Output 1.1

One advanced drug product for the treatment of Crohn's disease and ulcerative colitis

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Content

- Photo of a prototype
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- Report documenting the key properties of the system

Photo of a prototype

Optical microscopy picture of the innovative drug delivery systems allowing for colon targeting. The scale bar is in cm.

Report detailing the composition of the system and manufacturing procedure

Materials

5-aminosalicylic acid (5-ASA, Alfa Aesar, Kendel, Germany); BactoTM Tryptone, BactoTM 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 and pectin from apple (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, Siglufjourdur, Iceland); pea maltodextrin (LAB 4118), corn maltodextrin (Glucidex 17 D), cook-up maize starch (Clearam), pregelatinized maize starch (Lycatab C), 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).

Preparation of drug-loaded starter cores

5-aminosalicylic acid (5-ASA)-loaded starter cores were prepared by layering an aqueous drug-binder solution (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 coating was performed until a drug loading of 6 % (w/w) was obtained. The process parameters were as follows: inlet temperature = 50 °C, product temperature = 39 ± 2 °C, spray rate = 0.3 g/min, atomization pressure = 0.6 bar, air flow rate = 25 % (10 m3/h), batch size = 100 g, internal nozzle diameter = 0.8 mm, external nozzle diameter = 1.7 mm.

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 was prepared by dispersing 8.0 g of that compound in 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 D, SweetPearl P300 DC, LAB 4118, 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 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. The process parameters for polymeric film coating were as follows: inlet temperature = 50 °C, product temperature = 39 ± 2 °C, spray rate = 0.3 g/min, atomization pressure = 0.6 bar, air flow rate = 25 % (10 m3/h), batch size = 70 g, internal nozzle diameter = 0.8 mm, external nozzle diameter = 1.7 mm.

Report documenting the key properties of the system

<u>Inoculation of polysaccharides in culture medium +/- fecal samples</u>

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. 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 (106 CFU/mL bacteria) from inflammatory bowel disease 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. The rat feces were collected 1 d after TNBS administration. The tubes were incubated at 37 °C under anaerobic conditions (5 % CO2, 10 % H2, 85 % N2). 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 +/- standard deviations are reported.

Inoculation of Bacteriodes and Bifidus bacteria in culture medium +/- DBS

The kill-time test was applied to evaluate the antimicrobial activity of the plasticizer DBS by determining the reduction of the bacterial (Bacteroides Vulgatus and Bifidobacterium) population in terms of the exposure time to the plasticizers. 0.38 mg of plasticizer were added to 10.0 mL of bacteria suspension (1x105 CFU mL-1) and the tubes were incubated at 37 °C under anaerobic atmosphere. At predetermined time (0, 2, 6 and 24 h) 0.1 mL sample was collected and diluted to 10 mL of Cysteinated Ringer solution (CR). Successive 1/10 dilutions in (CR) were made up to 10−6 from the recovered bacterial suspension and 0.1 mL of each dilution was seeded onto Colombia Culture medium (CC). The plates are then incubated for 4 days at 37 °C. The number of viable bacteria (colony forming unit, CFU) was counted and expressed in Log CFU·mL−1 using the following formula: Viable bacteria (Log CFU·mL) log(1 n 0 10), 1 d where n represents the counted number of outgrown colonies and d represents the dilution.

Determination of the practical drug loading

Eighty mg 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, Schwabach, Germany). One (1.0) mL samples were withdrawn, filtered (0.2 µm) and their drug content determined by HPLC-UV analysis as follows: An UltiMate3000 HPLC apparatus (Thermo Fisher Scientific, Waltham, USA), equipped with a reversed-phase column [Gemini®, 5 µm C18, 150 x 4.6 mm (Phenomenex, Le Pecq, France)] was used. The mobile phase consisted of 10.0 % methanol and 90.0 % of an aqueous acetic acid solution (1.0 %). The flow rate was set to 1.0 mL/min, the column temperature to 25 °C. The drug was detected at 330 nm. All experiments were performed in triplicate. Mean values +/- standard deviations are reported.

In vitro drug release measurements

Eighty mg pellets were placed into 50 mL falcon tubes, filled with: (i) 42.5 mL culture medium inoculated with 2.5 mL fecal slurry (106 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 above. 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 pre-determined time points, 1.5 mL samples were withdrawn, filtered (0.2 µm) and their drug content was determined by HPLC-UV analysis, as described above. All experiments were performed in triplicate. Mean values +/- standard deviations are reported.

