AAPS PharmSciTech, 2000; 1 (3) article 22
(http://www.pharmscitech.com/)

The Potential of Organic-Based Amylose-Ethylcellulose Film Coatings as Oral Colon-Specific Drug Delivery Systems

Submitted: May 2, 2000; Accepted: July 20, 2000

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ABSTRACT Amylose-ethylcellulose film coatings obtained from organic-based solvents were investigated as potential vehicles for colonic drug delivery. Amylose, in the form of an amylose-butan-1-ol dispersion, and ethylcellulose, dissolved in either ethyl lactate, ethanol, or propanol and plasticized with dibutyl sebacate, were mixed in various proportions and applied using a fluidized bed coater to achieve a range of film thicknesses on 5-aminosalicylic acid pellets. Drug release from the coated pellets was assessed under gastric and small intestinal conditions in the presence and absence of pepsin and pancreatin using dissolution methodology, and also within a simulated colonic environment involving fermentation testing with human feces in the form of a slurry. Under upper gastrointestinal tract conditions, the rate and extent of drug release were found to be related to the thickness of the coating and the ratio of amylose to ethylcellulose within the film. Modeling of the drug release data revealed that the ratio was more important than coat thickness in controlling drug release, irrespective of the solvent used for coating. Coatings with a thick film and/or low amylose content were relatively impermeable and able to delay drug release under conditions mimicking the upper gastrointestinal tract. Furthermore, drug release was unaffected by the presence of pepsin and pancreatin and by long-term storage. Under simulated colonic conditions, drug release was more pronounced from coating formulations containing higher proportions of amylose. Colon-specificity can therefore be achieved using such systems by judicious choice of the appropriate ratio of amylose to ethylcellulose and coat thickness.

KEYWORDS: Amylose, Colonic Drug Delivery, Ethylcellulose, Film Coatings, Organic Solvents

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INTRODUCTION

Site-specific drug delivery to the colon would be particularly useful for local treatment of colonic disorders such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome. Furthermore, the colon may offer an environment that is more suited to the absorption of labile compounds, such as peptides and proteins, for systemic therapy.

A reliable colon-specific drug delivery system is necessary to achieve these goals. To this end, systems that utilize materials that are susceptible to degradation by bacterial enzymes within the colon are believed to hold more promise than pH- and time-dependent systems [1,2]. In addition, natural materials, such as

those found in the diet, are preferred over synthetic materials for colonic delivery because they are safer and more available [3].

Starch, a major constituent of the diet, is composed of 2 polysaccharides, amylose and amylopectin. Both are made up of α -(1,4)-linked D-glucose units, but amylose is essentially a linear molecule, while amylopectin is highly branched. Starch was initially believed to be completely hydrolyzed by pancreatic enzymes and absorbed within the small intestine, but it is now known that a substantial proportion of starch escapes digestion in the small intestine and undergoes fermentation by bacteria in the colon [4,5]. This resistant fraction of starch has properties similar to fiber and was found to be primarily composed of amylose [4,5]. Moreover, the glassy amorphous form of amylose is particularly resistant to pancreatic enzymes, but it is also susceptible to digestion by a group of amylase-producing bacteria [6], which make up over 50% of the bacterial count of human feces [7]. This form of amylose has therefore been exploited as a film-coating material for colonic drug delivery [6,8]. Amylose films, however, swell and become permeable in the presence of an aqueous environment. This problem was overcome by incorporating the water-insoluble polymer, ethylcellulose, in the form of an aqueous dispersion, into the coating formulation. These mixed coating formulations exhibited in vitro resistance to gastric and small intestinal conditions yet remained sensitive to digestion by enzymes of colonic bacterial origin [9,10]. Testing in human volunteers verified the results obtained in vitro [3, 11], thereby confirming the colon-specificity of these amylose-based delivery systems.

In line with current practice, film-coating operations employing aqueous conditions were used in the coating these amylose-ethylcellulose films. The use of organic coating conditions, on the other hand, would involve lower coating temperatures and shorter processing times, conditions that may be more desirable for water-sensitive and/or thermolabile drugs. A previous study has shown that amylose-ethylcellulose-free films can be prepared from the water-miscible organic solvent ethyl lactate, with sufficient physico-mechanical and digestion properties for colonic delivery [12]. The purpose of this study was to assess the in vitro colon-specificity of these films, and also films prepared from the solvents, ethanol or propanol, after application to conventional oral pellet dosage forms containing the model drug, 5-aminosalicylic acid.

