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In vitro evaluation of pectin-based colonic drug delivery systems

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Abstract

Pectin-based dosage forms with the potential for site-specific delivery to the colon have been evaluated using isolated enzymes and cultures of *Bacteroides ovatus*. The system used was designed and validated to allow systematic testing of colonic dosage forms which depend on the action of colonic bacteria for drug release. Preliminary experiments show the similarity in release patterns for the dosage form tested with isolated enzymes and bacterial cultures. This should enable further experiments to be performed to ascertain valid in vitro testing conditions.

Keywords: Colonic drug delivery; Pectin; *Bacteroides ovatus*; Colonic dosage form evaluation

1. Introduction

Colonic drug delivery is a relatively recent approach to the treatment of diseases such as ulcerative colitis, Crohn's disease and irritable bowel syndrome. Such local treatment has the advantage of requiring smaller drug quantities, possibly leading to a reduced incidence of side effects and drug interactions. Colon-specific drug delivery may also be useful for the oral delivery of peptides and proteins, for example insulin, which are degraded higher up in the GI tract (Saffran et al., 1986; Geary and Schlameus, 1993).

Several methods of colonic delivery have been developed, some of which are based on exploiting the enzymic actions of colonic bacteria. Natural polysaccharides such as pectin, xylan and guar gum are not digested in the human stomach or small intestine, but are degraded in the colon by resident bacteria (Salyers et al., 1977). They may therefore be useful in protecting drugs during their passage from mouth to large intestine. A recent γ-scintigraphy study (Ashford et al., 1993) showed that high methoxy pectin, used as a compression coat on tablets, had potential as a colonic drug delivery agent. Although such human studies are the ultimate test of a potential system, they are not of value in routine investigations. What is required here is an in vitro system that mimics in vivo conditions in terms of method and rate of breakdown of the delivery agent. This paper reports preliminary studies on drug release from a pectin-based colonic delivery system employing a method which can be used for both pure enzymes and bacterial cultures. Extended studies with this method should allow the choice of appropriate conditions for a valid in vitro test.

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2. Materials and methods

2.1. Equipment

The dissolution apparatus was designed to be capable of withstanding heat sterilisation to allow the use of pure bacterial cultures as well as isolated enzymes. Sterilisation was essential to avoid competition by other, perhaps more vigorous organisms. Two vessels were included to allow the concomitant use of a control. The nutrient dissolution medium was formulated to support all the nutritional requirements of the bacteria except for a carbon source; the only carbon source available was therefore in the dosage form. The dissolution apparatus comprises two glass USP dissolution vessels with perspex lids housed in a water bath maintained at 37°C (Fig. 1). Vessel contents are agitated using USP paddles or baskets powered by separate motors geared to operate at speeds between 25 and 100 rpm. Each motor is connected to its own variable speed controller. The two vessels, one test and one control, each contain two lengths of medical grade silicone tubing, one to facilitate sample removal, the other to permit the introduction of sterile nitrogen if required.

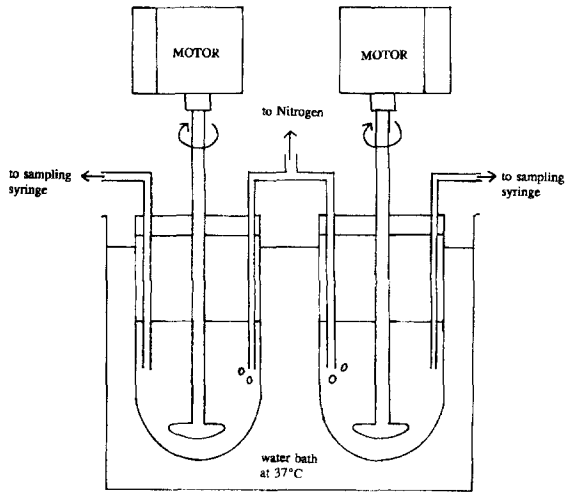

Fig. 1. Schematic diagram of dissolution apparatus.

2.2. Preparation of compression-coated tablets

Core tablets of lactose (Pharmatose 200M, DMV, The Netherlands) and Naphthol Green B (Sigma Chemical Company, UK) included as a model drug, were prepared by trituration in a mortar followed by mixing in a Turbula blender (type T2C, W.A. Bachofen, Switzerland) for 15 mins. Three hundred-mg portions were compressed in a 12-mm diameter die at 2000 kg (Beckman Model P16 hand press). Each core contained 50 mg of dye. One core was carefully placed on 300 mg (half the coat weight) of Pectin USP (HP Bulmer Pectin, UK) in a 16-mm die. A further 300 mg of pectin was placed on top of the core and the mixture compressed to 4000 kg.

