103, 687–696

138 Lamprecht, A. *et al.* (2005) A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles. *J. Control. Release* 104, 337–346

139 Sanka, K. *et al.* (2015) A pH-triggered delayed-release chronotherapeutic drug delivery system of aceclofenac for effective management of early morning symptoms of rheumatoid arthritis. *J. Microencapsul.* 32, 794–803

140 Touitou, E. and Rubinstein, A. (1986) Targeted enteral delivery of insulin to rats. *Int. J. Pharm.* 30, 95–99

141 Bazan, L. *et al.* (2016) Comparative pharmaceutical study on colon targeted micro-particles of celecoxib: in-vitro–in-vivo evaluation. *Drug Deliv.* 23, 3339–3349

142 Zhang, S. *et al.* (2016) Development of microbeads of chicken yolk antibodies against Clostridium difficile toxin A for colonic-specific delivery. *Drug Deliv.* 23, 1940–1947

143 Kendall, R.A. *et al.* (2009) Fabrication and *in vivo* evaluation of highly pH-responsive acrylic microparticles for targeted gastrointestinal delivery. *Eur. J. Pharm. Sci.* 37, 284–290

144 Pandey, S. *et al.* (2018) Multiple response optimisation of processing and formulation parameters of pH sensitive sustained release pellets of capecitabine for targeting colon. *J. Microencapsul.* 35, 259–271

145 Ali, A.S. *et al.* (2020) Colon-targeted therapy of tacrolimus (FK506) in the treatment of experimentally induced colitis. *Pharmacology* Published online January 5, 2020 https://www.karger.com/Article/Abstract/505101

146 Kankala, R.K. *et al.* (2015) Multi-laminated metal hydroxide nanocontainers for oral-specific delivery for bioavailability improvement and treatment of inflammatory paw edema in mice. *J. Colloid Interface Sci.* 458, 217–228

147 Xiao, B. *et al.* (2015) Oral administration of pH-sensitive curcumin-loaded microparticles for ulcerative colitis therapy. *Colloids Surf. B Biointerfaces*. 135, 379–385

148 Meissner, Y. *et al.* (2006) Nanoparticles in inflammatory bowel disease: Particle targeting versus pH-sensitive delivery. *Int. J. Pharm.* 316, 138–143

149 Pellequer, Y. *et al.* (2007) Epithelial heparin delivery via microspheres mitigates experimental colitis in mice. *J. Pharmacol. Exp. Ther.* 321, 726–733

150 Agostino, D. *et al.* (1959) Capsule implantation: method for establishing simulated colon carcinoma in rats. *Proc. Soc. Exp. Biol. Med.* 100, 717–718

151 Whiteley, H.W. (1967) Preoperative radiation therapy in simulated cancer of the colon in rabbits. *Dis. Colon Rectum* 10, 100–102

152 Sugimura, T. (1976) Experimental tumors in digestive organs. *Gastroenterol. Jpn.* 11, 265–266

153 Ward, J.M. (1975) Dose response to a single injection of azoxymethane in rats. *Vet. Pathol.* 12, 165–177

154 Yang, Q.Q. *et al.* (2017) Differential expression of microRNA related to irritable bowel syndrome in a rabbit model. *J. Dig. Dis.* 18, 330–342

155 Tanaka, T. *et al.* (2012) Effects of TAK-480, a novel tachykinin NK2-receptor antagonist, on visceral hypersensitivity in rabbits and ricinoleic acid-induced defecation in guinea pigs. *J. Pharmacol. Sci.* 120, 15–25

156 Tanaka, T. *et al.* (2012) Establishment and validation of a rabbit model for *in vivo* pharmacodynamic screening of tachykinin NK2 antagonists. *J. Pharmacol. Sci.* 118, 487–495

157 Fretland, D.J. *et al.* (1990) Effect of the leukotriene B4 receptor antagonist SC-41930 on colonic inflammation in rat, guinea pig and rabbit. *J. Pharmacol. Exp. Ther.* 255, 572–576

158 Morris, G.P. *et al.* (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 96, 795–803

159 Anthony, D. *et al.* (1995) The characterization of a rabbit model of inflammatory bowel disease. *Int. J. Exp. Pathol.* 76, 215–224

160 Rabin, B.S. (1980) Animal model: immunologic model of inflammatory bowel disease. *Am. J. Pathol.* 99, 253–256

161 Rabin, B.S. and Rogers, S.J. (1978) A cell-mediated immune model of inflammatory bowel disease in the rabbit. *Gastroenterology* 75, 29–33

162 Sundaram, U. and West, A.B. (1997) Effect of chronic inflammation on electrolyte transport in rabbit ileal villus and crypt cells. *Am. J. Physiol. Liver Physiol.* 272, G732–G741

163 Watt, J. and Marcus, R. (1970) Ulcerative colitis in rabbits fed degraded carrageenan. *J. Pharm. Pharmacol.* 22, 130–131

164 Hotta, T. *et al.* (1986) Lipopolysaccharide-induced colitis in rabbits. *Res. Exp. Med.* 186, 61–69

165 Cave, D.R. *et al.* (1973) Further animal evidence of a transmissible agent in Crohn's disease. *Lancet* 302, 1120–1122

166 Hodgson, H.J.F. *et al.* (1978) Immune-complex mediated colitis in rabbits. An experimental model. *Gut* 19, 225–232

167 Shanmugam, M. *et al.* (2005) Bacterial-induced inflammation in germ-free rabbit appendix. *Inflamm. Bowel Dis.* 11, 992–1926

168 Leonardi, I. *et al.* (2015) Oral administration of dextran sodium sulphate induces a cecum-localized colitis in rabbits. *Int. J. Exp. Pathol.* 96, 151–162