MATERIALS AND METHODS

MATERIALS

5-Aminosalicylic acid, certified as 95%-98% pure, was obtained from Sigma-Aldrich Co Ltd (Poole, UK) and microcrystalline cellulose (Avicel® PH101) was contributed by FMC Corp (Philadelphia, Pa). Lactose BP was purchased from Sheffield Products (Norwich, NY), and bentonite was obtained from Merck Ltd (Poole, UK). Amylose was extracted from pea starch and obtained in the form of an amylose-butan-1-ol complex aqueous dispersion [13] from the Institute of Food Research (Norwich, UK) and used at a concentration of 12% w/w. Ethylcellulose N-100 was obtained from Dow Chemical Co Ltd (Uxbridge, UK). The water-miscible organic solvent, ethyl lactate, and plasticizer, dibutyl sebacate, were purchased from Sigma-Aldrich. The other water-miscible organic solvents, ethanol (99.7%-100% v/v) and propanol (propan-1-ol) (Merck) were of AnalaR grade. Pepsin (1:2500 potency) and pancreatin (potency equivalent to USP specification) were purchased from Sigma-Aldrich. All other chemicals were of AnalaR grade and were obtained from Merck.

Preparation of 5-Aminosalicylic Acid Pellets

Pellets were prepared by extrusion and spheronization from a formulation comprising 10% 5-aminosalicylic acid, 55% microcrystalline cellulose, 30% lactose, and 5% bentonite. The formulation components were dry blended using a planetary mixer (A707A, Kenwood, Havant, UK) for 10 minutes. Distilled water, equivalent to 52.5% of the dry weight of powders, was added and mixing continued for 10 minutes. The wet powder mass was extruded using a ram extruder driven by an instrumented mechanical testing device (MX50, J.J. Lloyd, Southampton, UK) at a rate of 200 mm/min through a die of 1 mm diameter and 4 mm length. The resultant extrudate was processed using a 20.3 cm diameter spheronizer (G.B. Caleva Ltd, Sturminster Newton, UK) with a radial plate rotating at 1000 rpm for 30 minutes. The pellets formed were dried in a fluidized bed dryer (FDBL 70, P.R.L. Engineering Ltd, Flintshire, UK) for 30 minutes at 60° C. The dried pellets were then sieved and those ranging from 1.0 to 1.4 mm were used in further studies.

Preparation of Coating Formulations

Ethylcellulose was dissolved in either ethyl lactate, ethanol, or propanol to produce a 3% w/v solution. The plasticizer, dibutyl sebacate, was added to the solution based on the solid dry weight (35% w/w) of ethylcellulose present and mixed for 3 hours using a magnetic stirrer. Various quantities of the amylose-butan-1-ol complex aqueous dispersion were then added to the plasticized ethylcellulose solutions and stirred for another hour to produce coating formulations with different solid ratios of amylose and ethylcellulose (1:4, 1:2, 3:2, and 1:1). Preliminary experiments had established that the aqueous amylose dispersion was compatible with the different organic ethylcellulose solutions since precipitation of either polymer was not observed.

Film Coating

Coating was performed on 100-g batches of pellets using a fluidized bed spray coater (GPCG-1 Uni-Glatt, Glatt GmbH, Binzen, Germany). The coating procedure involved maintaining the bed temperature at 35 to 40° C, spray rate within 0.25 to 0.35 g/min, and atomizing air pressure in the region of 1.7 to 2.1 bar. A final drying stage was incorporated into the process by turning off the spray and keeping the coated pellets at the same bed temperature for 20 minutes. A series of coated batches with different film thicknesses were produced. The film thickness is expressed in terms of the percentage total weight gain (TWG), and products with a TWG of 3%, 6%, 10%, and 15% were obtained. These TWGs are approximately equivalent to film thicknesses of 10, 20, 32, and 48 μ m, respectively. Formulations were stored for up to 12 months in a dessicator containing a saturated salt solution of potassium carbonate (44% RH, 20° C) to assess the effect of long-term storage on stability.

