Preparation and Characterization of Poly(ethylene glycol) Crosslinked Chitosan Films

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ABSTRACT: Poly(ethylene glycol) (PEG) crosslinked chitosan films with various PEG to chitosan ratio and PEG molecular weight were successfully prepared via the epoxy-amine reaction between chitosan and PEG-epoxy. The thermal and mechanical properties and swelling behavior were studied for the PEG crosslinked chitosan films. The mechanical strength of chitosan films were greatly enforced by the introduction of PEG-epoxy, achieving an elongation of about 80%. It was found that the crosslinked chitosan films form hydrogel in water, achieving a swelling ratio higher than 20 times of original weight. The swelling behavior of chitosan films relied greatly on the molecular weight of the crosslinker PEG-epoxy and the weight percent of PEG-epoxy. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3823–3830, 2008

Key words: chitosan; poly(ethylene glycol); crosslinking; mechanical properties; hydrogel

INTRODUCTION

Hydrogels have the network structure of hydrophilic polymers, and have the capability of absorbing large amounts of water without losing their three-dimensional structure.[1,2] Many kinds of hydrogels are generally formed by chemically crosslinking water-soluble polymers via radical polymerization of hydrophilic monomers in the absence or in the presence of crosslinking agent.[3] Now, polymer gels are of great interest in various industrial, biotechnological, and biomedical applications. For example, polymer hydrogels are used as drug delivery matrices[4–9] and tissue engineering scaffolds.[10–13] In these applications, the gel characteristics are designed for particular applications. The changes in network swelling, network mesh size, and diffusion coefficient of a drug within the network are of interest.[14–17] In tissue engineering, polymer hydrogels provide a unique, largely aqueous environment for three-dimensional chondrocyte culture that facilitates nutrient transport, yet provide an elastic framework dictating tissue shape and supporting external loads.[18,19]

Hydrogels derived from naturally occurring polymers are able to address a number of interesting biomedical applications employing the materials as temporary implants. The degradation capability of biocompatible hydrogel can be engineered to produce optimum results in a particular application, and it provides another device parameter for engineers to tailor and optimize. Applications of the hydrogels in protein drug delivery and tissue engineering have been presented.[20–23]

Chitosan, poly[β(1-4)-2-amino-2-deoxy-D-glucose], is obtained via a deacetylation procedure from chitin, the most abundant natural structural polysaccharide after cellulose. Chitosan has been intensely investigated for the development of novel materials and sorption systems due to the presence of free amine groups in its structure, which allow chemical modification.[24] Recently, chitosan has attracted much interest in the biomedical industry because of its excellent biodegradability, biocompatibility, antimicrobial activity, and accelerated wound-healing properties.[25–28] Because of its unique polymeric cationic character, net negatively charged compounds such as DNA, glycosaminoglycans, and most of the proteins can bind to chitosan without the use of harsh and denaturing organic solvents, such as methylene chloride. Therefore, chitosan has been extensively examined in the pharmaceutical industry for its potential use in the development of a controlled release implant system.[28–30]

Poly(ethylene glycol) (PEG) is an effective biomaterial as it is hydrophilic, less toxic, and has good tissue biocompatibility.[31] PEG has been widely incorporated with hydrolysable polyrotaxane, proteins, poly(α-hydroxy acids), or gelatin to form hydrogels.[32–36] Bhattarai et al. has investigated the sol–gel transition property of PEG-grafted chitosan synthesized via an aldehyde–amine reaction.[37]

Chitosan is expected to be used in various medical applications because of the biocompatibility. But chitosan is difficult to apply to tissue engineering, and

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artificial scaffolds, because the chitosan film is very rigid and brittle. On the other hand, the hydrogel is flexible and is expected to be applicable to some medical devices.

The purpose of this study is to prepare the chitosan hydrogel with improved mechanical property. By forming the hydrogel, the application range of chitosan shall be expanded. Nowadays, many kinds of chitosan hydrogel have been developed, and they are mostly formed from grafted polymers. In general, the mechanical property of the grafted polymer hydrogel is not good because of the random structure. On the other hand, because the hydrogel formed from crosslinking polymer has well-regulated network structure, the mechanical property of the hydrogel is good. So the hydrogel formed from crosslinked polymer is expected to apply to artificial scaffolds, and so on.

In this work, the synthesis of a biodegradable chitosan-based hydrogel was achieved via a traditional epoxy-amine reaction. The crosslinking chitosan by PEG will be confirmed by differential scanning calorimetry (DSC) measurement. We shall investigate some important factors controlling the properties of chitosan hydrogel, such as the number of crosslinks and the chain-length of PEG. The effects of the molecular weight of PEG and the content of diepoxyPEG in the hydrogel on the properties of the hydrogel will be estimated. The thermal properties and the morphology of hydrogel samples are studied by thermal gravimetry analysis (TGA) and scanning electron microscopy (SEM). The swelling behavior of the hydrogels is estimated in aqueous buffer solution. The mechanical properties are determined by the tensile test.

