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International Journal of Pharmaceutics 315 (2006) 1–11
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

A thermo- and pH-sensitive hydrogel composed of quaternized chitosan/glycerophosphate

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Received 7 September 2005; received in revised form 18 January 2006; accepted 20 January 2006
Available online 17 April 2006

Abstract

The quaternized chitosan was synthesized by the reaction of chitosan and glycidyltrimethylammonium chloride (GTMAC) and named as *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC). A novel hydrogel system composed of HTCC/glycerophosphate (HTCC/GP) with thermo- and pH-sensitivity was synthesized and used as an intelligent drug carrier. The formulation was solution below or at room temperature, which allowed it injectable and to incorporate living cells, proteins, enzymes or other therapeutic drugs easily. Once the surrounding temperature was up to 37 °C, the system was transformed to a non-flowing hydrogel, and the formed hydrogel can release the trapped drug as a function of pH values. The swelling behavior of the system and the release profiles of doxorubicin hydrochloride (DX) as a model drug at different pH values were investigated. At acidic condition the hydrogel dissolved and released drug quickly, while it absorbed water and released drug slowly at neutral or basic conditions. Hydrogel composed of chitosan hydrochloride and glycerophosphate (CS/GP) was also prepared to compare with HTCC/GP hydrogel. The HTCC/GP hydrogel in this study was transparent which made it suitable for some specific uses such as ocular drug formulation.

Keywords: Quaternized chitosan; Hydrogel; Injectable implant; Thermosensitivity; pH-sensitivity; Controlled release

1. Introduction

In recent years, thermosensitive hydrogels especially which show sol–gel transition at body temperature have gained great interest in biomedical fields because the use of them can avoid the surgery and relieve the suffering of the patient (Eve and Leroux, 2004). A lot of new formulations have been proposed and used as drug delivery carriers, tissue engineering materials, cell encapsulation vehicles and so on. Jeong et al. (2002) reported PEG and PLGA graft copolymer systems, which gelled at 37 °C and were used to treat diabetes by sustained insulin delivery or repair cartilage by chondrocyte cell release. Jong et al. (2001) grafted lactic acid oligomers onto dextran and utilized the stereocomplex formation between the oligomers to form in situ crosslinked system. A hydrogel can be formed easily at room temperature by dissolving each dextran grafted D- or L-lactic acid oligomers in water respectively and mixing them. However, most of the in situ forming gels can't correspond the external or internal stimuli to release drug intelligently. Therefore, the systems both possessed injectability and pH-sensitivity have been studied and paid much attention because some diseases show changes in pH, which can be utilized to realize intelligent drug release. As an ideal drug release system, biocompatibility and biodegradability are also important properties to be used in clinic (Qiu and Park, 2001). So it's necessary to develop a new thermo- and pH-sensitive formulation, and it should be biodegradable to avoid the surgery after drug release.

Among the polysaccharide used in pharmaceutics, chitosan, composed of β-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, is a promising biomaterial. As one of polycationic biopolymers, chitosan has many unique advantages that attract scientific and industrial interests in pharmaceutics, biomedicine, food and cosmetics, such as biodegradability, biocompatibility, mucoadhesivity, non-toxicity, and antimicrobial activity. Chitosan-based drug delivery systems in various chemical and physical gel forms have been developed and studied in the past decades (Berger et al., 2004a,b). Eve et al. (2000) reported a thermosen-

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doi:10.1016/j.ijpharm.2006.01.045

sitive hydrogel composed of chitosan or chitosan hydrochloride and β-glycerophosphate (CS/GP). Glycerophosphate (GP) is an organic compound naturally found in the body, which is usually used as a source of phosphate in the treatment of unbalance of phosphate metabolism. Its veinal administration has been approved by FDA, and in this study GP represented the disodium salt of glycerophosphate. Eve et al. found that the CS/GP formulation was solution below or at room temperature and was transformed to hydrogel around body temperature. The author thought that the addition of GP to the chitosan solution promoted the protective hydration of the chitosan chains, so that it prevented the chitosan chains aggregation at low temperature even at neutral pH solution. Raising the temperature would strengthen the hydrophobic interaction between chitosan chains, which was the driving force in the gelation of CS/GP solution. As a result, the chitosan chains aggregated and the CS/GP solution was transformed to hydrogel. The thermosensitive CS/GP hydrogel can prolong the release of macromolecules, or low-molecular-weight hydrophilic compounds if they are contained in liposomes further to retard the drug delivery, but the pH-sensitivity of the hydrogel hasn't been studied (Eve et al., 2002).

Because of the existence of many NH_2 groups on chitosan chains, hydrogels composed of chitosan are also used as pH-sensitive drug carriers. Qu et al. (2000) developed physically crosslinked chitosan hydrogels by grafting D, L-lactic acid and/or glycolic acid. The physical crosslinking was formed by hydrophobic side chains aggregation and intermolecular interaction through hydrogen bonds between side and main chains. The formed hydrogel showed pH sensitivity that swelled under acidic condition due to protonation of the free amino groups on chitosan chains and shrank under basic condition. However, the hydrogel didn't show thermosensitivity and wasn't injectable.

In order to obtain an injectable and pH-sensitive drug delivery system, we investigated CS/GP system at the first, but it didn't behave appropriately as a pH-sensitive drug carrier. It showed high initial drug release at both acidic and basic conditions. So we modified chitosan by quaternization reaction and tried to develop a new hydrogel based on quaternized chitosan and GP. We suspected that since quaternary amino group showed stronger cationic property than amino group, the hydrogel prepared by quaternized chitosan would show more distinct pH-sensitivity. The quaternized chitosan, obtained by reacting chitosan with glycidyltrimethylammonium chloride (GTMAC) and named as *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC), is one of water-soluble chitosan derivatives (Lim and Hudson, 2004). Compared with chitosan, HTCC shows better hygroscopic property, moisture retentiveness, antibacterial activity, mucoadhesivity and permeability enhancing property (Jia et al., 2001; Xu and Lu, 1996; Xu et al., 2003).

Therefore, the main purpose of this study was to prepare a new thermo- and pH-sensitive hydrogel based on HTCC and GP and evaluate its potential as an 'intelligent' drug delivery carrier. Thermosensitivity and swelling behavior of the hydrogel at different pH values were investigated and compared with those of CS/GP formulation. Doxorubicin hydrochloride (DX), as a model drug, was entrapped into the hydrogel, and the release profiles in buffer solutions with different pH values were also investigated.

