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Research Paper

Enzymatically degraded Eurylon 6 HP-PG: ethylcellulose film coatings for colon targeting in inflammatory bowel disease patients

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Abstract

Objectives Film coatings based on blends of Eurylon 6 HP-PG (a hydroxypropylated and pregelatinized high amylose starch) and ethylcellulose were to be evaluated as promising coating materials for site-specific drug delivery to the colon of patients suffering from inflammatory bowel diseases.

Methods Pellet starter cores containing 60% 5-aminosalicylic acid were prepared by extrusion/spheronization and coated with different Eurylon 6 HP-PG : ethylcellulose blends at various coating levels. Drug release was measured in media simulating the contents of the upper gastrointestinal tract (in the presence and absence of enzymes) as well as in media simulating the contents of the colon.

Key findings 5-Aminosalicylic acid release could effectively be suppressed in 0.1 N HCl and phosphate buffer pH 6.8, optionally containing pepsin or pancreatin, but occurred as soon as the pellets came into contact with culture medium inoculated with faecal samples from inflammatory bowel disease patients. This can be attributed to the partial degradation of the starch derivative by enzymes secreted by bacteria present in the colon of these patients.

Conclusions The presented drug delivery system is adapted to the pathophysiological conditions in inflammatory bowel disease patients. Furthermore, drug release remained unaltered upon 1 year open storage.

Keywords colon; colon targeting; controlled release; film coating; starch derivative

Introduction

The site-specific delivery of a drug to the colon can offer major advantages for a pharmacotherapy.[1–5] This includes, for example, improvement of local treatments of inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. The suppression of premature drug release in the upper part of the gastrointestinal tract (GIT) avoids drug absorption into the systemic circulation and thus minimizes undesired side-effects. Once the colon is reached, the drug is released, ideally in a time-controlled manner, in order to ensure optimized drug concentration–time profiles at the site of action, leading to improved therapeutic effects.[6] Different strategies have been reported in the literature aiming at site-specific drug delivery to the colon.[7,8] Generally, the drug is embedded within a matrix former (matrix systems),[9] or a drug reservoir is surrounded by a film coating (reservoir devices).[10] Immediate drug release upon contact with aqueous body fluids is avoided by the presence of the matrix former/film coating. The latter should be insoluble and impermeable for the drug in the stomach and small intestine, but should dissolve or become permeable as soon as the target site is reached.[11,12] Often, polymers are used as matrix formers and film coating materials.[13–15] To provide the required site-specific system properties, one of the following four approaches is generally used. (1) The polymer exhibits pH-dependent solubility: it is water insoluble at low pH but becomes water soluble at neutral pH values (e.g. poly(acryl methacrylates)). The drawback of these systems is the fact that the

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pH of the contents of the GIT of an IBD patient can significantly vary from day to day and be very different from physiological pH values.[16] Thus, intra- and intervariability of product performance might be considerable.[17] (2) The dosage form is surrounded by a polymeric film, which is impermeable for the drug as long as the film is intact. Upon contact with aqueous body fluids, water penetrates into the system and creates a steadily increasing hydrostatic pressure, which acts against the film coating. After a predetermined lag time, the film coating cannot withstand the increasing mechanical stress and crack formation occurs.[18,19] The drug is subsequently released through the newly created, water-filled channels.[20–22] The lag time prior to drug release should be chosen in such a way that the release occurs as soon as the colon is reached. However, the transit times in the various GIT segments can significantly vary in a patient suffering from Crohn's disease or ulcerative colitis, as well as from patient to patient. Diarrhoea can significantly accelerate GIT transit. (3) The dosage form releases the drug right from the beginning but at a rate that is sufficiently low to ensure that drug release still continues once the colon is reached. Pentasa is an example of a commercial product based on this principle (ethylcellulose coated pellets). Again, variable transit times can affect the performance of the system. Furthermore, parts of the drug are prematurely released in the upper GIT. (4) The polymer is degraded by enzymes secreted by bacteria of the colonic microflora. The high concentrations of these bacteria in the colon allow for the site specificity of the release.[23–25] Nevertheless, great care should be taken, because the microflora in the colon of an IBD patient might be very different from that in a healthy subject (in quality and quantity).[26–28] Often, the concentration of bacteria is reduced compared with a healthy subject. Thus, the polymer should be degraded by enzymes that are present in the disease state in sufficient amounts.

In this study, a colon-targeting approach based on specific enzymatic degradation (strategy 4 above) was investigated. To ensure that the respective enzymes are indeed present in the colon of IBD patients and at appropriate concentrations, a particular starch derivative was used for film coating: Eurylon 6 HP-PG, a hydroxypropylated and pregelatinized high amylose starch. This polysaccharide has recently been reported to serve as substrate for the bacteria present in faecal samples from Crohn's disease and ulcerative colitis patients.[29] It was the aim of this work to evaluate the suitability of Eurylon 6 HP-PG based film coatings to allow for site-specific drug delivery to the colon. As this polysaccharide significantly swells in water at 37°C, it was blended with ethylcellulose (being water-insoluble, poorly swellable and poorly permeable for many drugs throughout the GIT).[14,30–33] 5-Aminosalicylic acid (5-ASA) was chosen as model drug, because it is frequently used for the local treatment of IBD.[34–36]

