Preparation of Chitosan-Based Thermosensitive Hydrogels for Drug Delivery

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ABSTRACT: The thermosensitive material that could be transformed into gel at 37°C was prepared from chitosan (dissolved in acetic acid/sodium acetate buffer solution) and a mixture of α- and β-glycerophosphate (αβ-GP). The thermosensitive characteristics, appearance, and structure of the hydrogel were all affected by the pH, ionic strength, and CS/αβ-GP ratio. The optimal conditions for the preparation of a transparent CS-αβ-GP thermosensitive hydrogel were pH 4.6, ionic strength 0.15 mol/L, and a CS/αβ-GP ratio of 8.8/1.2 (v/v). The hydrogel was stable for at least 3 months at 4°C. We believe that hydrogen bonding interactions between the *N*—H (and C=O) groups of chitosan and the O—H groups of αβ-GP play an important role during the process of sol-to-gel transition. The cumulative release of adriamycin from the CS-αβ-GP hydrogel, measured in PBS at pH 7.4, reached only 60 to 70% over 24 h, indicating that this material could be potentially used in a sustained drug delivery system. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 1509–1515, 2009

Key words: chitosan; αβ-GP; thermosensitive hydrogel; gelation; drug delivery system

INTRODUCTION

Chitosan is a polysaccharide composing of glucosamine and can be derived by partial deacetylation of chitin, which is obtained from crustacean shells, some microorganisms, and fungi such as yeast. Chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of controlled release of drug delivery systems because of its excellent biocompatibility, biodegradability, bioactivity, and nontoxicity.[1,2] Various sustained release drug carriers have been made from chitosan such as microparticles,[3,4] tablets,[5,6] gel,[7,8] and beads.[9,10]

Chitosan thermosensitive hydrogels prepared with different methods were of great interest in drug delivery,[11] cell encapsulation,[12] tissue engineering,[13] and so on. Dang et al.[12] described thermosensitive hydroxybutyl chitosan as a biomaterial for cell encapsulation and culture. Cho et al.[13] reported a chitosan gel prepared by grafting chitosan with Poly(*N*-isopropylacrylamide) in which human mesenchymal stem cells (MSCs) could differentiate into chondrocytes, and the combination (chondrogenic differentiated cells from MSCs with a thermosensitive polymer) could be used as an injectable cell-polymer complex. Bhattarai et al.[14] described PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release, which could be prepared in solutions with a physiological pH, allowing the safe incorporation of bioactive molecules for a broad range of medical applications, particularly for sustained drug release and tissue engineering. Chung et al.[15] reported water-soluble thermosensitive chitosan copolymers, which prepared by coupling Pluronic onto chitosan using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide as coupling agents and attested to the usefulness as an injectable material for cell and drug delivery.

Chitosan solutions containing β-glycerophosphate (β-GP) that has temperature-controlled solution-gel transition at a temperature close to 37°C had recently been proposed as a suitable vehicle for the extravascular parenteral administration of drugs.[16–19] Berger et al.[20] reported that the deacetylation degree of chitosan might modulate the turbidity of chitosan/β-GP hydrogel. Crompton et al.[21,22] described the morphology and gelation of thermosensitive chitosan/β-GP hydrogels and examined the procedure of injecting the hydrogel to the brain to form a gel

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TABLE I
Characteristics of CS-αβ-GP Hydrogel Prepared at Different Conditions

