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Research paper

Starch-based coatings for colon-specific delivery. Part II: Physicochemical properties and *in vitro* drug release from high amylose maize starch films

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ABSTRACT

This work reports an investigation into free-film properties of a high amylose maize starch-based film coating that has been used in the preparation of formulations for drug delivery to the colon (WO 2008/012573 A1) and relates these properties to *in vitro* drug release from pellets.

Maize starch/ethylcellulose free films were prepared and characterised by scanning electron microscopy (SEM), light microscopy, modulated differential scanning calorimetry (mDSC), Fourier-transform infrared (FT-IR), X-ray and % swelling in aqueous fluids with pH conditions similar to the stomach and small intestine. 5-ASA release from film-coated pellets was tested in enzyme free simulated gastric fluid and phosphate buffer pH 7.2. Selected formulations were further assessed in simulated gastric and intestinal fluids containing pepsin and pancreatin, respectively.

The free films prepared were smooth and homogeneous in their appearance. The two polymers are immiscible, and neither mDSC nor FT-IR could detect interactions between them. Films made from high amylose starches were found to have a considerably lower swelling ability than high amylopectin-based films, and they suppressed drug release in the enzyme free media successfully.

5-ASA release from pellets coated with mixtures of high amylose starches (Hylon® VII, Hylon® V or LAPS) and Surelease® in a ratio of 1 to 2 w/w was found to be minimal in simulated gastric and intestinal fluids. This suggests that these mixed films provide starch domains that are resistant to the enzymes present in the upper GI tract and thus can potentially be used in the preparation of colon-specific delivery devices. Starches with a minimum amylose content of 56% such as the starches used in this study (Hylon® VII and Hylon® V) are preferred, and although pure amylose can also be used this is not essential.

1. Introduction

Colon-specific delivery of drugs can be achieved with dosage forms coated with biopolymers that are metabolised selectively by the colonic microflora and yet resistant to enzymatic digestion in the small intestine. Research has focused mainly on polysaccharides such as pectin [1,2], guar gum [3], chitosan [4], inulin [5] and glassy amylose [6]. However, polysaccharides are hydrophilic compounds and when used on their own form brittle and fragile membranes. As a result, polysaccharide-based coatings lack sufficient resistance to overcome the conditions found in the upper gastrointestinal tract and often release the drug prematurely before reaching the colon.

Many strategies have been described to overcome these limitations either by changing directly the polysaccharide compound or by mixing it with polymers with reduced water solubility. For instance, the calcium salt of pectin [7] and high methoxy pectins [2] are less water soluble. Also, pectin has been used in conjunction with hydroxypropyl methylcellulose [8,9], ethylcellulose [10,11], Eudragit® [12,13] and chitosan [14].

Starch is a complex polysaccharide composed mainly of essentially linear amylose and branched amylopectin [15]. Starch films are used primarily in the plastics and food industries and to a lesser extent in the pharmaceutical industry [16–18].

Coating compositions intended for colon-specific drug delivery, based on glassy amylose, are known [6]. The amylose component is extracted from amylose-rich pea starch through a complex and lengthy procedure which ultimately yields an amylose-butan-1-ol complex. The glassy form of the amylose is then believed to be attained by controlling the drying rate of the amylose film. Whether the conversion of the amylose into its glassy form or simply the change from a crystalline type B into type V i.e. from a double helix into a single helix crystal form due to complexation with

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n-butanol [19,20] is responsible for the change in digestibility, and whether the extraction process is reproducible on a large scale have never been shown in practice. It has, however, been found that such process yields amylose in a form that is able to resist degradation by pancreatic α-amylases for a sufficient length of time for a solid oral dosage form to reach the colon where the amylose then undergoes fermentation by colonic enzymes to release the drug through the film coating [6].

In a recent patent (WO 2008/012573 A1), it has been shown that high amylose maize starch-based films can be used for colon-specific drug delivery successfully without the need to extract the amylose into a butanol complex. In the first part of this study [21], the effect of heat treatment received during the film coating process on the physicochemical properties of the high amylose maize starches, and in particular their digestibility by various types of α-amylases was investigated. It was found that the well-defined heat treatment of the starches during the preparation of the coating dispersion, and during the film coating and drying process yields starches less digestible by pancreatic α-amylases present in the small intestine. This was attributed to the formation of retrograded forms, as identified by the highly ordered arrangement of the macromolecules of the high amylose starches in the X-ray and FT-IR studies. As has been reported by Liu et al. [22], considerable retrogradation takes place at processing temperatures of 80 °C and is further enhanced by shear forces, lending support to the above statement.

The aim of the present study was to demonstrate the feasibility of using high amylose maize starch-based film coatings produced by a spray coating process without pre-treatment of the starch samples by an extraction process in colon-specific drug delivery. Free films comprising maize starches and a pre-plasticized aqueous dispersion of ethylcellulose (Surelease® E-7-7050) were prepared. Ethylcellulose was used to enhance the film formation and to control the swelling of the films. The following pre-requisites for the mixed polymeric films to be used successfully in colon-specific drug delivery were investigated: (1) interaction between starch and ethylcellulose; (2) immiscibility of the two polymers; (3) swelling behaviour in aqueous fluids and (4) drug permeability in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Cast films were thoroughly characterised by a range of techniques such as SEM, light microscopy, mDSC, X-ray diffraction, FT-IR and % of swelling in aqueous media. Finally, 5-ASA-loaded pellets were film coated with selected formulations of starch/ethylcellulose mixtures and the drug release assessed in different aqueous media.

