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Research Paper

# Assessment of the in-vivo drug release from pellets film-coated with a dispersion of high amylose starch and ethylcellulose for potential colon delivery

Cristina Freire[a], Fridrun Podczeck[b], Dinora Ferreira[c], Francisco Veiga[a], João Sousa[a] and Angelina Pena[a]

[a]Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde Azinhaga de Santa Comba, Coimbra, Portugal, [b]Dept of Mechanical Engineering, University College London, London, UK and [c]Institute of Hygiene and Tropical Medicine, Lisbon, Portugal

## Abstract

**Objectives** The aim of this study was to test the ability of a colon targeting system comprising pellets film-coated with a dispersion of high amylose starch (Hylon VII) and ethylcellulose (Surelease) (1 : 2 w/w) to deliver a model drug (5-aminosalicylic acid; 5-ASA) *in vivo* into the colon of rabbits. An uncoated pellet formulation was used as a control.

**Methods** Six New Zealand female rabbits, approximately 2 kg, were randomly divided into two groups. Pellet formulations containing 50 mg/kg of 5-ASA were filled into hard gelatin capsules size 4, and were administered orally using a cannula. The rabbits were fasted for 12 h before, and throughout, the study but had free access to water. Blood samples were collected, through a catheter inserted into the marginal vein of the ear, at pre-determined times and the plasma analysed by a validated HPLC method with fluorescence detection.

**Results** Analysis of the 5-ASA plasma levels following administration of the uncoated pellets showed a $C_{max}$ of 2.38 ± 0.49 $\mu$g/ml at 2 h post administration confirming that this system released the drug at an unspecific site, most likely in the rabbits' stomach and proximal small intestine. On the other hand, the coated formulation showed a delayed drug absorption ($C_{max}$ 0.22 ± 0.19 $\mu$g/ml and $t_{max}$ of 8 h), suggesting that the coating is able to prevent drug release in the stomach and small intestine, but allowing drug release in the colon. The coated pellets were retrieved from the rabbits' faeces after the 24-h study. They had a drug content of < 40%, suggesting that the film-coating had been digested by the bacterial amylases of the colon and the drug was released specifically in the colon of the rabbits.

**Conclusions** Results from this study showed that the proposed drug delivery system has the potential to deliver drugs specifically into the colon.

**Keywords** 5-aminosalicylic acid; colon-specific drug delivery; high amylose starches; *in vivo* drug release

## Introduction

The colon is susceptible to a number of conditions and diseases such as inflammatory bowel syndrome (IBS). The oral treatment of IBS with anti-inflammatory drugs, such as 5-aminosalicylic acid (5-ASA), is more effective if the drug is specifically targeted to the colon.

In humans, the lower gut contains about $10^{11}$–$10^{12}$ microorganisms/g, which represent 300–500 different species[1] and approximately 30% of the dry weight of faeces[2]. The number of microorganisms is much lower in the stomach (0–1000) and small intestine ($10^3$–$10^7$).[1] Thus, systems that release the active compound in the colon following degradation by the colonic microflora are considered highly specific on the site of drug release.

Recently a novel colon-specific drug delivery system based on the use of high amylose starch-based coatings[3,4] was described and successfully tested *in vitro*. Before the film coating procedure, the high amylose starches were subjected to heat treatment at 80 ± 5°C in the presence of 86% (w/w) of water using well-defined time-spans of heating. This

**Correspondence:** Cristina Freire, Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde Azinhaga de Santa Comba, 300-548 Coimbra, Portugal.
E-mail: anacfreire80@hotmail.com

treatment was found to yield starches presenting a crystalline or retrograded form, which were resistant to enzymatic digestion by enzymes resembling those present in the upper gastrointestinal tract in-vivo. The technological approach makes the system different to that of Milojevic *et al.*,[5] where the film coating was produced from an extracted fraction of pure amylose.