	Different preparation conditions			Characteristics of CS-αβ-GP thermosensitive hydrogel		
Symbol	Ionic strength (mol/L)	CS/αβ-GP	pH value	pH values	OD values	Viscosity (Pa·S)
A	0.20	9.0/1.0	3.81	4.44 ± 0.02	0.041 ± 0.002	0.742 ± 0.022
B	0.20	9.0/1.0	4.20	4.93 ± 0.01	0.046 ± 0.003	0.919 ± 0.032
C	0.20	9.0/1.0	4.41	5.25 ± 0.02	0.045 ± 0.002	1.180 ± 0.034
D	0.20	9.0/1.0	4.59	5.48 ± 0.01	0.049 ± 0.004	1.925 ± 0.045
E	0.20	9.0/1.0	4.80	5.77 ± 0.03	0.138 ± 0.008	18.55 ± 0.102
F	0.05	9.0/1.0	4.60	6.13 ± 0.03	2.154 ± 0.028	2.078 ± 0.033
G	0.10	9.0/1.0	4.59	5.84 ± 0.02	0.342 ± 0.002	3.138 ± 0.045
H	0.15	9.0/1.0	4.62	5.70 ± 0.01	0.084 ± 0.004	3.352 ± 0.043
I	0.20	9.0/1.0	4.61	5.53 ± 0.02	0.054 ± 0.002	3.127 ± 0.048
J	0.25	9.0/1.0	4.60	5.49 ± 0.01	0.053 ± 0.003	1.069 ± 0.024
K	0.15	9.5/0.5	4.61	5.24 ± 0.03	0.044 ± 0.002	2.307 ± 0.035
L	0.15	9.2/0.8	4.60	5.29 ± 0.01	0.041 ± 0.003	4.117 ± 0.043
M	0.15	9.0/1.0	4.59	5.54 ± 0.01	0.064 ± 0.004	2.147 ± 0.043
N	0.15	8.8/1.2	4.62	5.59 ± 0.02	0.040 ± 0.001	1.261 ± 0.023
O	0.15	8.5/1.5	4.58	5.88 ± 0.01	0.152 ± 0.015	2.598 ± 0.033

Data shown are the mean ± SD values, $N = 3$.

track. Cho et al.[23] demonstrated the characterized key physicochemical and rheological properties of chitosan/β-GP systems as a function of temperature. Jarry et al.[24,25] studied the effects of steam sterilization and γ-irradiation on chitosan and thermogelling chitosan-β-GP solution.

However, there were no reports on the preparation and influence of preparation conditions of chitosan-αβ-glycerophosphate (CS-αβ-GP) thermosensitive hydrogel. The αβ-glycerophosphate (αβ-GP) is a mixture of β-glycerophosphate and α-glycerophosphate while α-glycerophosphate has linear chain structure. Wu et al.[26] had reported a thermosensitive hydrogel of quaternized chitosan and αβ-GP and concluded that αβ-GP had better gelation capacity compared with β-GP. So, it is necessary to study the preparation and characteristics of CS-αβ-GP hydrogel.

In this article, the thermosensitive hydrogel of CS-αβ-GP was prepared with chitosan and αβ-GP by dissolving chitosan in acetic acid/sodium acetate buffer solution. The effect of different conditions on the characteristics and sol-to-gel transition of hydrogel were investigated. In addition, the effect of the CS-αβ-GP hydrogels on sustained release was studied. Adriamycin was chosen as a model drug because it is a chemotherapy drug, which is mainly used in the treatment of breast cancer, ovarian cancer, lung cancer, and so on.

MATERIALS AND METHODS

Materials

Chitosan, derived from crab shell, molecular weight: 1360 KD; deacetylation degree 75%, prepared in our laboratory. αβ-glycerophosphate (αβ-GP), acetic acid glacial, and sodium acetate were all chemical reagents of analytical grade provided by Sigma (St. Louis, MO). Adriamycin was purchased from Zhejiang Hisun Pharmaceutical.

Preparation of CS-αβ-GP thermosensitive hydrogel

Chitosan (2 g) was added into the acetic acid/sodium acetate buffer solution (100 mL) in given pH values (seen in Table I) with stirring until it dissolved thoroughly. Then, the chitosan solution was chilled in ice bath for 20 min. A 50% (w/v) αβ-GP aqueous solution was prepared in distilled water and chilled along with the chitosan solution in ice bath. Predetermined value of αβ-GP solution was added dropwise to the chitosan solution with stirring, and the final chitosan-αβ-GP solution was mixed for another 20 min. Finally, the CS-αβ-GP thermosensitive hydrogel was obtained and stored at 4°C or room temperature (25°C). Different pH values, ionic strength, and ratio of chitosan to αβ-GP (CS/αβ-GP, v/v) were chosen to prepare CS-αβ-GP thermosensitive hydrogel, which were shown in Table I. Furthermore, CS-αβ-GP thermosensitive hydrogel loaded adriamycin was made with the same method. Adriamycin was added into chitosan solution with agitating until it was dissolved thoroughly.

