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Research paper

Colon targeting with bacteria-sensitive films adapted to the disease state

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ABSTRACT

The aim of this study was to identify novel polymeric films allowing for the site-specific delivery of drugs to the colon of patients suffering from inflammatory bowel diseases. Ethylcellulose was blended with different types of bacteria-sensitive starch derivatives. The water uptake and dry mass loss kinetics of the systems were monitored upon exposure to media simulating the contents of the stomach, small intestine and colon (including fresh fecal samples from Crohn's Disease and Ulcerative Colitis patients). Importantly, ethylcellulose:Nutriose FB 06 and ethylcellulose:Peas starch N-735 films showed highly promising water uptake and dry mass loss kinetics in all the investigated media, indicating their potential to minimize premature drug release in the upper gastro-intestinal tract, and allowing for controlled release once the colon is reached. This can be attributed to the fact that the starch derivatives serve as substrates for the enzymes, which are secreted by the bacteria present in the colon of inflammatory bowel disease patients. Thus, the identified new polymeric films are adapted to the pathophysiological conditions in the gastro-intestinal tract in the disease state. Furthermore, Nutriose is known to provide pre-biotic effects, which can be of great benefit for these patients.

1. Introduction

Colon targeting can be very helpful for many pharmaco-therapies, including the treatment of inflammatory bowel diseases, such as Crohn's Disease (CD) and Ulcerative Colitis (UC) [1]. If a locally acting drug is orally administered using a conventional pharmaceutical dosage form, the latter rapidly disintegrates in the contents of the stomach and the drug is released and likely to be absorbed into the blood stream. This leads to elevated systemic drug concentrations and, thus, to an increased risk of undesired side effects and at the same time to low drug concentrations at the site of action in the colon, resulting in poor therapeutic efficiency [2,3]. These restrictions can be overcome if drug release is suppressed in the stomach and small intestine and time-controlled in the colon. This type of site-specific drug delivery to the colon might also offer an interesting opportunity for protein and peptide drugs to get absorbed into the systemic circulation upon oral administration [4].

To allow for colon targeting, the drug can, for instance, be embedded within a polymeric matrix former or drug-loaded tablets or pellets (spherical beads, approximately 0.5–1 mm in diameter) can be coated with a polymeric film [5–7]. In the *upper* gastro-intestinal tract (GIT), the permeability of the polymeric networks for the drug should be low, whereas the macromolecular barriers must become permeable once the colon is reached. This increase in drug permeability of the polymeric networks at the site of action might be induced by: (i) a change in the pH of the contents of the GIT, (ii) a change in the quality and/or quantity of enzymes along the GIT [8–11], or (iii) significant structural changes within the dosage form occurring after a predetermined lag-time (e.g. crack formation in poorly permeable film coatings providing pulsatile drug-release patterns) [12–15]. Interestingly, it has recently been shown in healthy volunteers that bacterially triggered drug delivery systems seem to be more reliable in vivo than pH-triggered devices [16]. Furthermore, a combination of a pH-sensitive and a bacteria-sensitive polymer has been proposed [17]. Alternatively, drug release might already start in the stomach and continue throughout the GIT, at a rate that is sufficiently low to assure that drug is still inside the dosage form once the colon is reached.

However, great care must be taken, because the conditions in the gastro-intestinal tract of patients suffering from inflammatory bowel diseases (e.g. Crohn's Diseases and Ulcerative Colitis) can significantly differ from those in a healthy subject [18]. The intra- and inter-individual variability can be substantial with respect to

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the pH of the GIT contents, types and concentrations of enzyme-secreting bacteria as well as to the transit times within the various GIT segments. For instance, considerable amounts of bifidobacteria are generally present in the colon of healthy subjects and are able to degrade complex polysaccharides due to multiple extracellular glycosidases [19,20]. However, in the disease state, their concentration can be significantly reduced [21,22]. For example, it was shown that fecal glycosidase activity (especially that of β-D-galactosidase) is decreased in patients suffering from Crohn's Disease and that the metabolic activity of the colonic flora is strongly disturbed in the active disease state [23,24]. A review on the alterations of the bacterial flora in patients suffering from inflammatory bowel diseases has been provided by Linskens et al. [25]. Thus, the impact of the *pathophysiology* can be crucial and lead to the failure of the pharmaco-treatment [26]. To avoid such treatment failures, the site-specific drug delivery system must be adapted to the conditions given in the patients' colon. For instance, polymeric film coatings might be used that are degraded by enzymes, which are present in the feces of Crohn's Disease and Ulcerative Colitis patients in sufficient amounts. However, yet it is unclear which type(s) of polymers fulfil(s) these prerequisites.