2. Materials and methods

2.1. Materials

Hylon® VII (Batch No. FG 5514), Hylon® V (Batch No. BJ 9960), acetate maize starch with a degree of substitution (DS) of 1.5 (IM-DS acetate starch, Batch No. 78-0469) and low amylopectin maize starch (LAPS, Batch No. 374964) were donated by the National Starch & Chemical Company, Bridgewater, NJ, USA. The amylose content of these starches is 69%, 56%, 71% and 95%, respectively. Amylopectin (Batch No. 9561E) was supplied by ICN Biomedicals Inc., Aurora, OH, USA. Surelease® E-7-7050 (Surelease® E-7-7050, Batch No. 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-Aminosalicylic acid (5-ASA) was supplied by Avocado Research Chemicals, Ltd. (Batch No. J 3433 B) and Avicel PH 101 was obtained from FMC Corporation, Cork, Ireland (Batch No. 6842C).

Pepsin from porcine gastric mucosa (Batch No. 095K0151) was purchased from Sigma Chemicals (St. Louis, MO, USA). Pancreatin with an activity at least equivalent to the USP specifications (Batch No. 093K0661) was supplied by Sigma-Chemicals (St. Louis, MO, USA). Sodium taurocholate hydrate (Batch No. 10111536) was supplied by Avocado Research Chemicals Ltd. (Lancaster, UK).

2.2. Preparation of free films

Free films were prepared by a casting/solvent evaporation technique. Three grams of starch were dispersed in 20 ml of distilled water and heated at 80 ± 5 °C for 30 min. The attained dispersion was allowed to cool down and admixed with the commercially pre-plasticized aqueous ethylcellulose dispersion (Surelease® E-7-7050). Complete mixing of the two polymers was achieved by stirring the dispersion for further 30 min. Surelease® was added in different ratios corresponding to the dry weight of both polymers. The attained dispersions were poured onto 90 mm teflon discs and dried in an air-oven at 60 °C. After drying, the films were peeled from the teflon surface and kept for subsequent studies in closed glass jars at ambient conditions (22 ± 2 °C and 50–55% RH). The film thicknesses were measured using a digital micrometer, type 293-766-30 (Mitutoyo Corporation, Kawasaki, Japan). Films with thicknesses between 60 and 80 μm were used. Free films of Surelease® were also prepared and stored under the same conditions.

2.3. Physicochemical characterisation of the free films

2.3.1. Scanning electron microscopy (SEM)

Surelease® films and selected formulations of mixed starch/Surelease® films 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, Newhaven, UK) with an emission of 5 kV and a magnification of 1500×.

2.3.2. Light microscopy

Surelease® films and selected formulations of mixed starch/Surelease® films were cut into small sections (1 × 1 cm^2), placed on a glass slide and stained with an iodine solution (0.5 N). The surface of the stained free films was analysed with a Leica DMIL inverted microscope (Leica Microsystems, Inc., Germany) using a magnification of 400×. Images were captured with a Canon Power Shot S45 digital camera.

2.3.3. Modulated differential scanning calorimetry (mDSC)

Thermal behaviour of Surelease® and starch-based free films was studied by means of mDSC (Q1000, TA Instruments, Waters LCC, Delaware, USA). Small fragments of the free films were accurately weighed (4–6 mg) into aluminium pans (TA Instruments, Delaware, USA), hermetically sealed and scanned between 10.0 and 250.0 °C with an optimized modulation temperature amplitude of ±2.0 °C, modulation time of 40 s and a ramp rate of 3.0 °C/min ($n = 3$. These conditions were shown to offer an adequate number of modulation cycles (more than four modulation cycles) over the temperature range of the transition. A nitrogen gas supply with a flow rate of 50 ml/min and a refrigerating cooling system (RCS 90, TA Instruments, Delaware, USA) with a temperature range from −90 to 550 °C was used. The mDSC cell conditioning and calibration were described previously [21]. Relevant endothermic or exothermic transitions and enthalpy of transition were analysed with the use of the TA universal analysis® 2000 soft-

ware. Glass transition temperatures were determined on the reversible signal as the inflection point, i.e. the portion of the curve between the first and the third tangent with the steepest slope.

2.3.4. X-ray diffraction

X-ray powder diffraction analysis of the free films was performed in a Philips X' Pert. Model PW 3040/00 equipped with a monochromatic Co-Kα radiation (1.78897 Å). Samples were placed into rectangular aluminium cells and exposed to an X-ray beam with a voltage of 40 kV and a current of 35 mA. Other test conditions were: scanning range at 2θ of 5–60°; step size 0.025°, acquisition time 30 min, divergence slit 1°, receiving slit 0.25° and scattering slit 1°.

