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Rheological characterisation of a novel thermosensitive chitosan/poly(vinyl alcohol) blend hydrogel

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Abstract

Thermosensitive hydrogels that are triggered by changes in environmental temperature thus resulting in in situ hydrogel formation have recently attracted the attention of many investigators for biomedical applications. In the current work, the thermosensitive hydrogel was prepared through the mixture of chitosan (CS), poly(vinyl alcohol) (PVA) and sodium bicarbonate. The mixture was liquid aqueous solutions at low temperature (about 4 °C), but a gel under physiological conditions. The hydrogel was characterized by FTIR, swelling and rheological analysis. The effect of hydrogel composition and temperature on both the gel process and the gel strength was investigated from which possible hydrogel formation mechanisms were inferred. In addition, the hydrogel interior morphology as well as porosity of structure was evaluated by scanning electron microscopy (SEM). The potential of the hydrogels as vehicles for delivering bovine serum albumin (BSA) were also examined. In this study, the physically crosslinked chitosan/PVA gel was prepared under mild conditions without organic solvent, high temperature or harsh pH. The viscoelastic properties, as investigated rheologically, indicate that the gel had good mechanical strength. The gel formed implants *in situ* in response to temperature change, from low temperature (about 4 °C) to body temperature, which was very suitable for local and sustained delivery of proteins, cell encapsulation and tissue engineering.

Keywords: Chitosan; Poly(vinyl alcohol); Hydrogel; Thermosensitivity

1. Introduction

Hydrogels that are hydrophilic three-dimensional polymeric networks capable of absorbing large quantities of water have become increasingly important in the biomedical field. One of the recent trends in hydrogel research is *in situ*-forming systems for various biomedical applications, including drug delivery (Gariepy, Chenite, Chaput, Guirguis, & Leroux, 2000; Hsiue, Chang, Wang, & Lee, 2003) and tissue engineering (Anseth et al., 2002; Shu, Liu, Palumbo, Luo, & Prestwich, 2004). *In situ*-forming systems are liquid aqueous solutions before administration, but gel under physiological conditions. There are several possible mechanisms that lead to in situ gel formation (Gariepy & Leroux, 2004): solvent exchange, UV-irradiation, ionic cross-linkage, pH change, and temperature modulation. These approaches, which do not require organic solvents, copolymerization agents, or an externally applied trigger for gelation, have gained increasing attention, such as a thermosensitive approach for in situ hydrogel formation (Jeong, Kim, & Baeb, 2002). The temperature responsive hydrogel, which is triggered by changes in environmental temperature thus resulting in in situ hydrogel formation, has caused the interest of many investigators for biomedical applications.

Recently, polysaccharides have been extensively studied for the development of thermosensitive *in situ*-forming hydrogel systems because they are suitably biodegradable, a quality not possessed by most synthetic polymers. Chitosan, a polysaccharide derived from naturally abundant chitin, is currently receiving a great deal of interest. Chenite,

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Chaput, Wang, Combes, and Buschmann (2000) developed a novel approach to produce thermosensitive neutral hydrogel based on chitosan/polyol salt combinations that could undergo sol–gel transition at a temperature close to 37 °C. Other researchers also evaluated the hydrogel for use in pharmaceutical applications (Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002; Gariepy et al., 2004) and cartilage repair (Hoemann et al., 2001, 2003). Many modified chitosan copolymers also have thermosensitive characteristics. Bhattaraim, Ramay, Gunn, Matsen, and Zhang (2005) found the aqueous solution of the PEG-grafted chitosan, which is an injectable liquid at low temperature and transforms to a semisolid hydrogel at body temperature, has a broad range of medical applications, particularly for sustained *in vivo* drug release and tissue engineering. Dang et al. (2006) have shown that a 3.8% w/w hydroxybutyl chitosan solution forms a gel within 60 s when it is exposed to a 37 °C environment, which indicates the potential of hydroxybutyl chitosan gel as an injectable carrier for future applications of delivering therapeutics to encourage a biologically relevant reconstruction of the degenerated disk. Chung, Bae, Park, Lee, and Park (2005) prepared two kinds of water soluble thermosensitive chitosan copolymers by graft polymerization of *N*-isopropylacrylamide and by coupling monocarboxy Pluronic® with chitosan. The resulting copolymers formed thermally reversible hydrogels with a lower critical solution temperature of 34 °C, and they could be used as injectable cell-polymer complexes.

Poly(vinyl alcohol) (PVA), a water-soluble polyhydroxy polymer, has been frequently explored as implant material for drug delivery systems and surgical repairs because of its excellent mechanical strength, biocompatibility and nontoxicity (Martien, 1986). Therefore, it is promising to blend chitosan with PVA to produce a new biosynthetic polymer applicable for a variety of purposes. Koyanoa, Koshizakib, Umeharab, Nagurac, and Minourab (2000) and Minoura et al. (1998) prepared a blend of chitosan with PVA to study the surface properties and elucidate the relationship between these properties and the cell attachment/growth behavior. Many researchers explored such blends to determine the properties changes in response to the external stimuli such as pH change (Wang, Turhan, & Gunasekaran, 2004) and the electrical charges (Kim, Park, Kim, Shin, & K, 2002). In addition, a blend of chitosan with PVA has been evaluated as a drug delivery agent (Kurkuri & Aminabhavi, 2004; Sugimoto, Yoshida, Yata, Higaki, & Kimura, 1998).