Animal models, such as rat,[6,7] mouse,[8] guinea-pig,[9] rabbit,[10] pig[11] and dog,[12,13] have been used for the testing of colon-specific drug delivery systems. It is generally recognised that there are important differences between the human gastrointestinal tract and that of most laboratory animals,[14] which contribute to noticeable differences in the performance of drug delivery systems. For example, whereas rat and mouse have total intestinal transit times similar to humans in the order of 20–30 h, beagle dogs present considerably shorter transit times of about 6–8 h.[15]

The aim of this study was to test the ability of the developed colon targeting system[3,4,16] comprising pellets film-coated with a dispersion of high amylose starch (Hylon VII) and ethylcellulose (Surelease) to deliver a model drug (5-ASA) *in vivo* into the colon of rabbits to demonstrate that the developed coating method provides a system that can be used for drug targeting specifically to the colon. This work is not intended to study the biological activity of the model drug, but uses it merely as a marker for the performance of the novel film-coating. Rabbits and humans alike have fewer bacteria in the stomach and small intestine than rats and guinea-pigs[1,17] and for that reason the rabbit can be seen as a potential animal model for testing colon-specific drug delivery systems. Another advantage of using rabbits over rats or guinea-pigs is that their higher body weight allows the administration of a higher quantity of 5-ASA, which translates into higher plasma levels of the drug. This is particularly important for drugs such as 5-ASA, as most analytical techniques reported in the literature can only detect this drug in plasma at concentrations of 20 ng/ml or higher.[18,19]

## Materials and Methods

### Materials

5-ASA (certified 95% purity) was used for the production of pellets and was supplied by Avocado Research Chemicals Ltd (Morecambe, UK; batch No. J 3433 B). Avicel PH 101 was obtained from FMC Corporation (Cork, Ireland; batch No. 6842C). Surelease E-7-7050 was a gift from Colorcon Ltd (Dartford, UK; batch No. 600092); this product was plasticized with dibutyl sebacate and the total nominal solids content was 25%w/w, 70% of which was ethylcellulose. Hylon VII was donated by National Starch & Chemical Company (Bridgewater, USA; batch No. FG 5514). 5-ASA (99% purity) was used for the preparation of the standard solutions and was purchased from Sigma Chemicals Co. (St Louis, USA; batch No. 095K1861). Methanol HPLC grade was obtained from Panreac Química Sau (Barcelona, Spain).

Reagents used for the preparation of phosphate-buffered saline (PBS) solution were: potassium chloride (Merck & Co, Inc., Whitehouse Station, USA; batch No. 846936), sodium chloride (Baker Ltd, Dagenham, UK; batch No. 7057), anhydrous disodium hydrogen phosphate (Fluka Biochemika GmbH, Buchs, Switzerland; batch No. 1338489) and potassium dihydrogen phosphate (Merck & Co, Inc., Whitehouse Station, USA; batch No. 421073).

Tetrabutylammonium hydroxide (TBA) solution (1.0 M in water) was purchased from Fluka Biochemika GmbH (Buchs, Switzerland; batch No. 1327731).

Ultra-purified water was prepared using a Milli-Q System (Millipore, Bedford, USA).

### Preparation of 5-aminosalicylic acid loaded pellets

Pellets containing 5-ASA and Avicel PH 101 (1 : 1 w/w) were produced by an extrusion and spheronisation process. The wet mass comprising 5-ASA, Avicel PH 101 and distilled water (40% w/w based on the total weight of the dry mass) were mixed for 15 min in a planetary mixer (Kenwood Chef Model A901E; Kenwood, Havant, UK) at a minimum speed setting. The wet mix attained was extruded through a radial screen extruder (Model 10 Extruder; Caleva, Sturminster Newton, UK) with a screen mesh size of 1 mm diameter and 1 mm thickness. Finally, the extrudate was spheronised for 10 min on a 228 mm diameter plate with cross-hatch geometry rotating at a speed setting of 1000 rev/min (Joshua Greaves & Sons Ltd, Ramsbottom, UK). The final pellets were dried on a porous tray at room temperature for a period of 48 h and sieved to obtain pellets with a size range of 1.00–1.40 mm.[4]

### Film coating of 5-aminosalicylic acid pellets

Batches of 30 g of the 5-ASA pellets were coated in a Uni-Glatt fluidized bed coater with Wurster column and a bottom spray nozzle with a diameter of 1 mm and an 8 cm diameter perforated bottom plate. The thickness of the film coating was determined by laser scanning confocal microscopy as described previously.[4]

The coating operation conditions used in this study were 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. Pellets were cured at 60°C in an oven for 1 h.