2. Materials and methods

2.1. Materials

Chitosan (MW ~7.8×10^5, degree of deacetylation (DD) was 89%) was purchased from Putian Zhongsheng Weiye Co. Ltd. (Fujian, China). DX was obtained from Zhejiang Hisun Pharmaceutical Co. Ltd. (Zhejiang, China). β-GP was purchased from Fluka (Switzerland). The mixture of α-GP and β-GP (α-β-GP) was provided by Kaiyuan Pharmaceutical & Chemical Co. Ltd. (Shanxi, China). GTMAC was obtained from Dongying Guofeng Fine Chemical Co. Ltd. (Shandong, China). All other reagents were of analytic reagent grade.

2.2. Preparation of HTCC

The HTCC was prepared by a modified method proposed by Viviane et al. (2004). The reaction scheme for synthesizing HTCC is presented in Fig. 1. Chitosan was modified by GTMAC in neutral aqueous system. Chitosan (3.0 g, 18.6 mmol) was dispersed in deionized water (30.0 mL) at 80 °C. GTMAC (11.29 g, 74.4 mmol) was dissolved in aqueous solution and added to chitosan suspension. The mol ratio of GTMAC to amino groups of chitosan was 4. After reaction for 4 h at 80 °C, the turbid and yellowish reaction solution was poured into cold acetone and stirred in refrigerator overnight. After washed by acetone several times, the white precipitated product was collected by fil-

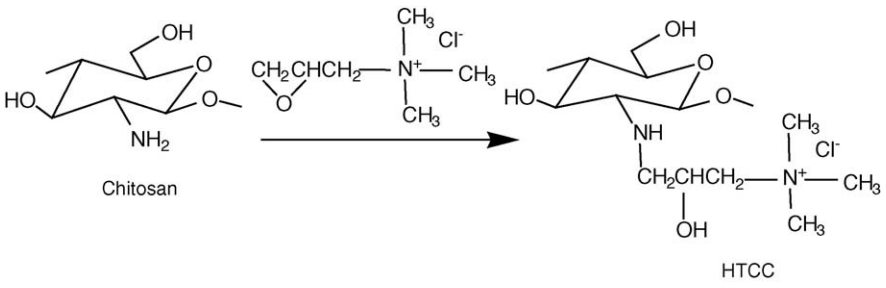

Fig. 1. Synthesis scheme of HTCC.

tration. To obtain more purified HTCC, the product was washed with hot EtOH using a Soxhlet extractor for 24 h and dried at 60 °C.

2.3. Characterization of HTCC

IR spectra of chitosan and HTCC were measured with KBr pellets on FT/IR-660 plus fourier transform infrared spectrometer (JASCO, Japan). Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was used to confirm substitutions of quaternary amino groups on the amino sites of chitosan at 400 MHz (Bruker DMX-300 NMR spectrometer). Chitosan and HTCC were dissolved in 2% (w/w) DCl/D_2O at room temperature. As an internal reference, sodium 2,2-dimethyl-2-silapentanoate-5-sulfonate (DSS) was used. HTCC was dissolved in 0.1 mol/L HAc solution and the degree of quaternization (DQ) was determined by titrating the amount of Cl^- ions on the HTCC with aq $AgNO_3$ solution according to Fan et al. (2003). DQ is calculated as following equation:

$$\mathrm{DQ}\,(\%) = \frac{Vc/1000}{[(Vc/1000)+W_1(1-\mathrm{DD})/M_1+(W_1\mathrm{DD}-W_2)/M_2]} \times 100$$

where V and c are the volume and concentration of used $AgNO_3$ solution, respectively. DD is the degree of deacetylation of the test sample. M_1 and M_2 are the molecular weight of *N*-acetyl-glucosamine and *N*-deacetyl-glucosamine unit, respectively. W_1 is the weight of the test sample and W_2 is calculated by the following equation:

$$W_2 = \frac{VcM_3}{1000}$$

where M_3 is the molecular weight of the monomeric unit of quaternized chitosan.

Dried HTCC was accurately weighed and dissolved in 0.1 mol/L CH_3COONa–0.2 mol/L CH_3COOH solution and the intrinsic viscosity defined as $[\eta] = c(\eta_{\mathrm{red}}) \rightarrow 0$ was obtained with Ubbelohde-type viscometer at 25 ± 0.5 °C.

2.4. Preparation of HTCC/GP and CS/GP hydrogel

HTCC/GP hydrogel was prepared by the following steps. Briefly, HTCC (360.0 mg) was dissolved in 5.0 mL of 0.1 M aqueous lactic acid solution at room temperature. This solution was co-cooled in a refrigerator at 4 °C with a solution of 1000.0 mg of α-β-GP or β-GP in 5.0 mL of deionized water. Then the GP solution was dropped into the stirring HTCC solution in an ice bath. The obtained solution was stirred for 10 min to gain homogeneous mixture. The hydrogel was formed by heating HTCC/GP solution in a water bath at 37 °C for several minutes.

CS/GP hydrogels were prepared according to Chenite et al. (2000). Briefly, 200.0 mg of chitosan hydrochloride was dissolved in 9.0 mL of deionized water. A solution of 560.0 mg of β-GP in 1.0 mL water was dropped into the stirring chitosan solution in an ice bath. The subsequent procedures were the same as stated in preparation of HTCC/GP hydrogel.

2.5. Characterization of formed hydrogel

2.5.1. Characterization of thermosensitivity

The gelation point was determined by test tube inverting method (Chung et al., 2002). The obtained formulation in solution state (2.0 mL) was added into a tube (10 mL) with a rubber cap and kept in a water bath at 37 °C. At predetermined interval, the tube was take 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. The sol–gel transition behavior of HTCC/GP and CS/GP systems were further illustrated by viscosity measurement at 37 °C by Brookfield DV-E viscometer. Shear viscosity measurements were made at a fixed shear rate of 1 min^{-1} and the acquisition rate was set up at one point per 1 min.

2.5.2. Turbidity determination

The turbidity of HTCC/GP or CS/GP hydrogel was determined according to a modified method described by Berger et al. (2005). Briefly, the HTCC/GP or CS/GP solution was added into a quartz cell and absorbance was then measured at 620 nm with a SP-721 visible spectrophotometer (Shanghai Spectrum Instruments Co. Ltd., Shanghai, China) as a function of time at 37 °C. The turbidity was calculated from standard absorbance–turbidity curve with formazin suspension as standards. Formazin suspension was prepared by reacting hydrazine sulfate with hexamethylenetetrammonium, and standards of formazin turbidity units (FTU) was prepared by appropriate dilution.

