



HAL
open science

Colon targeting in rats, dogs and IBD patients with species-independent film coatings

Fabiana Ferraro, Lisa Sonnleitner, Christel Neut, Séverine Mahieux, Jérémy Verin, Juergen Siepmann, Florence Siepmann

► **To cite this version:**

Fabiana Ferraro, Lisa Sonnleitner, Christel Neut, Séverine Mahieux, Jérémy Verin, et al.. Colon targeting in rats, dogs and IBD patients with species-independent film coatings. *International Journal of Pharmaceutics*: X, 2024, *International Journal of Pharmaceutics*: X, 7, pp.100233. 10.1016/j.ijpx.2024.100233 . hal-04504251

HAL Id: hal-04504251

<https://hal.univ-lille.fr/hal-04504251>

Submitted on 14 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Colon targeting in rats, dogs and IBD patients with species-independent film coatings

F. Ferraro^a, L.M. Sonnleitner^a, C. Neut^{b,1}, S. Mahieux^b, J. Verin^a, J. Siepmann^{a,*}, F. Siepmann^a

^a Univ. Lille, Inserm, CHU Lille, U1008, F-59000 Lille, France

^b Univ. Lille, Inserm, CHU Lille, U1286, F-59000 Lille, France

ARTICLE INFO

Keywords:

Colon targeting
Polymeric film coating
Rat
Dog
IBD patient

ABSTRACT

Polysaccharides were identified, which allow for colon targeting in human Inflammatory Bowel Disease (IBD) patients, as well as in rats and dogs (which are frequently used as animals in preclinical studies). The polysaccharides are degraded by colonic enzymes (secreted by bacteria), triggering the onset of drug release at the target site. It has to be pointed out that the microbiota in rats, dogs and humans substantially differ. Thus, the performance of this type of colon targeting system observed in animals might not be predictive for patients. The aim of this study was to limit this risk. Different polysaccharides were exposed to culture medium inoculated with fecal samples from IBD patients, healthy dogs and “IBD rats” (in which colonic inflammation was induced). Dynamic changes in the pH of the culture medium were used as an indicator for the proliferation of the bacteria and, thus, the potential of the polysaccharides to serve as their substrate. Fundamental differences were observed with respect to the extent of the pH variations as well as their species-dependency. The most promising polysaccharides were used to prepare polymeric film coatings surrounding 5-aminosalicylic acid (5-ASA)-loaded starter cores. To limit premature polysaccharide dissolution/swelling in the upper gastro intestinal tract, ethylcellulose was also included in the film coatings. Drug release was monitored upon exposure to culture medium inoculated with fecal samples from IBD patients, healthy dogs and “IBD rats”. For reasons of comparison, also 5-ASA release in pure culture medium was measured. Most film coatings showed highly species-dependent drug release kinetics or limited colon targeting capacity. Interestingly, extracts from aloe vera and reishi (a mushroom) showed a promising potential for colon targeting in *all* species.

1. Introduction

Site specific drug delivery to the colon (“colon targeting”) can offer key advantages for advanced medical therapies (Awad et al., 2022), including: (i) the *local* treatment of diseases of this organ (McCoubrey et al., 2023) and (ii) the possibility to protect protein and peptide drugs against enzymatic degradation & denaturation in the upper gastro intestinal tract (Yadav et al., 2016; Wang et al., 2015a; Wang et al., 2015b; Bak et al., 2018). Combined with strategies enhancing drug transport across the colonic mucosa, this might help paving the way for the oral administration of peptide/protein drugs in the future. At present, only the first type of applications (local treatments of diseases/disorders of the colon) is used in clinical practice. Several drug products are available on the market, e.g. Octasa, Pentasa, Salofalk, Asacol MR, Budenofalk, Lialda/Mezavant (Fadda and Basit, 2005; Goyanes et al., 2015;

Yoshimura et al., 2011; Merchant et al., 2009; Schellekens et al., 2010; Kamm et al., 2007). However, the development of this type of advanced drug delivery systems is cumbersome, and the resulting release kinetics at the target site are difficult to control. This is in part due to the substantial variability in the conditions encountered by the dosage form in the gastro intestinal tract of a patient (Vinarov et al., 2021; Ibekwe et al., 2008a).

Examples for widespread colonic diseases, which can benefit from efficient colon targeting, include ulcerative colitis and Crohn’s disease (Actis et al., 2019). Both are chronic Inflammatory Bowel Diseases (IBDs). In the case of ulcerative colitis, mainly the colon and rectum are affected (Porter et al., 2020; Ordás et al., 2012). In the case of Crohn’s disease, patients generally suffer from lesions in the distal ileum and proximal colon, but any segment of the gastrointestinal tract might be affected (Petagna et al., 2020). The local treatment of these diseases is

* Corresponding author at: University of Lille, College of Pharmacy, INSERM U1008, 3, rue du Professeur Laguesse, 59006 Lille, France.

E-mail address: juergen.siepmann@univ-lille.fr (J. Siepmann).

¹ Deceased.

not straightforward, because most drugs are absorbed into the bloodstream upon oral administration. Thus, the resulting concentrations at the site of action are low and the treatment is a failure, although well-known drugs could potentially provide promising therapeutic efficacy. In addition, the high drug amounts, which are “lost” into the rest of the human body, can cause serious undesired side effects. Providing *site-specific* drug delivery to the colon can overcome these two hurdles: If the drug is not released from the dosage form in the stomach and small intestine, it is not absorbed into the systemic circulation from the upper gastro intestinal tract. Once the system reaches the colon, drug release sets on and allows to provide therapeutic concentrations at the site of action.

Different formulation strategies have been proposed aiming at site-specific drug delivery to the colon (Awad et al., 2022; McCoubrey et al., 2023; Gazzaniga et al., 2006; Amidon et al., 2015). Often, polymeric film coatings surround drug-loaded cores, e.g. tablets or pellets. In most cases, the film coatings are not permeable for the drug in the upper gastro intestinal tract, but become permeable as soon as the colon is reached. Generally, one of the following strategies is used to trigger the onset of drug release at the target site:

- The film coating exhibits *pH-dependent solubility* (Fadda and Basit, 2005; Maroni et al., 2017; Yoshida et al., 2013; Maderuelo et al., 2019): At low pH (in the content of the stomach), the film is insoluble and impermeable, effectively trapping the drug. Once the small intestine is reached, the coating starts to dissolve. Depending on the pH threshold value for the dissolution of the film and the latter's thickness, this process can take more or less time. If desired, this lag-time matches the transit time through the small intestine, so that drug release sets on at the entry of the colon. For certain treatments, drug release is desired to start already in the distal ileum.
- Drug release sets on after a pre-programmed lag time: *time-dependent* drug delivery systems (Gazzaniga et al., 2006; Bussemer et al., 2003). For example, an outer polymeric film coating prevents drug release as long as it is intact. Another, *swellable* polymeric film layer is located directly below the outer layer. Upon contact with aqueous fluids, water penetrates into the system and the swellable layer increases in volume. Thus, a steadily increasing mechanical pressure acts against the outer film coating. At a certain time point, cracks are created in the latter, allowing for drug release. If the lag-time corresponds to the transit time through the stomach & small intestine, drug release sets on in the colon. Also, an outer enteric coating can aim at minimizing water penetration into the system in the stomach, so that the “clock starts” once the small intestine is reached.
- The film coating is degraded by *colonic bacterial enzymes* (Karrout et al., 2010; Karrout et al., 2015; Karrout et al., 2009a): This type of system benefits from the fact that the amounts of bacteria in the stomach and small intestine are low compared to the colon. Bacteria secrete enzymes, which degrade certain substrates, in particular polysaccharides. Film coatings based on such compounds can remain intact and poorly permeable for the drug in the *upper* gastro intestinal tract. However, once the colon is reached, they are enzymatically degraded and become permeable.