The coating dispersion comprising Hylon VII and Surelease was prepared as described previously[4] (i.e. 3 g of Hylon VII were dispersed in 20 ml of distilled water and heated at 80 ± 5°C for 30 min). Once the attained dispersion had cooled down, Surelease E-7-7050 was admixed in a ratio of 1 : 2 (w/w). Complete dispersion of the two polymers was achieved by stirring the suspension for a further 30 min.

### In-vivo study

This study was approved by the local ethical committee and conducted in accordance with the European Directive (86/609/EEC) for the accommodation and care of laboratory animals and the experimental procedures, and according to the Portuguese Veterinary General Division.

Six white New Zealand female rabbits, approximately 2 kg, were used in a two-way randomised crossover study. Rabbits were housed in separate standard cage racks in a humidity- and temperature-controlled room and were kept on a standard diet.

The bottom of the cages was perforated, leading into a different compartment, used for the collection of the rabbits' faeces.

For the study, rabbits were randomly divided into two groups and administered the two different pellet formulations. On the first day of the study, the uncoated formulation was given to group I, while group II received the coated pellets, and vice-versa on the second day of the study. A washout period of 15 days was allowed between administrations.

Rabbits were fasted for 12 h before the study and throughout the study but had free access to water. Pellet formulations (coated or uncoated) containing 50 mg/kg of 5-ASA were filled into hard gelatin capsules (size 4) and administered orally to rabbits using a cannula. To facilitate the ingestion of the capsule, rabbits were given 5 ml of water through a syringe after administration of the capsules. The rabbits were observed to ensure that the capsule had been swallowed easily, without any damage to the capsule or pellets induced by chewing, and that the rabbits had not regurgitated the capsules.

Blood samples (700–800 $\mu$l) were collected through a catheter (Abbocath-T, 26 gauge; Abbot Laboratories, Queenborough, Kent, UK) inserted into the marginal vein of the ear of the rabbits, at pre-determined times (0, 1, 2, 3, 4, 6, 8, 10 and 24 h) and rapidly transferred into 1 ml blood collection tubes containing lithium–heparin (Aquisel S.L., Abrera, Spain). The plasma was obtained after centrifugation of the blood samples at 13 800 rev/min (Minispin; Eppendorf, Hamburg, Germany) for 15 min and kept at −80°C (UF 240; Snijiders Scientific, AR Tilburg, Netherlands) until analysis.

## Quantification of 5-aminosalicylic acid in plasma samples by high-performance liquid chromatography with fluorescence detection

5-ASA quantification in plasma samples was done by high-performance liquid chromatography (HPLC) with fluorescence detection in a Manometric Module Gilson International model 805 system, equipped with a Gilson pump (model 305), a fluorescence detector (model LC 305; Lab alliance, State College, USA) and connected to a SP 4270 integrator (Spectra Physics, Mountain View, USA). The method employs a tandem column system, in which two columns, a Chromolith Flash column (rp 18e, 100 × 4.6 mm i.d.; batch No. UM 6072/028; Merck, Darmstadt, Germany) and a Chromolith Flash column (rp 18e, 50 × 4.6 mm i.d.; batch No UM 7038/118; Merck) are connected in series with a guard cartridge (batch No. HX785819; Merck).

