Selected properties of pH-sensitive, biodegradable chitosan–poly(vinyl alcohol) hydrogel
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Abstract: Chitosan and poly(vinyl alcohol) (PVA) were used to form a semi-interpenetrating polymeric network with glutaraldehyde as the cross-linker. The molecular weight and degree of deacetylation of the chitosan were 612 kDa and 72 %, respectively. The chemical bonds formed by the cross-linking reaction and transition of these bonds in different pH media were investigated. The gelation property of the chitosan–PVA pregel solution and mechanical properties of the hydrogel were studied. The FTIR spectra of the hydrogel before and after swelling at pH 3 and pH 7 indicated formation of Schiff’s base (C=N) and —NH3+. They also showed pH-induced transition of C=N to C—N, and —NH3+ to —NH2, as well as the instability of the Schiff’s base. The chitosan is essential for hydrogel formation through Schiff’s base reaction between the amino groups of the chitosan and the aldehyde groups of the glutaraldehyde. The addition of PVA improved the mechanical properties of the hydrogel. However, PVA tends to leach out at longer swelling times in the acidic medium due to hydrolysis of the gel networks, Schiff’s base.

Keywords: hydrogels; chitosan; poly(vinyl alcohol); biodegradable; gels; pH-sensitive

NOTATIONS
a,k constants of Mark–Houwink equation (eqn (1))
CS/PVA chitosan/poly(vinyl alcohol) molar ratio
DD degree of deacetylation
Mw molecular weight
[η] intrinsic viscosity (ml g−1)
G’ storage modulus (Pa)
G″ loss modulus (Pa)
δ phase angle (◦)
tgel gelation time (min)
tcom time for complete gelation (min)
CpVA solution PVA concentration (mg 100 ml−1)

INTRODUCTION
Hydrogels can be defined as polymeric networks that can retain a significant amount of water within their structures, and swell without dissolving in water. Relatively high water content, hydrophilicity, expandability, selective permeability, soft rubbery consistency and low interfacial tension are among the advantages of hydrogels, enabling them to resemble soft living tissues.

The response of hydrogels to environmental changes, simulating changes in biological systems, such as pH, temperature, electric field, ionic strength, salt type, etc, is an active area of research. Swelling, shrinking, bending and degrading are among the unique responses of environment-sensitive hydrogels.1

A significant body of research has focused on developing and characterizing novel hydrogels. The pH-sensitivity, ie the change in volume in response to changes in surrounding medium pH, is one such characteristic of the hydrogel.

Biodegradable pH-sensitive hydrogels are formed by cross-linking at least one ionic biopolymer to form a three-dimensional (3-D) network. The major disadvantage of pH-sensitive hydrogels is their low mechanical strength due to their high water content, particularly after swelling. The use of an interpenetrating (IPN) agent, however, has been shown to alleviate this problem. The IPN agent diffuses into the already formed hydrogel and polymerizes to form a double cross-linked or a hybrid polymeric network structure. The character of the network can be a covalent bond, an electrostatic or hydrophobic interaction, etc. The IPN polymer has better mechanical properties than the principal gel and has also been shown to improve the mechanical properties of the final hydrogel.2

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In this paper, we describe the synthesis of a biodegradable, pH-sensitive hydrogel using chitosan as the base polymer, poly(vinyl alcohol) (PVA) as the IPN agent, and glutaraldehyde as the cross-linker.

Chitin is the raw material of chitosan and has a structure similar to natural glycosaminoglycans. Chitosan is the principal deacetylated derivative of chitin with polymorphism forms of antiparallel fashion similar to that of α-chitin. Due to the high density of primary amino groups in its molecular structure, it is distinguished from most polymers as a linear-cationic polysaccharide. Commercial chitosan is mainly produced by the deacetylation of chitin extracted from crustacean shell waste of the seafood industry. Many manufacturing-related factors can significantly affect the properties of chitosan, and these include purity, M, and enzyme treatments are two major methods for chitin deacetylation. The former takes place under harsh conditions, while the latter is achieved under relatively mild conditions at a higher cost and less degradation and pollution.