Scanning electron microscopy

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

Polysaccharide degradation in fecal samples from different specifies

Figures 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 amount is high, the pH rapidly drops due to the generation of short chain fatty acids [39]. 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 IBD rats, healthy dogs and IBD patients. As it can be seen, a variety of behaviors were observed, differing in the importance of the pH drop and degree of dependence on the species.

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 an important decrease in pH upon 24 h incubation with fecal slurries from IBD patients (the difference in pH “t = 0” versus “IBD patient 24 h” was equal or larger than 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 Figure 1), the second type of polysaccharides exhibits a less promising potential (shown in Figure 2).

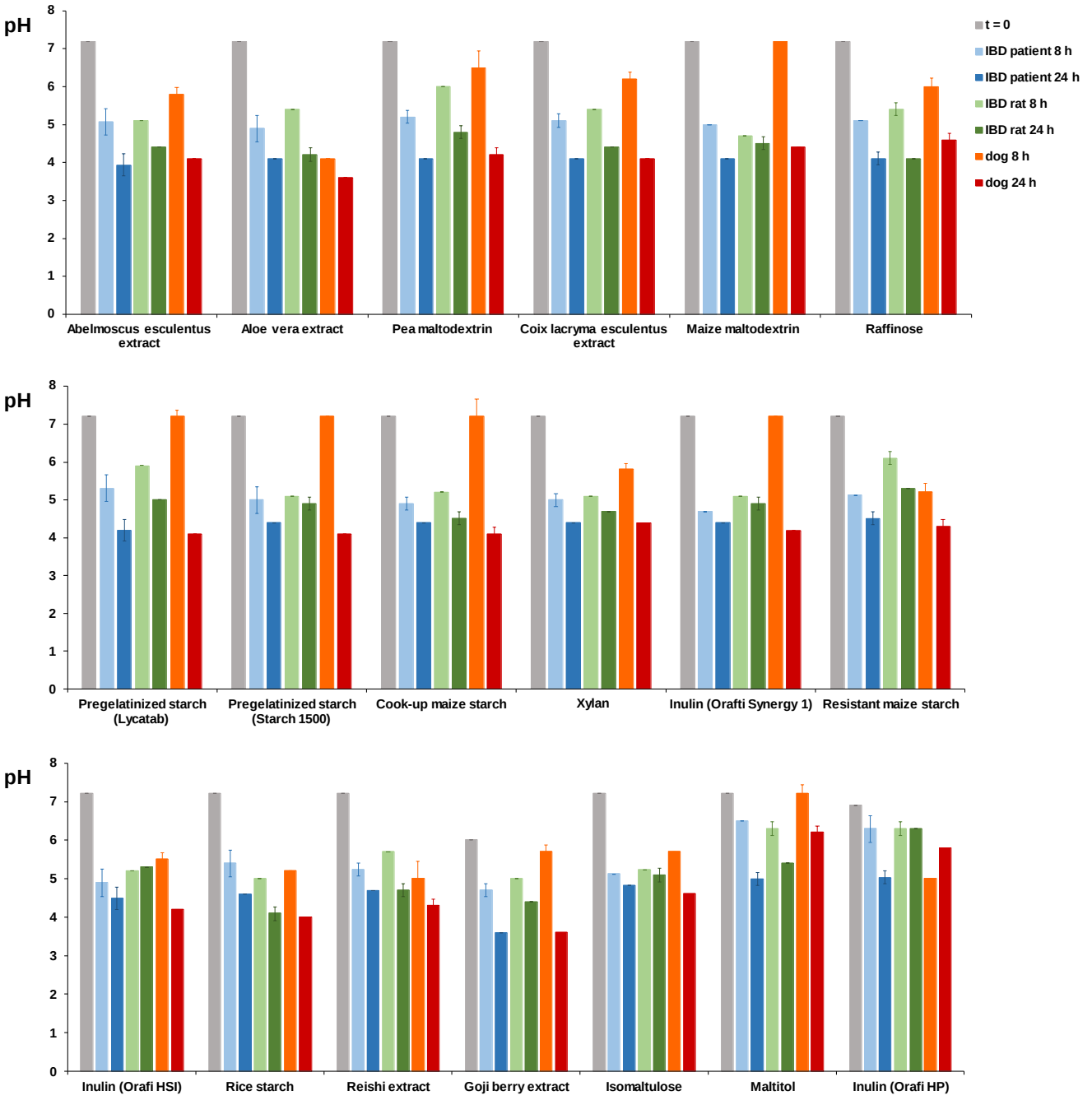

Fig. 1 pH values of different polysaccharide solutions/dispersions, inoculated with fecal slurries from inflammatory bowel disease patients (IBD patient), inflammatory bowel disease model rats (IBD rat) or healthy dogs (dog) for 8 or 24 h (as indicated). For reasons of comparison, also the pH values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

