Ileo-colonic drug delivery
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DOI:
10.33612/diss.214243319

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Broesder, A. (2022). Ileo-colonic drug delivery: coating the way to novel formulations. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. https://doi.org/10.33612/diss.214243319

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Chapter 3
pH-dependent ileo-colonic drug delivery, part II: pre-clinical evaluation of novel drugs and novel excipients

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\textit{Drug Discov. Today} 2020
CHAPTER 3

ABSTRACT

Safety issues require that novel drugs and novel excipients, formulated in pH-dependent ileo-colonic drug delivery systems, are tested in appropriate animal species before they are evaluated in humans. In a literature search, we found that, of the most frequently used laboratory animal species, the rabbit is the best choice. Its gastrointestinal (GI) pH values most resemble those in humans. In cases where rabbits cannot be used, pigs, dogs, and rats can serve as an alternative provided that GI pH values of individual animals are checked. Various methods to investigate the performance of ileo-colonic drug delivery systems are available. We recommend testing novel drugs with non-invasive imaging techniques in combination with plasma sampling, whereas novel excipients are best tested with the theophylline-sulfasalazine method.
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 ileo-colonic drug targeting. When evaluating the ileo-colonic targeting ability of a system or the therapeutic efficacy of an ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 humans. We aim to determine which animal species, if any, could best be used to test novel drugs or novel excipients in pH-dependent ileo-colonic drug delivery systems. Subsequently, in vivo methods used in preclinical evaluations to investigate and verify ileo-colonic drug delivery are discussed.
CHAPTER 3

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 ileo-colonic 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 the pH, other factors can play a role in the performance of pH-dependent ileo-colonic 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 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 x 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 are possible [16–23]. A drawback of this method is that only a mean pH of the collected fluid is determined. In addition, the pH should be measured immediately after collection to prevent possible post sampling 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 figure 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
| Species | Breed | Stomach | Duodenum | Jejunum | Ileum | Cecum | Colon | pH measurement | Fasted/fed | References |
|---------|-------|---------|----------|---------|-------|-------|-------|----------------|------------|------------|
| Monkey  | Cynomolgus | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Pig     | From farms | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Pig     | Cross-breed of large white and Landrace | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Pig     | Cross-breed of large white and Landrace | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Pig     | Large white, Landrace and Essex | X | X | X | X | X | X | Ex vivo | Fasted for 1 hour | [17] |
| Mouse   | Balb/c | X | X | X | X | X | X | Ex vivo | Fasted and fed | [23] |
| Mouse   | House  | X | X | X | X | X | X | In situ | NA | [27] |
| Mouse   | House  | X | X | X | X | X | X | Ex vivo | Fasted and fed | [22] |
| Mouse   | White  | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Rat     | White  | X | X | X | X | X | X | Ex vivo | Fasted/fed | [16] |
| Rat     | Wistar | X | X | X | X | X | X | Ex vivo | Fasted and fed | [23] |
| Rat     | Wistar | X | X | X | X | X | X | Ex vivo | Fasted and fed | [16] |
| Rat     | Albino Norwegian | X | X | X | X | X | X | In situ | Fasted and fed | [28] |
| Rat     | Wistar | X | X | X | X | X | X | In situ | Fasted | [30] |
| Rat     | Porton-Wistar | X | X | X | X | X | X | In situ | Fasted and fed standardized | [29] |

Table 1. Overview of studies investigating the pH values of the GI tract in laboratory animals and healthy human individuals.
### Table 1. Continued

| Animal | Species | Breed | Segment gastrointestinal tract | Method | pH measurement | References |
|--------|---------|-------|-------------------------------|--------|----------------|------------|
| Rat    | Porton-Wistar | X | Stomach | In situ | Fed | [26] |
| Rabbit | New Zealand Whites | X | Duodenum | Ex vivo | Fed | [16] |
| New Zealand Whites | X | Jejunum | Ex vivo | Fed | [18] |
| New Zealand Whites | X | Ileum | In situ | Fed | [25] |
| New Zealand Whites | X | Cecum | N/A | Fed | [39] |
| Guinea pig | N/A | X | Stomach | X | Ex vivo | [16] |
| Dog | From domestic households | X | Duodenum | In situ | Fed | [25] |
| Beagle | Dunkin-Hartley White | X | Jejunum | Ex vivo | Fed | [21] |
| Beagle | From domestic households | X | Ileum | Ex vivo | Fed | [21] |
| Beagle | Labrador | X | Cecum | N/A | Fed | [9] |
| Beagle | Beagles | X | Stomach | X | Aspiration | Fed | [8] |
| Beagle | Beagles | X | Duodenum | X | Aspiration | Fed | [31] |
| Beagle | Beagles | X | Jejunum | X | Aspiration | Fed | [10] |
| Beagle | Beagles | X | Ileum | X | Aspiration | Fed | [11] |
| Beagle | Beagles | X | Cecum | N/A | Fed | [19] |
| Beagle | Beagles | X | Stomach | X | Fasted | Fed | [19] |
| Beagle | Beagles | X | Duodenum | X | Fasted | Fed | [19] |
| Beagle | Beagles | X | Jejunum | X | Fasted | Fed | [19] |
| Beagle | Beagles | X | Ileum | X | Fasted | Fed | [19] |
| Beagle | Beagles | X | Cecum | N/A | Fasted | Fed | [19] |
| Beagle | Beagles | X | Stomach | X | Fed | Fed | [19] |
| Beagle | Beagles | X | Duodenum | X | Fed | Fed | [19] |
| Beagle | Beagles | X | Jejunum | X | Fed | Fed | [19] |
| Beagle | Beagles | X | Ileum | X | Fed | Fed | [19] |
| Beagle | Beagles | X | Cecum | N/A | Fed | Fed | [19] |
## Table 1. Continued

| Animal | Segment gastrointestinal tract | Method | References |
|--------|-------------------------------|--------|------------|
| Species | Breed | Stomach | Duodenum | Jejunum | Ileum | Cecum | Colon | pH measurement | Fasted/fed | |
| Dog | Labrador | x | x | x | x | x | Ex vivo | Fasted and fed | [20] |
| Human | x | x | x | x | x | x | Telemetry capsule | Fasted food when capsule left stomach | [35] |
| | x | x | x | x | x | x | Bravo™ capsule | Fasted food 30 min or 4 hours after ingestion | [36] |
| | x | x | x | x | x | x | In situ | Fasted | [37] |
| | x | x | x | x | x | x | Telemetry capsule | Fasted food allowed when capsule left stomach | [38] |
| | x | x | x | x | x | x | Intellicap® capsule | Fasted food allowed 4 hours after ingestion | [39] |
| | x | x | Heidelberg capsule | Fasted and fed | [40] |
| | x | x | Aspiration | Fasted | [41] |
| | x | x | Aspiration | Fasted | [42] |
| | x | x | SmartPill | Fed FDA standard breakfast | [43-44] |
| | x | x | SmartPill | Fasted after 4.5 hours standardized lunch | [45] |
| | x | x | x | Telemetry capsule | Fed food allowed after ingestion of capsule | [46] |
| Animal | Species | Method | Refereces |
|--------|---------|--------|-----------|
| Human  |         | IntelliCap® | [46] |
|        |         | Fasted; food and 10 hours after ingestion | |
|        |         | SmartPill | [47] |
|        |         | Heidelberg capsule | [9] |
|        |         | In situ | Fasted and fed standard meal | [48] |
|        |         | Aspiration | Fasted | [49] |
|        |         | In situ | Fasted and fed | [50] |
|        |         | Aspiration | Fasted | [51] |
|        |         | In situ | Fasted and fed | [52] |
|        |         | Heidelberg capsule | Fasted; standardized breakfast | [53] |
|        |         | In situ | Fasted and fed | [54] |
|        |         | In situ | Fasted and fed | [55] |
|        |         | Telemetry capsule | Fasted; food allowed when capsule left stomach | [56] |
|        |         | Telemetry capsule | Fasted; food allowed when capsule left stomach | [57] |
Table 1. Continued

| Animal | Segment gastrointestinal tract | Method | Fasted/fed | References |
|--------|-------------------------------|--------|------------|------------|
| Species Breed | Stomach | Duodenum | Jejunum | Ileum | Cecum | Colon |
| Human | X | X | | | | |
| | X | X | | | | |
| | X | | | | | |
| | X | | | | | |
| | X | | | | | |
| | X | | | | | |
| | X | X | X | X | Telemetry capsule | Fasted; food allowed when capsule left stomach |
| | X | X | X | X | SmartPill | Fasted; food allowed 6 hours after ingestion |
| | X | X | X | X | SmartPill | Fasted; standardized meal when capsule left stomach |
| | X | X | | | SmartPill | Fed test meal after 6 hours normal diet |

\[ ^1 \text{ Measured with a pH-electrode} \]
\[ ^2 \text{ Capsule attached to stomach} \]
\[ ^3 \text{ Colorimetric and with a pH-electrode} \]
\[ ^4 \text{ Capsule attached to duodenal bulb} \]
\[ ^5 \text{ Not available} \]
Figure 1. Luminal gastrointestinal (GI) pH values of laboratory animals (dark-green, yellow, orange, or red bars) and healthy human individuals (light-green bars). The different pH values of the GI tract of the (a) rabbit [16,18,25,34], (b) pig [16–18,25], (c) dog [7,8,32,9–11,16,19–21,31], (d) rat [16,18,23,26,28–30], (e) mouse [16,22,23,27], (f) guinea pig [16,25], and (g) monkey [16,33] are given against the values in healthy human individuals [9,35,44–53,36,54–63,37,64–69,38–43]. 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, 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.
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 figure 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 (figure 1a, 1b, 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; figure 1c) and rats (pH 3.2-6.7; figure 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 (figure 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 (figure 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 (figure 1g) [16,34]. The pH increases to pH 6.0 in the ileum and then drops to 5.0 in the cecum and the 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.
CHAPTER 3

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. Given the working principle of pH-sensitive ileo-colonic 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 ileo-colonic drug delivery in the chosen animal species or novel pH-dependent excipients can be evaluated.

