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Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance in vitro

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Abstract

The purpose of this study was to develop an enzyme-based fermentation system for the in vitro assessment of colonic digestion of amylose films and coatings, and to compare its performance with a conventional fermentation model inoculated with human faecal bacteria. Amylose and ethylcellulose were mixed in different ratios and cast as isolated films, as well as spray coated onto drug-(5-aminosalicylic acid) loaded pellets. Four commercial amylase enzymes were individually screened for their ability to digest amylose cast films. The enzyme from the bacterium *Bacillus licheniformis* was found to be the most active against this substrate. Digestion of mixed amylose and ethylcellulose films was also observed, with the extent of digestion being proportional to the quantity of amylose present in the film. In terms of product performance, drug release from coated pellets was accelerated in the presence of the enzyme. The results with the enzyme system were comparable to those obtained from a faecal-based fermentation model, thereby suggesting that such a system has practical potential for in vitro screening of putative amylose formulations for colonic drug delivery.

Keywords: Amylose; Colonic delivery; Mesalazine; 5-Aminosalicylic acid; Gastrointestinal microflora; *Bacillus licheniformis*; Fermentation assessment

1. Introduction

The targeting and delivery of drugs to the colonic region of the gastrointestinal tract has been the focus of considerable research effort in recent years (Ashford and Fell, 1994; Kinget et al., 1998; Yang et al., 2002; Sinha and Kumria, 2003). A platform technology based on the biodegradable polysaccharide amylose shows particular promise as a means of effecting drug release in the colon (Milojevic et al., 1996; Rhodes and Porter, 1998; Fish and Bloor, 1999). In its glassy amorphous form, amylose is metabolised by bacterial amylase enzymes of colonic origin but is recalcitrant to mammalian enzymes secreted into the small intestine by the pancreas. Therefore, on passage through the gastrointestinal tract, glassy amylose will remain intact in the upper gut and then be fermented in the colon. This unique characteristic of amylose has been exploited as the basis for an orally administered carrier for colonic delivery (Basit, 2000). Amylose has the ability to form films through gelation, and can be applied using conventional processing technology to solid oral dosage forms. Such films are fragile and swell in water, although the addition of a structuring agent, ethylcellulose, from an aqueous dispersion or organic solution, has been found to improve the physical and mechanical properties of the film, without

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impairing the film's sensitivity to bacterial degradation (Milojevic et al., 1996; Siew et al., 2000a,b; Leong et al., 2002).

The imaging modality of gamma scintigraphy, in combination with pharmacokinetic analysis, has been used to confirm the colonic targeting behaviour of the amylose system in man (Cummings et al., 1996; Bloor et al., 2002; Tuleu et al., 2002; Basit et al., 2004).

While such in vivo assessments usually provide unequivocal evidence for delivery system functionality, they are expensive and time consuming and so are not suitable for routine use. A predictive and relatively straightforward in vitro test is therefore warranted for development and quality control purposes. Although conventional dissolution methodology can be used in this regard, these tests offer at best crude simulations of upper gastrointestinal conditions, e.g. pH, electrolyte concentration, fluid volume and hydrodynamics, and so provide only a measure of the delivery system integrity in the stomach and small intestine but no information on behaviour or drug release in the colon. To address this latter point, batch culture fermenters, comprising human faeces homogenised in physiological buffer medium of near neutral pH under an atmosphere of nitrogen, of total volume 100 ml, have been proposed as a model of the large intestine (Macfarlane et al., 1993; Silvester et al., 1995; Basit and Lacey, 2001). Although somewhat of an oversimplification, this model provides a reasonable simulation of the low-fluid, bacteria-rich, anaerobic environment of the colon. We have used such systems to investigate drug release from amylose-based delivery systems in vitro (Milojevic et al., 1996; Siew et al., 2000b). Moreover, these in vitro data correlate reasonably well with those obtained in vivo (Cummings et al., 1996; Tuleu et al., 2002). Such fermentation models, being biological in nature, can be fairly laborious and unpleasant to set up and require special containment facilities. An alternative, simple, clean, reproducible and user-friendly system is therefore desirable.

The aim of this study was to assess the potential of a series of commercial amylase enzymes to degrade amylose cast films and film coatings. The results were further compared with those obtained from a batch culture fermenter based on a population of human faecal bacteria to determine whether a cell-free enzyme-based system could serve as an in vitro colonic digestion model.

