Comparison of Morphology and Properties of Polyelectrolyte Complex Particles Formed from Chitosan and Polyanionic Biopolymers

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ABSTRACT: Beads containing a chitosan core and a polyelectrolyte complex (PEC) shell were formed by the dropwise addition of chitosan to solutions containing sodium alginate, gellan, pectin, κ-carrageenan, or poly(acrylic acid). Hydrogel cores were formed by crosslinking chitosan with genipin, a natural bifunctional crosslinker. The shell thickness was generally only a few molecules thick and was impermeable to the transport of macromolecules but not low molecular weight molecules. Increasing the number of anionic groups and the strength of the chitosan–polyanion interaction through selection of different anionic species increased the mechanical strength of the PEC shell by increasing the number of interaction points in the shell. Because the core and shell swelled differentially, with the shell able to swell much less than the core, increasing the shell strength increasingly constrained the degree of swelling that could be attained for the entire bead. The degree of swelling could therefore be controlled via the mechanical properties of the shell, which could in turn be explained by the molecular structure of the PEC shell. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1581–1593, 2005

Key words: biopolymers; hydrogels; mechanical properties; polyelectrolytes; swelling

INTRODUCTION

Polyelectrolytes are macromolecules containing covalently bound anionic or cationic charged groups,[1] for example, COO^- or NH_3^+, with associated low molecular weight counterions (e.g., H^+ or Cl^-). Chitosan, whose structure is shown in Figure 1(a), is a well-known natural cationic polyelectrolyte that possesses primary amine groups (NH_2) that become protonated in acidic environments to become NH_3^+. It is the partially deacetylated form of chitin, which is a structural polysaccharide found in crustacea, insects, and some fungi, and it has attracted interest as a biocompatible, stimulus-responsive, mucoadhesive material for use in biomedical applications.[2] However, most natural polyelectrolytes are polyanions and polysaccharides with carboxylate (COO^-) or sulfate (SO_4^-) groups.[1] Examples of these, which were used in the present study, are sodium alginate, pectin, gellan, and carrageenan, which are shown in Figure 2. Sodium alginate and carrageenan are obtained from brown and red seaweed, respectively; pectin is a plant cell wall polysaccharide, and gellan is obtained via fermentation from *Pseudomonas elodea*.[3] They are used extensively in the food industry as thickeners, stabilizers, and gelation agents.

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Polyelectrolyte complexes (PECs) are formed when oppositely charged polyelectrolytes are mixed and interact via electrostatic (Coulombic) interactions. They have attracted interest for their use as wound dressings, membranes to control the transport of cells, polypeptides, and enzymes and for their ability to form fibers by spinning insoluble PEC precipitates from solution.[4–13] PECs are generally soluble or insoluble, but those containing chitosan are often insoluble. Usually, the stoichiometry of the PEC can be controlled via pH and polyelectrolyte concentration.[14,15] Additional factors, such as chain conformation in the case of carrageenan,[16] may also be important.

Many studies have been published on individual chitosan–polyanion systems. In these studies a variety of morphologies has been produced, including films,[7,17,18] fibers,[10–12] and capsules,[4–6,8,14,16,19–21] with either a polyanion or chitosan core and a PEC shell. Although parameters such as the molecular weight, temperature, and pH have been studied to some extent,[14,17,18,22] most of these studies are performed on specific systems and under specific and limited conditions. Few attempts have been made to compare the properties of complexes formed between chitosan and different polyanions and to rationalize the results in terms of the molecular properties of the polyanion. In this report the results of an initial study on the properties of PECs formed between chitosan and different polyanions are presented. The similarities and differ-

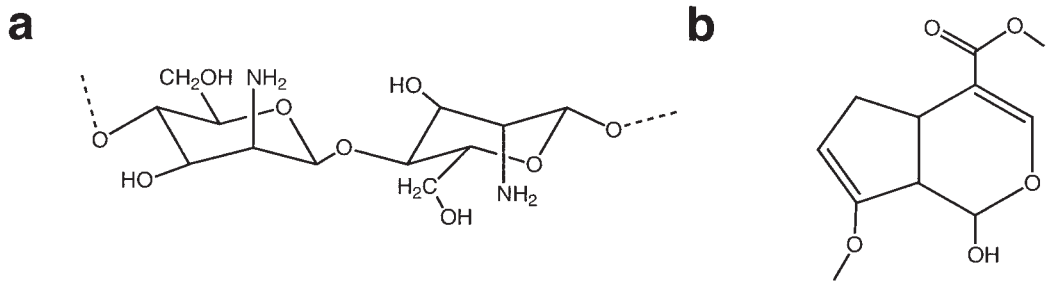

Figure 1 The molecular structures of (a) chitosan and (b) genipin, which are used to crosslink chitosan.

ences between the different PECs are explained in terms of the molecular structure of the polyanions that are used.

Sodium alginate, pectin, gellan, and κ-carrageenan were chosen as the natural polyanions and poly(acrylic acid) (PAA) was chosen as a synthetic polyanion, because they allow a study of the influence of anion species (carboxylate vs. sulfate), the amount of anionic groups (which decreases in the order PAA > sodium alginate > pectin > gellan), and the flexibility of the polyanion (synthetic PAA is expected to be more flexible than the bulkier polysaccharide molecules). In the present study, the influence of crosslinking of the chitosan core was also investigated, because crosslinking the core improves the mechanical stability of the beads containing PEC shells.[5] Genipin, whose structure is shown in Figure 1(b), is a natural molecule that reacts with primary amine groups to form crosslinks between chitosan molecules.[23–25] It was used because of its markedly lower cytotoxicity compared to commonly used crosslinkers such as glutaraldehyde.

EXPERIMENTAL

Materials and characterization

The following polymers were used: chitosan (Chitoclear, Primex Ingredients) with a 90% degree of