Characterization of the CS-αβ-GP thermosensitive hydrogel

The pH value, turbidity, and viscosity of CS-αβ-GP thermosensitive hydrogel were measured with pH meter (Delta pH meter, Mettler Toledo), UV-vis spectroscopy (Tu-1800 uv-vis spectrophotometer, Beijing Purkinje General Instrument) at 600 nm, and

viscometer (NDJ-8S digital viscometer' Scientific instrument of Shanghai, China), respectively.

Sol-to-gel study of CS-αβ-GP thermosensitive hydrogel

A simple test tube inverting method was employed to determine the occurrence of sol-to-gel transition.[27,28] The sol phase was defined as flowing liquid and the gel phase as nonflowing gel when the hydrogel solution in the test tube was inverted. CS-αβ-GP thermosensitive hydrogel (3 mL) was added into 10 mL tube to study sol-to-gel transition characteristics in a water bath of 37°C ± 0.5°C. At predetermined interval, the tube was taken out and inverted to observe the state of the sample. The gelation point was determined by flow or no-flow criterion over 30 s with the test tube inverted.

Measurement of viscosities of different samples

Sol-to-gel behavior of CS-αβ-GP thermosensitive hydrogel was further studied by measuring the solution viscosity of the samples. Viscosities of CS-αβ-GP thermosensitive hydrogels were measured as a function of time and temperature using a viscometer (NDJ-8S). For the measurement of viscosity, the 3rd rotator was selected, and rotation speed was 1.5 rpm. Fifty milliliters of CS-αβ-GP hydrogel was added into a vessel, and the 3rd rotator was immerged into the hydrogel. Then, the vessel was put into a water bath or ice-water bath, which could be adjusted into different temperatures (37, 25 or 15°C). At predetermined time intervals, the viscosity of hydrogel was measured and recorded.

Scanning electron microscopy analysis

Samples (5 mL, in Cryogenic Vials) of hydrogel were incubated in water bath under the same conditions used for the sol-to-gel studies. When the hydrogel was transited into gel, the gel was frozen in liquid nitrogen and freeze-dried for 48 h (Christ Alpha 1-4 Freeze Dryer, Germany). Samples without being heated were also frozen in liquid nitrogen and freeze-dried for 48 h. The samples were coated with gold under vacuum, and the surfaces were investigated with a scanning electron microscopy (KYKY2800B, KYKY Technology Development, China).

FTIR spectrometry

Samples of CS-αβ-GP thermosensitive gel were prepared by the same method as microscopy analysis. The infrared spectra of chitosan, αβ-GP, and CS-αβ-GP thermosensitive gel were recorded on an FT/IR-430 Fourier transform infrared spectrometer (Jasco Co. Tokyo, Japan) based on the method of Shigemasa et al.[29] A pellet was formed from 2 mg sample and 100 mg of KBr.

Stability of CS-αβ-GP thermosensitive hydrogel

The CS-αβ-GP thermosensitive hydrogels were stored at 4 and 25°C, respectively. At different times, the pH value, turbidity, and viscosity of hydrogels were measured, respectively. According to the variation of these criterions, the stability of hydrogel was evaluated.

Evaluation of *in vitro* adriamycin release

The CS-αβ-GP thermosensitive hydrogel with or without model drug (adriamycin), 1 mL, were placed into dialysis membrane with a molecular weight cut-off of 8000–15,000, respectively.[30] One milliliter of adriamycin solution (0.2%, w/v) was prepared just as sample to measure dissociative drug release. The dialysis membranes were placed in 100 mL phosphate buffer solutions (PBS, pH 7.4) in Erlenmeyer flasks (250 mL). The Erlenmeyer flasks were closed with plastic membrane. The buffer solutions were stirred continuously at 160 rpm in a vibrating incubator at 37°C ± 0.5°C. The experiment was repeated in triplicate.

