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ORIGINAL ARTICLE

Influence of the coating formulation on enzymatic digestibility and drug release from 5-aminosalicylic acid pellets coated with mixtures of high-amylose starch and Surelease® intended for colon-specific drug delivery

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Abstract

Background: Colon-specific delivery of drugs can be achieved with dosage forms coated with biopolymers that are metabolized selectively by the colonic microflora and yet resistant to enzymatic digestion in the small intestine. *Aim*: The aim of this study was to study the influence of formulation factors on the performance of mixed films from high-amylose starches and Surelease®, applied using a spray-coating process, as potential colon-specific delivery devices. *Methods*: 5-Aminosalicylic acid-loaded pellets were prepared by an extrusion-spheronization process and film coated with mixtures of the starches and Surelease®. Optimization of the coating formulation, that is, starch-to-Surelease® ratio, film-coating thickness, and type of starch, was undertaken first in enzyme-free media resembling the conditions in the stomach and small intestine. The effect of curing of the film coating on the drug release profile upon storage was also evaluated. Optimized coating formulations were further assessed for enzymatic digestibility using artificial gastric and intestinal juices containing commercially available pepsin and pancreatin or α-amylase from hog pancreas, respectively. Finally, drug release was assessed in fluid-simulating conditions in the colon (SCF) containing *Bacillus licheniformis* α-amylase. *Results*: Film coatings comprising high-amylose starches and Surelease® in a ratio of 1:2 (w/w) and film thickness of approximately 45 μm were able to withstand the chemical and enzymatic environment of the upper gastrointestinal tract, in particular, resisted degradation by the pancreatic α-amylases. Stability of the coatings during storage was achieved with additional curing. In SCF, these coatings were susceptible to enzymatic degradation. *Conclusions*: This study showed that high amylose starch-mixed films can be successfully used as colon-specific delivery devices. The preparation of the coating dispersions described is simple and rapid, without the need to extract the amylose component of starch.

Key words: *α-Amylases; colon-specific delivery; film coating; high-amylose starch; in vitro drug release; pellets*

Introduction

Colon drug delivery systems were first proposed to overcome the boundaries associated with the oral treatment of diseases of the colon, such as inflammatory bowel diseases as reviewed elsewhere[1]. The colon targeting of proteins and therapeutic peptides, known to experience degradation in the acidic environment of the stomach[2] and/or enzymatic degradation in the small intestine[3], has also been proposed.

Colon drug delivery systems must ensure that the drug release is minimal during the transit of the dosage form through the stomach and small intestine and yet that the drug is released once these systems are in the

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colon. The earliest proposed colon drug delivery systems exploited pH variations along the gastrointestinal (GI) tract[4], the transit time of dosage forms[5,6], or the combination of both[7,8]. Despite their simplicity and ease of manufacturing, these systems lack specificity, resulting in high fluctuation of the level of drug at the site of drug release[9,10].

More specific systems can be achieved with the use of biopolymer-based dosage forms. The mechanism responsible for drug release is the specific breakdown of these compounds by the colonic microflora. The biodegradable polymers, mainly polysaccharides, can be part of the coating or a matrix-forming material. Coated systems (pellets, tablets) are preferred as much higher drug load can be achieved. In vitro investigations to find the polysaccharides that provide the best resistance to the environment of the upper intestines and that are specifically metabolized in the colon were undertaken. These include pectin[11-13], guar gum[14], chitosan[15], inulin[16], amylose[17], psyllium[18], and konjac glucomannan[19].

Coating compositions that have been shown in vivo to function effectively, based on the so-called glassy form of amylose, are known[20]. Although 'glassy' amylose has been used in various coating compositions, it must first be extracted from starch into a butanol complex and converted into a glassy or amorphous form, resistant to the pancreatic α-amylases, all of which require lengthy and complex processing. Amylose as such or as butanol complex acceptable for the use in medicinal products is not commercially available. Hence, there remains a need for coating compositions that provide specific release in the colon and that can be simply and readily prepared.

Recent investigations on colon targeting[21-23] have been mainly focused on the use of starch-based matrices and, in particular, modified starches. Modification of starches is used to suppress the natural swelling ability of the starch materials and/or to reduce their propensity to undergo degradation by the pancreatic α-amylases. The work by Chen et al.[24] investigated the use of acetylated starches as film-coating materials for the colon-specific delivery of proteins.

In our previous study[25], we found that the heat treatment of commercially available, pharmaceutically acceptable high-amylose starches at 80 ± 5°C, using well-defined time spans of heating, aqueous dispersions with 86% (w/w) of water and drying, yields starches in a crystalline, retrograded form as seen in X-ray and Fourier-transform infrared (FTIR) studies. In the presence of different amylases resembling in vivo conditions, these treated starches were able to resist digestion by pancreatic α-amylases. Free films were prepared[26] from aqueous dispersions using the heat treatment described before. The important difference of this process compared with the 'glassy' amylose approach mentioned above is that the starches were used as received, that is, not subjected to any pretreatment to isolate the amylose component of the starch. To enhance the film-forming properties and reduce the swelling of the starch films in aqueous media, a pre-plasticized ethylcellulose aqueous dispersion, Surelease®, was added. Physicochemical characterization of the starch/Surelease®-free films showed that the two polymers were immiscible, and no interactions could be detected between them when using modulated differential scanning calorimetry and FTIR studies. 5-Aminosalicylic acid (5-ASA)-loaded pellets coated with these coating dispersions were tested in artificial juices simulating the gastric and the small intestine conditions. The 5-ASA release in these media was insignificant attesting the resistant character of these treated starches. Thus, films comprising these resistant starches might show potential as colon-delivery devices.

The aim of this study was to investigate the influence of coating formulation factors on the enzymatic digestibility and 5-ASA release from pellets coated with the above-defined coating dispersions when using a spray-coating process. High-amylose maize starches used were Hylon® VII and Hylon® V with amylose contents of 69% and 56%, respectively[27], obtained from maize hybrid plants, as well as IM-DS acetate starch, which is an acetylated form of Hylon® VII with a degree of substitution of 1.5 and an amylose content of 71%. Optimization of the coating formulation, that is, starch-to-Surelease® ratio, film thickness, and type of starch, was undertaken first in enzyme-free media. Coating stability upon storage was monitored. The optimized coatings were then tested in simulated gastric and intestinal fluids containing various α-amylases to assess their digestibility, in particular, digestion taking place in the environment of the colon, and their potential for colon drug delivery.