The order of the polysaccharides in Figure 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 (Figure 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. This can be expected to cause highly species-dependent colon targeting performance. 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, likely fails in the preclinical phase of product development, although it has an interesting potential to treat patients.

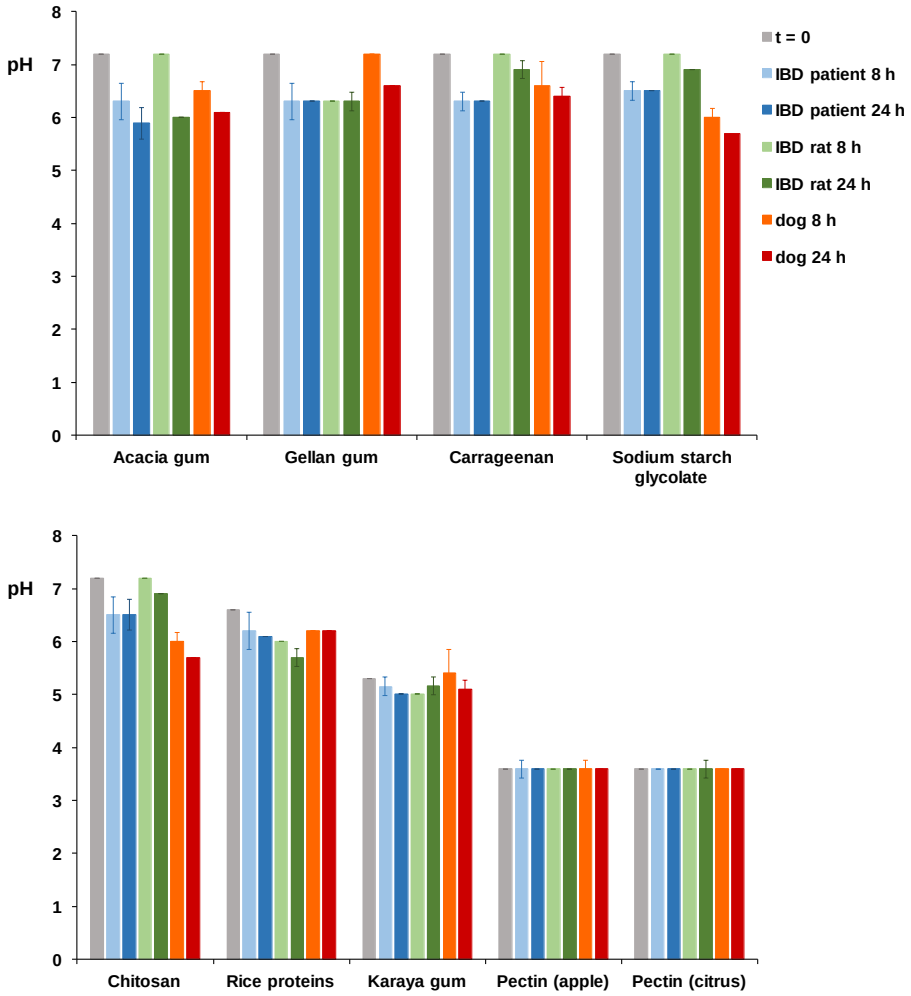

Fig. 2 pH values of different polysaccharide solutions/dispersions, inoculated with fecal slurries from inflammatory bowel disease patients (IBD patient), inflammatory bowel disease model rats (IBD rat) or healthy dogs (dog) for 8 or 24 h (as indicated). For reasons of comparison, also the pH values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

The polysaccharides shown in Figure 2 are "less promising candidates" in the light of the pH drops observed upon incubation with fecal slurries from IBD patients, IBD rats and healthy dogs in this study. 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 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 is about 4 right from the beginning and none of the conditions shows a pH drop. So, no hypothesis on the colon targeting potential of these compounds can be made based on the results obtained with this type of test.

