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Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system

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Abstract

A thermosensitive hydrogel has been prepared with chitosan and αβ-glycerophosphate (αβ-GP) which could be transited from solution into gel at 37 °C. The thermosensitive characteristics, appearance and structure of the hydrogel were affected by concentration, molecular weight and degree of deacetylation (DD) of chitosan. Chitosan, MW 1360 kDa, DD 75.4%, solution concentration 2% was optimal for preparation of chitosan-αβ-glycerophosphate (CS-αβ-GP) thermosensitive hydrogel. The appearance of the hydrogel became more compact and regular with increasing concentration and chitosan MW. Model drugs release from CS-αβ-GP hydrogel prepared by the drug being added into chitosan solution (method I) was slower than that from hydrogel prepared by the drug being directly added into chitosan hydrogel (method II).The release rate for both adriamycin and 6-mercaptopurine from CS-αβ-GP hydrogel decreased with the increase of MW of the chitosan. The hydrophilic model adriamycin was released 60–70% over 24 h which was slower than that of the hydrophobic model 6-mercaptopurine. Therefore it was projected that the CS-αβ-GP hydrogel should be an ideal sustained release system especially for hydrophilic drugs.

Keywords: Chitosan; CS-αβ-GP thermosensitive hydrogel; Molecular weight; Degree of deacetylation; In vitro release

1. Introduction

In recent years, great interest has focused on biomedical materials, especially on intelligent polymeric materials which exhibit response to the external stimuli changes, such as temperature (Uraki, Imura, Kishimoto, & Ubukata, 2004), photo field (Ninomiya & Kawaguchi, 2006), and antigen (Matzelle & Babensee, 2004), etc. Thermoreversible hydrogels are of great interest in drug delivery, cell encapsulation, and tissue engineering (Cho et al., 2004; Kang, Cheon, Khang, & Song, 2006a; Park & Song, 2006). Early research in the field focused on the synthesis of thermosensitive gel materials including poly(ethylene glycol)/poly(propylene glycol) block copolymers (poloxamers), poly(ethylene glycol)/poly(butylene glycol) block copolymers, poloxamer-*g*-poly(acrylic acid), and polymers of *N*-isopropylacrylamide that exhibited a sol-to-gel transition in aqueous solutions (Kiyotsukuri, Masuda, Tsutsumi, Sakai, & Nagata, 1995; Shi & Zhang, 2007; Yonga et al., 2001). Such materials are generally not biodegradable which limits their practicality for use in the clinic.

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Some natural polymers including agarose, gelatin, amylopectin, cellulose derivatives, carrageenans and so on exhibit thermoreversible gelation behavior (Babin & Dickinson, 2001; Dahmani, Ramzi, Rochas, & Guenet, 2003; Durrani & Donald, 1995; Mangione, Giacomazza, Bulone, Martorana, & Biagio, 2003; Richardson et al., 2006). Chitosan, a polysaccharide derived from naturally abundant chitin, is currently receiving a great deal of interest for medical and pharmaceutical applications in various chemical and physical gel forms. Chitosan thermosensitive hydrogels utilized in drug delivery (Barreiro-Iglesias, Coronilla, Concheiro, & Alvarez-Lorenzo, 2005), cell encapsulation (Lagarce et al., 2005), tissue engineering (Cho et al., 2004) have been prepared with different methods such as grafting chitosan with poly(*N*-isopropylacrylamide) (Cho et al., 2004), grafting chitosan with PEG (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005), mixing chitosan with poly(vinyl alcohol) and sodium bicarbonate (Tang, Du, Hu, Shi, & Kennedy, 2007) or coupling Pluronic (a block copolymers based on ethylene oxide and propylene oxide) onto chitosan using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide as coupling agents (Chung et al., 2005) and so on.

Chitosan solutions containing glycerol-β-phosphate (β-GP) which had temperature-controlled solution–gel transition at a temperature close to 37 °C have recently been proposed as a suitable vehicle for the extravascular parenteral administration of drugs (Chenite et al., 2000; Ruel-Gariépy, Leclair, Hildgen, Gupta, & Leroux, 2002). Berger et al. (2005) reported that the deacetylation degree of chitosan might modulate the turbidity of chitosan/β-GP hydrogel. Crompton et al. (2005, 2006) described the morphology and gelation of thermosensitive chitosan/β-GP hydrogels and examined the procedure of injecting the hydrogel to the brain in order to form a gel track. Cho, Heuzey, Bégin, and Carreau (2005) demonstrated the characterized key physicochemical and rheological properties of chitosan/β-GP systems as a function of temperature. Jarry (Jarry, Leroux, Haeck, & Chaput, 2002; Jarry et al., 2001) studied the effects of steam sterilization and γ-irradiation on chitosan and thermogelling chitosan-β-glycerophosphate solution. All reports demonstrated that the hydrogel prepared with chitosan and β-GP was thermosensitive near 37 °C.

Furthermore αβ-glycerophosphate (αβ-GP) is a mixture of α-glycerophosphate (α-GP) and β-glycerophosphate (β-GP), and α-GP has linear chain structure and shows less steric hindrance than β-GP. Wu, Su, and Ma (2006) had reported a thermosensitive hydrogel of quaternized chitosan and αβ-GP and concluded that αβ-GP had better gelation capacity compared with β-GP. However, there were no reports on the preparation and influence of chitosan characteristic of chitosan-αβ-GP (CS-αβ-GP) thermosensitive hydrogel. So it was necessary to study the thermosensitive characteristics of chitosan and αβ-GP.

