# Synthesis, Characterization, and Enzymatic Degradation of Chitosan/PEG Hydrogel Films

**Aylin Altinisik,[1] Kadir Yurdakoc[2]**

[1]*Dokuz Eylül University, Graduate School of Natural and Applied Sciences, Department of Chemistry, 35160 Izmir, Turkey*
[2]*Dokuz Eylül University, Faculty of Sciences, Department of Chemistry, 35160 Izmir, Turkey*

Received 22 November 2010; accepted 31 January 2011
DOI 10.1002/app.34278
Published online 26 May 2011 in Wiley Online Library (wileyonlinelibrary.com).

**ABSTRACT:** Poly(ethyleneglycol) (PEG)/tartaric acid (TA)-crosslinked chitosan hydrogel (CPT) films were prepared, and the formation of the PEG/TA-crosslinked structure was confirmed by Fourier transformed infrared (FTIR), nuclear magnetic resonance (NMR), and scanning electron microscope (SEM) measurements. The thermal properties of the crosslinked films were also determined with thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC) analysis. The swelling properties of the films were investigated at different temperature and pH values. It was found that the swelling ratio increased with the decrease of pH value of the surrounding buffer solutions, amount of PEG, and with the increase of temperature. Swelling behavior of the PEG/TA-crosslinked chitosan hydrogel films depended on pH and reversible with the temperature. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1556–1563, 2011

**Key words:** chitosan; hydrogel; PEG; temperature sensitive; pH sensitive; enzymatic degradation

## INTRODUCTION

Chitosan (CS), the only natural cationic polysaccharide obtained by deacetylation of chitin, has gained considerable attention in pharmaceutical and biomedical field owning to its favorable biological properties, such as low toxicity, good biocompatibility, biodegradability, accelerated wound healing properties, and antimicrobial activities.[1–3] However, CS is easily dissolved in acidic media, crosslinking of CS to form a network is necessary to solve this dissolution property. Conventional CS crosslinking agents including bifunctional reagents, such as formaldehyde, glutaraldehyde, or epiclorohydrin, cycloheptamylose were used in the synthesis of crosslinked CS.[4] On the other hand, chemical crosslinkers leaded to toxic effect in physiological environment, due to the presence of residual crosslinkers. Novel CS-based polymeric networks were synthesized using a naturally occurring crosslinking reagent—genipin.[5] Recently, new way to crosslink CS in aqueous solution was also discussed in details.[6]

Poly(ethyleneglycol) (PEG) is an attractive synthetic polymer. It shows biodegradability, biocompatibility, less toxicity, and hydrophilicity and has been used in many kinds of applications.[6] The mechanical properties of CS film were improved with the usage of PEG oligomer as plasticizer.[7] Several studies have been published on hydrogels containing CS and PEG. It had been also concluded from the viscometric studies that CS/PEG blends were compatible due to attractive intermolecular interactions between the polymer chains.[8] PEG-crosslinked CS with different molecular weight of diepoxy PEG was also synthesized and characterized in details.[9]

However, the toxicity of this crosslinker had an adverse effect on the biocompatibility of the obtained materials. Thus, there was a high demand for nontoxic and biocompatible crosslinking agents.[10] We have been studying CS-based hydrogels using tartaric acid (TA) as a crosslinking agent with PEG. It is known to be far less cytotoxic than ever used crosslinkers. TA is a molecule with at least two reactive functional groups that allow the formation of bridges between CS chains. TA crosslinks effectively with the amino groups of CS, and the crosslinking mechanism has been described.[11] Drug release mechanisms from CS-based hydrogels, design criteria for the hydrogels in drug delivery formulations, novel engineering of the CS polymer blended hydrogels for drug delivery (such as biodegradable hydrogels, smart hydrogels, and biomimetic hydrogels), and also molecule release mechanisms for hydrogel formulations have been recently reported.[12,13]

In this study, the PEG/TA-crosslinked CS hydrogel film samples (CPT) with several contents of PEG are prepared. For these samples, more direct method than previous thermal analysis, that is, Fourier transformed

*Correspondence to:* K. Yurdakoc (k.yurdakoc@deu.edu.tr).

infrared (FTIR) and nuclear magnetic resonance (NMR) will be applied to investigate the formation of the PEG/TA-crosslinked structure. It has been well-known that the swelling sensitive property to pH and temperature is considerably important capacity for the biomedical application such as drug delivery systems. To prove the swelling properties of the PEG/TA-crosslinked CS hydrogel films, swelling experiments were carried out at different temperatures and various pH values.

## MATERIALS AND METHODS

### Materials

CS (highly viscous) (Fluka Cat.No. 48165) and PEG ($M_n$ = 1450) (Cat.No. P5402) were purchased from Fluka and Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), respectively. L(+) TA (Cat.No. 411127) obtained from Carlo Erba (Rodano(MI), Italy). All other chemicals used in this work were analytical grade. Ultrapure water from Milli-Q water system was used to prepare the aqueous solutions. Lysozyme from chicken egg white (50,000 U/mg) was purchased from Sigma-Aldrich and was used without further purification.