The pH profile of the GI tract of mice, guinea pigs, and monkeys 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 ileo-colonic drug delivery systems (figure 1e, 1f, 1g). Dogs and rats might be suitable because the mean pH values in the various segments of their GI tract are generally comparable to those in humans, including a pH peak in the terminal ileum (figure 1c, 1d). 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 [70]. Rabbits and pigs have pH values comparable to those in humans, including the pH peak in the ileum above pH 7.2 (figure 1a, 1b). 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 ileo-colonic 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, ileo-colonic drug targeting might be proved with even more certainty. In cases where the pH values of the GI tract...
Table 2. Overview of pH-dependent ileo-colonic drug delivery systems tested in laboratory animals.

| Animal       | Rationale                     | pH values GI tract-mentioned | Drug delivery system | Drug/marker       | In vivo test                                      | Colon arrival determined with                           | References |
|--------------|--------------------------------|------------------------------|----------------------|-------------------|--------------------------------------------------|--------------------------------------------------------|------------|
| Dog          | N/A                            | Yes                          | Eudragit S (C)       | Mesalazine tegafur| Plasma samples                                    | Plasma concentrations compared with previously determined colon arrival time [71] |
| Dog          | Based on practical considerations | Yes                          | Eudragit FS 30 D (C) | Meloxicam         | Plasma samples                                    | Plasma concentrations and colon arrival time from literature [72] |
| Dog          | GI tract comparable to human   | Yes                          | Eudragit S (C)       | Mesalazine        | Tissue sections harvested; plasma samples         | Plasma and tissue concentrations                         | [73]       |
| Dog          | Pharmacokinetics               | No                           | Eudragit FS 30 D     | Lovastatin        | Plasma samples                                    | N/A                                                   | [74]       |
| 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 [75] |
| Dog and rat  | GI tract comparable to human   | Yes                          | Eudragit P-413SF (C); Eudragit L (C); Eudragit S (C) | Norfloxacin; fluorescein | Tissue sections harvested; plasma samples         | The sulfasalazine method                                 | [76]       |
| Rat          | N/A                            | No                           | Eudragit S (C)       | Mesalazine and/or curcumin | Colitis severity | N/A                                                   | [77]       |
| Rat          | N/A                            | No                           | N-succinyl chitosan/Zn²⁺ (M) | Mesalazine and/or zinc | Colitis severity | Colitis severity and in vitro release               | [78]       |
| Rat          | N/A                            | No                           | Acrylic acid and butyl methacrylate polymers (M) | Acetylsalicylic acid | Colitis severity | N/A                                                   | [79]       |
| Rat          | N/A                            | No                           | Eudragit S (M)       | Celecoxib and/or curcumin | Colitis severity | N/A                                                   | [80]       |
| Animal | Species | Rationale | pH values GI tract mentioned | Drug delivery system (M) and/or coating (C) | Drug/marker | In vivo test | Colon arrival determined with |
|--------|---------|-----------|-----------------------------|-----------------------------------------|-------------|-------------|-----------------------------|
| Rat    | N/A     | No        | Poly(starch/acrylic acid) (M) | Rutin                                    | Colitis severity | Colitis severity and in vitro release | [81] |
| Rat    | N/A     | No        | Eudragit P-4135F(M)          | Tacrolimus                                | Colitis severity | N/A         | [82] |
| Rat    | N/A     | No        | Eudragit P-4135F(M)          | Tacrolimus                                | Colitis severity | N/A         | [83] |
| Rat    | N/A     | No        | Eudragit S(M)                | Aceclofenac                               | Paw edema severity | N/A         | [84] |
| Rat    | N/A     | No        | Eudragit P-4135F(M)          | Calcitonin; carboxyfluorescein            | Plasma samples  | Plasma concentrations and colon arrival time from literature | [85] |
| Rat    | N/A     | No        | P(LEA-MEG) (M)               | Dexamethasone                             | Plasma samples  | Plasma samples and in vitro release | [86] |
| Rat    | N/A     | No        | Eudragit S (C); Eudragit L (C); Eudragit RS100 (C) | Insulin                                  | Plasma samples  | N/A         | [87] |
| Rat    | N/A     | No        | Eudragit L100-3.5(M); Eudragit L (M); Eudragit S (M) | Celecoxib                                | Plasma samples; colitis severity | N/A         | [88] |
| Rat    | N/A     | No        | Eudragit S (C)               | Budesonide                                | Tissue sections harvested | Tissue concentrations | [89] |
| Rat    | N/A     | No        | Eudragit S (C)               | Ginger extract                            | Tissue sections harvested | Visual observation of ileocecal junction | [90] |
Table 2. Continued

| Animal       | Drug delivery system | In vivo test | References |
|--------------|----------------------|--------------|------------|
| Rat          | Eudragit S/Compritol (M) | Tissue sections harvested; microscopy of plasma samples | [91]       |
| Rat          | Eudragit S (M)       | Tissue sections harvested; plasma samples | [92]       |
| Rat          | Eudragit R-4135F (M) | Tissue sections harvested; plasma samples | [93]       |
| Rat          | Eudragit S (C)       | Tissue sections harvested; plasma samples | [94]       |
| Rat          | Eudragit S (M); Eudragit L (M); Eudragit S/Eudragit L (M) | Insulin content in microspheres from harvested tissue sections | [95]       |
| Rat          | Eudragit SPLGA® (M); Eudragit S (M) | Tissue sections harvested; plasma samples; microscopy of colitis severity | [96]       |
| Rat          | Eudragit S (M); Eudragit L (M); Eudragit L:00:35 (M) | Prednisolone Plasma samples Not determined because of failure of the system (pH threshold not reached in rat) | [97]       |
| Rat          | Eudragit S/L and Surelease® (C) | Capecitabine Tissue sections harvested; Tissue concentrations | [98]       |
| Rat          | Eudragit S (C)       | Curcumin and cyclosporine | Colitis severity and in vitro release [99]       |
Table 2. Continued

| Animal | Species | Rationale | pH values GI tract mentioned | Drug delivery system (M) and/or coating (C) | Drug/marker | In vivo test | Colon arrival determined with |
|--------|---------|-----------|-----------------------------|---------------------------------------------|-------------|-------------|--------------------------------|
| Rat    | N/A     | No        | Eudragit S (M)              | Tacrolimus                                  | Colitis severity | In vivo release | [100]                         |
| Rat and mouse | N/A     | No        | Eudragit S (C)              | Budesonide DR; coumarin-6                   | Tissue sections harvested plasma samples | Visual observation of harvested tissue sections combined with plasma and colon concentrations | [101]                         |
| Rat and mouse | N/A     | No        | Pluronic/Polyacrylic acid (M) | Epibrain; Toluidine Blue O                   | Tissue sections harvested plasma samples; tumor size | Visual observation of harvested tissue sections | [102]                         |
| Rat and mouse | N/A     | No        | P(γC-MAA-MEG) (M)           | Dexamethasone                               | Tissue sections harvested plasma samples | Plasma and colon concentrations | [103]                         |
| Mouse  | N/A     | No        | Eudragit S/Eudragit L (C)   | Sulfasalazine                               | Paw edema severity | N/A          | [104]                         |
| Mouse  | N/A     | No        | P(CE-MAA-MEG) (M)           | Mesalazine                                  | Colitis severity | Colitis severity | [105]                         |
| Mouse  | Disease model available | No | Eudragit SPLGA (M)          | Curcumin                                   | Colitis severity | N/A          | [106]                         |
| Mouse  | N/A     | No        | Eudragit P-413SF (M)        | Tacrolimus                                  | Colitis severity | N/A          | [107]                         |
| Mouse  | Disease model available | No | Eudragit SPLGA (M)          | Curcumin                                   | Tissue sections harvested; colitis severity | Visual accumulation in the colon | [108]                         |
| Mouse  | N/A     | Yes       | Eudragit FS 30 D (C); Eudragit L100-55 (C) | FITC-BSA; luciferase DNA plasmid; CpgG vaccine | Tissue sections harvested | Cellular uptake in harvested tissue | [109]                         |
| Mouse  | Disease model available | No | Eudragit FS 30 D/PLGA (M)   | Cyclosporine; DiRc                          | Tissue sections harvested; colitis severity | Visual observation of harvested tissue sections | [110]                         |
Table 2. Continued

| Animal | Rationale | pH values GI tract mentioned | Drug delivery system | In vivo test | References |
|---|---|---|---|---|---|
| Mouse | Disease model available | No | Eudragit S (C) | Budesonide (DR); coumarin-6 | Near-infrared spectroscopy; tissue sections harvested; disease severity | Near-infrared spectroscopy and colon concentrations [111] |
| Mouse | Disease model available | No | Eudragit S (M) | Curcumin | Colon severity; plasma samples; fecal matter | Fecal concentrations [112] |
| 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 [113] |
| Rabbit | N/A | No | Eudragit SEthyl cellulose (C) | Metronidazole | Plasma samples | Plasma samples and colon arrival time from literature [114] |
| Rabbit | GI tract comparable to human | Yes | Eudragit SEthyl cellulose (C) | Theophylline | Radiography, plasma samples | Radiography and colon arrival time from literature [115] |