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 the preclinical phase, but fail in subsequent clinical trials.

Due to the less promising results obtained with the polysaccharides shown in Figure 2 in terms of potential clinical performance, they were not further investigated in this study. Instead, the more promising compounds shown in Figure 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.

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 starter cores in a fluidized bed (6 % drug loading). These starter cores were 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). It has to be pointed out that certain plasticizers have been reported to inhibit specific bacterial enzymes. Thus, 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. Figure 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 these 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.

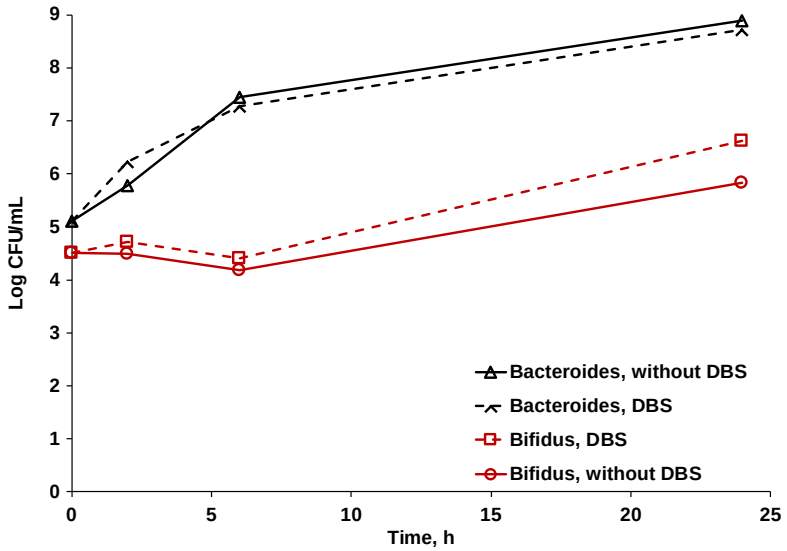

Fig. 3 Growth of Bacteroides and Bifidus bacteria in culture medium in the presence or absence of the plasticizer DBS.

Figure 4 shows examples of SEM pictures of surfaces of 5-ASA loaded starter cores and xylan:ethylcellulose coated beads (20 % coating level), respectively. As it can be seen, the drug-binder as well as the polysacharride: ethylcellulose layers completely cover the surfaces of the spheres and have a 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 stable during the observation period in all types of media, irrespective of the presence or absence of fecal slurries: Not more than 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.