### Synthesis of novel CS hydrogel

CS was dissolved in 2% (v/v) acetic acid. It was taken one night to dissolve CS completely, and the solution was filtered with cheesecloth to remove undissolved CS. TA was dissolved in water and then adjust pH = 6.5 with 0.1*M* NaOH solution because of tartarat form uses as crosslinker. After the addition of water soluble PEG, the reaction was stirred at 4°C for 30 min and subsequently mixed with CS solution. Then the reaction mixture was stirred further at room temperature for 24 h. After that, the mixture was castled on Petri dish, which was placed later in vacuum oven at 60°C for 24 h. Then, the dried samples were immersed in 1*M* NaOH solution for 5 h for the removal of residue (unreacted) acetic acid. In the preparation of the samples, the numbers of moles of $NH_2$ groups of CS were taken into account. In the preparation of the samples, 1 g of CS and 0.75 g of TA were remained constant. The amount of PEG was varied as 0.75, 1.25, and 2.5 g. The ratio of the components was 4:3:3; 4:5:3, and 4:10:3 for CP3T3, CP5 T3, and CP10T3 samples, respectively.

### Characterization techniques

#### FTIR measurements

FTIR spectra were recorded on the Perkin–Elmer FTIR spectrophotometer Spectrum BX-II to analyze the chemical structure of the PEG/TA-crosslinked CS. Before the FTIR measurements, crosslinked CS hydrogel film was brought to constant weight in a drying oven at 60°C for 24 h. The spectra were recorded with the sum of 25 scans at a resolution of 4 $cm^{-1}$ in the range of 4000–400 $cm^{-1}$. The sample for FTIR measurement was prepared by slicing the film along its thickness direction using microtome.

#### NMR spectroscopy analysis

$^{13}C$ and $^{1}H$ measurements were performed on Bruker DRX-300 and DRX-500 NMR spectrometers by Dr. R. Meusinger in Darmstadt Technical University/Germany. Samples (5–20 mg) were dissolved in 1.5 mL of 20 wt % $DCl/D_2O$ at 80°C.

#### Thermal properties

The thermal properties of crosslinked CS hydrogel films and CS film were investigated by thermogravimetric analysis (TGA). TGA was performed under nitrogen flow at a flow rate of 10°C/min from 30 to 500°C with a Perkin–Elmer Diamond TG/DTA instrument. The weights of the samples varied from 2 to 3 mg.

Thermal properties of the hydrogel films were also characterized by a differential scanning calorimeter (Perkin–Elmer Diamond DSC). The dried samples were heated first from 10°C and 300°C under nitrogen atmosphere at heating rate of 10°C/min and then cooled down to 0°C then heated again to 300°C.

#### Scanning electron microscope (SEM) morphology

The surface morphologies of the samples were studied using a SEM at an accelerating voltage of 10 kV. All samples were dried and coated with gold before SEM photographs were taken at different magnifications (in the range of 50–3000×) by using Jeol JSM 60 model SEM apparatus equipped with energy dispersive X-ray (EDX) in Metallurgy and Materials Engineering Department of Dokuz Eylül University/Izmir.

#### Enzymatic degradation

The *in vitro* degradation of the CPT hydrogel films was followed in 2 mL phosphate buffered solution (PBS, pH = 7.4) at 37°C containing 1 mg/mL lysozyme [hen egg white (HEW)]. The samples, after some minutes of degradation, were removed from the medium, dried overnight under vacuum oven at 60°C, and weighed. To distinguish enzymatic degradation from the dissolution, the control samples were tested under the same condition as described above, but without adding lysozyme.

### Swelling measurements

#### Swelling test

The swelling behavior of the dried sample films were observed in phosphate buffer saline solution at pH = 7.4 and in KCl/HCl buffer solution at pH = 1.2 at 37°C. After the dried films were weighed, they were conditioned at 37°C in the buffer solution at each pH condition. The samples were taken from the medium when they were reached to the equilibrium swelling, wiped with filter paper, and weighed. The water content of the film sample was determined according to the following equation:

$$S\% = \frac{M_s - M_d}{M_s} \times 100 \quad (1)$$

where $M_s$ and $M_d$ represent the weights of swollen and dried state samples, respectively.

#### pH-sensitivity of hydrogels

Dried hydrogels were left to swell in buffer solution of desired pH (2.6–8.3, $I = 0.01$) at 37°C. Swollen gels removed from the swelling medium at regular intervals were dried superficially with filter paper, weighed, and placed into the same bath. The measurements were continued until a constant weight reached for each sample. The swelling ratios were calculated on a dry basis by using eq. (1).

In addition, the reversible swelling behavior of hydrogels was also investigated. The samples that reached equilibrium at pH = 2.6 were allowed to reach pH = 8.3 and then back to pH = 2.6 medium again.