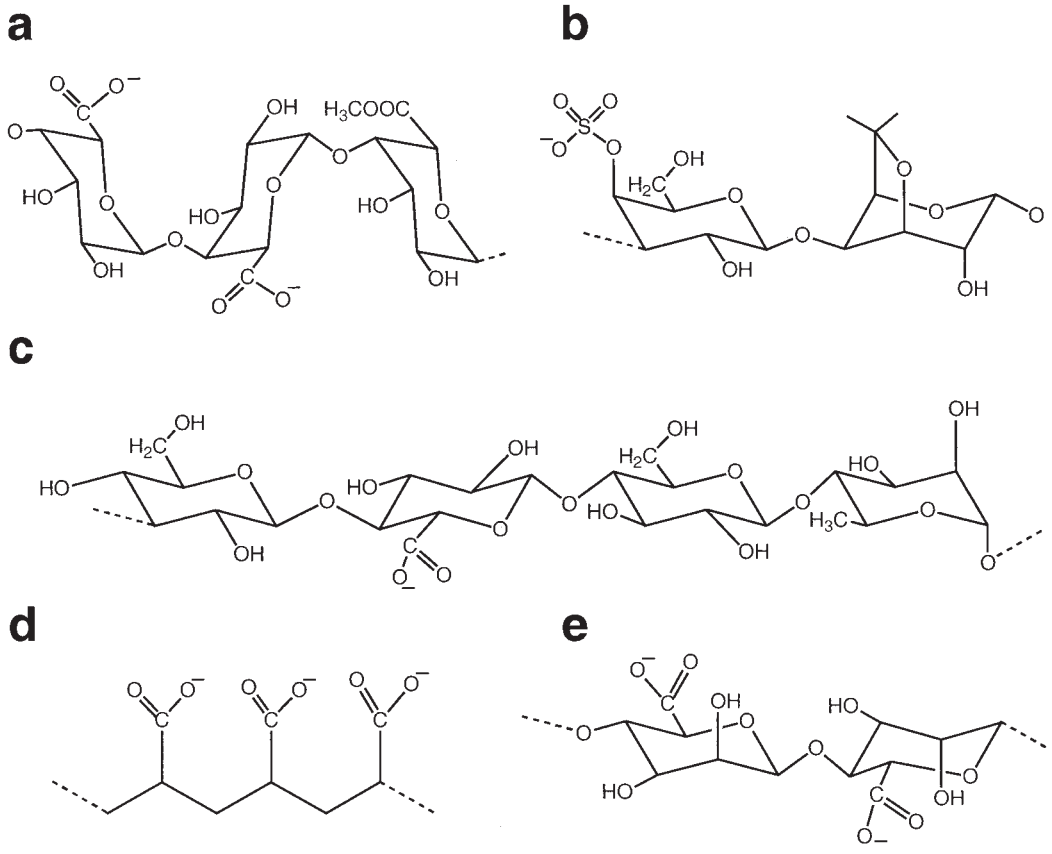

Figure 2 The molecular structures of polyanions: (a) pectin, (b) κ-carrageenan, (c) gellan, (d) PAA, and (e) alginate.

deacetylation; sodium alginate (Manugel DMB, Kelco) with a weight-average molecular weight of 241 kDa; κ-carrageenan (Genugel X0909, Kelco); gellan (Kelco) with a weight-average molecular weight of 109 kDa; low-methoxy pectin (LM12, Kelco) with a weight-average molecular weight of 130 kDa and a 35% degree of esterification; and PAA (Sigma–Aldrich) with a weight-average molecular weight of 450 kDa. Genipin, a crosslinking agent for chitosan, was supplied by Challenge Bioproducts. Hydrochloric acid and sodium hydroxide, which were used in making solutions with different pHs, were supplied by Sigma–Aldrich.

The viscosities of samples of PAA, sodium alginate, κ-carrageenan, and pectin (concentrations = 3–0.2% w/v) were measured using a Rheometrics Ltd DSR200 stress-controlled rheometer. The experiments were performed at 25°C in Couette geometry. To hinder water evaporation, the sample was covered with a layer of silicon oil. Stresses in the range 0.02–10 Pa were applied to the sample and the viscosity measured once per minute. The sample viscosity was taken to be the plateau value obtained at low stresses.

The viscosities of samples of chitosan and gellan were measured using the "falling ball" method. Briefly, a graduated cylinder was filled with chitosan or gellan solutions (concentration = 0.2–3% w/v), and the time taken for a glass ball to fall a distance of 20 cm was recorded. The terminal velocity was related to the viscosity (η) via the following expression:

$$\eta = \frac{2R^2}{9(\bar{u})} g(\rho - \rho_0)$$

where R is the glass ball radius, ρ is the sphere density, ρ_0 is the gellan density, u is the terminal velocity, and g is the gravitational force.

For all samples the plots of viscosity versus concentration were used to establish the critical entanglement concentration, beneath which the polymer solution was in the dilute regime and above which it was in the semiconcentrated regime.

The gel point for chitosan, crosslinked in the presence of genipin, was measured using a Rheometrics Ltd DSR200 stress-controlled rheometer. The sample was covered with a layer of silicon oil to hinder water evaporation. The gel points of samples containing 1.5% (w/v) chitosan and 5 or 25 m*M* genipin were established by measuring the changes in the storage modulus (G') and elastic modulus (G'') with time after mixing the chitosan and genipin. The G' and G'' were measured once per minute at a frequency of 0.1 Hz and with a stress of 0.02 Pa. The chitosan and genipin concentrations were chosen to be the same as those used in the formation of the polyelectrolyte beads.

Sample preparation and PEC formation

Stock solutions (3% w/v) of PAA, sodium alginate, gellan, κ-carrageenan, and pectin were prepared by dissolving all powders in deionized water. A 3% (w/v) stock solution of chitosan was prepared by dissolving the powder in 1% (v/v) acetic acid. When crosslinking of the chitosan was required, genipin powder was dissolved in the aqueous solutions of PAA, alginate, gellan, κ-carrageenan, and pectin to the required concentration of either 5 or 25 m*M*. The final concentration of PAA, sodium alginate, gellan, κ-carrageenan, and pectin used to make PECs with chitosan was 0.5% (w/v). The final concentration of chitosan used to make complexes was 1.5% (w/v). The pH values of the PAA, sodium alginate, gellan, κ-carrageenan, and pectin solutions were 2.9, 5.2, 5.6, 6.3, and 3.0, respectively.

Beads with a chitosan core and a PEC shell were spontaneously formed by dropping 1.5% (w/v) chitosan solution from a syringe with a needle diameter of either 0.8 or 1.1 mm into a continuously stirred solution containing 0.5% (w/v) polyanionic polymer. The bead sizes ranged from 3 to 9 mm, depending on the viscosity of the solution into which the beads were dropped and whether coalescence of droplets occurred in the initial stages of bead formation.

Some of the beads had interiors that were crosslinked with genipin and some were allowed to remain liquid. The beads were made at room temperature, which had a maximum variation of 5°C over the course of the experiments. Beads with genipin were crosslinked for 96 h in a closed vial, washed with deionized water, and stored in a refrigerator in a 0.5% (w/v) solution of the biopolymer contained in the bead shell.

Scanning electron microscopy (SEM)

The cross-sectional morphology of the PEC beads, including the core structure and shell thickness, was investigated using an SEM microscope. Samples were prepared via freeze-fracture at −98°C and coated with a gold/palladium mixture in an Oxford CP2000 preparation chamber at −110°C. Micrographs were obtained at various magnifications using a Jeol 6301 microscope equipped with a Cressington cold stage operated at −150°C.

Large deformation mechanical properties

The large deformation mechanical properties of the PEC beads were measured using a TA-XT2 texture analyzer equipped with a 5-kg load cell (TA Instruments). The compression force and displacement were measured while the beads were compressed with a

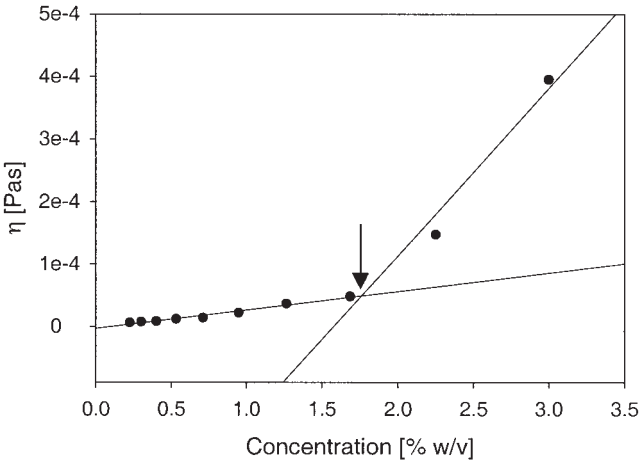

Figure 3 The viscosity versus concentration plot for sodium alginate, showing the entanglement concentration (c^*, arrow).

force of 0.981 N at a rate of 0.1 mm/s. All measurements were performed at room temperature.

Images of the beads were obtained every 0.5 s during compression using a CCD camera (Photonic Science Fast Digital Imager) equipped with a macrozoom lens.