In the previous study, the introduction of PVA to make blend fibers of chitosan and PVA improved tensile strength and water retention properties of the blend formations compared to those of pure chitosan (Zheng, Du, Yu, Huang, & Zhang, 2001). These properties are interesting for further study of the properties of blends of chitosan and PVA. Though some blends of PVA with chitosan have been prepared, there are scarcely any reports about the thermosensitive hydrogel nature of chitosan and PVA blends. This study aimed to develop an injectable and thermosensitive system based upon the blend of chitosan and PVA, and which can serve as a therapeutic drug-delivery system promoting tissue repair and regeneration through controlled release of loaded drugs. As Gariepy and Leroux (2004) reported, the system will be a solution that is a injectable liquid at ambient temperature and gel at body temperature. Moreover, loading with drugs or cells should be achieved by simple mixing. When administered parenterally, the system should exhibit a pH close to neutrality and should be biodegradable.

2. Materials and methods

2.1. Materials

Chitosan was obtained from Yuhuan Ocean Biochemistry Co. (Zhejiang, China). The DD as determined by elemental analysis was 92%, and the molecular weight calculated from the GPC method was about 2.7×10^5. Standard pullulans for GPC were purchased from Showa Denko, Tokyo, Japan. Poly(vinyl alcohol) with an average degree of polymerization of 2400–2500 were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). All other chemicals were of analytical grade.

2.2. Preparation of chitosan/PVA hydrogel

A clear solution of chitosan was obtained by dissolving chitosan (200 mg) in 0.1 M HCl (10 mL) and chilled in an ice bath for 15 min. PVA was added to deionized water and heated at 80 °C for 1 h to make solutions containing 2, 5 and 10% w/w PVA. 1.0 M $NaHCO_3$ (1 mL), and 2% w/w PVA solution (10 mL) were mixed and similarly chilled for 15 min. Then the PVA was slowly added to the chitosan solution in an ice bath under magnetic stirring for 10 min. The solution was degassed by centrifugation of samples at 3500*g* for 3 min at 5 °C. The gel was then formed in 30 min by keeping it at 37 °C.

2.3. Characterization of the gel by FTIR

FTIR spectra were recorded on an FTIR spectrometer (Nicolet, Model Impact 410, WI) at room temperature. Chitosan, PVA and the dried gel were triturated with KBr in the ratio of 1:100 and pressed to form pellet samples 400–4000 cm^{-1}.

2.4. Rheological measurement

The rheological properties were performed on a strain-controlled ARES rheometer (TA. Inc., New Castle, USA). The rheometer was equipped with two sensitive force transducers for torque measurements ranging from 0.004 to 100 g cm. A Couette (two concentric cylinders) cell geometry was used for monitoring the steady-state shear flow and dynamic rheology of the samples modulus. The rheometer is equipped with a thermo-bath with circulating

water that was calibrated to give a temperature in the sample chamber within ±0.5 °C of the set value. The degassed solution (10 mL) was heated or cooled to desired temperature directly in the rheometer (without shearing or oscillating) and then covered with mineral oil in order to prevent evaporation during the measurements. For the frequency and time sweep measurements, it was defined as time $t = 0$ s when the temperature reaches the desired temperature. The sweep of the frequency was from 0.1 to 200 rad/s, and each frequency sweep took 900 s to be completed. For the temperature sweep measurements to determine the gelation temperature, oscillatory measurements were performed at 1 Hz, while the temperature was increased at the rate of 1 °C /min between 5 and 60 °C. So the dynamic viscoelastic functions such as the dynamic shear storage modulus (G′) and loss modulus (G″) were measured as a function of time, temperature or angular frequency. The values of the strain amplitude were checked to ensure that all measurements were carried out within the linear viscoelastic regime, where the G′ and G″ are independent of the strain amplitude according to the result of dynamic strain sweep.

2.5. Morphological studies

Scanning electron microscopy was performed on hydrogels (freeze-dried to maintain the porous structure without any collapse) to obtain information on the pore structure of hydrogels. Then they were plunged in liquid nitrogen and the vitrified samples were cut with a cold knife. The samples were mounted on the base plate and coated with gold. The morphology was investigated using a Hitachi (Tokyo, Japan) S-570 Scanning Electron Microscope.

2.6. Drug load and release

Using a drug model (BSA) dissolved in PVA solution, the 'drug'-loaded hydrogels were fabricated following the same procedure as described in the preparation of chitosan/PVA hydrogel. The BSA-loaded hydrogels were immersed in 0.1M sodium phosphate pH 7.4, containing 0.145M NaCl [phosphate buffered saline (PBS), 100 mL] in a conical flask and incubated at 37 °C in a thermostated shaker rotating at 100 rpm. Aliquots (5 mL) of this solution were taken out at regular intervals and the release of BSA from them was estimated by UV spectrophotometry at 280 nm. With each sampling, the solution was changed with fresh medium, maintaining the total volume constant. The percentage of cumulative amount of released BSA was determined from standard calibration curves. The blank hydrogel was measured at the same way as comparable sample. All experiments were repeated in three times.

