

An Investigation into the Digestibility of Chitosan by Human Colonic Bacteria

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Abstract:

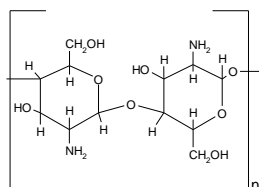
The suitability of chitosan (non-crosslinked and crosslinked by glutaraldehyde) for colonic drug delivery was assessed by incubation of chitosan films in human faecal slurry and assessment of the film's disappearance with time. It was found that non-crosslinked chitosan, was digested by colonic bacteria, but crosslinked chitosan was not.

Introduction:

Polysaccharides, such as amylose, guar gum, pectin and chitosan (Basit, 2005) are increasingly being investigated for the delivery of drugs to the colon. An essential feature of these potential drug delivery systems is non-degradation by small intestinal digestive enzymes, but digestion by the enzymes produced by the colonic microflora. Thus, they should prevent drug release in the small intestine, and allow drug release in the colon. Chitosan is being investigated as it is biodegradable, biocompatible and has low oral toxicity. There are several investigations into the suitability of chitosan for colonic delivery (Tozaki *et al.*, 1997; Zambito *et al.*, 2005) but all investigations to date have used rat caecal contents to assess the colonic release. This may not be directly comparable to human colonic contents or faecal material. Work has shown that chitosan is degraded to different extents in different species, such as dogs (Okamoto *et al.*, 2001), rabbits, hens and sheep (Hirano *et al.*, 1990). Hence, it cannot be assumed that chitosan is sufficiently digested by human colonic microflora. Before investigating the potential of chitosan as a colonic delivery system, it is essential to assess whether the human colonic microflora is capable of digesting the material. The process of microfloral digestion is one of fermentation, in which the anaerobic bacteria break down substrates to produce energy.

Chitosan, a weak base (pKa 6.2-7.0), is a [(1,4) 2-amino-2-deoxy-beta-d-glucan], whose structure is shown in Figure 1. It is obtained by the alkaline deacetylation of chitin, which is the second most abundant polysaccharide in nature, after cellulose. It is found in the exoskeletons of crustaceans and insects which are not substantial components of the human diet. Human colonic bacteria may not therefore normally produce enzymes capable of digesting chitin and chitosan. The latter is structurally similar to cellulose, which has been shown not to be fermented in the human colon.

Figure 1 Structure of Chitosan



When formulated as a drug delivery vehicle, chitosan is often crosslinked by agents such as glutaraldehyde, in order to reduce the swelling and dissolution in aqueous media. Examples are microparticles for colonic delivery (Rai *et al.*, 2005). It is not known whether crosslinked systems can be degraded by human colonic microflora.

The aim of this investigation was to therefore assess whether chitosan (crosslinked and non-crosslinked) can be digested by human colonic bacteria, and thus, whether they have potential as colonic delivery systems. Films of chitosan, and crosslinked chitosan, were prepared and the film loss in human faecal slurry was determined.

Experimental Methods:

Preparation of Chitosan Films

Chitosan of low and high molecular weights (LMW Chitosan and HMW Chitosan) (75-85% deacetylated) were used. Solutions of 1.5% w/v chitosan were prepared by dissolving in 5% v/v acetic acid with stirring overnight. The solution was passed through a 180 μm sieve. Forty millilitres of this solution were poured into Teflon plates (9 cm diameter) and dried for 48 hrs at room temperature, followed by 24 hr incubation in an oven at 50°C. The films were removed and stored at room temperature in 44% relative humidity (RH).

To prepare films crosslinked with glutaraldehyde, the same method was followed, except for the addition of 0.30 ml of glutaraldehyde solution (50% v/v) to 150 ml of chitosan solution in acetic acid, followed by stirring overnight, to allow the crosslinking to occur. The films were cast, dried, removed and stored as before.

Fermentation Studies

Preparation of faecal material and all fermentation studies were carried out under anaerobic conditions using an Electrotek Anaerobic Workstation AW500TG, at a temperature of 36.5°C, with a relative humidity of 70%.

The chitosan and crosslinked chitosan films were cut into sections, approximately 2.5 cm². Each section was weighed and placed into polyamide mesh bags (Nitex[®], Sefar), with a mesh size of 2000 μm .