2. Materials and methods

2.1. Materials

Amylose was extracted from pea starch and isolated in the form of an amylose-butan-1-ol complex aqueous dispersion from the Institute of Food Research, Norwich, UK. Ethylcellulose (grade N-100) was obtained from Dow Chemicals, Uxbridge, UK. The plasticiser, dibutyl sebacate, and water-miscible organic solvent, ethanol, were obtained from Sigma–Aldrich, Poole, UK. 5-Aminosalicylic acid (mesalazine) was purchased from Sigma–Aldrich, Poole, UK. Microcrystalline cellulose (Avicel PH101) (FMC Corporation, Brussels, Belgium), lactose BP (Sheffield Products, Philadelphia, USA) and bentonite (Merck, Poole, UK) were used as-received. Amylase enzymes from *Bacillus* sp. (Duramyl 300), *Bacillus licheniformis* (Termamyl 120), *Bacillus amyloliquefaciens* and *Aspergillus oryzae* (Fungamyl 800) were purchased from Sigma–Aldrich. All other reagents were of AnalaR grade and were obtained from Merck.

2.2. Preparation of isolated cast films

Ethylcellulose was dissolved in ethanol to produce a 3% (w/w) organic solution of the polymer. The plasticiser, dibutyl sebacate, was added to the ethylcellulose solution at a concentration of 35% (w/w) based on solid ethylcellulose content and mixed for 6 h. Amylose-butan-1-ol complex aqueous dispersion was added to the plasticised ethylcellulose solution, in various quantities, and mixed for an additional 1 h to produce mixtures with different amylose contents (0, 20, 25, 33, 40, 50 and 100%). The mixtures were poured into 9 cm diameter Teflon-coated moulds, which were then placed in a fan assisted oven at 40 °C for 3 h. Once dry, the films were removed from the moulds and allowed to stabilise in a desiccator at 20 °C and 44% RH for at least 7 days prior to testing. Film thickness was in the range of 50–100 μm.

2.3. Preparation and film coating of drug-loaded pellets

The model drug 5-aminosalicylic acid was formulated into pellets of size 1.0–1.4 mm by the process of extrusion–spheronisation (Siew et al., 2000b). The

formulation comprised 10% 5-aminosalicylic acid, 55% microcrystalline cellulose, 30% lactose and 5% bentonite.

The pellets (100 g batch size) were coated in a fluidised bed coater (GPCG-1 Uni Glatt, Glatt GmbH, Binzen, Germany) using an amylose–ethylcellulose mixture containing 50% amylose to a total weight gain of 15%. The mixture was sprayed at a rate of 0.3 g/min through a 1.1 mm nozzle under a pressure of 2 bar. The bed temperature was maintained at 40 °C.

2.4. Simulation of the colonic environment for in vitro digestibility studies

2.4.1. Batch culture fermenter utilising human faecal bacteria (cell-containing system)

Freshly voided faeces from three healthy human subjects were pooled and homogenised in a buffer medium of pH 7.2. The buffer components consisted of potassium dihydrogen orthophosphate (0.15%), dipotassium hydrogen orthophosphate (0.15%), sodium chloride (0.45%), magnesium chloride hexahydrate (0.05%), ferrous sulphate heptahydrate (0.005%) and calcium chloride dihydrate (0.015%). The fermenters were sealed under positive nitrogen pressure to attain an anaerobic environment, and maintained at 37 °C in an incubator. The total fermenter volume was 100 ml, and the concentration of faecal matter in the fermenter was 10% (w/w).

To assess the digestibility of the cast films, the films were first cut into strips of 1 cm × 3 cm size, accurately weighed and placed into individual nylon mesh bags (2 cm × 6 cm). These bags were then introduced into separate batch culture fermenters for a period of 24 h. In addition, control experiments in the absence of human faeces were run in parallel. At the end of the experiments, the bags were retrieved and the film fragments carefully removed. The fragments were washed with distilled water, dried between filter papers and then stored in a desiccator (20 °C, 44% RH). After seven days the films were removed and accurately reweighed to determine the level of film digestion. Each film formulation was assessed in triplicate.

To determine drug release from the coated pellets, 100 mg quantities were introduced into batch culture fermenters ($n = 3$). Two millilitre samples were removed from the fermenters at 0, 2, 4, 6, 8, 12 and 24 h. These were centrifuged at 13,000 rpm for 5 min, filtered through 0.2 μm filters and then analysed for 5-aminosalicylic acid concentration by high performance liquid chromatography (Siew et al., 2000b). Control experiments in the absence of faeces were also run in parallel.