2.3.5. Fourier-transform infrared spectroscopy (FT-IR)

Infrared spectra of the maize starch-based free films were recorded in the frequency range of 4000–550 cm^{-1} with a Spectrum BX series spectrophotometer (Perkin Elmer, High Wycombe, UK). For each sample, a total of 16 scans were performed at a resolution of 4 cm^{-1} and velocity of 0.30 cm/s. The spectra were corrected for baseline shifts and deconvoluted automatically with the use of Spectrum BX series software version 2.19, which was also used to determine peak positions.

2.3.6. Swelling of the free films

Free films were cut in 1.5×1.5 cm^2 sections ($n = 3$–5) and placed in petri-dishes with 25 ml of simulated gastric fluid without enzymes, pH 1.2 or Sörensen buffer pH 7.2 at 37 °C for 8 h. At predetermined times, i.e. 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min. The films were taken from the solution, the excess of water was removed carefully with filter paper and the films were weighed immediately.

The swelling index (SI) was calculated as the ratio between initial and final film weight in%.

2.4. Preparation and film coating of 5-ASA-loaded pellets

Pellets with a diameter ranging from 1.00 to 1.40 mm containing 50% of 5-aminosalicylic acid and 50% of Avicel® PH 101 were produced by an extrusion–spheronization process. The density of the pellets as determined by Helium Pycnometry was 1500 ± 10 kg m^{-3}.

Batches of 30 g of the 5-ASA pellets were subsequently coated in a Uni-Glatt fluidized bed coater with a bottom spray nozzle with a diameter of 1 mm and an 8 cm diameter perforated bottom plate. The coating operation conditions used in the present study are as follows: inlet temperature of 60 °C, spray rate of 0.7–0.8 ml/min, outlet temperature of 40–45 °C, product temperature of 45–50 °C, air velocity of 2–4 m/s and atomising air pressure of 2.0–2.2 bar.

Coating dispersions were prepared as described in Section 2.2.

The film coating thickness was determined with a Laser Scanning Confocal microscope 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, California, USA), Acousto-optic tuneable filter (AOTF) 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., Hamamatsu City, Japan). The software used was VoxCell, version 3.82 (VisiTech International, Sunderland, UK).

The laser intensity was adjusted to its maximum level during the procedure and two sets of filters (488 and 568 nm) were used.

Approximately, 0.001 g of sodium fluorescein (Sigma–Aldrich, St. Louis, MO, USA; Batch No. 113K0112) was added to the coating dispersions and mixed to achieve complete dissolution.

A total of 10 Pellets per batch were analysed. Pellets were carefully cut in half and the thickness was measured at 4 equidistant points. The average thickness in μm 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.

2.5. In vitro drug release studies

In vitro drug release studies were undertaken according to the USP method II (paddle method) in an Erweka DT 6R (Erweka, Heusenstamm, Germany). The pellets did not float, but remained at the bottom of the dissolution vessels at all times. 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. Enzyme free dissolution media used were the same as described in Section 2.3.6 for the swelling studies of the free films.

Simulated gastric fluid (SGF) was prepared according to the B.P. method (B.P. 2005, Appendix XII-B-A187).

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.

Samples were withdrawn at pre-determined intervals (10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min) diluted 1/5 and the absorbance was measured by UV (Cecil CE 594, UK) at 304 and 330 nm for the simulated gastric fluid and Sörensen phosphate buffer, respectively.

2.6. Statistical analysis

The results were analysed by one-way analysis of variance (ANOVA) using SPSS 14.0 for Windows (SPSS Inc., Woking, UK). A post hoc Scheffé test was employed when the overall *F*-test (ANOVA) indicated significant differences between samples.

3. Results and discussion

3.1. Surface morphology of the free films using SEM and light microscopy

SEM and light microscopy images of the surfaces of the free films obtained using different maize starches and Surelease® in a ratio of 1–2 (w/w) are represented in Fig. 1. Information regarding the miscibility of the two polymers and overall quality of the produced free films can be obtained qualitatively by SEM and light microscopy. SEM can provide a more detailed image of the surface of the films, whereas light microscopy of films that have been stained with iodine can be used to identify the starch domains within the free films. Amylose binds strongly to iodine, quickly developing a strong blue to purple color, whilst amylopectin gives a red iodine reaction [23]. Acetylated starches do not bind as efficiently to iodine [24] but also give a red to brown coloration in the presence of iodine.

The Surelease® film (Fig. 1A) is smooth and homogenous, with no detectable unfused particles, due to an optimal interaction between the ethylcellulose and the plasticizer, dibutyl sebacate [25].