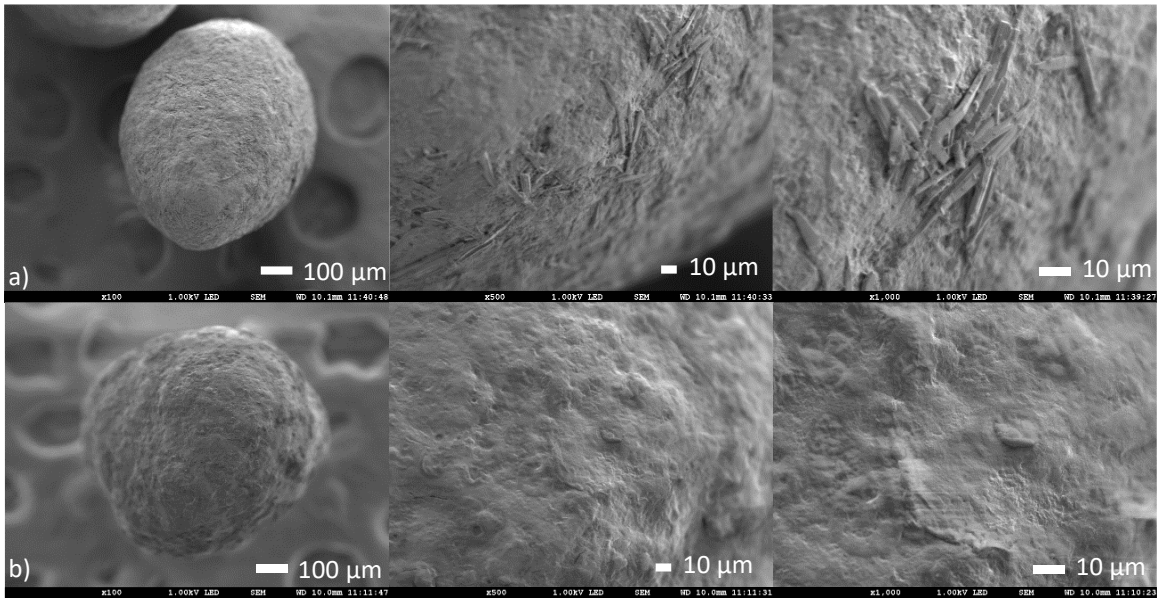

Fig. 4 SEM pictures of: a) 5-ASA-loaded starter cores, and b) pellets coated with a 2:3 xylan:ethylcellulose blend (20 % coating level).

The green and blue curves in Figure 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 show limited 5-ASA release during the observation period. As it can be seen in Figure 5, this is the case for all formulations. Unfortunately, several film coatings do not show very pronounced differences in the release rates in the presence of fecal slurries. For example, in the case of abelmoscus esculentus extract even after 24 h, the difference of drug release in the presence versus absence of fecal samples from IBD patients, IBD rats and healthy dogs was less than 30 %. 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 or without fecal slurries (Figure 1). The structure of the polymeric film coating (containing also water-insoluble ethylcellulose) might at least in part explain this observation: Eventually, the enzymes cannot freely attack the polysaccharide in the film coating, 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).