2.3. Validation of apparatus

The apparatus was validated by running five dissolution experiments in 900 ml of pH 7.0 BP phosphate buffer using USP paddles and pectin 170/Naphthol green B (NGB) matrix tablets. The tablets were prepared by triturating 7.6 g pectin 170 (HP Bulmer Pectin, UK) with 0.4 g NGB and mixing as above. Nine hundred-mg tablets were prepared by compression in a 16-mm die at 4000 kg force. Samples were taken every 30 min for 6.5 h, the time taken for approximately 100% dye release. The sample absorbance was measured at 715 nm ($\lambda_{max.}$ for NGB in pH 7.0 buffer) against a pH 7.0 buffer blank. Percentage dye release was calculated using regression data from a calibration plot. A one-way analysis of variance was performed on percentage dye released at all time points.

2.4. Nutrient dissolution medium

Sterile nutrient dissolution medium, similar to that described by Macfarlane et al. (1990), was prepared by autoclaving each component listed in Table 1 at 121°C for 15 min. All solutions were then transferred to a sterile container and mixed by gentle agitation.

Table 1
Components of nutrient dissolution medium

Components	Concentration (mg/l)
Potassium dihyrogen orthophospate[a]	4000
Sodium dihydrogen orthophosphate[a]	2000
Ammonium chloride[a]	750
Sodium hydrogen carbonate[a]	1500
Sodium chloride[a]	9000
Magnesium chloride hexahydrate[a]	150
Cobalt chloride hexahydrate[a]	100
Manganese chloride tetrahydrate	100
Calcium chloride dihydrate[a]	100
L-Cysteine[a]	800
Iron II sulphate heptahydrate[a]	1
Hemin[b]10	10
Resazurin[b]1	1
Vitamin B_{12}^{b}	5

[a]BDH Chemicals Ltd., UK.
[b]Sigma Chemical Co., UK.

2.5. Dissolution method using pectinolytic enzymes

A compression-coated pectin tablet was placed in each of two USP basket assemblies. Each basket was placed in 900 ml of 0.1 M HCl in two dissolution vessels and rotated at a speed of 50 ± 2 rpm for 2 h. The baskets were then removed, drained and placed in 900 ml of pH 7.4 Sorensen's phosphate buffer and rotated at 50 ± 2 rpm for a further 3 h. Samples of 1 ml were taken periodically and the absorbance of each measured at 713 nm ($\lambda_{max.}$ for NGB) against a pH 7.4 buffer blank. The baskets were removed, drained and replaced in vessels containing 900 ml of pH 6 Sorensen's phosphate buffer. The test vessel only contained 3 ml of pectinolytic enzymes (Pectinex Ultra SP-L, Novo Nordisk Ferment, Switzerland, activity 26 000 PG/ml at pH 3.5) in 900 ml of pH 6 Sorensen's phosphate buffer. Both baskets were rotated at 50 ± 2 rpm for several hours. Samples were taken periodically and assayed as before until approximately 100% dye release was detected, or absorbance readings reached a maximum. Percentage dye released was calculated as described above.

2.6. Dissolution method using B. ovatus

The method described above was used with the following modifications. All media and equipment were sterilised prior to use. The pH 6 buffer was replaced with nutrient dissolution medium containing approximately 10^9 cfu/ml *Bacteroides ovatus*. The bacteria had been grown to mid log. phase in the medium described by Macfarlane et al. (1990) with pectin USP as the sole carbon source. Cells were harvested by centrifugation at 8000 rpm (MSE High Speed Centrifuge, UK) for 20 min, and resuspended in nutrient dissolution medium. Sterile nitrogen was bubbled through both vessels to maintain anaerobic conditions. Samples of 1 ml were taken periodically and the pH measured. Each sample was subsequently centrifuged at 13 000 rpm (MSE Microcentaur Centrifuge, UK) for 5 min to precipitate cells and cell debris. The absorbance of the resulting supernatant was measured against a nutrient dissolution medium blank at 713 nm, and percentage dye released calculated as before.

2.7. Influence of Naphthol green B on B. ovatus viability

Twenty ml of standard (1.088 mg/ml) NGB in nutrient dissolution medium containing 5 g pectin USP/L was filter sterilised, the first 5 ml being discarded. The remaining 15 ml was used to prepare seven dilutions between 0.4 and 108.8 μg/ml NGB. Each dilution was inoculated with *B. ovatus* and incubated anaerobically with an uninoculated blank for 48 h at 37°C. The test tubes were examined for signs of growth by visual comparison with the blank.