Materials and methods

Materials

Hylon® VII (batch number FG 5514), Hylon® V (batch number BJ 9960), acetate maize starch with a degree of substitution of 1.5 (IM-DS acetate starch, batch number 78-0469), and low-amylopectin maize starch (LAPS, batch number 374964) were donated by the National Starch & Chemical Company (Bridgewater, NJ, USA). The amylose contents of these starches are 69%, 56%, 71%, and 95%, respectively. Amylopectin (batch number 9561E) was supplied by ICN Biomedicals Inc. (Aurora, OH, USA).

Surelease® E-7-7050 (Surelease® E-7-7050, batch number 600092) was a gift from Colorcon Ltd. (Kent,

UK). This product is plasticized with dibutyl sebacate and the total nominal solids content is 25% (w/w), 70% of which is ethylcellulose.

5-ASA was supplied by Avocado Research Chemicals, Ltd., Lancaster, UK (batch number J 3433 B), and Avicel PH 101 was obtained from FMC Corporation, Cork, Ireland (batch number 6842C). Pancreatin (batch number 105K0689, EC number 232-468-9) with an activity equivalent to at least the USP specifications was supplied by Sigma Chemicals Co. (St. Louis, MO, USA). Hog pancreas α-amylase with an activity of 53.2 units/mg (batch number 10080, EC number 232-565-6) was purchased from Fluka Biochemika GmbH (Buchs, Switzerland). *Bacillus licheniformis* α-amylase was supplied by Sigma Chemicals Co. as an aqueous suspension designed Type XII-A with 15% sodium chloride and 25% sucrose (batch number 025K1132, EC number 232-560-9). Its activity as determined by the Biuret method was 21 mg/mL (786 units/mg protein). For all enzymes used, one unit is defined as the amount of enzyme liberating 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20°C.

Sodium taurocholate hydrate (batch number 10111536) was supplied by Avocado Research Chemicals Ltd.

Preparation of 5-ASA-loaded pellets

Pellets containing 50% of the model drug, 5-ASA, and microcrystalline cellulose (Avicel® PH 101) were produced by an extrusion-spheronization process. The wet mass comprising 5-ASA, Avicel® PH101 (50:50), and distilled water (40%, w/w, based on the total weight of the dry mass) were mixed for 15 minutes in a planetary mixer (Kenwood Chef, model A901E; Kenwood, Croydon, UK) at a minimum speed setting. The wet mix attained was extruded through a radial screen extruder (model 10; G. B. Caleva Ltd., Extruder, Dorset, UK) with a screen mesh size of 1-mm diameter and thickness. Finally, the extrudate was spheronized for 10 minutes on a 228-mm diameter plate with cross-hatch geometry rotating at a speed setting of 1000 rpm (K.A. Geeves & Sons Ltd., UK). The final pellets were dried on a porous tray at room temperature for a period of 48 hours and sieved to obtain pellets with a size range of 1.00–1.40 mm.

Preparation of starch and starch–Surelease® aqueous coating dispersions

Approximately 0.001 g of sodium fluorescein (Sigma-Aldrich; batch number 113K0112) was added to all coating dispersions and mixed to achieve complete dissolution. This was necessary to be able to measure the film-coating thickness using a laser-scanning confocal microscope (LSCM) as described under Section *Determination of the film thicknesses with the laser-scanning confocal microscope*.

Hylon® VII aqueous dispersions

Aqueous dispersions of Hylon® VII were prepared as follows: Hylon® VII was dispersed in water in a ratio of 14:86 (w/w) and heated at 80 ± 5°C for 30 minutes. The dispersion was plasticized with 10% (w/w) dibutyl sebacate corresponding to the dry weight of the starch. Different coating thicknesses were achieved by varying the amount of starch in the coating dispersion.

Maize starch–Surelease® aqueous dispersions

The dispersions prepared as described above were allowed to cool down and mixed with the commercially available pre-plasticized aqueous ethylcellulose dispersion (Surelease® E-7-7050). Complete dispersion of the two polymers was achieved by mixing for a further 30 minutes at 70°C in magnetic stirrer thermostat hotplate (Gallenkamp, Loughborough, UK).

Different ratios of the high-amylose maize starch–Surelease® dispersions were prepared varying from 1:2 to 1:5, corresponding to the dry weight of both polymers. Within each ratio, different film-coating thicknesses were obtained by varying the amount of the two polymers in the coating dispersion.

Surelease® dispersions

Surelease®-coating dispersions were prepared by admixing the same volume of distilled water as used for the preparation of the high-amylose starch–Surelease® dispersions at 70°C for 30 minutes using a magnetic stirrer thermostat hotplate (Gallenkamp).

Film coating of the 5-ASA pellets

Batches of 30 g of 5-ASA pellets were coated in a Uni-Glatt fluidized bed Wurster column (6206; Dresden, Germany) with a bottom spray nozzle with a diameter of 1 mm and a 8-cm diameter perforated bottom plate. The coating operation conditions were carefully optimized resulting in an inlet temperature of 60°C, spray rate of 0.7–0.8 mL/min, outlet temperature of 45–50°C, product temperature of 43–47°C, air velocity 2–4 m/s, and atomizing air pressure of 2.2–2.4 bar. Following coating, the pellets were dried for 10 minutes in the coater at 60°C. Selected batches were further cured at 60°C in an oven for 1 hour.

Determination of the film thicknesses with the laser-scanning confocal microscope

The film-coating thickness was determined with an LSCM fitted with an Omnichrome-Ion laser power supply model 171 Krypton laser with a LaserPhysics model 150 m, argon

laser (Omnichrome Series 43, model 643R-NORN-E0; Omnichrome Corp., Chino, CA, USA), Acousto-optic tunable filter controller (VisiTech International, Sunderland, UK), Hamamatsu camera controller (model C4642-80-12AG; Hamamatsu Photonics K.K., Hamamatsu City, Japan), Nikon Eclipse TE2000-U inverted microscope (model T-DH; Nikon Corp., Tokyo, Japan), and a confocal head (Hamamatsu camera, model C4642-80-12AG; Hamamatsu Photonics K.K.). The software used was VoxCell (version 3.82; VisiTech International). The laser intensity was adjusted to its maximum level during the procedure and two sets of filters (488 and 568 nm) were used. A total of 10 pellets per batch were analyzed. Pellets were carefully cut in half using a razor blade and the film thickness measured at four equidistant points. The average thickness in micrometer was recorded. The calibration of the equipment was performed with a graticule (100 $\times$ 0.01 = 1 mm; Graticules Ltd., Tonbridge, UK, CS 809) for each single pellet tested.