Materials and Methods

Materials

Eurylon 6 HP-PG, a hydroxypropylated and pregelatinized high amylose starch, was obtained from Roquette Freres (Lestrem, France). Aquacoat ECD 30 (aqueous ethylcellulose dispersion) was from FMC Biopolymer (Brussels, Belgium). Triethylcitrate (TEC) was from Morflex (Greensboro, NC, US). 5-ASA (average particle size 10 μm) was from Sigma-Aldrich (Isle d'Abeau, Chesnes, France). Microcrystalline cellulose (Avicel PH 101) was from FMC Biopolymer. Bentonite and polyvinylpyrrolidone (Povidone K 30; PVP) were from Cooperation Pharmaceutique Francaise (Melun, France). Pancreatin (from mammalian pancreas; mixture of amylase, protease and lipase) and pepsin were from Fisher Bioblock (Illkirch, France). Extracts from beef and yeast as well as tryptone (pancreatic digest of casein) were from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). L-Cysteine hydrochloride hydrate was from Acros Organics (Geel, Belgium). Cysteinated Ringer solution was from Merck (Darmstadt, Germany).

Preparation of thin polymeric films

Thin polymeric films were prepared by casting blends of different amounts of Eurylon 6 HP-PG and aqueous ethylcellulose dispersion into Teflon moulds and subsequent drying for 1 day at 60°C. Eurylon 6 HP-PG was dispersed in purified water at 65–75°C (5% w/w). Aqueous ethylcellulose dispersion (15% w/w solids content) was plasticized for 24 h with 25% TEC (w/w, referred to as the solid content of the dispersion) under stirring. The Eurylon 6 HP-PG and plasticized ethylcellulose dispersions were blended at room temperature in the following ratios: 1 : 2, 1 : 3, 1 : 4 and 1 : 5 (polymer/polymer, w/w). The mixtures were stirred for 6 h prior to casting.

Film characterization

The thickness of the films was measured using a thickness gauge (Minitest 600; Erichsen, Hemer, Germany). The mean thickness of the films was over the range of 300–340 μm. The dry mass loss kinetics of the films was measured gravimetrically upon exposure to phosphate buffer pH 6.8 (USP 32), optionally containing 1% pancreatin as follows: pieces of 1.5 cm × 5 cm were placed into 120-ml plastic containers filled with 100 ml pre-heated medium, followed by horizontal shaking at 37°C (80 rev/min, GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, samples were withdrawn and dried to constant weight at 60°C (dry mass). The dry film mass (%) at time t was calculated as follows:

$$\text{dry film mass}\,(\%)(t) = \frac{\text{dry mass}\,(t)}{\text{dry mass}\,(t=0)} \times 100 \qquad (1)$$

Preparation of drug loaded starter cores

5-ASA loaded pellet starter cores (diameter: 0.7–1.0 mm; 60% 5-ASA, 32% microcrystalline cellulose, 4% bentonite, 4% PVP) were prepared via extrusion/spheronization. The respective powders were blended in a high-speed granulator (Gral 10; Collette, Antwerp, Belgium) and purified water was added until a homogeneous mass was obtained. The wetted mixture was passed through a cylinder extruder (SK M/R, holes: 1 mm diameter, 3 mm thickness, rotation speed: 96 rev/min; Alexanderwerk, Remscheid, Germany). The extrudates were subsequently spheronized at 520 rev/min for 2 min

(Spheronizer Model 15; Calveva, Dorset, UK) and dried in a fluidized bed (ST 15; Aeromatic, Muttenz, Switzerland) at 40°C for 30 min. The size fraction 0.7–1.0 mm was obtained by sieving.

Pellet coating

5-ASA loaded pellet starter cores were coated with aqueous ethylcellulose dispersion containing different amounts of Eurylon 6 HP-PG. The coating formulations were prepared in the same way as the dispersions used for film casting (described above). Pellets were coated in a fluidized bed coater, equipped with a Wurster insert (Strea 1; Aeromatic-Fielder, Bubendorf, Switzerland) until a weight gain of 5, 10, 15 or 20% (w/w) was achieved. The process parameters were as follows: inlet temperature 39 ± 2°C, product temperature 40 ± 2°C, spray rate 1.5–3 g/min, atomization pressure 1.2 bar, nozzle diameter 1.2 mm. Afterwards, the pellets were further fluidized for 10 min and subsequently cured in an oven for 24 h at 60°C.

Drug release measurements

5-ASA release from pellets (25 mg) was measured under sink conditions using media simulating the conditions in the upper GIT and the entire GIT.

Upper GIT

Pellets were placed into 120-ml plastic containers, filled with 100 ml dissolution medium: 0.1 N HCl (optionally containing 0.32% pepsin) during the first 2 h and phosphate buffer pH 6.8 (USP 32) (optionally containing 1% pancreatin) during the subsequent 9 h. The flasks were agitated in a horizontal shaker (80 rev/min; GFL 3033). At predetermined time points, 3-ml samples were withdrawn and analysed by UV-spectrophotometry for their drug content ($\lambda = 302.6$ nm in 0.1 N HCl; $\lambda = 330.6$ nm in phosphate buffer pH 6.8) (Shimadzu UV-1650; Shimadzu France, Champs sur Marne, France). In the presence of enzymes, the samples were centrifuged for 15 min at 6800*g* and subsequently filtered (0.2 μm) prior to UV measurements. Each experiment was conducted in triplicate. Alternatively, drug release was measured using the USP Apparatus 3 (Bio-Dis; Varian, Paris, France): pellets were placed into 250-ml vessels filled with 200 ml 0.1 N HCl. The dipping speed was 5 or 10 dips/min. After 2 h, the pellets were transferred into 200 ml phosphate buffer pH 6.8 (USP 32) and agitated at 5 or 10 dips/min for 9 h. At predetermined time points, 3-ml samples were withdrawn and analysed by UV-spectrophotometry as described above.