At predetermined time intervals, samples of 4 mL were taken out of the solution (stored at 4°C until analysis) and replaced by equal volume of PBS to maintain a constant volume. The samples were assayed by spectrophotometry at 233 nm with the sample of blank CS-αβ-GP hydrogel as control. The results were compared to evaluate the release rate of adriamycin. The standard curve of adriamycin was achieved in PBS (0.2 mol/L, pH 7.4).

RESULTS AND DISCUSSION

Characteristics of the CS-αβ-GP thermosensitive hydrogel

Characteristics of the CS-αβ-GP thermosensitive hydrogels prepared with different pH values, ionic strength, and ratio of CS/αβ-GP were shown in Table I. The pH values of hydrogel increased obviously with the increase of pH values of initial chitosan solution as seen in Table I. However, Table I showed that the pH values of hydrogel decreased slightly with the increase of ionic strength and the ratio of CS/αβ-GP. There was an abrupt change in turbidity when pH value of chitosan solution increased to 4.8, ionic strength decreased to 0.10*M*, and the ratio of CS/αβ-GP changed to 8.5/1.5, respectively. Viscosity of CS-αβ-GP hydrogel changed slightly and irregularly in all experiment formulations.

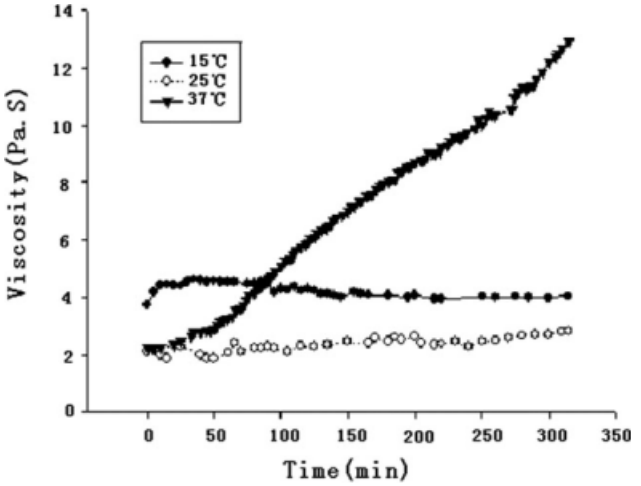

Figure 1 Viscosity of CS-αβ-GP hydrogel (sample D) as a function of time at different temperatures.

Thermosensitive gelation behavior

The CS-αβ-GP hydrogel underwent an apparent sol-to-gel transition at 37°C. Below the transition temperature of 37°C, the solutions were flowable viscous liquids and were injectable through a syringe. As the solution was heated to 37°C, it transformed into gel that was nonflowing.

The formed gel of CS-αβ-GP hydrogel prepared with chitosan and αβ-GP were transparent, whereas the gel of chitosan and β-GP was white and turbid as described in previous reports.[18,26] However, the transparent hydrogel is suitable for some specific uses such as ocular drug formulation. The hydrogel made from chitosan solution of pH 4.6, ionic strength 0.15 mol/L, and CS/αβ-GP ratio of 8.8/1.2 had the typical sol-to-gel transition time of about 10 min.

Sol-to-gel transition behavior of CS-αβ-GP hydrogel was further illustrated by rheological analysis. Figure 1 was the viscosity variety of hydrogel at different temperatures. Figure 1 showed that the viscosity of hydrogel at 37°C increased obviously and quickly with the incubated time lasting, however, that of hydrogel at 15 or 25°C was almost maintained the same as that of the beginning. Furthermore, Figure 1 showed that the viscosity of hydrogel at 15°C was a little higher than that at 25°C.

Figure 2 showed the variation of the viscosity as a function of time at temperature of 37°C. Figures 2(A–C) were the viscosity of hydrogel prepared with different pH values of chitosan solution, different ionic strength, and different ratio of CS/αβ-GP, respectively. Figure 2(A) showed that the viscosity of hydrogel made from pH 4.6 increased quickly with the time lasting, and others were increased slowly even maintained the same as that of beginning. So, the increase of pH values of chitosan solution was benefit to sol-to-gel transition. However, higher pH value was not practical because a small quantity of white precipitation appeared in the CS-