EXPERIMENTAL

Materials

Chitosan powder was kindly supplied by Yaidzu Suisan, Shizuoka, Japan, and used as received. The degree of deacetylation (GluN%) was determined to be 59% by FTIR spectroscopy. Three PEG samples, PEG1000 (M_w = 1000), PEG2000 (M_w = 2000), and PEG4000 (M_w = 4000), were purchased from Nacalai Tesque, Kyoto, Japan, and used without further purification. Sodium hydride (NaH) and epichlorohydrin were purchased from Kanto Kagaku, Japan and used as received. The diepoxyPEGs were synthesized according to the method reported by Laine et al.[38]

Synthesis of PEG crosslinked chitosan

The chitosan (1.0 g) was dissolved in 50 mL of 0.4% acetic acid. DiepoxyPEG 0.3–1.8 g dissolved in water was added into the chitosan solution drop wisely.

TABLE I
Sample Code, the Molecular Weight of DiepoxyPEGs, and the Amount of DiepoxyPEGs and Chitosan

Sample name	Molecular weight of diepoxyPEG (g/mol)	Amount of chitosan (g)	The amount of diepoxyPEG (g)
23%PEG2000-Chitosan	2,000	1.0	0.3
38%PEG2000-Chitosan	2,000	1.0	0.6
47%PEG2000-Chitosan	2,000	1.0	0.9
55%PEG2000-Chitosan	2,000	1.0	1.2
47%PEG1000-Chitosan	1,000	1.0	0.9
47%PEG4000-Chitosan	4,000	1.0	0.9
31%PEG1000-Chitosan	1,000	1.0	0.45
61%PEG4000-Chitosan	4,000	1.0	1.8

The chitosan and diepoxyPEG were reacted at 80°C for 24 h under the magnetic stirring. The sample code and the reactant compositions are shown in Table I. In this study, the effects of the molecular weight of PEG and the number of crosslinks on the properties of hydrogels were investigated. So, we studied the effects of diepoxyPEG/chitosan ratio and the molecular weight of diepoxyPEG on some physical properties of the hydrogel.

Preparation of shape-uniform hydrogel film

During the crosslinking reaction of diepoxyPEG with chitosan, the solution became viscous gradually by forming the hydrogel. The gelling solution was transferred into the glass dish covered and sealed by another glass dish with the same size. The dish was incubated in oven at 80°C for 24 h. After the reaction, the covering dish was detached and the hydrogel in the glass dish was dried at room temperature for 3 days. After the hydrogel was dried, the shape-uniform and transparent hydrogel film was obtained.

Characterization

^{1}H NMR spectra were measured at room temperature in $CDCl_3$ solution on a Bruker Avance 600 MHz spectrometer with 30° pulse, 3.7-s pulse repetition time, 32 K data points, and 256 FID accumulations.

DSC measurements were performed on a Seiko DSC-220U instrument with a SSC-5300 control system (Seiko Instruments, Tokyo, Japan). The sample (3–5 mg) was heated from −50 to 180°C at a heating rate 10°C/min. The melting enthalpy (ΔH) of PEG was determined from the first heating scan.

TGA measurements were carried out with a Seiko TG/DTA 220U with the Exstar 6000 Station (Seiko Instruments). The samples (5–10 mg) were heated from 25 to 500°C at a heating rate 5°C/min.

The swelling behavior of the hydrogel was observed in water and acetic acid buffer solution at

pH 5.0. The dried sheets with the size of about 20 × 20 mm^2 were conditioned in a vacuum oven for 24 h. After the dried sheets were weighed, they were conditioned at 20–25°C in the buffer solution and the time dependence of their weights were analyzed. The water content of the samples was determined according to the equation:

$$\text{Water content } (\%) = (W_t - W_0)/W_0 \times 100\ (\%) \quad (1)$$

Here, the W_t and W_0 represent the weights of swollen and dry state samples, respectively.

The tensile tests for the samples were carried out using a universal testing machine EZ Tester (Shimadzu, Kyoto, Japan). The gauge length and crosshead speed were 22.25 mm and 5 mm/min, respectively. The films were cut into the standard tensile samples from a dumbbell-shaped knife. The testing hydrogel films contained 15 ± 1% water at 22 ± 3°C.

SEM observation of the morphology for the hydrogel sample was carried out on a SEM JSM-5200 (JEOL, Japan). The SEM sample was prepared by freeze-drying method. The sample was frozen at −70°C in refrigerator and was dried under vacuum. After that, the sample was coated with gold.