Faecal samples were pooled from three volunteers and slurries at a concentration of 10% w/v in freshly boiled and cooled phosphate buffered saline (PBS pH 6.8 BP) were prepared, by homogenisation, and filtration through a 350 μm mesh to remove any unhomogenised material.

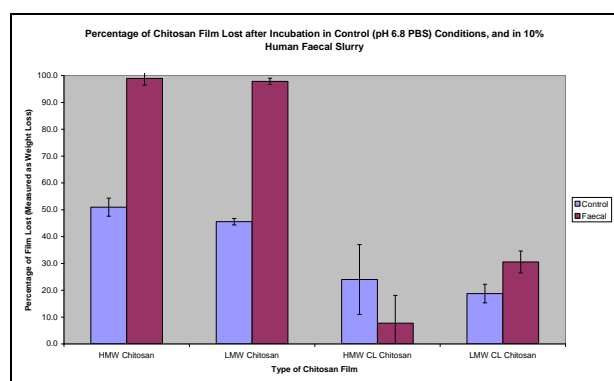
The faecal slurry was placed in 500 ml vessels, in which the film-containing mesh bags were suspended. A control vessel, also containing films, was filled with PBS pH 6.8 BP. These vessels were placed on a rocking

platform. At 4 and 18 hours, films were removed and dried in a hot air oven (50°C), overnight. The films were then stored at room temperature at 44% RH, for 24 hours after which they were weighed and the weight loss calculated. Each film and condition was studied in triplicate.

Results and Discussion:

There was a marked difference between the films produced with and without crosslinking. The chitosan solutions produced translucent white/ yellow films, which were somewhat flexible and easily torn. They had a paper like texture. These films swell in contact with water, but did not dissipate. The addition of crosslinking agent glutaraldehyde to the chitosan solutions produced films which were translucent and dark brown, and while they had flexibility, they were also tougher, with a plastic like texture. These did not swell in contact with water.

The weight loss of both chitosan and crosslinked (CL) chitosan films, after incubation in faecal slurry (10%), and control conditions (PBS pH 6.8) for 18 hours is shown in the Figure below. The results for both HMW chitosan and LMW chitosan films are shown.



In control conditions, non-crosslinked chitosan shows around 50% weight loss at 18 hours. At 4 hrs, there was seen to be less film loss (data not shown). This can be attributed to some dissolution of the chitosan gel, in the slightly acidic media of the control and the faecal slurry. The pH of the control and the faecal slurry before incubation were 6.84 and 6.93 respectively. After 4 hours, these had dropped to 6.0 and 6.4, which did not change further up to 18 hours. The drop in pH may be responsible for the dissolution of some of the chitosan films, especially in the control solution. In colonic conditions, almost complete loss of the low and high molecular weight chitosan films was seen. The extra loss of the films is likely to be due to bacterial degradation. This film loss in colonic conditions was seen to be almost complete at four hours (data not shown) and this may suggest that the in vivo site of degradation would be the proximal colon, the site most densely populated with polysaccharidase producing bacteria. These results confirm that degradation of chitosan by human colonic bacteria can occur, and hence it may be suitable for development as a colonic drug delivery system.

In contrast to the non-crosslinked chitosan films, the weight loss from crosslinked films occurred to a much

smaller extent. There was also no obvious difference between the control and faecal incubations, suggesting that degradation by colonic bacteria of the crosslinked material is minimal. The process of cross-linking may render the active sites inaccessible for the enzymes in question, and they are unable to use it as a substrate. Crosslinked chitosan may not then be suitable for a bacterially triggered colonic delivery system

Conclusions:

Non-crosslinked chitosan is degraded by the human colonic microflora. It may therefore be a potential candidate for colonic drug delivery systems and its digestibility in pancreatic enzymes should be assessed in further work.

In contrast to the suitability of non-crosslinked chitosan, those films prepared with crosslinked chitosan may not be appropriate candidates for colonic delivery systems. It will be important to assess the effects of varying the concentration of the crosslinking agents. The effect of other crosslinking agents, such as tripolyphosphate, will be investigated in future work.

These results highlight the potential of using chitosan as a microbially triggered colonic delivery system. However, they raise concerns for the use of crosslinked chitosan and indeed the use of other crosslinked polysaccharides for colonic delivery.

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Acknowledgements:

My colleagues in the research group, especially Tiago Sousa, for advice on the anaerobic work.

The School of Pharmacy, University of London for sponsorship.