1. Not available
2. Poly(lactic-co-glycolic acid)
3. 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine iodide
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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 ileo-colonic 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 potentially 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 [1,37,38,45,58,69]. 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 ileo-colonic 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.

Table 3. Examples of colonic disease models in rabbits.

| Disease                      | Disease induction                                                                 | References |
|------------------------------|----------------------------------------------------------------------------------|------------|
| Colon cancer                 | - VX2 carcinoma transplantation                                                  | [116,117] |
|                              | - Azoxymethane subcutaneously                                                    | [118,119] |
| Irritable bowel syndrome     | - Moist heat, stress, and low-dose laxatives                                     | [120]      |
|                              | - Colorectal distension with induced visceral hypersensitivity                   | [121,122] |
| Inflammatory bowel disease   | - Diluted acetic acid intrearectally                                             | [123]      |
|                              | - Hapten intrarectally (e.g. 2,4,6-trinitrobenzenesulfonic acid or dinitrochlorobenzene) | [124–127] |
|                              | - Inoculation with Eimeria magna oocytes intra-gastrically                        | [128]      |
|                              | - Degraded carrageenan orally                                                   | [129]      |
|                              | - Lipopolysaccharide intra rectally after a 1% formalin enema                  | [130]      |
|                              | - Inoculation with Crohn’s tissue homogenates intraileally                       | [131]      |
|                              | - Immune complex intravenously in combination with dilute formalin intrearectally | [132]      |
|                              | - Inoculation with Bacteroides vulgatus intra-appendiceal                        | [133]      |
|                              | - Dextran sodium sulfate orally                                              | [134]      |
IN VIVO METHODS TO INVESTIGATE OR VERIFY ILEO-COLONIC 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 ileo-colonic drug delivery in the chosen animal species. Different methods have been used to verify ileo-colonic 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.

| Method |
|--------|
| I      |
| IIa    |
| IIb    |
| IIIa   |
| IIIb   |
| IV     |

*The total drug content is not retrieved.

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 [73,89,92,101,103]. With this method, drug release is
indicated by the presence of the 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 [91,96]. 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 [96]. If the drug is not extracted from the dosage form (IIb), then the presence of the drug in the extract is indicative of drug release [91]. In the third method (III), only the drug content in the luminal content is measured while the tissue itself is not used [112]. 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. In case 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 [95]. In this method, incomplete recovery indicates drug release.

In three different approaches, the location of the release of drugs that are not absorbed into the systemic circulation can be 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 confirmation of drug release from the disappearance of the drug delivery system [75,76,90,93,102]. 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′-tetramethylindotricarbocyanine iodide (DiR), has been used [93,101,110,111]. Furthermore, cellular uptake of the marker/drug can be used to verify release [91,101,108,109]. 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.
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when the data are pooled. Moreover, the exact location of drug release cannot easily be determined, especially for multi-particulate 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 the 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 [135], is the use of non-invasive imaging techniques. Most frequently used are radiography and fluoroscopy, followed by γ-scintigraphy and NIR fluorescence imaging [115,136,145,146,137–144].

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 [147–149]. To visualize the dosage form in radiography or fluoroscopy, a contrast agent (e.g. barium sulfate) has to be integrated into the drug delivery system [148,149]. 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 [150]. With NIR fluorescence imaging no ionizing radiation is used and instead a fluorescent agent (e.g. DiR) is used as a marker compound [136,149]. The downside of NIR fluorescence imaging is that it suffers from a low resolution due to attenuation, scattering, and dispersion of the emitted light when it passes through tissues [136,149]. With all imaging techniques, the animals have to be restrained or brought under anesthesia to prevent blurred images, which causes discomfort to the animal [150]. 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...
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of the abdomen [115,136,145,146,148,137–144]. 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 [115,137,140,141,148,151]. 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. dorso-ventral) is often not given and the printed image quality and size are sometimes not optimal [115,136–144], which complicates the interpretation of these images by the readers. Furthermore, image exposure and animal positioning could be improved, which would simplify the interpretation of the images [115,136–138,140–143,145,146]. 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 ileo-colonic 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 [115,136,141,145]. 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 proof for ileo-colonic drug delivery in animal studies (table 2) [71,72,76,85,86,114]. However, plasma concentrations generally do not provide enough information to confirm ileo-colonic 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 [71,72,85,114,115]. Kennedy et al. developed a method to measure the colonic arrival time, also called mouth-to-cecal transit time, in humans, using sulfasalazine [152], which has been validated by others [153]. 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 [154,155]. Several researchers have used this method.
to circumvent interindividual variation in colon arrival times [76,156]. It is also possible to circumvent the intra-individual variation in colon arrival time by combining sulfasalazine with a release marker (e.g. theophylline) in the drug delivery system [157] [1]. 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 sulfasalazine-theophylline method. Furthermore, the pharmacokinetics and/or pharmacodynamics of 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 [152,158]. 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 [159,160]. 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, [1,46,161,162]. This principle could also be used in laboratory animals. The collection of urine in animals is possible by using a metabolic cage or by catheterization [163]. 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 [163,164]. The latter stressor can be prevented by using hydrophobic sand for urine collection [165], but individual housing would remain an issue. In addition, catheterization is problematic because, amongst other issues, the catheter can be removed by the animal, and inserting the catheter is a stressor on its own [166]. The collection of breath samples is also problematic because of stress caused by handling or individual housing in a breath-test system [163,167]. 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.
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THERAPEUTIC EFFECT

The last method, abundantly used to evaluate ileo-colonic drug delivery systems in laboratory animals, is to determine a therapeutic effect (table 2) [78,81,83,99,105]. However, the therapeutic effect as such does not directly answer the question whether or not the drug is actually targeted to, and released into, the ileo-colonic region. Therapeutic proteins or peptides given orally in an ileo-colonic 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 [168]. In other cases, a therapeutic effect should not be used as such to verify ileo-colonic drug targeting, but it 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 ileo-colonic delivery and/or for pH-dependent ileo-colonic drug delivery systems containing novel excipients, animals have to be used. To obtain ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 ileo-colonic 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 sulfasalazine-theophylline 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.
CHAPTER 3

REFERENCES

[1] Broesder, A.; Woerdenbag, H.J.; Prins, G.H.; Nguyen, D.N.; Frijlink, H.W.; Hinrichs, W.L. J. pH-dependent ileocolonic drug delivery, part I: in vitro and clinical evaluation of novel systems. Drug Discov. Today 2020, 25, 1362–1373, doi:10.1016/j. druds.2020.06.011.

[2] Huang, W.; Fercie du Sert, N.; Vollert, J.; Rice, A.S.C. Good research practice in non-clinical pharmacology and biomedicine. In Handbook of Experimental Pharmacology, Springer: Cham, 2019 ISBN 9783030336554.

[3] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Safety Guidelines Available online: https://www.ich.org/page/safety-guidelines (accessed on May 2, 2020).

[4] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline S7A Safety pharmacology studies for human pharmaceuticals Available online: https://database.ich.org/sites/default/files/S7A_Guideline.pdf (accessed on May 2, 2020).

[5] Hatton, G.B.; Yadav, V.; Basit, A.W.; Merchant, H.A. Animal farm: Considerations in animal gastrointestinal physiology and relevance to drug delivery in humans. J. Pharm. Sci. 2015, 104, 2747–2776, doi:10.1002/jps.24365.

[6] Sjögren, E.; Abrahamsson, B.; Augustijns, P.; Becker, D.; Bolger, M.B.; Breuwer, M.; Brouwers, J.; Flanagan, T.; Harwood, M.; Heinen, C.; et al. 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. 2014, 57, 99–151, doi:10.1016/j.ejps.2014.02.010.