RESULTS AND DISCUSSION

Figure 1 shows the shape-uniform chitosan hydrogel film, indicating high transparency. The shape of the chitosan hydrogel film, that ordinarily tends to be non-uniform, was succeeded to make uniform by our preparation method.

Figure 2 shows the ^1H NMR spectrum of diepoxyPEG with the molecular weight 2000 synthesized by the method of Laine et al.[38] The peaks in the NMR spectrum were ascribed according to the assignments made by Laine et al., indicated as a, b, c, d, e (Scheme 1 and Fig. 2), confirming the production of the diepoxy PEG.[38] The yield of this synthesis was as high as 95% and the conversion was almost 100%.

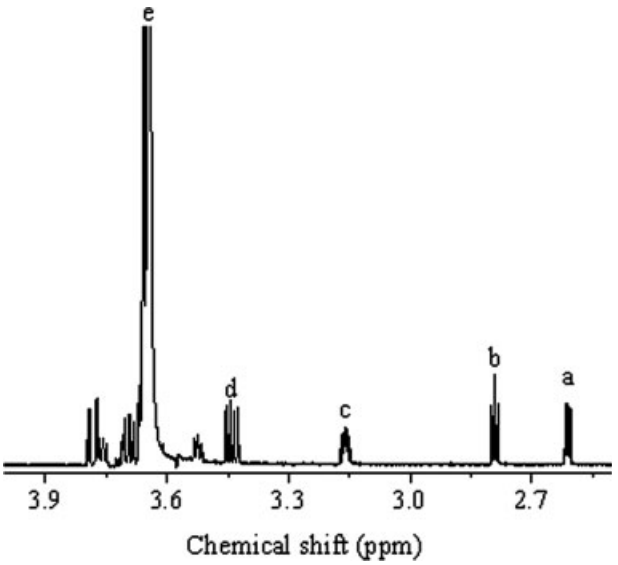
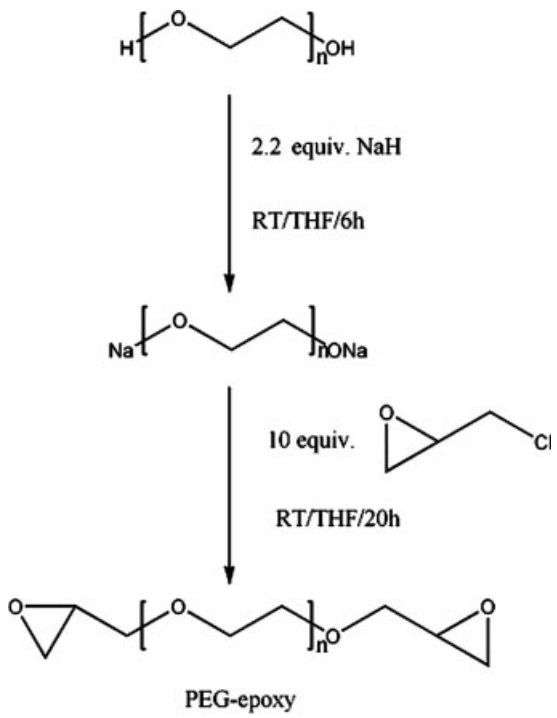


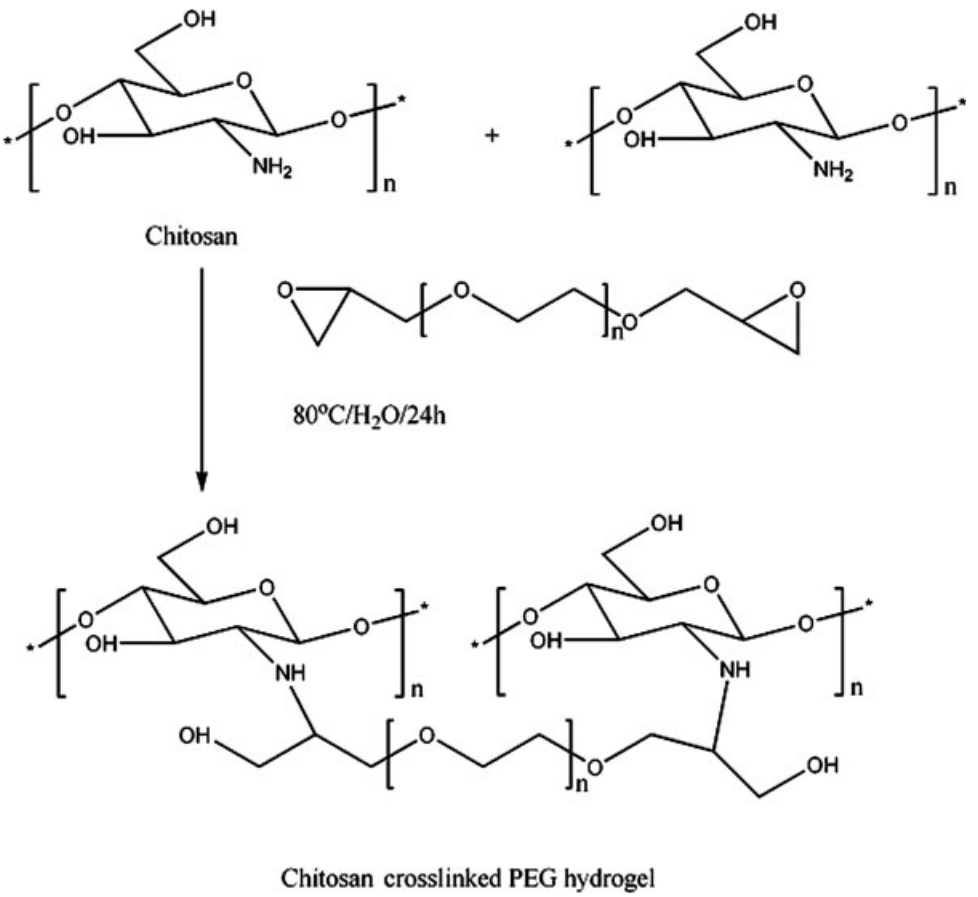
Figure 2 NMR spectrum of diepoxyPEG.