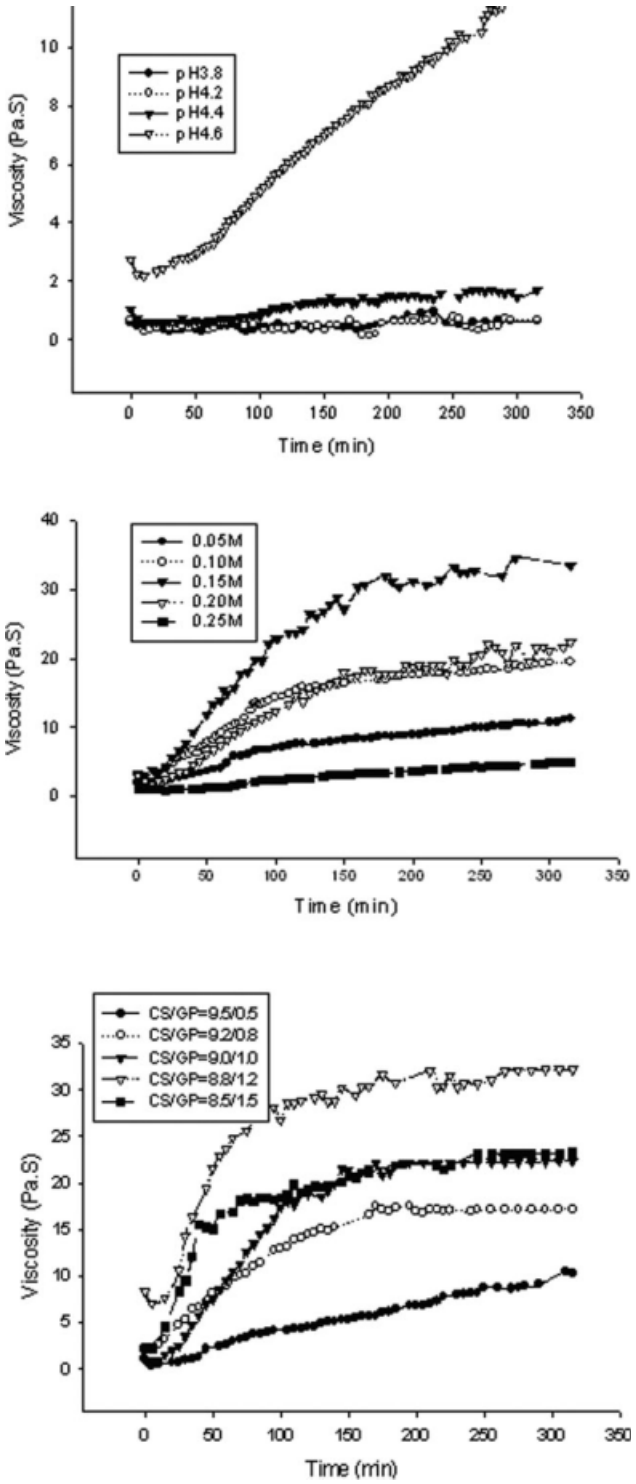

Figure 2 Viscosity of CS-αβ-GP hydrogel as a function of time at given temperature (37°C) (A) hydrogel prepared with different pH values of chitosan solution; (B) hydrogel prepared with different ionic strength of chitosan solution; and (C) hydrogel prepared with different ratios of CS/αβ-GP.

αβ-GP hydrogel when pH values increased to 4.8, which could be seen from the variety of turbidity of different samples (Seen in Table I).

It was shown in Figure 2(B) that the viscosity of hydrogel increased with ionic strength increasing from 0.05 to 0.15 mol/L and then decreased with it further increasing from 0.15 to 0.25 mol/L as a function of time at 37°C. The hydrogel prepared with higher or lower ionic strength all showed slower increase in viscosity with time lasting compared with that of hydrogel prepared with ionic strength of 0.15 mol/L. So, the optimal condition of ionic strength to form gelation was 0.15 mol/L, which was chosen for the following study.

The ratio of CS/αβ-GP affected the sol-to-gel transition of hydrogel as the same of ionic strength, which could be seen in Figure 2(C). The higher or lower of the CS/αβ-GP ratio were disadvantageous to the form of gelation. The optimal ratio of CS/αβ-GP was 8.8/1.2. It could be explained that the interactions of chitosan amino groups, phosphate moiety of αβ-GP, and ions in the solutions were changed with the variety of formulation, and there were optimal values to keep the hydrogel flowing at lower temperature and transfer to gel at 37°C.