[7] Arndt, M.; Chokshi, H.; Tang, K.; Parrott, N.J.; Reppas, C.; Dressman, J.B. Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state. Eur. J. Pharm. Biopharm. 2013, 84, 633–641, doi:10.1016/j.ejpb.2013.01.010.

[8] Akiyama, M.; Nagahata, N.; Furuya, A.; Fukushima, K.; Higuchi, S.; Suwa, T. Gastric pH profiles of beagle dogs and their use as an alternative to human testing. Eur. J. Pharm. Biopharm. 2000, 49, 99–102, doi:10.1016/S0939-6411(99)00070-3.

[9] Lui, C.Y.; Amidon, G.L.; Berardi, R.R.; Fleisher, D.; Youngberg, C.; Dressman, J.B. 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. 1986, 75, 271–274, doi:10.1002/jps.2600750313.

[10] Sagawa, K.; Li, F.; Liese, R.; Sutton, S.C. Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. J. Pharm. Sci. 2009, 98, 2494–2500, doi:10.1016/j.pis.21602.

[11] Mahar, K.M.; Portelli, S.; Coatney, R.; Chen, E.P. Gastric pH and gastric residence time in fasted and fed conscious Beagle dogs using the Bravo® pH system. J. Pharm. Sci. 2012, 101, 2439–2448, doi:10.1002/jps.23159.

[12] Steinberg, W.H.; Mina, F.A.; Pick, P.G.; Frey, G.H. Heidelberg capsule I. In vitro evaluation of a new instrument for measuring intragastric pH. J. Pharm. Sci. 1995, 84, 772–776, doi:10.1002/jps.2600540522.

[13] Dickman, R.; Fass, R. Ambulatory esophageal pH monitoring: New directions. Dig. Dis. 2006, 24, 313–318, doi:10.1159/000092885.

[14] Chotiprasiddhi, P.; Liu, J.; Carpenter, S.; Chuttani, R.; DiSario, J.; Hussain, N.; Somogyi, L.; Petersen, B.T. ASGE Technology Status Evaluation Report: wireless esophageal pH monitoring system. Gastrointest. Endosc. 2005, 62, 485–487, doi:10.1016/j.gie.2005.07.007.

[15] Becker, D.; Zhang, J.; Heimbach, T.; Penland, R.C.; Wanke, C.; Shimizu, J.; Kulmatycki, K. Novel orally swallowable IntelliCap® device to quantify regional drug absorption in human Gl tract using diltiazem as model drug. AAPS PharmSciTech 2014, 15, 1490–1497, doi:10.1208/s12249-014-0172-1.

[16] Smith, H.W. Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Pathol. Bacteriol. 1965, 89, 95–122, doi:10.1002/path.1700890112.

[17] Smith, H.W.; Jones, J.E.T. Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. J. Pathol.
PRE-CLINICAL EVALUATION

Bacteriol. 1963, 86, 387–412, doi:10.1002/path.1700860214.

[18] Merchant, H.A.; Alfonso-Pereira, F.; Rabbie, S.C.; Yousef, S.A.; Basit, A.W. Gastrointestinal characterisation and drug solubility determination in animals. J. Pharm. Pharmacol. 2015, 67, 630–639, doi:10.1111/jpp.12361.

[19] Gupta, P.K.; Robinson, J.R. Gastric emptying of liquids in the fasted dog. Int. J. Pharm. 1988, 43, 45–52, doi:10.1016/0378-5173(88)90057-9.

[20] Kalantzi, L.; Persson, E.; Polentarutti, B.; Abrahamsson, B.; Goumas, K.; Dressman, J.B.; Reppas, C. Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. Pharm. Res. 2006, 23, 1373–1381, doi:10.1007/s11095-006-0207-8.

[21] Grayzel, D.M.; Miller, E.G. The pH of the contents of the gastrointestinal tract in dogs, in relation to diet and rickets. J. Biol. Chem. 1928, 76, 423–436.

[22] Kohl, K.D.; Stengel, A.; Samuni-Blank, M.; Dearing, M.D. Effects of anatomy and diet on gastrointestinal pH in rodents. J. Exp. Zool. Part A Ecol. Genet. Physiol. 2013, 319, 225–229, doi:10.1002/jez.1786.

[23] McConnell, E.L.; Basit, A.W.; Murdan, S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J. Pharm. Pharmacol. 2008, 60, 63–70, doi:10.1211/jpp.60.1.0008.

[24] Cummings, J.H.; Macfarlane, G.T. The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. 1991, 70, 443–459, doi:10.1111/j.1365-2672.1991.tb02793.x.

[25] Merchant, H.A.; McConnell, E.L.; Liu, F.; Kamaswamy, C.; Kulkarni, R.P.; Basit, A.W.; Murdan, S. 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. 2011, 42, 3–10, doi:10.1016/j.ejps.2010.09.019.

[26] Ward, F.W.; Coates, M.E.; Walker, R. Nitrate reduction, gastro-intestinal pH and N-nitrosation in gnotobiotic and conventional rats. Food Chem. Toxicol. 1986, 24, 17–22, doi:10.1016/0278-6915(86)90258-9.

[27] Haiba, M.H. The pH of the alimentary tract in normal and Giardia-infected culture mice. Parasitology 1954, 44, 387–391, doi:10.1017/S0031182000019041.

[28] Haiba, M.H.; Williamson, J. The pH of the small intestine of normal, starved and Giardia-infected norway rats. Trans. R. Soc. Trop. Med. Hyg. 1952, 46, 85–93, doi:10.1016/0035-9203(52)90010-2.

[29] Ward, F.W.; Coates, M.E. Gastrointestinal pH measurement in rats: influence of the microbial flora, diet and fasting. Lab. Anim. 1987, 21, 216–222, doi:10.1258/002367787781268693.

[30] Eaintrakarn, S.; Itoh, Y.; Kishimoto, J.; Yoshikawa, Y.; Shibata, N.; Takada, K. Retention and transit of intestinal mucoadhesive films in rat small intestine. Int. J. Pharm. 2001, 224, 61–67, doi:10.1016/S0378-5173(01)00738-4.

[31] Mori, C.; Kondo, H. Effect of gastric acidity regulation on the gastrointestinal transit time and secretion of gastric fluids in beagle dogs. J. Drug Deliv. Sci. Technol. 2006, 16, 467–472, doi:10.1016/S1773-2247(06)50089-9.

[32] Yamada, I.; Haga, K. Measurement of gastric pH during digestion of a solid meal in dogs. Chem. Pharm. Bull. (Tokyo). 1990, 38, 1755–1756, doi:10.1248/cpb.38.1755.

[33] Chen, E.P.; Mahar Doan, K.M.; Portelli, S.; Coatney, R.; Vaden, V.; Shi, W. Gastric pH and gastric residence time in fasted and fed conscious cynomolgus monkeys using the Bravo® pH system. Pharm. Res. 2008, 25, 123–134, doi:10.1007/s11095-007-9358-5.

[34] Gidenne, T.; Lebas, F. Feeding behaviour in rabbits. In Feeding in domestic vertebrates: from structure to behaviour; CAB: Wallingford, 2006; pp. 179–194 ISBN 1845930630.

[35] Fallingborg, J.; Pedersen, P.; Jacobsen, B.A. Small intestinal transit time and intraluminal pH in ileocolic resected patients with Crohn’s disease. Dig. Dis. Sci. 1998, 43, 702–705, doi:10.1023/A:1018893409596.

[36] Ibekwe, V.C.; Fadda, H.M.; McConnell, E.L.; Khela, M.K.; Evans, D.F.; Basit, A.W. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic
CHAPTER 3

release systems. Pharm. Res. 2008, 25, 1828–1835, doi:10.1007/s11095-008-9580-9.

[37] Ovesen, L.; Bendtsen, F.; Tage-Jensen, U.; Pedersen, N.T.; Gram, B.R.; Rune, S.J. Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. Gastroenterology 1996, 90, 958–962, doi:10.1016/0016-5085(86)90873-5.

[38] Press, A.G.; Hauptmann, I.A.; Hauptmann, L.; Fuchs, B.; Fuchs, M.; Ewe, K.; Ramadori, G. Gastrointestinal pH profiles in patients with inflammatory bowel disease. Aliment. Pharmacol. Ther. 1998, 12, 673–678, doi:10.1046/j.1365-2036.1998.00358.x.

[39] Koziolek, M.; Grimm, M.; Becker, D.; Jordov, V.; Zou, H.; Shimizu, J.; Wanke, C.; Garbacz, G.; Weitschies, W. Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the IntelliCap® system. J. Pharm. Sci. 2015, 104, 2855–2863, doi:10.1002/jps.24274.

[40] Dressman, J.B.; Berardi, R.R.; Dermentzoglou, L.C.; Russell, T.L.; Schmaltz, S.P.; Barnett, J.L.; Jarvenpaa, K.M. Upper gastrointestinal (GI) pH in young, healthy men and women. Pharm. Res. 1990, 7, 756–761, doi:10.1007/BF01535781.