In certain drug products, several strategies are combined in order to limit the risk of system failure, if the conditions triggering the onset of release are not provided in the gastro intestinal tract of the patient. For example, it is well known that the pH values in the different segments of the patient's gastro intestinal tract can significantly vary *inter-* and *intra-*individually (Press et al., 1998; Nugent, 2001). If the critical threshold value for the dissolution of a *pH-sensitive* film coating is not reached, the drug remains entrapped and is excreted with the feces. It is also well known that the *types and amounts of bacteria* present in the colon of patients fundamentally vary. If the enzymes required for the degradation of the *bacteria-sensitive* film coatings are not present in sufficient amounts, the coatings remain impermeable and the drug is not released

throughout the entire gastro intestinal tract. To limit the risk of system failure, the Phloral technology combines the *pH-dependent solubility* and the *colonic bacterial enzymes* strategies in one film coating (Dodoo et al., 2017; Ibekwe et al., 2008b; Varum et al., 2020). The enteric polymer Eudragit S is blended with a resistant starch in a single layer. If the conditions are as expected, the enteric polymer starts dissolving once its pH threshold value is reached (pH 7) and the resistant starch is degraded by the bacterial enzymes in the colon. However, if the critical pH value is not reached, the starch can still be degraded and trigger the onset of drug release. And vice-versa: If the required colonic bacteria are not present in sufficient amounts, the dissolution of the Eudragit S can assure the onset of drug release. Thus, the delivery system has a “rescue back-up mechanism” for drug release, if one of the two strategies fails. Furthermore, the group of Andrea Gazzaniga proposed interesting colon targeting systems, based on a combination of different triggering strategies (Moutaharrik et al., 2021). For example, a delivery platform combining all 3 triggering mechanisms (pH-, microbiota- and time control) has been described and characterized *in vitro* and *in vivo* (Moutaharrik et al., 2023).

It has to be pointed out that the development of new drug products allowing for colon targeting is particularly cumbersome, because of the high risk of erroneous decisions in the preclinical phase: System performance is observed in animal models, serving as the basis to predict the outcome of clinical trials. But the conditions in the gastro intestinal tracts of animals are fundamentally different from those in human patients (Hatton et al., 2015; Mahar et al., 2012; Chen et al., 2008; Kararli, 1995). This concerns for instance the transit times through the different segments of the gastro intestinal tract, the variability of the pH of the contents as well as the qualitative & quantitative composition of the microbiota. A drug delivery system, which shows promising results in rats or dogs, can very well fail in patients, because the conditions triggering the onset of drug release are not provided in humans. On the other hand, film coatings which have the potential to reliably release the drug in the colon of IBD patients can fail in rats and dogs, e.g. because the required enzymes are not present in the animals. This leads to erroneous formulation selection and unsuccessful product development.

The aim of this study was to overcome this bottleneck and to identify polymeric film coatings, which are able to provide species-independent colon targeting: not only in IBD patients, but also in frequently used animal models, namely rats and dogs. 5-Aminosalicylic acid (5-ASA) was chosen as drug, being frequently used to treat chronic inflammatory bowel diseases (Desreumaux and Ghosh, 2006; Rousseaux et al., 2005). Seventeen polysaccharides were studied with respect to their capacity to provide enzymatically triggered colon targeting in the different species. In a first screening, the pure compounds were exposed to culture medium inoculated with fecal samples from rats, dogs and patients. Dynamic changes in the pH of the medium were used as an indicator for the proliferation of the bacteria and, thus, the capacity of the polysaccharides to serve as their substrates (Morrison and Preston, 2016). The most promising polysaccharide candidates were used coat 5-ASA-loaded starter cores. Drug release was monitored in culture medium inoculated with fecal slurries from rats, dogs and patients.

2. Materials and methods

2.1. Materials

5-aminosalicylic acid (5-ASA, Alfa Aesar, Kendel, Germany); Bacto™ Tryptone, Bacto™ desiccated Beef extract (Becton, Dickinson and Co., Le Pont de Claix, France); sodium chloride and dibutyl sebacate (DBS) (Acros organics, Geel, Belgium); yeast extract (Oxoid, Dardilly, France); L-cysteine hydrochloride, pectin from citrus, pectin from apple and 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma-Aldrich, Steinheim, Germany); aqueous ethylcellulose dispersion (Aquacoat ECD, FMC Corporation, Philadelphia, USA); sucrose starter cores (Suglets, mesh 30/35, 500–600 μm; Colorcon, Dartford, UK); partially

pregelatinized maize starch (Starch 1500) and hydroxypropyl methylcellulose (HPMC, Methocel K3 premium LV) (Colorcon, Kent, UK); aloe vera extract powder (aqueous extract from the leaves of *Aloe barbadensis* Mill., *Aloaceae*), reishi extract powder (ReISHIELD, aqueous extract from the fruiting body of *Ganoderma lucidum*, *Ganodermataceae*), goji berry extract powder, coix lacryma esculentus extract powder, and abelmoscus esculentus extract powder (Specialty Natural Products Co. Ltd., Chon Buri, Thailand); inulin (Orafti Synergy 1: oligofructose-enriched inulin; Orafti HIS: "standard inulin"; and Orafti HP: "long chain inulin"), isomaltulose (Palatinose PST-N) and rice protein (Remypro N80+) (Beneo-Orafti, Oreye, Belgium); low acyl gellan gum (Special Ingredients, Chesterfield, UK); spray-dried acacia gum and karaya gum powders (Alland & Robert, Saint-Aubin sur Gaillon, France); xylan from corn core (Tokyo Chemical industry, Zwijndrecht, Belgium); starch (Novelose 240; Ingredion, Hamburg, Germany) and carrageenan (Satia) (Ceca, Velizy-villacoublay, France); chitosan (Chitoclear; Primex, Siglufjörður, Iceland); corn maltodextrin (Glucidex 17), cook-up maize starch (Clearam), maltitol (SweetPearl P300 DC) and sodium starch glycolate (Glycolys) (Roquette Freres, Lestrem, France); raffinose [D-(+)-raffinose pentahydrate, Alfa Aesar, Kendel, Germany]; rice starch (Cooper, Melun cedex, France); hydrochloric acid (HCl), sodium hydroxide (NaOH, white pellets) and glacial acetic acid (Fisher Scientific, Loughborough, UK); methanol (Carlo Erba Reagents, Val de Reuil, France).

2.2. Inoculation of polysaccharides in culture medium \pm fecal samples

Culture medium was prepared by dissolving 1.5 g beef extract, 3 g yeast extract, 5 g tryptone, 2.5 g NaCl and 0.3 g L-cysteine hydrochloride in 1 L distilled water under heating (Karrout et al., 2009a). The pH was adjusted to 7.0 ± 0.2 with HCl or NaOH. The culture medium was sterilized in an autoclave at 115°C for 20 min, and stored at 4°C until use. Prior to inoculation with bacteria, the medium was heated to 100°C for 20 min to reduce the amount of oxygen and cooled to room temperature.

Two g polysaccharide were dissolved/dispersed in 100 mL culture medium, followed by sterilization in an autoclave at 115°C for 20 min. Eight mL of the solution/dispersion were inoculated in a glass tube with 2.0 mL fecal slurry (10^6 CFU/mL bacteria) from inflammatory bowel disease patients (Crohn's disease and ulcerative colitis patients), healthy dogs, or inflammatory bowel disease model rats. In the latter rat model, the disease was induced as follows: The animals were anesthetized for 90–120 min using pentobarbital (40 mg/kg) and received an intrarectal administration of TNBS (trinitrobenzene sulfonic acid, 250 μL , 20 mg/rat), dissolved in a 1:1 mixture of an aqueous 0.9% NaCl solution and ethanol (Karrout et al., 2015). The rat feces were collected 1 d after TNBS administration. The study was conducted respecting all governmental guidelines (including n° 2010/63/UE; Decret 2013–118) and ethical rules (APAFIS#201809281814746). The tubes were incubated at 37°C under anaerobic conditions (5% CO_2 , 10% H_2 , 85% N_2). The pH value of the bulk fluid was measured using pH indicator paper (Macherey-Nagel, Duren, Germany): immediately after fecal slurry addition, as well as after 8 and 24 h incubation. All experiments were performed in triplicate. Mean values \pm standard deviations are reported.

2.3. Inoculation of *Bacteroides Vulgatus* and *Bifidobacterium* in culture medium \pm DBS

To evaluate a potential antimicrobial activity of the plasticizer DBS on *Bacteroides Vulgatus* and *Bifidobacterium*, 0.38 mg DBS was added to 10.0 mL bacteria suspensions (1×10^5 CFU/mL) in tubes, which were incubated at 37°C under anaerobic conditions (5% CO_2 , 10% H_2 , 85% N_2). At predetermined time points, 0.1 mL samples were withdrawn and diluted with Cysteinated Ringer solution (CR) to 10 mL. Successive further 1:10 dilutions in CR were made up to $1:10^6$. At each stage of dilution, 0.1 mL samples were seeded onto Colombia Culture (CC)

plates, followed by incubation for 4 d at 37°C . The number of viable bacteria CFU was counted and expressed as log CFU/mL.