In vitro drug release studies in enzyme-free media

In vitro drug release studies were undertaken according to the USP method II (paddle method) in an Erweka DT 6R (Erweka, Heusenstamm, Germany). All tests were conducted in triplicate using 900 mL of dissolution media maintained at 37 ± 0.5°C with a paddle rotation speed of 100 rpm. Coated and uncoated formulations were first tested in enzyme-free simulated gastric fluid (pH 1.2), and Sörensen phosphate buffer (pH 7.2), over a period of 8 hours. Samples were withdrawn at predetermined intervals (10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, and 480 minutes), diluted 1/5, and the absorbance measured by UV (Cecil CE 594, Cambridge, UK) at 304 and 330 nm for the simulated gastric fluid and Sörensen phosphate buffer, respectively.

The mean dissolution time (MDT), area under the dissolution curve (AUC), and the relative dispersion coefficient (RDC) of the MDT were calculated and used to characterize the drug dissolution profiles for both uncoated and coated formulations.

In vitro drug release testing with enzymes

Selected formulations were further assessed in the presence of enzymes. Samples were filtered through cellulose nitrate membranes with a pore size of 0.2 μm (Whatman International Ltd., Maidstone, UK) and were analyzed for drug release as described previously. All the solutions containing enzymes were freshly prepared every day. Simulated gastric fluid was prepared according to the British Pharmacopoeia[28]. Simulated intestinal fluid (SIF) was prepared from phosphate buffer (pH 7.2) by adding 0.5 g of sodium taurocholate hydrate and 10.0 g of pancreatin per final volume of 1000 mL. The ability of the film formulations to resist digestion by mammalian α-amylases over a period of 8 hours was further confirmed with α-amylase from Hog pancreas origin (250 units/mL) dissolved in phosphate buffer (pH 7.2).

The enzymatic environment of the colon was simulated with the use of *B. licheniformis* α-amylase in concentrations of 50, 250, and 500 units/mL, in phosphate buffer (pH 7.2).

Scanning electron microscopy

Scanning electron microscopy (SEM) pictures of the surface of the pellets coated with different coating compositions were taken before and after the dissolution tests in order to establish the effect of the enzymes on the coating. The pellets were fixed onto specimen stubs by means of double-sided carbon conductive adhesive strips and vacuum coated with a standard mixture of gold and platinum in a sputter coater (Polaron SC7620; Quorum Technologies, Newhaven, UK). An approximate coating thickness of 11.5–14.5 nm was used.

Images were taken with a Hitachi S-3000N scanning electron microscope (Polaron SC7620; Quorum Technologies) with an emission of 5 kV and a magnification of ×500.

Results and discussion

Drug release in dissolution media simulating the upper GI tract

An ideal coating intended for colon targeting must prevent drug release under conditions resembling the stomach and small intestine. Drug release from dosage forms coated with starch/Surelease® films in the upper GI tract can be attributed to two main factors, that is, (1) extreme swelling of the starch moiety of the film and/or (2) digestion of the starch molecules by the pancreatic α-amylases. In either case, drug release will occur through the highly porous starch-based film coating.

To counteract this, higher film thicknesses and/or films with a higher content of Surelease® might be required. Drug release in the colon is expected to occur following digestion of the starch domains by the colonic enzymes. This is hence not an ad hoc process because enzymatic digestion can be assumed to follow the Michaelis–Menten mechanism[29] and is thus time- and enzyme concentration- and substrate concentration-dependent. In particular, very thick films or films with low-starch content can severely slow-down the beginning of the drug release process.

Hence, the optimization of the starch/Surelease®-mixed coatings intended for colon-specific delivery is of great importance and was here done by selecting the

starch-to-Surelease® ratio, film-coating thickness, and type of starch, which was able to suppress drug release in enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2). Coatings that showed the lowest 5-ASA release under these conditions were further tested in the presence of pancreatic α-amylases.

The effect of the starch-to-Surelease® ratio, film-coating thickness, and type of starch

Table 1 summarizes the percentage of 5-ASA release after 8 hours and the values of AUC and MDT, in enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2), for different Hylon® VII-to-Surelease® ratios and film-coating thicknesses.

5-ASA release from pellets coated with Hylon® VII, plasticized with dibutyl sebacate, without Surelease® was high in both media tested and similar to 5-ASA release from the uncoated pellets. This is explained by the fact that starch films are prone to swelling in aqueous media, allowing the drug to be released at a fast rate. To which degree starch-based films can provide a sustained release depends on the formulation and processing conditions. Palviainen et al.[30] prepared Hylon® VII films from solutions plasticized with sorbitol and glycerol and reported a sustained release effect for these films in purified water. Krogars et al.[31] prepared a hot solution of Hylon® VII starch in a high-pressure reactor at 160 ± 1°C. The solution was then cooled down to 95 ± 2°C and also plasticized with sorbitol and glycerol. Tablets containing theophylline and coated with films made from this starch solution did not provide a sustained release in an acidic medium.

As an attempt to reduce the swelling of the starches observed in the aqueous dissolution media[26], ethylcellulose, an aqueous insoluble polymer, in the form of Surelease®, was added to the film formulation, and this approach was also used in this study. Other strategies to overcome the intense swelling of polysaccharides reported in the literature are, for example, the use of the calcium salt of pectin[32] and high methoxy pectins[12]. Pectin has also been used in combination with hydroxypropyl methylcellulose[33,34], ethylcellulose[35,36], Eudragit®[37,38], and chitosan[15].