After the cross-linking reaction between diepoxyPEG and chitosan (Scheme 2), the melting enthalpy of PEG decreases, because the motion of PEG chains is confined by PEG attached to chitosan. It is consistent with the recent work of Laine et al.,[38] that the

Figure 1 The shape-uniform and transparent hydrogel film.



Scheme 1 The reaction of the addition of epoxy group to PEG.



Scheme 2 The reaction of crosslinked PEG hydrogel.

end-caps and the cross-linking points affect the segmental behavior, resulting in the decrease of the crystallinity. Thus, the reaction between diepoxyPEG and chitosan is confirmed by comparing the melting enthalpy of PEG/chitosan blend sample (un-modified PEG/chitosan blend) with that of PEG-chitosan sample (product of diepoxyPEG-chitosan reaction) by DSC measurements.

In the method to prepare the chitosan hydrogel reported in the previous reports,[39] chitosan was dissolved in 5% acetic acid. So, the cross-linking reaction of chitosan with diepoxyPEG was carried out in 5% acetic acid, but the reaction solution did not become gelling and the hydrogel was not formed. If the diepoxyPEG was reacted with chitosan, due to relatively high rigidity of the chitosan chain, the flexibility of the PEG chain was greatly confined, resulting in the decrease in the crystallinity as indicated by the decrease in the melting enthalpy. The melting enthalpy of this sample determined by DSC measurement was little different from that of the PEG/chitosan blend sample (Table II), suggesting that the extent of cross-linking reaction in 5% acetic acid was very low. This might be due to high concentration of acetic acid in the reaction medium. In the mechanism of this cross-linking reaction, the epoxy group of diepoxyPEG ring-opened by nucleophilic attack of the amino groups of chitosan. When the concentration of acetic acid is high, there is much nucleophilic acetate ion in the reaction medium, almost all the amino groups of chitosan are charged positively and they lost the nucleophilicity. When the cross-linking reaction was carried out in 0.4% acetic acid, that is, a lower limit concentration needed to dissolve chitosan, the reaction solution was gelling during the reaction and the hydrogel was formed. The progress of this reaction was confirmed by DSC measurement for the product after drying as shown in Figure 3 (Table II). When methanol was added into the reac-

TABLE II
The Melting Enthalpy of Chitosan Hydrogels (PEG : Chitosan = 0.6 : 1.0).

	Melting enthalpy (J/g)
PEG/Chitosan blend	49.801
PEG-Chitosan (prepared in 2% acetic acid)	33.274
PEG-Chitosan (prepared in 0.4% acetic acid, methanol)	9.608
PEG-Chitosan (prepared in 0.4% acetic acid, water)	10.286

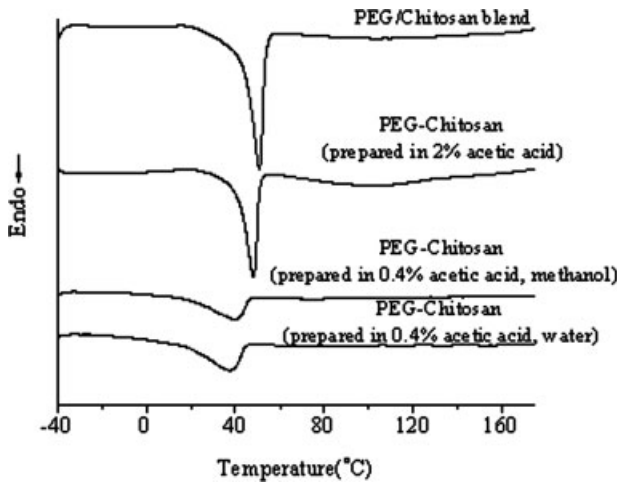


Figure 3 The DSC heating curves of chitosan hydrogels (PEG : chitosan = 0.6 : 1.0).

tion medium as described in the previous reports,[38] methanol caused the formation of air-bubbles in the hydrogel and the shape of the hydrogel became non-uniform. When the reaction was carried out without methanol, the rate of reaction was not much different from that with methanol, as confirmed by DSC measurement. Therefore, the cross-linking reaction, was carried out without methanol and using only 0.4% acetic acid as solvent.