Poly(vinyl alcohol) is one of the more widely used polymers because of its excellent mechanical properties. It is also biodegradable under suitable conditions. Commercial PVA is a mixture of different types of stereoregular PVA structures (isotactic, syndiotactic, and atactic). Its stereoregularity and physical and chemical properties are highly dependent on the preparation method used. Solubility of PVA in water depends on the degree of hydrolysis and polymerization. Usually, PVA with 98.5 % or higher can be dissolved in water at 70 °C, which is a common practice for preparing this solution. The relative viscosity of aqueous PVA solution within the range of 1–25 % and temperature range of 10−80 °C can be expressed as a linear function of concentration and Mwangle="915"\n
Glutaraldehyde is a common cross-linker used in polypeptide and protein cross-linking because of the high activity of the aldehyde groups, which readily form Schiff’s base with amino groups of proteins. Glutaraldehyde is also used as a cross-linking agent for PVA and some polysaccharides such as heparin, as well as hyaluronic acid and chitosan.

In our research directed toward developing a new practical hydrogel, we chose to use chitosan because of its biodegradability and unique cationic properties. PVA was chosen because of its good biodegradability and excellent mechanical properties. The chitosan and PVA combination in the presence of glutaraldehyde has shown promising attributes for practical applications, such as high swelling and shrinking ratio, high pH sensitivity and biodegradability. The objective of the research presented herein is to characterize particular chemical and physical properties of the pH-sensitive, biodegradable chitosan–PVA interpenetrating hydrogel.

**MATERIALS AND METHODS**

Two buffer solutions were used for sample preparation. They are designated as follows: Solvent A—0.1 M acetic acid/0.2 M NaCl, and Solvent B—0.75 % (v/v) acetic acid. These and other chemicals used were of analytical grade purchased from a commercial source (Aldrich Chemical, Milwaukee, WI).

**Molecular weight of chitosan**

The molecular weight (Mw) of chitosan was determined using the viscometric method of Mark and Houwink in which the intrinsic viscosity is measured and used to calculate Mw. Detailed methodology has been presented elsewhere. Low Mw chitosan (Cat No 44 886-9, Aldrich Chemical, Milwaukee, WI) was used to prepare 0.1 % chitosan solution in Solvent A. After maintaining it overnight at room temperature it was filtered through glass wool to remove insolubles. Four dilutions were made by completing 5, 10, 15, and 20 ml of the chitosan solution to 25 ml with Solvent A. The relative viscosities were calculated from the depletion times of the chitosan solutions and Solvent A using an Ostwald–Fensky viscometer (100 × 279 K, Cannon Instrument, State College, PA) and a stopwatch. Experiments were conducted in triplicate at 25 °C using a water bath (Neslab RTE 221, Thermolab, Portsmouth, NH).

**Degree of deacetylation of chitosan and FTIR spectra of chitosan film**

Infrared spectroscopy was used to quantify the degree of deacetylation of the chitosan using characteristic wavenumbers of 1650 cm\(^{-1}\) (amide I peak) and 3450 cm\(^{-1}\) (primary amine —NH\(_2\)) as given by Gupta and Kumar. Detailed calculation procedures are presented in Reference 8. Two milligrams of chitosan was ground into fine powder together with 100–200 mg of KBr (Cat No 22 186-4, Aldrich Chemical, Milwaukee, WI) in a glass mortar and kept in a desiccator. The powder was then loaded in an evacuable die and compressed to 13.8 MPa for 1 min to obtain a thin transparent tablet to be scanned in a FTIR spectrometer (Polaris\(^{TM}\), Mattson Instruments, Madison, WI). The FTIR spectrometer was calibrated by blank scanning between 400 cm\(^{-1}\) and 4000 cm\(^{-1}\) with resolution of 1 cm\(^{-1}\) and 32 scans/sample. The internal infrared standard was set at 3450 cm\(^{-1}\) with transmittance of 30 % after scanning several chitosan tablets.

To obtain the FTIR spectra of chitosan film, the chitosan was dissolved in Solvent B to prepare a 1 % solution. The solution was poured onto a plastic surface, dried in an oven at 80 °C, peeled off and stored in a desiccator until the FTIR tests were done. The resulting thickness of the chitosan film was between 0.5 mm and 1.0 mm as measured using a micrometer. The different regions of the film were directly mounted in the light path of the spectrometer for scanning.
Formation of Chitosan–PVA hydrogel