2.5.3. Characterization of pH-sensitive swelling

Swelling degree (SD) of the hydrogel was measured according to Chen et al. (2004). The obtained solution was poured into a cylindrical mold, kept in a water bath (37 °C) for 2 h to form hydrogel. Then, the formed hydrogel was cut into small disks with about 10 mm of diameter, weighed and immersed in different buffer solutions with desired pH values at room temperature, respectively. At predetermined time intervals, the disks were taken out from the solution, gently wiped with filter paper to remove the surface solution, weighed and returned to the same container until equilibrium was achieved. SD was calculated using:

$$\mathrm{SD} = W_t / W_0$$

where W_0 is the weight of original hydrogel put in buffer and W_t is the weight of gel at different swelling time. The ionic strength of buffer was justified to 0.2 M by adding the required amount of NaCl.

2.5.4. Morphological studies

The surface feature of formed hydrogel after lyophilization was observed by JEM-6700F scanning electron microscopy (SEM, JEOL, Japan). The sample was fixed on a metal stub with conductive tape, and was coated with platinum under vacuum by an ion sputter (JFC-1600, JEOL, Japan).

2.6. Incorporation of DX and in vitro release study

DX with different amount was dissolved in deionized water and added into the stirring HTCC or chitosan solution (10 mL) with GP. Each sample of about 1000 mg was placed into 10 mL glass tube and incubated at 37 °C for 2 h to form hydrogel, and then 4.0 mL of buffer with desired pH value was added to each tube. At predetermined intervals, 1.0 mL of the medium was collected and replenished by 1.0 mL fresh buffer with the same pH value. The amount of released DX was analyzed at 484 nm by UV spectrophotometer (Ultrospec 2100 pro, Amershan Biosciences, USA), using buffer from blank hydrogel as control to erase the disturbance of the hydrogel itself.

2.7. Mass loss

The mass loss measurement was carried out as follows. Each HTCC/GP solution of about 1000 mg was added into 10 mL glass tube and heated at 37 °C for 2 h. Then 4.0 mL of buffer with desired pH value was added to each tube. At predetermined intervals, the gels were taken out from the medium, gently blotted, weighted and dried in an oven at 80 °C until the constant weight was gotten.

3. Results and discussion

3.1. Synthesis of HTCC

Fig. 2 shows the IR spectra of chitosan and HTCC to indentify the existence of quaternary amino groups on HTCC chains. In the spectrum of HTCC, the characteristic peak (1597 cm^{-1}) representing NH_2 deformation was weakened and an new peak positioned at 1481 cm^{-1} was appeared, which corresponded to an asymmetric angular bending of methyl groups of quaternary hydrogen. The characteristic peaks of primary alcohol and secondary alcohol between 1102 and 1082 cm^{-1} didn't change in HTCC comparing with chitosan that proved the introduction of quaternary amino groups at NH_2 sites on chitosan chains (Viviane et al., 2004). The ^1H NMR analysis of chitosan and HTCC were also carried out to prove the quaternization reaction. The chemical shift at 3.2 ppm was methyl group on the quaternary nitrogen and wasn't found in the spectrum of chitosan (Xu et al., 1997). DQ, intrinsic viscosity and solubility of obtained HTCC were different according to reaction conditions and listed in Table 1. It was concluded that as the reaction time was prolonged or the ratio of GTMAC to chitosan was elevated, DQ and solubility were enhanced whereas the intrinsic viscosity was decreased. This was because more quaternary amino groups were introduced into chitosan molecules with longer reaction time or higher ratio of GTMAC to chitosan. Due to hydration and strong steric hindrance of quaternary amino groups, it resulted in much better water solubility and lower intrinsic viscosity. The low viscosity of the HTCC solution allowed it to be an ideal candidate as injectable material. However, excess substituted quaternary amino groups (DQ > 65%) suppressed hydrophobic interaction between chitosan chains, and resulted in non-gelling solution at 37 °C. However, HTCC with DQ < 52% (3.6 wt.%) didn't dissolve completely in acidic solution, and wasn't suitable for injection. Therefore, in present study HTCC with DQ ranging from 52% to 65% was chose to prepare hydrogel.

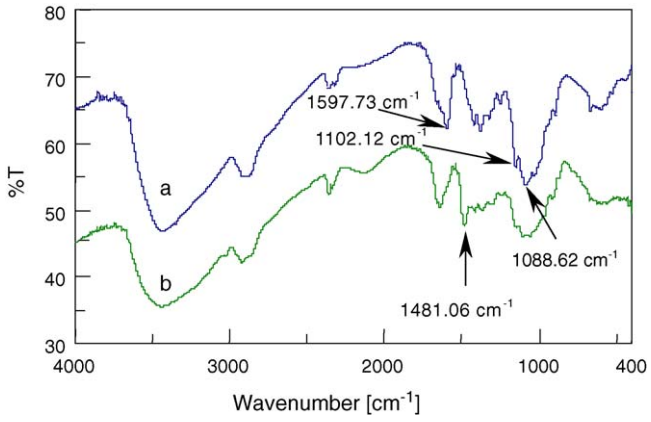

Fig. 2. FT-IR spectra for (a) chitosan and (b) HTCC.

3.2. Preparation and characterization of hydrogels

3.2.1. Thermosensitivity of the hydrogel

Fig. 3 shows the viscosity variation of HTCC/GP and CS/GP samples as a function of time at 37 °C. Both samples were solutions with low viscosity at room temperature, so they can be injected easily by a 20-gauge needle. When the solutions of HTCC/GP and CS/GP were heated to 37 °C, both of them were transformed to non-flowing hydrogels with increased viscosity in 10 ± 5 min. The gelation mechanism of CS/GP system was

Table 1
HTCC prepared with different reaction conditions

Sample no.	M_{GTMAC}/M_{CS}	Reaction temperature (°C)	Reaction time (h)	Intrinsic viscosity	DQ (%)	Solubility in 0.1 M acid solution[a]	
						Acetic acid	Lactic acid
1	4:1	80	4	436.42	51.21	+	−
2	5:1	80	4	436.02	53.71	+	+
3	6:1	80	4	435.02	52.58	+	+
4	5:1	70	4	442.06	45.62	−	−
5	5:1	90	4	347.68	61.81	+	+
6	5:1	80	2	380.09	50.59	+	−
7	5:1	80	6	346.72	65.89	+	+

[a] '+' means HTCC is soluble; '−' means HTCC is insoluble or partially soluble.