The mobile phase used was composed of methanol and PBS (16 : 84) containing 12 mM tetrabutyl ammonium hydroxide and adjusted to a final pH of 7.4. The injection volume was 100 $\mu$l and the flow rate was set at 0.8 ml/min. The mobile phase was filtered through polyamide membrane filters (0.2 $\mu$m, 50 mm; Whatman GmbH, Dassel, Germany) under vacuum and degassed by ultrasonication.

Fluorescence intensity was monitored at 360 nm and 470 nm, excitation and emission wavelengths, respectively.

Stock and intermediate solutions of 5-ASA were prepared in methanol at the concentrations of 20 $\mu$g/ml and 2 $\mu$g/ml, respectively. Standard working solutions were prepared daily in mobile phase at the following 5-ASA concentrations: 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 $\mu$g/ml.

### *Sample preparation*

Frozen plasma samples (250 $\mu$l) were allowed to thaw at room temperature and vortex mixed for 2 min. Five-hundred microlitres of methanol were added and the mixture was vortex mixed for 2 min, and allowed to stand for 15 min at 4°C before centrifugation for 15 min at 13 800 rev/min. The supernatant was removed and evaporated to dryness under a gentle nitrogen gas stream. The residue was reconstituted in 1 ml of mobile phase and centrifuged for 4 min at 13 800 rev/min before injecting into the HPLC system.

### *Validation of the analytical method*

The optimized analytical method was validated by the assessment of specificity, linearity, precision, accuracy, limits of detection (LOD) and quantification (LOQ).

Linearity was verified in the 5-ASA concentration range of 0.04–0.80 $\mu$g/ml. This was done with 5-ASA solutions prepared daily in plasma by spiking plasma with an adequate volume of standard working solutions (see above) followed by extraction of 5-ASA as described above.

The accuracy of the method was determined as the percentage of 5-ASA recovered from the plasma samples. Three different plasma concentration levels were investigated: 0.04, 0.20 and 0.80 $\mu$g of 5-ASA per ml. A minimum of three replicates was performed for each concentration level.

Intra and inter-day precision was determined as the % coefficient of variation (%CV) obtained from measuring three different 5-ASA fortification levels (0.040, 0.200 and 0.800 $\mu$g/ml) during the same day, or on three consecutive days, respectively.

The LOD of the method was determined as the lowest concentration of 5-ASA that could be detected, but not necessarily quantified in the plasma, whereas the LOQ was here defined as the lowest 5-ASA concentration quantifiable with suitable precision and accuracy.

## Determination of the drug content of the 5-aminosalicylic acid coated pellets retrieved from the faeces of the rabbits

The faeces of three rabbits that had been given the coated formulation were collected at the end of the 24-h study and the 5-ASA pellets retrieved from the faeces. 5-ASA pellets were ground in a ceramic mortar and the resultant powder was dispersed in methanol. The resulting suspension was ultrasonicated for 10 min, filtered through filter paper (Whatman No. 4, 150 mm; Whatman International Ltd, Maidstone, UK) and the filtrate diluted appropriately in mobile phase before analysing by HPLC. Experimental conditions for HPLC analysis were the same as described above.

The specificity of the method was tested for any chemicals interfering with the analysis of 5-ASA by HPLC, such as the components of the film coating (Hylon VII and Surelease). The method showed excellent specificity and

yielded a % of recovery of the 5-ASA from the pellets of 100.4 ± 2.30% ($n$ = 3).

### Pharmacokinetics analysis

The 5-ASA concentration in the plasma ($\mu$g/ml) as a function of the time of administration (in hours) was plotted for both uncoated and coated formulations. The maximum 5-ASA concentration in the plasma ($C_{max}$) is the arithmetic mean of values for the six rabbits in the two days of the study whereas the corresponding time ($t_{max}$) was read directly from the graph. The area under the 5-ASA plasma concentration–time curve ($AUC_{0\text{-}24h}$) was calculated using non-compartmental analysis (WinNonlin version 5.2.1, Pharsight, USA).