2.4. Preparation of drug-loaded starter cores

5-aminosalicylic acid (5-ASA)-loaded starter cores were prepared by layering an aqueous drug-binder dispersion (18.2% w/w 5-ASA, 0.9% w/w HPMC) onto drug-free sucrose starter cores in a fluidized bed coater (Solidlab 1, Bosch, Schopfheim, Germany). The process parameters were as follows: inlet temperature = 50°C , product temperature = $39 \pm 2^\circ\text{C}$, spray rate = 0.3 g/min, atomization pressure = 0.6 bar, air flow rate = 25% ($10 \text{ m}^3/\text{h}$), batch size = 100 g, internal nozzle diameter = 0.8 mm, external nozzle diameter = 1.7 mm. The coating was performed until a drug loading of 6% (w/w) was obtained.

2.5. Film coating of drug-loaded pellets

Three (3.0) g plasticizer (DBS) were dispersed in 40.0 g aqueous ethylcellulose dispersion (Aquacoat ECD). The formulation was stirred at room temperature for 24 h (magnetic stirrer, Heidolph, Schwabach, Germany).

A solution/dispersion of a second polysaccharide (ethylcellulose being the first polysaccharide) was prepared by adding 8.0 g of the compound to water, followed by stirring at room temperature for 3 h. The amount of water was adapted to the type of polysaccharide, assuring an appropriate viscosity of the liquid formulation for spraying: Twenty mL water were used in the case of Orafti Synergy 1, Orafti HSI, Orafti P95, Orafti HP, xylan from corn core, aloe vera extract powder, Novelose 240, Glucidex 17, SweetPearl P300 DC, Palatinose PST-N, Cleargum, rice starch, abelmoscus esculentus extract powder, Clearam and raffinose. Forty mL water were used in the case of reishi extract, goji berry extract powder and coix lacryma extract powder. Sixty mL water were used in the case of Starch 1500 and spray dried acacia gum.

The polysaccharide solution/dispersion was blended with the plasticized aqueous ethylcellulose dispersion. The final ratio of the 2nd polysaccharide: ethylcellulose (dry masses) was 2:3 (w/w). The blends were stirred for 1 h prior to coating. The drug-loaded starter cores were coated in a fluidized bed coater (Solidlab 1). The process parameters were as follows: inlet temperature = 50°C , product temperature = $39 \pm 2^\circ\text{C}$, spray rate = 0.3 g/min, atomization pressure = 0.6 bar, air flow rate = 25% ($10 \text{ m}^3/\text{h}$), batch size = 70 g, internal nozzle diameter = 0.8 mm, external nozzle diameter = 1.7 mm. The weight gain was 20% (w/w). After coating, the pellets were further fluidized for 10 min without spraying of liquid, and subsequently cured in an oven for 24 h at 60°C .

2.6. Determination of the practical drug loading

Forty mg pellets (drug-loaded starter cores or film-coated pellets) were manually ground for 5 min in a mortar with a pestle. The powder was dispersed in 50.0 mL deionized water, followed by stirring for 30 min at room temperature (magnetic stirrer, Heidolph). One (1.0) mL samples were withdrawn, filtered (0.2 μm) and their drug content determined by HPLC-UV analysis as follows (adapted from (Karrout et al., 2009b)): An UltiMate3000 HPLC apparatus (Thermo Fisher Scientific, Waltham, USA), equipped with a reversed-phase column [Gemini, 5 μm C18, 150×4.6 mm (Phenomenex, Le Pecq, France)] was used ($R > 0.9981$). The mobile phase consisted of 10.0% methanol and 90.0% of an aqueous acetic acid solution (1.0%). The injection volume was 30 μL , the flow rate was set to 1.0 mL/min. The column temperature was 25°C . The drug was detected at 330 nm. All experiments were performed in triplicate. Mean values \pm standard deviations are reported.

2.7. In vitro drug release measurements

Forty mg pellets (containing 1.9 mg drug) were placed into 50 mL falcon tubes, filled with: (i) 42.5 mL culture medium inoculated with

2.5 mL fecal slurry (10^6 CFU/mL bacteria) from inflammatory bowel disease patients, inflammatory bowel disease model (TNBS) rats or healthy dogs; or (ii) 45.0 mL culture medium free of feces, for reasons of comparison. The samples were incubated at 37 °C under horizontal agitation (80 rpm, mini orbital shaker; Stuart, Staffordshire, UK) and anaerobic conditions (5% CO₂, 10% H₂, 85% N₂). At predetermined time points, 3.0 mL samples were withdrawn, congealed and stored at -25 °C until further analysis. The samples were de-congealed at room temperature during 2 h, followed by centrifugation at 15,000 rpm for 10 min (Hettich fixed angle rotor, Tuttlingen, Germany) and filtration (0.2 μm PTFE mesh filter; Agilent Captiva Econofilters, Santa Clara, USA). The drug content was determined by HPLC-UV analysis, as described in section 2.6.

The stability of 5-ASA in the different release media was evaluated as follows: Five mg drug were dissolved in 50 mL culture medium free of

feces or culture medium inoculated with fecal slurries. At predetermined time points, 1.5 mL samples were withdrawn, filtered (0.2 μm) and their drug content was determined by HPLC-UV analysis, as described in section 2.6.

All experiments were performed in triplicate. Mean values ± standard deviations are reported.

2.8. Scanning electron microscopy

The morphology of drug layered starter cores and polymer coated pellets was observed using a JEOL field emission SEM (JSM-7800F, Tokyo, Japan) (magnification: x100, 500 or 1000, as indicated). Samples were fixed on the sample holder with a ribbon carbon double-sided adhesive and covered with a fine carbon layer.

