Controlled Preparation of Physically Crosslinked Chitosan-*g*-Poly(vinyl alcohol) Hydrogel

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ABSTRACT: Physically crosslinked chitosan-*g*-poly(vinyl alcohol) hydrogels with controllable graft percent were prepared in three steps. Poly(vinyl acetate) (PVAc) was first grafted onto chitosan via radical copolymerization. Then the copolymer was converted into chitosan-*g*-poly(vinyl alcohol) by alcoholysis reaction. The graft percent of poly (vinyl alcohol) (PVA) could be tailored by the reaction conditions such as the solvent composition, the concentration of initiator and the amount of monomer added. Finally, Chitosan-*g*-PVA hydrogels were formed by freezing-thawing cycles. The structure of graft copolymers was verified with FTIR. Both XRD analysis and contact angle test showed that the difference of crystallinity and hydrophilicity among chitosan, chitosan-*g*-PVAc and chitosan-*g*-PVA was evident. It was found that the maximum swelling ratios of chitosan-*g*-PVA hydrogels containing 37.6% and 46.2% PVA were 3.48 ± 0.11 and 4.40 ± 0.14 at pH 1.2, 1.90 ± 0.13 and 2.80 ± 0.15 at pH 7.4, respectively. This suggested the hydrogel was pH-sensitive, and its swelling behavior could be tailored with the graft percent of PVA. J Appl Polym Sci 117: 2946–2950, 2010

Key words: chitosan-*g*-poly(vinyl alcohol); controllable synthesis; physical crosslinking; hydrogel

INTRODUCTION

Hydrogels are hydrophilic networks and capable of imbibing large amounts of water or biological fluids but insoluble due to the presence of chemical or physical crosslinks. Hydrogels resemble natural living tissue more than other class of synthetic biomaterials.[1] Consequently, biodegradable hydrogels are important scaffolds for tissue engineering and widely utilized as a matrix for drug delivery systems.[2,3]

Chitosan is obtained by alkaline deacetylation from chitin, the second most abundant biopolymer in nature. Owing to its nontoxicity, biocompatibility, biodegradability, and low production cost, chitosan is one of the most promising biomaterials.[4,5] Chitosan can form chemical or physical hydrogels. Various bifunctional agents such as epichlorhydrine, glutaraldehyde, and diethyl squarate are used to crosslink chitosan.[6] Unfortunately, these crosslinking reagents are generally considered to be toxic. Another drawback of covalent crosslinking is most of the amino groups of chitosan have reacted with the crosslinker.[4] Therefore, physical chitosan hydrogels are much more desirable. Physical chitosan hydrogels can be produced via the formation of ionic crosslinks by electrostatic interaction with metal ions or other biopolymers. The gelation of chitosan can also be implemented by tailoring the degree of acetylation to achieve the balance between hydrophilic and hydrophobic.[7] However, it can be anticipated that such a chitosan gel is weak, fragile, and ready to dissociate when the medium changed.

As well known, poly(vinyl alcohol) (PVA) is water-soluble and biocompatible. In addition, the mechanical strength of PVA is well enough. The use of PVA in medical and pharmaceutical applications is well documented.[8,9] Hydrogels that composed of PVA and chitosan will combine the advantages of both components. Usually, hydrogels that contain PVA and chitosan are formed by chemical crosslinking.[10,11] An interesting approach to prepare physical crosslinking hydrogels of chitosan is the complexation of chitosan/poly(vinyl alcohol).[12,13] But it is found that chitosan component will concentrate on the surface of the chitosan/poly(vinyl alcohol) blend hydrogel membrane,[14] i.e., phase separation will happen.

PVA hydrogels can be prepared via freezing/thawing process,[15,16] which is mild in the sense that the use of crosslinking agents and organic solvents can be avoided.[17] Freezing/thawing technique has been successfully applied to prepare physically crosslinked starch-*g*-PVA hydrogel in our lab.[18] Thus, it is anticipated that the graft copolymer of chitosan and PVA can also form hydrogels in this way. Herein, chitosan-*g*-PVA of different graft percent is prepared and used to form physical chitosan-

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g-PVA hydrogel by the same process. The pH-responsive property of the chitosan-*g*-PVA hydrogels is also examined.