[41] Kalantzis, L.; Goumas, K.; Kalioras, V.; Abrahamsson, B.; Dressman, J.B.; Reppas, C. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. Pharm. Res. 2006, 23, 165–176, doi:10.1007/s11095-005-8476-1.

[42] Psachoulas, D.; Vertzoni, M.; Goumas, K.; Kalioras, V.; Beato, S.; Butler, J.; Reppas, C. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. Pharm. Res. 2011, 28, 3145–3158, doi:10.1007/s11095-011-0560-6.

[43] Schneider, F.; Grimm, M.; Koziolek, M.; Modeß, C.; Dokter, A.; Roustrom, T.; Siegmund, W.; Weitschies, W. Resolving the physiological conditions in bioavailability and bioequivalence studies: Comparison of fasted and fed state. Eur. J. Pharm. Biopharm. 2016, 108, 214–219, doi:10.1016/j.ejpb.2016.09.009.

[44] Koziolek, M.; Schneider, F.; Grimm, M.; Modeß, C.; Seekamp, A.; Roustrom, T.; Siegmund, W.; Weitschies, W. Intragastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. J. Control. Release 2015, 220, 71–78, doi:10.1016/j.jconrel.2015.10.022.

[45] Ewe, K.; Schwartz, S.; Petersen, S.; Press, A.G. Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. Dig. Dis. Sci. 1999, 44, 1434–1439, doi:10.1023/A:1026664105112.

[46] Maurer, J.M.; Schellekens, R.C.A.; van Rieke, H.M.; Wanke, C.; Jordov, V.; Stellaard, F.; Wutzke, K.D.; Dijkstra, G.; van der Zee, M.; Woerdenbag, H.J.; et al. Gastrointestinal pH and Transit Time Profiling in Healthy Volunteers Using the IntelliCap System Confirms Ileo-Colonic Release of ColoPulse Tablets. PLoS One 2015, 10, e0129076, doi:10.1371/journal.pone.0129076.

[47] Maqbool, S.; Parkman, H.P.; Friedenberg, F.K. Wireless capsule motility: Comparison of the SmartPill® GI monitoring system with scintigraphy for measuring whole gut transit. Dig. Dis. Sci. 2009, 54, 2167–2174, doi:10.1007/s10620-009-0899-9.

[48] Hila, A.; Bouali, H.; Xue, S.; Knuff, D.; Castell, D.O. Postprandial stomach contents have multiple acid layers. J. Clin. Gastroenterol. 2006, 40, 612–617, doi:10.1097/00004836-200608000-00010.

[49] Pedersen, P.B.; Vilman, P.; Bar-Shalom, D.; Müllertz, A.; Baldursdottir, S. Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities. Eur. J. Pharm. Biopharm. 2013, 85, 958–965, doi:10.1016/j.ejpb.2013.05.007.

[50] Savarino, V.; Mela, G.S.; Scalabrin, P.; Sumberaz, A.; Fera, G.; Colle, G. 24-hour study of intragastric acidity in duodenal ulcer patients and normal subjects using continuous intraluminal pH-metry. Dig. Dis. Sci. 1988, 33, 1077–1080, doi:10.1007/BF01535781.

[51] Pounder, R.E.; Williams, J.G.; Milton-Thompson, G.J.; Misiewicz, J.J. Effect of cimetidine on 24-hour intragastric acidity in normal subjects. Gut 1976, 17, 133–138, doi:10.1136/gut.17.2.133.

[52] Fimml, C.J.; Etienne, A.; Cilluffo, T.; von Ritter, C.; Gasser, T.; Rey, J.-P.; Caradonna-Moscatelli, P.; Sabbatini, F.; Pace, F.; Bühler, H.W.; et al. Long-term ambulatory gastric pH monitoring: Validation of a new method and effect of H2-antagonists.
PRE-CLINICAL EVALUATION

Gastroenterology 1985, 88, 1842–1851, doi:10.1016/0016-5085(85)90009-5.

[53] Mojaverian, P.; Vlasses, P.H.; Killner, P.E.; Rocci, M.L. Effects of gender, posture, and age on gastric residence time of an indigestible solid: pharmaceutical considerations. Pharm. Res. 1988, 5, 639–644.

[54] Simonian, H.P.; Vo, L.; Doma, S.; Fisher, R.S.; Parkman, H.P. Regional postprandial differences in pH within the stomach and gastroesophageal junction. Dig. Dis. Sci. 2005, 50, 2276–2285, doi:10.1017/s10620-005-0348-0.

[55] Vo, L.; Simonian, H.P.; Doma, S.; Fisher, R.S.; Parkman, H.P. 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. 2005, 21, 1321–1330, doi:10.1111/j.1365-2036.2005.02489.x.

[56] Falllingborg, J.; Christensen, L.A.; Ingeman-Nielsen, M.; Jacobsen, B.A.; Aibildgaard, K.; Rasmussen, H.H. pH-Profile and regional transit times of the normal gut measured by a radiotelemetry device. Aliment. Pharmacol. Ther. 1989, 3, 605–614, doi:10.1111/j.1365-2036.1989.tb00254.x.

[57] Bown, R.L.; Gibson, J.A.; Sladen, G.E.; Hicks, B.; Dawson, A.M. Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. Gut 1974, 15, 999–1004, doi:10.1136/gut.15.12.999.

[58] Pye, G.; Evans, D.F.; Ledingham, S.; Hardcastle, J.D. Gastrointestinal intraluminal pH in normal subjects and those with colorectal adenoma or carcinoma. Gut 1990, 31, 1355–1357, doi:10.1136/gut.31.12.1355.

[59] Perez de la Cruz Moreno, M.; Oth, M.; Deferme, S.; Lammert, F.; Tack, J.; Dressman, J.; Augustijns, P. Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. J. Pharm. Pharmacol. 2006, 58, 1019–1029, doi:10.1211/jpp.58.8.1009.

[60] Pozez, S.; Deferme, S.; Tack, J.; Augustijns, P. Gastrointestinal transit of a solid indigestible capsule as measured by radiotelemetry and dual gamma scintigraphy. Pharm. Res. 1989, 6, 719–724.

[61] Clarysse, S.; Tack, J.; Lammert, F.; Duchateau, G.; Reppas, C.; Augustijns, P. Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. J. Pharm. Sci. 2009, 98, 1177–1192, doi:10.1002/jps.21502.

Bratten, J.; Jones, M.P. Prolonged recording of duodenal acid exposure in patients with functional dyspepsia and controls using a radiotelemetry pH monitoring system. J. Clin. Gastroenterol. 2009, 43, 527–533, doi:10.1097/MCG.0b013e3181837ab.

[62] Annaert, P.; Brouwers, J.; Bijnen, A.; Lammert, F.; Tack, J.; Augustijns, P. 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. 2010, 39, 15–22, doi:10.1016/j.ejps.2009.10.005.

Brouwers, J.; Tack, J.; Lammert, F.; Augustijns, P. Intraluminal drug and formulation behavior and integration in in vitro permeability estimation: A case study with amprenavir. J. Pharm. Sci. 2006, 95, 372–383, doi:10.1002/jps.20353.

[63] Riethorst, D.; Mols, R.; Duchateau, G.; Tack, J.; Brouwers, J.; Augustijns, P. Characterization of human duodenal fluids in fasted and fed state conditions. J. Pharm. Sci. 2016, 105, 673–681, doi:10.1010/j.2460-3.

Evans, D.F.; Pye, G.; Bramley, R.; Clark, A.G.; Dyson, T.J.; Hardcastle, J.D. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 1998, 29, 1035–1041, doi:10.1136/gut.29.8.1035.

[64] Mikolajczyk, A.E.; Watson, S.; Surma, B.L.; Rubin, D.T. Assessment of tandem measurements of pH and total gut transit time in healthy volunteers. Clin. Transl. Gastroenterol. 2015, 6, e10, doi:10.1038/cgt.2015.22.

[65] Zarate, N.; Mohammed, S.D.; O'Shaughnessy, E.; Newell, M.; Yazaki, E.; Williams, N.S.; Lunniss, P.J.; Semler, J.R.; Scott, S.M. Accurate localization of a fall in pH within the ileocecal region: validation using a dual-scintigraphic technique. Am. J. Physiol. Liver Physiol. 2010, 299, G1276–G1286, doi:10.1152/ajpgi.00127.2010.

Farmer, A.D.; Mohammed, S.D.; Dukes, G.E.; Scott, S.M.; Hobson, A.R. Caecal pH is a biomarker of excessive colonic fermentation. World J. Gastroenterol. 2014, 20, 5000–7, doi:10.3748/wjg.v20.i17.5000.

