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A novel, biodegradable and reversible polyelectrolyte platform for topical-colonic delivery of pentosan polysulphate

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ABSTRACT

The goal of the present work was to develop a swellable hydrogel colonic delivery system, which would maximise the availability of the therapeutic agent at a site of inflammation, especially where the water is scarce. A novel method was developed to manufacture a biodegradable and reversible polyelectrolyte complex (PEC) containing chitosan and poly acrylic-acid (PAA). The PEC was analysed using FTIR and DSC, which confirmed the formation of non-permanent swollen gel-network at an alkaline pH. Pentosan polysulphate (PPS) was incorporated in a PEC and an activated partial thromboplastin time assay was developed to measure the release of PPS from PEC. *In vitro* studies suggested that the release of PPS was dependent on the initial drug loading and the composition of the PEC. The gel strength of the swollen network, determined using a texture analyser, was dependent on polymer composition and the amount of PPS incorporated. Bacterial enzymes were collected from the rat caecum and colon for the digestion studies and characterised for glucosidase activity, glucuronidase activity and protein content. The digestion of the reversible polyelectrolyte complexes was measured using a dinitro salicylic acid assay and an increased release of drug was also confirmed in the presence of bacterial enzymes.

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1. Introduction

Inflammation is a feature of many diseases and is associated with pain, swelling and tissue remodelling with the formation of non-functional scar tissue. In the gut, the inflammatory process involves the squeezing of pro-inflammatory leucocytes (especially basophils and neutrophils) through the space between endothelial cells (He, 2004). The process leads to the thickening of the bowel lining and disturbs the absorption and motility patterns. The defective endogenous sulphated glycosaminoglycans (GAG) are no longer able to provide a protective barrier following the leucocyte attachment to the gastrointestinal lining.

Yayon et al. (1991) showed exogenous heparin-induced accelerated-healing in mutant Chinese hamster ovary cell-lines suggesting that the presence of low affinity heparin sulphate proteoglycans (HSPGs) was necessary for the mitogenic action of basic-fibroblast growth factor. Papa et al. (2000) noticed an anti-inflammatory action of heparin, which affected the activity of cell adhesion molecules. Day and Forbes (1999) administered heparin to patients with inflammatory bowel disease (IBD) and found the accelerated ulcer healing of the inflamed bowel. Systemic administration of heparin by i.v. and s.c. route caused a significant reduction in stool frequency, C-reactive protein and improved endoscopic and histopathological scores in the IBD patients which strongly suggested its participation during the healing process (Ang et al., 2000). However, the parenteral administration of heparin is always associated with the risk of haemmorhage, suggesting the employment of less active short chain heparin-like molecules.

Törkvist et al. (1999) conducted a clinical trial utilising a low molecular weight heparin for the treatment of glucocorticosteroid-refractory ulcerative colitis and showed improved healing. Heparin and pentosan polysulphate, which has structural motifs similar to heparin, stimulated epithelial proliferation in cultured rat-intestinal epithelial cells (Flint et al., 1994). With regard to pharmacological activity, pentosan polysulphate has one tenth of the anticoagulant activity of heparin. Fischer et al. (1982) studied the inhibitory effects of pentosan polysulphate sodium (PPSNa) and heparin on thrombin, factor Xa and factor IXa in the presence and the absence of antithrombin III. PPSNa was approved as Elmiron® by the U.S. food and drug administration as an oral therapy for the treatment of interstitial cystitis. We investigated the design of a colon-targeted drug delivery platform for PPSNa, intended for the topical treatment of IBD.

Abbreviations: APTT, activated partial thromboplastin time; basic-FGF, basic-fibroblast growth factor; BCA, bicinchoninic acid; CP or Chi:PAA, chitosan:poly acrylic acid; DNSA, 3,5-dinitro-salicylic acid; GAG, glyscosaminoglycans; HSPGs, heparin sulphate proteoglycans; IBD, inflammatory bowel disease; PAA, poly acrylic acid; PEC, poly electrolyte complex; PPS or PPSNa, pentosan polysulphate sodium.

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The delivery of sulphated GAG by the topical route was suggested by Meissner et al. (2007), who entrapped low molecular weight heparin (enoxaparin) into Eudragit® microspheres. Polyelectrolyte complexes composed of chitosan and poly-acrylic acid (PAA), have also been prepared for several biomedical applications. Qu et al. (1997) described the dental applications of a covalently linked chitosan–polyacrylic acid complex. De la Torres et al. (2003, 2005) have prepared amoxicillin-loaded films of chitosan and PAA. The adhesiveness and tensile strength of a transmucosal cast-film system based on a chitosan–PAA complex containing triamcinolone was described by Ahn et al. (2001, 2002). A nanoparticulate ophthalmic drug delivery system, prepared by mixing chitosan solution in acetic acid and a PAA-dispersion in water, has been described for the delivery of pilocarpine (Kao et al., 2006).

The nature of the complex formed between PAA and chitosan described in the literature varies depending on the final usage of the polyelectrolyte complex and encompasses the range between a chemically bound strong-network to an ionically interacted non-permanent assembly. Several authors have described the polymerization of acrylic acid monomers in the presence of chitosan to obtain an inter-penetrated PEC (Lee et al., 1999; Peniche et al., 1999; Ahn et al., 2001; Nge et al., 2004). However, the chemically-linked PAA–chitosan may contain residual toxic acrylic-acid monomers and may display unpredictable swelling behaviour at different pHs (Wang et al., 1997).