EXPERIMENTAL

Materials

Chitosan (minimum 90% deacetylation) was purchased from Shanghai Chemical Agents Co. (Shanghai, China) and dried before use. Vinyl acetate (Shanghai Chemical Agents Co., Shanghai, China) was purified by distillation. Potassium persulphate was purified by recrystallization from distilled water. *N*,*N*-Dimethylformamide (DMF), acetic acid, benzene, methanol, 95% ethanol, and sodium hydroxide were all analytical grade reagents and used as received.

Controlled synthesis of chitosan-*g*-PVA

The pure chitosan-*g*-PVA was prepared according to the literature[19] with some major improvements. Four grams of chitosan was dissolved in the mixture of V mL N,N-dimethylformamide and $(100 - V)$ mL 5% acetic acid at 70°C. Nitrogen purging for 10 min was carried out to remove the dissolved oxygen from the solution. A predetermined amount of potassium persulfate was added and allowed to react for 20 min. The reaction temperature was modulated to 65°C. Then 4–10 mL vinyl acetate (VAc) was added in drops within 50 min and allowed to react for another 2 h. The reaction mixture was poured into a beaker. NaOH was added until the pH of solution was ~10–11 to precipitate the product completely. After washing with distilled water several times, it was filtered and dried. The pure chitosan-*g*-PVAc was obtained by extracting the crude product with benzene for 24 h. Then, chitosan-*g*-PVA was derived from alcoholysis of chitosan-*g*-PVAc. A mixture of 4.0 g dried chitosan-*g*-PVAc powder and 40 mL 3% NaOH/methanol was kept refluxing for 2 h. The product, yellow powder, was filtered and dried in an oven at 50°C to constant weight.

The graft percent of PVAc was calculated as $G\% = (W_1 - W_0)/W_0 \times 100$, where W_0 and W_1 were the weight of chitosan and chitosan-*g*-PVAc, respectively. And the graft efficiency of PVAc was calculated as $E\% = (W_1 - W_0)/(W - W_0) \times 100$, where W was the total weight of chitosan, homopolymer, and chitosan-*g*-PVAc.

Infrared spectroscopy

Powdered chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA were, respectively, mixed with dry KBr and compressed into disk. Then, Fourier Transform Infrared (FTIR) spectra of the samples were recorded using a Nexus 470 FTIR spectrophotometer.

X-ray measurements

X-ray diffraction profiles of chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA were collected with a Bruker D8-Advanced diffractometer using Nickel-filtered Cu Kα radiation ($\lambda = 0.15406$ nm) and scanned from 2° to 60° at a scan speed of 3°/min.

Hydrophilicity evaluation

Chitosan and chitosan-*g*-PVAc were, respectively, dissolved in 5% acetic acid, and chitosan-*g*-PVA was dissolved in the mixture of DMF and 5% acetic acid (1 : 7, *v* : *v*) to obtain 1 wt % solution. The solutions were cast onto glass, dried, extracted with 95% ethanol to remove the remained solvents and dried again.

The hydrophilic properties of chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA membranes were examined with water contact angle, which was measured with a JC2000A digital contact angle analyzer and an average of five measurements was taken.

Preparation of chitosan-*g*-PVA hydrogel

One gram of chitosan-*g*-PVA of different graft percent was dissolved in 10 mL 5% acetic acid and poured into a mold, respectively. Then the chitosan-*g*-PVA hydrogels were obtained by subjecting the solutions to seven repeated freeze/thaw cycles, 22 h at −12°C and 2 h at ambient temperature (25 ± 1°C). The hydrogels were soaked with distilled water for several times until it was neutral.