2. Materials and methods

2.1. Materials

Chitosan, derived from crab shell, molecular weight 1360 kDa; deacetylation degree 75.6%, prepared as previously by the method of degradation with acetic acid according to Chen, Zheng, Wang, Lee, and Park (2002). αβ-Glycerophosphate (αβ-GP), acetic acid glacial and sodium acetate were all analytical grade (Sigma Co., St. Louis, USA). Adriamycin (ADR) was from Zhejiang Hisun Pharmaceutical Co. Ltd. (Taizhou, China), 6-MP was kindly donated by the Xinhua Pharmaceutical Factory (Shijiazhuang, China).

2.2. Preparation of chitosan samples

2.2.1. Characteristics determination of chitosan samples

The viscosity and MW of chitosan were calculated using the Mark–Houwink equation: $[\eta] = kMW^{\alpha}$, where η is the intrinsic viscosity of chitosan samples derived using an Ubbelohde Viscosimeter at 30 °C; MW was the molecular weight of chitosan; $k = 1.64 \times 10^{-30} \times \mathrm{DD}^{14}$ and $\alpha = 1.02 \times 10^{-2} \times \mathrm{DD} + 1.82$ (Chen & Hwa, 1996); DD was the degree of the deacetylation of chitosan expressed as the percentage.

The DD was determined by the method of potentiometric titration (Jia & Li, 2001). The DD was calculated using following equation:

$$\mathrm{DD}\% = \frac{\Delta V \times C_{\mathrm{NaOH}} \times 10^{-3} \times 16}{W \times 0.994} \times 100\%$$

where ΔV was the volume of NaOH between two inflexion points, (one was the excess HCl and the second was the inflexion point of amino group of chitosan), C_{NaOH} was the concentration of the NaOH solution, W was the weight of chitosan sample, 16 g/mol is the molecular of the amino group and 0.0994 was the theoretical percentage of amino groups (no units) in chitosan.

2.2.2. Different MW chitosan preparations

Different MW chitosan with the same DD were prepared by the method of acetic acid hydrolysis of chitosan (Chen et al., 2002). Chitosan (10 g, 100 mesh power) was dissolved in 5% v/v aqueous acetic acid (190 ml), incubated at 50 °C for 0, 0.5, 1, 2.5, and 24 h, respectively, and then centrifuged (5000*g*) for 20 min. NaOH (4 M) was added into the supernatant to adjust the pH to within the range 7–9. The sediment was filtered off and sequentially rinsed in water and ethanol and dried at 50 °C. The MW of chitosan samples prepared was 1360 kDa, 1130 kDa, 499 kDa, 200 kDa and 88 kDa, respectively, with the almost same DD ranging from 75.6% to 74.2% ($p > 0.05$) of which the mean was 75.1%.

2.2.3. Different DD chitosan preparations

Chitosan with different DD's were prepared by the process of deacetylation and acetylation with acetic anhydride.

In order to obtain a more highly deacetylated chitosan, a method of repeated alkaline treatment was chosen (Wan, Creber, Peppley, & Bui, 2003). Chitosan (10 g) was treated in 50% w/v aqueous NaOH (100 ml) for 30 min at 100 °C. After washing with deionized water repeatedly and drying, the corresponding product was further deacetylated twice using the same conditions to obtain a chitosan with a DD of 90.3%. To obtain a series of chitosan samples with similar MW but varied DD, reacetylation of chitosan was introduced. Briefly, chitosan (5 g) was dissolved in 1% v/v aqueous acetic acid (250 ml). Methanol (250 ml) was added to each solution and stirred for a further 20 min. Then different amounts of acetic anhydride were added into the solutions and stirred for a further 20 h. At the end of the reaction, methanolic ammonia (1.0% ammonia in methanol, 1000 ml) was added to each solution in order to precipitate the chitosan. The precipitates were washed intensively with deionized water until neutrality and then dried at 50 °C. The DDs of the resultant chitosans were 90.3%, 85.5%, 75.4%, 69.2% and 56.5%, respectively, with the almost same MW ranging from 1380 to 1320 kDa ($p > 0.05$) of which the mean was 1340 kDa.

2.3. Preparation of CS-αβ-GP thermosensitive hydrogel

Chitosan (1.8 g) was added to 0.2 M acetic acid/sodium acetate buffer pH 4.6 (90 ml) with stirring until complete dissolution. Then the chitosan solution was chilled to 4 °C for 20 min. 50% w/v aqueous αβ-GP (10 ml) was prepared in distilled water and chilled along with the chitosan solution to 4 °C. Then the αβ-GP solution was added dropwise to the chitosan solution under stirring and the final chitosan-αβ-GP solution was mixed for another 20 min. Finally, the CS-αβ-GP thermosensitive hydrogel was obtained (chitosan-αβ-GP solution was mixed in the ratio of 9/1 at 4 °C to form hydrogel and stored at 4 °C). Different chitosan concentrations, different MWs and different DDs were chosen to prepare CS-αβ-GP thermosensitive hydrogels.