Park et al. (2008) manufactured carbopol–chitosan polyelectrolyte complex according to the patent reported in our laboratories (Wilson and Mukherji, 2003). Park et al. (2008) utilised FTIR and DSC to characterise the PEC and confirmed the chemical interaction between two oppositely charged polymers. De Lima et al. (2009) manufactured chitosan–PAA films by allowing diffusion of PAA molecules through chitosan membranes. The authors detected a new covalent linkage at 1560 cm^{-1} during FTIR analysis. These studies suggest that the covalent links between two oppositely charged polymers lead to the formation of a new chemical entity. The complexation of two polymers in solution has lower percentage yield compared to the theoretical yield, which makes it difficult to determine the proportion of individual polymers in the PEC. The unknown amount of individual polymers also makes it difficult to predict the degree of swelling and the mechanical properties of the PEC. The irreversible complex would also have different physico-chemical properties and possibly different physiological effects compared to the individual components.

It is desirable that the oppositely charged polymers in a PEC should not form the covalent bonds in order to utilise the PEC as a drug delivery system. Furthermore, the formulation should swell at neutral to alkaline pH and transport water from the upper gut into the colon to provide water for dissolution. Several hydrogel platforms have been described in the literature as the colonic delivery systems (Simonsen et al., 1995; Pitarresi et al., 2008; Ali and AlArifi, 2009). We utilised chitosan and PAA to form the polyelectrolyte complex for the colonic delivery. The reversible ionic interaction between chitosan and PAA is necessary in order to formulate the biodegradable PEC. The present work addresses these issues and experiments were conducted to formulate a reversible PEC. The bacterial enzymes from rat colon were also utilised to understand the digestion of PEC and its effect on the release of PPS from polyelectrolyte complex.

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight: 50,000–190,000; degree of deacetylation 75–85%, Sigma–Aldrich Co., UK) and poly-acrylic acid (M_v 4,000,000, Sigma–Aldrich Co., UK) were used to manufacture the polyelectrolyte complexes. Other ingredients were sourced as follows: N-acetyl glucosamine (Sigma–Aldrich Co., UK), pentosan polysulphate sodium (bene Arzneimittel GmbH, Germany), potassium dihydrogen orthophosphate (AnalaR, BDH Laboratories, UK), sodium hydroxide (BDH Laboratories, UK), glycine (99%, Sigma–Aldrich Co., UK), 3,5-dinitro salicylic acid (Sigma–Aldrich Co., UK), potassium sodium tartrate (Sigma–Aldrich Co., UK), phenolphthalein (Sigma–Aldrich Co., UK), phenolphthalein-β-D-glucuronide (Sigma–Aldrich Co., UK), p-nitrophenol (Sigma–Aldrich Co., UK), p-nitrophenol-β-D-glucoside (Sigma–Aldrich Co., UK), sodium chloride (BDH Laboratories, UK), EDTA (Sigma–Aldrich Co., UK), bicinchoninic acid kit (Sigma–Aldrich Co., UK), bovine serum albumin (ICN Biomedicals Inc., UK), kaolin/platelet substitute (Diagnostic Reagents Ltd., UK), lyophilised normal human plasma (Diagnostic Reagents Ltd., UK), calcium chloride (Sigma–Aldrich Co., UK).

2.2. Manufacturing of polyelectrolyte complexes (PEC)

Chitosan was dissolved in 0.1 M acetic acid to obtain 1% (w/v) solution. Chitosan was precipitated using acetone, dried overnight and milled to reduce the particle size below 180 μm. Poly-acrylic acid was dispersed in acetone, dried overnight and milled to reduce the particle size below 180 μm. Chitosan (precipitated) and poly-acrylic acid (precipitated) were mixed physically to obtain a PEC containing 1:1 (CP 1:1) and 1:2 (CP 1:2) mass ratios of chitosan and PAA, respectively.

2.3. Characterisation of poly-acrylic acid and chitosan

The samples of poly-acrylic acid and chitosan were characterised by FTIR spectroscopy using a KBr disc method (Genesis Series, FTIR™, ATI Mattson, USA).

The samples of poly-acrylic acid and chitosan were weighed between 3 mg and 5 mg and placed in hermetically sealed pans (Alumina crucible, 40 μL). DSC experiments were performed for PAA and for chitosan between 25 °C and 150 °C at 10 °C min^{-1} using TC 15 (Mettler, Switzerland).

2.4. Activated partial thromboplastin time (APTT) assay for the quantitative determination of PPS

An automatic haemostasis analyser (Amax CS190, *Amelung*®, Germany) was used for the APTT assay. The primary mix of 50 μL of kaolin/platelet substitute and 25 μL of plasma sample were dispensed and incubated automatically at 37 °C for 2 min. Following incubation, calcium chloride (25 μL, 0.025 M) was added automatically to promote the activation of the clotting pathway. Within the measurement cuvette, a small ball-bearing was stopped automatically during the rotation as a result of the clot formation and measuring the clotting time. The assay was controlled using a normal human plasma (clotting time range: 42–48 s) and an abnormal plasma sample (clotting time range: 82–100 s).

A calibration series of pentosan polysulphate (0.01–0.13 mg/mL) was made in phosphate buffer saline. Each calibration standard (100 μL) was aliquoted into 400 μL cups to which normal human pooled plasma (300 μL) was added. The cups were loaded on to the auto sampler and the APTT (sec) was recorded using the Amax CS190. The linear relationship of $\sqrt{\text{Clotting time}\,(s)}$ vs concentration (mg/mL) was plotted and the line of best fit was obtained. Using this reference curve, the activated partial thromboplastin time was measured for PPS containing systems to determine the release rates from PEC.

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