To illustrate the properties of the pellets, in this study, SEM images of the surfaces of the pellets coated with varying Hylon® VII-to-Surelease® ratios are shown in Figure 1. Film-coated pellets present an overall similar surface morphology, that is, film coatings appeared smooth and homogenous. In the film coating with the highest amount of starch (Figure 1A), starch particles can be seen, interdispersed in the Surelease® domains. Despite their similar surface morphology, film coatings with similar thicknesses but different percentage of Hylon® VII provided different 5-ASA release profiles. Coatings with higher

Table 1. Summary of the film thickness (in μm), percentage of 5-ASA released after 8 hours and the values of area under the curve (AUC), mean dissolution time (MDT), and relative dispersion coefficient (RDC) of MDT, of the Hylon® VII, Hylon® VII–Surelease®, and Surelease® film coatings with varying ratios and film thicknesses, in enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2).

Hylon® VII/ Surelease® ratio (w/w)	Film thickness (μm)[a]	Percentage of 5-ASA release (8 hours)[b]	AUC[b] (% minutes)	MDT[b] (minutes)	RDC[b]
Enzyme-free-simulated gastric fluid (pH 1.2)					
Uncoated	—	95.0 ± 0.40	7305 ± 674	81 ± 7.55	0.84 ± 0.39
1:0	23.8 ± 4.98	94.3 ± 4.23	9792 ± 933	104 ± 5.31	1.10 ± 0.09
1:2	16.8 ± 1.66	93.5 ± 3.00	15,065 ± 751	161 ± 3.91	0.64 ± 0.02
	27.3 ± 1.60	17.9 ± 2.13	4147 ± 445	232 ± 3.99	0.32 ± 0.04
	40.3 ± 3.32	17.2 ± 0.65	4211 ± 536	243 ± 21.7	0.25 ± 0.05
	47.9 ± 5.33	1.46 ± 0.11	291 ± 46	199 ± 27.0	0.54 ± 0.05
1:4	21.8 ± 0.58	7.13 ± 1.32	2099 ± 165	298 ± 32.4	0.20 ± 0.05
1:5	21.2 ± 1.59	11.6 ± 1.06	3142 ± 173	272 ± 13.5	0.25 ± 0.03
0:1	22.8 ± 3.42	3.67 ± 1.00	359 ± 78	382 ± 17.2	0.04 ± 0.02
Enzyme-free phosphate buffer pH 7.2					
Uncoated	—	90.0 ± 1.24	9821 ± 540	110 ± 5.34	1.07 ± 0.02
1:0	23.8 ± 4.98	88.6 ± 1.99	10,864 ± 452	122 ± 2.48	0.97 ± 0.04
1:2	16.8 ± 1.66	81.5 ± 2.80	14,949 ± 123	183 ± 8.65	0.51 ± 0.01
	27.3 ± 1.60	52.2 ± 0.60	11,337 ± 232	217 ± 4.12	0.35 ± 0.01
	40.3 ± 3.32	45.1 ± 3.13	10,260 ± 1216	226±11.12	0.31 ± 0.02
	47.9 ± 5.33	1.75 ± 0.22	679 ± 91	387 ± 6.21	0.02 ± 0.00
1:4	21.8 ± 0.58	19.31 ± 0.95	4687 ± 309	243 ± 10.4	0.28 ± 0.03
1:5	21.2 ± 1.59	21.3 ± 0.30	5428 ± 125	235 ± 8.17	0.30 ± 0.02
0:1	22.8 ± 3.42	4.6 ± 1.58	283 ± 165	380 ± 41.2	0.02 ± 0.03

[a]Values are the mean ± SD of 10 replicates. [b]Values are the mean ± SD of three replicates.

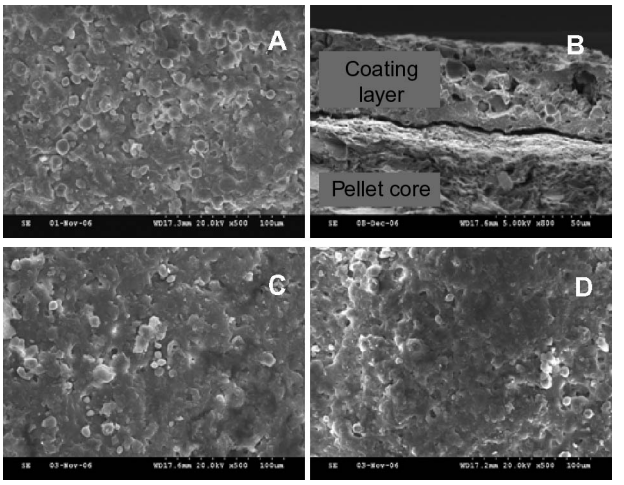

Figure 1. Scanning electron microscopy images of the pellet surfaces coated with varying ratios of Hylon® VII/Surelease® (w/w). Hylon® VII/Surelease® 1:2 surface (A), and cross-section (B), Hylon® VII/Surelease® 1:4 surface (C), Hylon® VII/Surelease® 1:5 surface (D).

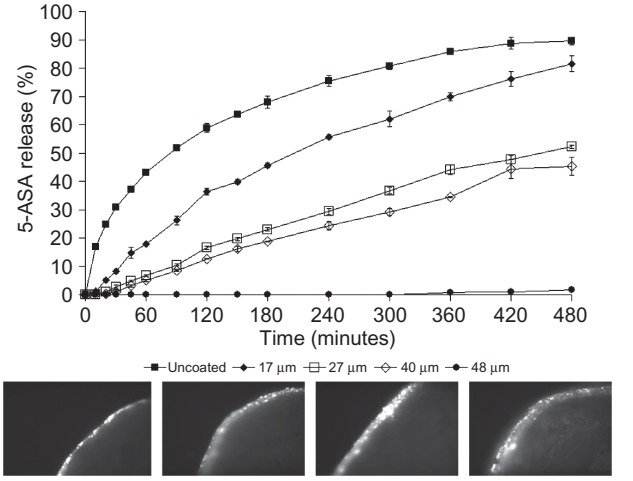

Figure 2. LSCM images of the cross-section of pellets coated with varying thicknesses of Hylon® VII/Surelease® (1:2, w/w) film and their corresponding 5-ASA drug release profile (mean and SD of three replicates) in phosphate buffer (pH 7.2), at 37°C over a period of 8 hours.

percentage of Surelease® were able to suppress 5-ASA release more efficiently. For example, 52% release of 5-ASA was achieved in phosphate buffer (pH 7.2) when the ratio of the two polymers within the film was 1:2 compared with approximately 20% for the ratios of 1:4 and 1:5 and >5% release for the Surelease® films (see Table 1). The analysis of the MDT and AUC confirm these findings, that is, the value of the MDT decreases while the AUC values increase with increasing percentage of Hylon® VII in the film coating in both media.