Entire GIT

Pellets were exposed to 0.1 N HCl for 2 h and subsequently to phosphate buffer pH 6.8 (USP 32) for 9 h in a USP Apparatus 3 (Bio-Dis; dipping speed: 10 dips/min). Then, the pellets were transferred into 120-ml flasks, filled with: (i) 100 ml culture medium inoculated with faeces from IBD patients; (ii) culture medium inoculated with a mixture of *Bifidobacterium*, *Bacteroides* and *Escherichia coli*; (iii) culture medium inoculated with *Bifidobacterium*; or (iv) culture medium free of faeces and bacteria for comparison. The samples were agitated (50 revs/min) at 37°C under anaerobic conditions (5% CO_2, 10% H_2, 85% N_2). 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. Faeces of patients (1 g) suffering from Crohn's disease or ulcerative colitis ($n = 5$) was diluted 1 : 200 with cysteinated Ringer solution; 2.5 ml of this suspension was diluted with culture medium to 100 ml. At predetermined time points, 2-ml samples were withdrawn, centrifuged at 9500*g* for 5 min, filtered (0.22 μm) and analysed by high-performance liquid chromatography for their drug content (ProStar 230; Varian, Paris, France). The mobile phase consisted of 10% methanol and 90% of an aqueous acetic acid solution (1% w/v).[31] Samples were injected into a Pursuit C18 column (150 × 4.6 mm; 5 μm); the flow rate was 1.5 ml/min. The drug was detected by UV-spectrophotometry at $\lambda = 300$ nm.

Storage stability

Coated pellets were stored in open glass vials at room temperature (23 ± 2°C) and ambient relative humidity (55 ± 5%) for 1 year. Drug release from the pellets was measured before and after 1 year of storage as described above.

Statistical analysis

Since experiments were conducted in triplicate, classical statistical methods could be used to assert the conclusions. The response variable was always a percentage (of 5-ASA release, mainly or dry film mass). The time was considered as a continuous covariable. Other categorical variables (e.g. presence/absence of enzymes, coating level, blend ratio) were taken as fixed factors. Statistical methods encompassed either slope comparisons within a log linear regression framework or the analysis of covariance scheme, when fixed factors were involved. Such a parametric framework was vindicated by the sufficient amount of data (many files exceeded a hundred observations) and by a residual analysis. When necessary, log transforms were performed.

Results and Discussion

Dry mass loss in media simulating the upper GIT

In order to avoid premature drug release in the stomach and small intestine, a polymeric film coating allowing for colon targeting should be poorly permeable for the incorporated drug upon exposure to media simulating the conditions in the upper GIT. Generally, the permeability of a polymeric film increases if the latter loses significant amounts of dry mass upon contact with the release medium. The decrease in film mass often leads to less dense macromolecular structure, which offers less hindrance for drug diffusion. Hence, the dry mass loss of a polymeric film aiming at colon targeting should be limited upon exposure to 0.1 N HCl and phosphate buffer pH 6.8 in the absence as well as in the presence of enzymes of the upper GIT. Importantly, the 'target-region sensitive' compound in the investigated film coatings (the starch derivative Eurylon 6 HP-PG, which is degraded by bacterial enzymes present in the colon of IBD patients) swells significantly in water at 37°C. Hence, film coatings containing significant amounts of this compound are likely to become highly

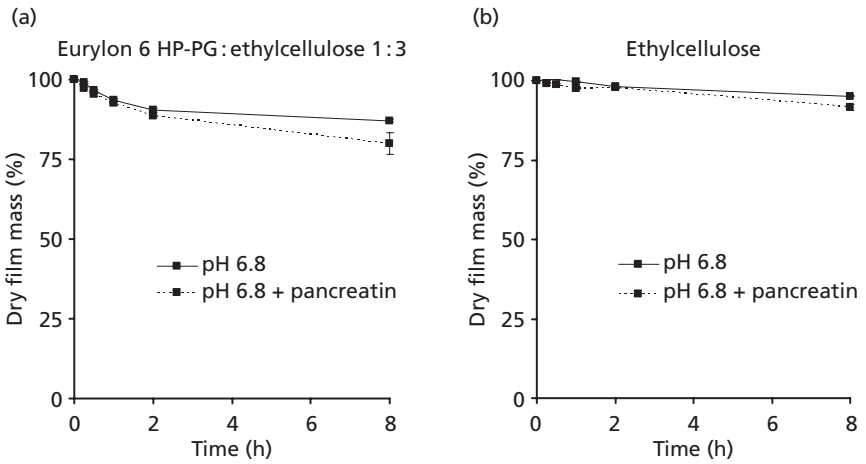

Figure 1 Dry mass loss kinetics of thin films consisting of Eurylon 6 HP-PG : ethylcellulose 1 : 3 or pure (plasticized) ethylcellulose upon exposure to phosphate pH 6.8 or phosphate buffer pH 6.8 containing 1% pancreatin.