2.4. Characterization of the CS-αβ-GP thermosensitive hydrogel

The pH, turbidity, UV–vis spectroscopy at 600 nm and viscosity (NDJ-8S digital viscometer, Scientific Instrument of Shanghai, Shanghai, China) of CS-αβ-GP thermosensitive hydrogel were measured.

2.5. 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 (Chung, Simmons, Gutowska, & Jeong, 2002). The sol phase was defined as flowing liquid and the gel phase as non-flowing gel when the hydrogel solution in the test tube was inverted. CS-αβ-GP thermosensitive hydrogel was added into a 5 ml tube to study sol-to-gel transition characteristics in a water bath of 37 ± 0.5 °C.

2.6. Viscosity measurements

Sol-to-gel behavior of CS-αβ-GP thermosensitive hydrogels was further studied by measuring the solution viscosity of the samples. Viscosities of CS-αβ-GP thermosensitive hydrogels (in 50 ml lots) were measured at predetermined time intervals as a function of time using a viscometer (NDJ-8S, Shanghai Cany Precision Instrument Co., Ltd., Shanghai, China) at 37 °C. Measuring the viscosity, the third rotator was selected and rotation speed was 1.5 rpm, the viscosity of hydrogel was measured and recorded.

2.7. Microscopy analysis

Samples of hydrogel (5 ml, in Cryogenic Vials) were incubated in a water bath under the same conditions used for the sol-to-gel studies. When the hydrogels were transformed into gels they were freeze dried (for 48 h). The samples were then coated with gold under vacuum using SCD 004 Balzers sputter coater (Balzers, Liechtenstein) and the surfaces were investigated by scanning electron microscopy (KYKY2800B, KYKY Technology Development Ltd., Beijing, China).

2.8. FT/IR spectrometry

Samples of CS-αβ-GP thermosensitive gel were prepared by the same method as used for microscopy analysis. The infrared spectra of chitosan, αβ-GP and CS-αβ-GP thermosensitive gel (2 mg sample in 100 mg of KBr) were recorded on a FT/IR-430 Fourier Transform Infrared Spectrometer (Jasco Co., Tokyo, Japan) (Shigemasa, Matsuura, Sashiwa, & Saimoto, 1996).

2.9. Evaluation of loading and in vitro release efficiency

CS-αβ-GP thermosensitive hydrogel loaded with ADR or 6-MP were prepared by two different methods. Method I was that ADR or 6-MP (10 mg) was added into chitosan solution (9 ml), respectively, under agitating until it was dissolved or dispersed thoroughly, then prepared thermosensitive hydrogel as forenamed. Method II was that model drug (10 mg) was added into CS-αβ-GP thermosensitive hydrogel (10 ml) directly while stirring in ice bath.

The CS-αβ-GP thermosensitive hydrogel (1 ml) with or without model drug (ADR or 6-MP), was placed in dialysis membrane (New Brunswick, New Jersey, USA) with a molecular weight cut-off of 8000–10,000 (Kang, Cheon, Khang, & Song, 2006b). ADR or 6-MP solution (1 ml, 0.2% w/v) was prepared for measuring dissociative drug release. The dialysis membranes were placed in 0.2 M sodium phosphate buffer pH 7.4 (100 ml) in Erlenmeyer flasks (250 ml) which were then closed with plastic

membranes. The buffer solutions were stirred continuously at 160 rpm in a vibrating incubator at 37 ± 0.5 °C.

At predetermined time intervals, aliquots (4 ml) were removed (stored at 4 °C until analysis) and replaced by an equal volume of the 0.2 M phosphate buffer pH 7.4 to maintain a constant volume. The samples were assayed spectrophotometrically at 233 nm (ADR) or 325 nm (6-MP), respectively, with a control of CS-αβ-GP hydrogel, to evaluate the release rate of the model drugs.

2.10. Statistical analysis

Assays were performed in triplicate, collected data is expressed as the mean value ± standard deviation. Statistical data were analyzed using SPSS13.0 and differences were considered to be significant at a level of $p < 0.05$, using a two tailed paired *t*-test.

3. Results and discussion

3.1. Characteristics of chitosan samples

The FT-IR spectra of the chitosan samples with different MW showed no obvious differences. There were strong characteristic amino peaks of chitosan at 3420, 1655, and 1325 cm^{-1}, and the peaks assigned to the saccharide structure were at 1152 cm^{-1} (C—H stretch), 1154 cm^{-1} (bridge-O-stretch), and 1094 cm^{-1} (C—O stretch). These results showed that chitosan samples made via acetic acid hydrolysis had no obvious change in their DD values and molecular structures. A similar result had been reported by Chen et al. (2002).

The infrared spectra of chitosan with different DD's (Fig. 1) show that the intensity of amide band I at 1650 cm^{-1} increased when the DD of chitosan was decreased, which clearly indicates N-acetylation of the amino groups in the chitosan molecule. These results are in accordance with those of Ren, Yi, Wang, and Ma, (2005).