The IM-DS acetate and amylopectin-based films present a very similar surface morphology (Fig. 1B and C) with no detectable granular structures. This could be explained by the lower gelatinisation temperatures of these powders. As a result, most granules fragment upon heating at 80 °C. However, the formation of a pre-

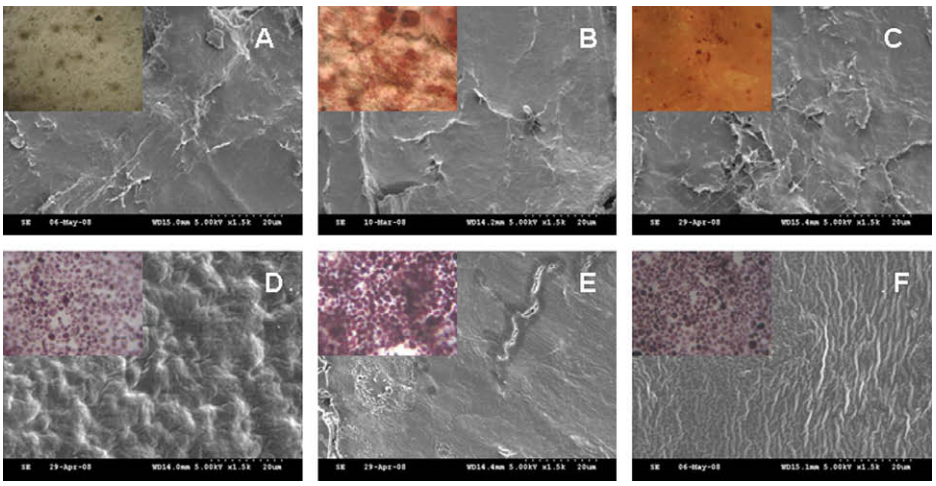

Fig. 1. SEM (1500×) and light microscopy images (400×), embedded into the left-hand corner of the SEM images) of the free films comprising different maize starches and Surelease® in a ratio of 1–2 (w/w). (A) Surelease® film without addition of starch, (B) amylopectin, (C) IM-DS acetate starch, (D) Hylon® VII, (E) Hylon® V, (F) LAPS.

dominantly red to brownish coloration denotes the presence of starch material homogenously dispersed in the Surelease® matrix.

The Hylon® VII and LAPS-based free films (Fig. 1D and F) present very different surface morphologies to that of the amylopectin and IM-DS acetate-based films. Light microscopy clearly shows the presence of intact starch granules inter-dispersed in the Surelease® domain as a result of the higher gelatinisation temperature of these starches.

During the manufacture of the films, the starch that posed the most problems was Hylon® V. These films were fragile and difficult to peel from the teflon surface. Given the fact that Hylon® V is composed of 44% of amylopectin and only 56% amylose, this starch has a low gelatinisation temperature and high swelling aptitude at 80 °C. Despite the intense swelling, most granules do not burst upon treatment at 80 °C; instead these highly swollen granules appear to form aggregates at the surface of the free films (Fig. 1E, embedded picture), forming a less well distributed starch phase. As these aggregates interfere with the coalescence of the latex polymer, a higher fragility of the films is observed. It is not surprising, therefore, that small cracks could be seen in the SEM picture (Fig. 1E).

3.2. Thermal behaviour of the free films as observed by modulated differential scanning calorimetry (mDSC)

Table 1 lists the relevant thermal transitions i.e. glass transition and endothermic loss of residual water over the temperature range of 10–250 °C of the starch-based free films.

Surelease® free films presented a weak glass transition at 41.0 °C, detected on the reversing signal. The glass transition of unplasticized ethylcellulose films has been reported to be approximately 135 °C [26]. However, it is well known that the incorporation of small amounts of plasticizers can significantly depress the glass transition temperature. For example, Surelease® films have been reported to have a glass transition temperature of approximately 35 °C [25,27]. The significantly higher value found in the present study could be due to the use of a more sensitive technique such as mDSC.

The thermal behaviour of the mixed polymeric films was characterised by the presence of a glass transition at around 40–43 °C reflecting the Surelease® moiety, and in most cases an endothermic peak at 130–160 °C reflecting the starch fraction. For IM-DS acetate starch-based free films, the first glass transition value is again related to the glass transition of Surelease®, whereas the additional second glass transition value reflects the starch fraction within the film, because IM-DS acetate starch on its own had previously been found to have a distinctive glass transition at 181 ± 1 °C [21]. However, for the IM-DS acetate-based free films the endothermic peak was not observed. This acetylated starch is highly hydrophobic in its character [28] and any endotherm associated with water loss is expected to be less pronounced or even non-existent.

These results confirm that the two polymers (maize starch and ethylcellulose) coexist in the film in an immiscible form, as would be expected from their individual properties. This is a major prerequisite for the use of such films in drug delivery to the colon,

Table 1
Summary of the relevant thermal transitions deduced from both reversing and non-reversing mDSC signals obtained from heat-processed maize starches and free films produced from these and Surelease® in different ratios (w/w). T_g, glass transition temperature; ΔH, enthalpy related to the endotherm associated with residual water loss; ND, undetectable.