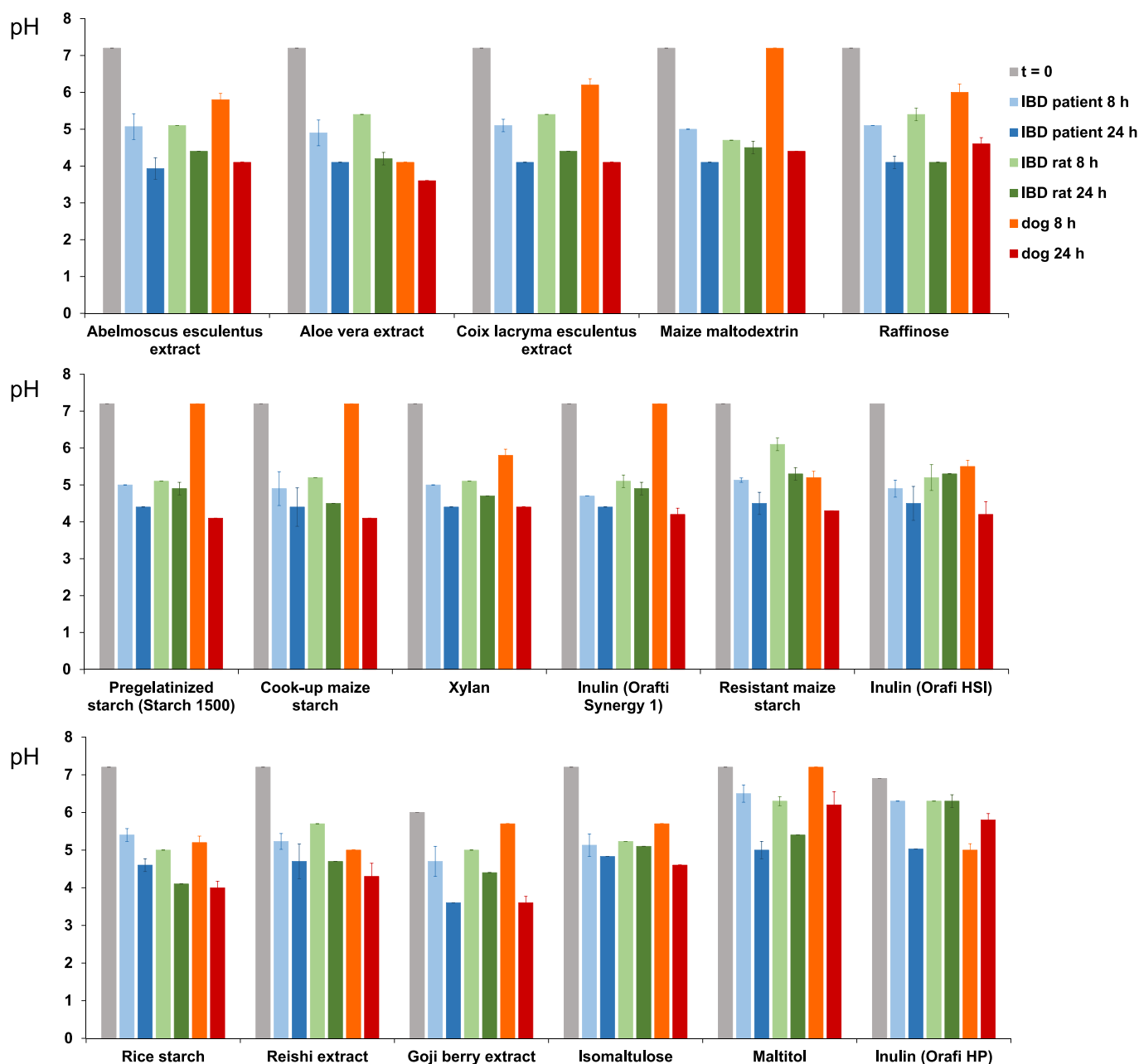


Fig. 1. pH values of different polysaccharide solutions/dispersions, inoculated with fecal slurries from inflammatory bowel disease patients (IBD patient), inflammatory bowel disease model rats (IBD rat) or healthy dogs (dog) for 8 or 24 h (as indicated). For reasons of comparison, also the pH values at time $t = 0$ are indicated. In all cases, the differences in pH between “ $t = 0$ ” and “IBD patient 24 h” are pronounced (≥ 2 units).

3. Results and discussion

The aim of this work was to identify polymeric film coatings allowing for colon targeting in different species, in particular: inflammatory bowel disease model rats (IBD rats), healthy dogs (dogs) and inflammatory bowel disease patients (IBD patients). The film coatings should be poorly permeable for the drug in the upper gastro intestinal tract, but should become permeable as soon as the colon is reached, due to degradation by enzymes secreted by colonic bacteria. These enzymes should be present in the different species in sufficient amounts to reliably trigger the onset of drug release. These key features were to be provided by a suitable polysaccharide. To avoid premature drug release due to the swelling and/or dissolution of the latter, the film coating consisted of a blend of two polymers: (i) the polysaccharide, which is degraded by bacterial enzymes in the colon of the different species, and (ii) ethylcellulose, which insoluble and non-degradable throughout the gastro intestinal tract. Thus, ethylcellulose traps the release rate triggering polysaccharide to avoid premature release in the stomach and small intestine. To identify a suitable release triggering polysaccharide, a variety of candidates was exposed to culture medium inoculated with fecal samples from the different species. Bacterial proliferation was monitored via dynamic changes in the pH of the medium.

3.1. Polysaccharide degradation in fecal samples from different species

Figs. 1 and 2 show the pH values of culture medium inoculated with fecal samples from IBD patients, IBD rats and healthy dogs after 8 and 24 h. For reasons of comparison, also the pH values at $t = 0$ (right after the addition of the polysaccharide to the medium) are illustrated. If the polysaccharide serves as a substrate for the bacteria present in these samples, the latter proliferate, and in case their concentration is high, the pH rapidly drops due to the generation of short chain fatty acids (Morrison and Preston, 2016). Thus, steep pH drops can serve as an indication for substantial polysaccharide degradation. A suitable polysaccharide candidate for species-independent colon targeting should show steep pH drops in all species: IBD rats, healthy dogs and IBD patients. As it can be seen, a variety of behaviors was observed, differing in the importance of the pH drop and degree of species-dependency. Please note that the pH of the fecal samples did not change to a noteworthy extent during the observation period, when no polysaccharide was added.

From a practical point of view, the capacity of the film coating to allow for colon targeting in *IBD patients* is most important. This is why the investigated polymers were divided into two groups: (i) Polysaccharides showing a pronounced decrease in pH upon 24 h incubation

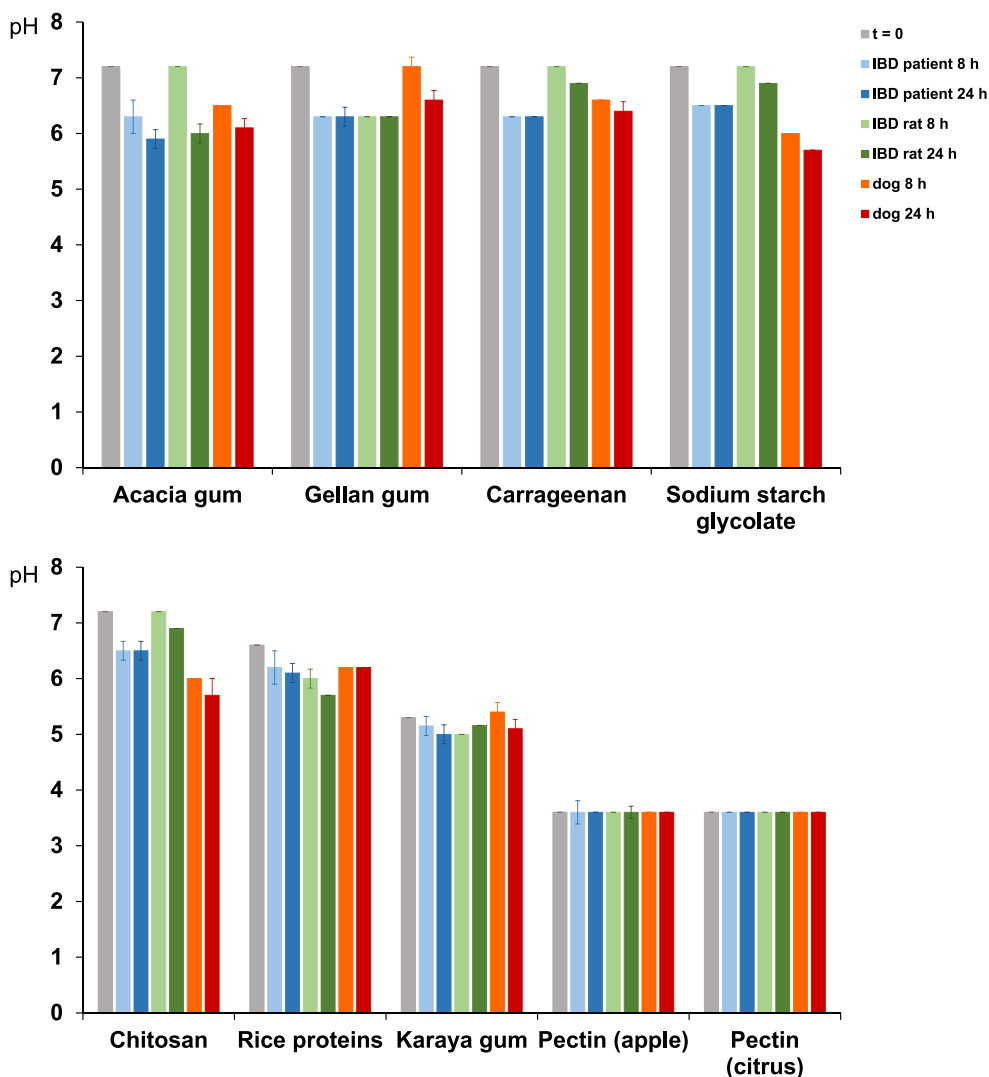


Fig. 2. pH values of different polysaccharide solutions/dispersions, inoculated with fecal slurries from inflammatory bowel disease patients (IBD patient), inflammatory bowel disease model rats (IBD rat) or healthy dogs (dog) for 8 or 24 h (as indicated). For reasons of comparison, also the pH values at time $t = 0$ are indicated. In all cases, the differences in pH between “ $t = 0$ ” and “IBD patient 24 h” are not very pronounced (< 1.5 units).

with fecal slurries from IBD patients (the difference in pH “ $t = 0$ ” versus “IBD patient 24 h” was ≥ 2 units). (ii) Polysaccharides showing a less pronounced difference (< 1.5 pH units) under these conditions. The first type of compounds is potentially promising for colon targeting (illustrated in Fig. 1), the second type of polysaccharides exhibits a less promising potential (shown in Fig. 2).