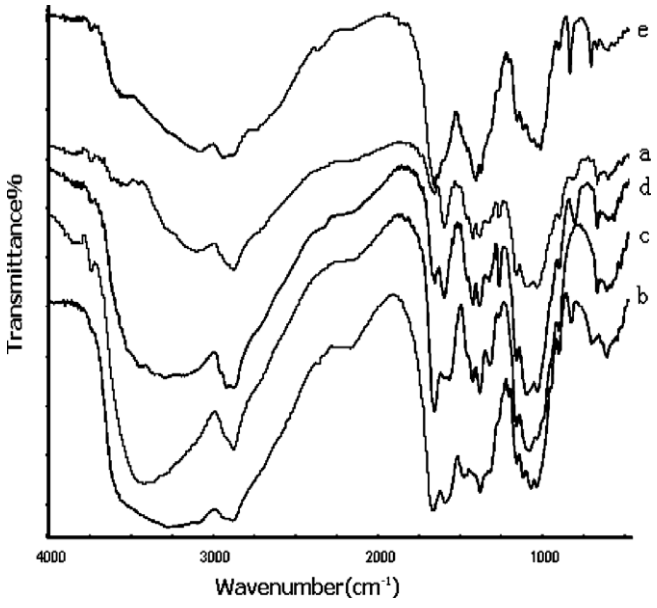

Fig. 1. Infrared spectra of chitosan samples with different DD (a) 56.5%; (b) 69.2%; (c) 75.4%; d: 85.5%; (e) 90.3%.

3.2. Characteristics of the CS-αβ-GP thermosensitive hydrogel

From the characteristics of the CS-αβ-GP thermosensitive hydrogels prepared with different formulations (Tables 1–3) the pH values of hydrogel were almost the same and increased only a little with increases of concentration, MW and DD of chitosan. The turbidity of the hydrogels did not vary with the increase of chitosan concentration. However the turbidity increased with increase of DD and decreased with increase of chitosan MW. The viscosities of the hydrogels increased greatly with the increase of chitosan concentration and increased to a high degree when the concentration of chitosan increased to 2.0 mol/L. The viscosity of chitosan increased slightly with increase of MW. However the viscosity increased when the DD of chitosan was 75.4% as seen in Table 3. Although the reason why the viscosity is affected by DD is not clear.

3.3. Thermosensitive gelation behaviour

The CS-αβ-GP hydrogel showed an apparent sol-to-gel transition at 37 °C, below which, the solutions were flowable viscous liquids and were injectable through a syringe. As the solution was heated to 37 °C, it transformed into gel which was non-flowing.

Amongst the CS-αβ-GP hydrogels prepared with different concentrations of chitosan the thermosensitive gels prepared with chitosan of lower concentration were transparent. However the gel was slightly white and turbid when the concentration of chitosan increased to 2.5%. The hydrogel made from of 2.0% w/v chitosan solution MW 1360 kDa and DD 75.1%, had a transparent appearance and 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. Figs. 2a, b, and c) show the change of viscosities of hydrogels prepared with different concentrations of chitosan, different MW's and different DD's, respectively. The hydrogel was maintained at 37 °C and the viscosity of the hydrogel was measured every 2–3 min, from which the curve of viscosity of hydrogel and time was produced.

When they were maintained at 37 °C, the viscosity of hydrogels made from chitosan of concentrations lower than 2.0% w/v were unchanged or increased slowly, whereas the viscosity of hydrogel prepared with a chitosan concentration of 2.0% w/v increased quickly. However, the viscosity became too high to maintain a flowable medium when the concentration increased to 2.5% w/v. So the increase of concentration of chitosan solution from 1.0% to 2.0% benefited sol-to-gel transition and above 2.5%

Table 1
Characteristics of CS-αβ-GP thermosensitive hydrogel prepared with different concentrations of chitosan

Symbol	Starting chitosan			Characteristics of CS-αβ-GP thermosensitive hydrogel		
	MW (kDa)	DD (%)	*C* (w/v)	pH values	OD values	Viscosity (Pa s)
K1	1360	75.1	1.0	5.68 ± 0.02	0.062 ± 0.002	0.203 ± 0.022
K2	1360	75.1	1.5	5.71 ± 0.01	0.048 ± 0.003	0.270 ± 0.032
K3	1360	75.1	2.0	5.73 ± 0.02	0.094 ± 0.002	2.589 ± 0.034
K4	1360	75.1	2.5	5.74 ± 0.01	0.061 ± 0.004	26.59 ± 0.045
K5	1360	75.1	3.0	5.75 ± 0.03	0.075 ± 0.008	non-flowing

Data shown are the means ± SD, $n = 3$.

Table 2
Characteristics of CS-αβ-GP thermosensitive hydrogel prepared with different MW of chitosan

Symbol	Different preparation conditions			Characteristics of CS-αβ-GP thermosensitive hydrogel		
	MW (kDa)	DD (%)	*C* (w/v)	pH values	OD values	Viscosity (Pa s)
L1	88	75.1	2.0	5.64 ± 0.03	1.035 ± 0.028	0.247 ± 0.033
L2	200	75.1	2.0	5.65 ± 0.02	1.128 ± 0.002	1.232 ± 0.045
L3	499	75.1	2.0	5.69 ± 0.01	0.178 ± 0.004	2.515 ± 0.043
L4	1130	75.1	2.0	5.70 ± 0.02	0.238 ± 0.002	3.384 ± 0.048
L5	1360	75.1	2.0	5.86 ± 0.01	0.077 ± 0.003	3.837 ± 0.024

Data shown are the means ± SD, $n = 3$.