#### Temperature sensitivity of hydrogels

To determine the temperature sensitivity of hydrogels, dynamic swelling measurements were done by gravimetric means at 4 and 37°C. The samples were dried to the constant weight in vacuum and than immersed in a constant temperature bath filled with PBS (pH = 7.4, $I = 0.2$). The samples were removed from the PBS at appropriate intervals, blotted with filter paper, weighed, and then returned to PBS. This procedure was repeated, until the equilibrium was reached. The swelling ratios were calculated on a dry basis using eq. (1). All swelling experiments were repeated in an incubator (GLF 1086) at least three times, and the results reported as averages.

## RESULTS AND DISCUSSION

### FTIR spectra of the samples

The structure of CPT hydrogels was analyzed by using FTIR spectroscopy. Figure 1 shows FTIR spectra of CS and its hydrogels. The spectrum of CS film exhibits an absorption around 1647 and 1555 $cm^{-1}$, which represent the amide I (C=O stretching) and amid II (N—H deformation) bands, respectively.[9,14] Although standard CS showed bands at 1309 $cm^{-1}$ and 1430 $cm^{-1}$ (C—H bending). The absorption bands at 1133 $cm^{-1}$ (asymmetric stretching of C—O—C bridge) and 1056 $cm^{-1}$ (C—O stretching) were characteristics of its saccharine structure.[15–17] Pure PEG has characteristic bands at 1280, 947, and 843 $cm^{-1}$.[18] Compared to the amide I band at 1647 $cm^{-1}$, the band intensity of amide II significantly decreased. This resultant spectrum showed that the $-NH_2$ groups of CS were partially interacted with PEG. The characteristic bands associated with PEG in CPT at 1280, 948, and 841 $cm^{-1}$ were significantly increased with the increasing amounts of PEG. The bands at 1120 and 2880 $cm^{-1}$ in CPTs were attributable to the superposition of C—O and C—H stretching vibrations of CS and PEG. In addition, the formation of the crosslinked structure was also confirmed by the absorption band at 1380 $cm^{-1}$ (Fig. 1). The intensity at 1380 $cm^{-1}$ increased with the content of PEG of the CPT hydrogel films. This 1380 $cm^{-1}$ absorption band caused by the PEG $CH_2$ deformation vibration, because the number of the $CH_2$ groups was increased with preceding the crosslinked reaction.

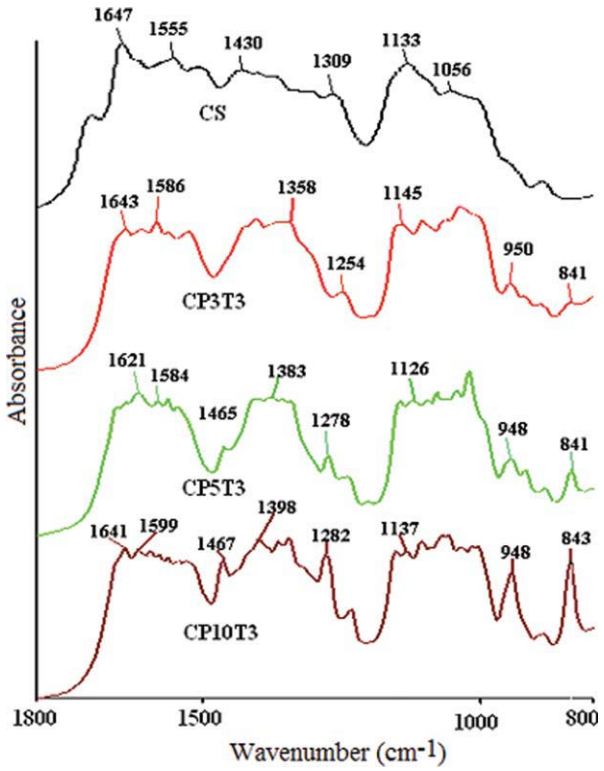


**Figure 1** FTIR spectra of chitosan and its hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**TABLE I**
**Thermal Analysis Report of the Samples**

| | | | | First stage | | Second stage | | Third stage | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | $T_e$ (°C) | $\Delta H_e$ (J/g) | $T_d$ (°C) | $T$ (°C) | $m$ (%) | $T$ (°C) | $m$ (%) | $T$ (°C) | $m$ (%) |
| CS | 35 | 0.7 | 198 | 25–105 | 13 | 230–270 | 38 | – | – |
| CP3T3 | 40.0 | 1.3 | 271 | 53–146 | 6 | 235–332 | 22 | 432–477 | 14 |
| CP5T3 | 40.4 | 27.3 | 274 | 68–144 | 10 | 235–285 | 33 | 370–402 | 33 |
| CP10T3 | 43.0 | 58.7 | 279 | 75–83 | 2 | 243–310 | 18 | 372–477 | 40 |

### NMR spectroscopy

Typical peaks at 3.4 ppm and 3.6 were assigned to the ring methane and methylene protons of CS saccharide units and methylene groups of PEG.[17] Peaks at 2.8 ppm and 2.1 ppm attributed to —$CHNH_2$ and —$COCH_3$, but these peaks were not observed at NMR spectrum. Methylene protons and —$OCH_3$ peaks from PEG were appeared at 1.6 ppm and 3.4 ppm, respectively.