The pH, ionic strength, CS/αβ-GP ratio, and temperature significantly affect the thermosensitive characteristics of CS-αβ-GP hydrogel. They affect the broad range of molecular interactions, which could occur in aqueous solutions of the cationic polyelectrolyte chitosan and the divalent anionic base glycerol phosphate including: (1) electrostatic repulsion between like-charged chitosan chains; (2) electrostatic attraction between oppositely charged chitosan and the phosphate moiety of αβ-GP; (3) attractive hydrophobic and hydrogen bonding between chitosan chains; (4) the hydrophobic character of the glycerol moity of αβ-GP; and (5) electrostatic interaction between ions (Na^+ or CH_3COO^-) in aqueous solutions and chitosan chains or phosphate moiety of αβ-GP. Upon heating the CS-αβ-GP hydrogel at 37°C, physical junction zones of chitosan chain segments throughout the solution occurs to form a hydrogel, necessarily by inducing a sudden preponderance of attractive hydrophobic and hydrogen bonding forces over interchain electrostatic repulsion. Furthermore, the hydrophobic interaction force in CS/αβ-GP hydrogel between chitosan molecules forms more easily because αβ-GP is a mixture of α-GP and β-GP, and α-GP has linear chain structure and shows less steric hindrance than β-GP.[26] So, the system with αβ-GP was favorable to gel forming at 37°C.

Stability of the CS-αβ-GP thermosensitive hydrogel

The CS-αβ-GP thermosensitive hydrogel, prepared with pH 4.6, 0.20 mol/L, and CS/αβ-GP 9.0/1.0 (sample D), was as an example to check the stability in storage at either 4 or 25°C for 3 months. The data of the variation of pH value, optical density (OD), and viscosity during storage time at 4°C were shown in Figure 3. The result showed that the three criterion, pH values, turbidity, and viscosity of hydrogel, were almost maintained the same during the storage time. So, the CS-αβ-GP thermosensitive hydrogel was stable for at least 3 months at 4°C. When the hydrogel was stored at 25°C, the pH values, turbidity, and viscosity were maintained the same in the first month. However, the viscosity became little lower than that of the beginning after first month (the date was not shown). The results were in accordance with Ruel-Gariépy et al.[19] who reported the stability of chitosan/β-GP hydrogel. So, the αβ-GP had the same effect on the stability of chitosan hydrogel as β-GP.

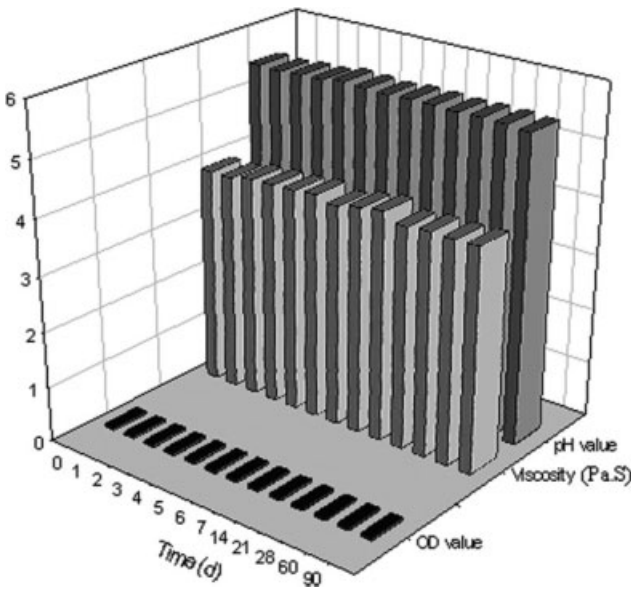

Figure 3 Variation of pH, OD, and viscosity of CS-αβ-GP hydrogel (sample D) during storage time at 4°C.