Table 3
Characteristics of CS-αβ-GP thermosensitive hydrogel prepared with different DD of chitosan

Symbol	Different preparation conditions			Characteristics of CS-αβ-GP thermosensitive hydrogel		
	MW (kDa)	DD (%)	*C*	pH values	OD values	Viscosity (Pa S)
M1	1340	56.5	2.0	5.42 ± 0.03	0.035 ± 0.002	0.112 ± 0.035
M2	1340	69.2	2.0	5.52 ± 0.01	0.036 ± 0.003	0.111 ± 0.043
M38	1340	75.4	2.0	5.54 ± 0.01	0.099 ± 0.004	3.728 ± 0.043
M4	1340	85.5	2.0	5.62 ± 0.02	2.345 ± 0.001	0.112 ± 0.023
M5	1340	90.3	2.0	5.90 ± 0.01	2.515 ± 0.015	0.309 ± 0.033

Data shown are the means ± SD, $n = 3$.

was not practical (as seen in Table 2). The concentration of 2.0% w/v was optimal to prepare CS-αβ-GP hydrogel.

The viscosity of hydrogels prepared with different MW of chitosan increased with MW increasing from 88 to 1360 kDa (Fig. 2b) when they were incubated at 37 °C. So the increase of MW was favorable for sol-to-gel transition and a high molecular chitosan was optimal for hydrogel preparation.

The DD of chitosan obviously affected the sol-to-gel transition (Fig. 2c). The viscosity of hydrogel prepared with DD of chitosan of 75.4% increased quickly at 37 °C whereas others increased either slowly or were unchanged and therefore the optimal DD for hydrogel preparation was 75.4%.

The concentration, MW and DD affected the thermosensitive characteristics of CS-αβ-GP hydrogel. The variations 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. Upon heating the CS-αβ-GP hydrogel at 37 °C, physical junction zones of chitosan chain segments occurs throughout the solution forming a hydrogel, necessarily by inducing a sudden preponderance of attractive hydrophobic and hydrogen bonding forces over interchain electrostatic repulsion. The optimal concentration, DD and high MW affected the attractive hydrophobic and hydrogen bonding forces and made the CS-αβ-GP hydrogel become quickly transformed into gel.

3.4. Characteristics of the CS-αβ-GP thermosensitive gel

The SEMs of CS-αβ-GP gels prepared with different formulations are shown in Fig. 3. In Figs. 3a and b, the SEM of hydrogel prepared with chitosan of different concentration but of the same MW (1360 kDa) and DD showed that the appearance of the hydrogel changed with the increasing of chitosan concentration.

The SEM micrographs clearly illustrate the dependence of hydrogel morphology on the characteristics of chitosan. The appearance of gel made from 1.0% w/v chitosan solution was loose and had a lot of ramified configuration (Fig. 3a) and the surface became more compact with the increasing of chitosan concentration (Fig. 3b). However, the appearance of hydrogel became more loose and some holes appeared when the MW of chitosan decreased (Fig. 3c). The DD of chitosan affected the morphology of

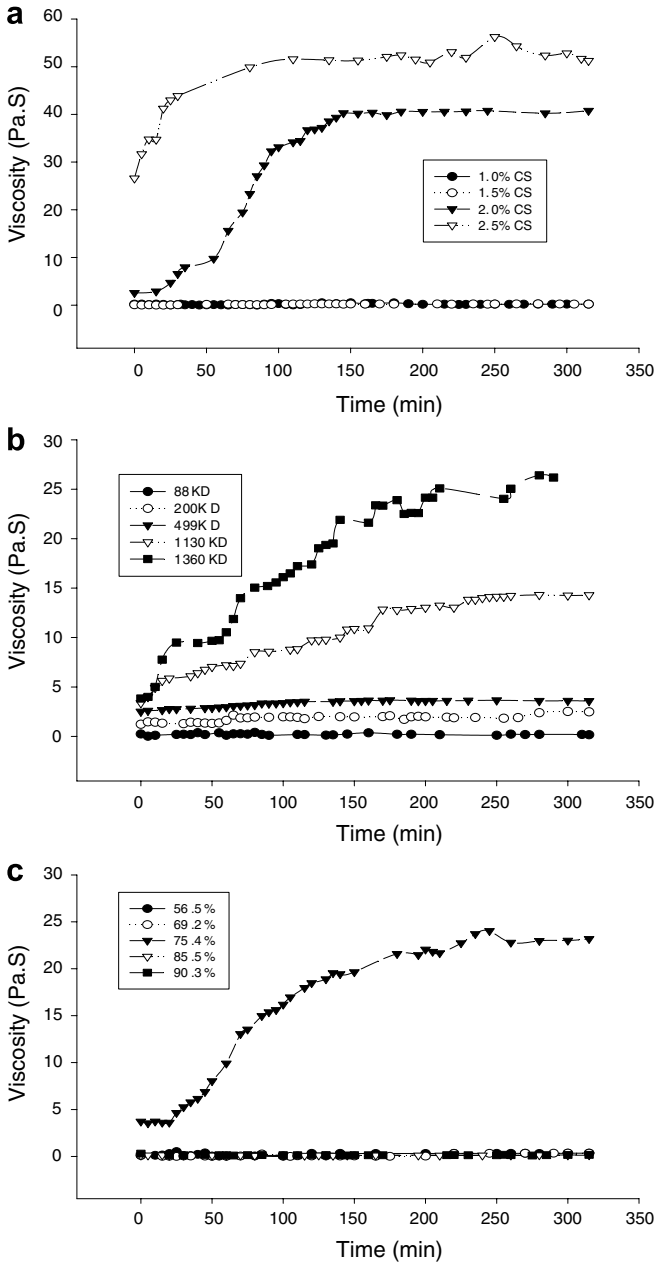

Fig. 2. Viscosity of CS-αβ-GP hydrogels as a function of time at given temperature. (a) Hydrogel prepared with different concentrations of chitosan solution; (b) hydrogel prepared with different MW's of chitosan; (c) hydrogel prepared with different DD's of chitosan.