It was the aim of this study to identify new polymeric film coatings that allow for site-specific drug targeting to the colon *under pathophysiological conditions*. Different types of starch derivatives (being partially acetylated and/or pre-gelatinized) have been studied for this purpose. Starch and starch derivatives are known to offer a great potential for the control of drug release [27,28] and a broad variety of micro-organisms present in the colon secrete enzymes, which are able to digest starch [29]. As the investigated polysaccharides are (partially) water-soluble, a second (water-insoluble) polymer was added: ethylcellulose to avoid premature film dissolution and swelling (and, thus, drug release) in the *upper* GIT [5,9,11]. Ethylcellulose is non-toxic, non-allergenic and non-irritant. Hence, the investigated polymeric networks consist of two compounds: (i) a polysaccharide, which should be preferentially degraded by the enzymes present in the colon of inflammatory bowel disease patients and (ii) ethylcellulose assuring that the film coatings do not spontaneously dissolve and does not significantly swell in the contents of the stomach and small intestine.

2. Materials and methods

2.1. Materials

Nutriose FB 06 [a branched dextrin with non-digestible glycoside linkages: α-1,2 and α-1,3], Peas starch N-735 (peas starch), Lycoat RS 780 (pregelatinized hydroxypropyl starch), Glucidex 1 (a maltodextrin), Eurylon 7 A-PG (an acetylated and pregelatinized high amylose starch), Eurylon 6 A-PG (an acetylated and pregelatinized high amylose starch) and Eurylon HP-PG (a hydroxypropylated and pregelatinized high amylose starch) (Roquette Freres, Lestrem, France); aqueous ethylcellulose dispersion (Aquacoat ECD 30; FMC Biopolymer, Philadelphia, USA); triethylcitrate (TEC; Morflex, Greensboro, USA); pancreatin (from mammalian pancreas = mixture containing amylase, protease and lipase; Fisher Bioblock, Illkirch, France); extract from rat intestine (rat intestinal powder, containing amylase, sucrase, isomaltase and glucosidase; Sigma–Aldrich, Isle d'Abeau Chesnes, France); Columbia blood agar, extracts from beef and yeast as well as tryptone (=pancreatic digest of casein) (Becton Dickinson, Sparks, USA); L-cysteine hydrochloride hydrate (Acros Organics, Geel, Belgium); McConkey agar (BioMerieux, Balme-les-Grottes, France); cysteinated Ringer solution (Merck, Darmstadt, Germany).

2.2. Film preparation

Thin polymeric films were prepared by casting blends of different types of starch derivatives and aqueous ethylcellulose dispersion into Teflon moulds and subsequent drying for 1 d at 60 °C. The starch derivatives were dissolved/dispersed in purified water (5% w/w, Nutriose FB 06: at room temperature, all other starch derivatives: at 65–75 °C). Aqueous ethylcellulose dispersion (15% w/w polymer content) was plasticized for 24 h with 25% TEC (w/w, referred to the solid content of the dispersion). The dissolved/dispersed starch derivative and plasticized ethylcellulose dispersion were blended at room temperature at a ratio of 1:3 (polymer:polymer w:w) and the mixture was stirred for 6 h prior to casting.

2.3. Film characterization

The thickness of the films was measured using a thickness gauge (Minitest 600; Erichsen, Hemer, Germany). The mean thickness of all films was in the range of 300–340 μm. The water uptake and dry mass loss kinetics were measured gravimetrically upon exposure to:

(i) simulated gastric fluid (0.1 M HCl),
(ii) simulated intestinal fluid [phosphate buffer pH 6.8 (USP 30) with or without 1% pancreatin or 0.75% extract from rat intestine],
(iii) culture medium inoculated with feces from healthy subjects,
(iv) culture medium inoculated with feces from inflammatory bowel disease patients,
(v) culture medium free of feces for reasons of comparison.

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 hydrate in 1 L distilled water (pH 7.0 ± 0.2) and subsequent sterilization in an autoclave. Fresh feces of patients with Crohn's Disease or Ulcerative Colitis as well as fresh feces of healthy subjects were diluted 1:200 with cysteinated Ringer solution; 2.5 mL of this suspension was diluted with culture medium to 100 mL in order to facilitate bacteria proliferation under the given in vitro conditions. Film pieces of 1.5 × 5 cm were placed into 120 mL glass containers filled with 100 mL pre-heated medium, followed by horizontal shaking at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). The incubation with fecal samples was performed under anaerobic conditions (5% CO_2, 10% H_2 and 85% N_2). At predetermined time points, samples were withdrawn and rinsed with water. Excess water was removed by careful blotting with Kimtech precision wipes (Kimberly Clark, Roswell, GA). The films were accurately weighed (wet mass) and dried to constant weight at 60 °C (dry mass). The water content (%) and dry film mass (%) at time t were calculated as follows:

$$\text{water content } (\%)(t) = \frac{\text{wet mass}(t) - \text{dry mass}(t)}{\text{wet mass}(t)} \cdot 100\% \quad (1)$$

$$\text{dry film mass } (\%)(t) = \frac{\text{dry mass}(t)}{\text{dry mass}(t=0)} \cdot 100\% \quad (2)$$