### Statistical analysis

All statistical calculations were undertaken using SPSS 17.0 (SPSS Inc., Woking, UK). The values for the $AUC_{0\text{-}24h}$ were compared using the GLM module considering that the two sets of data (i.e. data from uncoated and film-coated pellets) are dependent due to the experimental design being a two-way randomised crossover study using the same rabbits for either formulation (e.g. as is done in bioequivalence studies). The type of pellet formulation was treated as fixed factor, whereas the order of treatment and the individual rabbits were regarded as random factors, as described by Weber.[20] The analysis demonstrated that the random factors did not influence the results significantly, and hence the statistically significant difference between the $AUC_{0\text{-}24h}$ values ($F = 94.07$; $P < 0.001$) is the result of the different formulation principles (i.e. film-coating versus no coating).

In this study, a simple and fast HPLC technique using monolithic columns was employed wherein the 5-ASA is extracted from the plasma simply by adding methanol, which acts by precipitating the proteins of the plasma, as previously described.[18]

The specificity of a method is its ability to detect and separate impurities present in the plasma sample. In this method complete resolution of 5-ASA has been achieved. Moreover no other component of significant response was co-eluted, establishing the specificity of the method.

The various parameters used to validate the analytical method are summarised in Table 1.

The method was shown to have an excellent linearity within the concentration range of 0.04–0.8 $\mu$g/ml ($R^2$ adjusted = 0.996 and residual root mean square of 6.3%). The accuracy values obtained for the three different fortification levels ranged from 95 to 98%.

Intra-day precision values ranged from 2.50% to 8.23% whereas the inter-day precision values were below 5%. These results confirmed the high precision and accuracy of the analytical method optimised in this study.

A colon-specific drug delivery system should be able to minimise drug release in the stomach and small intestine. To be certain of this, the analytical method used for the quantification of the drug must have low limits of detection and quantification. The LOD and LOQ of the method used in this study were 0.02 $\mu$g/ml and 0.04 $\mu$g/ml, respectively, which is in line with previous studies.[18,19]

As all validation parameters were within the recommended limits, the analytical method was considered suitable for the quantification of 5-ASA in plasma samples.

## Results

### Validation of the analytical method

The choice of a proper analytical method for the quantification of drugs or their metabolites in biological samples plays a significant role in the interpretation of pharmacokinetic data. HPLC techniques have been widely used in the quantification of 5-ASA in plasma samples.[18,19,21] Given the polar character of this drug, its extraction from biomatrices is complicated. For that reason, some of these techniques have used derivatization of the parent drug, resulting in techniques that are more reliable although more complicated and lengthy.[19,21]

### 5-Aminosalicylic acid plasma levels

Figure 1 shows the 5-ASA plasma concentrations ($\mu$g/ml) obtained after oral administration of uncoated pellets and pellets coated with Hylon VII/ Surelease, 1 : 2 (w/w), administered in a dose of 50 mg/kg of 5-ASA.

Higher peak plasma concentrations of 5-ASA were reached faster after oral administration of the uncoated formulation (mean $C_{max}$ of 2.38 ± 0.49 $\mu$g/ml at 2 h post administration) than following the administration of the coated formulation (mean $C_{max}$ 0.22 ± 0.19 $\mu$g/ml and $t_{max}$ of 8 h).

**Table 1** Summary of the validation parameters of the HPLC method used for the quantification of 5-aminosalicylic acid in rabbit plasma samples

| Linearity[1,2] | LOD ($\mu$g/ml) | LOQ ($\mu$g/ml) | Accuracy mean[1] (%) | Intra-day precision (% CV) | Inter-day precision[a] (% CV) |
|---|---|---|---|---|---|
| $R^2$ adjusted (RMS)[3] | | | | | |
| 0.996 (6.3%) | | | 98.25 ± 0.84[a] | 8.23[a] | 0.85[a] |
| Y-intersect | 0.020 | 0.040 | 94.71 ± 4.19[b] | 4.96[b] | 4.43[b] |
| −2138.1 | | | 95.33 ± 1.36[c] | 2.50[c] | 1.43[c] |
| Slope | | | | | |
| 385578.4 | | | | | |

[1]Obtained during 3 consecutive days; [2]obtained for five different 5-ASA concentrations within the concentration range of 0.04–0.80 $\mu$g/ml; [3]root mean square deviation in residual analysis; [a]5-ASA concentration of 0.04 $\mu$g/ml; [b]5-ASA concentration of 0.20 $\mu$g/ml; [c]5-ASA concentration of 0.80 $\mu$g/ml.