[66] Polentarutti, B.; Albery, T.; Dressman, J.; Abrahamsson, B. Modification of gastric pH
CHAPTER 3

in the fasted dog. J. Pharm. Pharmacol. 2010, 62, 462–469, doi:10.1211/jpp.62.04.0008.
[71] Takaya, T.; Niwa, K.; Muraoka, M.; Ogita, I.; Nagai, N.; Yano, R-I.; Kimura, G.; Yoshikawa, Y.; Yoshikawa, H.; Takada, K. Importance of dissolution process on systemic availability of drugs delivered by colon delivery system. J. Control. Release 1998, 50, 111–122, doi:10.1016/S0168-3659(97)00123-5.
[72] Gao, C.; Huang, J.; Jiao, Y.; Shan, L.; Liu, Y.; Li, Y.; Mei, X. In vitro release and in vivo absorption in beagle dogs of meloxicam formulation of Eudragit® RS 100 D-coated pellets. Int. J. Pharm. 2006, 322, 104–112, doi:10.1016/j.ijpharm.2006.05.035.
[73] Hirayama, M.; Toda, R.; Ozaki, T.; Hasegawa, J.; Nakamura, T.; Naraki, Y.; Haraguchi, Y.; Hori, Y.; Tanaka, T.; Takei, M.; et al. Concentration dependence of 5-aminosalicylic acid pharmacological actions in intestinal mucosa after oral administration of a pH-dependent formulation. Mol. Pharm. 2011, 8, 1083–1089, doi:10.1021/mp100088z.
[74] Hubert, S.; Chadwick, A.; Wacher, V.; Coughlin, O.; Kokai-Kun, J.; Bristol, A. Development of a modified-release formulation of lovastatin targeted to intestinal methanogens implicated in irritable bowel syndrome with constipation. J. Pharm. Sci. 2018, 107, 662–671, doi:10.1016/j.xphs.2017.09.028.
[75] Geary, R.S.; Schlameus, W.H. Vancomycin and insulin used as models for oral delivery of peptides. J. Control. Release 1993, 23, 65–74, doi:10.1016/0168-3659(93)90071-C.
[76] Hu, Z.; Shimokawa, T.; Ohno, T.; Kimura, G.; Mawatari, S.-S.; Kamitsuwa, M.; Yoshikawa, Y.; Masuda, S.; Takada, K. Characterization of norfloxacin release from tablet coated with a new pH-sensitive polymer. P-413SF. J. Drug Target. 1999, 7, 223–232, doi:10.3109/107175499085505.
[77] Duan, H.; Lü, S.; Gao, C.; Bai, X.; Qin, H.; Wei, Y.; Wu, X.; Liu, M. Mucoadhesive microparticles based on polysaccharide for target dual drug delivery of 5-aminosalicyclic acid and curcumin to inflamed colon. Colloids Surfaces B Biointerfaces 2016, 145, 510–519, doi:10.1016/j.colsurfb.2016.05.038.
[78] Duan, H.; Lü, S.; Qin, H.; Gao, C.; Bai, X.; Wei, Y.; Wu, X.; Liu, M.; Zhang, X.; Liu, Z. Co-delivery of zinc and 5-aminosalicyclic acid from alginate/ N-succiynl-chitosan blend microspheres for synergistic therapy of colitis. Int. J. Pharm. 2017, 516, 214–224, doi:10.1016/j.ijpharm.2016.11.036.
[79] Arya, A.; Majumdar, D.K.; Pathak, D.P.; Sharma, A.K.; Ray, A.R. Design and evaluation of acrylate polymeric carriers for fabrication of pH-sensitive microparticles. Drug Dev. Ind. Pharm. 2017, 43, 305–318, doi:10.1080/03639045.2016.1239629.
[80] Gugulotu, D.; Kulkarni, A.; Patrawale, V.; Dandekar, P. pH-sensitive nanoparticles of curcumin–celecoxib combination: Evaluating drug synergy in ulcerative colitis model. J. Pharm. Sci. 2014, 103, 687–696, doi:10.1016/j.jps.2013.09.012.
[81] Abdul Ghaffar, A.M.; Radwan, R.R.; Ali, H.E. Radiation synthesis of poly(starch/ acrylic acid) pH sensitive hydrogel for rutin controlled release. Int. J. Biol. Macromol. 2016, 92, 957–964, doi:10.1016/j.ijbiomac.2016.07.079.
[82] Lamprech, A.; Yamamoto, H.; Takeuchi, H.; Kawashima, Y. A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles. J. Control. Release 2005, 104, 337–346, doi:10.1016/j.jconrel.2005.02.011.
[83] Lamprech, A.; Yamamoto, H.; Ubrich, N.; Takeuchi, H.; Mainent, P.; Kawashima, Y. FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression. Pharm. Res. 2005, 22, 193–199, doi:10.1007/s11095-004-1186-2.
[84] Sanka, K.; Pragada, R.R.; Veerareddy, P.R. A pH-triggered delayed-release chromotherapeutic drug delivery system of aceclofenac for effective management of early morning symptoms of rheumatoid arthritis. J. Microencapsul. 2015, 32, 794–803, doi:10.3109/02652048.2015.1081417.
[85] Lamprech, A.; Yamamoto, H.; Takeuchi, H.; Kawashima, Y. pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. J. Control. Release 2004, 98, 1–9, doi:10.1016/j.jconrel.2004.02.001.
[86] Dong, K.; Dong, Y.; You, C.; Xu, W.; Huang, X.; Yan, Y.; Zhang, L.; Wang, K.; Xing, J. Assessment of the drug loading, in vitro and in vivo release behavior of novel pH-sensitive hydrogel. Drug Deliv. 2016, 23, 174–184, doi:10.3109/10717544.2014.908329.
[87] Toutou, E.; Rubinstein, A. Targeted enteral delivery of insulin to rats. Int. J. Pharm. 1986,
PRE-CLINICAL EVALUATION

[97] Kendall, R.A.; Alhnan, M.A.; Nilkumhang, S.; Murdan, S.; Basit, A.W. Fabrication and in vivo evaluation of highly pH-responsive acrylic microparticles for targeted gastrointestinal delivery. Eur. J. Pharm. Sci. 2009, 37, 284–290, doi:10.1016/j.ejps.2009.02.015.

[98] Pandey, S.; Swamy, S.M.V.; Gupta, A.; Koli, A.; Patel, S.; Maulvi, F.; Vyas, B. Multiple response optimisation of processing and formulation parameters of pH sensitive sustained release pellets of capcetibine for targeting colon. J. Microencapsul. 2018, 35, 259–271, doi:10.1080/02652048.2018.14651 38.

[99] Desai, N.; Momin, M. Colon targeted bioadhesive pellets of curcumin and cyclosporine for improved management of inflammatory bowel disease. Drug Deliv. Transl. Res. 2020, 10, 893–900, doi:10.1007/s13346-020-00756-x.

[100] Ali, A.S.; Altayari, A.A.; Khan, L.M.; Alharthi, S.; Ahmed, O.A.; El-Shitany, N.A.; Ali, S.S.; Saadah, O.I. Colon-targeted therapy of tacrolimus (FK506) in the treatment of experimentally induced colitis. Pharmacology 2020, 1–9, doi:10.1159/000505101.

[101] Kim, H.Y.; Cheon, J.H.; Lee, S.H.; Min, J.Y.; Back, S.-Y.; Song, J.G.; Kim, D.H.; Lim, S.-J.; Han, H.-K. 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. Nanobiotechnology 2020, 18, 17, doi:10.1186/s12951-020-0579-7.

[102] Lo, Y.-L.; Hsu, C.-Y.; Lin, H.-R. pH-and thermo-sensitive pluronic/poly(acrylic acid) in situ hydrogels for sustained release of an anticancer drug. J. Drug Target. 2013, 21, 54–66, doi:10.3109/106118 6X.2012.725406.

[103] Dong, K.; Dong, Y.; You, C.; Xu, W.; Huang, X.; Yan, Y.; Zhang, L.; Wang, K.; Xing. J. Assessment of the safety, targeting, and distribution characteristics of a novel pH-sensitive hydrogel. Colloids Surfaces B Biointerfaces 2014, 123, 965–973, doi:10.1016/j.colsurfb.2014.10.049.

[104] Kankala, R.K.; Kuthati, Y.; Sie, H.-W.; Shih, H.-Y.; Lue, S.-I.; Kankala, S.; Jeng, C.-C.; Deng, J.-P.; Weng, C.-F.; Liu, C.-L.; et al. Multi-laminated metal hydroxide nanocontainers for oral-specific delivery of colitis rat model. Eur. J. Pharm. Biopharm. 2009, 72, 1–8, doi:10.1016/j.ejpb.2008.12.013.
CHAPTER 3

for bioavailability improvement and treatment of inflammatory paw edema in mice. J. Colloid Interface Sci. 2015, 458, 217–228, doi:10.1016/j.jcis.2015.07.044.

[105] Bai, X.Y.; Yan, Y.; Wang, L.; Zhao, L.G.; Wang, K. Novel pH-sensitive hydrogel for 5-aminosalicylic acid colon targeting delivery: in vivo study with ulcerative colitis targetting therapy in mice. Drug Deliv. 2016, 23, 1926–1932, doi:10.3109/10717544.2014.996924.

