Investigation of Ionically Crosslinked Chitosan and Chitosan–Bovine Serum Albumin Beads for Novel Gastrointestinal Functionality

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ABSTRACT: The objective of the work was to determine whether chitosan–tripolyphosphate (TPP) systems can be used to develop safe gel particles for *in vivo* applications. In particular, we are interested in the use of chitosan systems capable of swelling at low pH *in vitro* as potentially swellable, satiety-enhancing ingredients. The formation of homogeneous chitosan-TPP gel beads was improved by reducing the pH of the TPP gelling bath from 8.5 to 4.0 thus increasing the cationic nature of chitosan and the crosslink density. However, the mechanical strength of this system was reduced compared to the basic system. This has been related to tightening of the gel network by increased shrinking of this system. Although release studies have shown that decreasing the pore size of the gel, by increasing the crosslink density through increasing the TPP concentration, is a good way to trap our model release polymer (dextran), in simulated gastrointestinal conditions, these ionic gel beads showed marked shrinking. We propose that this is due to screening of the charge on the chitosan molecule in the presence of salt and/or an excess of TPP which encourages ionic interactions. We conclude that these ionic gels are an unfeasible alternative satiety-enhancing ingredient to covalently crosslinked systems. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 2876–2885, 2008

Key words: biopolymers; gel microstructure; mechanical properties; release properties; swelling

INTRODUCTION

Chitosan [poly(β-(1→4)-2-amino-2-deoxy-D-glucose)] is a copolymer of glucosamine and *N*-acetylglucosamine and is a natural cationic polysaccharide derived from chitin. Chitosan is a well-known natural cationic polyelectrolyte possessing primary amine groups (NH_2) that become protonated in acidic conditions to form NH_3^+ groups. The pK_a of the amine groups is 6.3, and therefore a pH $<$ 6.3 will result in an increase in the overall positive charge on the chitosan molecule.[1] This will favor a more extended molecular conformation of the chitosan molecule because of charge repulsion. At pH $>$ 6.3 chitosan will be less charged and will adopt a less extended molecular conformation due to charge screening.[1,2] Likewise, it is expected that in acidic conditions chitosan will be more likely to form ionic interactions due to increased charge density. The amine groups of the deactylated units of chitosan are capable of forming coordinate covalent bonds to various metal ions by complexation.[2] As such, the application of chitosan gels as absorbents for heavy metals is reported.[3]

The use of chitosan beads as controlled release systems is well established.[4–9] In many applications, the chitosan is chemically crosslinked using glutaraldehyde or ethylene glycol diglycidyl ether. Both these crosslinking agents are toxic, and the beads must be washed to remove any free crosslinker before consumption. Chitosan can also form gels with nontoxic multivalent anions such as tripolyphosphate (TPP) by ionic interactions.[2,7,10–12] Ionically crosslinked chitosan gels can be used in drug-delivery systems due to their increased biocompatibility over covalently crosslinked gels.[13] However, they are not as strong mechanically as covalent gels and can break down due to highly pH-sensitive swelling.[1,6,13]

Mi et al. have shown that chitosan may be used successfully for drug-delivery systems, depending on the pH of the curing solution.[2] The suggestion is that adjusting the pH of the curing agent (TPP) from basic to acidic would significantly increase the ionic crosslinking density and result in increased stability to pH-sensitive swelling. The idea of the current work is to investigate whether chitosan-TPP systems can be used to develop safe, gel particles for *in vivo* applications such as controlled release or enhancing

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satiety by using simulated gastrointestinal (GI) conditions. Previous work undertaken using a covalently crosslinked composite of chitosan and bovine serum albumin (BSA) has shown that this system swells at low pH (gastric) conditions in the presence of salt (NaCl) because of a conformational unfolding of the BSA molecule.[14] Therefore, this system has the potential to provide an enhanced satiety effect by gastric distension. This study also investigates the swelling behavior of ionically linked chitosan-BSA composite gels.

The mechanical properties, microstructure, and release characteristics of the chitosan-TPP complexes have been investigated and compared to the chitosan–genipin systems previously characterized.[14]

For the release study, fluorescently labeled dextran (fluorescein isothiocyanate, FITC) was used as our model molecule, since polymers with a relatively large molecular weight can be used for purely diffusion-controlled release applications.[15,16] Furthermore, a wide range of different molecular weight fluorescently labeled dextrans can be readily obtained, making a model experiment investigating the effect of size on the release of an encapsulated molecule readily performable. FITC-dextran has been used previously in a number of controlled release studies using chitosan systems.[17,18]

MATERIALS AND METHODS

Materials

Chitosan [1.5% (w/w), 90% deacetylated; Chitoclear Primex Ingredients] solution was prepared by dispersing the dry chitosan powder in water and then hydrated via the addition of 1% w/w glacial acetic acid (Fisher Chemicals Analytical A/0400/PB17). The final pH was $\sim$ 4.0. The solution was then stirred at room temperature for 3 h to ensure complete hydration. TPP (10%, w/v; Sigma Chemicals (Gillingham, UK); T5883) solution was prepared. The TPP solution was divided into two equal parts; one solution was left at $\sim$ pH 8.5, the other solution was adjusted to pH 4.0 using 2*M* hydrochloric acid (Fisher Chemicals, Loughborough, UK; J/4315/15).

For release experiments, fluorescently labeled dextran [(FITC); Sigma Chemicals (Gillingham, UK)] of 2000 kDa molecular weight was dissolved in the chitosan solutions to give a final concentration of 0.1% w/w. The chitosan concentration was varied between 1.5 and 3% w/w.

Experiments were also carried out with a composite system of chitosan and BSA (A3912; initial fraction by heat shock, Fraction V, minimum $>$ 98%, Sigma Chemicals). A 3% chitosan-15% BSA solution was prepared by first dissolving the BSA in deionized water with a magnetic stirrer at room temperature and then dispersing the chitosan powder into the BSA solution. The chitosan was then solubilized by the addition of 1% w/w glacial acetic acid (Fisher Chemicals Analytical A/0400/PB17).