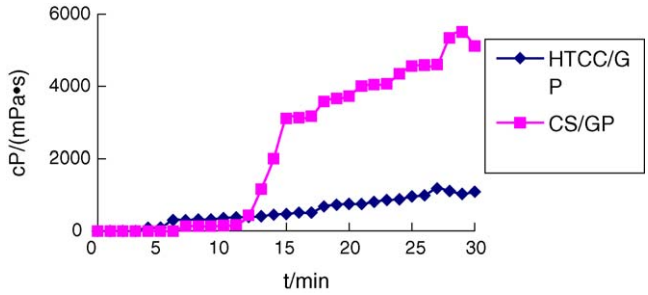

Fig. 3. Viscosities of HTCC/GP and CS/GP systems as a function of time at 37 °C.

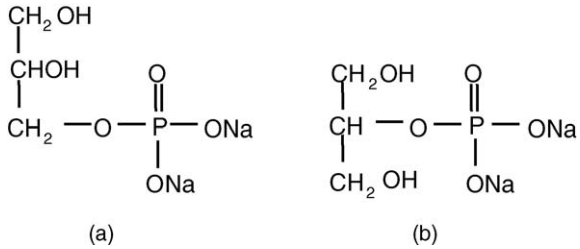

Fig. 4. Molecular structure of (a) α-GP and (b) β-GP.

already reported by Eve et al., which was mentioned in Section 1 (Eve et al., 2000). The gelation of HTCC/GP system should be similar with CS/GP, but it was more complex. In HTCC/GP system, the addition of GP into HTCC acid solution would neutralize the acid solution to around pH 7.4 because GP was a weak base. At this pH value, NH_2 groups on HTCC were unprotonated while quaternary amino groups were protonated. The static repulsive force between quaternary amino groups prevented the HTCC chains from aggregation. It is well known that polyols are able to stabilize some compounds in aqueous solutions and promote the formation of a shield of water around some macromolecules or polymer chains (Back et al., 1979). Glyceryl groups of GP also can promote the protective hydration of HTCC chains and keep the polymer chains stretched freely in solution at low temperature. Raising the temperature would increase the internal energy and break the hydrogen bonds between HTCC and water, so the water molecules bound to HTCC chains were released and moved with freedom. The movement of free water molecules increased the entropy in system, and in order to decrease the entropy change, hydrophobic side chains tended to aggregate and gelation happened. It was also showed in Fig. 3 that the viscosity of formed HTCC/GP hydrogel wasn't as high as that of CS/GP hydrogel. The result can be explained that the quaternization decreased crystallinity and improved water-solubility of chitosan which both contributed to reduce the viscosity. It should be mentioned that although the viscosity of HTCC/GP hydrogel decreased, it didn't damage its gelation capacity and the sustained drug release property.

In addition, it was observed that HTCC not only formed thermosensitive hydrogels with β-GP, but also with α-β-GP and showed similar thermosensitivity. The comparison of α-β-GP and β-GP in the hydrogel preparation was listed in Table 2. It was showed that α-β-GP had better gelation capacity compared with β-GP, in other words, the same amount of α-β-GP allowed the solution to gel quickerly at the same temperature. Because α-β-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, thus hydrophobic interaction force between chitosan molecules formed more easily. So the system with α-β-GP gelled quickerly at 37 °C. The molecular structures of α-GP and β-GP are sketched in Fig. 4. Therefore, α-β-GP was used to prepare HTCC/GP hydrogel system in the following experiments.

Table 2
Influence of α-β-GP and β-GP on HTCC/GP system thermosensitivity

HTCC concentration (wt.%)	GP type	GP concentration (wt.%)	pH	Gelation time at 37 °C (min)
3.6	α-β-GP	10.0	7.46	14
3.6	β-GP	10.0	7.40	33

The HTCC concentration affected the gelation time greatly (Table 3). The higher concentration resulted in gelation in shorter time. However, if the concentration was too high, HTCC was hard to completely dissolve and the solution was too viscous to be injected by syringe. Morever, the solution with high concentration of HTCC was unstable and gelled in a short period even when it was stored at room temperature or 4 °C. So 3.6 wt.% was chosen as an optimum concentration of HTCC. At this concentration, the formulation possessed proper thermosensitivity, good injectability and storage stability.

The influence of α-β-GP concentration on the thermosensitivity was also investigated. The result is showed in Table 4. With the increase of α-β-GP amount, more quaternary amino groups were combined with GP by ionic interaction. As a result, electrostatic repulsive force between quaternary amino groups was weakened, and the aggregation of polymer chains formed more easily. Therefore, the gelation time was markedly shortened. In the following experiments, α-β-GP with 10.0 wt.% was selected in gel preparation.

3.2.2. *Turbidity of the hydrogel*

Fig. 5 shows the formed HTCC/GP and CS/GP hydrogels in the tubes. HTCC/GP hydrogel was transparent and CS/GP hydrogel was white and turbid. The turbidity changes of both hydrogels at 37 °C as a function of time were investigated and are showed in Fig. 6. Turbidity of CS/GP hydrogel increased quickly at 4 min that showed the start of gelation, then slowed down at 10 min. The turbidity shifted from 83 to 1338 FTU in 1 h. The gelation of HTCC/GP hydrogel didn't cause great increase of turbidity, and the turbidity only increased from 80 to 286 FTU in 1 h.