2.4.2. Batch culture fermenter utilising commercial amylase enzymes (cell-free system)

Four commercial amylase enzymes from different bacterial and fungal species were separately introduced into the buffer medium described in Section 2.4.1. The total volume of each fermenter was 100 ml and the temperature was maintained at 37 °C. The type and concentration of enzyme in each fermenter were both varied. The enzymes were oxygen stable, and so anaerobic conditions were not required.

These enzyme-based fermentation models were used to assess film digestion and drug release from coated pellets, identical to the procedures described in Section 2.4.1.

3. Results and discussion

The extent of digestion of amylose cast films in the presence of amylase enzymes from four different bacterial and fungal sources, each at an enzyme concentration of 1000 units/ml, can be seen in Fig. 1. The enzyme from *B. licheniformis* degrades more than 60% of the amylose film in 24 h, whereas the other amylase enzymes digest less than 50% in the same time frame. A number of factors are known to affect enzyme activity, including temperature, pH and electrolyte composition of the medium. Under the present test conditions, the amylase enzyme from the bacterium *B. licheniformis* would appear to be the most active in terms of amylose digestion. This amylase was therefore considered the most suitable for further investigation.

The effect of varying the amylase enzyme concentration on the extent of degradation of amylose cast films is presented in Fig. 2. The level of film digestion increases with increasing amylase concentration. The film was totally fermented when the concentration of amylase in the system was greater than or equal to 2500 units/ml. Within the same time frame, equivalent amylose films were also completely digested in

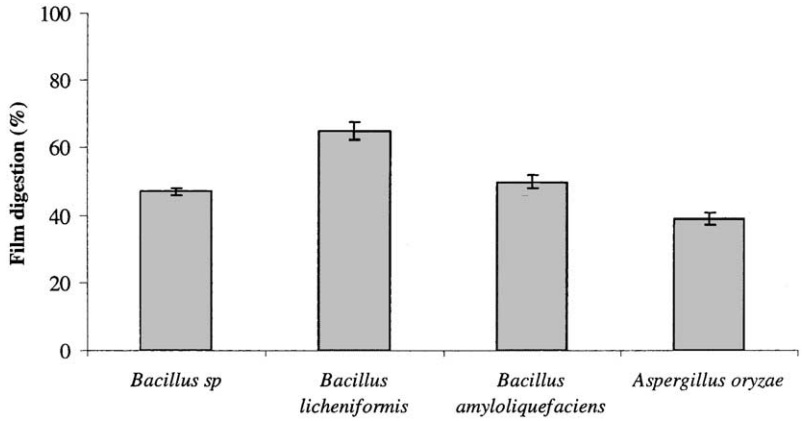

Fig. 1. Influence of type of amylase enzyme, at a concentration of 1000 units/ml, on the extent of digestion of amylose cast films (mean ± S.D.).

the batch culture fermenter inoculated with human faecal bacteria. The faecal fermenter is populated with numerous bacteria and their respective enzymes since more than 400 different species of bacteria are present in the human gastrointestinal tract (Finegold et al., 1983). The total metabolic activity of the faecal fermenter will therefore be very different to the single-enzyme-based fermentation system. Nevertheless, at least in terms of digestive activity against amylose cast films, the cell-free enzyme fermentation system, at a concentration of 2500 units/ml, would appear to be comparable to the faecal fermenter.

The extent of digestion of mixed cast films of amylose and ethylcellulose is shown in Fig. 3. Similar digestion trends can be observed in both the cell-free and cell-containing fermentation systems. A relationship exists between the initial proportion of amylose present in the film and the level of film digestion, since increasing the amylose component of the film increases the level of film digestion. This indicates that the digestible fraction of the film is amylose. In contrast, films prepared from ethylcellulose alone remained intact, thereby confirming its resistance to enzymatic attack. The fact that the amylose component of the mixed films was susceptible to colonic digestion also implies that amylose and ethylcellulose are immiscible and phase separated within the film structures. No change in film weight was noted for any of the films after 24 h incubation in the control buffer medium.