Preparation of chitosan-TPP beads

A 1.5% chitosan solution (20 mL) was dropped into gently agitated 10% TPP solution at pH 8.5 and 4.0, respectively. Both systems were observed to look quite similar in terms of size, shape, and opacity when first formed. The beads were gently stirred for 1.5 h to harden and then stored in the TPP at 5°C until required. The experiment was also repeated using 5% TPP solution, and then a 3% chitosan-10% TPP system was investigated.

The 3% chitosan-15% BSA solution was too viscous to extrude to prepare beads, and therefore further experiments were undertaken using a 1.5% chitosan-15% BSA solution prepared as described previously. Shiraishi et al. used higher chitosan concentrations (4–25% w/v), although they used lower molecular weight hydrolysates of chitosan (Kurita Water Industries, Japan).[11] The properties of these beads were compared to that of chitosan gels covalently crosslinked with genipin. Experimental details of the preparation of the covalently crosslinked gels are given in Butler et al.[14]

Beads were prepared for the release experiments by extruding chitosan solutions (1.5–3% w/w) into TPP solutions between 0.5 and 5% w/w, and the pH was not adjusted. Within 1 min of formation, the beads were removed from the TPP bath using a nylon tea strainer and left on plastic weighing boats to dry. During this time, the leakage of FITC-dextran into the TPP bath was negligible, as shown by the bath remaining clear and colorless at this time. After drying overnight, in darkness, the beads were stored in glass vials in a dark cupboard until the release measurements were made.

Infrared spectroscopy

The experiments were carried out using a Biorad FTS 6000 FTIR spectrometer. The samples were measured using a diamond ATR ''golden gate'' (Geasby Specac, UK). Spectra were taken at a resolution of 4 cm^{-1}, and each spectrum consisted of 256 scans coadded and ratioed against an air-background spectrum. A pure-water spectrum was subtracted where necessary.

Gel microstructure analysis

The gel beads were prepared for transmission electron microscopy (TEM) by first fixing in 0.1% aq. ruthenium tetroxide for 90 min. The beads were then rinsed using distilled water for 20 min and this was

repeated. The beads were then stained in 1% aq. uranyl acetate overnight. The beads were dehydrated in ethanol and infiltrated with epoxy resin, which was polymerized at 60°C for 48 h. Sections of ~ 100-nm thickness were prepared and stained in lead citrate for conventional imaging. The sections were then examined in a Jeol 1200 TEM at 100 kV.

Mechanical testing

Following overnight storage at 5°C, the mechanical properties of the beads were determined using the texture analyser (TA) apparatus (TA XT plus, Stable Micro Systems, UK). The start distance of the TA probe was 5 mm with experimental movement of 0.1 mm sec^{-1}. Five beads were measured, and the Young's modulus was calculated as the initial slope of a force (N)-dimensionless approach [(compressive displacement)/(initial bead diameter)] to the power 3/2 plot (for deformations between 5 and 25%).[19]

Swelling behavior

The beads were tested for their swelling characteristics in gastric and intestinal conditions *in vitro*. Approximately 10 g of microparticles was filtered from the TPP storage solutions, weighed, and placed into beakers in duplicate. The beads were tested under gastric conditions (2.86 g NaCl, 0.865 g KCl, 0.4 g $CaCl_2$/L; all Sigma-Aldrich Analytical Grade Chemicals) for 2 h and then intestinal conditions (6.5 g NaCl, 0.835 g KCl, 0.22 g $CaCl_2$, 1.386 g $NaHCO_3$/L; all Sigma-Aldrich Analytical Grade Chemicals) for 3 h at 37°C. These GI solutions were prepared such that they simulated gastric and intestinal solutions physiologically similar to gastric juice for a human who has fasted.[20] Both of these solutions were made fresh, 24 h before the experiment. The beads were also tested under intestinal conditions only for 3 h. The pH of the gastric solution was reduced to 2.0 using 4*M* hydrochloric acid (Fluka 84435) after 15 min to simulate *in vivo* acidification. The beads were filtered, weighed, and returned to the GI solutions at 37°C every 15 min. The swell ratio was determined using the following equation:

$$\text{Swell ratio} = 100 \times \left(\frac{m_f - m_i}{m_i}\right)$$

where m_f = final bead mass; m_i = initial bead mass.

Release properties

All release measurements were carried out using the same batch of beads for each concentration of TPP and chitosan.

A fixed amount of beads (~ 0.15 g) was dispersed in 100 mL simulated gastric fluid (SGF; 2 g NaCl, 3.2 g pepsin, 7 mL conc. hydrochloric acid made up to 1 L using distilled, deionized water; pH = 1.2) contained in a 250-mL glass jar and stirred at a rate of 120 rpm using a magnetic stirrer (length = 2 cm). The supernatant was continuously sampled by means of a tube, pumped by a peristaltic pump, which passed into a flow cell that was located inside a fluorimeter (Perkin Elmer, UK), and then back into the stirred jar. The end of the tube was covered by a piece of nylon to prevent the beads from being sucked into the flow cell.

The fluorescence intensity was measured at 490 nm, at intervals of 60 s, for times up to about 10 h to follow the release kinetics. The readings were normalized to the initial mass of the beads used in the release experiment.

RESULTS

Bead preparation

Both 1.5% chitosan-10% TPP systems, prepared in basic and acidic conditions, were observed to look quite similar in terms of size, shape, and opacity when first formed. However, after continued mixing, the beads at pH 8.5 coalesced and disintegrated. The experiment was repeated at lower stirring speeds to minimize disruption after initial formation. This improved the viability of the beads at pH 8.5, although some disruption was still observed. After storage at 5°C overnight, the beads were examined. The pH 8.5 beads had shrunk massively, and the beaker contained a large volume of precipitated biopolymer on the bottom of the beaker. The pH 4.0 beads remained structurally intact and had become more opaque on storage.