Various amounts of PVA (Cat No 34158-4, Aldrich Chemical, Milwaukee, WI) with a hydrolytic degree greater than 99 % and a $M_w$ in the range of 89–98 kDa were added into the 1 % chitosan solution to obtain chitosan/PVA molar ratios of 1/0, 1/5 and 1/10. The mixture was kept at 80 °C for 5 min till the PVA completely dissolved and formed a clear blend. During heating the container was covered with a polyethylene film to prevent evaporation. The blend was cooled to 25 °C and glutaraldehyde (25 % v/v, Cat No G400-4, Aldrich Chemical, Milwaukee, WI) was slowly added under constant stirring. The final concentration of glutaraldehyde in the pregel solution varied from 3 to 25 µM to 83.2 µM, which roughly equals an amine to aldehyde mole ratio of 2.3 to 0.46. The pregel solution in a cylindrical mold (diameter = 1 cm; height = 3 cm) for 12 h at 25 °C, as to stay within the linear limit of the gel system during the entire gelation process. The storage modulus ($G'_s$), loss modulus ($G''_s$), and phase angle ($\delta = \tan^{-1} (G''/G')$) were recorded as a function of time till $\delta \approx 0$ (ie $G' \gg G''$), which is considered to signify the completion of the gelation process.

Mechanical properties of chitosan–PVA hydrogel

Samples for this test were prepared by gelling the pregel solution in a cylindrical mold (diameter = 1 cm; height = 3 cm) for 12 h at 25 °C with a final glutaraldehyde concentration of 33.3 µM in the hydrogel gel. The compressive strength of the hydrogel samples was determined using a uniaxial compression testing device equipped with a 50 N load cell (Synergie 200, MTS Microsystems, Eden Prairie, MN). After 12 h of preparation and swelling in pH 3 buffer for 24 h, the hydrogels samples were tested until they fractured. The data acquisition rate was 20 Hz, the surface detection load was 0.0196 N, and the cross-head speed was 1 mm s$^{-1}$.

Leaching of PVA from chitosan–PVA hydrogel

The chitosan–PVA hydrogels with chitosan/PVA molar ratios of 1/0, 1/4, 1/7, 1/10 and 1/15 were prepared as previously described. The final glutaraldehyde concentration was 33.3 µM in all hydrogels. Hydrogel (2.5 g) was placed in a 10 ml of pH 3 buffer solution for swelling in 50-ml beakers covered with parafilm to prevent evaporation. At various time intervals, the relative viscosity of the swelling solution was measured using an Ostwald–Fensky viscometer at 25 °C. To eliminate any possible error, the swelling medium for the chitosan–PVA molar ratio 1/0 the hydrogel was used as the reference for determining the relative viscosity. The relative viscosity (repeated three times) of the swelling media was used to quantify the extent of PVA leached using a linear relationship between relative viscosity of PVA solution ($\eta_r$) versus solution PVA concentration ($C_{PVA}$, mg (100 ml)$^{-1}$) with an $R^2$ of 0.9973:

$$\eta_r = 0.0096 C_{PVA} + 0.9851 \quad (1)$$

RESULTS AND DISCUSSION

Molecular weight and degree of deacetylation of chitosan

Molecular weight is one of the key indices governing the functional properties of chitosan. As has been well documented, viscometry is the simplest and the most effective method for determining $M_w$ of polymers.$^{11,12}$ For a linear chain polymer the relationship between intrinsic viscosity, $[\eta]$ and $M_w$, is shown by the Mark–Houwink equation:$^{13,14}$

$$[\eta] = k M_w^a \quad (2)$$

where, $k$ and $a$ are constants independent of $M_w$ over a wide range. They are affected by solvent conditions such as temperature, pH and ionic strength. For chitosan in Solvent A, $k$ and $a$ values have been reported as 1.81 $\times$ 10$^{-3}$ and 0.93 at 25 °C, respectively.$^{8,15}$ The intrinsic viscosity of chitosan was experimentally determined using the intercept of reduced viscosity versus concentration plot.$^3$ This value was 436 ml g$^{-1}$, and $M_w$ of the chitosan was estimated as 612 kDa through Eqn. (2). The $M_w$ of natural chitin is usually larger than 1000 kDa, while that of commercial chitosan products are between 100 kDa and 1200 kDa. This is because the harsh deacetylation processes used on chitin result in the degradation of chitin and chitosan molecules.