The RDC values can be used to assess the mechanism of 5-ASA dissolution[39]. RDC values of 1 describe first-order release mechanisms whereas an RDC of 0.8 corresponds to a pseudo first-order release mechanism. Cube root and zero-order release mechanism are associated with RDC values of 0.6 and 0.3, respectively. Most film coatings with similar film thicknesses but different Hylon® VII-to-Surelease® ratios provided zero-order release, as opposed to first-order or pseudo first-order release found for uncoated or pure Hylon® VII-coated formulations. These results suggest that the rate of drug release in the media is controlled by the addition of Surelease®, changing the drug release of the uncoated pellets or pellets coated with pure Hylon® VII to the desired zero-order process.

It was possible to adjust the 5-ASA release profile by changing the film-coating thickness. Figure 2 represents the 5-ASA release profile from pellets coated with Hylon® VII and Surelease® (ratio 1:2, w/w) with thicknesses varying from 17 to 48 µm, in enzyme-free phosphate buffer (pH 7.2). An increase in the film-coating thickness results in a lower 5-ASA release (Figure 2), lower values of AUC, and increased values of MDT (Table 1). A thickness of approximately 48 µm was required to prevent the 5-ASA release completely in both enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2; >2% release; see Figure 2 and Table 1). The 5-ASA release mechanisms proposed in this case vary from cube root for the lower film thickness tested to zero order for the remaining thicknesses. Drug release from the formulations with lower film-coating thickness occurs mainly through diffusion through pores. These pores result simultaneously from starch and plasticizer particles that are leached out from the film coating. In this context, Hylon® VII can act as a pore former and be used to enhance drug release through diffusion from hydrophobic coatings such as ethylcellulose-based coatings. Drug release through diffusion is suppressed with increasing film thickness as seen by the change to a zero-order release mechanism in the remaining film-coating formulations presenting a higher thickness.

The higher film thickness of approximately 48 µm (Hylon® VII-to-Surelease® ratio of 1:2) and the pure Surelease® film-coated formulations with a thickness of 23 µm provided virtually no drug release until approximately 5 hours into the dissolution test in any tested media; therefore the value of RDC does not correspond to any of the models described. The only exception was the film coating with a thickness of approximately 48 µm comprising one part of Hylon® and two parts of Surelease®, which provided a cube root release mechanism in simulated gastric fluid without enzymes.

Another important factor in controlling the drug release profile is the type of starch used in the film coating. Different starches exhibit different swelling abilities that are related to the amylose-to-amylopectin ratio of the starches[25]. Hence, starches with varying

amylose contents were tested. In addition to the three main types of starch studied in this work (Hylon V®, Hylon VII®, and IM-DS acetate starch), pure amylopectin and low-amylopectin starch (LAPS) were used for comparative reasons. The two materials were added to help establishing which starches, in terms of their amylose content, are better suited to be used as film-coating materials in colon-specific drug delivery devices.

The percentage of 5-ASA release for different starch/Surelease®-based coatings in two different ratios (1:2 and 1:5) in enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2) are compared in Table 2. The results show that amylopectin-based coatings were unable to prevent 5-ASA release even when the amylopectin-to-Surelease® ratio was 1:5. This can be attributed to the high-aqueous solubility of amylopectin and its intense swelling[25]. 5-ASA release from Hylon® V-based coatings was the highest when comparing the remaining starch-based films, because of this starch having a higher amylopectin content (44%). Nearly 20% of 5-ASA was released after 8 hours in phosphate buffer (pH 7.2) when the Hylon® V-to-Surelease® ratio was 1:2. Hylon® VII, and IM-DS acetate-based coatings with a film thickness of approximately 45 μm (see Table 2) prevented the 5-ASA release even when the percentage of Surelease® was low. LAPS-based films produced similar drug release profiles to those of the other high-amylose starches, suggesting that although pure amylase-based films can be used this is not essential.

These results also show that Surelease® can control the swelling of the high-amylose maize starches sufficiently resulting in a low 5-ASA release in enzyme-free media. Finding the optimum amount of Surelease® required to minimize drug release depends on both the film-coating thickness and the type of starch used in the coating. Coatings comprising high-amylose starches such as Hylon® VII, Hylon® V, or IM-DS acetate and Surelease® in ratios of 1:2 or 1:5 and film thicknesses of approximately 45 μm can suppress drug release in the enzyme-free media resembling gastric or intestinal conditions.

Table 2. Percentage of 5-ASA release after 8 hours and film thicknesses (in μm) of the film coatings with different compositions (different starch types and starch-to-Surelease® ratios of 1:2 or 1:5) in enzyme-free simulated gastric fluid and phosphate buffer (pH 7.2).

Type of starch	Percentage of 5-ASA release (8 hours)[a]	Film thickness (μm)[b]
Enzyme-free simulated gastric fluid (pH 1.2)		
Starch-to-Surelease® ratio 1:2		
Hylon® VII	<2.00	47.9 ± 5.33
Hylon® V	2.97 ± 1.24	44.8 ± 5.73
IM-DS acetate	<1.00	45.2 ± 7.24
LAPS	<1.00	44.1 ± 3.12
Amylopectin	89.75 ± 4.24	44.2 ± 3.94
Starch-to-Surelease® ratio 1:5		
Hylon® VII	<3.00	45.4 ± 2.79
Hylon® V	2.33 ± 0.137	42.7 ± 2.94
IM-DS acetate	<2.00	41.7 ± 3.67
LAPS	<1.00	40.0 ± 5.18
Amylopectin	66.45 ± 0.72	37.1 ± 5.74
Enzyme-free phosphate buffer (pH 7.2)		
Starch-to-Surelease® ratio 1:2		
Hylon® VII	<2.00	47.9 ± 5.33
Hylon® V	18.83 ± 3.82	44.8 ± 5.73
IM-DS acetate	<1.00	45.2 ± 7.24
LAPS	<2.00	44.1 ± 3.12
Amylopectin	77.13 ± 8.48	44.2 ± 3.94
Starch-to-Surelease® ratio 1:5		
Hylon® VII	< 2.00	45.4 ± 2.79
Hylon® V	6.71 ± 0.701	42.7 ± 2.94
IM-DS acetate	< 1.00	41.7 ± 3.67
LAPS	< 1.00	40.0 ± 5.18
Amylopectin	75.35 ± 2.88	37.1 ± 5.74

[a]Values are the mean ± SD of three replicates. [b]Values are the mean ± SD of 10 replicates.