Furthermore, the analysis of CPT $^{13}$C-NMR spectrum showed the peak at 71 ppm attributed to —$CH_2$— groups of the —O—$CH_2$—$CH_2$—O— chain.[20] Also the peaks at 173 ppm and 184 ppm referred to —NH—CO— and C=O groups of TA.

### Thermal analysis

Differential scanning calorimeter (DSC) and TGA were selected to characterize the thermal properties of CPT film. Thermal decomposition behaviors of CPT films and CS films were performed by TG/differential thermogravimetric (DTG) analysis. There were three degradation stages in the thermograms. The first stage showed decomposition of water at 56°C, 119°C, 122°C, and 76°C for CS, CP3T3, CP5T3, and CP10T3, respectively. The temperature of degradation ($T_d$) of CS film was 250°C. In the thermograms, CPT films have two stages of mass losses, which were due to the degradation of the samples. The first stage mass loss was observed at the temperature range of 257–286°C with a total of 33–18% mass loss. On the other hand, the samples were then completely degraded at 402–473°C, which might be attributed to the PEG and TA onto CS.

It has been observed in the DSC profiles of CS that it did not melt or degraded between 20 and 200°C as shown.[21] However, in this study, a broad endothermic peak centered at the temperatures between 40 and 130°C were observed for the DSC curves of the CS film and CP3T3 samples. This peak was caused by the overall endothermic process connected with the evaporation of bound water. In general, polysaccharides have a strong affinity for water and can be easily hydrated in the solid state.[22] For this reason, the broad peak was also observed for the hydrogel films. Whereas, CPT film samples also showed a sharp endothermic peak centered at the temperatures between 40 and 50°C. This may be attributed to the melting of the PEG moiety. The intensity of this endothermic peak of the CPT hydrogel films was increased with the increase in the amounts of PEG. The endothermic enthalpy ($\Delta H$) values were calculated from the area under the endothermic peak caused by the melting of the CPT hydrogels. The melting enthalpies of these samples were determined by DSC, measurements were different in some respect from that of the PEG/CS blend samples. TG/DTG and DSC analysis results were summarized in Table I.

### SEM images

SEM images of CS and CPT hydrogel films were given in Figure 2. As shown in Figure 2a, CS on the surface has a spherical structure and a regular surface. On the other hand, SEM images of the CP3T3 in Figure 2b showed increasing amounts of roughness. At the same time, the small holes on the surface were disrupted, and different structures, such as fibrous morphology, have begun to create. As shown in Figure 2c and d, by the increase in the amounts of PEG caused fibrous structure. In addition, these fibrous structures may be an indication of the interaction between PEG and CS.

### Swelling measurements

Swelling test

To investigate the time-dependent swelling behavior of CPT hydrogels in solutions with different pH, we performed dynamic swelling studies. The swelling $S\%$ is calculated from the following relation:

$$S\% = \frac{M_s - M_d}{M_d} \times 100$$

where $M_d$ is the mass of dry gel at time 0, $M_s$ is the mass of swollen gel at time $t$. Swelling curves of CPT hydrogels in solutions with pH = 1.2 and pH = 7.4 at 37°C were shown in Figure 3 A and B, respectively.