Figure 4(a) shows the thermogravimetric curves of PEG, chitosan, PEG/chitosan blend sample, and PEG-chitosan sample. Although PEG and chitosan were thermally degraded, respectively, from 280 to 240°C, thermal degradation temperatures of PEG and chitosan in the PEG/chitosan blend sample and the PEG-chitosan sample were 380 and 260°C, respectively. Thus, it was indicated that the thermal degradation temperatures of both PEG and chitosan in the PEG/chitosan blend and the PEG-chitosan samples are much higher than those of respective raw materials, because of the interaction between PEG and chitosan. Also, it was found that the extent of weight loss in the PEG-chitosan during the thermal degradation was lower than that in the PEG/chitosan blend sample at the same temperature. Thus, the thermal stability of both PEG and chitosan increased by the presence of the cross-link. Here, the thermal degradation behavior of PEG is quite different from that of chitosan, the thermal degradation behavior of the PEG-chitosan sample with different constitution cannot be compared with each other.

Figure 4(b) shows the thermogravimetric curves of 47%PEG1000-chitosan, 47%PEG2000-chitosan, and 47%PEG4000-chitosan. The numbers of cross-links in these samples were different from each other, because the molecular weights of diepoxyPEG of these samples were different from each other, but the ratios of PEG to chitosan were the same. There were no clear correlations between the thermogravimetric curves of these samples and the numbers of cross-links or the molecular weight of diepoxyPEG. Therefore, the numbers of cross-links were not directly related to the increase of the thermal stability, but the presence of the cross-link definitely increased the thermal stabilities of the hydrogel.

Figure 5(a) shows the results of the tensile tests for the chitosan, PEG/chitosan blend film, and PEG-chitosan films with different content of diepoxyPEG. The value of maximum elongation at the yield of chitosan and PEG/chitosan blend sample were 20% and the tensile strength of PEG/chitosan blend sample was lower than that of chitosan. For the hydrogel films, the higher the PEG content in the hydrogel was, the more the tensile strength of the hydrogel film decreased and the strain increased. Only, the maximum elongation at the yield of 55%PEG2000 (62%) was lower than that of 47%PEG2000 (82%). Therefore, it was suggested that the more diepoxyPEG is

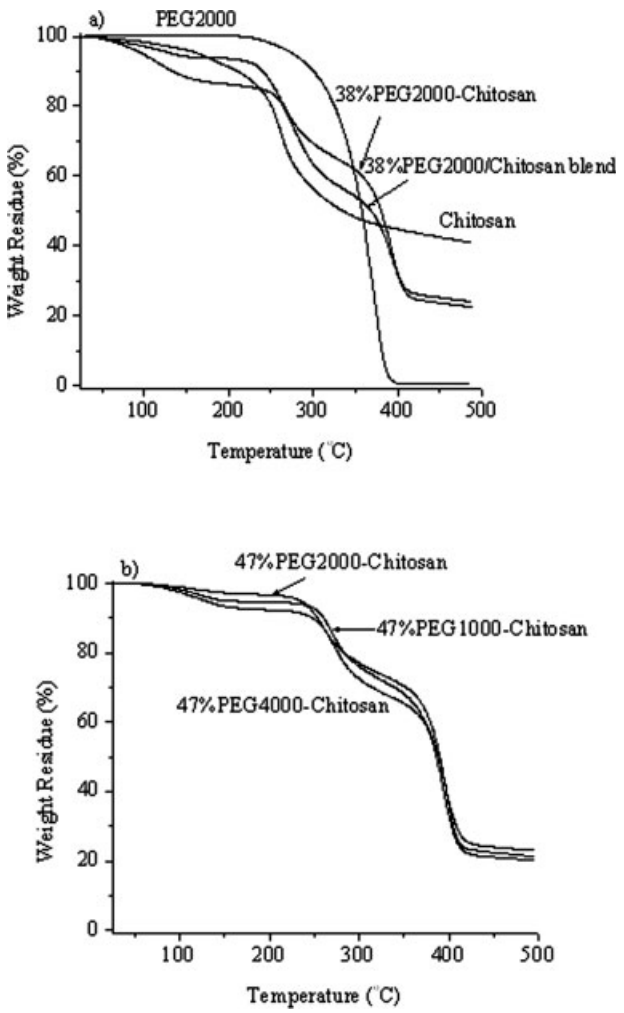


Figure 4 Thermogravimetric curves of (a) PEG, Chitosan, PEG/Chitosan blend, and PEG-Chitosan and (b) 47%PEG1000-Chitosan, 47%PEG2000-Chitosan, and 47%PEG4000-Chitosan.