Determination of swelling ratio

Three beads of each type of PEC in which the core could be crosslinked were weighed before and after 24-h immersion in different pH solutions to obtain an average swelling ratio at a variety of pH values. The swelling media were hydrochloric acid (1, 0.1, 0.001, and 0.0001*M* concentrations) and sodium hydroxide (0.001 and 0.000026*M* concentrations). The degree of swelling was calculated with the following equation:

$$\mathrm{SR} = \frac{w_t - w_0}{w_0}$$

where w_0 is the bead weight before and w_t is the bead weight after 24 h in the acid or alkaline solution. Before weighing the beads, excess water was removed from the bead surface using filter paper.

RESULTS

Materials characterization

A typical viscosity versus concentration plot is shown in Figure 3 for sodium alginate, showing the clear transition from the dilute solution regime to the semiconcentrated solution regime where the viscosity increases much more rapidly with concentration. The entanglement concentration, shown in Table I for all of the biopolymers in the study, varied for the biopolymers but was never lower than 1.1% (w/v). These values supplied the rationale for the concentrations of biopolymers used in the formation of the PEC beads. The value of 0.5% (w/v, dilute) was chosen for the polyanions so that they would be unhindered by interactions between themselves in the presence of chitosan and would therefore be free to interact with chitosan. The value of 1.5% (w/v, semientangled) for chitosan was chosen so that there would be a sufficient degree of interaction between chitosan molecules for the crosslinking reaction to form a mechanically stable hydrogel in a reasonable amount of time.

The cure curves for chitosan crosslinked with 5 and 25 m*M* genipin are shown in Figure 4. The time required to form a gel, defined as the time at which the value of the elastic modulus (G') exceeded the G'', and the value of the G' at the gel time both depended on the genipin concentration, as in previous studies.[25] Table II shows the values of the gel time and elastic modulus at the gel time for both samples. The sample with less crosslinker took longer to form a gel. At the beginning of the experiment the mixture of chitosan with genipin was clear and slightly yellow. At the end of the experiment the hydrogel formed was a distinct blue. The sample containing 25 m*M* genipin was a much darker blue than the sample containing only 5 m*M* genipin.

PEC beads

Beads containing a chitosan core and a chitosan–polyanion shell were formed immediately on dropping chitosan into the polyanion solution containing genipin. After several hours the interior of the beads developed a light green color that gradually became dark blue and became smaller. They also became substantially tougher and less prone to damage while handling. Although the majority of the changes were observed before washing to remove the excess genipin, the beads slowly changed color (became darker) and size (became slightly smaller) during storage in biopolymer solution after washing. Differences were also observed between samples made with different polyanions. Beads made with sodium alginate were darkest and the most dense, followed by gellan and

TABLE I
Entanglement Concentrations for Biopolymers

Biopolymer	c^* (% w/v)
Chitosan	1.20
Poly (acrylic acid)	1.40
Alginate	1.75
Gellan	1.12
κ-carrageenan	1.75
Pectin	1.83

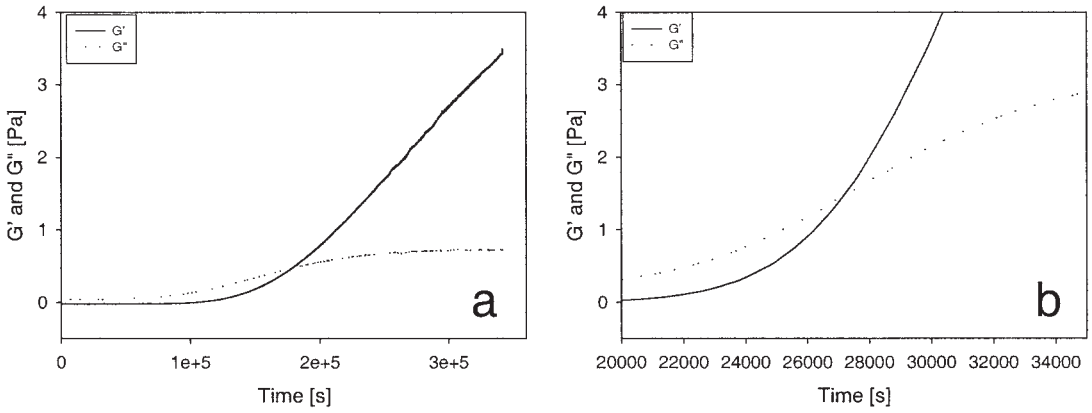

Figure 4 The loss modulus (G'') and storage modulus (G') showing the G'–G'' crossover at the gel point in 1.5% (w/v) chitosan mixed with genipin with concentrations of (a) 5 and (b) 25 m*M*.

κ-carrageenan. The chitosan core of PAA and pectin-containing beads remained liquid inside even in the presence of genipin, although the pectin-containing beads were much more fragile and tended to disintegrate during washing whereas the PAA-containing beads remained intact.

SEM

SEM images of the core–shell morphology are shown in Figure 5 for crosslinked beads made with shells containing PAA, sodium alginate, gellan, κ-carrageenan, and pectin, with a genipin concentration of 25 m*M* in the core. The lower part of the SEM images shows the chitosan core, the lighter part above the core [Fig. 5(a)] is the shell, and the biopolymer solution surrounding the bead is above that. The cell-like morphology observed in the upper part of the images is an artifact induced by the freezing step in the sample preparation procedure.

The samples containing sodium alginate, gellan, and κ-carrageenan possessed shells with thicknesses of approximately 100 nm. Samples containing PAA and pectin had much thicker shells of approximately 6000 and 1330 nm, respectively.

Figure 6 shows a comparison of the core–shell morphology for samples that were crosslinked with different amounts of genipin. Although there was no significant difference in shell thickness, clear differences between the cores and shell topographies of beads containing gellan and κ-carrageenan were observed. The cores were much less dense in the samples containing only 5 m*M* genipin, evidenced by the much more open network structure that can be seen; and the shell was less convoluted in the samples containing only 5 m*M* genipin. Beads containing sodium alginate and different amounts of crosslinker were much more similar. As for the other samples, the surface was less convoluted in the sample containing 5 m*M* genipin, but the shell thickness and core morphology appeared to be similar.

The effect of swelling on the bead morphology is illustrated in Figure 7 for beads made with shells containing κ-carrageenan as an example. Lowering the pH altered both the core and the shell morphology, but not the shell thickness. The disruption of the core at low pH that is due to swelling is clearly shown by the amount of structure induced in the core during the freezing stage of the sample preparation process. The decrease in convolution of the shell as the sample reached a maximum degree of swelling around pH 3 [Fig. 7(b)] followed by an increase in convolution as the sample shrunk at even lower pH values around 0.4 [Fig. 7(c)] can be clearly seen.

TABLE II
Critical Gel Modulus (G_c') and Gel Time (t_c) for Mixture of 1.5% (w/v) Chitosan and Genipin at Different Genipin Concentrations

Genipin concn (m*M*)	G_c' (Pa)	t_c (h)
5	0.47	50
25	1.47	7

Large deformation mechanical properties

When compressed, the beads did not break until they had reached about 70% of their original diameter, as shown in Figure 8 for a bead made using sodium alginate. For beads containing liquid cores and beads containing weakly crosslinked cores, the observed rupture corresponded with the yield point in the load-displacement curve. For the cores crosslinked with 25 m*M* genipin, the rupture occurred before the final maximum load was achieved.