Film-coating stability: effect of curing on the 5-ASA release profile

The effect of curing of the film coating on the 5-ASA release profile was investigated after a storage period of 3 months. The initial release from cured and uncured film-coated pellets with Hylon® VII–Surelease® dispersions (1:2, w/w) was found to be the same (see Figure 3). After 3 months the uncured film-coated pellets stored in closed glass jars at ambient conditions (22 ± 2°C and 50–55% RH) showed an increased drug release. Curing at 60°C for 1 hour was found to produce a more stable film coating. This was also observed in the case of Hylon® VII/Surelease® (1:5, w/w) and pure Surelease® coatings, suggesting that this effect could not be due to

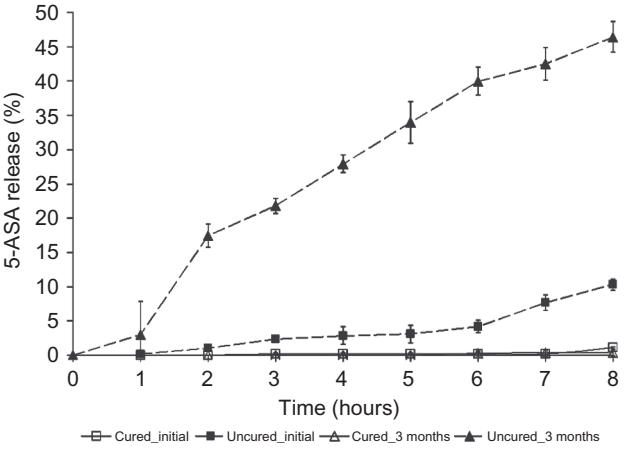

Figure 3. Effect of curing of film coatings on the percentage of 5-ASA release (mean and SD of three replicates) upon storage for 3 months, tested in phosphate buffer (pH 7.2), at 37°C over a period of 8 hours.

the presence of the starch in the coating. This is not in agreement with previous studies[40-42], which have suggested that an inlet temperature of 60°C during the film-coating process should be sufficient to allow the polymer particles of a Surelease® dispersion to undergo coalescence and attain a reproducible drug release profile, without the need for further curing. The results from this study, however, indicate that additional thermal treatment upon coating is important for complete curing and stability of the high-amylose maize starch-based coatings because of the presence of Surelease®.

The effect of the pancreatic α-amylases

The effect of pancreatic enzymes on the 5-ASA release from pellets coated with starch–Surelease® films was studied in the previously optimized formulations, that is, formulations that showed the lowest drug release in enzyme-free media, in particular those with the higher amount of starch dispersed in the film coatings.

Pancreatin is commonly used to represent digestion taking part in this portion of the GI tract. However, pancreatin is a complex enzyme mixture comprising not only amylases but also lipases, proteases, and other minor impurities. Hence, its activity toward starch materials is lower than that of a pure hog pancreas α-amylase. For that reason, formulations were tested in the presence of both pancreatin and hog pancreas α-amylase using the same concentration (250 units/mL).

Figures 4 and 5 show the 5-ASA release profiles in SIF containing pancreatin and hog pancreas α-amylase, respectively, over a period of 8 hours from coated formulations comprising Hylon® VII, Hylon® V, or IM-DS acetate starch and Surelease® in a ratio of 1:2 (w/w) and film thicknesses of approximately 45 μm. Drug release was minimal in either SIF for all formulations. Hylon® VII and

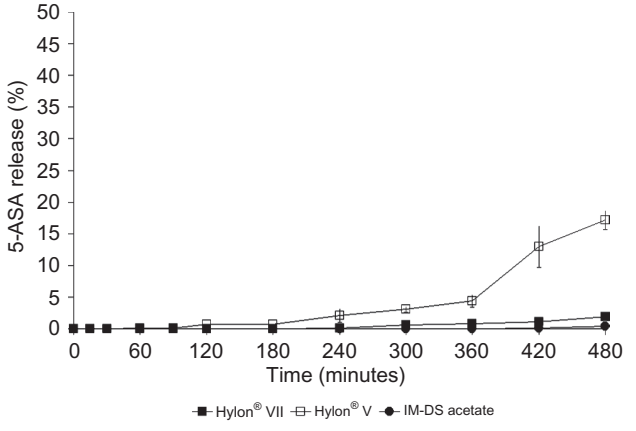

Figure 4. Percentage of 5-ASA release (mean and SD of three replicates) from Hylon® VII, Hylon® V, and IM-DS acetate-based mixed films (1:2, w/w, and film thickness of approximately 45 μm) in simulated intestinal fluid containing pancreatin, at 37°C over a period of 8 hours.

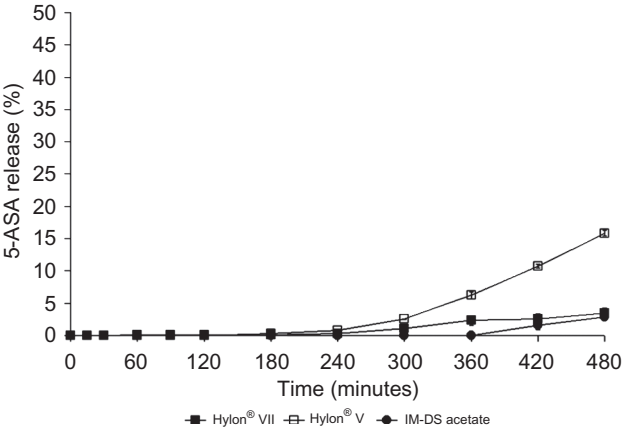

Figure 5. Percentage of 5-ASA release (mean and SD of three replicates) from Hylon® VII, Hylon® V, and IM-DS acetate-based mixed films (1:2, w/w, and film thickness of approximately 45 μm) in simulated intestinal fluid containing hog pancreas α-amylase, at 37°C over a period of 8 hours.

IM-DS acetate-based coatings released >2% of 5-ASA during the 8-hour dissolution test. The Hylon® V-based coating showed an increased drug release after 3 hours. However, drug release was already high in the enzyme-free phosphate buffer (pH 7.2; see Table 1), presumably because of the higher swelling of this type of starch[25,26] and thus not directly related to the presence of the enzymes.