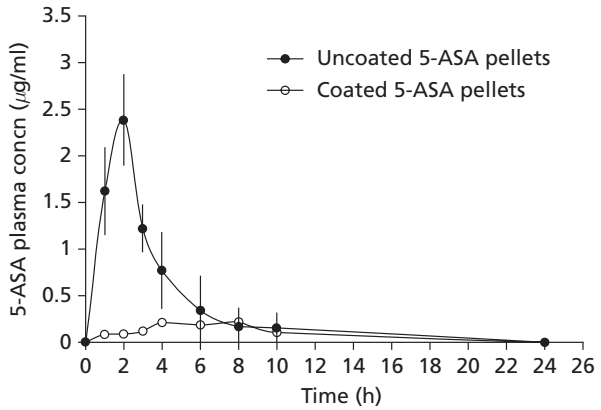


**Figure 1** 5-Aminosalicylic acid plasma levels. Plasma concentrations of 5-aminosalicylic acid (ASA; μg/ml) obtained after oral administration of uncoated pellets (formulation 1) and pellets coated with Hylon VII/Surelease, 1 : 2 (w/w) (formulation 2) containing 50 mg/kg of 5-ASA to rabbits. Each point represents the mean ± SD of six replicates.

The mean $AUC_{0\text{-}24h}$ values obtained with the uncoated and coated formulations were 7.83 and 1.29 μg h/ml, respectively.

Given the low 5-ASA plasma concentration attained following administration of the coated formulation, the coated pellets were retrieved from the faeces of three rabbits, 24 h post dosing and analysed for drug content. The average percentage of 5-ASA contained in the pellets was 38.3%, which means that more than 60% of the 5-ASA had been released from the pellets.

## Discussion

The in-vitro behaviour of the coated pellets has been reported in a previous study[16] in the presence of pancreatin and *Bacillus licheniformis* α-amylase to simulate the digestion of the starch moiety of the coating in the small intestine and colon, respectively. 5-ASA release was found to be significantly increased in the presence of *Bacillus licheniformis* α-amylase, suggesting that the starch can be digested under conditions simulating the colon, permitting drug release into the colon. In the presence of pancreatin, however, the drug release was only marginal, thus confirming the site specificity of the proposed drug delivery system.

In this study, further proof of concept was obtained *in vivo* in rabbits for both uncoated and coated 5-ASA pellet formulations. The in-vivo behaviour of both formulations was very different. The uncoated formulation showed high plasma concentrations of 5-ASA after just 1 h post administration, suggesting that the drug was released from the pellets without any delay. For the coated formulation, drug release was much lower and only increased 8 h post administration (Figure 1). Mouth to ileum transit times of cell wall material in rabbits was found to vary between 6 to 7.9 h.[22] In another study, transit of food in the form of individual particles through the stomach of rabbits was found to be in the range of 3–6 h, and shorter transit times were found in the small intestine (10–20 min in the jejunum and 30–60 min in the ileum).[23] No reference has been made in the literature as to whether the transit times in the stomach and small intestine of rabbits differ according to the size of the food particles.

The retention time in the colon of rabbits, however, appears to be related to size of the particles. Smaller particles of food are delayed in the colon as they are sent backwards into the caecum to undergo fermentation. The larger particles move more quickly through the colon and are eliminated first in the faeces.[22,24,25] In one study,[22] ileum-to-rectum transit of particles with a size 1–3 mm was 14.1 h. When the same particles were ground to a particle size < 1 mm the ileum to rectum transit time was increased to 15.9 h.