Depending on the state of the crosslinker concentration in the core of the bead (i.e., whether the core was crosslinked or remained liquid and, if crosslinked, the

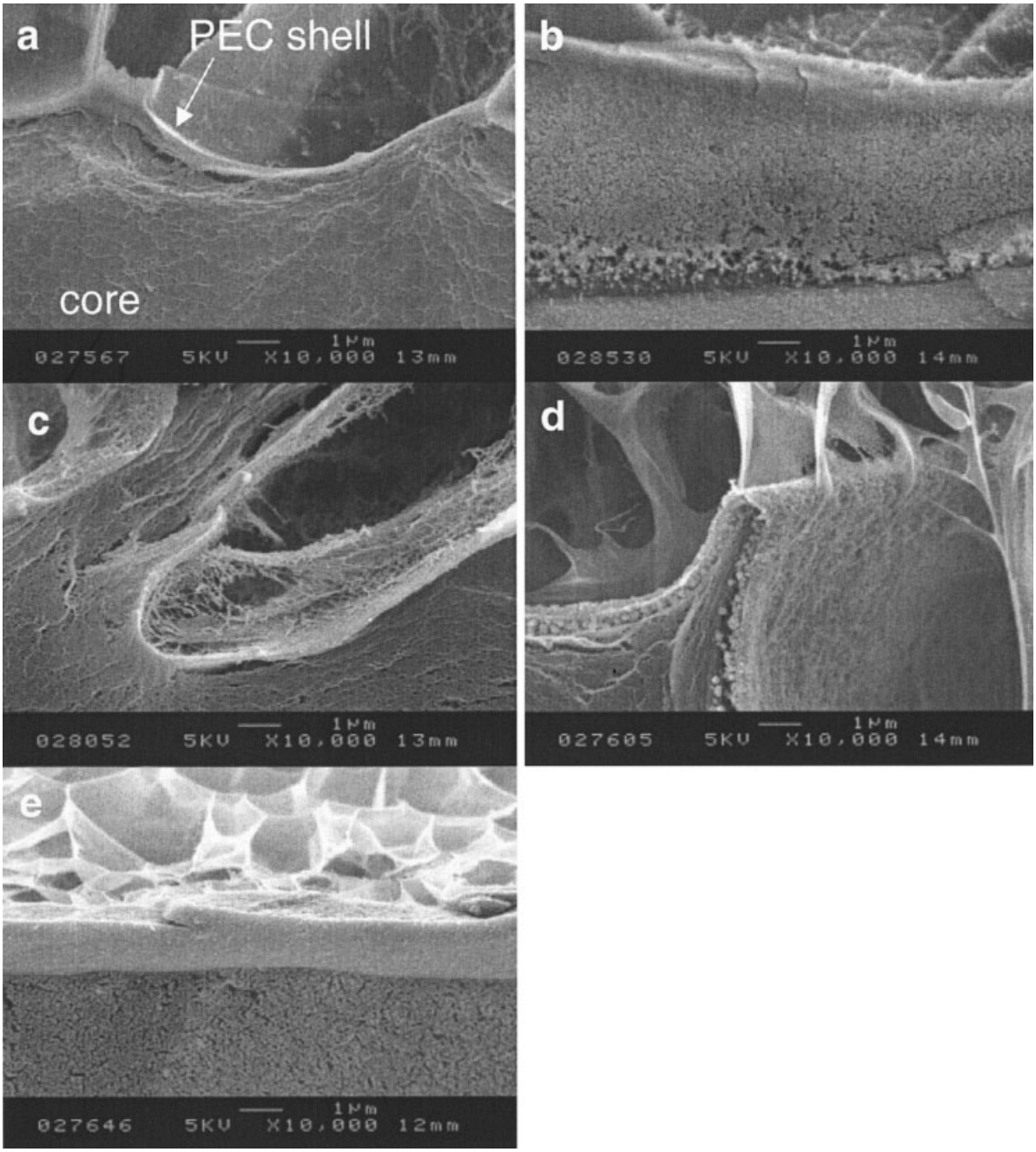

Figure 5 The influence of the polyanion type on PEC bead morphology shown by SEM images of beads crosslinked with 25 m*M* genipin. Polyanions: (a) PAA, (b) alginate, (c) gellan, (d) κ-carrageenan, and (e) pectin.

crosslinker concentration), different mechanical responses were measured with the texture analyzer. The influence of the crosslinker concentration is shown in Figure 9, which provides the load-displacement curves for a sodium alginate containing bead made with 0, 5, and 25 m*M* genipin. Only one peak, for a maximum compressive force of 0.13 N, was measured for the bead without genipin. The bead failed at the maximum load and the measured applied force subsequently dropped rapidly. Compression of beads containing 5 m*M* genipin also yielded a single peak, albeit with a larger maximum compressive force of 3.2 N. However, the load-displacement curve for beads containing 25 m*M* genipin displayed two peaks. The load initially reached a maximum force of 3.8 N, which was followed by an abrupt decrease in the applied load (which did not reach zero) before rising to a second peak value (also around 3.8 N) and then rapidly dropping to zero as the bead catastrophically collapsed.

Similar results were obtained for PEC beads made with the other polyanions, which are summarized in