2.4. Bacteriological analysis

For the bacteriological analysis of fecal samples, the latter were diluted 1:10 with cysteinated Ringer solution. Eight further tenfold dilutions in cysteinated Ringer solution were prepared, and 0.1 mL of each dilution was plated onto non-selective, modified Columbia blood agar [30] (for total cultivable counts) and McConkey agar (being selective for enterobacteria). Columbia blood agar plates

were incubated during 1 week at 37 °C under anaerobic conditions (5% CO_2, 10% H_2 and 85% N_2). Colonies were outnumbered, and the predominant colonies were subcultured and identified based on phenotypic identification criteria [30]. McConkey agar plates were incubated during 48 h at 37 °C in air. The colonies were outnumbered and identified using the API 20E system (BioMerieux, Balme-les-Grottes, France). Counts were expressed as log CFU/g (Colony Forming Units per gram) of fresh feces.

For the bacteriological analysis of the microflora developed upon *film incubation with fecal samples*, photomicrographs were taken after Gram staining with an Axiostar plus microscope (Carl Zeiss, Jena, Germany), equipped with a camera (Unit DS-L2, DS camera Head DS-Fi 1; Nikon, Tokyo, Japan). Incubation was performed in a glucides-free culture medium containing only small amounts of polypeptides (thus, favoring the use of the investigated polysaccharides as substrates) under *anaerobic* conditions.

3. Results and discussion

3.1. Film properties in the upper GIT

The permeability of a polymeric system for a drug strongly depends on its water content and dry mass, which determine the density and mobility of the macromolecules [31]. For instance, in dry hydroxypropyl methylcellulose (HPMC)-based matrix tablets, the apparent diffusion coefficient of a drug approaches zero, whereas in a completely hydrated HPMC gel, diffusivities can be reached, which are in the same order of magnitude as in aqueous solutions [32]. With increasing water content, the macromolecular mobility significantly increases and, thus, the free volume available for diffusion [33]. In some systems, the polymer undergoes a glassy-to-rubbery phase transition as soon as a critical water content is reached. This leads to a significant, stepwise increase in polymer and drug mobility. Thus, the water content of a polymeric film coating can give important insight into the macromolecular mobility and, hence, permeability for a drug. Fig. 1a and b show the water uptake kinetics of thin films consisting of various types of starch derivative:ethylcellulose blends in 0.1 N HCl and phosphate buffer pH 6.8, respectively. The presence of ethylcellulose in all films allows avoiding premature dissolution in the *upper* GIT. The investigated starch derivatives are (partially) water-soluble and aim at providing the sensitivity of the coatings' drug permeability to the surrounding environment: once the colon is reached, the starch derivatives are to be enzymatically degraded and drug release to be started. The starch derivative:ethylcellulose blend ratio in Fig. 1 is constant: 1:3. Clearly, the water uptake *rates*