Dissolution studies

Drug release studies were performed using a method 2 dissolution test apparatus (PTWS, Pharma Test, Hainburg, Germany) as described in the USP 23. Tests were conducted in 900 mL of dissolution medium maintained at 37 ± 0.5° C with a paddle rotation speed of 100 rpm. The pH of the medium was varied over the course of the experiment: 0.1 N hydrochloric acid (pH 1.2) was used for the first 3 hours and 0.05 M phosphate buffer (pH 7.2) was used for the next 3 hours. For each test, 300 mg of coated pellets was used. Three mL samples were withdrawn at predetermined times using an automated sampler (PTFC-1, Pharma Test, Hainburg, Germany). The 5-aminosalicylic acid concentration in each sample was determined using an UV-Vis spectrophotometer (554, Perkin Elmer, Ueberlingen, Germany) at wavelengths of 302

nm and 332 nm for the pH 1.2 and pH 7.2 dissolution media, respectively. The percentage of 5-aminosalicylic acid released over time was calculated and plotted as an average of 6 runs using calibration curves consistent with Beer's law.

Selected formulations were further evaluated under physiological conditions that more closely resemble those in the upper gastrointestinal tract. In this case, freshly prepared simulated gastric fluid (0.1 N hydrochloric acid containing 0.32% w/v pepsin) was used as the dissolution medium for the first 3 hours. This medium was then replaced by freshly prepared simulated intestinal fluid (0.05 M phosphate buffer containing 1% w/v pancreatin) for an additional 3 hours. Samples were withdrawn at specific times, centrifuged at 9600*g* for 15 minutes, filtered using 0.2 µ m filters, and analyzed as described previously.

Fermentation studies

Drug release from the pellets was also assessed under simulated colonic conditions using a batch culture fermentation system, which was slightly modified from a previously described system [7]. One hundred mg of coated pellets were presoaked in 100 mL of 0.1N hydrochloric acid (pH 1.2). After 30 minutes the pellets were removed and introduced into 100-mL batch culture fermenters inoculated with human feces (10% w/v). The fermenters were prepared by homogenizing freshly voided human feces in a buffer medium comprising 0.15% potassium dihydrogen orthophosphate, 0.15% dipotassium hydrogen orthophosphate, 0.45% sodium chloride, 0.05% magnesium chloride hexahydrate, 0.005% ferrous sulphate heptahydrate, 0.015% calcium chloride dihydrate and enough sodium hydroxide to obtain a pH of 7.2. Unhomogenized fibrous material was removed by passing the slurry through a 500-µ m sieve. The fermenters were sealed under positive nitrogen pressure to establish an anaerobic environment and then placed in an incubator at 37° C and shaken at 100 rpm (for 12 hours). Control experiments using buffer medium without feces were run in parallel. Two-mL samples were removed at predetermined times over a 12-hour period, centrifuged at 13 000 rpm for 5 minutes, and filtered through 0.2 µ m filters prior to analysis for 5-aminosalicylic acid by high-performance liquid chromatography (HPLC; Series 400, Perkin Elmer, Norwalk, Conn). The mobile phase consisted of 10% methanol and 90% of 1% w/v acetic acid pumped at 1.5 mL/min through a 5 µ m Techsphere ODS (25 cm x 4.6 mm) column (Jones Chromatography, Gwent, UK). The detection wavelength was set at 300 nm.

RESULTS AND DISCUSSION

Thirteen batches of pellets were satisfactorily coated from each of the 3 organic-based solvent systems (ethyl lactate, ethanol, and propanol) at temperatures less than 40° C with amylose-ethylcellulose coatings of different compositions and thicknesses. These coating systems, although not strictly nonaqueous because of the presence of amylose, are predominantly organic in nature due to the greater proportion of organic solvent in the mixed dispersions. These conditions and formulations are particularly suited to coating drugs that are sensitive to water and/or heat.

The effect of coating thickness and ratio of amylose to ethylcellulose, from the ethyl lactate– based solvent system, on 5-aminosalicylic acid release are shown in **Figures 1 through 3**.