Characteristics of the CS-αβ-GP thermosensitive gel

The SEM of hydrogel (made from the formulation of pH 4.6, 0.15 mol/L, and CS/αβ-GP 9.0/1.0) with or without incubated at 37°C were shown in Figure 4. Figure 4 showed that there were some granules on the appearance of hydrogel without heating as shown in Figure 4(A). However, the appearance of hydrogel incubated at 37°C became more corrugated, and the granule was almost disappeared as seen in Figure 4(B). This might be explained by the fact that the interaction and configuration of hydrogel were changed by incubation at 37°C.

The infrared spectra of chitosan, αβ-GP, and CS-αβ-GP thermosensitive gel were shown in Figure 5.

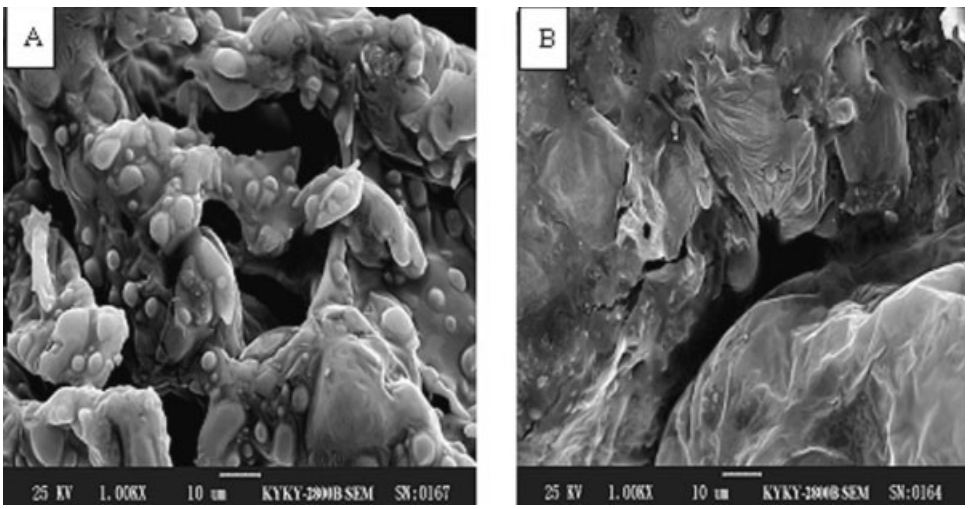

Figure 4 Effect of heating (37°C) on the scanning electron micrograph of CS-αβ-GP hydrogel (sample D) (A) SEM of sample D without heating; (B) SEM of sample D heated at 37°C.

The basic characteristics of chitosan (spectrum a) were at the O—H and *N*—H stretching bands overlapped in the 3000–3600 cm^{-1} region, amide and amine bands of chitosan appeared at 1630 and 1524 cm^{-1}, respectively, and the saccharide ether peaks of the skeletal vibrations involving the C—O stretching appeared between 1152 and 1090 cm^{-1}.[31] Spectrum b in Figure 5 was the infrared spectra of αβ-GP and spectrum c was that of CS-αβ-GP gel. Figure 5 showed that the peak strength of C=O stretching band of chitosan (1630 cm^{-1}), O—H and *N*—H stretching bands were decreased after the formation of gelation. Former might be an indication of the occurrence of hydrogen bonding between C=O of chitosan and —OH of αβ-GP, and the latter might be due to the junction of *N*—H of chitosan and O—H of αβ-GP. These results indicated that the gelation was formed because of the interactions between chitosan and αβ-GP.

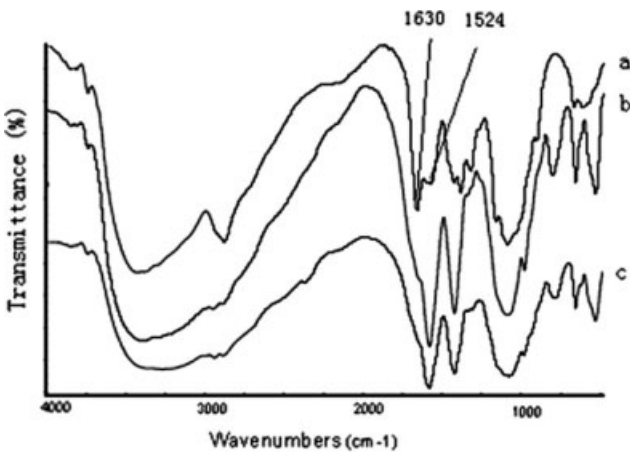

Figure 5 Infrared spectrometry of chitosan, αβ-GP, and CS-αβ-GP hydrogel. (a) chitosan; (b) αβ-GP; (c) CS-αβ-GP hydrogel.