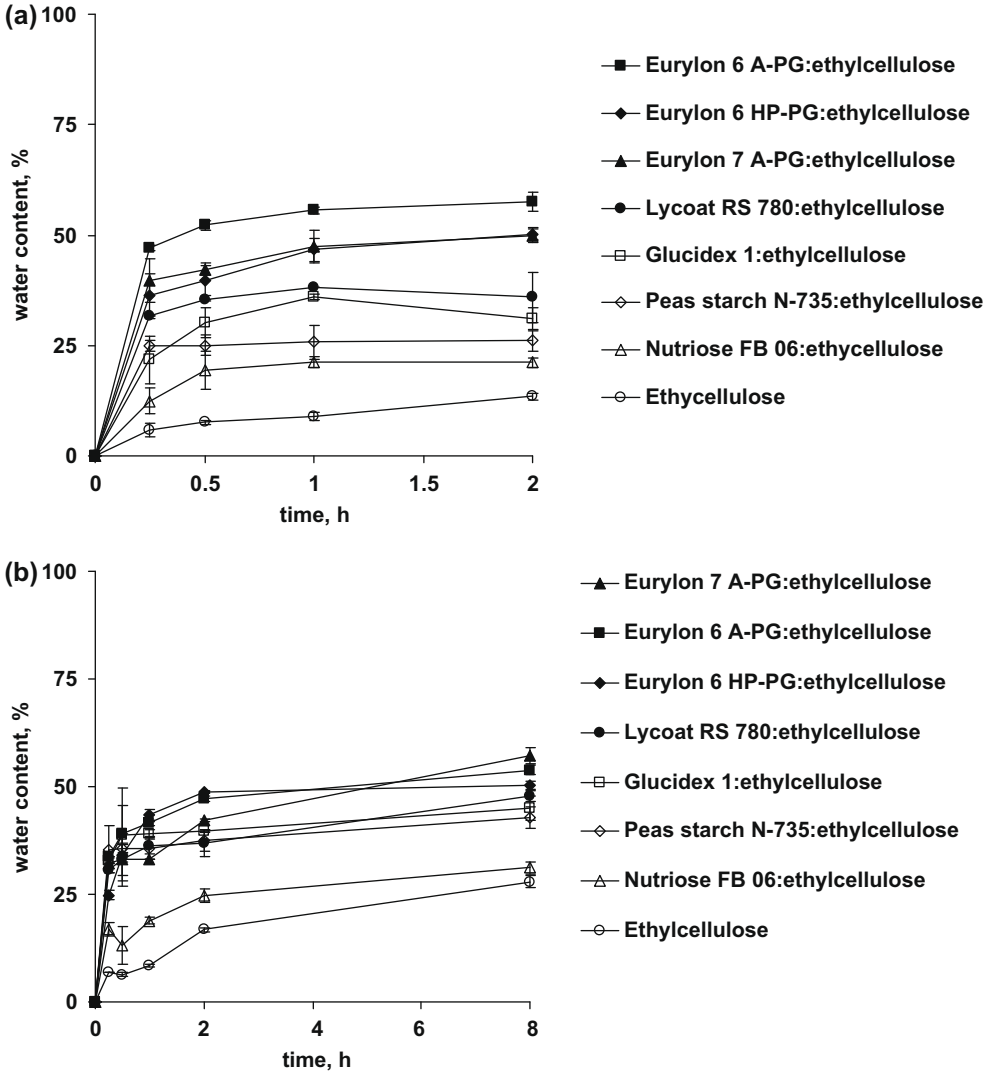

Fig. 1. Water content of thin films consisting of different types of polymer blends (indicated in the figures) upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.

and *extents* significantly depend on the type of starch derivative. The ideal film coating allowing for colon targeting should take up only small amounts of water at a low rate in both media in order to prevent premature drug release in the *upper* GIT. As it can be seen, blends of ethylcellulose and Nutriose FB 06 or Peas starch N-735 are most promising for this purpose. Polymeric coatings with similar water uptake kinetics have been shown to effectively minimize drug release at appropriate coating levels [34]. Plasticized ethylcellulose films without (partially) water-soluble polysaccharide take up only low amounts of water (empty circles).

In addition to the water uptake kinetics also, the *dry mass loss behavior* of thin polymeric films serves as an indicator for the coatings' permeability for the drug [34–36] and, hence, potential to suppress premature release within the *upper* GIT. If the films loose significant amounts of dry mass upon exposure to the release media, the coatings can be expected to become permeable for many drugs, in particular, those with a low molecular weight such as 5-aminosalicylic acid (5-ASA, 153.1 Da). Fig. 2a and b illustrate the experimentally determined dry mass loss of thin films consisting of various starch derivative:ethylcellulose blends (constant ratio = 1:3) upon exposure to 0.1 N HCl and phosphate buffer pH 6.8, respectively. The ideal film looses only minor amounts of dry mass at a low rate (or no mass at all), assuring dense polymeric networks which are poorly permeable for the incorporated drug under these conditions. As it can be seen, the dry mass loss of Peas starch N-735- and Nutriose FB 06-containing films is very low, even after up to 8 h exposure to these release media. The observed decrease in dry mass can at least partially be attributed to the leaching of the water-soluble plasticizer triethyl citrate (TEC, used to plasticize the aqueous ethylcellulose dispersion) into the bulk fluid. In addition, parts of the starch derivative might leach out of the films. Pure (plasticized) ethylcellulose films loose only very small amounts of dry mass, irrespective of the type of release medium (empty circles). The permeability of intact ethylcellulose films is known to be very low for many drugs [34,35], which can at least partially be attributed to the low water uptake and dry mass loss rates and extents of these systems. For this reason, intact ethylcellulose films are also used as moisture protective coatings. Please note that the loss of the water-soluble plasticizer TEC into the bulk fluids can be expected to be much more pronounced in films containing 25% (w/w) (partially) water-soluble polysaccharides compared to pure (plasticized) ethylcellulose films, because the