The different turbidity changes can be explained according to Chen et al. (2004). Chitosan hydrochloride was the salt form of commercial available chitosan. The commercial chitosan was prepared by deacetylation of solid chitin particles, because the deacetylation was processed under heterogeneous condition, the product showed a block-type distribution which would form microdomains and induce light-scattering. And chitosan was a semicrystalline polymer due to strong hydrogen bond between the OH and NH_2 groups of chitosan, which showed reflection

Table 3
Influence of HTCC concentration on HTCC/GP system thermosensitivity

HTCC concentration (wt.%)	HTCC solution viscosity (mPa s)	α-β-GP concentration (wt.%)	pH	Gelation time at 37 °C (min)
4.0	148.34	10.0	7.62	12
3.6	55.73	10.0	7.46	14
3.0	28.36	10.0	7.48	17
2.5	18.58	10.0	7.46	37

Table 4
Influence of α-β-GP concentration on HTCC/GP system thermosensitivity

HTCC concentration (wt.%)	α-β-GP concentration (wt.%)	pH	Gelation time at 37 °C (min)
3.6	10.0	7.46	14
3.6	8.0	7.41	16
3.6	6.0	7.28	25

Fig. 5. The formed CS/GP and HTCC/GP hydrogels at 37 °C. Left: CS/GP hydrogel; right: HTCC/GP hydrogel.

at 19.7° and a relatively weak reflection at 10.2° by wide-angle X-ray diffraction measurement (Narayan et al., 2005). The chitosan molecules in the hydrogel tended to form microcrystalline domains that also caused light-scattering.

On the other hand, HTCC was prepared by reacting chitosan with GTMAC in water. GTMAC can easily react with compounds containing amino groups to introduce in quaternary amino groups (Xu et al., 1997). The quaternary amino groups had high steric hindrance that decreased the crystallinity and restricted the formation of microdomains. When light passed through HTCC/GP hydrogel, it wasn't scattered too much, allowing the hydrogel to be transparent. Further more, HTCC had good water solubility which would favor the hydrogel transparency. Narayan et al. (2005) developed PEG-grafted chitosan, in which the crystalline structures of chitosan and PEG were disrupted by the chemical bond between the two polymers and thus the water solubility of the material was improved. The hydrogel prepared from PEG-g-chitosan was also transparent, and the result proved that the decrease of crystallinity and increase of water solubility helped to reduce turbidity. The transparent hydrogel is suitable for some specific uses such as ocular drug formulation.

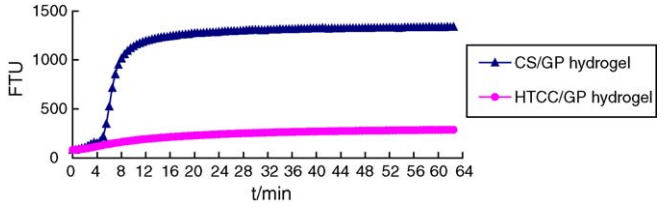

Fig. 6. Turbidity graphs of HTCC/GP hydrogel and CS/GP hydrogel as a function of time at 37 °C.

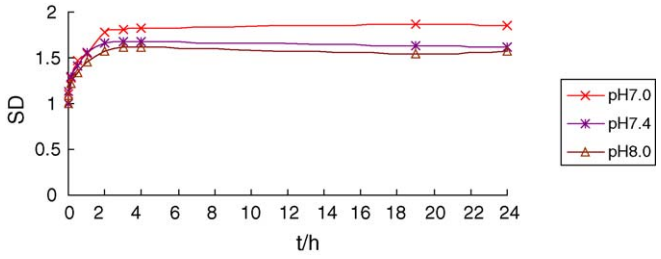

Fig. 7. Swelling degree (SD) of HTCC/GP hydrogels at different pH (in the case of pH 5.0, 6.0 and 6.4, hydrogels dissolved quickly and weren't showed in this figure).

3.2.3. *pH-sensitivity of the hydrogel*

The pH sensitivities of hydrogels formed by HTCC and chitosan hydrochloride were compared, and the results are showed in Figs. 7 and 8, respectively. HTCC/GP hydrogel dissolved promptly in acidic solution while it nearly kept original state in neutral or basic conditions. This phenomenon was understood easily because the quaternized chitosan chains bore positive charges, the hydrogel swelled quickly. The swelled network allowed more acid solution to enter the interior, therefore the protonated quaternized chitosan chains dissolved quickly. On the other hand, CS/GP hydrogels behaved quite different, they shrank at low pH. This phenomenon can be explained according to Chenite et al. (2001). Because excess GP was needed to

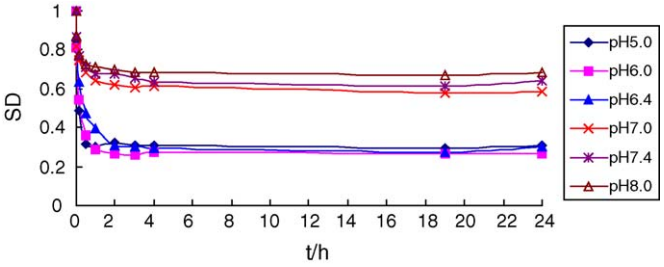

Fig. 8. Swelling degree (SD) of CS/GP hydrogels at different pH.

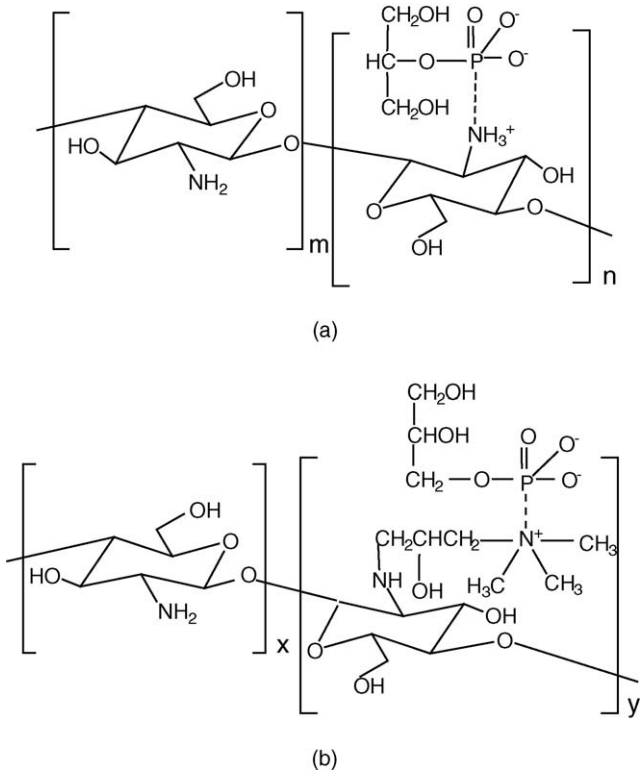

Fig. 9. Illustration of ionic interactions of (a) chitosan–β-GP and (b) HTCC–α-β-GP.

increase the gelation rate, Chenite et al. found part of β-GP was freely diffusible even after gelation in CS/GP system. The amino groups in CS/GP hydrogel were protonated and interacted with the free GP when the hydrogel was dipped in acid solution, that resulted in shrinkage of hydrogel. SD decrease of CS/GP hydrogels at basic solution might due to the weight loss of the hydrogel in solution, because ionic interaction between chitosan chloride and GP was destroyed in basic condition. The ionic interactions of chitosan–GP and HTCC–GP are showed in Fig. 9. SD of HTCC/GP and CS/GP hydrogels at different pH after immersion in buffer for 24 h are plotted in Fig. 10. It is clearly showed that HTCC/GP hydrogel has better pH-sensitivity.