The average transit time of a dosage form in the small intestine is fairly constant and has been claimed to be about 3 ± 1 hours[43]. Thus, any of the tested coatings can potentially withstand the chemical and enzymatic environment of the small intestine, hence minimizing the drug release until the dosage form reaches the colon.

5-ASA release in fluid resembling enzymatic activity of the colon

Being able to minimize the drug release in the upper GI tract is only one of the requirements for a colon-specific delivery device to work. In addition, drug release needs to be initiated upon entering the colon. High-amylose maize starches/Surelease®-based film coatings potentially provide starch domains that serve as a substrate to the enzymes of the colon. Hence, once in the colon, the starch moiety of the film is expected to be digested and leached out from the film, opening pores through which drug can be released. The long transit time in the colon also allows a prolonged contact time between the starch and the colonic enzymes. Some authors suggested that the transit time of a solid oral dosage form, in particular pellets, through the colon, although highly variable, can be as long as 72 hours[44].

Colonic conditions were simulated in vitro by using *B. licheniformis* α-amylase[45], as it resembles the properties of colonic amylases to some degree. Figure 6

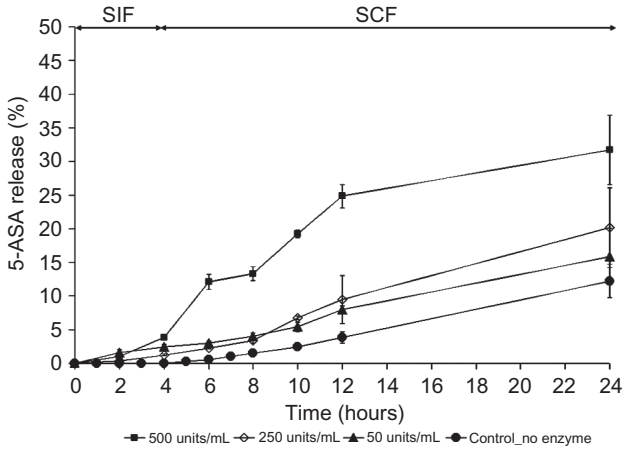

Figure 6. Percentage of 5-ASA release (mean and SD of three replicates) from Hylon® VII/Surelease ® (1:2, w/w, and film thickness of approximately 48 μm) in simulated intestinal fluid containing pancreatin for 4 hours followed by 18 hours in fluid-simulating colon conditions (SCF) by containing varying concentrations of *B. licheniformis* α-amylase (50–500 units/mL).

represents the 5-ASA release profile from Hylon® VII/Surelease® film-coated pellets [ratio 1:2 (w/w) and film thickness of 48 μm] first immersed in pancreatin containing SIF for 4 hours, followed by immersion for 18 hours in SCF containing different concentrations of *B. licheniformis* α-amylase, ranging from 50 to 500 units/mL. Drug release was very low in the presence of pancreatin as discussed previously. In the colonic fluids, 5-ASA release was low when the concentrations of *B. licheniformis* α-amylase were 50 or 250 units/mL. However, increasing the enzyme concentration to 500 units/mL resulted in a considerably faster drug release. SEM images of the surface of these coated pellets after the dissolution study (Figure 7) corroborate these findings. In the presence of the higher enzyme concentration the film-coating surface appears porous and shows several cracks because of the enzymatic digestion of the starch domains within the film (Figure 7A and B). When the enzymatic concentration was reduced to 250 units/mL (Figure 7C) the film coating appeared to be less affected

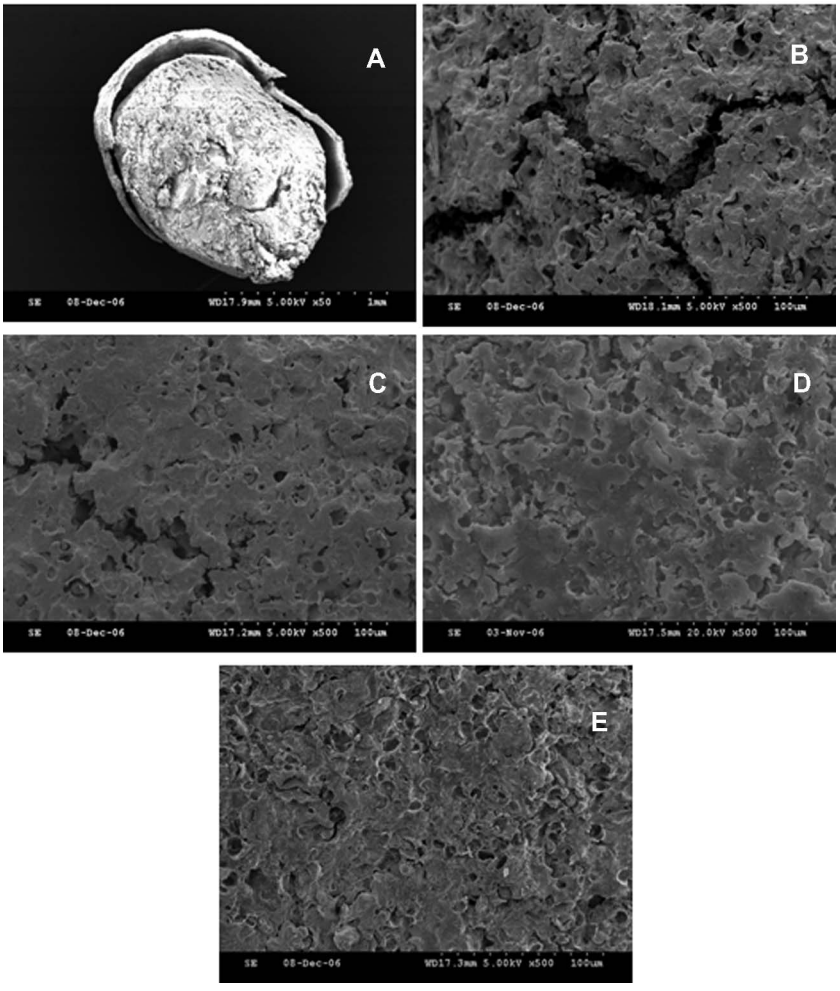

Figure 7. Scanning electron microscopy images of the surface of 5-ASA pellets coated with Hylon® VII/Surelease® (1:2, w/w) after 2 hours in SIF containing pancreatin followed by 18 hours in SCF with *B. licheniformis* α-amylase at varying concentrations. 500 units/mL (A, B); 250 units/mL (C); 50 units/mL (D); reference sample without enzymes (E).

by the enzyme activity and only some small cracks could be differentiated. At a concentration of 50 units/mL, the effect of the enzyme could not be observed (Figure 7D) and the coating presented a very similar morphology to that attained under reference conditions (Figure 7E).