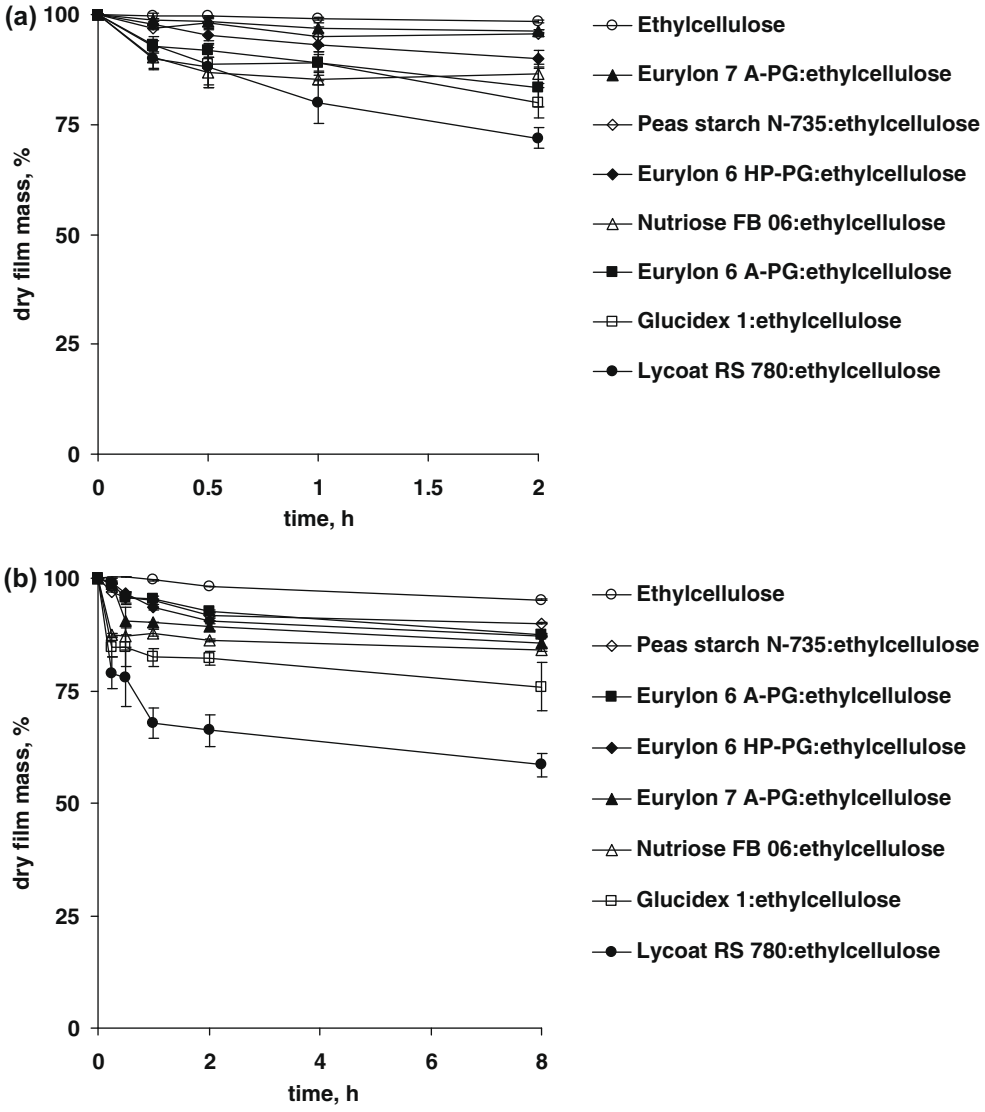

Fig. 2. Dry mass of thin films consisting of different types of polymer blends (indicated in the figures) upon exposure to: (a) 0.1 M HCl, and (b) phosphate buffer pH 6.8. Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.

increased water uptake rates and extents (Fig. 1) of the blended systems lead to much higher polymer chain mobility and, thus, also increased TEC mobility.

It has to be pointed out that the results shown in Fig. 2 were obtained in the *absence* of any enzymes. It is well known that pancreatic enzymes can degrade certain polysaccharides and, thus, potentially induce significant mass loss and water uptake under *in vivo* conditions, resulting in increased film permeability for the drug. To clarify the importance of this phenomenon, the water uptake kinetics and dry mass loss behavior of the thin films were also measured in the *presence* of pancreatin (=mixture containing amylase, protease and lipase) and of an extract from rat intestine (containing amylase, sucrase, isomaltase and glucosidase) in phosphate buffer pH 6.8 (Fig. 3). Clearly, the addition of these enzymes did not significantly affect the resulting water uptake and dry mass loss kinetics of the investigated films. Thus, the latter do not serve as substrates for these enzymes under these conditions.