The SEM photographs of the originally formed hydrogel are showed in Fig. 11, and those of the hydrogels after dipping in

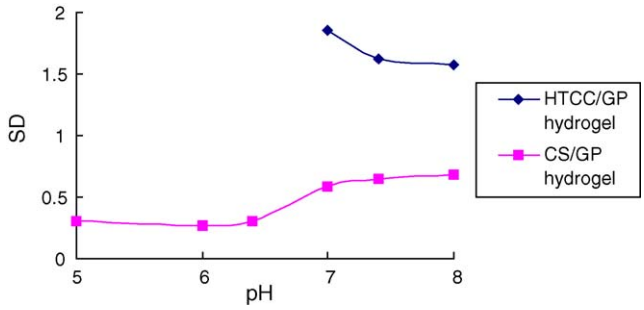

Fig. 10. Swelling degree (SD) of HTCC/GP and CS/GP hydrogels at different pH after immersion in buffer for 24 h (in the case of pH 5.0, 6.0 and 6.4, HTCC/GP hydrogels dissolved quickly and weren't showed in this figure).

buffer with different pH for 1 h are showed in Fig. 12. It was clear that the original HTCC/GP hydrogels showed more compact structure than CS/GP hydrogels. This was probably due to the decreased crystallinity and increased uniformity of HTCC than CS as stated in Section 3.2.2, which resulted in more uniform morphology in HTCC/GP hydrogel without microcrystalline domains and phase separation. Due to good hydrophilicity of HTCC, the cationic quaternized chitosan dissolved quickly in acidic solution. It was observed the HTCC/GP hydrogel dipped in acidic solution had large 'pores' in the network as showed in Fig. 12(a), which was formed by dissolution of HTCC. The morphology of HTCC/GP hydrogel dipped in basic solution didn't change apparently as showed in Fig. 12(b). However, the CS/GP hydrogel shrank and ejected most of the entrapped water in acidic condition. Therefore, the CS/GP hydrogel showed more compact morphology and smaller holes at lower pH (Fig. 12(c)) than at higher pH (Fig. 12(d)). In basic solution, the CS/GP hydrogel had porous structure due to the dissolution of chitosan as described before.

3.3. Incorporation of drug and its release in vitro

3.3.1. Effect of pH of the release buffer

A model drug DX was trapped in HTCC/GP and CS/GP gels respectively, and their cumulative release properties were investigated. DX is a cytotoxic anthracycline antibiotic widely used in the treatment of non-Hodgkin's lymphoma, acute lymphoblastic leukemia, breast carcinoma and several other types of cancers.

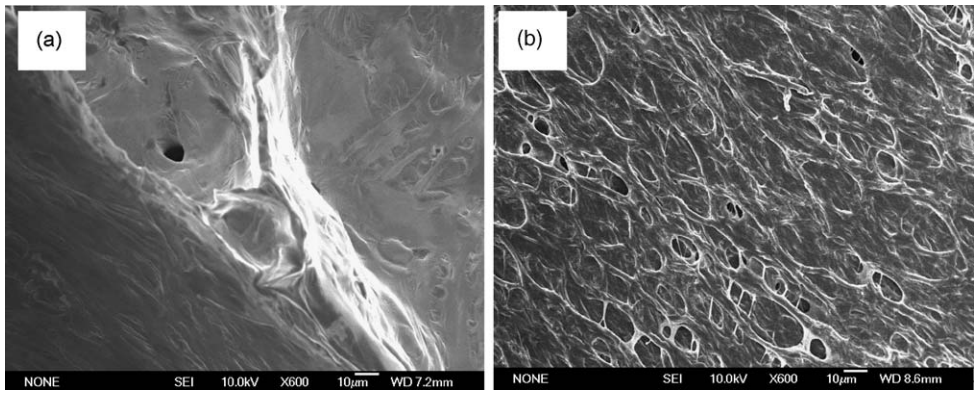

Fig. 11. SEM micrographs (×600) of originally formed hydrogels after heated at 37 °C for 2 h. (a) HTCC/GP hydrogel; (b) CS/GP hydrogel.