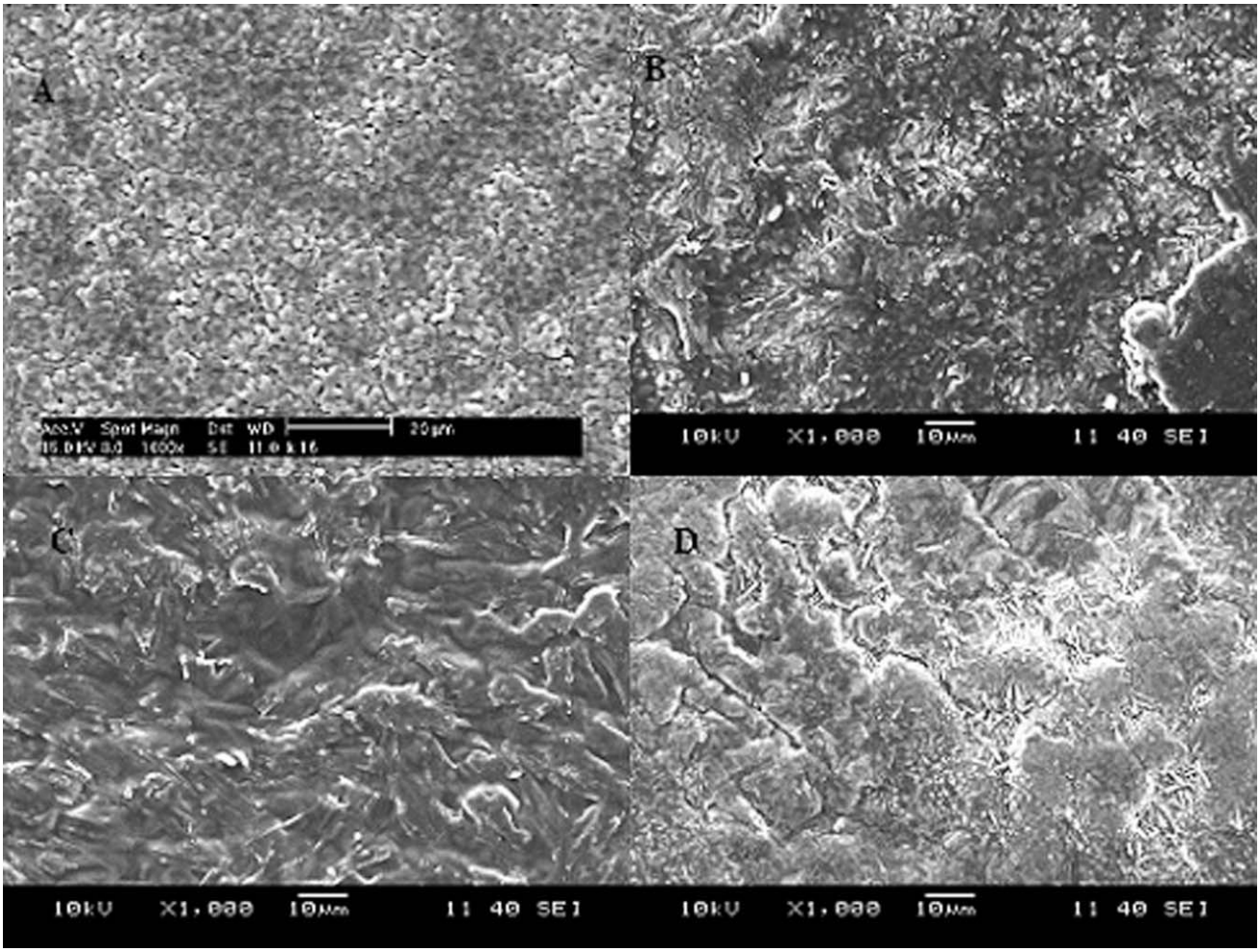


**Figure 2** The SEM images of chitosan and CPT hydrogel films. (A) Chitosan, (B) CP3T3, (C) CP5T3, and (D) CP10T3.

As can be seen in these figures, the values of equilibrium swelling of CPT hydrogels have decreased with increasing PEG concentrations in hydrogels. The swelling degree of a hydrogel depends on its network structure, which is controlled by the concentration of the crosslinker. Also, the swelling of hydrogels are depended on the pH of the medium.

For extensive swelling of polymers, the following relation can be written as below[23];

$$\frac{t}{S} = A + Bt \tag{2}$$

where $S$ is the degree of swelling at time $t$, $B = \frac{1}{S_{\max}}$ is the inverse of the maximum swelling, $A = \frac{1}{(dS/dt)_0} = \frac{1}{S_{\max}^2 k_s}$ is the reciprocal of the initial swelling rate ($r_o$) the gel. The relation represents second-order kinetics. The values of the initial swelling rate, $r_o$ [g solution (g CPT)$^{-1}$ s$^{-1}$] and maximum swelling, $S_{\max}$ [g solution (g CPT)$^{-1}$] were calculated from the slope, and intersection of the lines and swelling rate constant, $k_s$ [g CPT (g solution)$^{-1}$ s$^{-1}$] were presented in Table II.

pH-sensitivity of hydrogels

A number of factors that influence the degree of swelling of hydrogel include the properties of the polymer and swelling medium. This is why experiments to determine the pH-dependent swelling kinetics were performed in buffer solutions which have various pHs between 2 and 8. Figure 4 represents the variation of the equilibrium swelling ratio of hydrogel CPT as a function of the pH of the swelling medium at 37°C. In all the hydrogels, maximum swelling was observed at pH = 2 and minimum at pH = 6. There was a decrease in the equilibrium swelling ratio of the samples with below pH = 6. However, from the pH = 6, CPT hydrogel exhibited an increase in equilibrium swelling ratio values. It can be seen that the hydrogels swelled mostly in acidic medium as compared with the neutral or basic medias.

Whereas, swelling ratio of the samples was decreased with the increasing of the amounts of PEG. On the other hand, the effect of pH on the swelling of the CS hydrogels was explained on the basis of protonation of the amino groups of CS. In