The rate of release is inversely proportional to the thickness of the coat, implying that the film coat was controlling the release process. The mechanism of release, although likely to be via diffusion through the plasticized amylose and ethylcellulose phases of the coat, will be influenced by the amount of amylose present

within the film. The presence of amylose results in a porous, heterogeneous film structure [12]. In the presence of an aqueous medium, the swelling of amylose will lead to a disruption in the structure of the film and the formation of aqueous filled pores through which diffusion can also occur. Film coatings containing higher concentrations of amylose are therefore more permeable to 5-aminosalicylic acid release. One of the key requirements of a colon-specific delivery system is that it must delay drug release until it passes through the upper gastrointestinal tract. The 6-hour dissolution test used in these studies should be sufficient to assess this, since mouth to colon transit times of pellet dosage forms have been found to be of this order [14]. Formulations with a thicker film coat and/or low amylose content appear to comply with this requirement.

To further elucidate the influence of coating thickness and amylose to ethylcellulose ratio on drug release, a model was developed from the dissolution data using regression analysis (Statistical Package for Social Sciences, Version 8, SPSS, Woking, UK):

Solvent: Ethyl lactate

$$\% \text{ 5-ASA released} = 5.901 + 73.434 \times (\text{A:EC})^2 - 5.185 \times \text{TWG} \times \text{A:EC} \quad (1)$$

($RMS = 2.19\%$, $R^2 = 0.935$)

(% 5-ASA released = % 5-aminosalicylic acid released after 6 hours, A:EC = amylose to ethylcellulose ratio, TWG = total weight gain)

This equation shows that the amylose to ethylcellulose ratio has a greater influence on drug release than the coating thickness. Information derived from this equation on the effect of various theoretical amylose to ethylcellulose ratios and coating thicknesses on drug release is depicted in **Figure 4**.

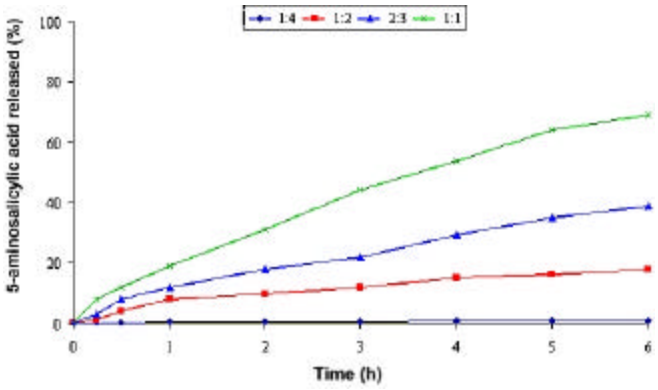

Figure 1. Effect of amylose to ethylcellulose ratio on 5-aminosalicylic acid release from pellets coated to a TWG of 3%.

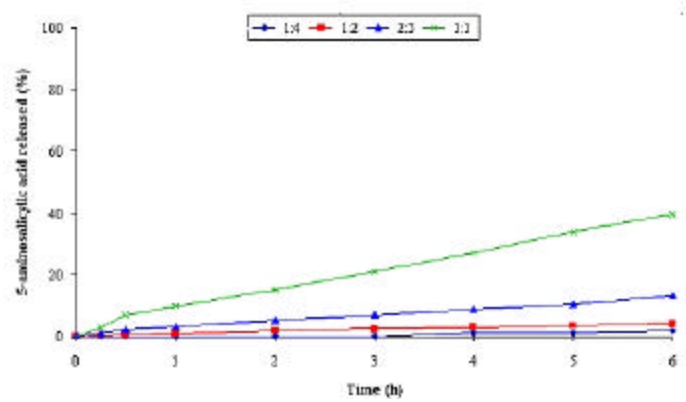

Figure 2. Effect of amylose to ethylcellulose ratio on 5-aminosalicylic acid release from pellets coated to a TWG of 6%.