[106] Xiao, B.; Si, X.; Zhang, M.; Merlin, D. Oral administration of pH-sensitive curcumin-loaded nanoparticles for ulcerative colitis therapy. Colloids Surfaces B Biointerfaces 2015, 135, 379–385, doi:10.1016/j.colsurfb.2015.07.081.

[107] Meissner, Y.; Pellequer, Y.; Lamprecht, A. Nanoparticles in inflammatory bowel disease: Particle targeting versus pH-sensitive delivery. Int. J. Pharmac. 2006, 316, 138–143, doi:10.1016/j.ijpharm.2006.01.032.

[108] Belouqui, A.; Coco, R.; Memvanga, P.B.; Ucakar, B.; des Rieux, A.; Préat, V. pH-sensitive nanoparticles for colonic delivery of curcumin in inflammatory bowel disease: Particle targeting versus pH-sensitive delivery. Int. J. Pharmac. 2014, 473, 203–212, doi:10.1016/j.ijpharm.2014.07.009.

[109] Zhu, Q.; Talton, J.; Zhang, G.; Cunningham, T.; Wang, Z.; Waters, R.C.; Kirk, J.; Eppler, B.; Kliman, D.M.; Sui, Y.; et al. Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitourinary viral infection. Nat. Med. 2012, 18, 1291–1296, doi:10.1038/nm.2866.

[110] Naeem, M.; Bae, J.; A. Oshi, M.; Kim, M.-S.; Moon, H.R.; Lee, B.L.; Im, E.; Jung, Y.; Yoo, J.W. Colon-targeted delivery of cyclosporin A using dual-functional Eudragit® FS30D/PLGA nanoparticles ameliorates murine experimental colitis. Int. J. Nanomedicine 2018, 13, 1225–1240, doi:10.2147/IJN.S157566.

[111] Naeem, M.; Oshi, M.A.; Kim, J.; Lee, J.; Cao, J.; Nuthasiri, H.; Im, E.; Jung, Y.; Yoo, J.W. pH-triggered surface charge-reversal nanoparticles alleviate experimental murine colitis via selective accumulation in inflamed colon regions. Nanomaterials Nanotechnol. Biol. Med. 2018, 14, 823–834, doi:10.1016/j.nano.2018.01.003.

[112] Kesharwani, S.S.; Ahmad, R.; Bakkari, M.A.; Rajput, M.K.S.; Dachinieni, R.; Valiveti, C.K.; Kapur, S.; Jayarama Bhat, G.; Singh, A.B.; Tummala, H. Site-directed non-covalent polymer-drug complexes for inflammatory bowel disease (IBD): Formulation development, characterization and pharmacological evaluation. J. Control. Release 2018, 230, 165–179, doi:10.1016/j.jconrel.2018.08.004.

[113] Pellequer, Y.; Meissner, Y.; Ubrich, N.; Lamprecht, A. Epithelial heparin delivery via microspheres mitigates experimental colitis in mice. J. Pharmacol. Exp. Ther. 2007, 321, 726–733, doi:10.1124/jpet.106.117226.

[114] Shah, N.; Sharma, O.P.; Mehta, T.; Amin, A. Design of experiment approach for formulating multi-unit colon-targeted drug delivery system: in vitro and in vivo studies. Drug Dev. Ind. Pharm. 2016, 42, 825–835, doi:10.3109/03639045.2015.1082581.

[115] Patel, M.M.; Amin, A.F. Design and optimization of colon-targeted system of theophylline for chronotherapy of nocturnal asthma. J. Pharm. Sci. 2011, 100, 1760–1772, doi:10.1002/jps.22406.

[116] Agostino, D.; Seal, S.H.; Nickson, J.J. Capsule implantation: Method for establishing simulated colon carcinoma in rats. Proc. Soc. Exp. Biol. Med. 1959, 100, 717–718, doi:10.3181/00379727-100-24754.

[117] Whiteley, H.W. Preoperative radiation therapy in simulated cancer of the colon in rabbits. Dis. Colon Rectum 1967, 10, 100–102, doi:10.1007/BF02617354.

[118] Sugimura, T. Experimental tumors in digestive organs. Gastroent. Jpn. 1976, 11, 265–6.

[119] Ward, J.M. Dose response to a single injection of azoxymethane in rats. Vet. Pathol. 1975, 12, 165–177, doi:10.1177/030098587501200302.

[120] Yang, Q.Q.; Xu, X.P.; Zhao, H.S.; Cai, Y.Q.; Pan, Y.M.; Xu, J.Q.; Ma, Q.X.; Chen, M.L. Differential expression of microRNA related to irritable bowel syndrome in a rabbit model. J. Dig. Dis. 2017, 18, 330–342, doi:10.1111/1751-2980.12485.

[121] Tanaka, T.; Tanaka, A.; Nakamura, A.; Matsushita, K.; Imanishi, A.; Matsumoto-Okano, S.; Inatomi, K.; Miura, K.; Toyoda, M.; Mizojiri, G.; et al. 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. 2012, 120, 15–25, doi:10.1254/jphysiol.1208SFP.

[122] Tanaka, T.; Matsumoto-Okano, S.; Inatomi, N.; Fujioka, Y.; Kamiguchi, H.; Yamaguchi, M.; Imanishi, A.; Kawamoto, M.; Miura, K.; Nishikawa, Y.; et al. Establishment and
validation of a rabbit model for in vivo pharmacodynamic screening of tachykinin NK2 antagonists. J. Pharmacol. Sci. 2012, 118, 487–495, doi:10.1254/jphs.121245FF.

[132] Freetland, D.J.; Widomski, D.; Tsai, B.S.; Zemaitis, J.M.; Levin, S.; Djuric, S.W.; Stone, R.L.; Gagnella, T.S. Effect of the leukotriene B4 receptor antagonist SC-41930 on colonic inflammation in rat, guinea pig and rabbit. J. Pharmacol. Exp. Ther. 1990, 255, 572–6.

[124] Morris, G.P.; Beck, P.L.; Herridge, M.S.; Depew, W.T.; Szewczuk, M.R.; Wallace, J.L. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 1989, 96, 795–803, doi:10.1016/S0016-5085(89)80079-4.

[125] Anthony, D.; Savage, F.; Sams, V.; Boulou, P. The characterization of a rabbit model of inflammatory bowel disease. Int J Exp Pathol 1995, 76, 215–224.

[126] Rabin, B.S. Animal model: immunologic model of inflammatory bowel disease. Am. J. Pathol. 1980, 99, 253–6.

[127] Rabin, B.S.; Rogers, S.J. A cell-mediated immune model of inflammatory bowel disease in the rabbit. Gastroenterology 1978, 75, 29–33, doi:10.1016/0016-5085(78)93759-9.

[128] Sundaram, U.; West, A.B. Effect of chronic inflammation on electrolyte transport in rabbit ileal villus and crypt cells. Am. J. Physiol. Liver Physiol. 1997, 272, G732–G741, doi:10.1152/ajpli.1997.272.4.G732.

[129] Watt, J.; Marcus, R. Ulcerative colitis in rabbits fed degraded carrageenan. J. Pharm. Pharmacol. 1970, 22, 130–131, doi:10.1111/j.1365-2184.1970.tb08406.x.

[130] Hotta, T.; Yoshida, N.; Yoshikawa, T.; Sugino, S.; Kondo, M. Lipopolysaccharide-induced colitis in rabbits. Res. Exp. Med. 1986, 186, 61–69, doi:10.1007/BF01851834.

[131] Cave, D.R.; Kane, S.P.; Mitchell, D.N.; Brooke, B.N. Further animal evidence of a transmissible agent in Crohn’s disease. Lancet (London, England) 1973, 302, 1120–2, doi:10.1016/S0140-6736(73)9036-7.

[132] Hodgson, H.J.F.; Potter, B.J.; Skinner, J.; Jewell, D.P. Immune-complex mediated colitis in rabbits. An experimental model. Gut 1978, 19, 225–232, doi:10.1136/gut.19.3.225.

[133] Shanmugam, M.; Sethupathi, P.; Rhee, K.-J.; Yong, S.; Knight, K.L. Bacterial-induced inflammation in germ-free rabbit appendix. Inflamm. Bowel Dis. 2005, 11, 992–996, doi:10.1097/01.MIB.0000182869.74648.0f.

[134] Leonardi, I.; Nicholls, F.; Atrott, K.; Cee, A.; Teves, B.; Greinwald, R.; Rogler, G.; Frey-Wagner, I. Oral administration of dextran sodium sulphate induces a caecum-localized colitis in rabbits. Int. J. Exp. Pathol. 2015, 96, 151–162, doi:10.1111/iep.12117.

[135] Russell, W.M.S.; Burch, R.L. The principles of humane experimental technique. Methuen & Co. Ltd.: London, 1959.

[136] Hou, L.; Shi, Y.; Jiang, G.; Liu, W.; Han, H.; Feng, Q.; Ren, J.; Yuan, Y.; Wang, Y.; Shi, J.; et al. Smart nanocomposite hydrogels based on azo crosslinked graphene oxide for oral colon-specific drug delivery. Nanotechnology 2016, 27, 315105, doi:10.1088/0957-4484/27/31/315105.