2.7. Swelling properties

To measure the equilibrium water content (EWC), the freeze-dried samples, which have different contents of PVA and CS, (for 2% w/w, 5% w/w and 10% w/w PVA, the preweighed samples were about 0.44, 0.86 and 1.32 g, respectively when the CS content was 2% w/w) were immersed in distilled water (50 ml) at 20 °C. After excess surface water had been removed with filter paper, the weight of swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase to i.e. equilibrium swelling of the samples had been reached. EWC was determined according to the following equation:

$$\mathrm{EWC}\ (\%) = ((W_s - W_d)/W_d) \times 100,$$

where W_s and W_d represent the weight of swollen and dry states samples, respectively.

3. Results and discussion

3.1. Gel formation

Chitosan is a pH-dependent cationic polymer. When the pH value of chitosan solution is below its pK_a (pH < 6.2), chitosan is water-soluble and positively charged. The free amino groups (–NH_2) are protonated as –NH_3^+, which causes electrostatic repulsion between the protonated amino group. Then chitosan–NH_3^+ is considered as a weak acid and the addition of a weak base ($NaHCO_3$) will obviously increase the pH. At the same time, the basification can also reduce the electrostatic repulsion between chitosan molecules, which subsequently allows for extensive hydrogen bonding and hydrophobic interactions between chains, and eventually leads to the formation of a white-like precipitate above pH 6.2. In the present study, the precipitates could be prevented by the addition of PVA and the hydrogel will be formed by adjusting the environment temperature from 4 to 37 °C. When the PVA and $NaHCO_3$ were added to the chitosan solution, the temperature was controlled at about 4 °C in ice bath. Then the mixed solution was placed at 37 °C, the gel was formed after 30 min. The high temperature can reduce the intermolecular hydrogen bonding interactions and accelerate the mobility of CS molecules. So the energized water molecules surrounding the CS chains are removed. The dewatered hydrophobic chitosan chains associate with each other. As a result, a gel is formed.

The mechanism of the thermosensitive sol–gel transition for the chitosan/PVA blend system is illustrated in Fig. 1. As known, this hydroxy polymer can stabilize certain compounds and promote the formation of a shield of water around some macromolecules in aqueous solutions (Back, Oakenfull, & Smith, 1979). Therefore, when the mixture solution of PVA and $NaHCO_3$ is added at low temperature, hydrogen bonds exist not only between the OH group of PVA and the OH and NH_2 groups of chitosan but also between PVA and water due to the high hydrophilicity of PVA, which can lead to the dissolution of chitosan chains (Fig. 1a). At the same time, the low temperature can also reduce the mobility of chitosan molecules, which further prevents the association of chitosan chains. It is thus a

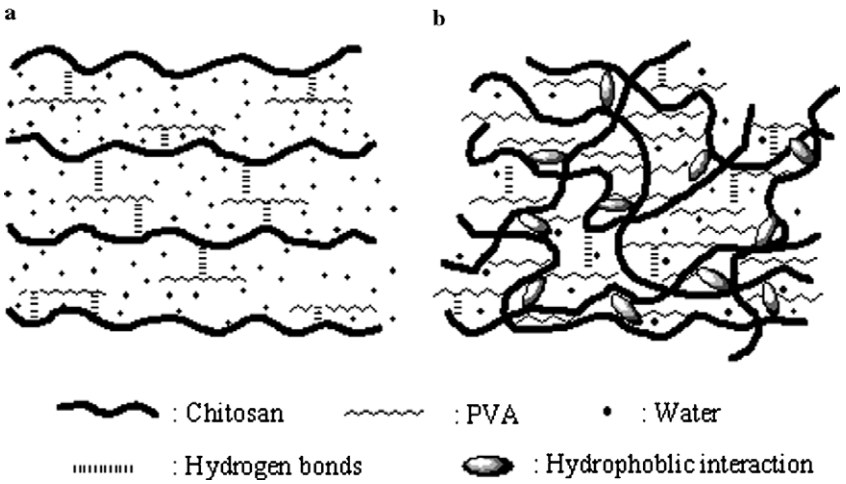

Fig. 1. Formation mechanism of chitosan/PVA gel (a) solution at low temperature; (b) gel at high temperature.

poor conformation or shape to build up a 3D network structure because of the difficulty of creating contacts between the junction chains. When temperature is elevated, the intermolecular hydrogen bonding interactions are reduced and the energized water molecules surrounding the polymer are removed. The dewatered hydrophobic chitosan chains associate with each other. As a result, a gel is formed (Fig. 1b). This type of thermosensitive gelation has also been observed in other cases (Bhattaraim et al., 2005; Chenite et al., 2000).Therefore, hydrophobic interactions are assumed to be the main driving force to form the gel consisting of chitosan and PVA at high temperature. In the experiment, the blend solution of CS and PVA was homogeneous, transparent and flowing at 4 °C in ice bath. Then the mixed solution was placed at 37 °C, the gel was formed as a transition from the solution to a semi-transparent elastomer after 30 min.