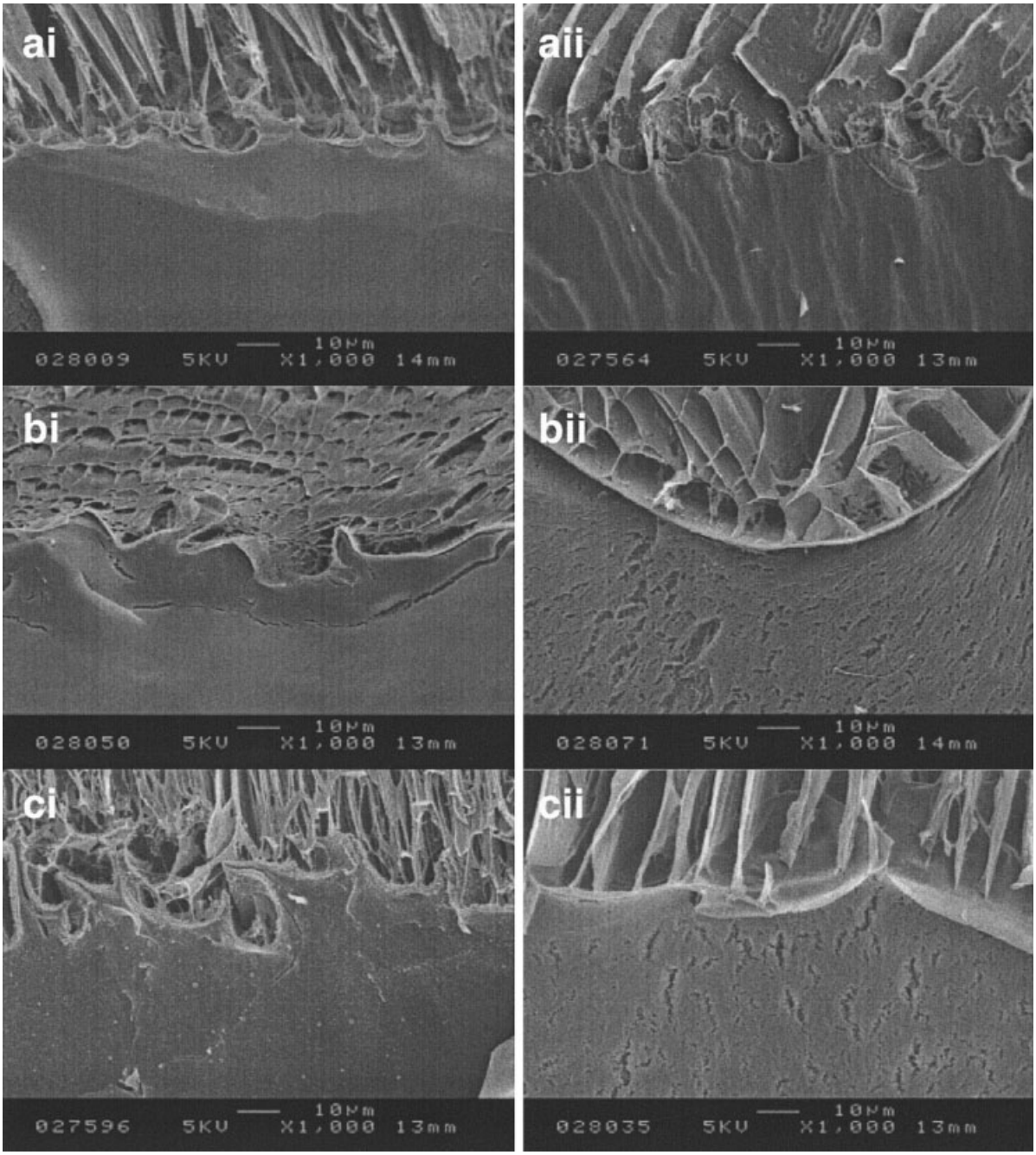

Figure 6 The influence of the core crosslinker concentration on PEC bead morphology shown by SEM images of chitosan cores and PEC shells containing (a) alginate with (i) 25 or (ii) 5 m*M* genipin; (b) gellan with (i) 25 or (ii) 5 m*M* genipin; and (c) κ-carrageenan with (i) 25 or (ii) 5 m*M* genipin.

Figure 10. At least two peaks were measured for the samples containing 25 m*M* genipin. Only one peak was measured in samples containing only 5 m*M* genipin. The samples whose core remained liquid (such as those made with PAA), regardless of the crosslinker concentration, displayed only one peak, as did beads made with sodium alginate in the absence of any core crosslinker. Bead shells containing PAA and sodium alginate were the strongest, because they were the only ones that could be handled with only a liquid core. The relative heights of the peaks in the load-extension curve depended on the polyanion used to make the bead shell. Whereas the first and second peaks were roughly equal in height for beads made with sodium alginate and 25 m*M* genipin, the maximum load for the first peak in beads containing gellan and κ-carrageenan was always lower than the maximum load for the second peak. The second peak height was roughly the same for all of the samples that contained a crosslinked core and therefore the main

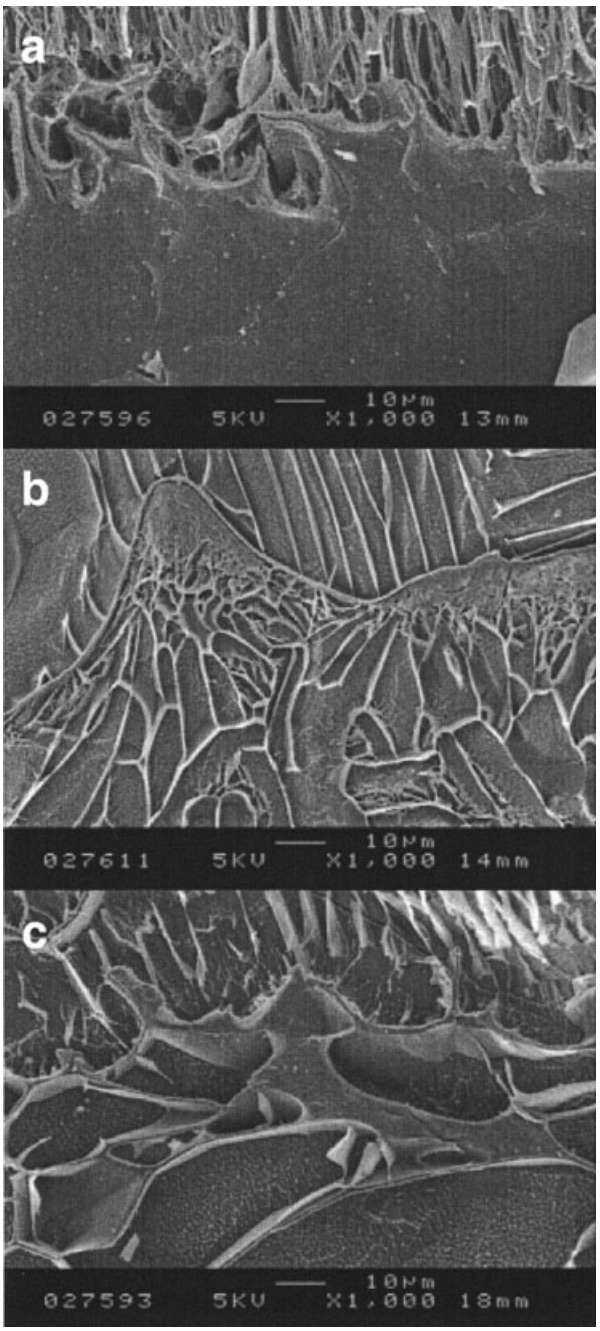

Figure 7 The influence of pH on PEC bead morphology shown by SEM images of chitosan cores and PEC shells for beads made with κ-carrageenan beads immersed in solutions with pH values of (a) 6.3, (b) 3.0, and (c) 0.4.

difference between them was the height of the first peak. As shown in Table III, the maximum force of the first peak for beads with 25 m*M* genipin decreased in the following order with polyanion type: sodium alginate, gellan, κ-carrageenan, and PAA. The force decreased in the same order for PEC beads containing 5 m*M* genipin. No measurements were performed on beads containing pectin for any genipin concentration because of difficulties with handling them.

Swelling ratio

Volume changes of beads started within a few minutes after they were added to different pH solutions and reached equilibrium within 1 h. No swelling ratio measurements were made on samples containing pectin or PAA because they did not form chitosan hydrogel cores. For these samples the shell ruptured at low pH values and the liquid core flowed out.

The variation in the swelling ratio with the pH is shown in Figures 11 and 12 for samples containing sodium alginate, gellan, and κ-carrageenan and crosslinked with 5 and 25 m*M* genipin, respectively. In all cases, a maximum value of the swelling ratio was achieved in the pH range between 3 and 4. At neutral pH very low swelling ratios were recorded whereas at very low and very high pH values the beads actually shrank. Beads containing 25 m*M* genipin achieved lower swelling ratios for a given pH than the beads crosslinked with 5 m*M* genipin. The beads containing gellan and κ-carrageenan became so swollen that the shell often split. For the other samples the shell was distended but remained intact. The maximum swelling ratio is shown for all of the samples in Table IV. For the beads crosslinked with 25 m*M* genipin, samples made with κ-carrageenan swelled the most, followed by gellan and then sodium alginate. For the beads crosslinked with 5 m*M* genipin, κ-carrageenan also swelled the most, followed by sodium alginate and then gellan.

DISCUSSION

PEC formation and morphology

Beads were formed as soon as chitosan, which is cationic, was dropped into the solutions containing the various polyanions because the interaction between

Figure 8 Images of a bead crosslinked with 25 m*M* genipin and containing sodium alginate in the shell (a) before compression or during compression in the texture analyzer (b) just prior to rupture, and (c) after rupture. Scale bar = 5 mm.