The experiment was repeated using 5% w/v TPP in order that the ratio of chitosan and TPP was similar to that reported by Mi et al.[2] This provided increased stability of the system at pH 8.5, which was displayed by a lack of precipitation of the biopolymer after 3 h of gentle agitation. The beads were stored overnight at 5°C and examined. The pH 8.5 beads had shrunk massively with less precipitated biopolymer present. The pH 4.0 beads remained structurally intact and had become more opaque on storage. The beads were harvested and, during handling, it was observed that the beads at pH 8.5 were mechanically stronger than the beads at pH 4.0. The mechanical properties of the beads were determined and are discussed later.

The experiment was repeated using 3% chitosan solution dropped into 10% w/v TPP solution. Because of the highly viscous nature of the chitosan solution, the height of the syringe above the beaker

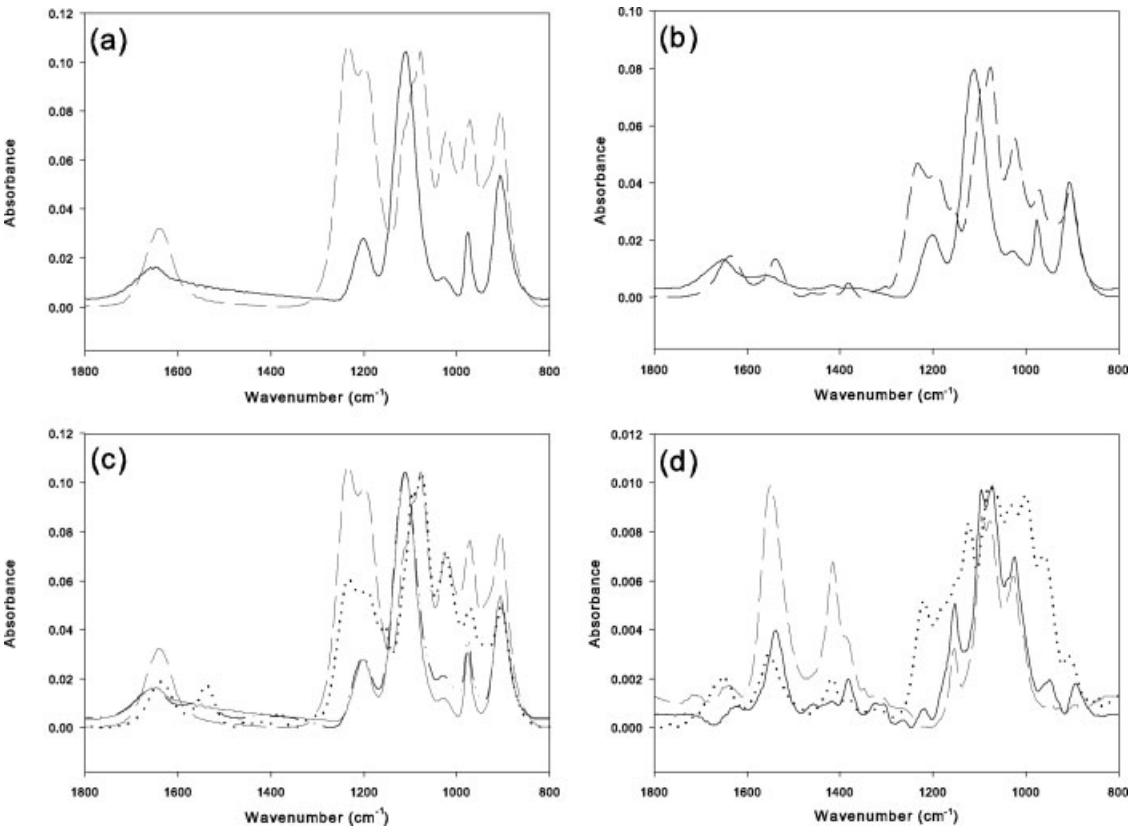

Figure 1 IR spectra of (a) 10% TPP solution at pH 8.5 (solid), 10% TPP solution at pH 4 (dashed). (b) 3% chitosan-10% TPP beads at pH 8.5 (solid), 3% chitosan-10% TPP beads at pH 4 (dashed). (c) 10% TPP solution at pH 8.5 (solid), 3% chitosan-10% TPP beads at pH 8.5 (dot-dashed), 10% TPP solution at pH 4 (dashed) and 3% chitosan-10% TPP beads at pH 4 (dotted); (d) 3% chitosan-10% TPP beads at pH 8.5 subtracted (dotted), 3% chitosan-10% TPP beads at pH 4 subtracted (solid) and 3% chitosan/1% acetic acid (pH 4) solution (dashed). The figures are scaled for clarity.

was increased to allow formation of spherical beads. Within 5 min of formation, the beads at both pH 8.5 and 4.0 became more opaque as gelation occurred. However, the beads at pH 8.5 were less stable as, with minor agitation, the TPP became cloudy, and the beads coalesced and in some cases disintegrated completely.

Following gentle stirring overnight, the beads were observed to be similar to the 1.5% chitosan-5% TPP beads. The pH 8.5 beads had shrunk and agglomerated within a slightly cloudy TPP solution. The pH 4.0 beads were much bigger and remained as structurally intact individual beads within a clear TPP solution.

The 1.5% chitosan-15% BSA-10% TPP beads were prepared in a similar fashion and examined after gentle stirring overnight. The beads prepared at pH 4.0 were more opaque following overnight incubation, and the surrounding solution was straw-colored. The beads were too weak to handle, and therefore could not be investigated. The beads prepared at pH 8.5 had shrunk in a similar manner to the chitosan only beads, with the presence of precipitated biopolymer present in the surrounding TPP solution. The swelling behavior of these beads was determined.