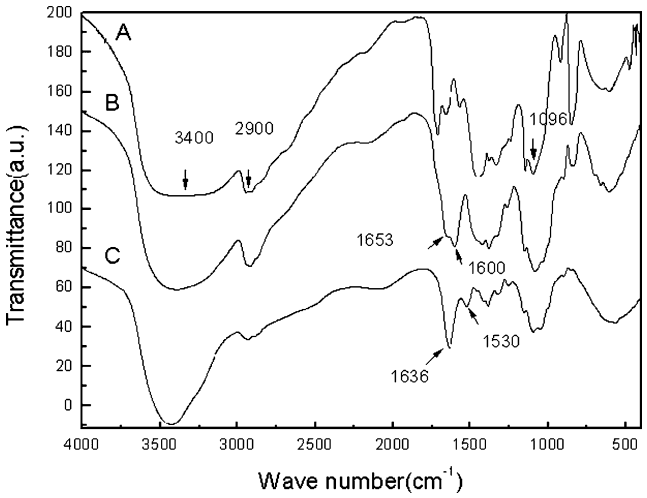

Fig. 2. FTIR spectra of PVA (A), 1% w/w CS/PVA gel (B) and CS-HCl (C).

3.2. FTIR analysis

An FTIR spectrum of pure PVA (Fig. 2 curve A), shows –OH stretching at about 3400 cm^{-1}, –CH stretching as in alkanes at 2900 cm^{-1} and –C–O stretching at about 1096 cm^{-1}. In the spectrum of freeze-dried chitosan–HCl (curve C), peaks at the 1200–1000 cm^{-1} region are assigned to polysaccharide structure. The sharp peaks at 1383 and 1423 cm^{-1} are due to the $–CH_3$ symmetrical deformation mode. The absorption band of chitosan at 1636 and 1530 cm^{-1} reflects the interaction between the amino groups and the HCl. Peaks at 2900 cm^{-1} are due to the C–H stretch vibrations. The broad peaks at 3400 cm^{-1} are caused by amine N–H symmetrical vibration. In the FTIR spectrum of hydrogels (curve B), the characteristic absorption bands of chitosan at about 1653 (AmideI) and 1600 cm^{-1} ($–NH_2$ bending) appear again, indicating the transition of $–NH_3^+$ to $–NH_2$ when $NaHCO_3$ is added to the chitosan–HCl solution. The reaction between chitosan and PVA is not confirmed by FTIR, as evidenced by the lack of observed peaks on the FTIR spectrum. Thus, chitosan chains are believed to be essential for the formation of 3D structure and the PVA molecules are only physically entangled inside the chitosan networks. The PVA has proven to have a synergistic effect on the network by essentially improving the network density. This result has been verified by SEM.

3.3. Rheological analysis

The dynamic mechanical characterization is useful for understanding the formation mechanism of the hydrogels and consequently their possible applications. Samples are first subjected to a strain sweep test to define the linear viscoelastic region in which the modulus G′ and G″ are independent of the applied strain. In our experiment, the strain amplitude was set as 20%.

3.3.1. Gel temperature

Dynamic mechanical experiments are carried out as a function of temperature. Fig. 3 shows the temperature dependence of the storage modulus G′ and the loss modu-

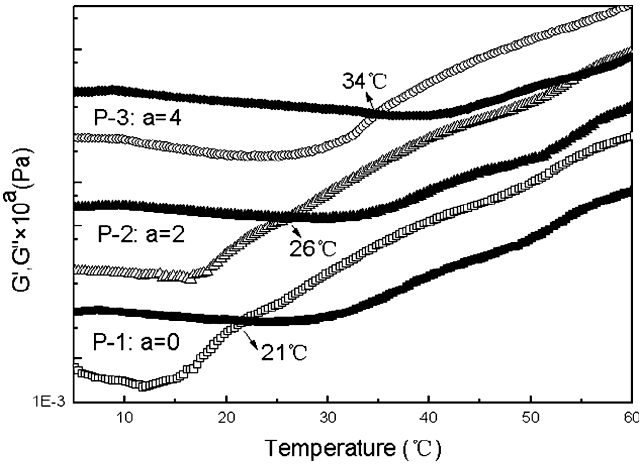

Fig. 3. Temperature dependence of storage modulus G′ and loss modulus G″ of chitosan/PVA aqueous solution with different content at a heating rate of 1 °C/min and at a frequency of 1 rad/s. Open and closed symbols denote G′ and G″, respectively. For P-1, P-2 and P-3, the chitosan content is 1% w/w and the PVA concentration is 1, 2.5 and 5% w/w, respectively. The data is shifted along vertical axes by 10^a with given a values to avoid overlapping.

lus G″ for the P-1, P-2 and P-3 chitosan/PVA solutions at a frequency of 1 rad/s. The temperature was increased from 5 to 60 °C at a rate of 1 °C/min. The storage modulus G′ reflects the solid-like component of the rheological behaviour, which is thus low at solution stage but increases drastically at the gelation temperature. Gelation temperature is usually defined as the sol/gel transition temperature at which G′ is equal to G″. The thermosensitive property of gels is evaluated by it. For the 1% w/w PVA (P-1) solution, the gel temperature is about 21.0 °C, so the curves are divided into two parts. The first region is below 21 °C, where G′ is lower than G″ and decreases as the temperature increases, showing the common viscoelastic behavior of a liquid. Subsequently, the sharp increase in G′ is considered as a result of the partial formation of chitosan clusters through hydrophobic interaction. However, when the temperature is very close to the gel temperature 21 °C, a few chitosan chains become formed into clusters. But these clusters are too slight to form a completely mature gel network. In the second region, G′ and G″ increases dramatically with an increase of temperature and G′ is higher than G″, indicating that an elastic gel network has formed. The curves of 2.5% w/w (P-2) and 5% w/w PVA (P-3) solutions shows a similar feature, but the gelation temperatures are elevated, which are 26 and 34.0 ° C for P-2 and P-3, respectively. This indicates that the increase of PVA content results in the increase of the gelation temperature, which is corresponding to the gelation mechanism. The higher PVA content can produce the more hydrogen-bonding interactions, which needs the higher gelation temperature to reduce the hydrogen bonds and promote the gel formation.