permeable for small, water-soluble molecules, leading to premature drug release under conditions simulating the upper GIT. In order to avoid this, Eurylon 6 HP-PG was blended with ethylcellulose, which is water-insoluble and poorly swellable in aqueous media. The addition of ethylcellulose is also intended to limit the leaching of water-soluble film compounds into the surrounding bulk fluids. As can be seen in Figure 1a, this strategy worked very well in the investigated systems. The mass loss of thin Eurylon 6 HP-PG : ethylcellulose 1 : 3 films upon exposure to phosphate buffer pH 6.8 is shown as an example. For comparison, the dry mass loss of pure (plasticized) ethylcellulose films upon exposure to phosphate buffer pH 6.8 is also shown (Figure 1b). Clearly, the dry mass loss is not zero despite the water-insolubility of this polymer, because the water-soluble plasticizer can (at least partially) leach out into the release medium.[22,37]

It has to be pointed out that the presence of pancreatin *in vivo* might significantly increase the extent and the rate of the dry mass loss of a polymeric film due to polymer degradation (and subsequent diffusion of degradation products into the bulk fluid). In order to estimate the impact of such enzymatic degradation, 1% pancreatin was added to the phosphate buffer. As can be seen in Figure 1a, the dry mass loss of the investigated Eurylon 6 HP-PG : ethylcellulose 1 : 3 films increased under these conditions. Comparing the slopes of the log-transformed curves gave a significant *P value* of 1.4×10^{-6} (the value was 2×10^{-8} for Figure 1b). The films lost 20% of their initial dry mass upon 8 h exposure to phosphate buffer pH 6.8 containing 1% pancreatin. Thus, the presence of 25% of the starch derivative in the film coating is likely to result in too rapid drug release in the upper GIT at commonly used coating levels.

Drug release in media simulating the upper GIT

Figure 2 shows the release of 5-ASA from pellets coated with Eurylon 6 HP-PG : ethylcellulose blends into 0.1 N HCl and phosphate buffer pH 6.8 (without enzymes). The coating level was varied from 5 to 20%, the polymer/polymer blend ratio from 1 : 2 to 1 : 5, as indicated. Clearly, drug release was too rapid at all coating levels for the Eurylon 6 HP-PG : ethylcellulose blend ratios 1 : 2 and 1 : 3. This can at least partially be attributed to the dry mass loss of the film coatings (Figure 1a), resulting in increased permeability for the drug. As expected, the release rate decreased with increasing ethylcellulose content (because ethylcellulose is water-insoluble and poorly permeable for the drug) and with increasing coating level (because of the increasing length of the diffusion pathways). Importantly, undesired 5-ASA release into 0.1 N HCl and phosphate buffer pH 6.8 could effectively be limited at a coating level of 15 and 20% at the Eurylon 6 HP-PG : ethylcellulose blend ratios 1 : 4 and 1 : 5 (Figure 2). Thus, in these cases, the polymeric networks remain sufficiently dense and are sufficiently thick to effectively hinder drug diffusion into the bulk fluid during the observation period.

As the dry mass loss of thin Eurylon 6 HP-PG : ethylcellulose films significantly increased in the presence of upper GIT enzymes (Figure 1a), it was important to measure 5-ASA release also in the presence of: (i) 0.32% pepsin in 0.1 N HCl; and (ii) 1% pancreatin in phosphate buffer pH 6.8. In Figure 2, it can be seen that the drug release rate significantly increased in the presence of these enzymes at high and intermediate Eurylon 6 HP-PG contents and intermediate coating levels (covariance analysis showed a significant enzyme effect on the percentage of released 5-ASA, with time taken as a continuous covariate); at low coating levels, drug release was rapid in all cases. However, this phenomenon had only limited impact at a coating level of 15 and 20% for the Eurylon 6 HP-PG : ethylcellulose blend ratios 1 : 4 and 1 : 5. For instance, less than 7% 5-ASA was released after 2 h exposure to 0.1 N HCl containing 0.32% pepsin, followed by 9 h exposure to phosphate buffer pH 6.8 containing 1% pancreatin in the case of Eurylon 6 HP : ethylcellulose 1 : 4 blends and a coating level of 20%. Thus, under these conditions the presence of ethylcellulose can effectively avoid significant degradation of the starch derivative by enzymes present in the upper GIT.