Sample	Reversing signal (T_g)	Non-reversing signal	
		Endotherm (°C)	ΔH (J/g)
Surelease® films	41.0 ± 0.8	–	–
Hylon® VII/Surelease® film (1:2)	40.5 ± 0.4	129.5 ± 3.8	47.7 ± 2.6
Hylon® VII/Surelease® film (1:3)	40.1 ± 0.7	141.8 ± 7.1	37.2 ± 8.8
Hylon® VII/Surelease® film (1:4)	40.5 ± 0.6	130.6 ± 2.5	32.9 ± 5.3
Hylon® VII/Surelease® film (1:5)	40.3 ± 0.2	146.5 ± 3.5	14.0 ± 1.4
Hylon® V/Surelease® film (1:2)	42.6 ± 0.0	146.3 ± 2.7	52.5 ± 2.3
IM-DS/Surelease® film (1:2)	41.3 ± 0.6 179.3 ± 0.9	ND	ND
LAPS/Surelease® film (1:2)	41.1 ± 0.5	166.8 ± 2.1	44.8 ± 5.8
Amylopectin/Surelease® film (1:2)	42.0 ± 0.9	131.8 ± 0.8	42.1 ± 1.8

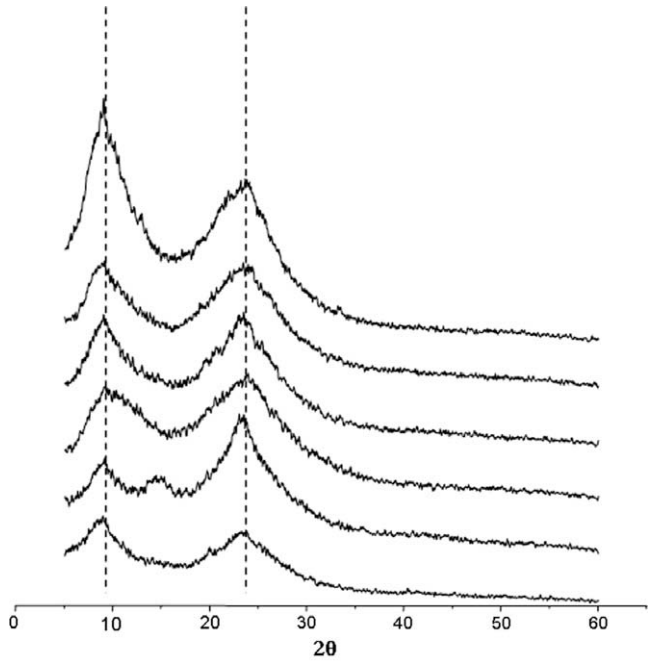

Fig. 2. X-ray diffractograms of free maize starch/Surelease® films in a ratio of 1–2 (w/w). From top to bottom: Surelease® film, amylopectin/Surelease® film, LAPS/Surelease® film, IM-DS acetate starch/Surelease® film, Hylon® V/Surelease® film and Hylon® VII/Surelease® film.

as starch domains should be readily accessible for enzymatic digestion by bacterial enzymes in the colon.

Comparing the values of the glass transition temperatures of all films, it can be concluded that the T_g of the Surelease® fraction of the films was only marginally altered by the presence of starch. Hence, the temperatures required for the production of the Surelease® and maize/Surelease® films are essentially the same, without the need for the addition of extra plasticizer.

3.3. X-ray diffraction patterns of the free films

The X-ray diffractograms of the Surelease® films and the mixed maize starch/Surelease® films are compared in Fig. 2. Surelease® films can be considered essentially as amorphous as seen by the lack of diffraction peaks. The X-ray diffractograms of the starch/Surelease® (1:2) mixed films also denoted an amorphous character. In the first part of this study, heat treatment of maize starches was shown to result in a decrease of their degree of crystallinity (%) when determined as described by Nara and Komiya [29]. This decrease was different according to the starch type. The amylopectin and IM-DS acetate starches showed a more pronounced decrease, whereas LAPS, Hylon® VII and Hylon® V starches retained part of their crystallinity, preserving some clear diffraction peaks at 2θ of 19° and 22°. Despite this, the X-ray patterns of the various starch-based films were very similar to each other probably due to the overall amorphous character of the ethylcellulose domain.

3.4. Fourier-transform infrared analysis (FT-IR) of the free films

Fig. 3A compares the FT-IR spectra of the various maize starch/Surelease® films (ratio 1–2 w/w) in the spectral region of 1300–800 cm^{-1}. The spectrum of the Surelease® film presents intense peaks at 1003, 1030, 1054, 1088 and 1104 cm^{-1}. Heat-treated starches have intense bands in the same spectral region and for this reason the analysis of these spectra offers no evidence regarding the crystalline or amorphous state of the starch within the film. The IM-DS acetate-containing film was clearly differentiated from all other films due to the presence of a peak at 1240 cm^{-1} resulting from a C–O stretching band, typical for acetylated starches [30].

Fig. 3B shows the FT-IR spectra of the Hylon® VII/Surelease® films with varying ratios (w/w) in the spectral region of 1300–800 cm^{-1}. A heat processed Hylon® VII powder sample and a Surelease® film were used as a comparison. The spectra of the mixed films are the sum of the spectra of the heat processed Hylon® VII and Surelease® films. No new peaks could be detected, thus confirming the immiscibility and lack of interactions between the two polymers.