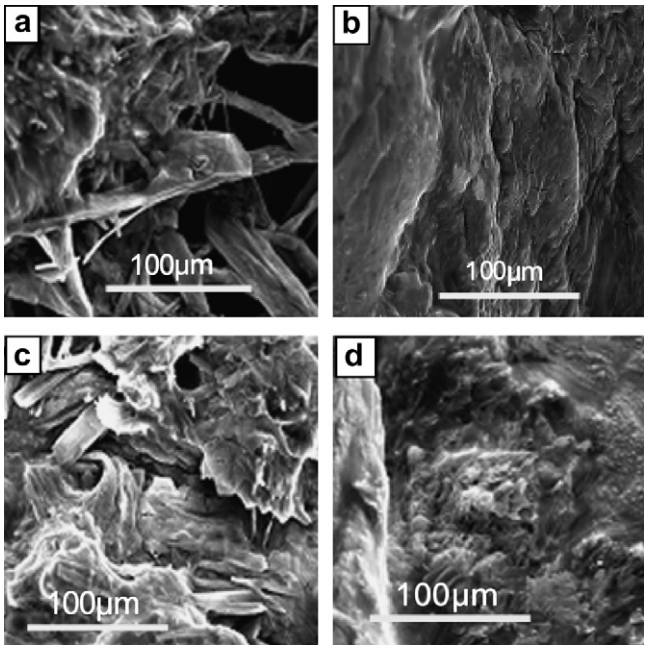

Fig. 3. Scanning electron micrograph of CS-αβ-GP gelation with different formulation (500×). (a) MW 1360 kDa, DD 75.6% and concentration 1% of chitosan; (b) MW 1360 kDa, DD 75.6% and concentration 2% of chitosan; (c) MW 499 kDa, DD 75.4% and concentration 2% of chitosan; (d) MW 1340 kDa, DD 85.5% and concentration 2% of chitosan.

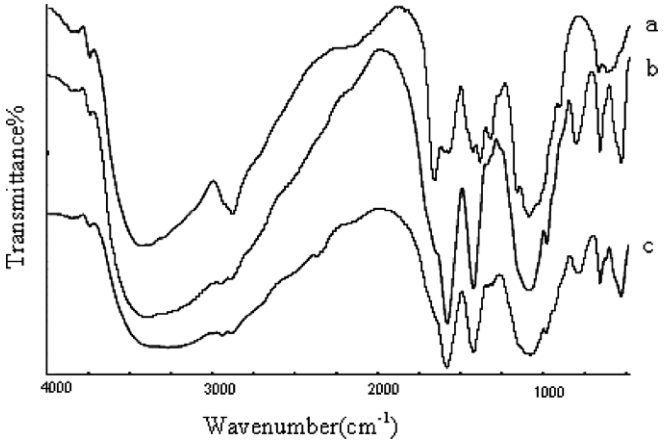

Fig. 4. Infrared spectra of CS-αβ-GP hydrogel. (a) chitosan; (b) αβ-GP; (c) CS-αβ-GP gel.

gel and the appearance of gel became more compact but not regular when the DD of chitosan increased from 75.4% to 85.5% (Figs. 3b and d).

In the chitosan FT-IR spectrum (Fig. 4a) 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 oxygen bridge peaks of the skeletal vibrations involving the C—O stretching which appeared between 1152 and 1090 cm^{-1} (Ruel-Gariépy et al., 2002). It can be seen in Fig. 4b and c that the C=O stretching band of chitosan (1630 cm^{-1}) and the O—H and N—H stretching bands were decreased after the formation of gel. The former band 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 —OH of αβ-GP. These results could indicate that the gel was formed because of the interactions between chitosan and αβ-GP.

3.5. In vitro release of model drugs

The release rates of ADR and 6-MP from the loaded hydrogels prepared by different methods (Fig. 5) show that