3.2. Film properties in the colon

Once the colon is reached, the polymeric film coatings should become permeable for the drug; this can, for instance, be induced by (partial) enzymatic degradation. Importantly, the concentrations of certain enzymes are much higher in the colon than in the *upper* GIT. This includes enzymes, which are produced by the natural microflora of the colon (this part of the GIT contains much more bacteria than the stomach and small intestine) [19,20]. However, great caution must be paid when using this type of colon targeting approach, because the microflora of patients suffering from inflammatory bowel diseases can be significantly different from the microflora of healthy subjects. Thus, the drug delivery system must be adapted to the disease state of the patient. Table 1 shows, for instance, the concentrations of the bacteria determined in the fecal samples of the healthy subjects as well as of the Crohn's Disease and Ulcerative Colitis patients included in this study. Importantly, there were significant differences, in particular with respect to the concentrations of *Bifidobacterium* (being able to degrade complex polysaccharides due to multiple extracellular glycosidases) and *Escherichia coli*, which were present at much higher concentrations in the feces of healthy subjects compared to the feces of the inflammatory bowel disease patients. In contrast, the fecal samples of the Crohn's Disease and Ulcerative Colitis patients contained *lactose-negative E. coli, Citrobacter freundii, Klebsiella pneumoniae, Klebsiella oxytoca and Enterobacter cloacae*, which were not detected in healthy subjects. Thus, there are fundamental differences in the *quality* and *quantity* of the microflora, which must be taken into account. Polymeric film coatings, which allow for colon targeting under physiological conditions in a healthy volunteer, might fail under the pathophysiological conditions in the disease state of a patient. To address this very crucial point, which is generally neglected, the water uptake and dry mass loss of thin films consisting of various types of starch derivative:ethylcellulose blends were determined upon exposure to fecal samples from Crohn's Disease and Ulcerative Colitis patients as well as to the feces of healthy subjects and to pure culture medium for reasons of comparison (Fig. 4). Appropriate films should take up considerable amounts of water and show significant dry mass loss upon exposure to patients' feces in order to induce drug release at the site

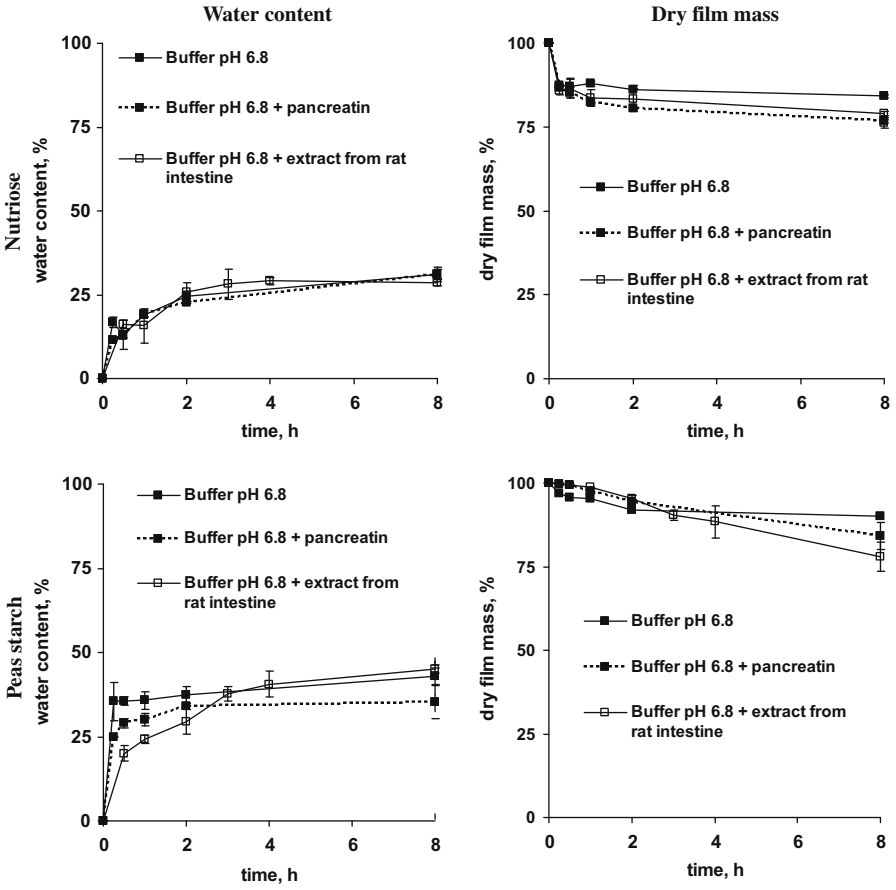

Fig. 3. Water uptake kinetics and dry mass loss kinetics of thin films consisting of Nutriose FB 06:ethylcellulose or Peas starch N-735:ethylcellulose upon exposure to pure phosphate buffer pH 6.8, phosphate buffer pH 6.8 containing pancreatin, or phosphate buffer pH 6.8 containing extract from rat intestine (as indicated).

Table 1
Concentrations of bacteria [log CFU/g] in the investigated fecal samples of healthy subjects and inflammatory bowel disease patients.