The order of the polysaccharides in Fig. 1 corresponds to the importance of the decrease in pH “ $t = 0$ ” versus “IBD patients 24 h”. The first compounds show the highest pH drop and can be considered as the “most promising” polysaccharides in the light of these results. However, it has to be pointed out that the polymers exhibiting promising potential for colon targeting in IBD patients did not necessarily lead to steep and rapid decreases in the pH upon incubation with fecal slurries from IBD rats and/or healthy dogs (Fig. 1). This can probably be attributed to the fact that in the colon of IBD patients, IBD rats and healthy dogs the types and amounts of bacteria secreting enzymes (which are able to degrade the polysaccharides) substantially differ. Consequently, the colon targeting performance is likely highly species-dependent in these cases. For instance, a dosage form coated with a polymeric film containing a polysaccharide, which exhibits a steep pH drop in IBD patients, but not in healthy dogs, probably fails in the preclinical phase of product development using a dog model, although it has an interesting potential to treat patients.

The polysaccharides shown in Fig. 2 are “less promising candidates” in the light of the pH drops observed upon incubation with fecal slurries from IBD patients. The differences in pH at “ $t = 0$ ” and after 24 h incubation with feces from IBD patients are inferior to 1.5 units. Again, the order of the compounds in the figure corresponds to the importance of the respective difference in pH (highest differences are shown at the beginning). However, great caution must be paid in the case of polysaccharides, which acidify the culture medium themselves, for example pectin from apple and citrus: In these cases, the pH was about 4 right from the beginning (which is at the lower limit of pH values measurable with the applied pH paper). So, no hypothesis on the colon targeting potential of these compounds can be made based on the obtained results.

It is worth noting that certain polysaccharides (e.g., chitosan) showed a relatively pronounced decrease in pH of the culture medium

after inoculation with fecal samples from healthy dogs. Thus, film coatings based on these compounds might allow for colon targeting in these animals. However, the pH drop was not very pronounced upon incubation with fecal samples from IBD patients. Consequently, the systems might show promising results in a preclinical phase using dogs, but fail in subsequent clinical trials.

Due to the less promising results obtained with the polysaccharides shown in Fig. 2 in terms of potential clinical performance, they were not further investigated in this study. Instead, the more promising compounds shown in Fig. 1 were used to coat 5-ASA-loaded starter cores and the resulting drug release kinetics were measured in culture medium inoculated with fecal samples from IBD rats, healthy dogs and IBD patients.

3.2. Species-dependent drug release from coated pellets

5-ASA-loaded starter cores were prepared by layering an aqueous drug-binder (hydroxypropyl methylcellulose) solution onto sucrose cores in a fluidized bed (6% drug loading). These starter cores were subsequently coated with 20% of a blend of “colon targeting polysaccharide”: ethylcellulose (2:3, w/w, based on dry polymer masses). The ethylcellulose trapped the colon targeting polysaccharide to limit premature swelling and/or dissolution of the film coating in the upper gastro intestinal tract. An aqueous ethylcellulose dispersion was used for this purpose. Thus, a plasticizer was added to facilitate polymer particle coalescence: dibutyl sebacate (DBS). For this reason, it was important to evaluate the potential impact of the presence of this plasticizer on the growth of the bacteria in fecal samples from IBD patients. Fig. 3 shows the growth of *Bacteriodes* and *Bifidus* species in culture medium in the presence and absence of 38 mg/L DBS (dashed and solid curves, respectively). As it can be seen, no inhibitory effect of DBS was observed on the growth of these bacteria under the given conditions. Thus, the capacity to allow for colon targeting in IBD patients using the investigated polymeric film coatings is probably not affected by the presence of this plasticizer.

Fig. 4 shows examples of SEM pictures of surfaces of a 5-ASA loaded starter core and a xylan:ethylcellulose coated bead (20% coating level),

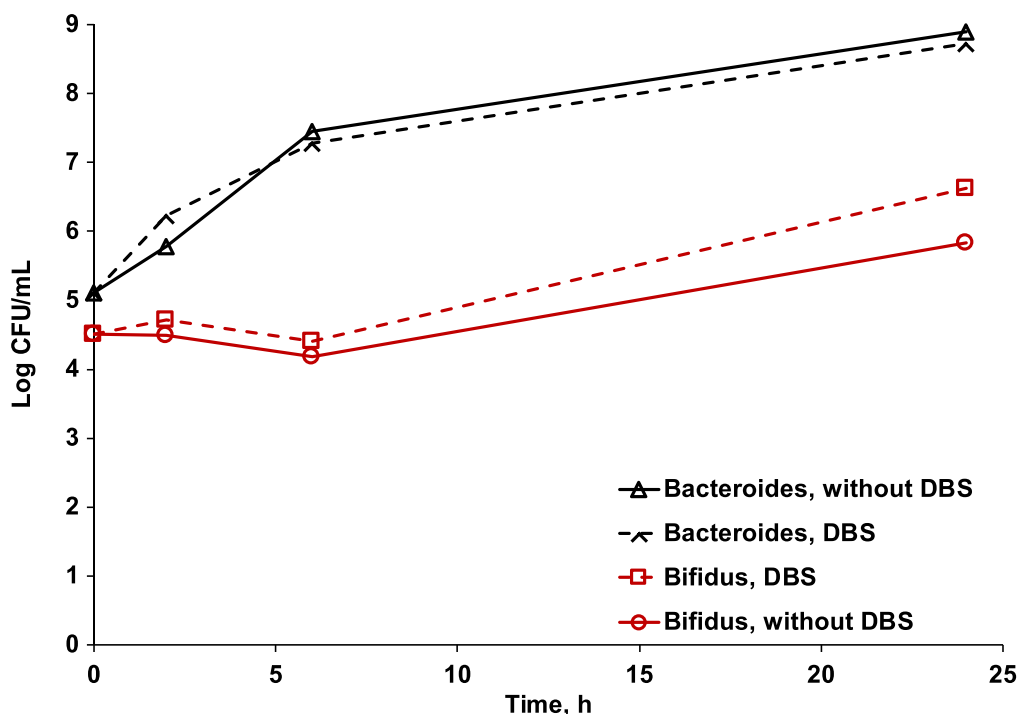


Fig. 3. Growth of *Bacteriodes* and *Bifidus* bacteria in culture medium in the presence or absence of the plasticizer DBS.

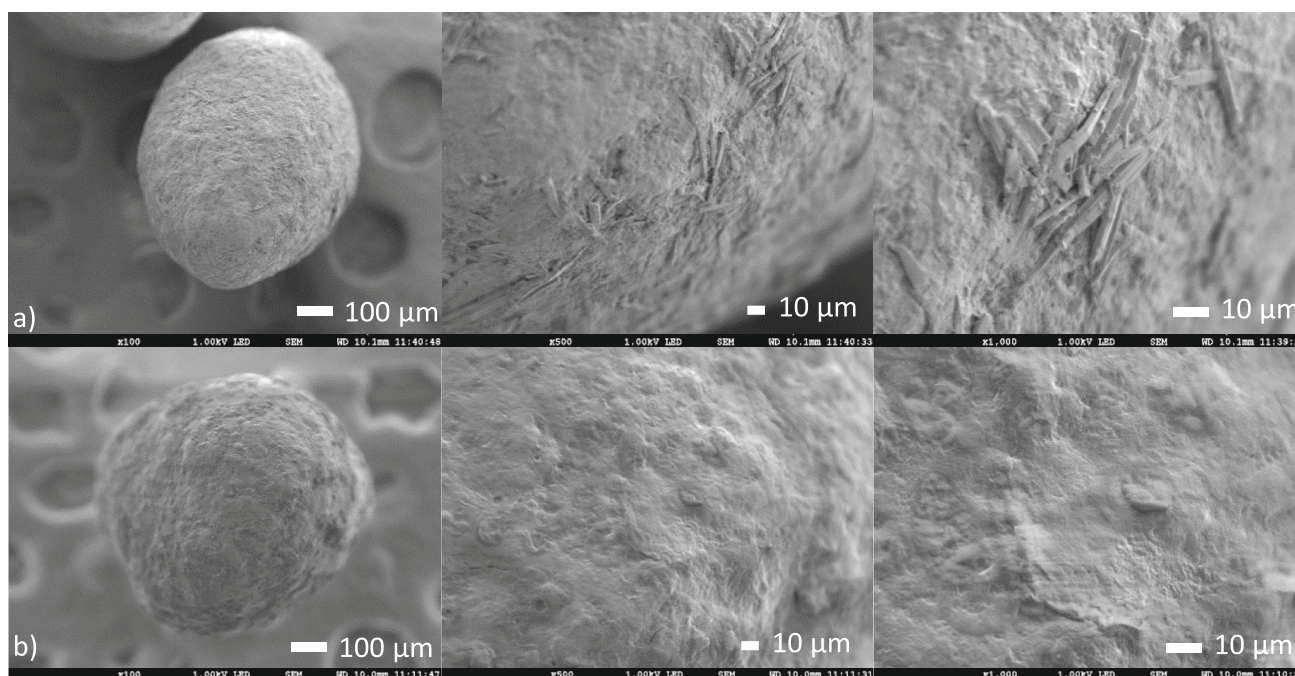


Fig. 4. SEM pictures of: a) a 5-ASA-loaded starter core, and b) a pellet coated with a 2:3 xylan:ethylcellulose blend (20% coating level).