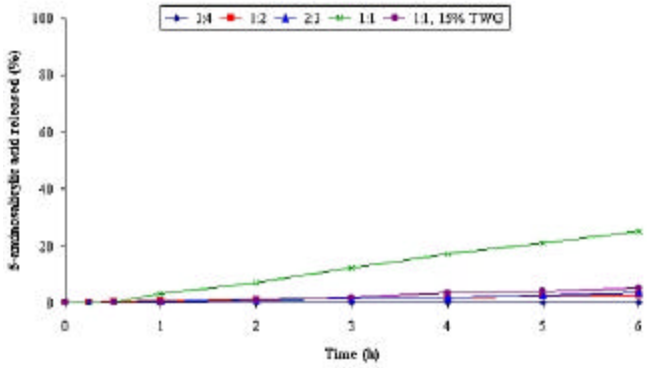

Figure 3. Effect of amylose to ethylcellulose ratio on 5-aminosalicylic acid release from pellets coated to a TWG of 10% and 15%.

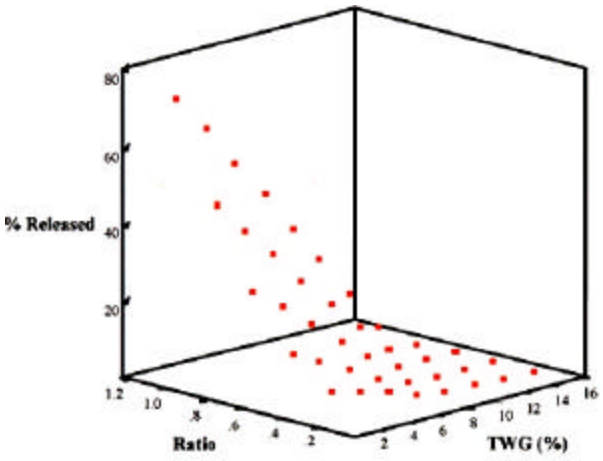

Figure 4. Relationship between amylose to ethylcellulose ratio, coat thickness (TWG), and 5-aminosalicylic acid release from coated pellets after 6 hours [solvent: ethyl lactate]. The amylose to ethylcellulose ratio is represented by a single figure, for example 1 part amylose:4 parts ethylcellulose ≡ 0.25:1 ≡ 0.25.

The trend to note here is that drug release is minimized by a film coat with low amylose content and/or high thickness, as suggested earlier. The drug release models and graphs for coatings obtained from the ethanol- and propanol-based coating systems are presented below and in **Figures 5** and **6**.

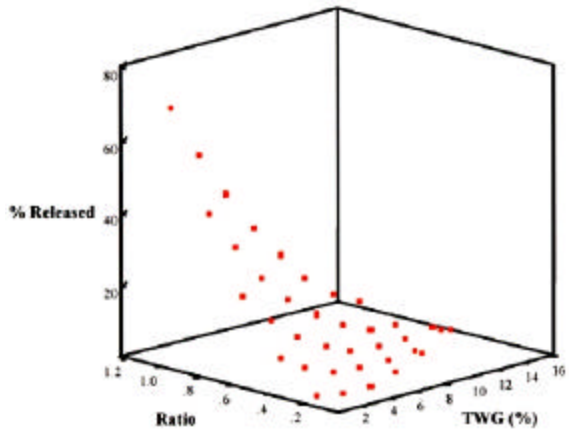

Figure 5. Relationship between amylose to ethylcellulose ratio, coat thickness (TWG), and 5-aminosalicylic acid release from coated pellets after 6 hours [solvent: ethanol]. The amylose to ethylcellulose ratio is represented by a single figure, for example 1 part amylose: 4 parts ethylcellulose ≡ 0.25:1 ≡ 0.25.