	Healthy subjects	Crohn's Disease	Ulcerative Colitis
Number	10	11	5
Mean age	40+/−15	32+/−12	36+/−20
Columbia blood agar			
Mean total counts [log UFC/g]	9.88+/−0.48	9.15+/−1.30	9.88+/−0.57
Number of strains	28	34	14
Mean	2.8	3.1	2.8
Anaerobes			
Bacteroides	9	10	3
Prevotella	2	2	2
Fusobacterium	3	3	2
Veillonella	0	0	1
Clostridium	0	5	1
Bifidobacterium	9	3	1
Other Gram + rods	3	2	2
Gram + cocci	1	2	0
Aerobes			
Enterobacteria	1	3	2
Escherichia coli	1	2	1
Citrobacter freundii	0	1	1
Lactobacillus	0	2	0
Streptococcus	0	2	0
McConkey agar			
Mean counts	6.30+/−1.19	7.16+/−1.48	8.01+/−1.06
Number of strains	10	14	7
Escherichia coli	10	6	4
E. coli lac-	0	1	0
Citrobacter freundii	0	3	1
Klebsiella pneumoniae	0	1	1
Klebsiella oxytoca	0	2	0
Enterobacter cloacae	0	1	0
Other Gram – rods	0	0	1

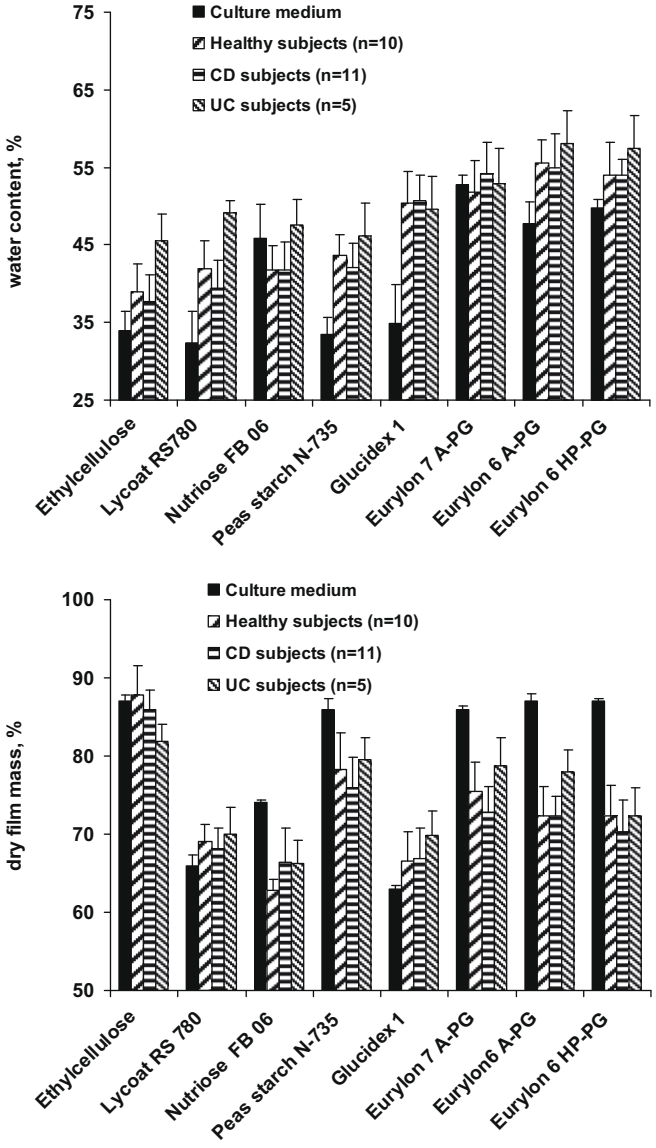

Fig. 4. Water content and dry mass of thin films consisting of different types of polysaccharides blended with ethylcellulose upon exposure to culture medium, culture medium inoculated with feces of healthy subjects and culture medium inoculated with feces of Crohn's Disease (CD) patients and Ulcerative Colitis (UC) patients (as indicated in the figures). Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.

of inflammation in the colon. As it can be seen in Fig. 4, films based on ethylcellulose: Nutriose FB 06 and ethylcellulose:Peas starch N-735 (which are the two most promising types of polymer blends based on the above described results obtained in media simulating the contents of the *upper* GIT) show pronounced water uptake and dry mass loss upon exposure to the feces of Crohn's Disease patients and Ulcerative Colitis patients as well as of healthy subjects. The fact that no major difference was observed between inflammatory bowel disease patients and healthy subjects might serve as an indication for the suitability of these films not only in the disease state, but also under physiological conditions. Most importantly, the enzymes present in the disease state are able to degrade these polymers. Thus, colon targeting approaches based on this type of polymeric films are adapted to the disease state. It has to be pointed out that also other types of polymer blends look promising with respect to the presented films' water uptake and dry mass loss behavior upon exposure to fecal samples (or even more appropriate than ethylcellulose:Nutriose FB 06 and ethylcellulose:Peas starch N-735 blends). However, these systems already take up considerable amounts of water and remarkably loose in dry mass upon contact with media simulating the contents of the *upper* GIT (Figs. 1 and 2).