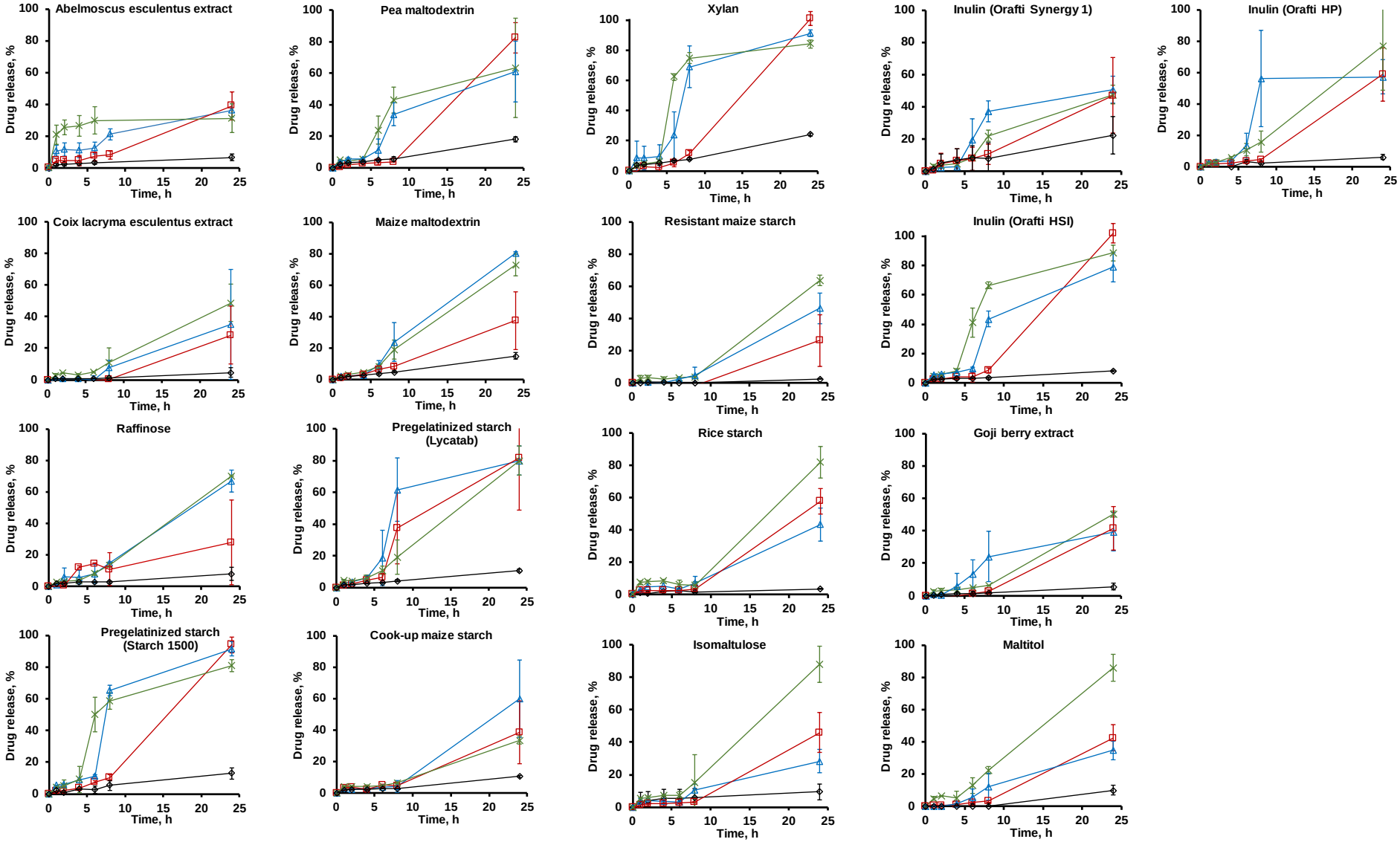

Fig. 5 5-ASA release from pellets coated with different types of polysaccharide: ethylcellulose blends (2:3 w/w blend ratio; 20 % coating level) in culture medium inoculated with fecal samples from IBD patients, IBD rats or dogs (as indicated). For reasons of comparison, also drug release in pure culture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

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 Figure 5, this was not the case for the illustrated polysaccharide: ethylcellulose blends. In certain cases, drug release was much faster in the presence of feces from IBD rats compared to IBD patients (e.g., isomaltulose and rice starch), in others it was the opposite (e.g., pregelatinized starch- Lycatab). In many cases, 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 (Figure 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, or show only limited colon targeting potential in IBD patients.

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: Figure 6 illustrate 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 investigated fecal samples: from IBD rats, healthy dogs and IBD patients. Thus, results observed in the preclinical development 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 probably 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.

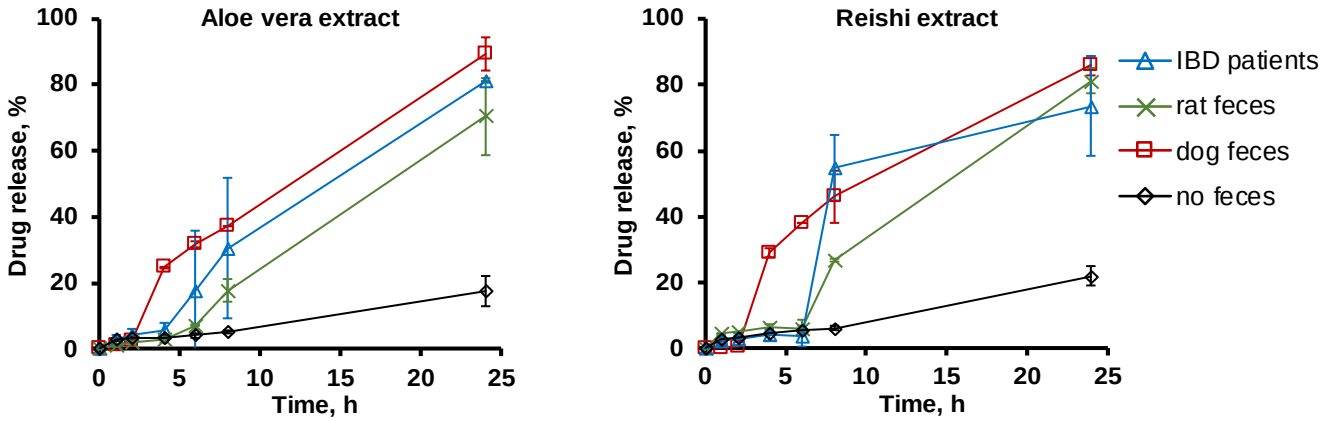

Fig. 6 5-ASA release from pellets coated with aloe vera extract: ethylcellulose blends or reishi extract: ethylcellulose blends (2:3 w/w blend ratio; 20 % coating level) in culture medium inoculated with fecal samples from IBD patients, IBD rats or dogs (as indicated). For reasons of comparison, also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