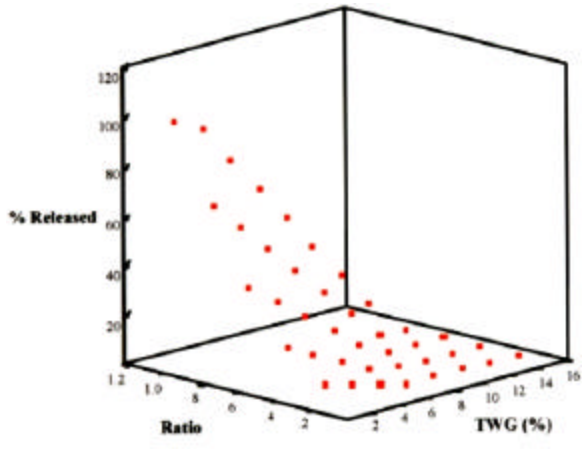

Figure 6. Relationship between amylose to ethylcellulose ratio, coat thickness (TWG), and 5-aminosalicylic acid release from coated pellets after 6 hours [solvent: propanol]. The amylose to ethylcellulose ratio is represented by a single figure, for example 1 part amylose: 4 parts ethylcellulose ≡ 0.25:1 ≡ 0.25.

Solvent: Ethanol

$$\% \text{ 5-ASA released} = -0.815 + 78.953 \times (\text{A:EC})^2 + 0.212 \times (\text{TWG})^2 - 8.191 \times \text{TWG} \times \text{A:EC} \quad (2)$$

(RMS = 0.63%, $R^2 = 0.972$)

Solvent: Propanol

$$\% \text{ 5-ASA released} = 5.325 + 110.272 \times (\text{A:EC})^2 - 7.319 \times \text{TWG} \times \text{A:EC} \quad (3)$$

(RMS = 1.58%, $R^2 = 0.966$)

Here, once again, the amylose to ethylcellulose ratio has a more profound effect on drug release than the thickness of the coating. Although the drug release equation for the propanol system is almost identical to that for ethyl lactate, the ethanol system is somewhat different from the other two, which suggests that the films produced from ethanol are structurally different from those obtained from ethyl lactate or propanol. This could be because ethanol is a more polar solvent than ethyl lactate and propanol and may interact with the

aqueous amylose phase to a greater extent than the other 2 solvents, thereby leading to the formation of films with dissimilar properties. Regardless of the reason, the overall drug release trend is similar among the 3 solvents (**Figures 4 to 6**).

Coated pellets that satisfactorily retarded drug release were further tested for resistance under physiological conditions more closely resembling those of the stomach and small intestine. Pepsin and pancreatin (a complex mixture of lipases, proteases, and amylases) were found to have no apparent influence on drug release (data not shown), confirming the resistance of the coatings to enzymatic degradation in the upper gastrointestinal tract. Further, no appreciable difference in dissolution performance was seen for the different formulations over 12 months (data not shown), thereby confirming stability of the amylose-ethylcellulose coatings on storage.

The dissolution testing methodology utilized above assesses only the resistance of the coating to drug release under conditions found in the upper gastrointestinal tract, but it provides no information about the coating's digestibility or permeability within the colonic environment. A batch culture fermentation system was used to obtain such information. **Figure 7** highlights the effect of colonic conditions on the release of 5-aminosalicylic acid from pellets coated with 1 part amylose and 4 parts ethylcellulose from an ethyl lactate solvent system to a TWG of 3%.

Surprisingly, drug release was very slow and only marginally faster than in the control phosphate buffer. The slow release may be related to the limited amount of amylose present within the film, which would prevent the formation of a continuous amylose network throughout the film structure, leading to the amylose being less accessible to enzymatic attack and hence hindering digestion of the coating. This result contrasts with the rapid and complete drug release achieved from coated pellets with the same amylose and ethylcellulose composition but obtained from aqueous-based solvent systems [9]. Such findings may be attributed to differences in the formation, structure, and properties of films prepared from organic and aqueous conditions. Increasing the film's amylose component improved drug release. For example, a coating formulation comprising 1 part amylose and 1 part ethylcellulose with a TWG of 15% displayed a markedly faster drug release in simulated colonic conditions than in the control (**Figure 8**).

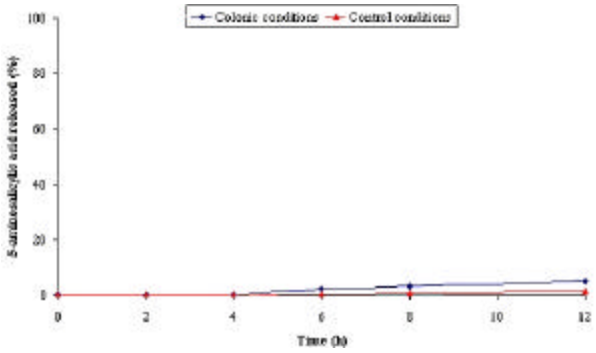

Figure 7. Effect of simulated colonic conditions on 5-aminosalicylic acid release from pellets coated with 1 part amylose and 4 parts ethylcellulose to a TWG of 3%.