The fact that the investigated polymeric films serve as substrates for the bacteria in feces from inflammatory bowel disease patients could be further confirmed by the analysis of the microflora developed upon film exposure to fecal samples under anaerobic conditions at 37 °C (Fig. 5). Clearly, specific types of bacteria proliferated upon incubation with the blended films. Importantly, this phenomenon can be expected to be highly beneficial for the ecosystem of the GIT of the patients in the disease state, normalizing the microflora in the colon. This very positive, pre-biotic effect comes in addition to the drug targeting effect. Biological samples incubated without any polymeric films or with pure (plasticized) ethylcellulose films showed much less bacterial growth (Fig. 5).

4. Conclusions

Novel polymeric film coatings for colon targeting have been identified, which are adapted to the disease state of the patients. Importantly, low water uptake and dry mass loss *rates* and *extents* in media simulating the contents of the *upper* GIT can be combined with elevated water uptake and dry weight loss upon contact with feces from inflammatory bowel disease patients. Changes in the composition of the flora in the colon of patients indicate that these polysaccharides serve as substrates for colonic bacteria in the *disease state* and are likely to exhibit beneficial effects on the ecosystem of the GIT of the patients. The obtained new knowledge, thus, provides the basis for the development of novel polymeric film

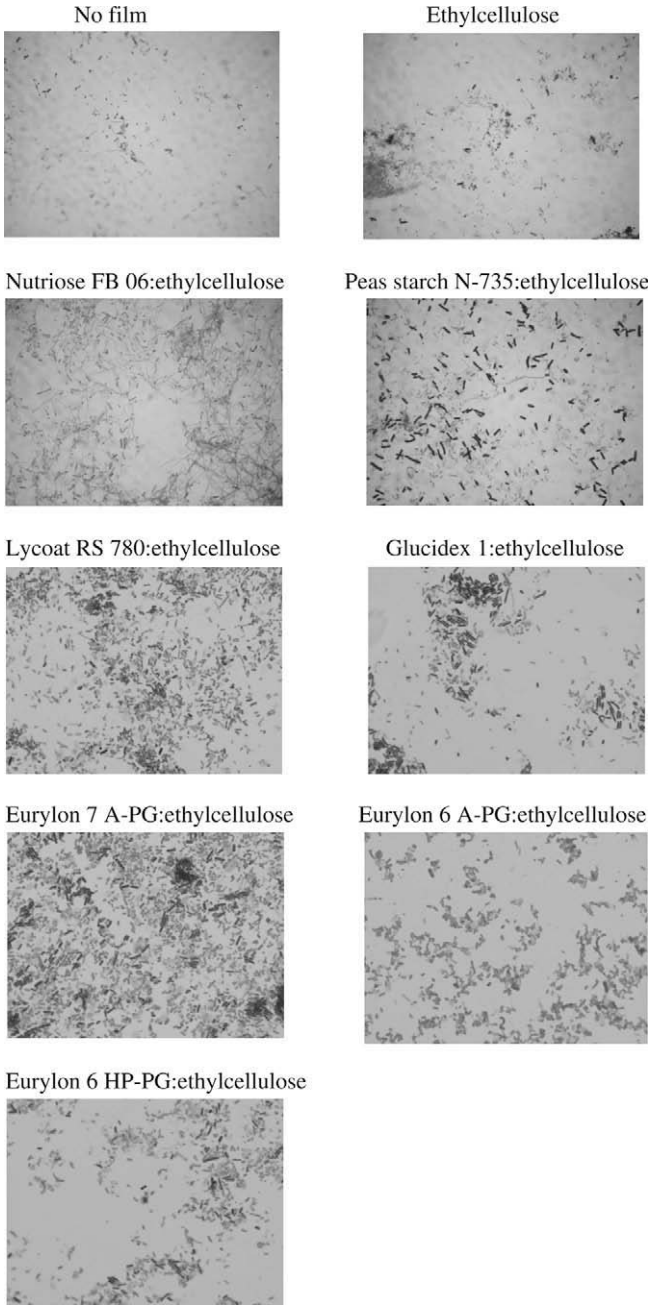

Fig. 5. Pictures of the microflora developed upon incubation of thin, polymeric films of different composition (indicated in the figure) with fecal samples of inflammatory bowel disease patients.

coatings able to deliver drugs specifically to the colon. Importantly, these polymeric barriers are adapted to the conditions at the target site in the disease state.

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