Infrared spectroscopy

Figure 1(a) shows the IR spectra for the TPP solutions at pH 8.5 and 4.0. The TPP solution spectra differed substantially. As sodium TPP is salt of the triphosphate oxy acid, changes in the IR spectra with pH as the ionization changes are not surprising. This is similar to the relationship between carboxylic acids and their carboxylate salts.[21,22] The spectra highlights the differences between 800 and 1800 cm^{-1}, since this incorporates peaks assigned to saccharide structure, amino and amide groups, and reflects the P—O and P=O absorbance frequency, which were used to explain changes in the interaction between chitosan and TPP.[1,2] Figure 1(b) shows the IR spectra for the chitosan-TPP beads at pH 8.5 and 4.0 and, by comparing the two figures [Fig. 1(c)], it can be seen that the TPP spectrum dominates the chitosan spectra, particularly for the system at pH 8.5. Subtraction of the TPP spectra is necessary

to determine whether any changes are seen in the chitosan spectra.

Figure 1(d) gives the IR spectra of the chitosan-TPP bead systems after subtraction of the TPP solution spectra and compares with the original 3% chitosan/1% acetic acid solution spectra. It can be seen that the spectrum of the chitosan-TPP beads at pH 4.0 (solid line) is similar to the spectrum of the original chitosan solution (before gelation; dashed line), which suggests that the conformation of the chitosan could be similar in both cases.

Gel microstructure analysis

Microstructural analysis of the gel network was undertaken using TEM and the results are displayed in Figure 2.

The gel microstructure of the 3% chitosan-10% TPP beads prepared at pH 8.5 [Fig. 2(a)] was very difficult to fix with ruthenium tetroxide, presumably because of the denser gel network obtained following shrinkage. The section was very fragile as can be seen by the presence of holes in Figure 2(a). The microstructure of the 3% chitosan-10% TPP beads prepared at pH 4.0 is depicted by a fine arrangement of chitosan gel surrounding a very porous, open network [Fig. 2(c)]. This porous structure can be seen to penetrate into the center of the bead [Fig. 2(b,d)].

The gel microstructure of the ionically gelled chitosan-TPP beads is compared with that of a covalently crosslinked chitosan gel in Figure 3.

At higher magnification, it is clear that the microstructure of the gels prepared using an ionic or covalent crosslinker is substantially different [Fig. 3(a,b)]. The covalently crosslinked chitosan gel appears to be much denser than the ionically crosslinked gel despite the lower chitosan concentration used in this system.

Mechanical testing

A number of 1.5% chitosan-10% TPP beads were harvested but were very weak, breaking on handling,

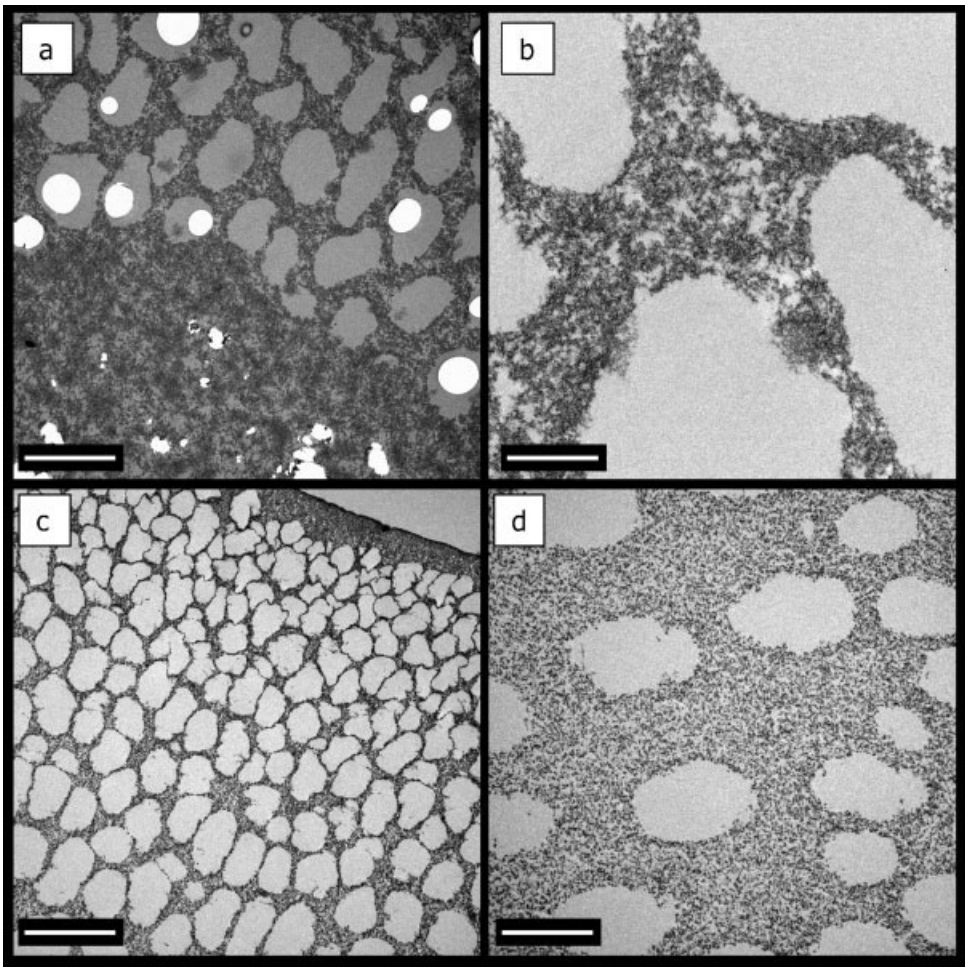

Figure 2 3% chitosan-10% TPP beads TEM at (a) pH 8.5 (toward edge of bead), scale bar 5 μm; (b) pH 8.5 (towards center of bead), scale bar 1 μm; (c) pH 4 (edge of bead), scale bar 5 μm; and (d) pH 4 (towards center of bead), scale bar 5 μm.