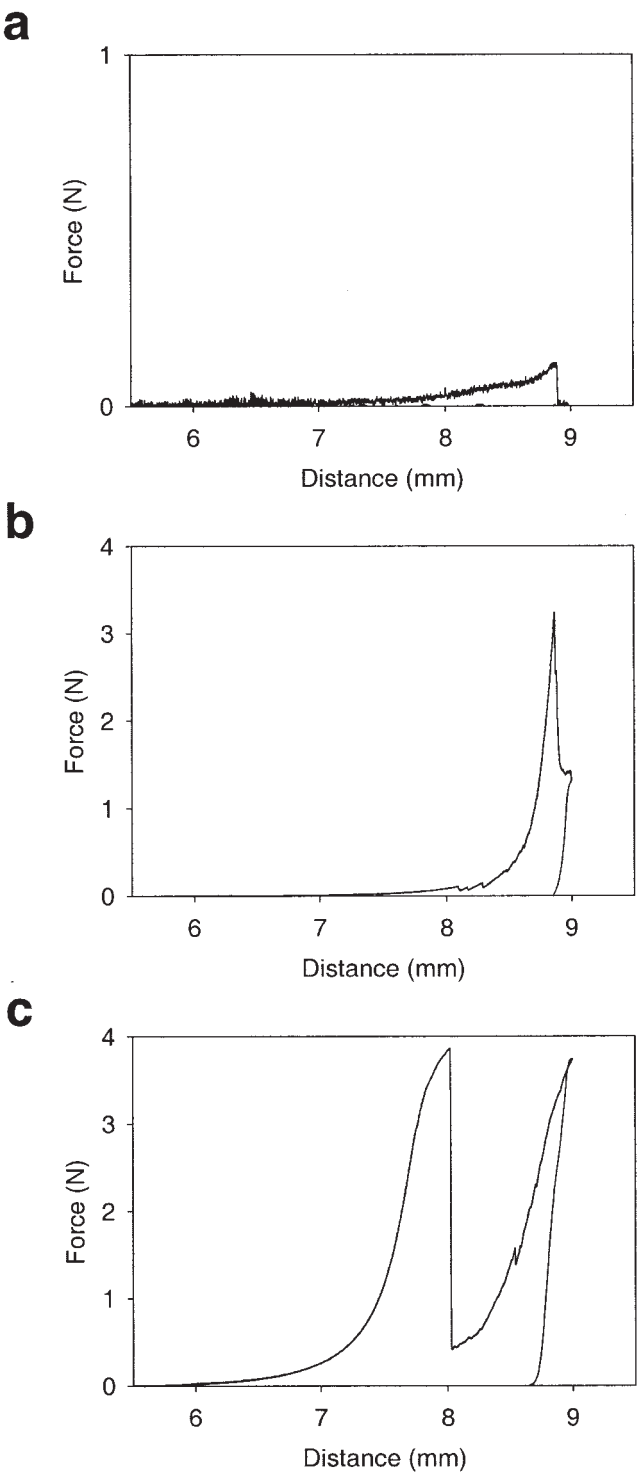

Figure 9 The effect of the crosslinker concentration on the load-displacement curves of PEC beads made with sodium alginate. The concentrations of genipin are (a) 0, (b) 5, and (c) 25 m*M*.

the oppositely charged functional groups (amine in the case of chitosan and either carboxylic acid, for PAA, sodium alginate, gellan, and pectin, or sulFate, for κ-carrageenan) formed a PEC membrane (Fig. 13) that enclosed the chitosan droplet. Exposure to genipin enabled crosslinks to form between the amine groups in chitosan via a reaction that described previously,[24] which led to the formation of a hydrogel core.

The absence of crosslinking in the systems containing PAA and pectin was due to the low pH of the solutions containing these biopolymers. These two polymer solutions possessed the lowest pH values. The large degree of protonation of the chitosan amine groups therefore inhibited the nucleophilic substitution reaction that was involved in the crosslinking reaction by removing the lone pair of electrons on the amine group required to perform this reaction.

The different pH conditions during the formation of the PEC shells also provide one explanation for the difference in the measured shell thickness. The PEC shell was the thin, light layer observed in the SEM images that was about 100 nm thick for the polymers in solutions with pH >5 but several hundred nanometers thick for pectin and PAA that were in solutions with pH ≈3. In the former case the shell thickness would have been on the order of only a few molecules whereas in the latter case it would have been many molecules thick. In the higher pH conditions many of the carboxylate or sulfate groups on the polyanion will be charged and therefore able to interact with the chitosan molecule. A self-supporting membrane can therefore be made with a shell that is only a few molecules thick, because the majority of the anionic groups in the shell will be involved in ionic interactions with the chitosan cationic groups, resulting in a denser membrane. At the lower pH, however, there will be a greater concentration of uncharged carboxylic acid groups that are unable to interact with chitosan. Loops of uncharged polymer may therefore exist in the shell, leading to a thicker and less dense membrane.[22,26,27]

However, Gaserod et al. showed that chain extension and flexibility is also important when considering the ability of oppositely charged polyelectrolytes to form membranes of particular thickness.[19] Chitosan exists in solution as a stiff coil[28] and will therefore have a large radius of gyration, hindering its ability to diffuse through the membrane and increasing the likelihood of interactions occurring between the chitosan and polyanions. This effect favors the formation of thin membranes. However, experiments comparing the binding ability of lysine and chitosan, which are both cationic but exhibit different flexibilities, have shown that the more flexible lysine molecules are able to bind more effectively to polyanions.[19] The different membrane thicknesses may therefore also be affected by the flexibility of the polyelectrolytes that are present. The thicker membranes formed in the presence of pectin and PAA may thus have resulted from these molecules being more flexible than the others and hence being able to diffuse more readily into the

