pH-Responsive Release Behavior of Genipin-Crosslinked Chitosan/Poly(ethylene glycol) Hydrogels

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ABSTRACT: Chitosan/poly(ethylene glycol) blends were crosslinked by the nontoxic genipin as a crosslinker to produce the pH-responsive hydrogels. The pH-responsive swelling and releasing behavior of chitosan/poly(ethylene glycol) hydrogels was studied by varying the contents of poly(ethylene glycol) (PEG) and genipin, and the molecular weights of PEG. The drug release increased as the pH of the medium decreased, because the protonation of amino acids in chitosan chains at the acidic condition caused the higher swelling of hydrogels. The drug release increased as the content of PEG increased, because the larger pores were formed in the hydrogel and the higher swelling of matrix was obtained. The drug release decreased with the increase of the content of genipin, because the higher degree of crosslinking impeded the diffusion of drug from the hydrogel matrix. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: pH-response; chitosan; poly(ethylene glycol); hydrogel; drug release

INTRODUCTION

Hydrogels have the three-dimensionally crosslinked networks that can swell dramatically in the presence of aqueous medium and retain a large amount of water while maintaining their structures. Hydrogels have found various applications in the fields of tissue engineering and controlled drug release.[1–3] One of the most interesting hydrogels is the intelligent hydrogel. Such hydrogels have the ability to respond to the external stimuli such as temperature, pH, and electric and photo fields.[4–12] Because of their diverse functions, the temperature and pH-sensitive hydrogels have received the most extensive investigations during the past decades.[2,13–15]

Chitosan is a cationic biopolymer obtained by full or partial N-deacetylation of chitin, which is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans.[16] Chitosan has been evaluated for various uses in food, medical, pharmaceutical, agricultural, and chemical industries because of its nontoxic, biocompatible, mucoadhesive, and biodegradable properties.[17–19] In addition, chitosan is dissolved in acidic aqueous solutions due to its free amino groups and is fabricated into various forms such as gels, films, sutures, beads, and fibers. Chitosan has long been known and used in pharmaceutical and biomedical applications due to its intriguing biological properties.[20,21] In recent decades, chitosan works as a candidate for drug carriers and it has attracted the attention of many researchers. Since the amine group $-NH_2$ can be protonated to $-NH_3^+$, which may endow chitosan with a favorable gel forming property, chitosan has been investigated extensively in the pharmaceutical industry as the potential use in pH-sensitive drug release systems.

However, chitosan film is too rigid to be applied to the tissue engineering and the artificial scaffolds.[22] Therefore, it is very important to improve its ductility for the extensive applications. Poly(ethylene glycol) (PEG) is frequently used in the production of polymer blends. PEG can improve the flexibility and ductility of the blended films. PEG also has many attractive properties, such as excellent solubility in an aqueous medium, low toxicity, and chain flexibility. PEG is readily excreted from the body via kidneys and forms nontoxic metabolites. The incorporation of PEG was reported to improve the biocompatibility of the blended film.[23] PEG is often blended or compounded with other polymers to be used in the field of controlled drug release.

Genipin can be prepared from geniposide, one of the glucosides, using b-glucosidase. Genipin is isolated in large quantity by a microbiological process involving *Penicillium nigricans* that produces b-glucosidase, which in turn hydrolyzes the geniposide into the aglycone genipin. Genipin and its related iridoid glucosides have been widely used as an antiphlogistic and cholagogue in the herbal medicines.[24] Genipin was known to have about 5000–10,000 times less

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cytotoxicity than glutaraldehyde.[25] It has been used as a crosslinking agent for the fixation of biological tissues in the bioprostheses.[26,27] The biocompatibility of the genipin-fixed tissues was evaluated in several animal studies.[28,29] It was consistently noted that the inflammatory reaction of the genipin-fixed tissues was significantly less than their glutaraldehyde-fixed counterparts.

The physical properties of chitosan can be improved by blending with PEG. Chitosan contributes to the improvement in mechanical properties, while PEG contributes to the improvement in flexibility and ductility of chitosan. Chitosan/PEG blends had a reduced level of crystallinity due to the favorable interactions between components, which results in the facilitated diffusion of water into the blends to give a higher swelling ratio. Among various stimuli-responsive materials, pH-sensitive polymers have been widely used to design novel stimuli-responsive delivery systems. pH-dependent conformational change of the copolymer chains could give a mechanical stress to the liposomal membrane, leading to a pH-sensitive release.

This study aims to develop a chitosan-based crosslinked system that can serve as a therapeutic drug delivery system promoting tissue repair and regeneration through the controlled and sustained release of the loaded drugs. The nontoxic genipin-crosslinked chitosan (GC)/PEG film can be applied for the localized drug delivery *in vivo* or *in vitro* environment. In this work, we prepared the GC/PEG films with various blend compositions to find out the optimal properties for the pH-responsive drug release. The drug release behavior of GC/PEG film was studied in terms of the pH-responsive swelling characteristics and the morphologies of the blends. The release behavior of drug, rhodamine B, from GC/PEG films was evaluated with varying the pH of releasing medium.