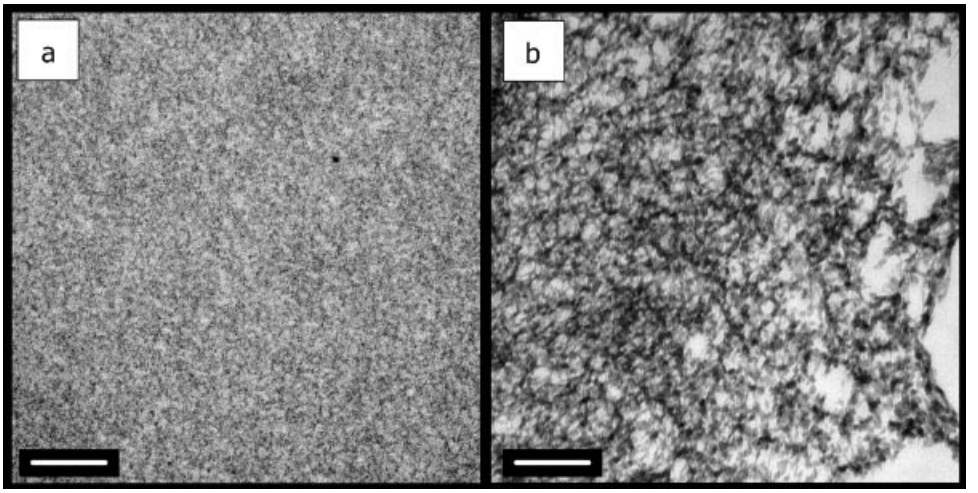

Figure 3 Comparison of microstructure of covalently and ionically crosslinked chitosan gels. (a) 1.5% chitosan gel crosslinked with 25 m*M* genipin, scale bar 0.2 μm; (b) 3% chitosan-10% TPP beads at pH 4.0, scale bar 0.2 μm.

and therefore could not be tested using the TA apparatus.

The force–displacement behavior of the 1.5% chitosan-5% TPP beads is displayed in Figure 4. The beads prepared at pH 8.5 had a Young's modulus of 187 kPa. The beads at pH 4.0 were very soft, and the Young's modulus was difficult to determine because of the large scatter of data at low deformations [Fig. 4(b)].

The force–displacement behavior of the 3% chitosan-10% TPP beads is shown in Figure 5(a,b). Figure 5(c,d) illustrates the calculation of the Young's modulus as the initial slope of a force (N)-dimensionless approach [(compressive displacement)/(initial bead diameter)] to the power 3/2 plot (for deformations between 5 and 25%).[19]

The 3% chitosan-10% TPP beads prepared at pH 4.0 were mechanically weaker than the beads prepared at pH 8.5, with a Young's modulus of 57.4 ± 5.37 kPa compared with 96.3 ± 14.8 kPa, respectively.

Swelling behavior

The swelling behavior of the 3% chitosan-10% TPP beads prepared at pH 8.5 and 4.0 was determined in GI conditions *in vitro*. These results are displayed in Figure 6. All the beads shrunk in GI conditions. The most significant point to note was that the low-pH, gastric condition had a more significant effect on shrinking of the beads prepared at pH 8.5 than the beads prepared at pH 4.0 [Fig. 6(a)]. The beads at pH 8.5 have a swell ratio of $\sim -45\%$ after 2 h in gastric conditions compared to $\sim -10\%$ for the beads prepared at pH 4.0. In both cases, there was a further reduction in the swell ratio of $\sim 20\%$ following the 3-h incubation in intestinal conditions.

Figure 6(b) shows the swell ratio of the beads tested in intestinal solution only, i.e., they are not subjected to preliminary incubation at low pH (gastric) conditions. Both chitosan-TPP complexes shrink although not to as great an extent as previously displayed, and there is less difference between the beads prepared at different pHs.

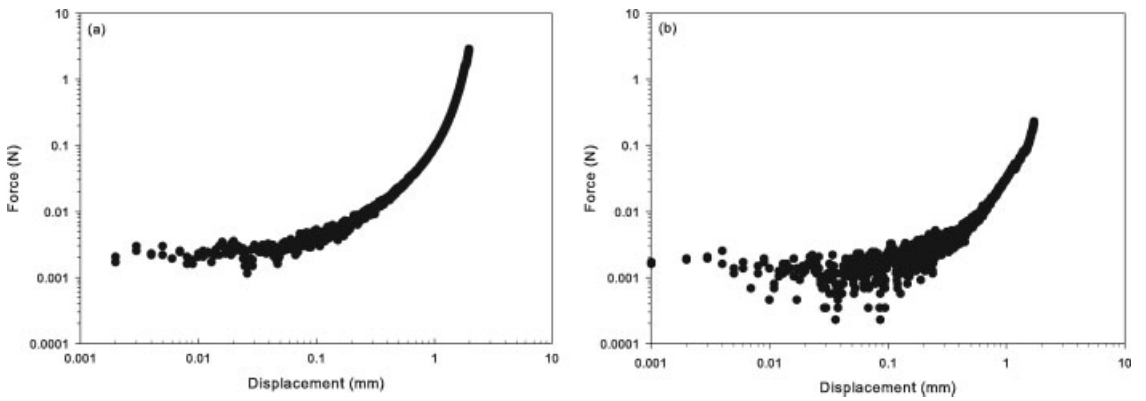

Figure 4 Force–displacement curves for 1.5% chitosan-5% TPP beads at (a) pH 8.5 and (b) pH 4.