[137] Sharma, P.; Pathak, K. Inulin-based tablet in capsule device for variable multipulse delivery of aceclofenac: Optimization and in vivo roentgenography. AAPS PharmSciTech 2013, 14, 736–747, doi:10.1208/s12249-013-0995-8.

[138] Omar, S.; Aldosari, B.; Refai, H.; Al Gohary, O. Colon-specific drug delivery for mebeverine hydrochloride. J. Drug Target. 2007, 15, 691–700, doi:10.1080/10611860701603281.

[139] Avachat, A.M.; Shinde, A.S. Feasibility studies of concomitant administration of optimized formulation of probiotic-loaded Vancomycin hydrochloride pellets for colon delivery. Drug Dev. Ind. Pharm. 2015, 42, 80–90, doi:10.3109/03639451.2015.10299 39.

[140] Yassin, A.E.B.; Alsarra, I.A.; Alnazi, F.K.; Al-Mohizea, A.M.; Al-Robayan, A.A.; Al-Obeed, O.A. New targeted-colon delivery system: in vitro and in vivo evaluation using X-ray imaging. J. Drug Target. 2010, 18, 59–66, doi:10.1080/10611860903165022.

[141] Patel, M.M.; Amin, A.F. Formulation and development of release modulated colon targeted system of meloxicam for potential application in the prophylaxis of colorectal cancer. Drug Deliv. 2011, 18, 281–293, doi:10.3109/10717544.2011.538447.

[142] Moghimipour, E.; Dorkoosh, F.A.; Rezaei, M.; Kouchak, M.; Fatahiad, J.; Angali, K.A.; Ramezani, Z.; Amini, M.; Handali, S. In vivo evaluation of pH and time-dependent polymers as coating agent for colonic delivery using central composite design.
CHAPTER 3

**[143]** Amrutkar, J.R.; Gattani, S.G. A novel hydrogel plug of Sterculia urens for pulsatile delivery: in vitro and in vivo evaluation. *J. Microcapsul.* **2012**, 29, 72–82, doi:10.3109/02652048.2011.629789.

**[144]** Mastiholimath, V.S.; Dandagi, P.M.; Jain, S.S.; Gadad, A.P.; Kulkarni, A.R. Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma. *Int. J. Pharm.* **2007**, 328, 49–56, doi:10.1016/j.ijpharm.2006.07.045.

**[145]** Patel, M.M. Formulation and development of di-dependent microparticulate system for colon-specific drug delivery. *Drug Deliv.* **2017**, 7, 312–324, doi:10.1007/s13346-017-0358-7.

**[146]** Hong, S.; Yum, S.; Yoo, H.-J.; Kang, S.; Yoon, J.-H.; Min, D.; Kim, Y.M.; Jung, Y. Colon-targeted cell-permeable NfκB inhibitory peptide is orally active against experimental colitis. *Mol. Pharm.* **2012**, 9, 1310–1319, doi:10.1021/mp200391q.

**[147]** Perkins, A.C.; Frier, M. Radionuclide imaging in drug development. *Curr. Pharm. Des.* **2004**, 10, 2907–2921, doi:10.1017/S1381620433834746.

**[148]** Owens, J.M.; Biery, D.N. *Radiographic interpretation for the small animal clinician*; Second.; Williams & Wilkins: Baltimore, 1998; ISBN 0683006846.

**[149]** Kiesling, F.; Pichler, B.J.; Hauff, P. Small intestinal imaging; Kiesling, F., Pichler, B.J., Hauff, P., Eds.; Second.; Springer Nature: Cham, 2017; ISBN 978-3-319-42200-8.

**[150]** University of British Columbia Animal Care Committee UBC ACC. Guideline on imaging of rodents Available online: https://animalcare.ubc.ca/sites/default/files/documents/Guideline - Rodent Imaging %282014%29.pdf (accessed on Feb 27, 2020).

**[151]** Hardy, J.G.; Healey, J.N.C.; Reynolds, J.R. Evaluation of an enteric-coated delayed-release 5-aminosalicylic acid tablet in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **1987**, 1, 273–280, doi:10.1111/j.1365-2036.1987.tb00627.x.

**[152]** Kennedy, M.; Chinwah, P.; Wade, D. A pharmacological method of measuring mouth caecal transit time in man. *Br.

**[153]** Kellow, J.E.; Borody, T.J.; Phillips, S.F.; Haddad, A.C.; Brown, M.L. Sulfapyridine appearance in plasma after salicylazosulfapyridine. *Gastroenterology* **1986**, 91, 396–400, doi:10.1016/0016-5085(86)90574-3.

**[154]** Dahan, A.; Amidon, G.L. 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.* **2009**, 297, G371–G377, doi:10.1152/ajplip.00102.2009.

**[155]** Schröder, H.; Lewkonia, R.M.; Price Evans, D.A. Metabolism of salicylazosulfapyridine in healthy subjects and in patients with ulcerative colitis; Effects of colectomy and of phenobarbital. *Clin. Pharmacol. Ther.* **1973**, 14, 802–809, doi:10.1002/cpt1973145802.

**[156]** Takaya, T.; Ikeda, C.; Imagawa, N.; Niwa, K.; Takada, K. Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rh-CSF) in Beagle dogs. *J. Pharm. Pharmacol.* **1995**, 47, 474–478, doi:10.1111/j.1365-2009.1995.tb05834.x.

**[157]** Ishibashi, T.; Ikegami, K.; Kubo, H.; Kobayashi, M.; Mizobe, M.; Yoshino, H. Evaluation of colonic absorbability of drugs in dogs using a novel colon-targeted delivery capsule (CTDC). *J. Control. Release* **1999**, 59, 361–376, doi:10.1016/S0168-3659(99)00005-X.

**[158]** Khan, A.K.A.; Piris, J.; Truelove, S.C. An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* **1977**, 310, 892–895, doi:10.1016/s0140-6736(77)90831-5.

**[159]** Uchida, M.; Yoshida, K. Non-invasive method for evaluation of gastrocecal transit time by using a breath test in conscious rats. *J. Pharmacol. Sci.* **2009**, 110, 227–230, doi:10.1254/jpsy.090408C.

**[160]** Uchida, M.; Yoshida-Iwasawa, K. Simultaneous measurement of gastric emptying and gastrocecal transit times in conscious rats using a breath test after ingestion of [1-13C] acetic acid and lactose-[13C] ureide. *J. Smooth Muscle Res.* **2012**, 48, 105–114, doi:10.1540/jsmr.48.105.

**[161]** Maurer, M.J.M.; Schellekens, R.C.A.; Wutzke, K.D.; Stellard, F. Isotope-labelled urea to test colon drug delivery devices in vivo: principles, calculations and
interpretations. *Isotopes Environ. Health Stud.* 2013, 49, 473–491, doi:10.1080/10256016.2013.803099.

[162] Maurer, J.M.; Schellekens, R.C.A.; van Rieke, H.M.; Stellaard, F.; Wutzke, K.D.; Busurman, D.J.; Dijkstra, G.; Woordenbag, H.J.; Feijlink, H.W.; Kosterink, J.G.W. 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* 2013, 172, 618–624, doi:10.1016/j.jconrel.2013.09.021.

[163] Principles of laboratory animal science. In: van Zutphen, L.F., Baumans, V., Beynen, A.C., Eds.; Elsevier, 2001; p. 428 ISBN 9780444506122.

[164] Whittaker, A.L.; Lymn, K.A.; Howarth, G.S. Effects of metabolic cage housing on rat behavior and performance in the social interaction test. *J. Appl. Anim. Welf. Sci.* 2016, 19, 363–374, doi:10.1080/10888705.2016.1164048.

[165] Hoffman, J.F.; Fan, A.X.; Neuendorf, E.H.; Vergara, V.B.; Kalinich, J.F. Hydrophobic sand versus metabolic cages: A comparison of urine collection methods for rats (Rattus norvegicus). *J. Am. Assoc. Lab. Anim. Sci.* 2018, 57, 51–57.

[166] Horst, P.-J.; Bauer, M.; Veelken, R.; Unger, T. A new method for collecting urine directly from the ureter in conscious unrestrained rats. *Kidney Blood Press. Res.* 1988, 11, 325–331, doi:10.1159/000173171.

[167] Uchida, M.; Endo, N.; Shimizu, K. Simple and noninvasive breath test using 13C-acetic acid to evaluate gastric emptying in conscious rats and its validation by metoclopramide. *J. Pharmacol. Sci.* 2005, 98, 388–395, doi:10.1254/jps.FP0050153.

[168] Aguirre, T.A.S.; Teijeiro-Osorio, D.; Rosa, M.; Coulter, I.S.; Alonso, M.J.; Brayden, D.J. Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials. *Adv. Drug Deliv. Rev.* 2016, 106, 223–241, doi:10.1016/j.addr.2016.02.004.