3.5. 5-ASA release profile from pellets film coated with starch/Surelease® coatings

3.5.1. The effect of starch type and concentration of ethylcellulose on the swelling of the films

To be used as coating material for colonic drug delivery systems, the swelling of starch films must first be controlled, as otherwise drug will be released at a fast rate before the dosage form is reaching the colon. In this study, as an attempt to control the starch swelling, starch-based films were prepared by mixing it with a water insoluble polymer i.e. ethylcellulose. An effective control of the swelling of the starch films by the ethylcellulose domains should result in a low drug release in enzyme free media.

The 5-ASA release profiles from starch/Surelease® coated formulations (1–2 w/w) in simulated gastric and simulated intestinal

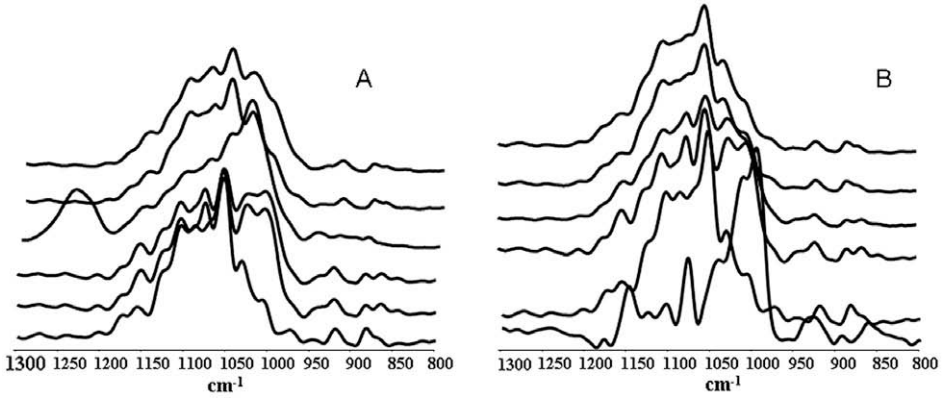

Fig. 3. FT-IR spectra of the free films in the region 1300–800 cm^{-1}. (A) Maize starch/Surelease® mixed films in a ratio of 1–2 (w/w). From top to bottom: amylopectin/Surelease® film, LAPS/Surelease® film, IM-DS acetate starch/Surelease® film, Hylon® V/Surelease® film, Hylon® VII/Surelease® film and Surelease®. (B) Hylon® VII/Surelease® films with varying ratios (w/w). From top to bottom: starch to Surelease® ratio of 1:5, 1:4, 1:3, 1:2, 1:0 and 0:1.

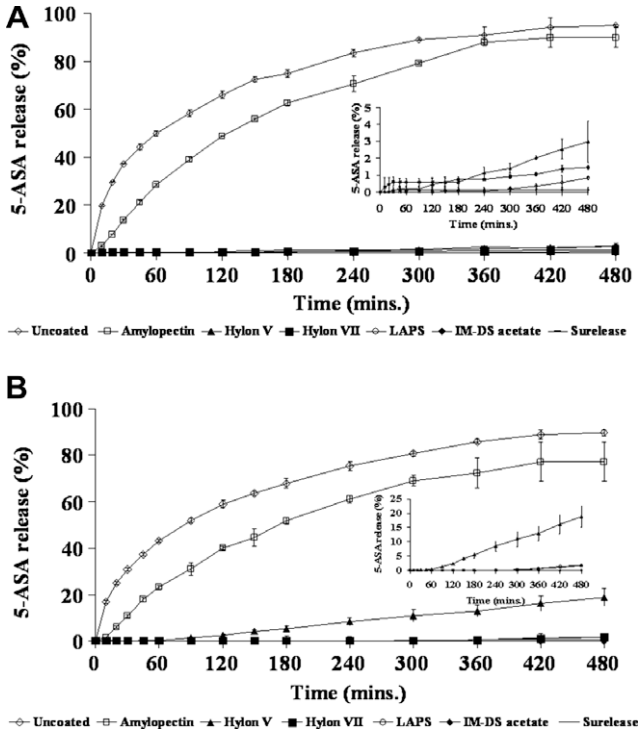

Fig. 4. % of 5-ASA release from formulations coated with various maize starch/Surelease® mixtures (1:2 w/w and film thickness of approximately 45 μm) in (A) simulated gastric fluid without enzymes pH 1.2 and (B) phosphate buffer pH 7.2, at 37 °C over a period of 8 h.