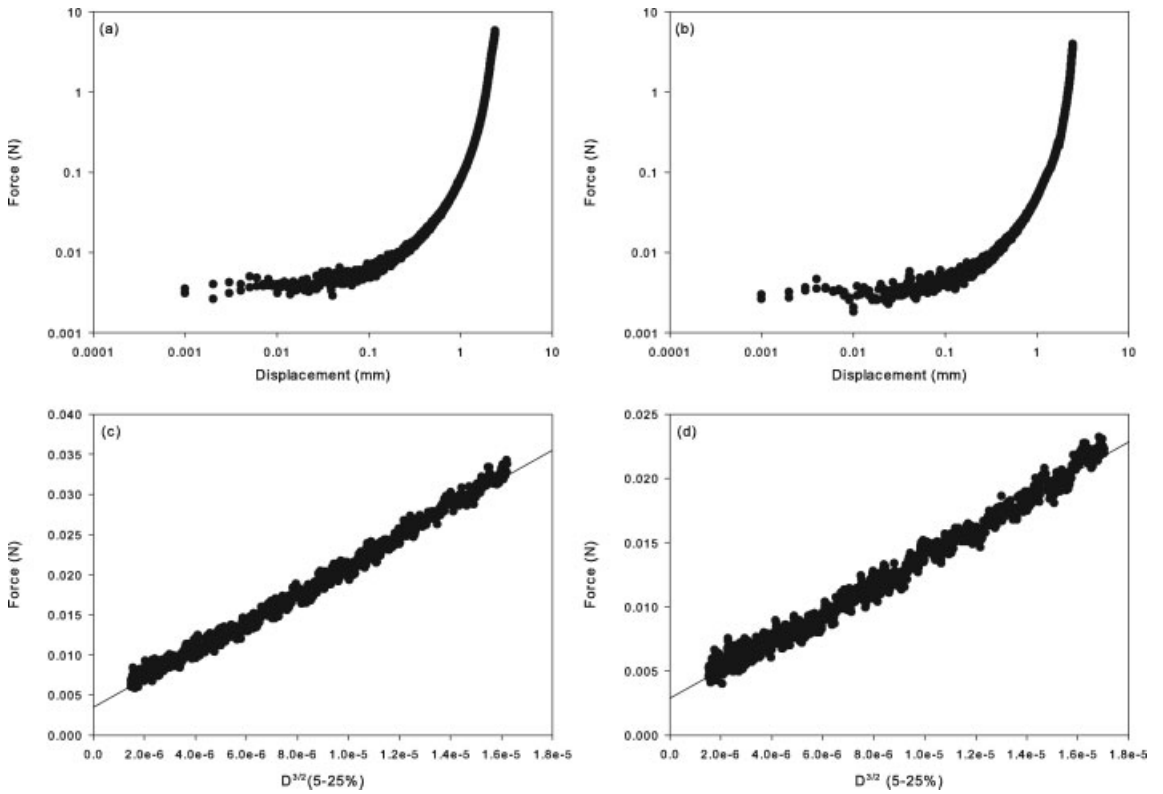

Figure 5 Force–displacement curves for 3% chitosan-10% TPP beads at (a) pH 8.5 and (b) pH 4.0. Young's modulus determination for 3% chitosan-10% TPP beads at (c) pH 8.5 (slope = 1778.508; r^2 = 0.991) and (d) pH 4.0 (slope = 1108.256; r^2 = 0.985).

The swelling behavior of the 1.5% chitosan-15% BSA beads was determined in GI conditions *in vitro*. The beads prepared at pH 4.0 were too weak to handle and could not be tested. Because of the concentration of the chitosan and BSA on shrinking, the pH 8.5 beads were mechanically robust enough to test. The results are displayed in Figure 7.

The beads containing BSA behaved in a similar way to the chitosan-only beads, with shrinking occurring in low pH (gastric) conditions [Fig. 7(a)]. Likewise, shrinking was reduced in intestinal only conditions [Fig. 7(b)].

Release measurement

Release curves of 2000 kDa FITC-dextran from chitosan beads gelled with different concentrations of TPP are shown in Figure 8.

The data clearly show that the increase in crosslinker concentration decreased the amount of dextran

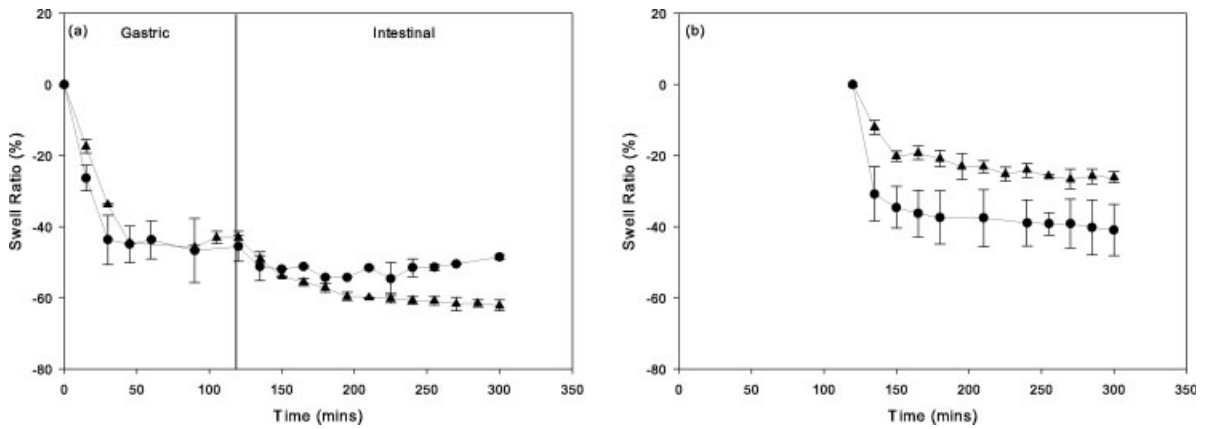

Figure 6 Swell ratio of 3% chitosan-10% TPP beads at pH 8.5 (circles) and pH 4.0 (triangles) (a) gastric to intestinal and (b) intestinal only.