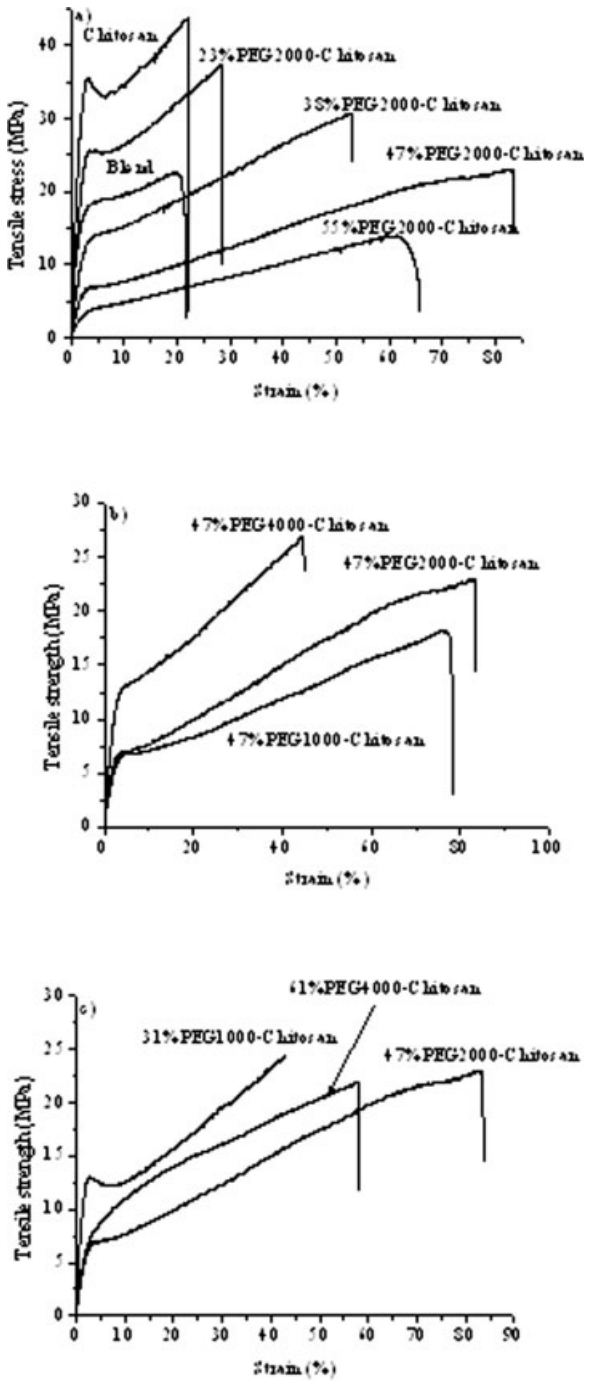


Figure 5 Tensile properties of the hydrogel films contained 15 ± 1% water at 22 ± 3°C. (a) The hydrogel films with different diepoxyPEG content, (b) the hydrogel films with different PEG molecular weight but with the same diepoxyPEG content, and (c) the hydrogel films with different PEG molecular weight but with the same diepoxyPEG content.

added to the hydrogel, the more elastic the chitosan hydrogel becomes. And also, the tensile strength decreased and the maximum elongation at the yield increases, because of the relatively soft nature of the PEG chain and the network formation. When the content of PEG in the hydrogel is too much, such as 55%PEG2000, the strain is decreased, because the plasticization effect of PEG is too much.

Figure 5(b) shows the results of tensile tests for the hydrogel films with different PEG molecular weight but the same PEG content. The lower the molecular weight of diepoxyPEG was, the more the tensile strength of the hydrogel film decreased. The highest strain value of 82% was observed for 47%PEG2000 samples. Therefore, it was suggested that the lower the molecular weight of PEG in the hydrogel film is, the larger were the plasticization and flexibility of PEG chains.

Figure 5(c) shows the results of the tensile test for the hydrogel films with different PEG molecular weight but with the same number of diepoxyPEG molecules. The maximum elongation at the yield of 47%PEG2000 (82%) was the best among the three samples. Both the data of the tensile strength and the strain indicate that 31%PEG1000 was not flexible, as the content of diepoxyPEG was too low and the mechanical property of this hydrogel film was similar to that of chitosan. Also, 61%PEG4000 did not show notable flexibility, because PEG4000 has poor plasticization effect and the content of diepoxyPEG was too high. The property of its hydrogel film reflected the brittleness of PEG itself. Therefore, the mechanical property of the hydrogel films with different molecular-weight diepoxyPEG was mostly affected by the contents of PEG and chitosan in the hydrogel, but affected little by the number of and the length of the cross-links.