A previous study[45] reported the effect of *B. licheniformis* α-amylase concentration on the extent of digestibility of amylase-based cast films with a thickness of approximately 50–100 μm, based on films cast from organic solvents. It was found that an enzyme concentration of 500 units/mL would result in >40% film digestion. An enzyme concentration of 2500 units/mL was required to obtain 100% digestion.

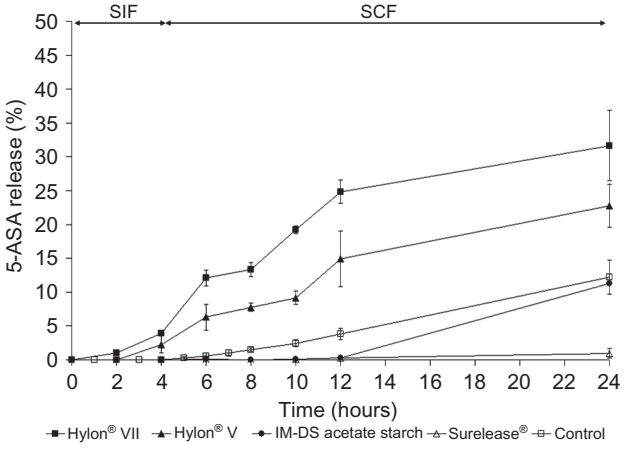

Figure 8. Percentage of 5-ASA release (mean and SD of three replicates) from Hylon® VII, Hylon® V, or IM-DS acetate and Surelease® (1:2, w/w, and film thickness of approximately 45 μm) in simulated intestinal fluid (SIF) containing pancreatin for 4 hours followed by 18 hours in fluid-simulating colon conditions (SCF) by containing *B. licheniformis* α-amylase at a concentration of 500 units/mL.

The digestive activity of a human batch fecal fermenter was found to be comparable to that of *B. licheniformis* α-amylase at a concentration of 2500 units/mL[45]. Thus, the limited drug release attained in the current study (32% 5-ASA release after 24 hours) could be due to the low enzyme concentration used.

The 5-ASA release profile from Hylon® VII, Hylon® V, or IM-DS acetate and Surelease® film-coated pellets [ratio 1:2 (w/w) and film thickness of approximately 45 μm] in pancreatin containing SIF for 4 hours followed by an 18-hour test in SCF containing *B. licheniformis* α-amylase at a concentration of 500 units/mL is shown in Figure 8. The coating comprising Hylon® V showed an increased drug release under the simulated conditions of the colon, further supporting the susceptibility of these high-amylase-based coatings to undergo fermentation in the colon environment. However, the IM-DS acetate starch-based coating showed a very low-drug release comparable with that of the pure Surelease® coating. This might be explained by a lower efficacy of this enzyme with respect to the digestion of the acetylated starch. SEM images of the pellet surfaces before and after the dissolution test in the simulated colon conditions (Figure 9) lend support to the previous observations. Hylon® V comprising coatings appeared leaky with cracks clearly visible, whereas the pure Surelease® and IM-DS acetate-based coatings remained intact.

Conclusions

This study describes a simple and rapid method for the preparation of starch-based coatings for solid dosage forms intended for colon-specific drug delivery. These coatings are produced by heating an aqueous dispersion of high-amylose starch, followed by mixing

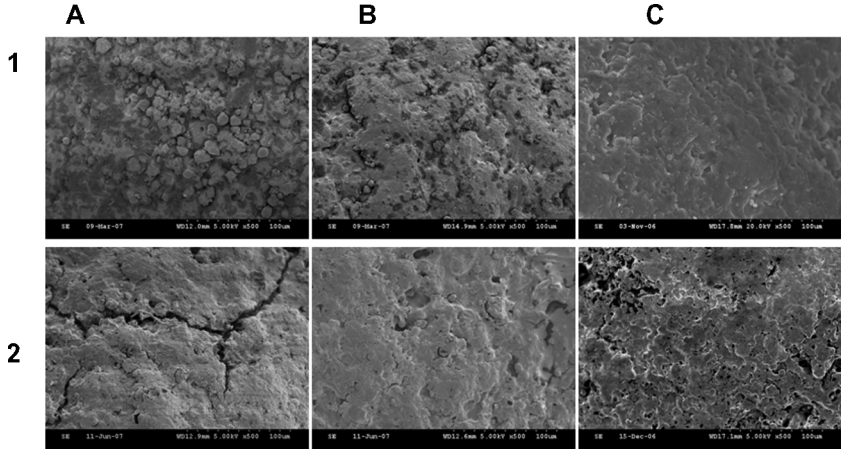

Figure 9. Scanning electron microscopy images of the surface of 5-ASA pellets coated with Hylon® V/Surelease® (1:2, w/w) (A), IM-DS acetate/Surelease® (1:2, w/w) (B), or Surelease® without addition of starch (C), before 1 or after 2 hours in SIF containing pancreatin followed by 18 hours in SCF with *B. licheniformis* α-amylase at a concentration of 500 units/mL (2).

with insoluble polymer latex, here Surelease®. The advantage of this method over the production of so-called glassy amylose is that it avoids the complex extraction procedure to isolate the amylose before film coating. Hence it allows the use of commercially available, pharmaceutically acceptable raw materials, resulting in a conventional film-coating process.

Optimized film coatings comprising high-amylose starches and Surelease® in a ratio of 1:2 (w/w) and film thickness of approximately 45 μm were able to withstand the chemical and enzymatic environment of the upper GI tract, and in particular were able to resist degradation by pancreatic α-amylases. Stability during storage can be achieved with additional curing of the films. The coatings were shown to be susceptible to enzymatic degradation in artificial fluids simulating the conditions of the colon, permitting drug release into the colon.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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