3. Results and discussion

Since the vessels were totally independent, it was therefore necessary to show there were no differences between them. The validation experiments showed that with (1,4) degrees of freedom there was no evidence to suggest a difference between the two vessels at any time point (99% level of significance). This showed that there was

no disparity affecting dye release. In each case approximately 100% dye release was obtained indicating that Naphthol green B does not bind significantly to the pectin.

The system was designed to mimic in vivo conditions both prior to and including the large intestine. After exposure to 0.1 M HCl and pH 7.4 phosphate buffer to mimic gastric and small intestinal conditions the dosage form may be exposed to either rat caecal contents, human faeces or pure enzymes specific for one polysaccharide. In addition, pure cultures of colonic bacteria may be used. In this study pectin was selected for the model dosage form since it has successfully been used to protect drugs in previous studies (Ashford et al., 1993). The pure enzymes used were therefore pectinolytic, and the colonic bacterium, *Bacteroides ovatus*, selected for its known pectinolytic activity (Salyers et al., 1977; Salyers, 1979; Salyers and Leedle, 1983). Preliminary studies showed that tablets compression-coated with pectin exposed only to bacteria in nutrient dissolution medium released no dye in 8.5 h, but that those pre-treated in 0.1 M HCl and pH 7.4 buffer started to release dye soon after transferring to a bacterial environment. Hydration is an important consideration with polysaccharide-based dosage forms since they must absorb water to swell before they are open to attack by bacterial enzymes. Bacteria ferment polysaccharides to gases such as methane, carbon dioxide and hydrogen, and to short chain fatty acids, accounting for the drop in pH from the small intestine to the colon (Englyst et al., 1987). pH control must therefore be a major consideration of any bacterial system. The pH of the bacterial environment used in this study was found to be between 6.22 and 6.20 over the period of 7 h, compared with 6.23–6.22 in the control. This lack of variation was attributed to the isolation of the bacteria from their growth medium and resuspension in a large volume of fresh medium. pH control was therefore felt to be unnecessary.

Naphthol green B was chosen as the marker substance since it absorbs at wavelengths away from medium components, making a simple spectrophotometric assay possible. By monitoring NGB concentration in the presence of a dissolving pectin USP matrix tablet over a period of 6 h, pectin was shown to have a negligible effect on this assay (concentration range 8.96–9.04 μg/ml). Bacterial cells were shown to affect absorbance readings, and were therefore removed by centrifugation.

Some dyes, for example methylene blue, are known to have bactericidal properties (Gurr, 1971). It was therefore necessary to investigate the influence of NGB on *B. ovatus* viability. Growth was present in all tubes except the blank, indicating that *B. ovatus* is unaffected by NGB up to a concentration of 108.8 μg/ml. It was therefore possible to use up to 97 mg of dye per tablet. Spread plates prepared from test vessel contents at the end of one experiment showed growth only when incubated anaerobically, indicating a lack of aerobic contaminants. The system therefore supported the growth of *B. ovatus* over the duration of the experiment. Similar plates prepared from control vessel contents showed no growth under aerobic or anaerobic conditions indicating that sterility is maintained.

The results of the dissolution experiment using pectinolytic enzymes (Fig. 2) show that NGB is rapidly released from the pectin-coated tablet in the presence of enzymes compared with the control. A similar result was obtained from the bacterial experiment (Fig. 3). In each case no dye was released from the dosage form before enzymes or bacteria were introduced emphasising the potential of pectin in colonic drug delivery. Both enzyme and bacterial studies show a high degree of variation compared with standard dissolution profiles which is typical of biological systems. The clear difference between test and control profiles demonstrates that the system can be used to monitor the enzymic degradation of a colonic dosage form.

The similarity in the timing and profile of the release of dye in the enzyme and bacterial experiments indicates a similarity in the mode of breakdown of the pectin. Further experiments using a standardised system as described and other single or mixed bacterial cultures, including faecal samples, should help in the identification of conditions for a simple, valid in vitro test.