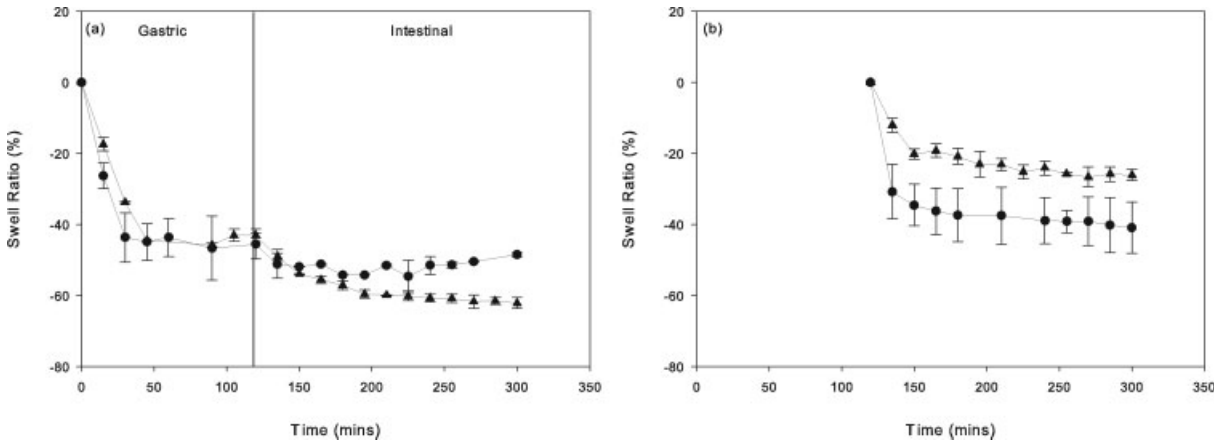

Figure 7 Swell ratio of 1.5% chitosan-15% BSA-10% TPP beads at pH 8.5 (circles) and 3% chitosan-10% TPP beads at pH 8.5 (triangles) (a) gastric to intestinal and (b) intestinal only.

released over the timescale of the experiment. The initial kinetics of release (shown by the gradient of the curves in the first 30–60 min) was relatively similar, although the higher crosslinker concentrations permitted release of dextran at slightly slower rates. The disruption in the 5% TPP data between 6 and 8 h was an experimental artefact because of gel material passing through the nylon filter and becoming temporarily embedded in the flow cell. This caused a locally higher concentration of fluorescent label to be measured, but was observed for the 5% TPP experiment only.

Release curves of 2000 kDa FITC-dextran from different concentration chitosan beads gelled with 1% (w/w) TPP are shown in Figure 9.

In this case, the final released amount of dextran appeared to be reaching the same amount, regardless of chitosan concentration. The main effect of increasing chitosan concentration was to reduce the release kinetics of dextran.

DISCUSSION

Bead preparation and storage

The results showed that decreasing the pH of the curing agent (TPP) increased the ease of preparation of the beads, but that at lower pH beads were mechanically weaker after storage.

This observation can be explained by the polyelectrolyte nature of chitosan. At low pH, the chitosan molecules possess greater positive charge owing to protonation of the amine sidegroups, and they will adopt a more extended conformation. This will lead to a greater number of sites for ionic interactions to occur between chitosan and TPP, leading to increased stability of the beads during preparation. The IR results support the argument for the gelled chitosan being in different conformational states at the different pH values, since the spectra were significantly different at pH 4.0 and 8.5. From their

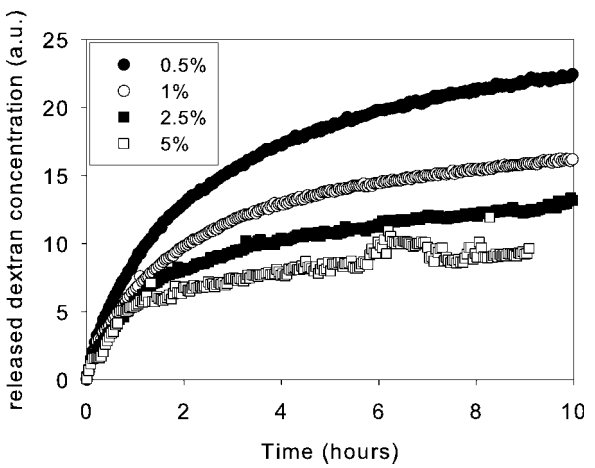

Figure 8 Release curves for 2000 kDa FITC-dextran released from beads made from 1.5% (w/w) chitosan gelled with different concentrations of TPP.

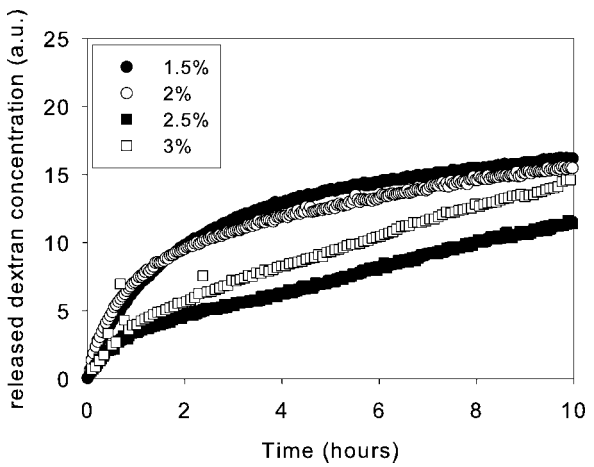

Figure 9 Release curves for 2000 kDa FITC-dextran released from beads made from different concentrations of chitosan gelled with 1% (w/w) TPP.

study of chitosan/TPP gelation at different pH, Mi et al. suggested that chitosan possessed significant ''loop-like'' regions at high pH, where a large amount of the molecule was uncharged and therefore unable to bind to TPP, and ''ladder-like'' structures at low pH, where there were a greater number of TPP-mediated intermolecular interactions.[2] However, these authors failed to take into account the changes in the state of the TPP. As such, to be able to observe the spectrum due to chitosan, the spectrum of TPP at the right pH has been subtracted. Such spectra are shown in Figure 1(d) and compared with that of chitosan at pH 4. These spectra show that at pH 4 the chitosan spectra are similar, with only few minor differences. At pH 8.5, the spectrum is significantly different. However, as we are unable to compare the spectrum at the same pH since chitosan itself is not soluble at pH 8.5, it is difficult to make any firm conclusions about how the crosslinking of the TPP is effecting the state of chitosan.