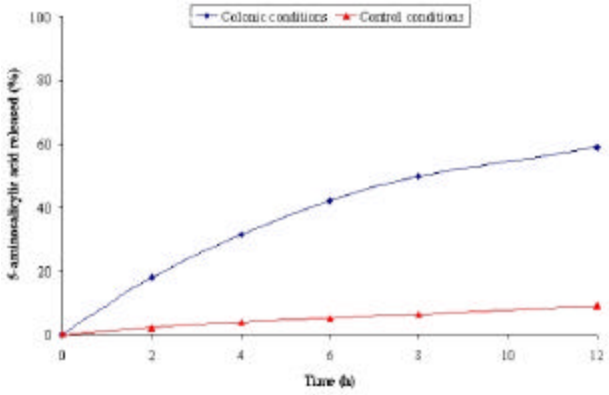

Figure 8. Effect of simulated colonic conditions on 5-aminosalicylic acid release from pellets coated with 1 part amylose and 1 part ethylcellulose

This high proportion of amylose within the film also necessitated an increase in coat thickness

to overcome swelling of the polysaccharide and prevent premature drug release in the upper gastrointestinal tract. In spite of this increase in film thickness, accelerated drug release under simulated colonic conditions indicates digestion of the amylose component of the coating by bacterial enzymes. **Table 1** shows that the drug release trend is similar regardless of the solvent system used.

Table 1. Influence of Coat Thickness (TWG), Ratio of Amylose to Ethylcellulose, and Coating Solvent on 5-Aminosalicylic Acid Release from Coated Pellets After Incubation in Simulated Colonic/Control Conditions for 12 Hours

5-Aminosalicylic Acid Released (%)							
Coat Characteristics		Ethyl lactate		Ethanol		Propanol	
TWG (%)	Ratio	Colonic	Control	Colonic	Control	Colonic	Control
3	1:4	5	1	3	1	12	5
6	1:2	18	8	10	5	18	4
10	2:3	20	6	17	6	28	9
15	1:1	59	9	50	10	41	10

In other words, drug is released more rapidly from coatings that have a greater proportion of amylose. Although the paucity of data precludes modeling these results, it appears that the ratio of amylose to ethylcellulose in the film is more important than coat thickness both for retarding drug release under simulated upper gastrointestinal conditions and allowing release under colonic conditions.

CONCLUSIONS

This study confirms the possibility, as previously suggested [12], of employing coatings of amylose and ethylcellulose mixtures prepared under predominantly organic-based conditions for colon-specific drug targeting. The coated pellets demonstrated reproducible drug release rates that were unaffected by upper gastrointestinal pH and enzymes and also long-term storage. Drug release was modified by varying parameters such as the ratio of amylose to ethylcellulose in the film and the coat thickness. Modeling of the resultant data found that the ratio was more important than coat thickness in controlling drug release, irrespective of the solvent used for coating. Formulations comprising 1 part amylose and 1 part ethylcellulose of coat thickness, 15% TWG, successfully resisted 5-aminosalicylic acid release in the upper gastrointestinal tract yet gave a relatively rapid onset of release in simulated colonic conditions. Such organic-based systems offer a practical means of delivering drugs to the colon, particularly those that are water-sensitive and/or thermolabile.

ACKNOWLEDGEMENTS

The authors would like to thank Glatt GmbH and the Overseas Research Students (ORS) scholarship scheme for their financial support. Dr F. Podczeck at the School of Pharmacy is warmly acknowledged for her assistance with the statistical analysis of the data. The assistance of Dr J.H. Cummings is greatly appreciated in providing facilities at the MRC Dunn Clinical Nutrition Centre, Cambridge, for the fermentation studies. Professor M.C. Allwood and colleagues at the Medicines Research Unit, University of Derby, are kindly thanked for conducting the HPLC experiments.

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