The degree of deacetylation (DD) is another key factor affecting the functional properties of chitosan, which is indicated as a percentage of primary amino group in total glycosaminoglycans after the chemical or enzymatic N-deacetylation of chitin amide bonds.
High DD usually means good acid solubility, high reaction activity and high heavy metal ion chelating ability. This is because the primary amino groups of chitosan are relatively active and are readily available for many chemical modifications, such as salt formation and chelation. Infrared spectroscopy is one of the methods used to quantify the DD of chitosan. This is performed on the basis of the characteristic wavenumbers of $1650 \text{ cm}^{-1}$ for the amide I peak and $3450 \text{ cm}^{-1}$ for the primary amine $-\text{NH}_2$. The DD of the chitosan used was estimated to be around 73% using the FTIR spectroscopy.

**IR spectra of chitosan, chitosan–PVA film and hydrogel**

The IR spectrum of chitosan (Fig 1) shows peaks around $905 \text{ cm}^{-1}$ and $1157 \text{ cm}^{-1}$ corresponding to saccharide structure. In spite of several peaks clustering in the amide II peak range from $1515 \text{ cm}^{-1}$ to $1570 \text{ cm}^{-1}$, there still were absorption peaks at $1650 \text{ cm}^{-1}$ and $1322 \text{ cm}^{-1}$, which are characteristic of chitin and chitosan and have been reported as amide I and III peaks, respectively. The sharp peaks at $1383 \text{ cm}^{-1}$ and $1420 \text{ cm}^{-1}$ were assigned to the $\text{CH}_3$ symmetrical deformation mode. The broad peak at $1083 \text{ cm}^{-1}$ indicates the $\text{C—O}$ stretching vibration in chitosan. Another broad peak at $3450 \text{ cm}^{-1}$ is caused by amine $\text{N—H}$ symmetrical vibration, which is used with $1650 \text{ cm}^{-1}$ for quantitative analysis of deacetylation of chitosan. Peaks at $2800 \text{ cm}^{-1}$ and $2900 \text{ cm}^{-1}$ are the typical $\text{C—H}$ stretch vibrations.

The IR spectrum of the chitosan–PVA film (Fig 2) is different than that of the chitosan because of the ionization of the primary amino groups in the chitosan–PVA films. There are two distinguishing peaks at $1408 \text{ cm}^{-1}$ and $1548–1560 \text{ cm}^{-1}$. Formation of the $1548–1560 \text{ cm}^{-1}$ peak is the symmetric deformation of $-\text{NH}_3^+$ resulting from ionization of primary amino groups in the acidic medium whereas the peak at $1408 \text{ cm}^{-1}$ indicates the presence of carboxylic acid in the polymers. The peaks at $1700–1725 \text{ cm}^{-1}$ are characteristic of the carboxylic acid dimer. In our samples, the presence of carboxylic dimer was due to the acetic acid used for dissolving the chitosan. The peak at $1267 \text{ cm}^{-1}$ is due to the $\text{C—H}$ vibration.

A significant peak can be found at $\sim 1643 \text{ cm}^{-1}$ (Fig 3) corresponding to the formation of imine bond ($\text{C=}=\text{N}$), ie Schiff’s base structure by the reaction of amino groups of chitosan and aldehyde groups of glutaraldehyde. When the hydrogel was swollen at pH 3, this peak was observed for up to 3 days, then it gradually diminished with time. This suggested the instability of Schiff’s base ($\text{C=}=\text{N}$ bond) and possible hydrolysis under acidic conditions. The characteristic peaks between $1548$ and $1560 \text{ cm}^{-1}$ indicated the presence of $-\text{NH}_3^+$ groups in the swollen hydrogel. One important reason for the swelling is the formation of $-\text{NH}_3^+$ groups in the hydrogel at the environmental medium (pH 3) below the acid dissociation constant ($pK_a$) which is 6.3.

![Figure 1. FTIR spectrum of the chitosan (see Table 3 for the peak assignments).](image)

![Figure 2. FTIR spectrum of the chitosan-PVA film (see Table 3 for the peak assignments).](image)

![Figure 3. FTIR spectrum of the Schiff’s base (C= N at 1643 cm⁻¹) of the chitosan–PVA hydrogel during swelling in the pH 3 buffer (see Table 3 for the peak assignments).](image)
for amino groups of chitosan.22 The ionic —NH$_3^+$ groups formed cause the migration of counter ions into the hydrogel, thus changing the osmotic pressure of the network and inducing water transfer from the exterior buffer solution, resulting in the swelling of the hydrogel. Another significant peak at 1700 cm$^{-1}$ indicates the presence of carboxylic acid.