Swelling behavior of chitosan-*g*-PVA hydrogels

Dried rectangular chitosan-*g*-PVA hydrogels (0.020 g) with graft percent of 37.6% and 46.2% were placed in vials that contained 3 mL HCl (0.1 *M*, pH 1.2) and maintained at 37°C until the weight of samples were constant. Then the samples were removed and immersed into phosphate buffer saline (PBS) (0.1 *M*, pH 7.4) at the same temperature. At timed intervals, the samples were removed, blotted up the surface liquid of the samples with filter paper and weighed. The swelling ratio (SR) of chitosan-*g*-PVA hydrogels could be calculated as $SR = W_{wet}/W_{dry}$, where W_{wet} and W_{dry} were the weight of swollen and dry chitosan-*g*-PVA hydrogels, respectively. An average of triplicate measurements was taken.

RESULTS AND DISCUSSION

Controlled preparation of chitosan-*g*-PVA

To enhance the graft copolymerization of VAc onto chitosan chain, the monomer should be dispersed in the solution uniformly. In view of VAc was hydrophobic, DMF was added to promote the miscibility between VAc and acid aqueous chitosan solution. Since chitosan could not be dissolved in DMF, less DMF was preferred. As expected, it was found that the graft percent increased to a maximum as the volume of DMF increased to 10 mL and then decreased (Fig. 1). More DMF would limit chitosan dispersed homogeneously in the solution, which would hinder the graft copolymerization while benefit to the homopolymerization of VAc. In other words, the solvent components could be used to adjust the graft percent.

To ensure enough radicals produced on the macromolecule chain, chitosan should be initiated for some time with proper concentration of initiator. Then the active center formed initiated VAc to perform the graft copolymerization.[20,21] However, more initiator would also increase the probability of homopolymerization. In consequence, the graft percent decreased (Fig. 2). Thus, 0.0372 mol/L was adopted as the optimal concentration of initiator, potassium persulphate.

It was well known that the concentration of monomer would affect the graft percent obviously. As expected, the graft percent increased with the increase of the concentration of VAc (Fig. 3). Meanwhile, the probability of homopolymerization was increased, which resulted in the decrease of graft efficiency.

Because the alcoholysis reaction of chitosan-*g*-PVAc was the conversion of the side groups,[22] the graft percent of chitosan-*g*-PVA could be regarded as the same to that of chitosan-*g*-PVAc. In other words, the factors mentioned above were still effective to tailor the graft percent of chitosan-*g*-PVA.

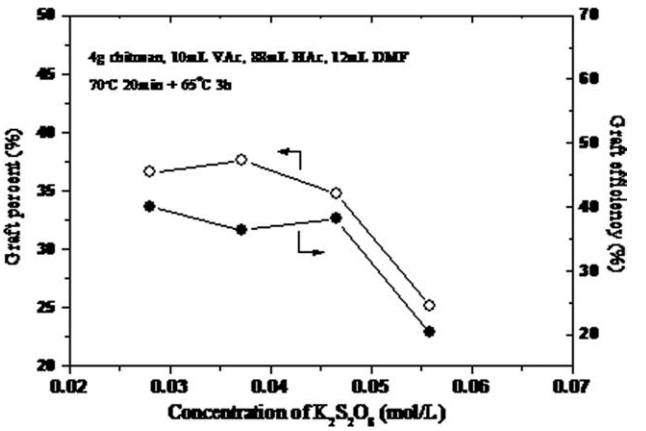

Figure 2 The effect of the concentration of initiator on the graft copolymerization.

Characterization of chitosan-based copolymer

The structure of the graft copolymers was analyzed with FTIR. Figure 4 showed the FTIR spectra of chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA. On the FTIR spectra of chitosan, the characteristic peaks of amide group exhibited at 3401, 1654, and 1600 cm^{-1}. While the bands showed at 2922, 2860, 1423, and 1380 cm^{-1} were attributed to the absorption of C—H bonds on the chitosan chain. It was found that two additional characteristic peaks appeared at 1739 and 1246 cm^{-1} on the FTIR spectra of the extracted graft copolymer of chitosan and VAc, which was attributed to the absorption of carbonyl group and C—O bond, respectively. In other words, FTIR analysis results demonstrated that chitosan-*g*-PVAc was obtained.[19] In addition, an absorption peak appeared at 3401 cm^{-1} on the FTIR spectra of chitosan-*g*-PVAc, which indicated that the graft copolymer still contained $-NH_2$ groups.