Figure 6(a) shows the results of the swelling tests for the films of chitosan, the PEG/chitosan blend and the PEG-chitosans with different diepoxyPEG content. The chitosan and PEG/chitosan blend samples dissolved after swelling, so the swelling tests were impossible for these films. The water content of the PEG/chitosan blend film, $\sim$ 1500%, was lower than that of the chitosan film, $\sim$ 2100%, because the content of chitosan in the hydrogel was different, indicating that the water content of chitosan is much more than that of PEG. There are many hydrophilic groups in this hydrogel, but the swelling behavior of this hydrogel mostly depended on the ionic bond between the water molecule and the amino groups with positive charge of chitosan. While the hydrogen bonds between the water molecule and the ether oxygen of PEG is weak. On the other hand, the hydrogels were not dissolved. Thus, it was indicated that the cross-links help the hydrogel to retain their shapes after swelling.

Here, if the diepoxyPEG content in the hydrogel is increased, the chitosan content in the hydrogel decreases, so the swelling behavior of the hydrogel was changed with the composition of PEG and chitosan in the hydrogel. To remove this effect on the

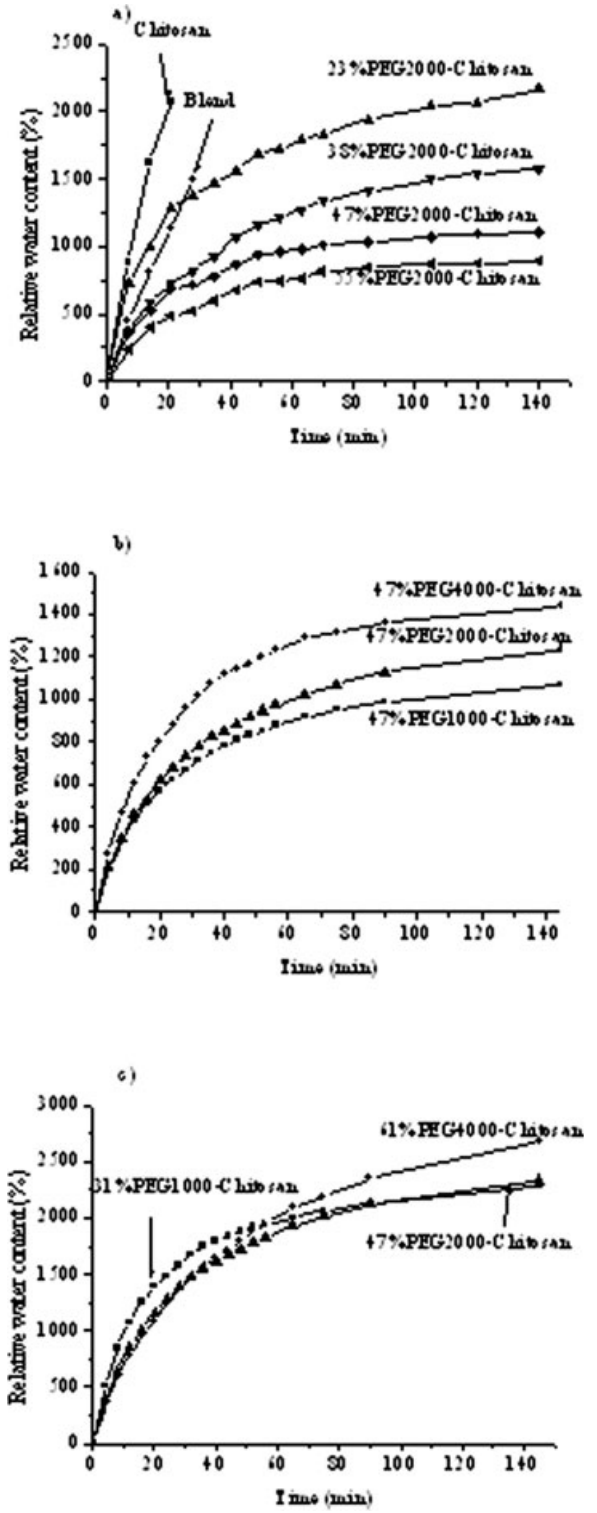


Figure 6 Swelling behavior of the hydrogel films in the buffer solution (pH 5) for (a) the hydrogel films with different diepoxyPEG content (relative water content), (b) the hydrogel films with different PEG molecular weight but with the diepoxyPEG content, and (c) for the hydrogel films with different PEG molecular weight but with the same diepoxyPEG content (relative water content).