3.3.2. Gel time

Gelation time is defined as the time when the storage modulus become higher than the loss modulus. It reflects the changes of G′ and G″ during the gelation process, indicating the gelation speed and the gel intensity. There are many factors that have effects on gel time for chitosan/PVA hydrogel, such as environment temperature, and chitosan and PVA contents.

In the study of the influence of temperature on the gelation process for 1% w/w chitosan/PVA solution at a fixed frequency of 1 rad/s, Fig. 4 shows the gel temperature is about 21 °C. Therefore, the gel is only formed in seconds when the experiment temperature is 20 °C which is close to the gel temperature. The gel forms very quickly when the experiment temperature is elevated to 40 or 60 °C. The higher the temperatures, the faster the formation speed of the gel. During the gelation process, both G′ and G″ increase gradually and eventually reach the onset of a plateau, in which G′ is largely higher than G″. This indicates that the elastic response of the material is stronger than the viscous response. The present hydrogel system displays a predominantly solid-like behavior. As known, the storage modulus can be considered as a measure of the extent of gel network formation. The higher G′ value of the gel means the stronger gel intensity. Cabane, Lindell, Engstrom, and Lindman (1996) have explained that raising the temperature causes some macromolecules or some sections of macromolecules to dump into lumps of a polymer-rich phase, where there are always interactions of hydrophobic groups.

The influence of CS or PVA concentration on the gelation process, as shown in Figs. 5 and 6, indicates that an increase of chitosan content from 0.5% to 1% w/w at 40 °C, the gel time decreases and gel intensity is enhanced. However, for an increase of PVA content from 1% to 5% w/w at 40 °C, the phenomenon is the reverse. These results are consistent with the gel mechanism. The chitosan is responsible for the hydrophobic interactions at high temperature. When its content is increased, the entanglements among chitosan chains are heightened leading to the

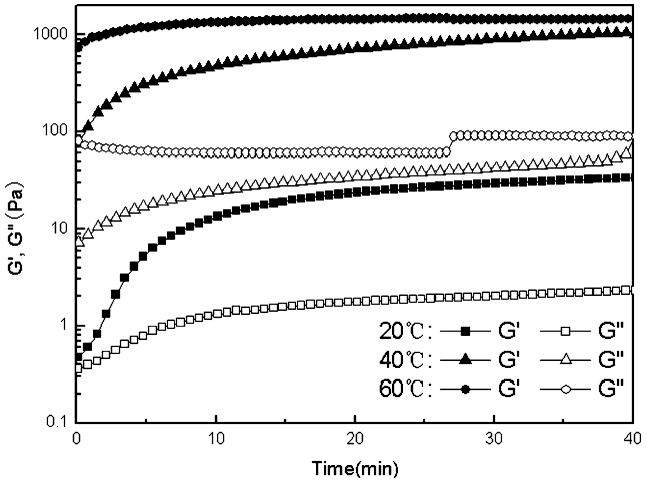

Fig. 4. Time dependence of storage modulus G′ and loss modulus G″ of 1% w/w CS/PVA solution for different temperature at a frequency of 1 rad/s. Closed and open symbols denote G′ and G″, respectively.

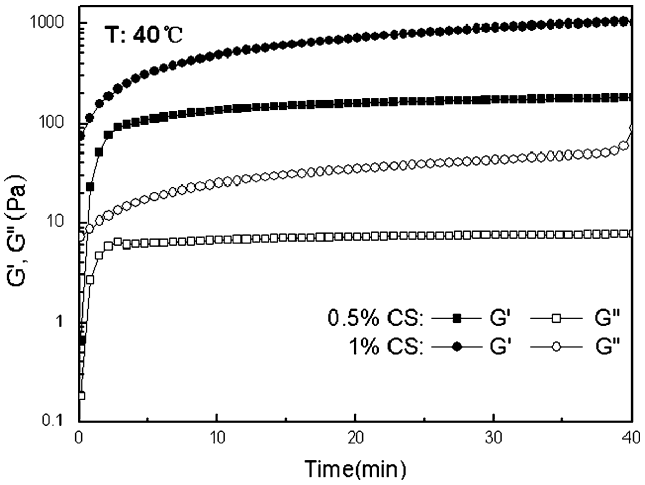

Fig. 5. Time dependence of storage modulus G′ and loss modulus G″ of CS/PVA solution for different content of CS at 40 °C and at a frequency of 1 rad/s. Closed and open symbols denote G′ and G″, respectively. The PVA content is 1% w/w.