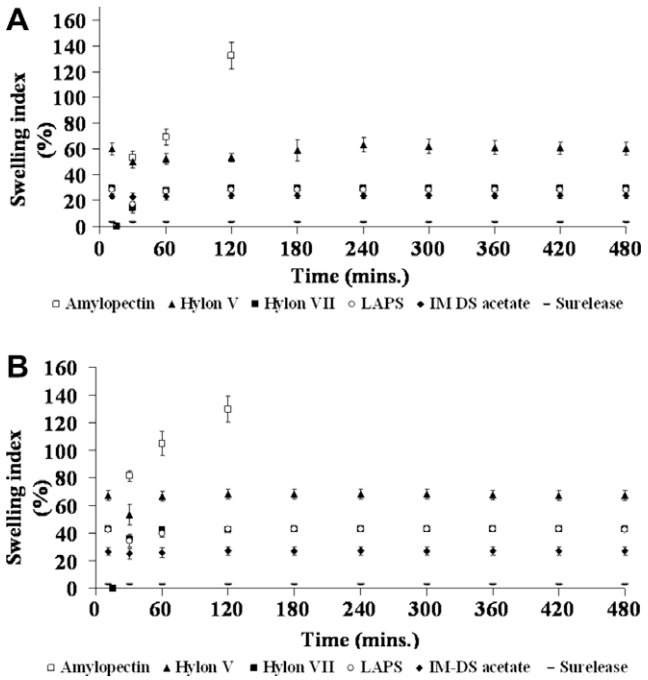

Fig. 5. Swelling index (%) of the various maize starch/Surelease® mixed films (1:2 w/w) in (A) simulated gastric fluid without enzymes pH 1.2 and (B) phosphate buffer pH 7.2, at 37 °C over a period of 8 h.

fluids without enzymes are shown in Fig. 4A and B, respectively. In both media drug release was considerably higher for the amylopectin-based film. In the simulated gastric fluid, nearly 90% of the 5-ASA was released after 8 h and approximately 77% was released in phosphate buffer. The higher release in simulated gastric fluid was also observed for the uncoated formulation suggesting that drug release is governed by the 5-ASA solubility in the different media. However, Hylon® V coating suppressed drug release more efficiently in simulated gastric fluid than at the higher pH value, i.e. 3% drug release compared with 18% release, indicating that for this coating formulation the drug solubility is not the only factor involved in controlling the drug release. This effect was not observed for the remaining coating formulations. The formulations presenting the lowest drug release were the pure Surelease® and IM-DS acetate-based coatings with 0% drug release in both media. Hylon® VII and LAPS coatings presented very similar drug release profiles with less than 2% 5-ASA release.

As the film thicknesses of all the formulations were the same (approximately 43–48 μm), drug release will only depend on the type of starch used in the coating formulation and, in particular, the swelling ability of the starch film in the different media.

The swelling of the Surelease® and maize starch/Surelease® films (ratio 1–2 w/w) in simulated gastric fluid without enzymes and phosphate buffer pH 7.2 is presented in Fig. 5A and B, respectively.

Surelease® films are insoluble and do not swell at contact with aqueous fluids. The films comprising amylopectin and Hylon® V showed the highest % swelling in both media. After only one hour, the amylopectin films disintegrated due to an increase in swelling and solubility in the aqueous media, whereas the other films showed considerably less swelling and remained intact over the full test period of 8 hours. The swelling of the Hylon® VII and LAPS-containing films was very similar (overlapping of symbols in both figures), whilst the IM-DS acetate starch presented a further decreased swelling ability. These results correlated well with the obtained *in vitro* drug release results and show that in the formulations with the same amount of ethylcellulose the drug release is dependent on the swelling ability of the starches. For the amylopectin-based films the 5-ASA is mainly released by diffusion through the highly swollen domains of the hydrophilic amylopectin. Therefore, drug release is fast and almost complete after 8 h.

The pH-dependent release behaviour of the Hylon® V-based films might be explained by the slightly higher swelling index of this film in pH 7.2 (67%) than in simulated gastric conditions (60%). This results in films that are more porous at higher pH, which release the drug at a faster rate.

The IM-DS acetate films are almost impermeable to the 5-ASA, which is related to the considerably lower swelling ability of the films and to their highly hydrophobic character as seen in the mDSC analysis.

LAPS and Hylon® VII-based films were also able to prevent the drug release in the enzyme free media resembling the upper GI tract conditions despite presenting a higher % swelling than the pure Surelease® and IM-DS acetate-based films.

From the results presented above, it can be concluded that the starches with a higher content of amylose provide coatings with a superior resistance to the conditions of the upper gastrointestinal tract. Therefore, further investigations were undertaken using Hylon® VII (69% of amylose)-based coatings to identify the proportion of ethylcellulose required to control the starch swelling to prevent excessive drug release sufficiently in the upper intestinal tract. The % of 5-ASA release from the pellets coated with varying amounts of ethylcellulose at the same coating thickness (approximately 25 μm) was assessed in phosphate buffer pH 7.2 and is represented in Fig. 6.

As the amount of ethylcellulose in the films increases, the drug release decreases. The decrease in drug release is proportional to the amount of ethylcellulose in the film, decreasing from 42% to 27% when the Hylon® VII/Surelease® ratio changed from ratio 1:2 to 1:4. The decrease in drug release was less pronounced for the reduction of ethylcellulose from ratio 1:4 to 1:5. The swelling of

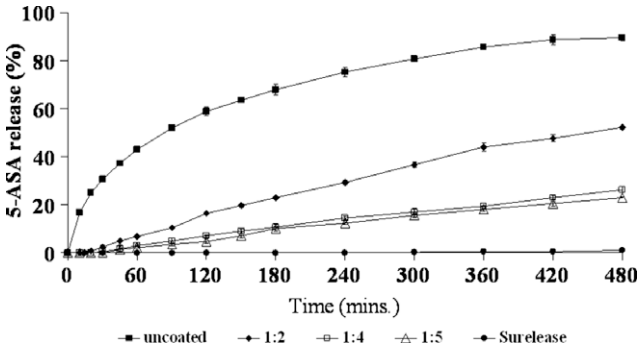

Fig. 6. % of 5-ASA release from formulations coated with Hylon® VII/Surelease® with varying ratios and film thickness of approximately 25 μm, in phosphate buffer pH 7.2, at 37 °C over a period of 8 h.