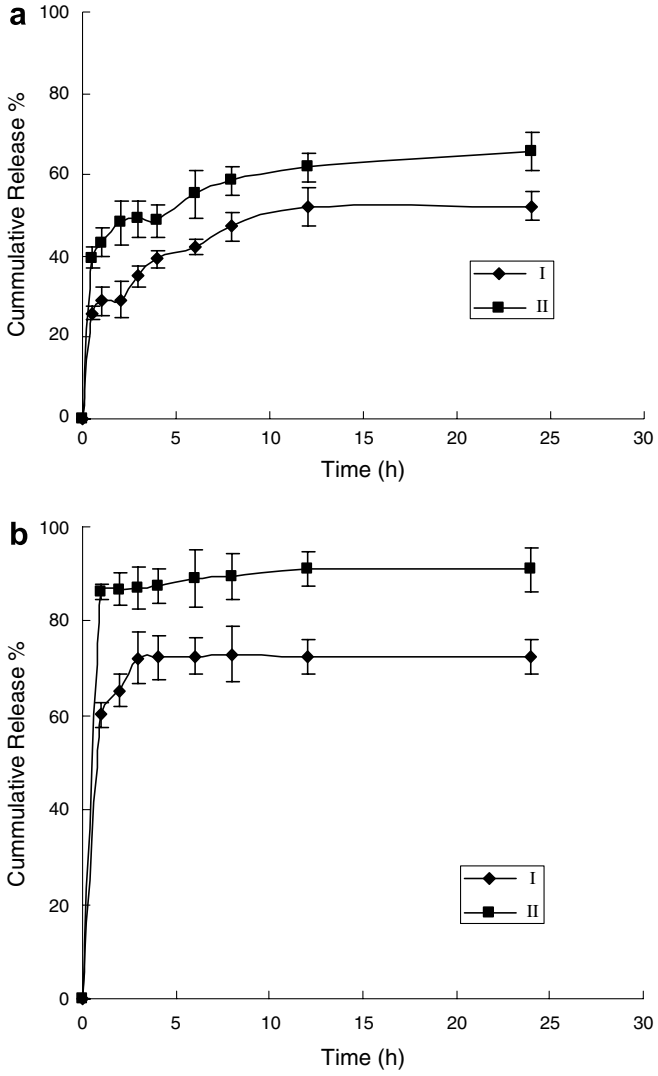

Fig. 5. Cumulative release of different model drugs from CS-αβ-GP hydrogels of MW 1360 kDa, DD 75.4%, 2.0% w/v chitosan prepared by method I and II, respectively). (a) ADR; (b) 6-MP.

the preparation method affected the release efficiency. It is clear that the hydrogel prepared by method I released model drugs much slower than that prepared by method II. This might be explained by Fig. 6 which shows the SEM of gels loaded with ADR but prepared by different methods. There was little granule (small grain or pellet) in the appearance of gel prepared by method I (as seen in Fig. 6a) while there were many granules in that of gel prepared with method II (Fig. 6b). The granules might be crystals of the added model drug.

This illuminated that the model drug which has been added into chitosan hydrogel directly as in method II distributed mostly in the exterior of the gel whereas that in method I distributed mostly in the interior of the gel. The model drugs in the exterior of the gel released more quickly than those in the inner of gel. Model drugs might be connected with chitosan or αβ-GP during the process of hydrogel preparation as in method I, whereas it was only

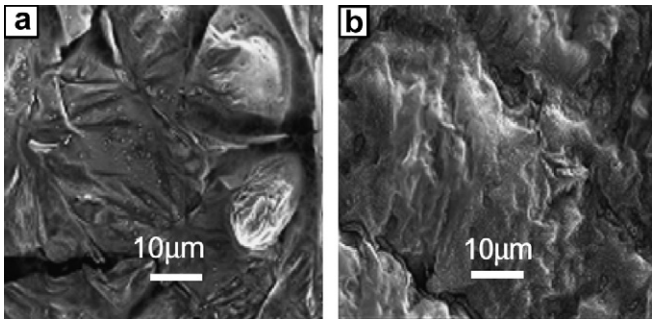

Fig. 6. The SEM of CS-αβ-GP hydrogel loaded ADR prepared with different methods (1500×). (a) Gel prepared with method I; (b) gel prepared with method II.

dispersed in the hydrogel as in method II. So method I was optimal for the sustained release of hydrogel and the hydrogels loaded model drugs fabricated for in vitro release studies were therefore all prepared by method I.

The release rate of ADR from CS-αβ-GP thermosensitive hydrogel which had been prepared with different MW's of chitosan in phosphate buffer pH 7.4 (Fig. 7a). It can be seen from Fig. 7a that the ADR released from dissociative ADR solution was almost 100% while ADR released from CS-αβ-GP hydrogel was only 70% during the same time. In the meantime, the release rate of ADR from CS-αβ-GP hydrogel made from higher MW chitosan was slower than that made from lower MW chitosan. It might be explained that gel formation retarded the release of ADR. The hydrogel of higher MW chitosan transformed into gel more quickly than that of lower MW chitosan, so it had a slower release rate of ADR of 50%. The release profile of 6-MP from CS-αβ-GP thermosensitive hydrogel prepared with different MW of chitosan in phosphate buffer pH 7.4 (Fig. 7b) shows that the release rate of 6-MP from CS-αβ-GP hydrogel made from higher MW chitosan was slower than that made from lower MW chitosan and the release rate of 6-MP from hydrogel made from lower MW of chitosan was almost the same as that of dissociative 6-MP.

The sustained release effect of hydrogel on 6-MP was not as good as that on ADR (Fig. 8). The release rate of 6-MP from CS-αβ-GP hydrogel made from higher MW chitosan was almost 80% and that of ADR from the same hydrogel was 50%. The reason might be that the hydrophilic ADR dissolved in the gel and formed interactions with the gel while the hydrophobic 6-MP only dispersed in the gel and connected loosely with the gel. 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.