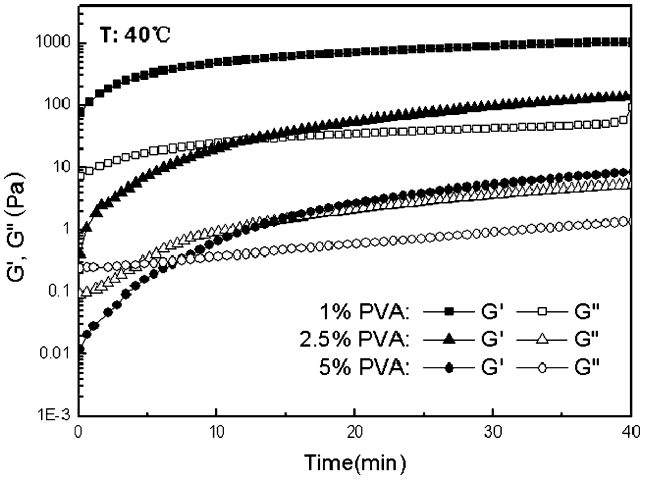

Fig. 6. Time dependence of storage modulus G′ and loss modulus G″ of CS/PVA solution for different concentration of PVA at 40 °C and at a frequency of 1 rad/s. Closed and open symbols denote G′ and G″, respectively. The CS content is 1% w/w.

increase on the gel intensity. The PVA content is related to the hydrogen-bonding interactions at low temperature. The higher PVA content, the longer gel time is needed to produce enough energy in order to reduce the hydrogen bonds and promote the gel formation at the stated temperature.

3.3.3. Gel frequency

The frequency dependence of the viscoelastic properties of P-1, P-2 and P-3 hydrogels is revealed at different temperatures in Fig. 7. For each sample, the G′ and G″ are measured as a function of the frequency from 0.1 to 200 Hz. The data are shifted along vertical axes by 10^a with given a value to avoid overlapping.

The different viscoelastic properties of three hydrogels at different temperatures might be attributed to the different gelation temperatures (Fig. 3). When the ambient

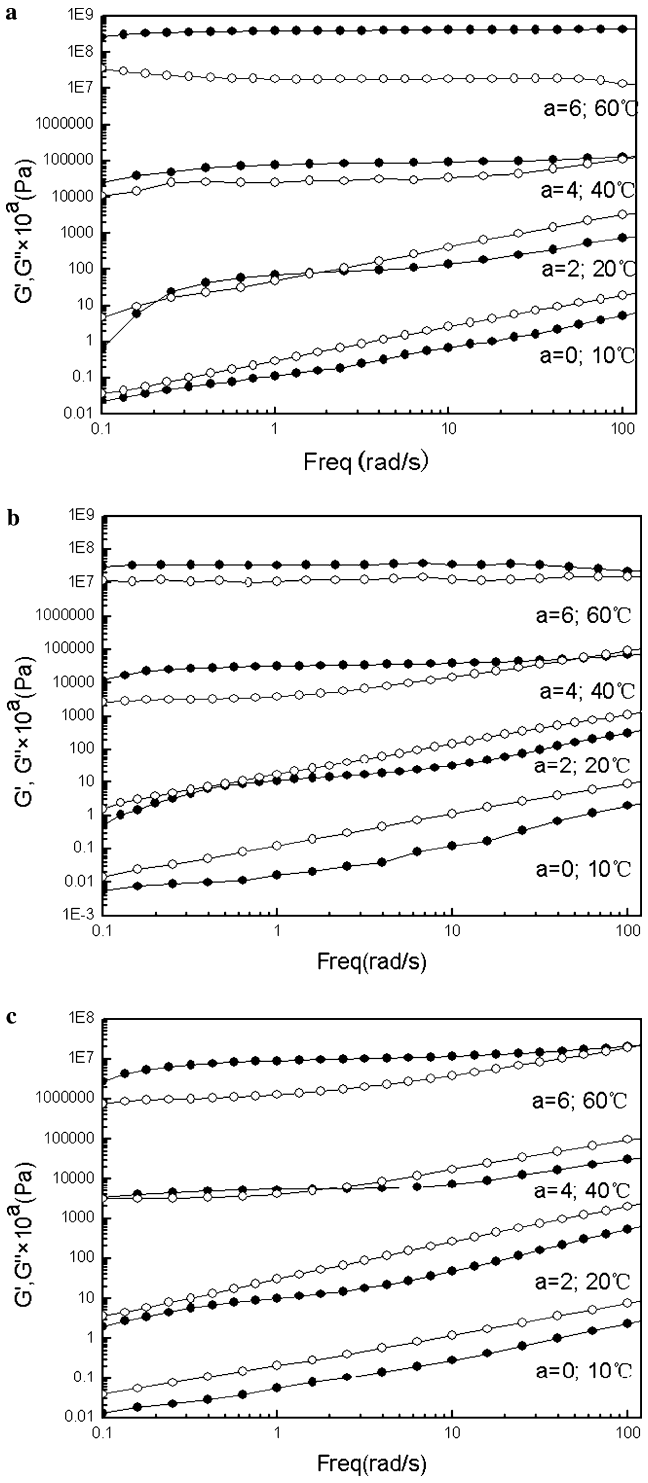

Fig. 7. Frequency dependence of storage modulus G′ and loss modulus G″ of 1% w/w CS/PVA aqueous solutions at different temperature. The data is shifted along vertical axes by 10^a with given a value to avoid overlapping.