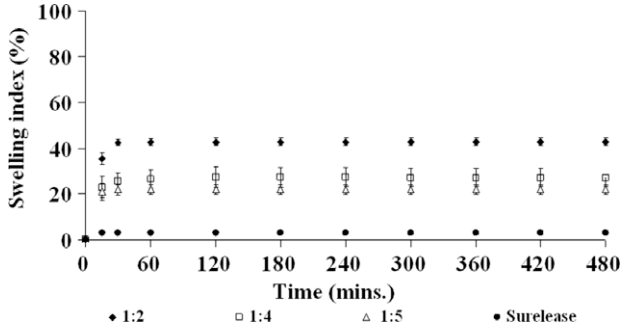

Fig. 7. Swelling index (%) of the Hylon® VII/Surelease® mixed films with varying ratios (w/w) in phosphate buffer pH 7.2, at 37 °C over a period of 8 h.

these films (Fig. 7) is also suppressed proportionally with the increase in ethylcellulose concentration. The results thus indicate that the suppression of swelling is the main factor in the control of drug release from Hylon® VII/Surelease® films.

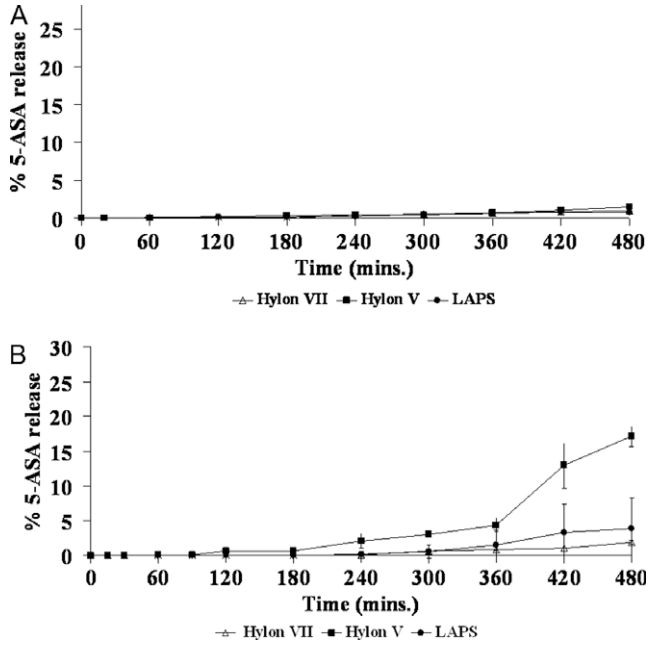

Fig. 8. % of 5-ASA release from Hylon® VII, Hylon® V and LAPS-based mixed films (1:2 w/w and film thickness of approximately 45 μm) in (A) simulated gastric fluid and (B) Simulated intestinal fluid, at 37 °C over a period of 8 h.

3.5.2. 5-ASA release in simulated gastric and intestinal fluids using enzymes

Figs. 8A and B represent the drug release profiles of the Hylon® VII, Hylon® V and LAPS-based mixed films (ratio 1:2 w/w and film thickness of approximately 45 μm) in SGF and SIF, containing pepsin and pancreatin, respectively.

The 5-ASA release was very low for all formulations and in both media, suggesting that the starch present in the films is resistant to digestion by the enzymes present in the upper GI tract. The slightly higher drug release observed in the case of the Hylon® V-based coatings was the result of the higher swelling ability of this type of starch, rather than the result of enzymatic digestion, since 5-ASA release was already high (see Fig. 4B) in enzyme free phosphate buffer pH 7.2.

These results confirm the findings reported in the first part of the study [21], where it was found that heat treatment received by the high amylose starches during the film coating preparation yields starches less digestible by the α-amylases present in the upper GI tract.

4. Conclusions

High amylose starch/Surelease® mixed films intended for colon-specific drug delivery were successfully prepared and characterised. The surface of the films was smooth and homogeneous with starch domains clearly identified. The lack of interaction between the two polymers was confirmed by mDSC and FT-IR analysis.

High amylose starch mixed films were found to have a significantly lower swelling aptitude in the aqueous media than the amylopectin-based films and were able to suppress the 5-ASA release in the enzyme free media successfully.

The coatings comprising the high amylose starches resisted digestion by the pepsin enzymes and pancreatic α-amylases, thus confirming the enzyme resistant form of these starches. This study shows that high amylose maize starch-based films can be used potentially in the development of colon-specific delivery devices. Starches with a minimum amylose content of 56% such as the ones used in this study are preferred, and although pure amylose can also be used this is not essential. The extraction of the amylose into a butanol complex as claimed elsewhere [6] is also not required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2009.02.010.

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