swelling behavior, the water content of the hydrogels was divided by the weight of chitosan in the respective PEG-chitosan hydrogels. The value of water content for all the hydrogels is increased by this calculation. The lower the content of chitosan in PEG-chitosan hydrogel is, the more the water content value of hydrogel increased. Then, the difference of the raw data on water content among the PEG-chitosan hydrogels decreased when the content of chitosan in the hydrogel is different. After calculation, the water content was defined as the relative water content. On the time dependence of relative water content, the more the diepoxyPEG was increased, the lower was the relative water content in the hydrogel. The reason why the amount of water molecule included in the structure of the hydrogel decreases is that too much cross-link confine the elasticity of the films, so that the network structure is too tight to hold water.

Figure 6(b) shows the result of the swelling test for the hydrogel films with different molecular weight of diepoxyPEG but with the same diepoxyPEG content. It is obvious that, the higher the molecular weight of diepoxyPEG is, the higher is the water content of the hydrogel. This is due to the fact, when the molecular weight of diepoxyPEG in the hydrogel with the same content is lower, the number of molecules and cross-links of PEG in the hydrogel are lower, thus, the hydrogel containing high-molecular weight PEG shows high water content. It is suggested that the length of PEG chain in the hydrogel also affects the swelling behavior.

Figure 6(c) shows the result of the swelling test for the hydrogel films with different molecular-weight diepoxyPEG but the same molar content of PEG molecules. There was no dependence on a number of cross-links because the molar content of diepoxyPEG was the same. The water content of these samples was also divided by the weight of chitosan in the hydrogels because of the difference in their composition. In short time, as the molecular weight of diepoxyPEG decreased, the relative water content of the hydrogels increased. The reason for this result is that the hydrogel containing low-molecular weight PEG includes a lot of chitosan, so it swells more quickly than the others, because the ability to swell of chitosan is higher than that of PEG. On the other hand, in long time, as the molecular weight of diepoxyPEG increased, the relative water content of the hydrogels increased. It is suggested that the longer the PEG chain is, the higher is the freedom of chitosan chain, so the ability to swell of the hydrogel containing high-molecular weight PEG is higher than the others at last. After the swelling test, the hydrogel formed in water were freeze-dried for the observation of SEM.

The SEM photographs of the morphology of the hydrogel formed in water are shown in Figure 7,

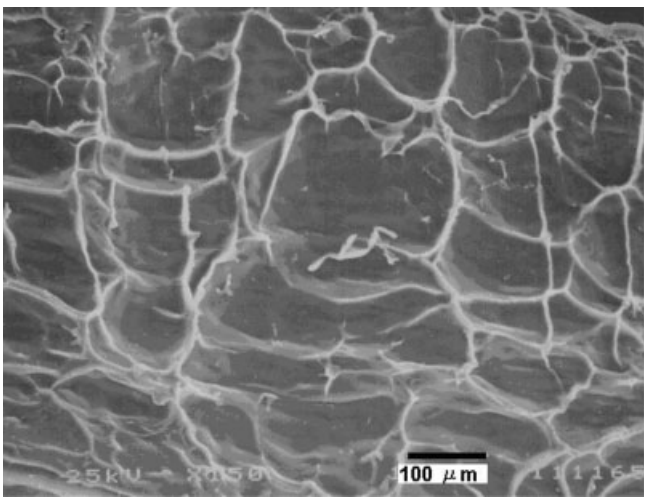


Figure 7 The SEM photograph of the hydrogel.

indicating that this hydrogel is porous membrane and the average mesh size of the hydrogel is about 200 μm.

CONCLUSIONS

The diepoxyPEGs crosslinked chitosan films were successfully prepared via the epoxy-amine reaction. In order to balance the solubility of chitosan with the reaction yield, the concentration of acetic acid in the reaction solution was adjusted to optimum concentration about 0.4% estimated by DSC study. The thermal stabilities of the crosslinked chitosan samples were higher than those of corresponding PEG/chitosan blend samples. The maximum elongation at the yield of the crosslinked chitosan films was higher than that of PEG/chitosan blend films, achieving a fracture strain about 80%. There is an optimum diepoxyPEG content to achieve highest fracture strain for certain molecular weight diepoxyPEG, as for diepoxyPEG with the PEG molecular weight 2000, the optimum content is the 47%diepoxyPEG. The swelling behavior of the diepoxyPEG crosslinked chitosan was found to greatly rely on the molecular weight of PEG in diepoxyPEGs and weight percent of the diepoxyPEGs. The higher the diepoxyPEG weight percent is, the lower is the equilibrium swelling ratio for certain diepoxyPEG. The higher the molecular weight of PEG in diexpoxyPEG is, the higher is the equilibrium swelling ratio for the same weight percent of the diepoxyPEGs.

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