Increasing the concentration of chitosan increased the crosslink density and thereby increased the stability of the beads.

The difference in strength after storage can be explained by the shrinkage of the beads with time. Owing to the smaller number of charged groups at higher pH, beads stored at pH 8.5 were able to shrink to a greater extent, as observed. The more compact nature of these beads, and lower degree of freedom of the collapsed chitosan chains, therefore, led to a greater strength in compression. It is also possible that an increased amount of hydrolytic degradation of the beads at lower pH decreased their strength upon storage. Durkat and Elçin showed that degradation rates of the chitosan/TPP system increased at lower pH.[13] Studies describing more stable chitosan-based microparticles generally use chemical crosslinkers instead of polyanions, or use chemically modified chitosan or chitosan composites.[1,8,9,23] Comparison of the TEM images of the TPP/chitosan gel with a covalently crosslinked chitosan gel indeed showed that the microstructure of the TPP/chitosan gels at pH 4.5 and 8.0 was much more open and porous in nature than that of the covalently crosslinked gel. Unfortunately, the poor staining and weakness of the chitosan/TPP gels do not permit even reliable qualitative comparison of the differently prepared chitosan/TPP gels.

Behavior in *in vitro* GI conditions

Similar explanations, related to the charge density of chitosan at different pH values, can be used to explain the greater shrinkage of the pH 8.5 beads when exposed to simulated gastric (low pH) conditions. In low pH, and in the presence of excess TPP, additional ionic binding sites are formed on the loop-like regions of the chitosan in the pH 8.5 prepared gels. Further gelation can therefore occur, which leads to shrinking of the beads. The pH 4.0 prepared gels have no further sites available for crosslinking when placed in gastric conditions, in excess TPP, and therefore experience no driving force for further gelation and therefore shrinkage. The lack of swelling, in either case, is believed to be a result of the excess TPP and salts in the gastric solution. In excess TPP, there will be very few charged amine groups remaining to cause electrostatic repulsion. Additionally, as shown in previous studies on chitosan gels, the presence of salts will screen any charges that do remain on the chitosan chains.[14] The gel therefore experiences no internal electrostatic repulsive forces and therefore cannot swell.

The further shrinkage that was observed in intestinal conditions (pH 7.5–8.0) subsequent to the gels being immersed in gastric solution was most likely due to further dehydration of the gel structure at increased salt concentrations. The similar degree of shrinkage observed in the gels prepared at pH 4.0 and pH 8.5 when immersed only in the intestinal solution was largely due to less shrinking of the beads prepared at pH 8.5. The pH of the intestinal solution is $\sim$ 8.0 and therefore above the pK_a of the amine groups on the chitosan ($\sim$ 6.3).[1] This would result in a reduction in the positive charge on the chitosan and would not favor any further interactions with excess TPP in the system. Similarly for the chitosan-BSA composite system, shrinking was observed in gastric conditions suggesting the presence of excess TPP and salts screened for any available amine groups.

These findings are contrary to the results of Mi et al., and several others that study the swelling behavior of chitosan-based gels.[1,7,8,16,23] It is believed that the difference is due to the nature of the swelling solution: in many of the previous studies, the swelling solutions did not contain salt or possessed much lower ionic strength than used in the present study. In the present study, swelling was performed in a gastric solution that contains a high concentration of added salt. The materials used in previous studies would therefore not have experienced the charge screening that suppresses swelling that is believed to occur in the present system or would have experienced it to a lesser extent.

In summary, then, it is clear that chitosan/TPP gels shrink rather than swell when exposed to GI conditions. We believe that this is due to the presence of both excess TPP and salts in our system that can appropriately cause both further crosslinking and suppress electrostatic repulsion in the chitosan/TPP gel particles.

Release studies

Increasing crosslinker concentration (0.5–5% TPP) caused a higher amount of residual dextran to be trapped in the gel. The reason for this is likely to be related to the decreasing pore size in the gel with increasing crosslinker concentration. Because the dextran is polydisperse, there will be a pore size below which the higher molecular weight dextran molecules cannot pass through the structure and therefore remains trapped. As crosslinker concentration increases, the molecular weight limit above which the dextran is trapped becomes smaller, and therefore more dextran is trapped in the structure.

In this case, it is likely that the pore size was not sufficiently small to trap dextran inside the gelled structure. However, the increased entanglement of dextran with chitosan brought about by the increase in chitosan concentration, leading to a greater number of chitosan–dextran interactions, will lead to the diffusion of dextran through the gel being hindered, accounting for the slower release kinetics. This is similar to results from Shiraishi et al.[11] who found that the release of an acidic anti-inflammatory drug, indomethacin, decreased with increasing molecular weight of chitosan, which related to the porosity, tortuosity, and surface area of the matrix.

CONCLUSIONS

The formation of homogeneous, spherical ionic chitosan-TPP gel beads is improved by reducing the pH of the gelling bath from 8.5 to 4.0, thus increasing the cationic nature of chitosan and the crosslink density. However, we did determine that the beads prepared at basic pH were stronger due to increased shrinkage of this system and due to a reduction in charge repulsion on deprotonation, which resulted in a denser gel network. However, these ionic systems showed marked shrinking in GI conditions, presumably because of the presence of salts within the GI solutions, which effectively screen the positive charge and/or an excess of TPP which encourages ionic interactions. This makes these systems unfeasible as an alternative to the covalently crosslinked gels studied previously. These swell at low pH, and therefore provide a potential satiety enhancement *in vivo* by gastric distension. The release characteristics of these ionic chitosan gels can be enhanced by reducing both chitosan and TPP concentration, suggesting an increased porosity of the gel microstructure.

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