4. Conclusions

In the preparation of CS-αβ-GP thermosensitive hydrogel from chitosan and αβ-GP the concentration, MW and DD of the chitosan all affected the pH values, turbidity,

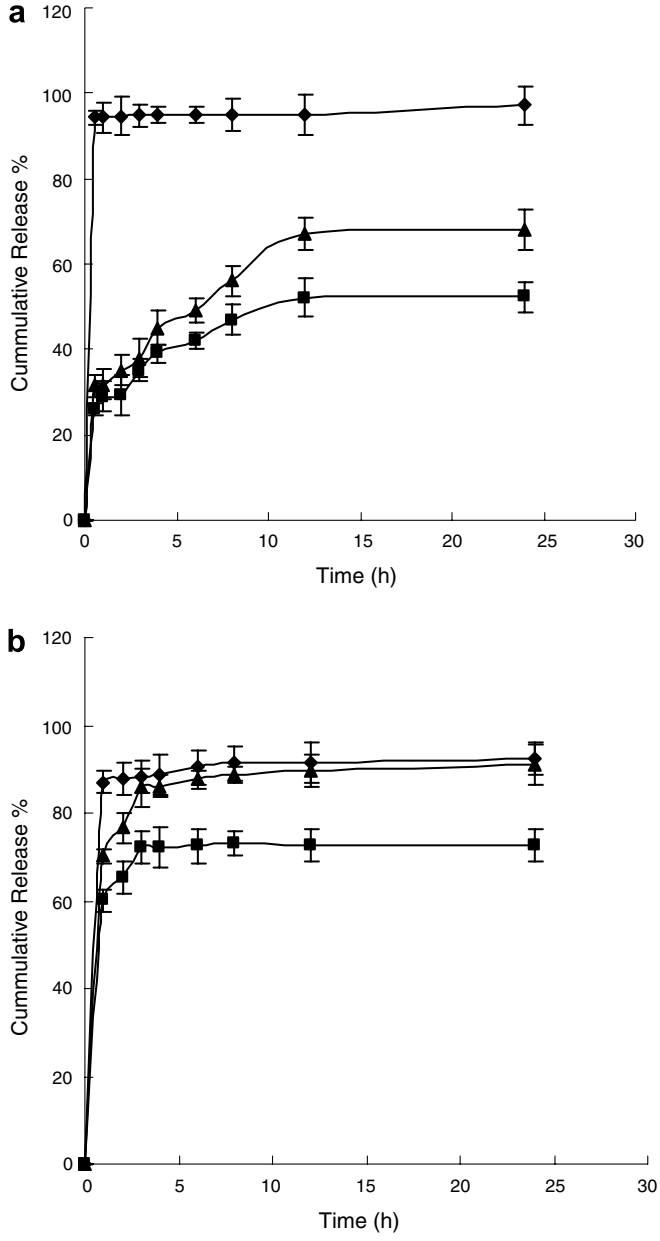

Fig. 7. Cumulative release of drugs from CS-αβ-GP hydrogel of 2.0% w/v of chitosan MW 1360 kDa, DD 75.4% in 0.2 M sodium phosphate buffer pH 7.4 at 37 °C. (a) ADR –♦–: dissociative ADR; –▲–: 1130 kDa; –■–: 1360 kDa; (b) 6-MP –♦–: dissociative 6-MP; –▲–: 1130 kDa; –■–: 1360 kDa.

viscosity, and thermosensitive characteristics of the product hydrogels. The chitosan solution concentration of 2.0% w/v, MW 1360 kDa and DD of 75.4% was optimal for preparation of CS-αβ-GP thermosensitive hydrogel. The appearance of the hydrogel became more compact and regular with increase of chitosan concentration and MW. However it became more compact and irregular when the DD of chitosan increased from 75.4% to 85.5%. The FT-IR spectra illuminated the formation of the bonding between N—H of chitosan or C=O of chitosan and —OH of αβ-GP. The CS-αβ-GP hydrogel loaded with model

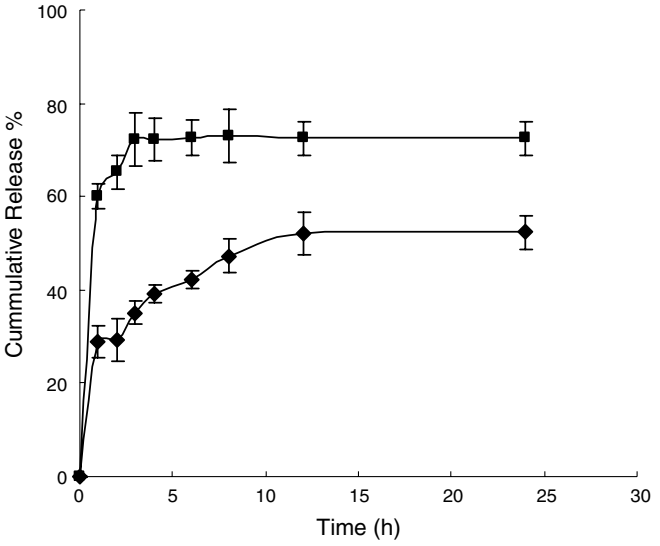

Fig. 8. Cumulative release of different model drugs from CS-αβ-GP hydrogel (–♦–: ADR; –■–: 6-MP).

drugs prepared by method I released model drugs much slower than hydrogel prepared by method II. Also the release of ADR, the hydrophilic model drug was slower than that of 6-MP the hydrophobic model drug. In general, the release rate became slower when the MW of chitosan increased. The CS-αβ-GP hydrogel was an ideal sustained release system for hydrophilic drug according to the results of in vitro release from the hydrogel which was only 60–70% for ADR during 24 h. Furthermore, the drug release rate could be controlled by changing the formulation of hydrogel prepared.

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