respectively. As it can be seen, the drug-binder as well as the polysaccharide: ethylcellulose layers completely cover the surfaces of the spheres and have a rather homogeneous appearance. At higher magnification, needle-shaped 5-ASA crystals are visible in the case of the drug-layered starter cores, but not in the case of the xylan:ethylcellulose coated beads. This can serve as an indication for the fact that the drug layer is fully surrounded by the outer “colon targeting layer”.

Drug release from the polymer-coated beads was measured in culture medium inoculated with fecal samples from IBD rats, healthy dogs and IBD patients under anaerobic conditions. For reasons of comparison, 5-ASA release was also monitored in pure culture medium. Importantly, the drug was sufficiently stable during the observation period in all types of media, irrespective of the presence or absence of fecal slurries: 4.2–4.5% 5-ASA was degraded after 48 h (at 0.1 mg/mL). Thus, the enzymes present in the fecal samples do not chemically attack the drug to an important extent.

The green, red and blue curves in Fig. 5 show the observed 5-ASA release kinetics from pellets coated with different polysaccharide: ethylcellulose blends in culture medium inoculated with fecal samples from IBD rats, healthy dogs and IBD patients. The black curves illustrate drug release in the absence of fecal slurries. A promising colon targeting system should show clear differences in the release rates in the presence versus absence of fecal samples: The presence of the colonic bacteria should trigger film coating degradation and result in faster drug release. Hence, promising film coating candidates show important differences between the colored and the black curves. The black curves should only show limited 5-ASA release during the observation period. As it can be seen in Fig. 5, this is the case for all formulations. However, several film coatings do not show very pronounced differences in the release rates in the presence vs. absence of fecal slurries. For example, in the case of *abelmoscus esculentus* extract the difference in drug release was only about 30% after 24 h. Please note that this polysaccharide showed the most promising results with respect to the steepness in the pH drop upon incubation of the pure compound in culture medium with vs. without fecal slurries (Fig. 1). The structure of the polymeric film coating (containing also water-insoluble ethylcellulose) might at least in part explain this observation: Potentially, the enzymes cannot attack the polysaccharide in the film coating very easily, because it is too effectively trapped in the ethylcellulose matrix. The inner film coating structure

depends on a variety of parameters, including the miscibility of the two polymers and their behavior during film formation (e.g., precipitation rate of dissolved polymer chains and phase separation).

Furthermore, ideal film coatings allowing for colon targeting should be species-independent and exhibit similar drug release kinetics upon incubation in culture medium containing fecal samples from IBD rats, healthy dogs and IBD patients. As it can be seen in Fig. 5, this was not the case for many of the illustrated polysaccharide: ethylcellulose blends. Often, drug release was slower in the presence of fecal samples from healthy dogs compared to fecal samples from IBD rats or IBD patients (e.g., raffinose, xylan, resistant maize starch). This is consistent with the observation that the pH drop upon incubation of several pure polysaccharides with fecal slurries from healthy dogs was limited after 8 h compared to the other species (Fig. 1). So, it seems that the colon of a dog does not contain the same types and amounts of enzymes needed to rapidly degrade these compounds.

Thus, film coatings based on these polysaccharides: ethylcellulose blends might lead to erroneous decisions during the preclinical development phase of novel colon targeting products using rat or dog models, or show only limited colon targeting potential in IBD patients.

3.3. Species-independent colon targeting

Interestingly, two of the investigated film coatings showed a highly promising potential to allow for colon targeting in IBD patients and exhibit only limited species dependence: Fig. 6 illustrates the release of 5-ASA from pellets coated with aloe vera extract: ethylcellulose (2:3 w/w) and reishi extract: ethylcellulose (2:3 w/w) blends (20% coating level in both cases). Again, the green, red and blue curves show drug release in the presence of fecal samples from IBD rats, healthy dogs and IBD patients, while the black curves illustrate 5-ASA release in culture medium free of feces. Reishi extract is obtained from the fruiting body of a mushroom: *Ganoderma lucidum*, *Ganodermataceae*. Clearly, for both types of extracts (aloe vera and reishi) drug release was much faster in the presence of fecal slurries compared to pure culture medium. Thus, these film coatings offer an interesting potential to allow for colon targeting. Importantly, the observed release kinetics were rather similar for all types of the investigated fecal samples: from IBD rats, healthy dogs and IBD patients. Thus, results observed in the preclinical development

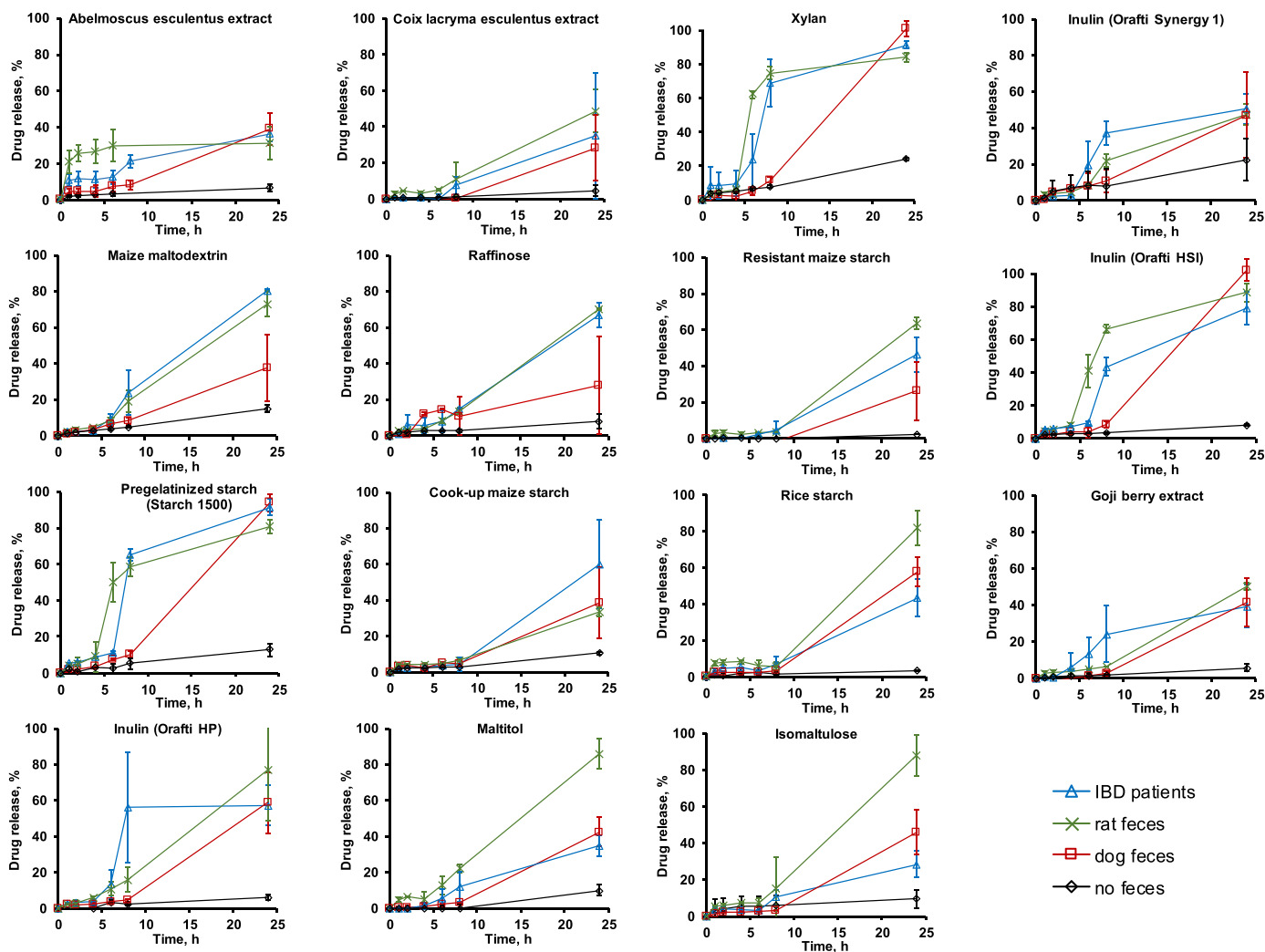


Fig. 5. 5-ASA release from pellets coated with different types of polysaccharide: ethylcellulose blends (2:3 w/w blend ratio; 20% coating level) in culture medium inoculated with fecal samples from IBD patients, IBD rats or dogs (as indicated). For reasons of comparison, also drug release in pure culture medium is illustrated.