temperature is largely lower than the gel temperature, the loss modulus G″ is higher than the storage modulus G′ within the frequency range. Those features are characteristic of the stable viscous liquid. With an increase of the temperature close to the gel temperature, the difference

between G′ and G″ becomes small, that is close to gelation. Furthermore, when the ambient temperature is equal to and even slightly higher than the gel temperature, G′ is gradually increased to exceed G″. It means the gel has been formed. However, the further increase in frequency causes a significant decrease in storage modulus, which is indicative of structure break by mechanical shear. The storage modulus becomes lower than the loss modulus and the system behaves like a solution. Their rheological behavior is typical of a ''weak gel'', which has the frequency dependence. At last, when the ambient temperature is obviously higher than the gel temperature, G′ is always higher than G″ over the whole frequency range. It is evident that the storage modulus G′ shows almost no dependence with frequency. These features are characteristic of a ''strong gel''.

3.4. Scanning electron microscopy

The effect of PVA content on the morphology of CS/PVA hydrogels as examined by SEM is shown in Fig. 8(a–d). The SEM micrographs clearly illustrate the dependence of hydrogel morphology on the PVA content. Firstly, the gel networks are not so evident when PVA content is very low (0.2% w/w), as shown in Fig. 8a. Furthermore, for Fig. 8b–d, the PVA contents are 0.5%, 1% and 5% w/w, respectively. These show that the pore structures of the resulting gels dramatically change from macroporous to microporous with an increase of PVA content, the porous structure becoming more compact and the pore size smaller. This is mainly explained by the entanglings between PVA and chitosan being enhanced with the increase of PVA content. For samples of 1% and 5% w/w PVA content, the SEM micrographs are further magnified to clearly show microstructure in Fig. 8c and d. The chitosan concentration is 1% w/w in all samples.

3.5. Swelling experiments

In the results of swelling experiments shown in Fig. 9, the pattern obtained points to a limited but very rapid

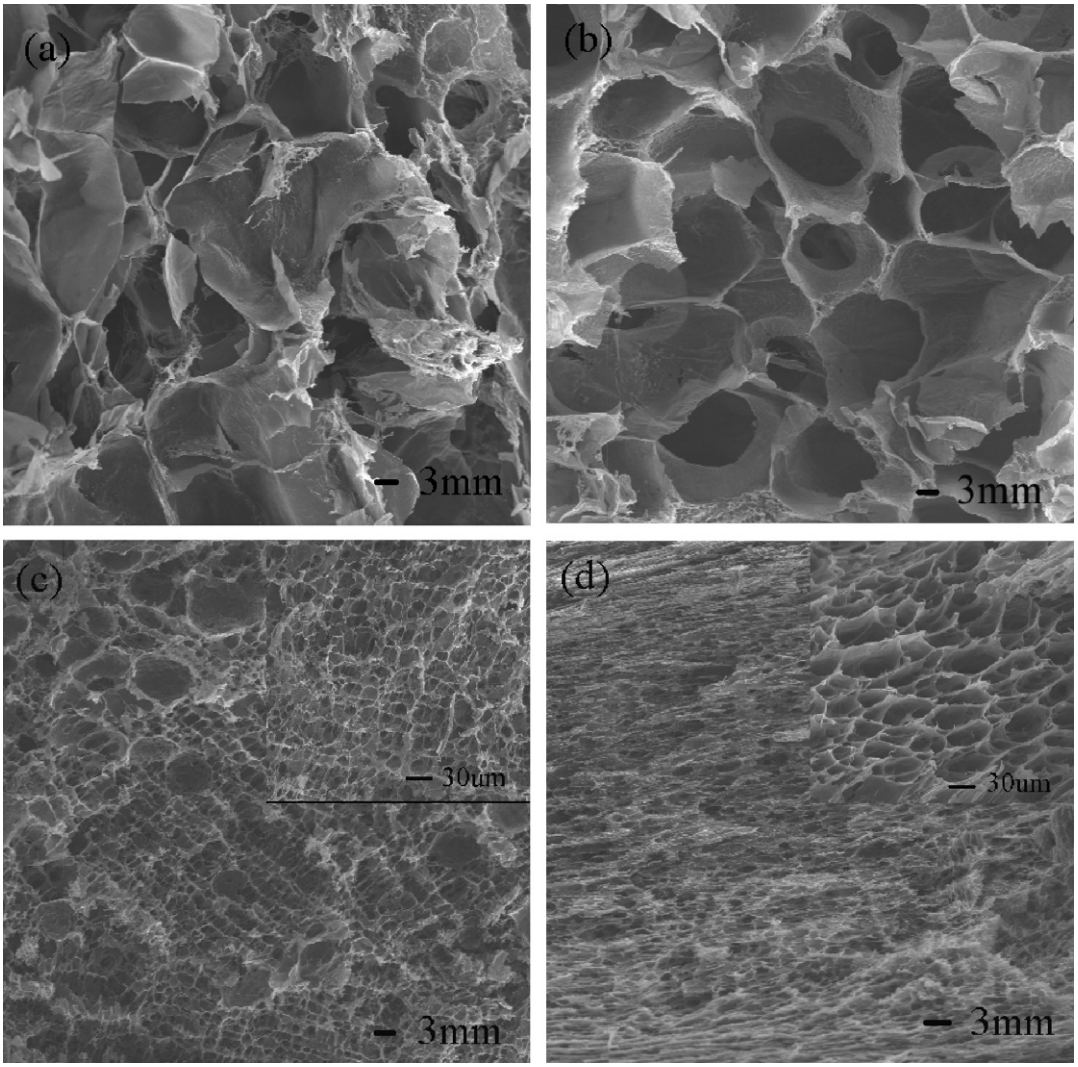

Fig. 8. SEM of different content CS/PVA gel [CS – 1% w/w; PVA – (a) 0.2% w/w, (b) 0.5% w/w; (c) 1% w/w, (d) 5% w/w].