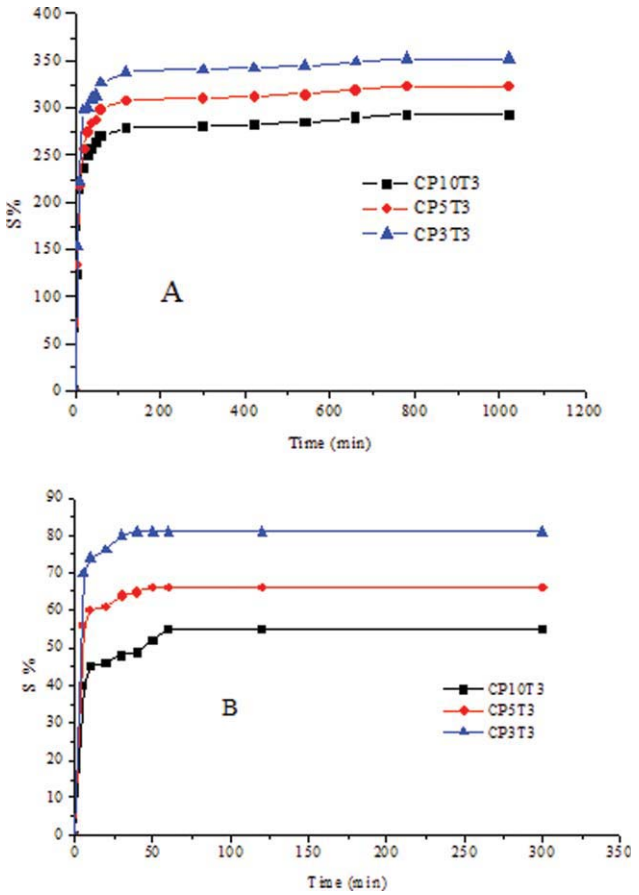


**Figure 3** Swelling ratio of CPT at 37°C (A) pH = 1.2 and (B) pH = 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the acidic medium, the protonation of the amino groups leads to repulsion in the polymer chains, thus allowing more water in the hydrogel network. At higher pH, deprotonation of the amino groups takes place, and repulsion in polymer chains is reduced. This results in the shrinking of the gels and therefore, the amount of water in the gel decreases. Swelling is important properties for drug release study for hydrogel. The sudden and rapid swelling causes degradation and rapid release. At acidic medium, swelling ratio of a gel which 960% and 840% were synthesized.[24] These hydrogels were broken after the immersion in the soaking phase. However, maximum swelling ratio of the hydrogels was observed as 250%, whereas its minimum was 189% in acidic medium. Furthermore, reversible swelling characteristics and low response time of the samples may be an advantage for drug release study. As can be seen from Figure 5A, repeated changes in pH for the CPT hydrogels responded reversible pattern with a faster response in swelling than in deswelling.

**TABLE II**
**Swelling Parameters of CPT at 37°C for Different pH Values**

| Sample | $S_{eq}$ (%) | $S_{max}$ (%) | $k_s \times 10^5$ [g CPT (g solution)$^{-1}$ s$^{-1}$] | $r_o$ [g solution (g CPT)$^{-1}$ s$^{-1}$] |
|---|---|---|---|---|
| *pH = 7.4* | | | | |
| CP3T3 | 81 | 82 | 2091 | 141 |
| CP5T3 | 66 | 68 | 1718 | 79 |
| CP10T3 | 55 | 56 | 565 | 18 |
| *pH=1.2* | | | | |
| CP5T3 | 353 | 355 | 42 | 53 |
| CP10T3 | 323 | 324 | 44 | 46 |
| CP3T3 | 293 | 295 | 48 | 42 |

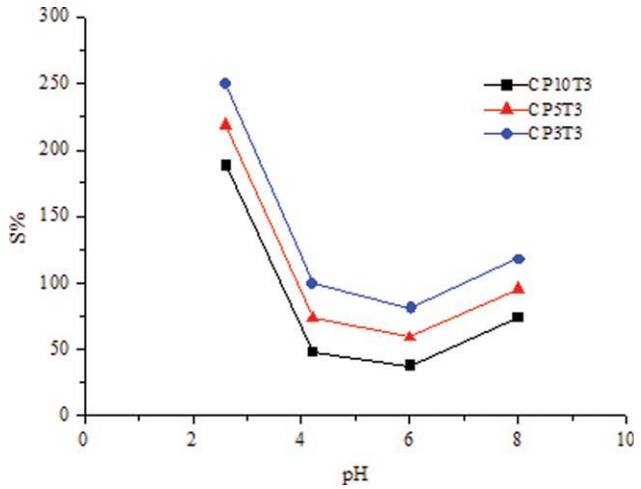


**Figure 4** Effect of pH on the equilibrium swelling ratio of the samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

## Temperature sensitivity of hydrogels

Temperature-dependent reversible swelling behavior was found, as shown in Figure 5B. At the first run, the equilibrium swelling ratio was measured at 37°C. Then, the equilibrium swelling experiment was done at 4°C for the same film sample. The equilibrium swelling ratio increased with decreasing the temperature to 4°C. When the temperature was raised to 37°C again (3rd run), the equilibrium swelling ratio increased to the level of the first run at 37°C. These heating/cooling runs were repeated two times. The similar reversible and reproducible temperature dependence of swelling–deswelling behavior was observed for all samples. Thus, all samples have almost the same temperature dependency, higher swelling ratio was observed at higher temperature and lower at lower temperature. It has been well-known that the swelling–deswelling behavior is mainly due to the interaction between polymer and water molecules. In general, the strength and extent of this interaction decrease with increasing the temperature. The sample with higher PEG content was more sensitive to the temperature changes, showing more distinctive temperature-dependent swelling–

deswelling response. It would be a desirable character for controlled-drug release system with swelling property controllable by pH and temperature. So, it can be expected that these hydrogel films are of great interest for biomedical application, such as artificial muscles or switches and drug delivery systems.