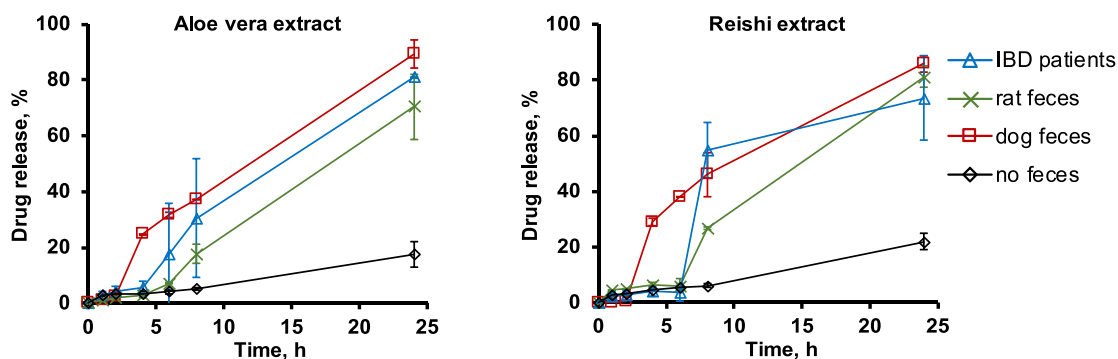


Fig. 6. 5-ASA release from pellets coated with aloe vera extract: ethylcellulose blends or reishi extract: ethylcellulose blends (2:3 w/w blend ratio; 20% coating level) in culture medium inoculated with fecal samples from IBD patients, IBD rats or dogs (as indicated). For reasons of comparison, also drug release in pure culture medium is illustrated.

phase of a new drug product aiming at colon targeting are likely predictive for the performance of the system in subsequent clinical trials, in terms of drug release. This is very important from a practical point of view, to minimize the risk of erroneous decisions at this early stage of product development. In addition, these film coatings also offer an interesting potential to allow for colon targeting in dogs as advanced

veterinary medicines. The reason for the promising performance of these film coatings is likely the fact that the enzymes, which are required to degrade the compounds in aloe vera extract and reishi extract are present in sufficient quantities in the colon of IBD rats, healthy dogs and IBD patients.

4. Conclusions

Great caution must be paid when studying the performance of bacteria-sensitive film coatings aiming at colonic drug delivery in animals in order to predict their performance in human patients. The types and amounts of bacteria substantially differ between species and erroneous conclusions during the preclinical development phase can easily be drawn. Interestingly, film coatings containing aloe vera extract or reishi extract show a promising potential for colon targeting in IBD patients and exhibit similar release patterns upon exposure to fecal samples from IBD rats, healthy dogs and human patients.

CRedit authorship contribution statement

F. Ferraro: Investigation, Methodology, Validation, Visualization, Writing – original draft. **L.M. Sonnleitner:** Investigation. **C. Neut:** Conceptualization, Investigation, Methodology, Resources, Supervision, Validation. **S. Mahieux:** Investigation. **J. Verin:** Investigation. **J. Siepmann:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **F. Siepmann:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

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

Juergen Siepmann reports financial support was provided by Interreg 2 Seas programme 2014–2020. Juergen Siepmann reports financial support was provided by European Regional Development Fund. Juergen Siepmann has patent pending to Assignee. Florence Siepmann has patent pending to Assignee. Christel Neut has patent pending to Assignee. Fabiana Ferraro has patent pending to Assignee. The Editor-in-Chief of the journal is one of the co-authors of this article. The manuscript has been subject to all of the journal's usual procedures, including peer review, which has been handled independently of the Editor-in-Chief. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This project has received funding from the Interreg 2 Seas programme 2014-2020, co-funded by the European Regional Development Fund under subsidy contract "Site Drug 2S07-033".

References

- Actis, G.C., Pellicano, R., Fagoonee, S., Ribaldone, D.G., 2019. History of inflammatory bowel diseases. *J. Clin. Med.* 8, 1970 fasc. 11. nov. <https://doi.org/10.3390/jcm8111970>.
- Amidon, S., Brown, J.E., Dave, V.S., 2015. Colon-targeted oral drug delivery systems: design trends and approaches. *AAPS PharmSciTech* 16, 731–741 fasc. 4. ago. <https://doi.org/10.1208/s12249-015-0350-9>.
- Awad, A., et al., 2022. Clinical translation of advanced colonic drug delivery technologies. *Adv. Drug Deliv. Rev.* 181, 114076 feb. <https://doi.org/10.1016/j.addr.2021.114076>.
- Bak, A., Ashford, M., Brayden, D.J., 2018. Local delivery of macromolecules to treat diseases associated with the colon. *Adv. Drug Deliv. Rev.* 136–137, 2–27 nov. <https://doi.org/10.1016/j.addr.2018.10.009>.
- Bussemer, T., Peppas, N.A., Bodmeier, R., 2003. Time-dependent mechanical properties of polymeric coatings used in rupturable pulsatile release dosage forms. *Drug Dev. Ind. Pharm.* 29, 623–630 fasc. 6. lug. <https://doi.org/10.1081/ddc-120021311>.
- Chen, E.P., Mahar Doan, K.M., Portelli, S., Coatney, R., Vaden, V., Shi, W., 2008. Gastric pH and gastric residence time in fasted and fed conscious cynomolgus monkeys using