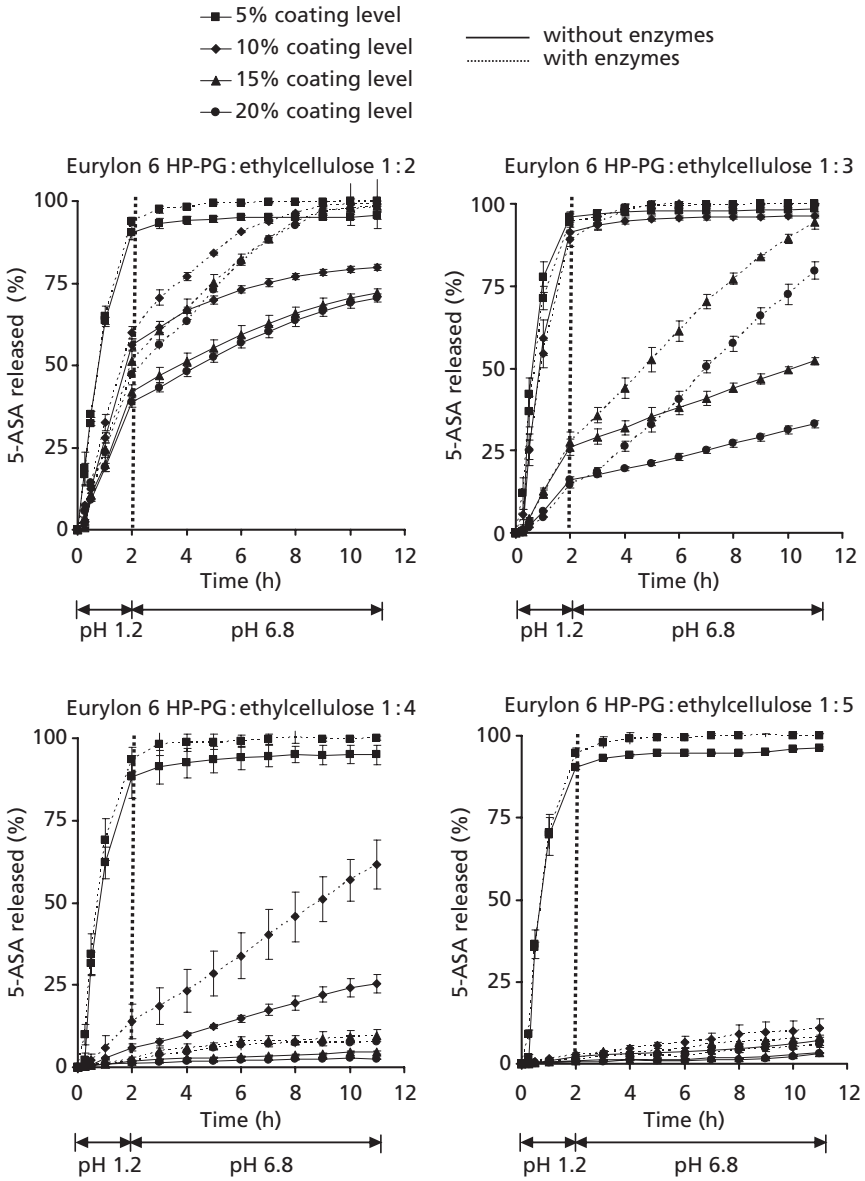

Figure 2 Effects of the Eurylon 6 HP-PG : ethylcellulose blend ratio and coating level on 5-aminosalicylic acid (5-ASA) release from pellets under conditions simulating the transit through the upper gastrointestinal tract. Release medium: 0.1 N HCl for 2 h, followed by phosphate buffer pH 6.8 for 9 h. Solid/dotted lines indicate the absence/presence of upper gastrointestinal tract enzymes (0.32% pepsin at low pH, 1% pancreatin at high pH).

It has to be pointed out that all the experiments shown in Figures 1 and 2 were conducted in flasks that were horizontally agitated at 80 rev/min (at 37°C). Thus, the mechanical stress the pellet coatings were exposed to due to bulk fluid agitation during drug release was limited. *In vivo*, this stress might be more important in the upper GIT (due to the motility of the stomach and small intestine). If the film coating is not sufficiently mechanically stable, crack formation can occur, resulting in rapid release through water-filled channels.[21,37] In order to evaluate the sensitivity of the presented film coatings to an increase in the mechanical stress exerted by the bulk fluid, drug release from the pellets was also studied using the USP Apparatus 3 (Bio-Dis) at a dipping speed of 5 and 10 dips/min. Figure 3 shows the resulting 5-ASA release rates from pellets coated with 15 or 20% Eurylon 6 HP-PG : ethylcellulose 1 : 4. The release medium was 0.1 N HCl during the first 2 h, followed by phosphate buffer pH 6.8 during the subsequent 9 h. For comparison, drug release in flasks horizontally shaken at 80 rev/min is also illustrated. As can be seen, the resulting drug release patterns were not significantly affected by the type and degree of agitation, irrespective of the coating level. The same was true for pellets coated with 10, 15 or 20% Eurylon 6 HP-PG : ethylcellulose 1 : 5 (data not shown). Thus, undesired crack formation and subsequent premature drug release in the upper GIT *in vivo* is unlikely from this type of formulation.

Drug release in media simulating the entire GIT

Based on the results obtained under conditions simulating the upper GIT, 5-ASA loaded pellets coated with 15 and 20%