As the hydrogel contracted at pH 7, the characteristic peak of —NH$_3^+$ at 1548 cm$^{-1}$ diminished (Fig 4), indicating the transition of —NH$_3^+$ to —NH$_2$ under neutral or alkaline conditions. The C=N bond of the Schiff’s base structure (at 1643 cm$^{-1}$) was still present in the hydrogel which is assumed to be the major covalent bond maintaining the 3-D structure of the hydrogel. A significant peak at 1573 cm$^{-1}$ was also observed, especially as the contraction time increased. Because of the instability of the imine bond (C=N) of the Schiff’s structure, the hydrogen could undergo structural changes depending on reactant structure, temperature, and pH of the solution, especially when hydrolyzed at high pH.23 The appearance of the new peak at 1573 cm$^{-1}$ indicates the structure transition from C=N to C—N. This transformation is more likely to occur under alkali condition of the surrounding media.20 Compared with the swelling of the hydrogel in pH 3, there was no such a transition took place at pH 7. In addition, the contraction of the hydrogel in pH 7, with characteristic peaks at 1700 cm$^{-1}$ and 1715 cm$^{-1}$ of carboxylic acid dimer diminished gradually.

The acetal and hemiacetal products of possible cross-linking reaction between the PVA and glutaraldehyde were also examined. The characteristic peaks of these products should be strongly located at 1140–1190 cm$^{-1}$, 1060–1100 cm$^{-1}$ and 1035–1060 cm$^{-1},21$ but these could not be identified in all the hydrogel samples. This suggests that the cross-linking reaction may occur only between the chitosan molecules. The mechanism of this cross-linking reaction is schematically illustrated in Fig 5. The nucleophilic nitrogen of the amino group (—NH$_2$) attacks the carbon of the aldehyde, which displaces the oxygen of the aldehyde and results in the loss of one water molecule, thus forming the C=N bond, the Schiff’s base. When the pH of the reaction environment is neutral or alkaline, it facilitates the formation of the —NH$_2$ of chitosan (pKa = 6.3), which is essential for the formation of Schiff’s base. In an acidic environment, however, —NH$_3^+$ is more likely to form, which significantly decreases the nucleophilicity of nitrogen, thereby lowering the activity of the Schiff’s base reaction.

The main FTIR peak assignments for the chitosan, chitosan film and chitosan hydrogel are summarized in Table 1.

**Gelation of chitosan–PVA pregel solution**

The cross-linking reaction among the —NH$_2$ groups is regarded as the typical Schiff’s base reaction, which is believed to be dominant in giving the three-dimensional structure of the hydrogel.24 The typical evolution of $G'$ and $G''$ of the gel as function of time are presented in Fig 6. The $G'$ increases substantially after a specific time, indicating the rapid formation of a 3-D gel structure. The point at which the discontinuity on the $G'$ versus time curve is observed is often referred to as gelation time ($t_{gel}$) which is normally the time at which $G'$ versus time, and $G''$ versus time curves cross over.25 The $t_{gel}$ values for all gelation conditions are listed in Table 2. These values decreased substantially with an increase in cross-linker concentration and the PVA composition in the gel.