## Enzymatic degradation

In human body, CS is mainly degraded by lysozyme. But the degradation of CS film was not so clearly, because its degradation was very slow.[22] The *in vitro* degradation behavior of CS has been usually investigated using HEW lysozyme,[25–27] because HEW lysozyme as well as human lysozyme cleavages the b(1–4)-linked GlcNAc and GlcN subunits of CS.[9]

In this study, the degradation behavior of the hydrogel film in the presence and in the absence of lysozyme was investigated in PBS at 37°C (Fig. 6). Figure 6(a) showed the degradation behavior of the CP3T3 film sample. The mass loss of CP3T3 at most

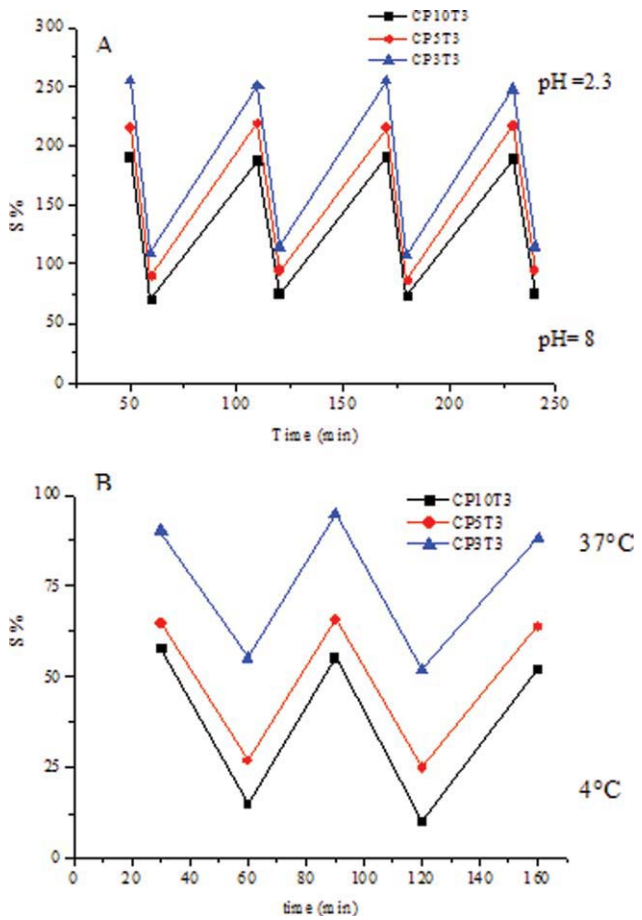


**Figure 5** Swelling and deswelling behavior of CPT hydrogel as a function of time. (A) pH (pH = 8.3 and 2.3, at 37°C) and (B) temperature (at 37°C and 4°C in buffer at pH = 7.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

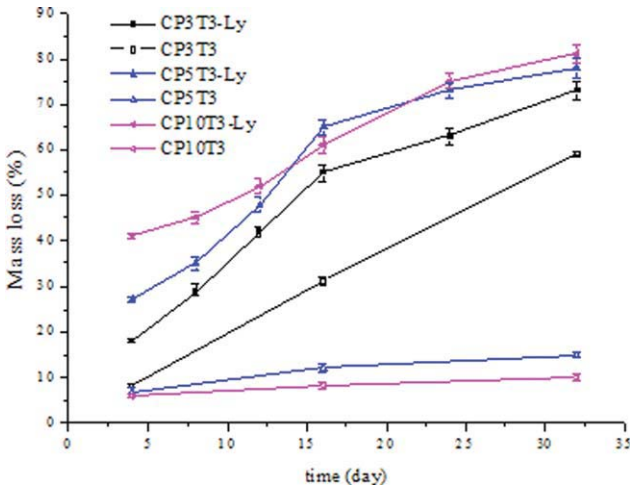


**Figure 6** Mass loss of hydrogel films in 1 mg/mL lysozyme/PBS and in PBS without lysozyme at 37°C as a function of time (Ly refers to with lysozyme). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

59 even after 32 days in PBS without lysozyme, whereas in the presence of 1 mg/mL lysozyme, the mass loss reached to more than 73% after 32 days. Similarly, the mass loss of CP5T3 at most 15% even after 32 days in PBS without lysozyme, whereas in the presence of 1 mg/mL lysozyme, the mass loss was more than 78% after 32 days, and it was proportional to the degradation time. The degradation behavior of CP10T3 was shown in Figure 6(c). The mass loss of CP10T3 degraded with 1 mg/mL lysozyme was more than 81% after 32 days.

From these results, the tendency of the enzymatic degradation rates the samples was found to be paralleled with that of the swelling ratio. On the other hand, this tendency was not the same as far as the samples with different ratio of PEG. This was due to the fact that the CP10T3 has more dispersed crosslinked point and lysozyme may easily access the binding site of CS molecular chain. Thus, the swelling ratio and the internal structure were influenced the enzymatic degradation.