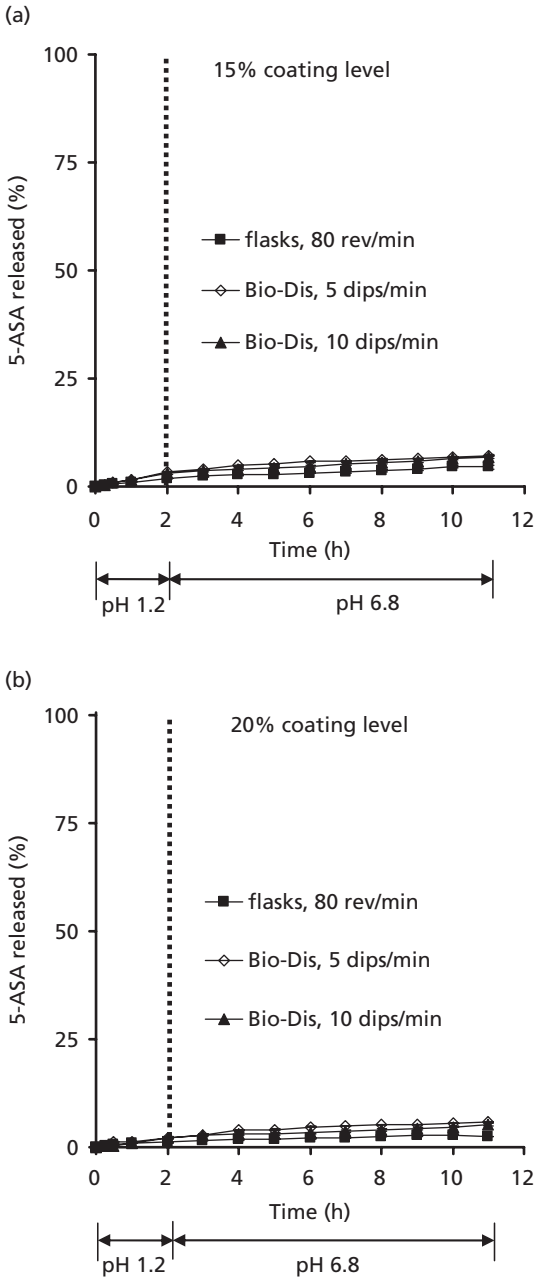

Figure 3 5-Aminosalicylic acid (5-ASA) release from pellets coated with Eurylon 6 HP-PG : ethylcellulose 1 : 4 at a coating level of 15 or 20%, under conditions simulating the transit through the upper gastrointestinal tract using a USP 3 Apparatus (Bio-Dis). The release medium was 0.1 N HCl during the first 2 h, followed by 9 h phosphate buffer pH 6.8. The dipping speed was 5 or 10 dips/min. For comparison, drug release in agitated plastic flasks (horizontally shaken at 80 rev/min) is also illustrated.

Eurylon 6 HP-PG : ethylcellulose 1 : 4 and 1 : 5 were chosen for further experiments. Once the colon of patients suffering from IBD is reached, the polymeric film coatings should become permeable for the drug and provide time-controlled release. When measuring drug release *in vitro*, ideally culture medium inoculated with fresh faecal samples from IBD patients should be used to simulate the contents of the colon,

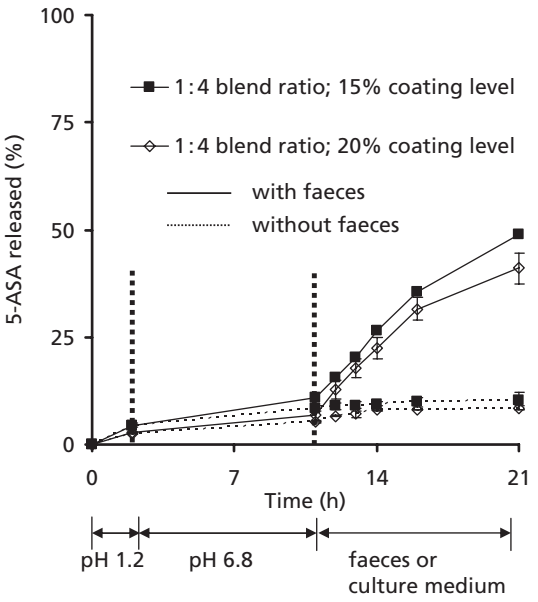

Figure 4 5-Aminosalicylic acid (5-ASA) release from pellets coated with Eurylon 6 HP-PG : ethylcellulose 1 : 4 under conditions simulating the transit through the entire gastrointestinal tract, in the presence and absence of faeces from inflammatory bowel disease patients. The coating level was 15 or 20%.

especially the given microflora.[10] This is particularly important if drug release is triggered by enzymatic degradation. Furthermore, the pellets should be exposed to bulk fluids simulating the transit through the upper GIT prior to exposure to a medium simulating the contents of the colon. The composition and properties of the film coatings might significantly change (e.g. dry mass loss, crack formation, water uptake), resulting in altered drug release kinetics. Figure 4 shows the experimentally measured release of 5-ASA from pellets coated with 15 and 20% Eurylon 6 HP-PG : ethylcellulose 1 : 4 into: (i) 0.1 N HCl (during the first 2 h); (ii) phosphate buffer pH 6.8 (during the following 9 h); and (iii) culture medium inoculated under anaerobic conditions with faeces from IBD patients (during 10 h). For comparison, drug release into culture medium free of faeces is also illustrated. Clearly, undesired drug release in the upper GIT is effectively minimized, whereas 5-ASA release occurs as soon as the pellets come into contact with the medium simulating the contents of the colon of IBD patients. This can be attributed to the, at least partial, degradation of Eurylon 6 HP-PG by enzymes secreted by the bacteria present in the faeces of patients suffering from ulcerative colitis and Crohn's disease.[29] This site-specific film degradation renders the coatings much more permeable for the drug, resulting in time-controlled 5-ASA release. Importantly, this phenomenon does not occur in culture medium free of faeces. A significant difference was found at both coating levels between the release patterns in the presence and absence of faeces (P values: 2×10^{-20} and 2×10^{-15}). Thus, drug release from this type of pellet only occurs once the target site is reached and the system is adapted to the conditions in the colon of the patients. The observed decrease in the drug release rate with time under conditions simulating

the transit within the colon might, at least partially, be attributable to the limited viability of the bacteria under the given conditions.