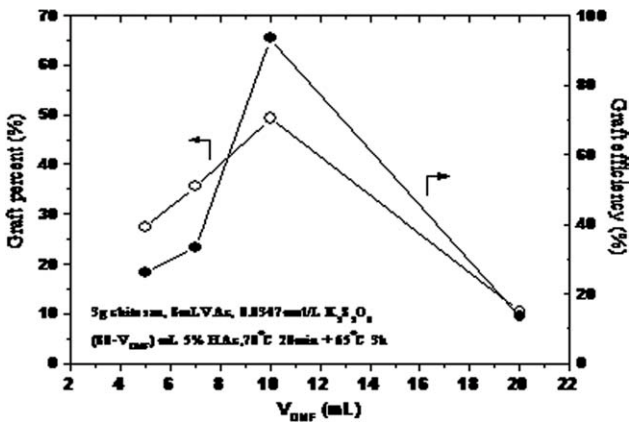

Figure 1 The effect of solvent composition on the graft copolymerization.

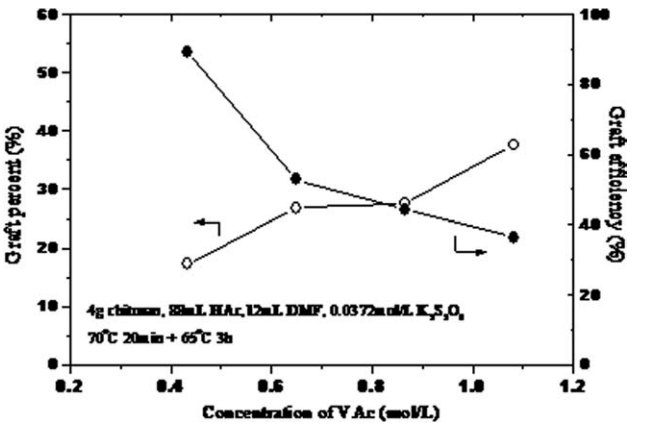

Figure 3 The effect of the concentration of monomer on the graft copolymerization.

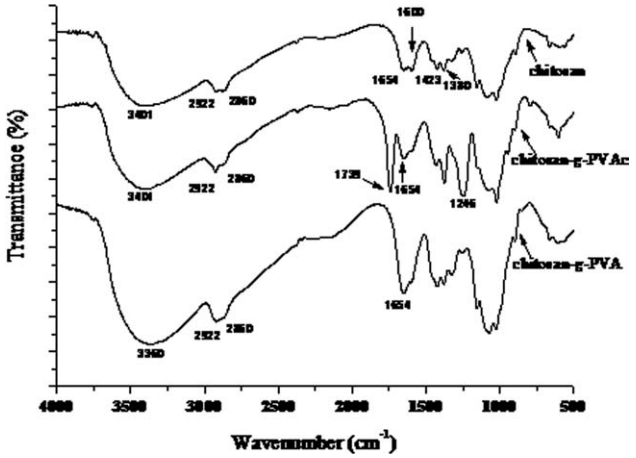

Figure 4 FTIR spectra of chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA.

After alcoholysis of chitosan-*g*-PVAc, the absorption band appeared at 1739 cm^{-1} disappeared, which suggested that the —$OCOCH_3$ groups were completely converted into hydroxyl groups. A wide peak showed at 3360 cm^{-1} on the FTIR spectrum of the alcoholysis derivate also indicated that hydroxyl groups were formed. Thus, the expected chitosan-*g*-PVA was prepared.