In vitro release of adriamycin

The adriamycin release profiles from CS-αβ-GP thermosensitive hydrogel prepared with different conditions in pH 7.4 PBS medium were shown in Figure 6. Figure 6 showed that the adriamycin release from hydrogel prepared with different formulations were only more than 10% during the first hour which were all relatively slower than that from dissociative adriamycin solution, which was almost 100% during the same time. The release rate of adriamycin from hydrogel prepared with pH 4.2 chitosan solution was more than 70% during 24 h and that from hydrogel prepared with pH 4.6 chitosan solution was 63% during the same period. The release rate became slower with the increase of pH of chitosan

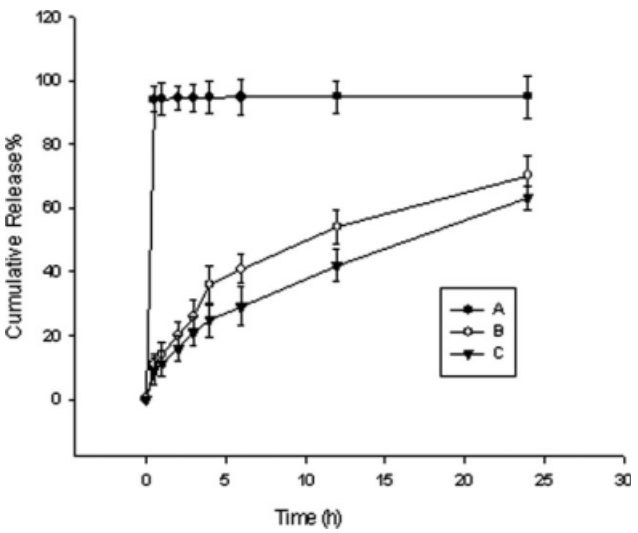

Figure 6 Cumulative release of adriamycin in pH 7.4 PBS medium at 37°C (data shown are the mean ± S.D., $N = 3$) (A) dissociative adriamycin; (B) Hydrogel prepared in pH 4.2; (C) Hydrogel prepared in pH 4.6.

solutions. The result might be explained by the sol-to-gel transition when it was incubated at 37°C. The formation of gel retarded the diffusion of the drug through the gel into the release medium. The release rate from hydrogel of higher pH (pH 4.6) was slower than that of pH 4.2 for the pH 4.6 was advantageous in formation gel than pH 4.2. So the CS-αβ-GP hydrogel was an ideal sustained release system and the drug release rate could be controlled by changing the formulation of hydrogel prepared.

CONCLUSIONS

The novel CS-αβ-GP thermosensitive hydrogels were prepared with chitosan (dissolved in the acetic acid/sodium acetate buffer solution) and αβ-GP. The hydrogel could be transited into gel at 37°C. Different pH of chitosan solution, different ionic strength, and different ratio of CS/αβ-GP all affected the pH values, turbidity, viscosity, and thermosensitive characteristics of CS-αβ-GP hydrogel. It could be concluded that the optimal conditions to prepare CS-αβ-GP thermosensitive hydrogel were: pH 4.6, ionic strength 0.15 mol/L, and CS/αβ-GP ratio of 8.8/1.2. The hydrogel was stable for at least 3 months at 4°C. The appearance of hydrogel had incubated at 37°C became more corrugated and had almost no granule compared with that of hydrogel without heated. The FTIR spectra illuminated the formation of the bonding between *N*—H or C=O group of chitosan and O—H group of αβ-GP, which played an important role during the process of sol-to-gel transition. The cumulative release of adriamycin from the CS-αβ-GP hydrogel, measured in PBS at pH 7.4, reached only 60 to 70% over 24 h, indicating that this material could potentially be used in a sustained drug delivery system.

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