Since gels evolve continually over a long time, it is rather difficult to pinpoint the specific time for complete gelation ($t_{com}$) from the $G'$ versus time curve. For this purpose, we presented the evolution of phase angle versus time during gelation (Fig 7). As gelation progresses, the phase angle decreases and eventually reaches plateau value. The time needed to reach the onset of the phase angle plateau value may
Table 1. Main IR peak assignments for the chitosan and its derivatives (see Reference 21 for assignment details)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak position (cm(^{-1}))</th>
<th>Peak type</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan, chitosan film</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic saccharide</td>
<td>905</td>
<td>Medium</td>
<td>Aliphatic aldehydes</td>
</tr>
<tr>
<td>structure</td>
<td>1153–1158</td>
<td>Strong</td>
<td>Primary or secondary alcohol</td>
</tr>
<tr>
<td>Characteristic primary</td>
<td>1580–1650</td>
<td>Strong</td>
<td>NH(_2) deformation vibrations</td>
</tr>
<tr>
<td>amine</td>
<td>3450</td>
<td>Board</td>
<td>NH(_2) symmetric stretching vibrations</td>
</tr>
<tr>
<td>Characteristic chitin</td>
<td>1322–1325</td>
<td>Weak</td>
<td>Amide III: OH and CH deformation</td>
</tr>
<tr>
<td>(secondary amide)</td>
<td>1515–1570</td>
<td>Medium</td>
<td>Amide II: N(\ldots) H deformation and C—N stretching vibration</td>
</tr>
<tr>
<td></td>
<td>(1554)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1649–1655</td>
<td>Strong</td>
<td>Amide I: C=O stretch vibration</td>
</tr>
<tr>
<td>Other characteristic peaks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1083</td>
<td>Strong</td>
<td>C—O stretching vibration</td>
</tr>
<tr>
<td></td>
<td>1263–1267</td>
<td>Weak</td>
<td>CH wag (ring) vibration</td>
</tr>
<tr>
<td></td>
<td>1377–1383</td>
<td>Strong</td>
<td>CH(_3) deformation (bend) vibration</td>
</tr>
<tr>
<td></td>
<td>1420</td>
<td>Medium</td>
<td>CH and CH deformation (ring)</td>
</tr>
<tr>
<td></td>
<td>1700–1725</td>
<td>Strong</td>
<td>Aliphatic carboxylic acid dimer</td>
</tr>
<tr>
<td></td>
<td>2800–2900</td>
<td>Weak</td>
<td>C—H stretch vibration</td>
</tr>
<tr>
<td></td>
<td>3200–3600</td>
<td>Strong</td>
<td>OH stretch vibration</td>
</tr>
<tr>
<td></td>
<td>1409</td>
<td>Strong</td>
<td>COOH C—O stretch and O—H deformation</td>
</tr>
<tr>
<td>Chitosan hydrogel</td>
<td></td>
<td>Weak</td>
<td>NH(_3)+ deformation vibration</td>
</tr>
<tr>
<td></td>
<td>1510–1570</td>
<td>Medium</td>
<td>NH(_3)+ deformation vibration</td>
</tr>
<tr>
<td></td>
<td>1573</td>
<td></td>
<td>Symmetric and asymmetric deformation</td>
</tr>
<tr>
<td></td>
<td>1640–1690</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1643</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1500–1625</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1548, 1560</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Progress of the elastic modulus (\(G'\)) and the viscous modulus (\(G''\)) during the hydrogel formation (chitosan/PVA molar ratio = 1/10, glutaraldehyde concentration = 16.6 \(\mu\)M).

be considered a relative indicator of the \(t_{com}\). This can be graphically determined by charting the time corresponding to the point of intersection between two tangents drawn to the plateau region and pre-plateau region on the curve. The effect of the PVA and glutaraldehyde concentration on \(t_{com}\) is summarized in Table 2. As noted previously, the \(t_{gel}\) and \(t_{com}\) values also decreased with increasing cross-linker concentration and PVA content, although it was most dramatic at lower cross-linker concentrations. Thus, the addition of glutaraldehyde and PVA contribute both to the initiation and to the completion of gel formation.

<table>
<thead>
<tr>
<th>Glutaraldehyde concentration ((\mu)M)</th>
<th>(t_{gel}) (min)</th>
<th>(t_{com}) (min)</th>
<th>(t_{gel}) (min)</th>
<th>(t_{com}) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/1</td>
<td>1/5</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.6</td>
<td>320</td>
<td>825</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
<td>74</td>
<td>186</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>58.8</td>
<td>22</td>
<td>48</td>
<td>23</td>
</tr>
</tbody>
</table>

The acetal and hemiacetal reaction among the —OH of the PVA, as well as the reaction between the —NH\(_2\) of the chitosan and the —OH of the PVA was not confirmed by FTIR, as evidenced by the lack of observed peaks on the FTIR spectrum. Thus, chitosan is believed to be essential for the formation of 3-D structure. The PVA has proven to have a synergistic effect on the network by essentially improving the network density. This has been verified both rheological data reported and drug release kinetics. Thus, PVA addition helps to significantly
decrease the amount of the cross-linker needed for hydrogel formation.

**Mechanical properties of the chitosan-PVA hydrogel**

Fracture tests for different chitosan–PVA hydrogels before and after swelling exhibited significant differences. The compression fracture stress, toughness (area under the stress–time curve till fracture) and fracture strain of hydrogels decreased after swelling compared with their unswollen counterparts (Fig 8A and B). This is due to the significant increase in water content of the hydrogel after swelling. However, fracture stress and toughness of both the unswollen and swollen hydrogels tended to increase with PVA content in the gel (Table 3).