- the Bravo® pH system. *Pharm. Res.* 25 (fasc. 1), 123–134 gen. <https://doi.org/10.1007/s11095-007-9358-5>.
- Desreumaux, P., Ghosh, S., 2006. Review article: mode of action and delivery of 5-aminosalicylic acid - new evidence. *Aliment. Pharmacol. Ther.* 24 (Suppl. 1), 2–9 set. <https://doi.org/10.1111/j.1365-2036.2006.03069.x>.
- Dodoo, C.C., Wang, J., Basit, A.W., Stapleton, P., Gaisford, S., 2017. Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation. *Int. J. Pharm.* 530, 224–229 fasc. 1. set. <https://doi.org/10.1016/j.ijpharm.2017.07.068>.
- Fadda, H.M., Basit, A.W., 2005. Dissolution of pH responsive formulations in media resembling intestinal fluids: bicarbonate versus phosphate buffers. *J. Drug Deliv. Sci. Technol.* 15 (fasc. 4), 273–279. [https://doi.org/10.1016/S1773-2247\(05\)50049-2](https://doi.org/10.1016/S1773-2247(05)50049-2).
- Gazzaniga, A., Maroni, A., Sangalli, M.E., Zema, L., 2006. Time-controlled oral delivery systems for colon targeting. *Expert Opin. Drug Deliv.* 3, 583–597 fasc. 5. set. <https://doi.org/10.1517/17425247.3.5.583>.
- Goyanes, A., Hatton, G.B., Merchant, H.A., Basit, A.W., 2015. Gastrointestinal release behaviour of modified-release drug products: Dynamic dissolution testing of mesalazine formulations. *Int. J. Pharm.* 484, 103–108 fasc. 1–2. apr. <https://doi.org/10.1016/j.ijpharm.2015.02.051>.
- Hatton, G.B., Yadav, V., Basit, A.W., Merchant, H.A., 2015. Animal farm: considerations in animal gastrointestinal physiology and relevance to drug delivery in humans. *J. Pharm. Sci.* 104, fasc. 9, 2747–2776. <https://doi.org/10.1002/jps.24365>.
- Ibekwe, V.C., Fadda, H.M., McConnell, E.L., Khela, M.K., Evans, D.F., Basit, A.W., 2008a. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. *Pharm. Res.* 25 (fasc. 8), 1828–1835 ago. <https://doi.org/10.1007/s11095-008-9580-9>.
- Ibekwe, V.C., Khela, M.K., Evans, D.F., Basit, A.W., 2008b. A new concept in colonic drug targeting: a combined pH-responsive and bacterially-triggered drug delivery technology. *Aliment. Pharmacol. Ther.* 28, 911–916 fasc. 7. ott. <https://doi.org/10.1111/j.1365-2036.2008.03810.x>.
- Kamm, M.A., et al., 2007. Once-daily, high-concentration MMX mesalazine in active ulcerative colitis. *Gastroenterology* 132, fasc. 1, 66–75 quiz 432–433, gen. <https://doi.org/10.1053/j.gastro.2006.10.011>.
- Kararli, T.T., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* 16 (fasc. 5), 351–380 lug. <https://doi.org/10.1002/bdd.2510160502>.
- Karrou, Y., et al., 2009a. Colon targeting with bacteria-sensitive films adapted to the disease state. *Eur. J. Pharm. Biopharm.* 73, 74–81 fasc. 1. set. <https://doi.org/10.1016/j.ejpb.2009.04.003>.
- Karrou, Y., Neut, C., Wils, D., et al., 2009b. Novel polymeric film coatings for colon targeting: Drug release from coated pellets. *Eur. J. Pharm. Sci.* 37, 427–433. <https://doi.org/10.1016/j.ejps.2009.03.014>.
- Karrou, Y., et al., 2010. Enzymatically activated coated multiparticulates containing theophylline for colon targeting. *J. Drug Deliv. Sci. Technol.* 20, 193–199 fasc. 3. gen. [https://doi.org/10.1016/S1773-2247\(10\)50029-7](https://doi.org/10.1016/S1773-2247(10)50029-7).
- Karrou, Y., et al., 2015. In vivo efficacy of microbiota-sensitive coatings for colon targeting: a promising tool for IBD therapy. *J. Control. Release* 10.
- Maderuelo, C., Lanao, J.M., Zarzuelo, A., 2019. Enteric coating of oral solid dosage forms as a tool to improve drug bioavailability. *Eur. J. Pharm. Sci.* 138, 105019 ott. <https://doi.org/10.1016/j.ejps.2019.105019>.
- Mahar, K.M., Portelli, S., Coatney, R., Chen, E.P., 2012. Gastric pH and gastric residence time in fasted and fed conscious beagle dogs using the Bravo® pH system. *J. Pharm. Sci.* 101 (fasc. 7), 2439–2448 lug. <https://doi.org/10.1002/jps.23159>.
- Maroni, A., Moutaharrik, S., Zema, L., Gazzaniga, A., 2017. Enteric coatings for colonic drug delivery: state of the art. *Expert Opin. Drug Deliv.* 14, 1027–1029 fasc. 9. set. <https://doi.org/10.1080/17425247.2017.1360864>.
- McCoubrey, L.E., Favaron, A., Awad, A., Orlu, M., Gaisford, S., Basit, A.W., 2023. Colonic drug delivery: Formulating the next generation of colon-targeted therapeutics. *J. Control. Release* 353, 1107–1126 gen. <https://doi.org/10.1016/j.jconrel.2022.12.029>.
- Merchant, H., Fadda, H.M., Arafat, B.T., Basit, A.W., 2009. Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. *Int. J. Pharm.* 382, 56–60 fasc. 1–2. dic. <https://doi.org/10.1016/j.ijpharm.2009.08.003>.
- Morrison, D.J., Preston, T., 2016. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* 7 (fasc. 3), 189–200 mag. <https://doi.org/10.1080/19490976.2015.1134082>.
- Moutaharrik, S., Maroni, A., Melocchi, A., et al., 2021. Oral colon delivery platform based on a novel combination approach: Design concept and preliminary evaluation. *J. Drug Deliv. Sci. Tech.* 66, 102919 <https://doi.org/10.1016/j.jddst.2021.102919>.
- Moutaharrik, S., Maroni, A., Neut, C., et al., 2023. In vitro and in vivo evaluation of a pH-, microbiota- and time-based oral delivery platform for colonic release. *Eur. J. Pharm. Biopharm.* 183, 13–23. <https://doi.org/10.1016/j.ejpb.2022.12.01>.
- Nugent, S.G., 2001. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosaliclates and other drugs. *Gut* 48, 571–577 fasc. 4. apr. <https://doi.org/10.1136/gut.48.4.571>.
- Ordás, I., Eckmann, L., Talamini, M., Baumgart, D.C., Sandborn, W.J., 2012. Ulcerative colitis. *Lancet* 380, 1606–1619 fasc. 9853. nov. [https://doi.org/10.1016/S0140-6736\(12\)60150-0](https://doi.org/10.1016/S0140-6736(12)60150-0).
- Petagna, L., et al., 2020. Pathophysiology of Crohn's disease inflammation and recurrence. *Biol. Direct* 15, 23 fasc. 1. dic. <https://doi.org/10.1186/s13062-020-00280-5>.
- Porter, R.J., Kalla, R., Ho, G.-T., 2020. Ulcerative colitis: recent advances in the understanding of disease pathogenesis. *F1000Research* 9, 294 apr. <https://doi.org/10.12688/f1000research.20805.1>.
- Press, A.G., et al., 1998. Gastrointestinal pH profiles in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 12, 673–678 fasc. 7. lug. <https://doi.org/10.1046/j.1365-2036.1998.00358.x>.

- Rousseaux, C., et al., 2005. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor- γ . *J. Exp. Med.* 201 (fasc. 8), 1205–1215 apr. <https://doi.org/10.1084/jem.20041948>.
- Schellekens, R.C.A., Stellaard, F., Olsder, G.G., Woerdenbag, H.J., Frijlink, H.W., Kosterink, J.G.W., 2010. Oral ileocolonic drug delivery by the colopulse-system: a bioavailability study in healthy volunteers. *J. Control. Release* 146, 334–340 fasc. 3. set. <https://doi.org/10.1016/j.jconrel.2010.05.028>.
- Varum, F., Freire, A.C., Fadda, H.M., Bravo, R., Basit, A.W., 2020. A dual pH and microbiota-triggered coating (PhloralTM) for fail-safe colonic drug release. *Int. J. Pharm.* 583, 119379 giu. <https://doi.org/10.1016/j.ijpharm.2020.119379>.
- Vinarov, Z., et al., 2021. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review. *Eur. J. Pharm. Sci.* 162, 105812 lug. <https://doi.org/10.1016/j.ejps.2021.105812>.
- Wang, J., Yadav, V., Smart, A.L., Tajiri, S., Basit, A.W., 2015a. Stability of peptide drugs in the colon. *Eur. J. Pharm. Sci.* 78, 31–36 ott. <https://doi.org/10.1016/j.ejps.2015.06.018>.
- Wang, J., Yadav, V., Smart, A.L., Tajiri, S., Basit, A.W., 2015b. Toward oral delivery of biopharmaceuticals: an assessment of the gastrointestinal stability of 17 peptide drugs. *Mol. Pharm.* 12, 966–973 fasc. 3. mar. <https://doi.org/10.1021/mp500809f>.
- Yadav, V., Varum, F., Bravo, R., Furrer, E., Basit, A.W., 2016. Gastrointestinal stability of therapeutic anti-TNF α IgG1 monoclonal antibodies. *Int. J. Pharm.* 502, 181–187 fasc. 1–2. apr. <https://doi.org/10.1016/j.ijpharm.2016.02.014>.
- Yoshida, T., Lai, T.C., Kwon, G.S., Sako, K., 2013. pH- and ion-sensitive polymers for drug delivery. *Expert Opin. Drug Deliv.* 10, 1497–1513 fasc. 11. nov. <https://doi.org/10.1517/17425247.2013.821978>.
- Yoshimura, N., Tadami, T., Kawaguchi, T., Sako, M., Saniabadi, A., Takazoe, M., 2011. The efficacy of asacol, a pH-dependent release mesalamine formulation in patients with ulcerative colitis who showed inadequate response to the time-dependent release mesalamine formulation, Pentasa. *Gastroenterology* 140, S-394–S-395 fasc. 5. mag. [https://doi.org/10.1016/S0016-5085\(11\)61618-1](https://doi.org/10.1016/S0016-5085(11)61618-1).