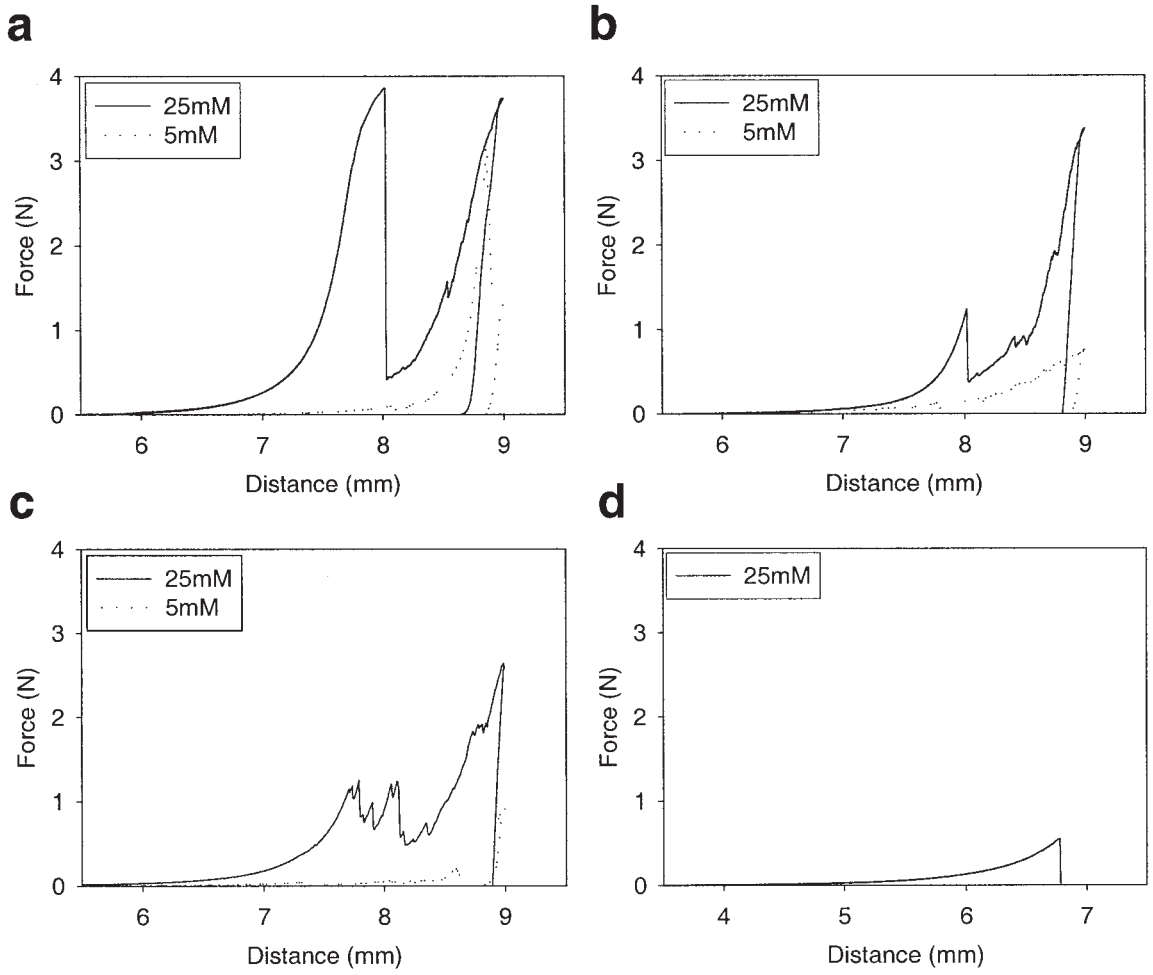

Figure 10 Load–compression curves of PEC beads made with different polyanions and concentrations of crosslinker. Polyanions: (a) 5 m*M* alginate, (b) gellan, (c) κ-carrageenan, and (d) PAA.

bead. Certainly, PAA is expected to be less rigid than the polysaccharides, demonstrated by the lower Mark–Houwink parameter measured from viscometry for PAA compared to polysaccharides.[29,30] Of the polysaccharides used in the present study, pectin has the lowest Mark–Houwink parameter (and is therefore the most flexible),[30] followed by gellan and sodium alginate, which correlates with the decreasing membrane thickness formed in the presence of these polyanions, in that order. Chain flexibility may therefore have played a role in determining the diffusivity of the polyanion in the membrane and hence the membrane thickness.

TABLE III
Maximum Compressive Force for First peak Measured in Samples Containing 25 m*M* Genipin and for Only Peak in Samples Containing 5 m*M* Genipin

Polyanion	Genipin concn (m*M*)	Max force of first peak (N)
Alginate	5	3.4
Gellan	5	0.8
κ-carrageenan	5	0.5
Alginate	25	3.9
Gellan	25	1.3
κ-carrageenan	25	1.1

The permeability of the membranes to low molecular weight organic molecules was shown by the ability of the genipin to continuously diffuse from the surrounding biopolymer solution into the core, which was revealed by the continual darkening and shrinking of the beads with time as they became increasingly crosslinked and therefore denser. The impermeability of the membrane to macromolecules was shown by the formation of a very thin membrane that retarded the formation of further complexation between chitosan and polyanion in the core. When chitosan is exposed to a low molecular weight anion, such as tripolyphosphate, a PEC is formed throughout the entire bead.[31]

The increased crosslink density in the core with increasing genipin concentration was shown by the

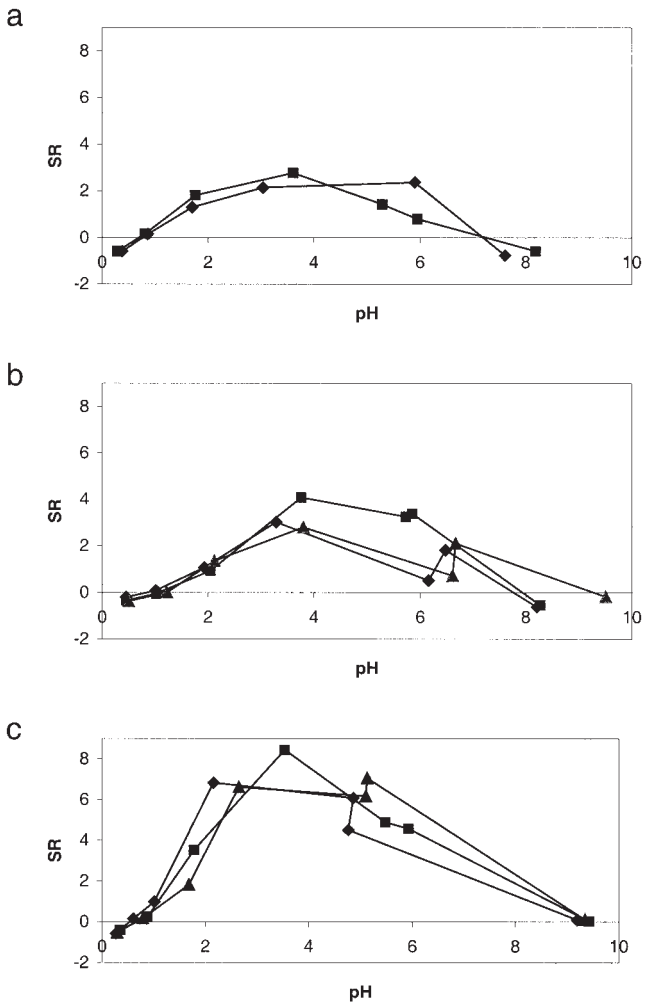

Figure 11 The variation of the swelling ratio with the pH for beads containing chitosan cores crosslinked with 5 m*M* genipin and PEC shells containing (a) sodium alginate, (b) gellan, and (c) κ-carrageenan. The different symbols in each plot refer to separate samples, and the lines are intended as a guide to the eye.

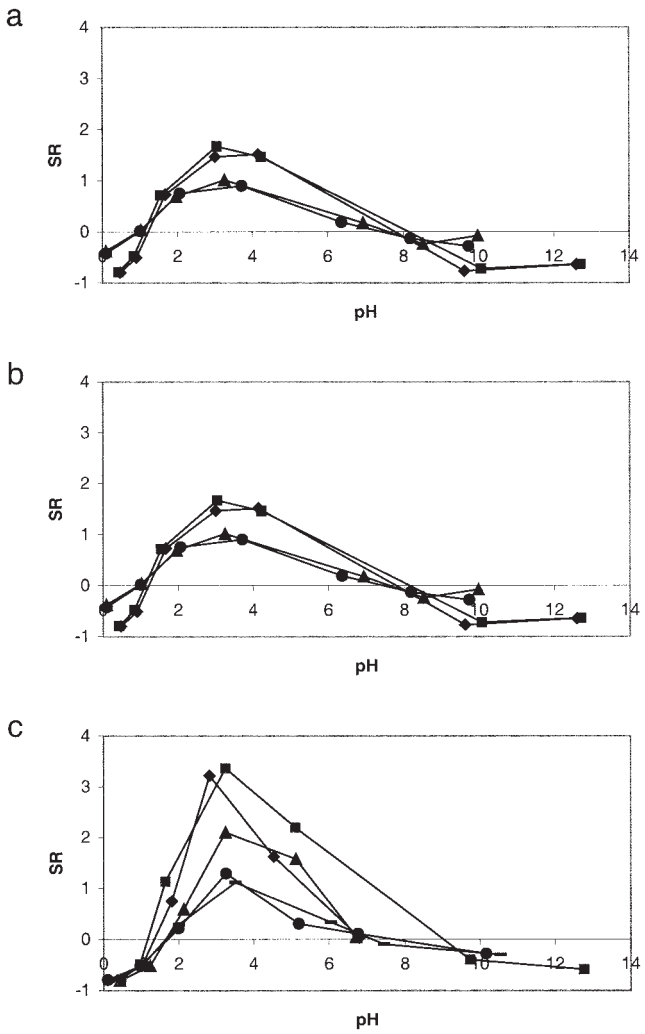

Figure 12 The variation of the swelling ratio with the pH for beads containing chitosan cores crosslinked with 25 m*M* genipin and PEC shells containing (a) sodium alginate, (b) gellan, and (c) κ-carrageenan. The different symbols in each plot refer to separate samples, and the lines are intended as a guide to the eye.

reduced amount of freezing damage in the core of the beads containing 25 m*M* genipin. The increase in density of the core due to the increased amount of crosslinking in the 25 m*M* genipin beads was confirmed by the shrinking of the beads, which was observed by eye and by the increased contortion of the PEC shell attached to the core observed via SEM. The SEM images also confirmed that the shell became stretched as the beads became swollen, because less contortion of the shell was observed in the swollen beads. The decrease in density of the core in the swollen beads was shown by the increased amount of freezing damage in the swollen beads.