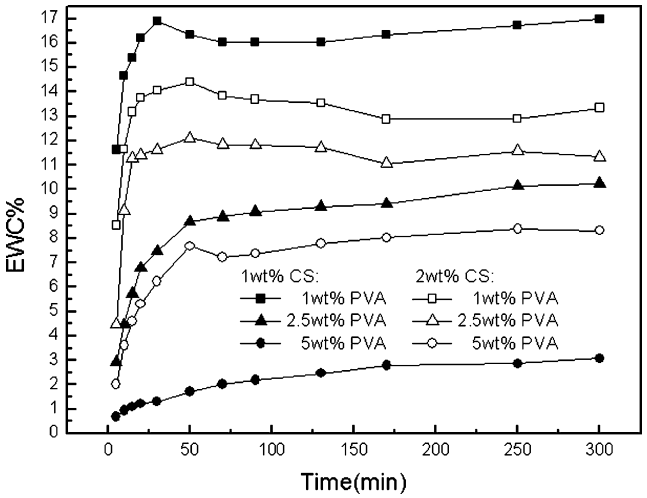

Fig. 9. Swelling ratio of different content CS/PVA gel in distilled water at 20 °C.

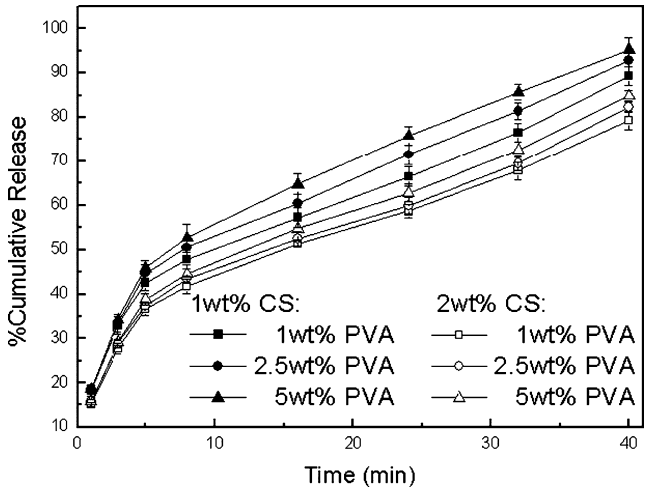

Fig. 10. Drug delivery from different content CS/PVA gel at pH 7.4 in phosphate buffered saline at 37 °C.

uptake of water by the gel during the initial 20 min. Visual inspection of the samples also shows no appreciable volume increase. For samples 1a, 1b and 1c, the chitosan content is 1% w/w and the PVA contents are 1%, 2.5% and 5% w/w, respectively. For samples 2a, 2b and 2c, the chitosan content is elevated to 2% w/w and the PVA contents are also 1%, 2.5% and 5% w/w, respectively.

The swelling ratio decreases with the increase of PVA content when the chitosan content is the same. This is mainly due to the fact that the higher PVA content results in the more compacted gel structure, which is unfavorable to the swelling rate. However, when the PVA content is the same, the swelling ratio is different. For example, the swelling rate of 1a is higher than that of 2a, but the swelling rate of 1b is lower than that of 2b and the swelling rate of 1c is also lower than that of 2c. These results are corresponding to the gel mechanism. The PVA chains are only physically entangled inside the chitosan networks. When the PVA content is largely higher than CS, it is easy for PVA to leak from the gel, which results in the lower swelling rate.

3.6. Drug delivery

With BSA as a model protein, cumulative release of BSA from the thermosensitive hydrogel is shown in Fig. 10. The release profiles exhibited a fast release rate in the first 5 h, followed by a virtually linear release over 40 h period. The BSA release rate reduced with an increase of the chitosan content. It is mainly because that the higher chitosan content can maintain the stronger gel structure at the same gel temperature and time, as shown in Fig. 4. However, the release rate is slightly elevated with an increase of the PVA content. According to the rheological and swelling experiments, the higher PVA content resulted in the weaker gel and the faster PVA leakage from the hydrogel. Therefore, the BSA was easily released due to the collapse of gel framework.

4. Conclusions

An injectable, thermoreversible hydrogel was formed by hydrogen bonds between PVA and chitosan chains and hydrophobic interactions of chitosan chains. The hydrogel is liquid in aqueous solutions at low temperature (about 4 °C), but gel under physiological conditions, at which bioactive species can be safely and uniformly incorporated. Since all the component materials involved have been proven to be biocompatible, the copolymer hydrogel is potentially suited for a wide range of *in vivo* biomedical applications, such as drug release, tissue repair and regeneration.

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