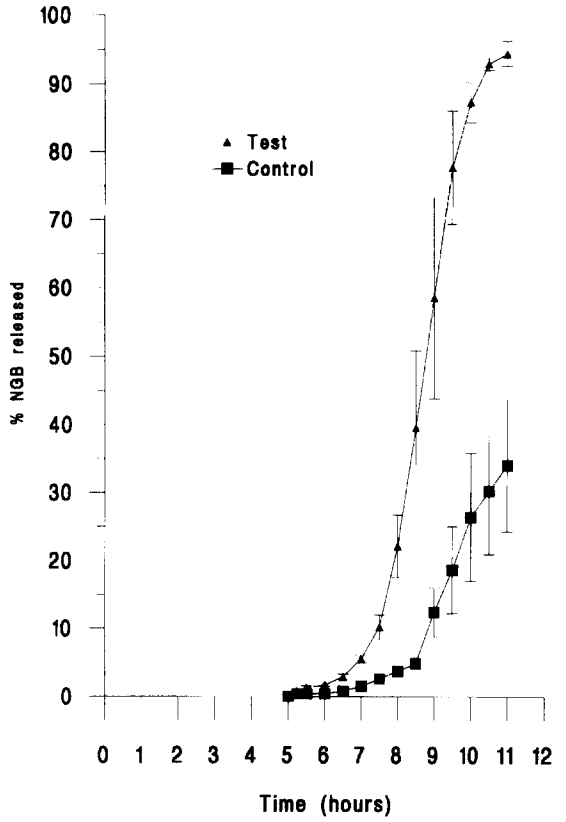

Fig. 2. Release of Naphthol green B (NGB) from tablets-compression coated with pectin USP in the presence (▲) and absence (■) of pectinolytic enzymes. Error bars = mean ± SE.

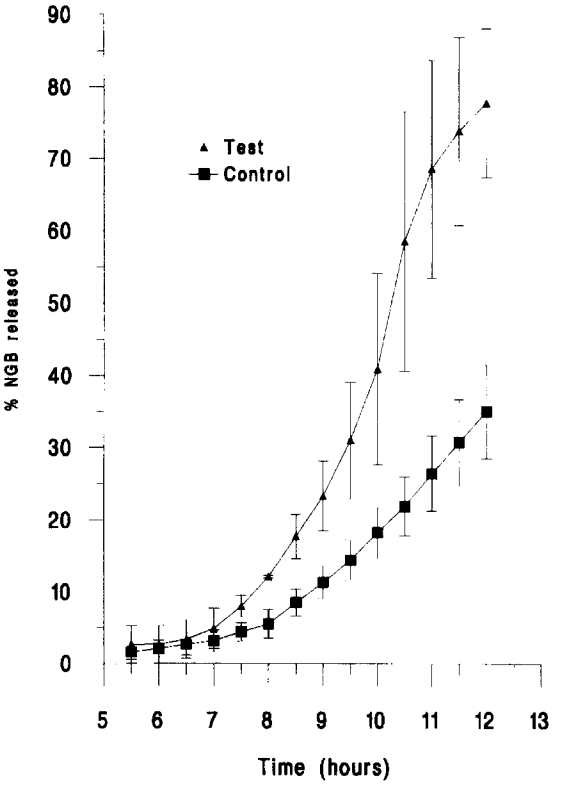

Fig. 3. Release of Naphthol green B (NGB) from tablets compression-coated with pectin USP in the presence (▲) and absence (■) of *Bacteroides ovatus*. Error bars = mean ± SE.

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References

Ashford, M., Fell, J., Attwood, D., Sharma, H. and Woodhead, P., An evaluation of pectin as a carrier for drug targeting to the colon. *J. Control. Release*, 26 (1993) 213–220.

Englyst, H.N. Hay, S. and Macfarlane, G.T., Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiol. Ecol.*, 95 (1987) 163–171.

Geary, R.S. and Schlameus, H.W., Vancomycin and insulin used as models for oral delivery of peptides. *J. Control. Release*, 23 (1993) 65–74.

Gurr, E., *Synthetic Dyes in Biology, Medicine and Chemistry*. Academic Press, London, 1971.

Macfarlane, G.T., Hay, S., Macfarlane, S. and Gibson, G.R., Effect of different carbohydrates on growth, polysaccharidase and glycosidase production by *Bacteroides ovatus*, in bath and continuous culture. *J. Appl. Bacteriol.*, 68 (1990) 179–187.

Saffran, M., Kumar, G.S., Savariar, C., Burnham, J.C., Williams, F. and Neckers, D.C., A new approach to the oral administration of insulin and other peptide drugs. *Science*, 233 (1986) 1081–1084.

Salyers, A.A., Energy sources of major intestinal fermentative anaerobes. *Am. J. Clin. Nutr.*, 32 (1979) 158–163.

Salyers, A.A. and Leedle, J.A.Z., *Human Intestinal Microflora in Health and Disease*, Academic Press, London, 1983.

Salyers, A.A., Vercellotti, J.R., West, S.E.H. and Wilkins, T.D., Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl. Environ. Microbiol.*, 33 (1977) 319–322.