Two peaks exhibited at 9.7° and 20.0° on the XRD patterns of chitosan, chitosan-*g*-PVAc (graft percent of 37.6%) and its derivate chitosan-*g*-PVA (Fig. 5). The crystallinity of chitosan, chitosan-*g*-PVAc, and chitosan-*g*-PVA, could be calculated as the ratio of the intensity of sharp peaks to the total intensity, were approximately 63.0%, 41.8%, and 66.4%, respectively. Chitosan and PVAc were semicrystalline and amorphous, respectively. Thus the crystallinity of chitosan-*g*-PVAc was lower than that of chitosan. PVA was semicrystalline.[23] A strong

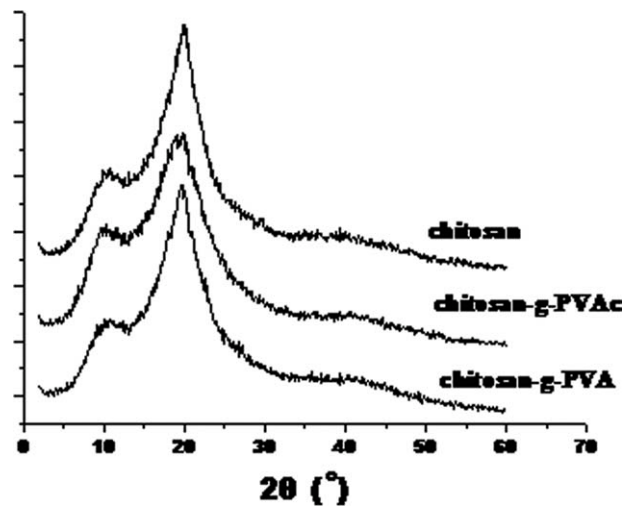

Figure 5 XRD profiles of chitosan, chitosan-*g*-PVAc and chitosan-*g*-PVA.

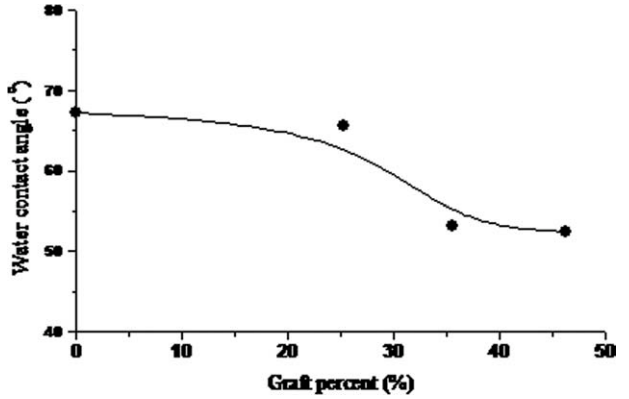

Figure 6 The effect of graft percent on the hydrophilicity of chitosan-*g*-PVA.

interaction would be formed between —OH and —NH_2 groups in the chains. Both of them rendered the crystallinity of chitosan-*g*-PVA were higher than that of chitosan.

As it was known, both chitosan and PVA were hydrophilic while PVAc was hydrophobic. It could be anticipated that chitosan-*g*-PVA was hydrophilic, while chitosan-*g*-PVAc was more hydrophobic than chitosan. The water contact angles of chitosan, chitosan-*g*-PVAc (graft percent of 25.2%), and the corresponding chitosan-*g*-PVA were 67.2, 73.2, and 65.5°, respectively. It was the introduced PVAc side chains that decreased the hydrophilicity of chitosan. Chitosan-*g*-PVA was more hydrophilic than chitosan, which was consistent with the fact that chitosan was less hydrophilic than PVA.[15] In addition, the hydrophilicity of chitosan-*g*-PVA increased with the increase of graft percent (Fig. 6).

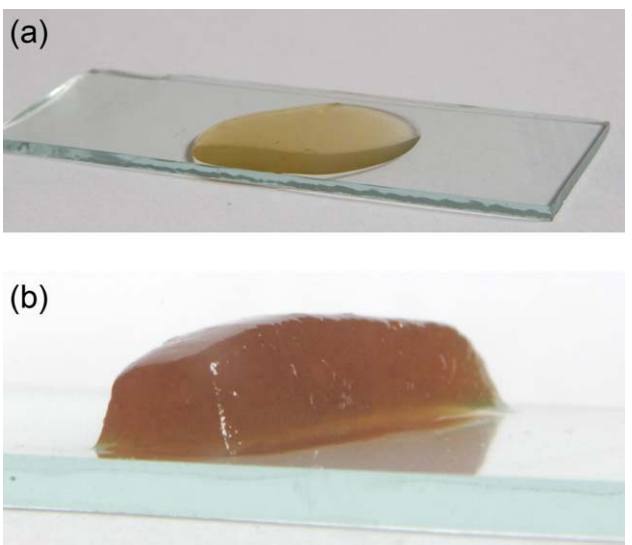

Figure 7 The feasibility of forming hydrogel of chitosan (a) and chitosan-*g*-PVA (b). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