PEC physical properties

Samples that contained a liquid, or very weakly crosslinked, core displayed only one maximum in the load-displacement curve whereas the highly crosslinked samples contained several maxima. For the liquid samples the maximum load must have been the rupture of the PEC membrane surrounding the bead because this is the only part of the sample that can provide any resistance to deformation.

TABLE IV
Maximum Swelling Ratios for All Swellable Beads

	Maximum swelling ratio	
Polyanion	5 m*M*	25 m*M*
Alginate	2.5	1.4
Gellan	3.1	1.7
κ-carrageenan	7.5	2.2

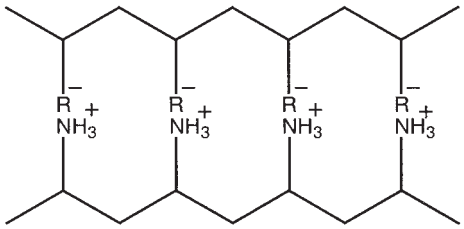

Figure 13 A schematic depiction of the polyelectrolyte complex between chitosan and biopolymer; R = COO^- (PAA, alginate, gellan, and pectin) or OSO_3^- (κ-carrageenan).

For the weakly crosslinked, 5 m*M* genipin samples the hydrogel was too weak to provide much resistance to deformation once the shell had ruptured. Nevertheless, the core must have provided some additional resistance because the maximum force was higher for samples containing 5 m*M* genipin than those containing no genipin. Therefore, for these samples the maximum load corresponded to the rupture of the PEC shell supported by some resistance from the core.

For the strongly crosslinked, 25 m*M* genipin samples, however, resistance to deformation did not cease with the visually observed shell rupture and the load recovered as the crosslinked core experienced further deformation. The final maximum in the load displacement curve was similar for all of the crosslinked samples, as expected from a contribution solely from the core that would have been crosslinked to a similar extent in the presence of sodium alginate, gellan, and κ-carrageenan, which possessed similar pH values in solution. Therefore, the initial maxima in the load-displacement curves, which were dependent on the polyanion type, for the 25 m*M* genipin samples were attributable to rupture of the shell supported by some resistance from the core and the final maximum was due to failure of the core only. In some cases there was a series of smaller maxima before the final yield point. It is likely that these smaller maxima were due to multiple failure points in the PEC membrane and that it did not lose all of its strength in one yielding event.

The main influence on the PEC shell strength was the number of interactions between the protonated chitosan amine groups and the polyanion acidic groups. Beads made with PAA and sodium alginate possessed the strongest shells. They also had the most carboxylic acid groups per monomer unit and the lowest pH values (thus the greatest degrees of chitosan amine protonation) of the different polyanion solutions that were used. Gellan and κ-carrageenan possessed fewer functional groups and their solutions exhibited higher pH values in that order, which corresponded to decreasing shell strength. A greater number of interactions between the oppositely charged polymers in the PEC shell is likely to promote the formation of a denser network of connected chains in the PEC layer, which will increase the mechanical strength of the shell. The exception for pectin, which possessed a low pH in solution and possessed more anionic functional groups than gellan or κ-carrageenan but formed weaker beads, may have been because pectin forms complexes with divalent anions more easily than monovalent ones, such as the protonated amine group. The distribution of anionic groups on pectin may also have been important. If the pectin possessed a highly nonrandom (i.e., blocky) distribution of carboxylic acid groups, then there could have been long sequences without groups that could interact with chitosan. This would lead to the presence of weaker regions in the PEC shell that would be more prone to rupture.

The swelling ability of the beads is governed by the uptake of solvent into the bead as a result of the initial osmotic imbalance between the solvent concentration inside and outside the bead and the Donnan equilibrium that depends on the polyelectrolyte nature of the polymer chains. The maximum in swelling at pH ≈3 can be explained by the increasing amounts of chitosan amine groups becoming protonated and experiencing electrostatic repulsion forces between each other, leading to extension of the chitosan molecules between crosslinks and hence swelling of the beads. At very low pH the excess counterions from the acid overcome the repulsive forces between the protonated amine groups by screening the charges, and the chitosan gel is therefore unable to swell any further. The rigidity and strength of both the crosslinked core and the PEC shell counteract this swelling effect, however. The more highly crosslinked cores containing 25 m*M* genipin swell to a lesser extent than the 5 m*M* genipin cores because the greater number of crosslinks retard the extension of the chitosan molecules. Moreover, a rigid shell will prevent osmotic swelling if it is strong but will break if it is weak. The mechanical properties of the different PEC systems are therefore directly linked to the maximum swelling ratio that was observed. In the current systems, the PECs containing the polyanions that formed shells with the highest mechanical strength (i.e., alginate) were those that constrained the maximum swelling to the lowest value. Conversely, the weakest shells, which contained κ-carrageenan, swelled the most because they exhibited the least resistance.

Many studies of PEC swelling have been performed on systems containing a polyanion core and a PEC shell containing chitosan[18,20] or on samples consisting only of the PEC itself.[32–34] At low pH, the swellability will be dominated by the chitosan core in the present system, constrained by the shell. At higher pH, the beads are expected to swell to a greater extent than a sample containing only chitosan because the anionic groups in the PEC shell may become charged.[18,20,32–34] At alkaline pH, in systems containing a chitosan–

pectin PEC, some dissociation of the PEC has been proposed and the charging of the free anionic groups has led to quite large degrees of swelling.[32–34] In the current study the lack of swelling at alkaline pH indicated that no dissociation occurred. It also supported the interpretation for the thin PEC shells that were observed using SEM, which was that the majority of the anionic groups in the samples containing sodium alginate, gellan, and κ-carrageenan were ionically bonded to chitosan and were therefore unavailable for swelling.

CONCLUSIONS

When cationic chitosan solution is added dropwise to solutions containing polyanions, beads containing a chitosan core (which may be crosslinked with the bifunctional crosslinker genipin, provided it is not in an environment with a pH that is too low) and a PEC shell are formed. The shell thickness is generally only a few molecules thick and is impermeable to the transport of macromolecules but not low molecular weight organic molecules. Increasing the number of anionic groups and the strength of the polycation–polyanion interaction through selection of different anionic species increases the mechanical strength of the PEC shell by increasing the number of interaction points in the PEC shell. Because the core and shell swell differentially, with the shell able to experience much lower swelling ratios than the core, increasing the shell strength increasingly constrains the degree of swelling that may be attained. Knowledge of the number and strength of the molecular interactions in the PEC shell will therefore enable greater control of the mechanical and swelling properties of beads formed from chitosan cores and PEC shells.

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