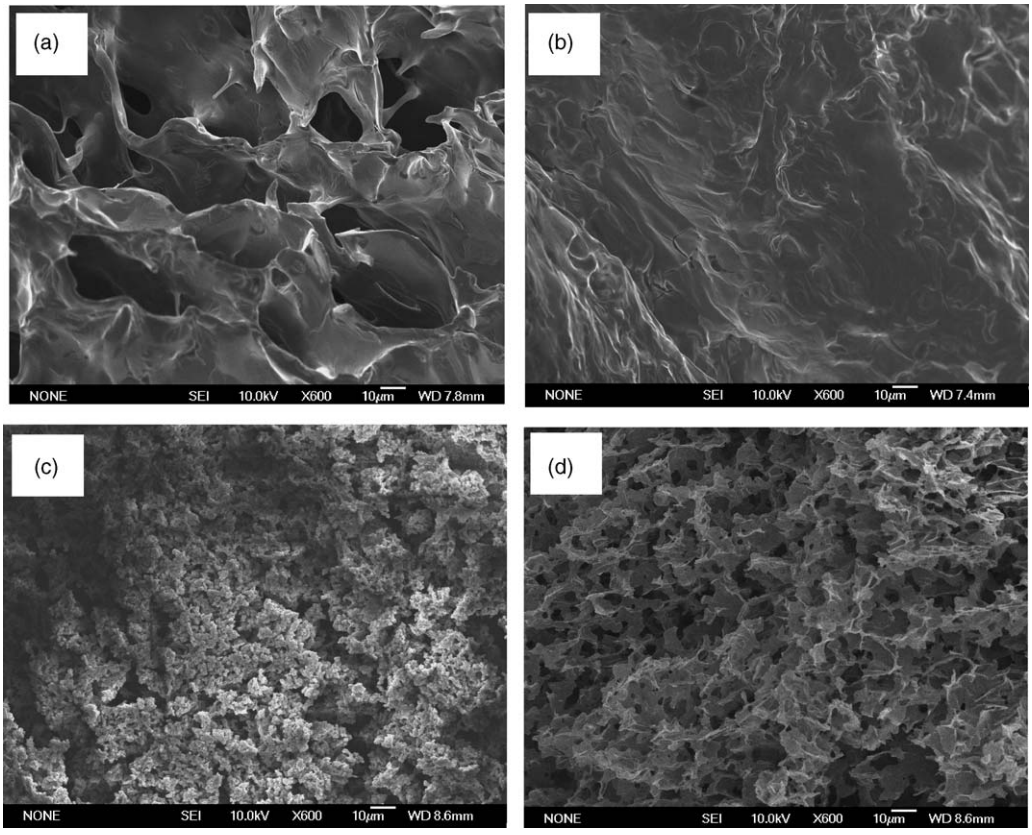

Fig. 12. SEM micrographs (×600) of formed hydrogels after immersion in buffer (pH 5.0 or pH 7.4, 37 °C) for 1 h. (a) HTCC/GP hydrogel at pH 5.0; (b) HTCC/GP hydrogel at pH 7.4; (c) CS/GP hydrogel at pH 5.0; (d) CS/GP hydrogel at pH 7.4.

DX easily dissolved in buffer solution within the pH range (pH 5–8) used in this study at 37 °C, whose molecular weight is 579.99 and half-life time in blood is 16.7–30 h. The chemical structure of DX is showed in Fig. 13. In clinic, its curative effect has been restricted by its dose-limited cardiotoxicity and the resistance developed by the tumor cells to this molecule after some time of treatment. In order to optimize its curative effect and reduce side-effect, it has been studied to deliver by several pH-sensitive formulations (Lee et al., 2005; Kang et al., 2003; Etrych et al., 2001; Leroux et al., 2001).

Due to the pH-sensitivity of HTCC/GP hydrogel, DX showed different accumulative release properties from it as illustrated in Fig. 14. At acidic condition, especially at pH 5 or pH 6, drug was released rapidly from the hydrogel, and almost 80% DX was released in the first 6 h at pH 5 buffer solution. The hydrogel completed the drug release at pH 5 between 1 and 2 days and at pH 6 about 4 days. However, at basic or neutral condition the drug was released slowly during the first 6 h and no apparent release was observed thereafter. At pH 7.4 buffer solution, about 13% loaded drug was released during the first 6 h, and only 9% was released in the following 4 days. When pH value of the buffer was lifted to 8, much slower release tendency was observed, only 9% DX was released during the first 6 h and after that 13% was released into medium in the following 4 days. The above release behaviors corresponded well with the pH-sensitivity of the hydrogel.

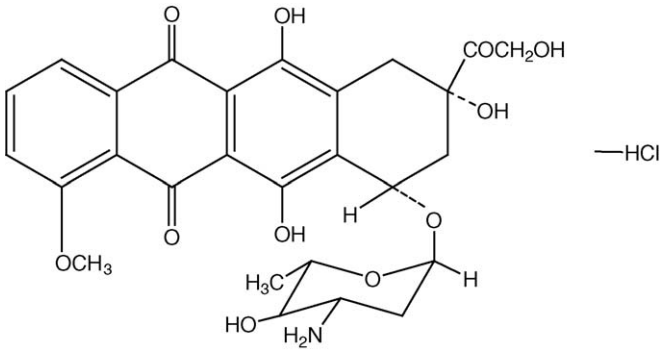

Fig. 13. The chemical structure of DX.

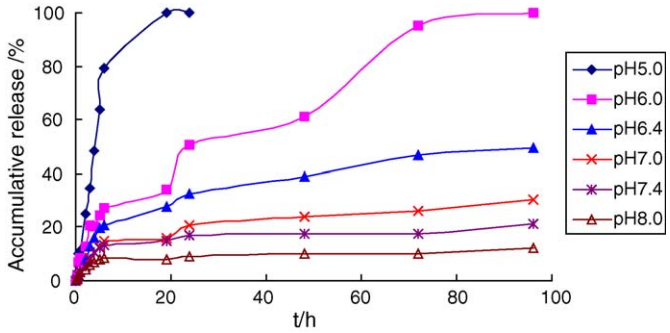

Fig. 14. Accumulative release of DX from HTCC/GP hydrogel in different pH.

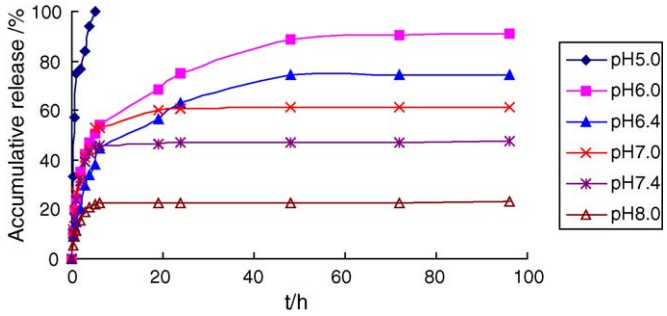

Fig. 15. Accumulative release of DX from CS/GP hydrogel in different pH.

The release behavior of CS/GP hydrogel is showed in Fig. 15. DX was released with high initial release and didn't show apparent release difference in acidic and basic circumstances during the first 6 h. After initial release, the release rate slowed down in basic condition and kept high in acidic solution. These phenomena also corresponded with the pH-sensitivity of the CS/GP hydrogel. The CS/GP hydrogel shrank quickly in acidic solution, so the trapped drug was ejected to outside with water together. In basic condition hydrogels had porous structures as showed in Fig. 12, which would favor drug diffusion from the inside to outside. Because diffusion was slower than ejection, after the initial release CS/GP hydrogel released drug with lower rate in basic solution than in acidic solution. Since drug was embeded in hydrogel, part of drug wouldn't be released until hydrogel was degraded. Compared with CS/GP hydrogel, HTCC/GP hydrogel behaved better as pH-sensitive drug carrier by lower initial release and more apparent pH-sensitive release property.