**Leaching of PVA from the chitosan-PVA hydrogel**

If a significant degree of cross-linking occurs between the chitosan and the PVA, the PVA and the PVA, or if the PVA molecules are large enough or no hydrolysis of Schiff’s base formed takes place, the relative viscosity of the swelling medium is not supposed to change with time. The percentage of PVA leached out of the

<p>| Table 3. Fracture test results for the chitosan–PVA hydrogel$^{a}$ |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Chitosan/PVA molar ratio</th>
<th>State$^{b}$</th>
<th>Fracture stress (kPa)</th>
<th>Peak modulus (kPa)</th>
<th>Toughness (MJ m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/0</td>
<td>Unswollen</td>
<td>81.08 ± 11.17</td>
<td>23.62 ± 3.96</td>
<td>5.46 ± 0.11</td>
</tr>
<tr>
<td>1/0</td>
<td>Swollen</td>
<td>57.99 ± 16.77</td>
<td>18.73 ± 6.23</td>
<td>0.63 ± 0.08</td>
</tr>
<tr>
<td>1/4</td>
<td>Unswollen</td>
<td>87.82 ± 25.93</td>
<td>27.51 ± 7.67</td>
<td>7.62 ± 0.34</td>
</tr>
<tr>
<td>1/4</td>
<td>Swollen</td>
<td>63.01 ± 17.29</td>
<td>23.47 ± 3.01</td>
<td>0.63 ± 0.08</td>
</tr>
<tr>
<td>1/7</td>
<td>Unswollen</td>
<td>134.00 ± 30.95</td>
<td>41.18 ± 11.74</td>
<td>9.44 ± 0.77</td>
</tr>
<tr>
<td>1/7</td>
<td>Swollen</td>
<td>73.23 ± 25.99</td>
<td>31.35 ± 9.41</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>1/10</td>
<td>Unswollen</td>
<td>147.33 ± 46.31</td>
<td>46.08 ± 16.63</td>
<td>11.88 ± 0.58</td>
</tr>
<tr>
<td>1/10</td>
<td>Swollen</td>
<td>91.48 ± 25.59</td>
<td>40.59 ± 6.35</td>
<td>1.11 ± 0.14</td>
</tr>
<tr>
<td>1/15</td>
<td>Unswollen</td>
<td>196.82 ± 56.75</td>
<td>66.49 ± 25.30</td>
<td>13.34 ± 0.86</td>
</tr>
<tr>
<td>1/15</td>
<td>Swollen</td>
<td>100.81 ± 18.90</td>
<td>43.83 ± 8.43</td>
<td>1.76 ± 0.11</td>
</tr>
</tbody>
</table>

$^{a}$Glutaraldehyde concentration = 33.3 µM.
$^{b}$The gels were swollen at pH 3.
hydrogel as a function of time is presented in Fig 9. The extent of PVA leaching was calculated according to eqn (1). In all chitosan–PVA hydrogels the PVA leached out. The extent of PVA leaching increased considerably with time, reaching about 25 % after 120h in pH 3 buffer. This suggests that the PVA molecules are only physically entangled inside the chitosan networks, but are free to leave the hydrogel if any significant changes in the network take place, such as the hydrolysis of Schiff’s base of the hydrogel. This agrees with the FTIR spectrum analysis of the pH 3 swollen gels (Fig 3). There is no obvious effect of the chitosan/PVA molar ratio on the rate and extent of PVA leaching during swelling of the hydrogel.

We acknowledge that $M_c$ distribution of PVA and $M_w$ between PVA–chitosan crosslinks could affect the leaching of PVA. Furthermore, the interaction between PVA and glutaraldehyde could also contribute to leaching of PVA by altering the overall $M_c$ of PVA in the network. These effects are yet to be investigated.

CONCLUSIONS

The formation of chitosan–PVA semi-interpenetrating pH-sensitive hydrogel is due to the Schiff’s base reaction between amino groups of chitosan, the base polymer and aldehyde groups of glutaraldehyde, the cross-linker. The addition of PVA decreased the gelation rate and time for complete gelation, and increased the mechanical strength of the hydrogel, especially at low cross-linker concentrations. Increasing the cross-linker concentration decreased the gelation rate and the complete gelation time of the hydrogel. Up to about 25 % concentration, the PVA leached out from the hydrogel at pH 3 at long swelling times due to hydrolysis of the Schiff’s base. Thus, the lower mechanical strength of the swollen hydrogel is due not only to increased water content but also to loss of PVA from its network.

REFERENCES