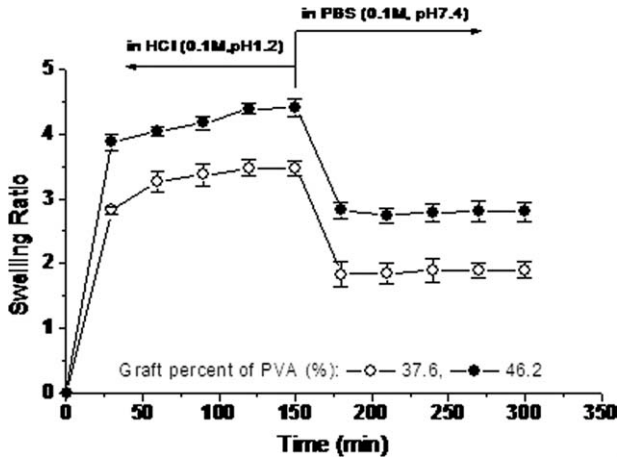

Figure 8 Swelling behavior of chitosan-*g*-PVA hydrogels in hydrochloric acid (HCl); (0.1 *M*, pH 1.2) and phosphate buffer saline (PBS); (0.1 *M*, pH 7.4) at 37°C.

Preparation of chitosan-*g*-PVA hydrogel

To prepare a novel physically crosslinked hydrogel, an aqueous solution of chitosan-*g*-PVA was subjected to seven freeze/thaw cycles. As expected, soft and elastic chitosan-*g*-PVA hydrogels were formed. On the contrary, chitosan alone remained viscous liquid after similar treatment (Fig. 7). As chitosan-*g*-PVA still contained $-NH_2$ groups, the swelling behavior of the gels was investigated in acidic and neutral mediums.[24] It was found that the maximum swelling ratios of chitosan-*g*-PVA hydrogels containing 37.6% were 3.48 (standard deviation 0.11, $n = 3$) at pH 1.2 and 1.90 (standard deviation 0.13, $n = 3$) at pH 7.4 (Fig. 8), respectively, which suggested the hydrogel was pH-responsive. The $-NH_2$ groups of chitosan-*g*-PVA would transfer into $-NH_3^+$ groups in acidic medium, and the electrostatic repulsion force between cationic chains resulted in higher swelling ratio. In addition, the swelling ratios of chitosan-*g*-PVA hydrogels of containing 37.6 and 46.2% PVA were 3.48 and 4.40 at pH 1.2, 1.90, and 2.80 at pH 7.4, respectively, which indicated the swelling behavior relied on the graft percent of PVA. The repeated freezing-thawing gelation process resulted in the formation of a porous network in which PVA crystallite domains acted as junction points.[23] Thus, the swelling ratio of the hydrogel increased with the increase of graft percent of PVA.

CONCLUSIONS

A novel physically crosslinked chitosan-*g*-PVA hydrogel with controllable graft percent has been prepared via radical graft copolymerization, alcoholysis reaction and freezing-thawing gelation.

The graft percent of PVA can be tailored with the conditions such as the solvent composition, the concentration of initiator, and the amount of monomer. The aqueous solution of chitosan-*g*-PVA of different graft percent, derived from chitosan-*g*-PVAc, is subjected to several freezing-thawing circles to form physically crosslinked hydrogel. It is found that the swelling-property of the chitosan-*g*-PVA hydrogel depended on the graft percent of PVA and shows pH-sensitive.

Chitosan-*g*-PVA hydrogels will keep the advantages of the components such as biocompatible, pH-responsive, mechanical, and antibacterial properties. As chitosan is biodegradable, the chitosan-*g*-PVA hydrogel will be a good candidate for biomedical applications if the side chains of PVA are short enough.

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