Potential alternative colonic release media

The use of fresh faecal samples from IBD patients is obviously a very suitable way to simulate the conditions to which the pellets are exposed at the target site, in particular with respect to the quality and quantity of the given microflora. However, in practice it is not straightforward to ensure a regular supply with these types of samples, which cannot be deep-frozen or freeze-dried without significant damage. Thus, for routine applications (e.g. quality control during production) the application of this test is challenging. Nevertheless, drug release in the colon is one of the key features of this type of advanced delivery system. Thus, it is highly desirable to provide alternative release media, which are more readily accessible and appropriately simulate the critical properties of the contents of the colon. If drug release from the dosage form is triggered by enzymatic degradation (with enzymes secreted by the bacteria present in the colon), it is of major importance to ensure that the decisive types of microorganisms are present at appropriate concentrations in the release medium. For these reasons, two types of bulk fluids have been tested as potential alternatives to culture medium inoculated with fresh faecal samples from IBD patients: (i) culture medium inoculated with a cocktail of *Bifidobacterium*, *Bacteroides* and *E. coli* (because these bacteria are present in significant amounts and are able to ferment carbohydrates); and (ii) culture medium inoculated with *Bifidobacterium*. Figure 5a shows the experimentally measured in-vitro release kinetics of 5-ASA from pellets coated with 15 and 20% Eurylon 6 HP-PG : ethylcellulose 1 : 4 and 1 : 5 into 0.1 N HCl (for 2 h), phosphate buffer pH 6.8 (for 9 h) and culture medium inoculated under anaerobic conditions with a cocktail of *Bifidobacterium*, *Bacteroides* and *E. coli* (for 10 h). Clearly, drug release was minimized in the media simulating the upper GIT, and occurred as soon as the pellets came into contact with the bacterial cocktail. Comparing Figures 5a and 4, it becomes obvious that the proposed combination of bacteria is likely to be a suitable substitute for fresh faecal samples from IBD patients for routine use. Comparing the slopes at both coating levels yielded high *P* values (40 and 48%), indicating no statistically significant difference. Figure 5b shows the respective 5-ASA release kinetics upon exposure to 0.1 N HCl, phosphate buffer pH 6.8 and culture medium inoculated (under anaerobic conditions) only with *Bifidobacterium*. Comparing Figures 4, 5a and 5b, it can be seen that the use of the bacterial cocktail seems to better mimic the fresh faecal samples from patients than the single-bacterium inoculum, but the latter can give some valuable indication of whether or not drug release is likely to occur in the colon. Moreover, there was no statistical difference between the three release patterns at a coating level of 20%.

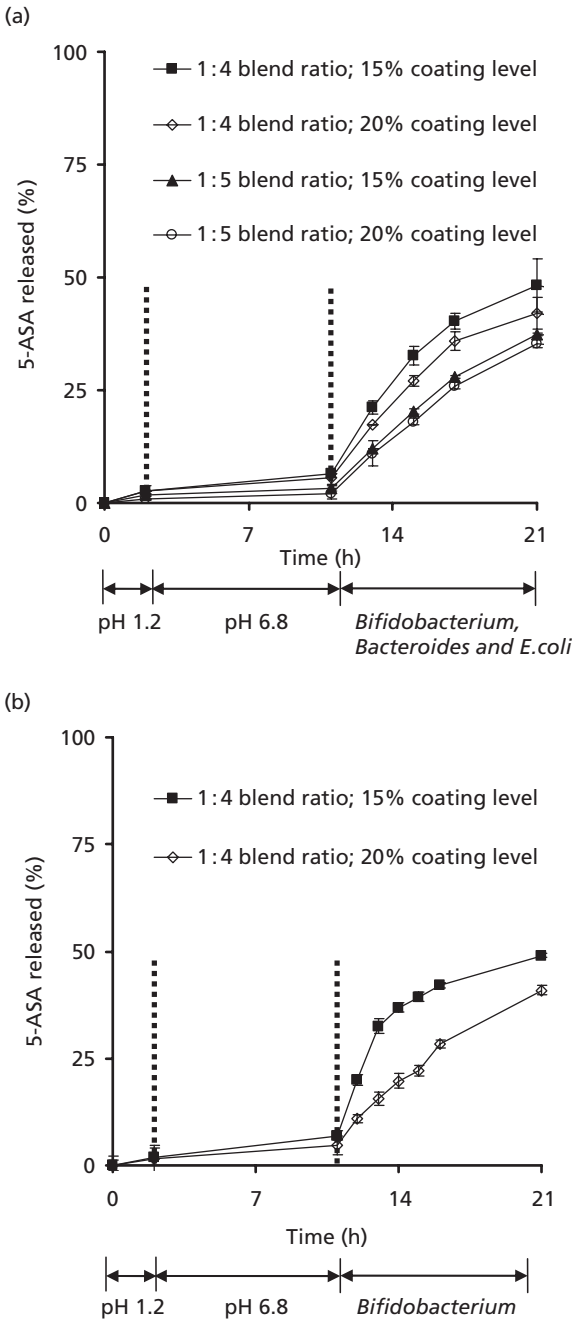

Figure 5 Potential alternatives to culture medium inoculated with fresh faecal samples from inflammatory bowel disease patients simulating the conditions in the colon. 5-Aminosalicylic acid (5-ASA) release from pellets coated with Eurylon 6 HP-PG : ethylcellulose blends into 0.1 N HCl (for 2 h) and phosphate buffer pH 6.8 (for 9 h), followed by culture medium inoculated with a cocktail of bacteria (*Bifidobacterium*, *Bacteroides* and *Escherichia coli*) or culture medium inoculated with *Bifidobacterium* (for 10 h). The coating level was 15 or 20%.