## CONCLUSIONS

The PEG–TA crosslinked CS hydrogel films with different contents of PEG were prepared successfully. The formation of the PEG-crosslinked structure was confirmed by comparing absorption bands of amide I (1650 $cm^{-1}$) and amide II (1580 $cm^{-1}$). By measuring the glass transition temperature by DSC, the internal three dimensional structure of crosslinked CS hydrogel film was turn out to become dense as the increase of PEG content. The thermal stabilities of the crosslinked CS samples were higher than CS film sample.

Swelling properties of the hydrogels were studied at different temperatures as well as in media of different pH values. The swelling ratio increased with the decrease of pH value of the surrounding buffer solution. All films also showed similar reversible temperature-dependent swelling behavior; the high swelling ratio under high temperature. The swelling rate would be controllable by changing the content of PEG. These PEG/TA-crosslinked CS hydrogel films will be useful in the field of controlled-drug release studies. We can conclude that the hydrogel sample with higher content of PEG was more sensitive to the enzymatic degradation.

## References


1. Muzzarelli, R.; Baldassarre, V.; Conto, F.; Ferrara, P.; Biagini, G.; Gazzanelli, G.; Vasi, V. Biomaterials 1988, 9, 247.
2. Kurita, K. Polym Deg Stab 1998, 59, 117.
3. Rinaudo, M. Prog Polym Sci 2006, 31, 603.
4. Wang, J.; Hon, M. J Mater Sci: Mater Med 2003, 14, 1706.
5. Mi, F.-L.; Sung, H.-W.; Shyu, S. –S. J Polym Sci A: Polym Chem 2000, 38, 2804.
6. Roberts, M. J.; Bentley, M. D.; Harris, J. M. Adv Drug Del Rev 2002, 54, 459.
7. Suyatma, N. E.; Tighzert, L.; Copinet, A. J Agric Food Chem 2005, 53, 3950.
8. Jiang, W. H.; Han, S. J. J Polym Sci B: Polym Phys 1998, 36, 1275.
9. Tanuma, H.; Saito, T.; Nishikawa, K.; Dong, T.; Yazawa, K.; Inoue, Y. Carbohydr Polymers 2010, 80, 260.
10. Hennink, W. E.; van Nostrum, C. F. Adv Drug Del Rev 2002, 54, 13.
11. Bodnar, M.; John, F. H.; Janos, B. Biomacromolecules 2005, 6, 2521.
12. Lin, C.-C.; Metters, A. T. Adv Drug Del Rev 2006, 58, 1379.
13. Bhattarai, N.; Gunn, J.; Zhang, M.,Adv Drug Del Rev 2010, 62, 83.
14. Cao, W.; Cheng, M.; Ao, Q.; Gong, Y.; Zhao, N.; Zhang, X. J Biomater Sci Polymer Ed 2005, 16, 791.
15. Kurita, K. Prog Polym Sci 2001, 26 1921.
16. Qu, X.; Wirsén, A.; Albertsson, A.-C. Polymer 2000, 41, 4589.
17. Kolhe, P.; Kannan, R. M. Biomacromolecules 2003, 4, 173.
18. Bhattarai, N.; Ramay, H. R.; Gunn, J.; Matsen, F. A.; Zhang, M. J Controlled Release 2005, 103, 609.
19. Kong, X. Y.; Li, X. Y.; Wang, X. H.; Liu, T. T.; Gu, Y. C.; Guo, G.; Luo, F.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. Carbohydr Polymers 2010, 79, 170.
20. Sugimoto, M.; Morimoto, M.; Sashiwa, H.; Saimoto, H.; Shigemasa, Y. Carbohydr Polymers 1998, 36, 49.
21. XiangYe, K.; XingYi, L.; XiuHong, W.; TingTing, L.; YingChun, G.; Gang, G.; Feng, L.; Xia, Z.; YuQuan, W.; ZhiYong, Q. Carbohydr Polymers 2010, 79, 170.
22. Tanuma, H.; Kiuchi, H.; Kai, W.; Yazawa, K.; Inoue, Y. J Polym Sci 2009, 114 1902.
23. Katime, I.; Valderruten, N.; Quintana, J. R. Polym Int 2001, 50, 869.
24. Ramos, M.; Rodríguez, N. M.; Henning, I.; Díaz, M. F.; Monachesi, M. P.; Rodríguez, M. S.; Abarrategi, A.; Correas-Magan, V.; López-Lacomba, J. L.; Agullo, E. Carbohydr Polymers 2006, 64, 328.
25. Etienne, O.; Schneider, A.; Taddei, C.; Richert, L.; Schaaf, P.; Voegel, J. C.; Egles, C.; Picart, C. Biomacromolecules 2005, 6, 726.
26. Freier, T.; Koh, H. S.; Kazazian, K.; Shoichet, M. S. Biomaterials 2005, 26, 5872.
27. Neamnark, A.; Sanchavanakit, N.; Pavasant, P.; Bunaprasert, T.; Supaphol, P.; Rujiravanit, R. Carbohydr Polymers 2007, 68, 166.