3.3.2. *Effect of DX concentration*

The effect of DX concentration trapped in hydrogel on release profile was investigated in pH 7.4 buffer at 37 °C. As showed in Fig. 16, the accumulative release profiles were similar except the initial burst. The initial burst decreased with increase of DX concentration. Approximately 23.86% of the drug was released from the hydrogel with 0.5 mg/mL concentration during the first 6 h. When the trapped drug concentration increased to 1 and 2 mg/mL, only 13.39% and 9.79% were detected in the supernatant during the first 6 h. The above phenomenon that the

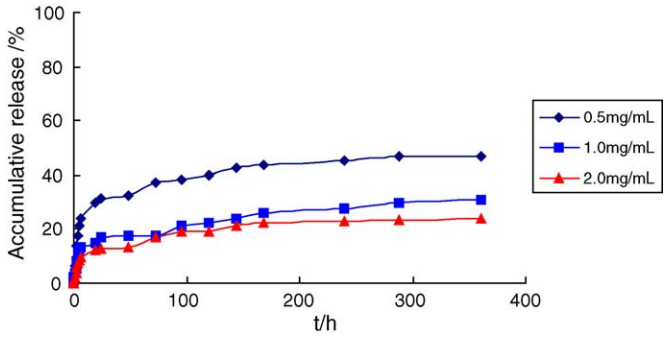

Fig. 16. Accumulative release of DX from HTCC/GP hydrogel with different drug concentration.

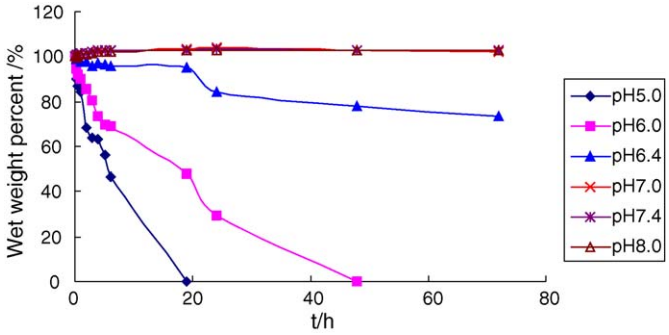

Fig. 17. Wet weight percent of HTCC/GP hydrogels as a function of immersion time in buffer with different pH at 37 °C.

release reate declined with the increase of drug concentration was contrary to usual concentration-dependence release profiles. In this study, because DX bore positive charged amino group, which might take part in the hydrogel formation by ionic interaction with phosphate groups. The reaction strengthened the crosslinked network and retarded drug release. In preparation process, it was found the system containing drug gelled in shorter time than the control without drug, which also proved DX participated in gel formation. Therefore, with more amount of DX added, more compact hydrogel structure was obtained and much slower drug release profile was observed.

3.3.3. *Mass loss of the hydrogel*

There were a few differences between pH-sensitivity graphs and drug release profiles of HTCC/GP hydrogel. For example, HTCC/GP hydrogel dissolved entirely at pH 5.0 within 0.5 h. However, drug wasn't released completely at pH 5.0 during this period. There are several reasons responsible for these differences. First, the drug release was studied at 37 °C that the heat would strengthen the hydrophobic interaction between chitosan chains and make the hydrogel more stable. Further more, the drug participated in hydrogel formation as mentioned above, which slowered dissolution rate. We investigated the mass loss of HTCC/GP hydrogel in different pH buffer at 37 °C to illuminate release profiles more accurately, and found that the mass loss graphs accorded well with drug release profiles. Figs. 17 and 18

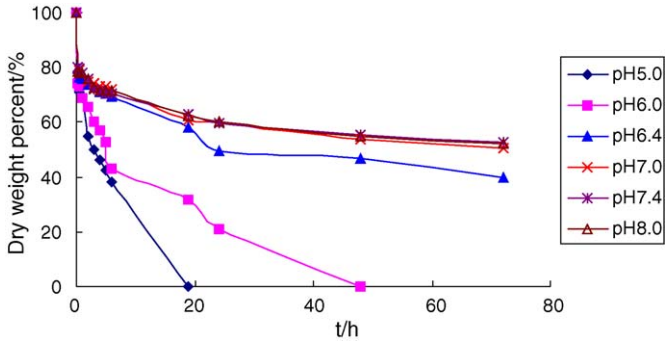

Fig. 18. Dry weight percent of HTCC/GP hydrogels as a function of immersion time in buffer with different pH at 37 °C.

demonstrate the wet weight percent and dry weight percent changes of HTCC/GP hydrogels as a function of time, respectively. Approximately 62% of the dry weight and 53% of the wet weight was lost in the first 6 h at pH 5.0. At this condition, 79% DX was released into the medium. At pH 7.4, hydrogels swelled to 102% with 30% dry weight loss, and only 13% of the drug was released from the hydrogel. The correspondence between mass loss curve and drug release profiles suggested that drug release in acidic circumstance was dominated by gel erosion, and in basic condition diffusion dominated the drug release.

4. Conclusion

The hydrogel was prepared by HTCC and β-GP or α-β-GP, which was injectable by thermosensitive sol–gel transition at body temperature. The gel can incorporate drug easily in solution state and was stable below or at room temperature, while it was transformed to transparent hydrogel at body temperature (37 °C) and released drug responding to surrounding pH values. DX as a model drug was incorporated in this hydrogel and its release profiles from HTCC/GP hydrogel were compared with those from CS/GP hydrogel. CS/GP hydrogel released drug with high initial release rate at both acidic and basic circumstances without apparent pH-sensitivity. HTCC/GP hydrogel showed better pH-sensitivity. The trapped drug was released quickly in acidic condition and quite slowly with low initial release in basic condition. HTCC/GP hydrogel can be used as pH-sensitive drug carriers or other stimuli-sensitive materials if it is combined with some reagents. For example, HTCC/GP hydrogel entrapped glucose oxidase may be used as glucose-sensitive insulin release system. At high glucose concentration, glucose oxidase transforms glucose to gluconic acid. As a result of low pH in the hydrogel, HTCC/GP hydrogel will dissolve and release trapped insulin quickly. Thus, the pH-sensitive HTCC/GP hydrogel containing glucose oxidase can control insulin release in response to the glucose concentration. The formed hydrogel is biocompatible and biodegradable, which will favor its use in pharmaceutical and medical fields. Moreover, the HTCC/GP hydrogel is transparent that facilitates its use in optical drug delivery systems or other special fields.

Acknowledgements

We would like to thank the financial support of the National Nature Science foundation of China under Contract 20125616 and 20221603. The support from Chinese Academy of Sciences is gratefully acknowledged.

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