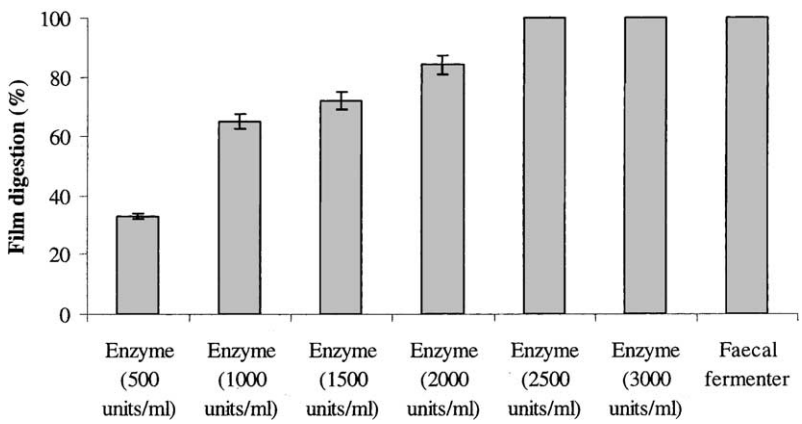

Fig. 2. Influence of concentration of amylase enzyme from *B. licheniformis* on the extent of digestion of amylose cast films (mean ± S.D.).

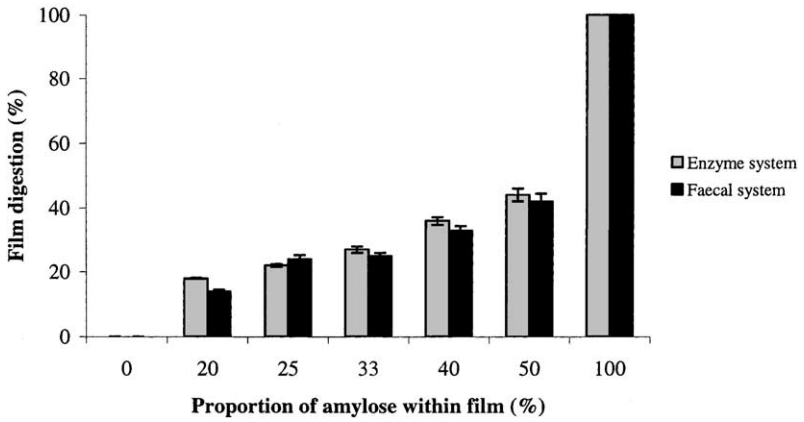

Fig. 3. Influence of type of batch fermentation system (enzyme and faecal) on the extent of digestion of mixed amylose and ethylcellulose cast films (mean ± S.D.).

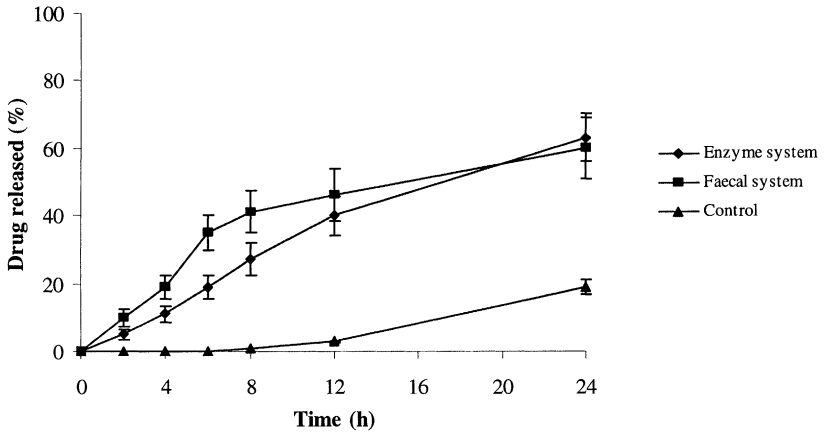

Fig. 4. 5-Aminosalicylic acid release from amylose–ethylcellulose coated pellets in batch culture fermentation systems (enzyme and faecal) and control (mean ± S.D.).

The in vitro performance of the amylose–ethylcellulose coated pellet formulation in the cell-free and cell-containing fermentation systems are shown in Fig. 4. The rate of release of 5-aminosalicylic acid was slightly faster in the faecal fermentation system than in the enzyme system, although release in both systems was substantially faster than in the control. The extent of release in both fermenters was similar at 24 h. The accelerated rate of release from the coated pellets in the fermentation systems can be attributed to the generation of pores in the coat via enzymatic degradation of the amylose component of the film through which the drug can be released.

4. Conclusions

This study has explored the feasibility of utilising enzyme systems as an alternative to conventional faecal bacteria fermentation models to assess the in vitro digestion of amylose–ethylcellulose films and coatings. A cell-free system based on the commercially available amylase from *B. licheniformis* shows promise in this respect. Such a model should provide the basis for a more straightforward and user-friendly tool to aid the development of amylose-based colonic delivery systems.

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