Following administration of the uncoated formulation and based on the previously documented transit times, 5-ASA release and absorption should have occurred mainly in the rabbit's stomach and proximal small intestine. On the other hand, following administration of the coated formulation, 5-ASA release and subsequent absorption were more significant upon entering the rabbit's caecum. In the literature, the upper intestines are reported to be the preferential site within the gastrointestinal tract for 5-ASA absorption. For example, in humans 5-ASA absorption through the duodenum was reported to be 5-fold higher than in the ileum.[26] Therefore, the finding that the plasma level of 5-ASA after administration of the coated formulation remained low for 8 h (i.e. during the transit of the pellets through the stomach and small intestine) indicates that the coating was able to prevent drug release at these sites. Moreover, the increased drug release on entering the colon confirms that 5-ASA release from this system was the result of the specific degradation of the starch by the colon microflora. However, the plasma levels of 5-ASA were relatively low ($AUC_{0\text{-}24h}$ = 1.29 μg h/ml), suggesting that either the drug was not entirely released from the pellets or the absorption through the colon mucosa was limited. Comparatively low drug plasma levels were also reported in humans following administration of a colon-specific delivery system comprising ranitidine.[27]

The average drug content of the pellets after the 24-h study was 38.3%, therefore release of 5-ASA, although incomplete, was accomplished in the rabbit's colon. Lower absorption of 5-ASA (< 25%) from the human colon mucosa has been previously described and reviewed by Klotz.[28] Different studies found that a very high amount of 5-ASA is excreted in the faeces, either unchanged or in its acetylated form. Acetylation of the 5-ASA in the faeces is carried out by a high proportion of the faecal microflora.[29,30] When given orally in the form of the pro-drug sulfasalazine in humans, the amount of 5-ASA excreted in the faeces varied from 50% in one study[31] to as high as 80% in another study.[32] In both cases, 5-ASA was reported to be released in the colon as solid particles due to disintegration of the dosage form. Therefore, to be absorbed, it needs to be dissolved. In the colon, the volume of luminal fluids is rather low as re-absorption of water occurs primarily from the colon mucosa. For example, the free fluid volume in the colon of humans is just 13 ± 12 ml in the fed state and 11 ± 26 ml in the fasted state compared with 105 ± 72 ml and 54 ± 41 ml in the small intestine, in the fed and fasted state, respectively.[33]

The exact luminal fluid volume in the colon of rabbits has not yet been described. However, in rats, the colon luminal fluids were found to be 7.8 ± 1.5 ml in the fed state and 3.2 ± 1.8 ml under fasted conditions.[34] This considerably low volume of fluids available for dissolution of 5-ASA can impair the drug absorption though the colon mucosa. Furthermore, when solutions of 5-ASA were directly instilled into the colon in six healthy humans, drug absorption was still very low,[35] pointing to a very limited permeability of the colon mucosa for 5-ASA. These results suggest that the low 5-ASA plasma level attained in our study, following the administration of the coated formulation, supports the concept of the topical mode of action of 5-ASA in the colon. An earlier report[36] suggested that in humans 5-ASA acts from the luminal side by penetrating the colon mucosa at high concentrations.

## Conclusions

The in-vivo behaviour of a colon-specific drug delivery system was tested in rabbits. The system comprises 5-ASA pellets coated with a mixture of Hylon VII and Surelease in a 1 : 2 ratio and film thickness of approximately 48 $\mu$m. In this system the starch is used as received, without extracting the amylose content, employing a well-defined thermal treatment and coating procedure to achieve crystallisation or retrogradation of the starch component. The 5-ASA absorption was significantly delayed until 8 h post administration. Therefore, the coating was able to prevent the drug release in the stomach and small intestine, and release started only in the lower intestine. More than 60% of the 5-ASA was released from the coated pellets in the colon.

This study showed that the newly developed high amylose starch-based coatings are a potential method of accomplishing drug delivery specifically into the colon.

## Declarations

### Conflict of interest

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

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