Longterm stability

When using polymeric film coatings to control drug release from a dosage form, it is important to determine the longterm stability of these delivery systems. This is particularly true for film coatings applied from aqueous dispersions, because incomplete film formation upon coating and/or curing might lead to further polymer particle coalescence during storage, resulting in decreasing drug release rates with increasing storage time.[38] Figure 6 shows the release profiles of 5-ASA from pellets coated with 15 or 20% Eurylon 6

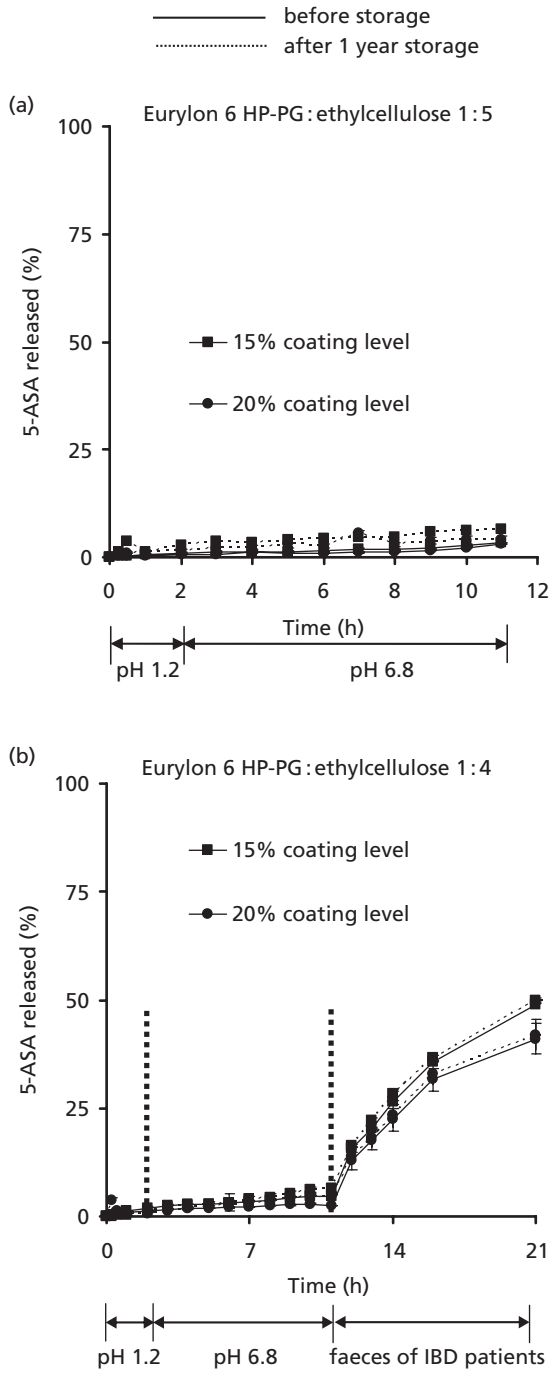

Figure 6 Longterm storage stability of pellets coated with Eurylon 6 HP-PG : ethylcellulose 1 : 4 or Eurylon 6 HP-PG : ethylcellulose 1 : 5. Drug release before and after 1 year storage (no packaging material) is indicated. The pellets were exposed to 0.1 N HCl for 2 h and to phosphate buffer pH 6.8 for 9 h to simulate the transit through the upper gastrointestinal tract. After 1 year storage, the pellets were subsequently exposed to culture medium inoculated with fresh faecal samples from inflammatory bowel disease (IBD) patients for 10 h.

HP-PG : ethylcellulose 1 : 4 or 1 : 5 before and after 1 year open storage (no packaging material) upon exposure to 0.1 N HCl (for 2 h), followed by phosphate buffer pH 6.8 (for 9 h) and culture medium inoculated with fresh faecal samples from IBD patients (for 10 h). Importantly, either drug release was negligible or, if it was not (Figure 6 upon exposure to media simulating the contents of the colon), no significant difference was observed between the release patterns before and after 1 year storage (*P* values: 41 and 40% at 15 and 20% coating levels, respectively). Thus, this type of dosage form seems to be stable during longterm storage, which is an important prerequisite for its practical use.

Conclusions

The proposed film coatings show a promising potential for colon targeting under the pathophysiological conditions in IBD patients. Drug release is effectively minimized in media simulating the conditions in the upper GIT, and occurs as soon as the pellets come into contact with culture medium inoculated with faeces from IBD patients. Importantly, drug release remained unaltered upon 1 year open storage. Furthermore, a cocktail of *Bifidobacterium*, *Bacteroides* and *E. coli* is a promising potential alternative to fresh faecal samples from IBD patients for routine in-vitro drug release measurements under simulated colonic conditions.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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