EXPERIMENTAL

Materials

Chitosan (75–85% deacetylated, Brookfield viscosity of 200–800 cps for 1 wt % solution in 1 wt % acetic acid) was purchased from Aldrich (St. Louis, MO). PEG (Mw = 750, 2000, 4000, and 8000), acetic acid, and rhodamine B were purchased from Sigma (St. Louis, MO). The buffer solutions of pH 2.0, 4.0, 7.0, and 10.0 were purchased from Samchun Pure Chemical (Seoul, Korea). Genipin was obtained from Aldrich (St. Louis, MO).

Preparation of GC/PEG hydrogel films

Aqueous chitosan solution of 1.5% (w/v) was prepared by dissolving chitosan powder in aqueous 1% (v/v) acetic acid solution. The viscous chitosan solution was filtered through filter paper to remove any undissolved gel. The clear, light yellow chitosan solution was then mixed with an aqueous PEG solution. Mixtures were stirred overnight at 25°C. Genipin was dissolved in water and then was added to the chitosan/PEG mixture of various compositions. The cross-linking reaction of the chitosan/PEG/genipin mixture lasted for 2 h before it was cast on Petri dish to form the film-shaped hydrogel.

Rhodamine B (0.5 wt %), a model drug, was dissolved in 100 mL of each blend solution to make the completely homogeneous mixtures. The blend solutions started to turn light blue and became more viscous after 2 h. The blend solution was filtered through a glass Buchner funnel, cast onto Petri dish, and dried in vacuum for 2 days at room temperature. The drug-loaded GC/PEG hydrogel films were stored in a desiccator at room temperature for further experiments. The formulation of GC/PEG preparation is shown in Table I.

TABLE I
Feed Compositions of GC/PEG (PEG: Mw = 4000) Hydrogel Films Containing Rhodamine B (0.5 wt %)

Sample name	Chitosan (g)	PEG (g)	Genipin (g)
GC/PEG-1	0.45	0.05	0.003
GC/PEG-2	0.40	0.10	0.003
GC/PEG-3	0.35	0.15	0.003
GC/PEG-4	0.30	0.20	0.003
GC/PEG-5	0.20	0.30	0.003
GC/PEG-6	0.40	0.10	0.0005
GC/PEG-7	0.40	0.10	0.001
GC/PEG-8	0.40	0.10	0.003
GC/PEG-9	0.40	0.10	0.006

FTIR analysis

The characteristic functional groups of GC/PEG hydrogel films were studied with Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR, FTS 175C, Bio-Rad) in the range of 4000–500 cm^{-1} with the resolution of 2 cm^{-1} at room temperature.

DSC measurement

The thermal properties of GC/PEG hydrogel films were measured by differential scanning calorimetry (DSC, 2010, TA instruments). The films were cut into small pieces and about 10 mg of samples were placed inside an aluminum sample pan. The thermal analysis was performed from −20 to 150°C at the heating rate of 10°C/min under dry nitrogen atmosphere with a flow rate of 20 mL/min.

Morphological study

The variations in surface morphology of GC/PEG hydrogel films at various pHs were studied using a scanning electron microscope (SEM, JEM-1010, Jeol). Small pieces of films were cut and immersed in the distilled water for 8 h. The swollen films were freeze-dried prior to SEM observation. The specimens were mounted onto the aluminum stubs with double-sided carbon tape and sputter-coated with gold for 5 min.

Partial dissolution of PEG in water

The dried GC/PEG hydrogel films were immersed in 40 mL methanol for 24 h to remove any acetic acid remained in the films, and then were taken out and dried in the vacuum oven for 24 h at room temperature. The dried films were weighed (W_1), immersed in distilled water for 8 h, dried in a vacuum oven for 24 h at room temperature, and then stored in a desiccator for 5 h for weight balance. The equilibrium weights of the residual GC/PEG hydrogel films were weighed (W_2). The dissolution ratio of GC/PEG hydrogels in water (W_s) was calculated with the following equation:

$$W_S = (W_1 - W_2)/W_1 \times 100\%$$

Swelling of GC/PEG hydrogels

The swelling ratios of GC/PEG hydrogels were determined gravimetrically. GC/PEG hydrogels were kept in the buffer solution for 8 h at 37°C to reach the equilibrium swelling. The swollen hydrogel films were removed at predetermined intervals and weighed immediately after the excess water on the film surface was wiped off with a filter paper. The swelling ratio was calculated with the following equation:

$$\text{Swelling ratio } (\%) = (W_t - W_0)/W_0 \times 100$$

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

Release of rhodamine B from GC/PEG hydrogels

The drug-loaded GC/PEG hydrogel films were put in glass vessels containing 50 mL of buffer solutions under shaking at 37°C. Parts of buffer solutions were taken at several time intervals and the amount of rhodamine B released from the drug-loaded GC/PEG hydrogel films was evaluated by UV/vis spectrophotometer (UV-1201, Shimadzu) at 552 nm. Then after the measurement of the buffer solution, it was returned to maintain the constant volume of the parent solutions. The various buffer solutions of pH 2, 4, 7, and 10 were used as the media for the controlled release studies. All the release experiments were done in triplicate. The mean values were used in the figures and the deviations were used for the error bars in figures.

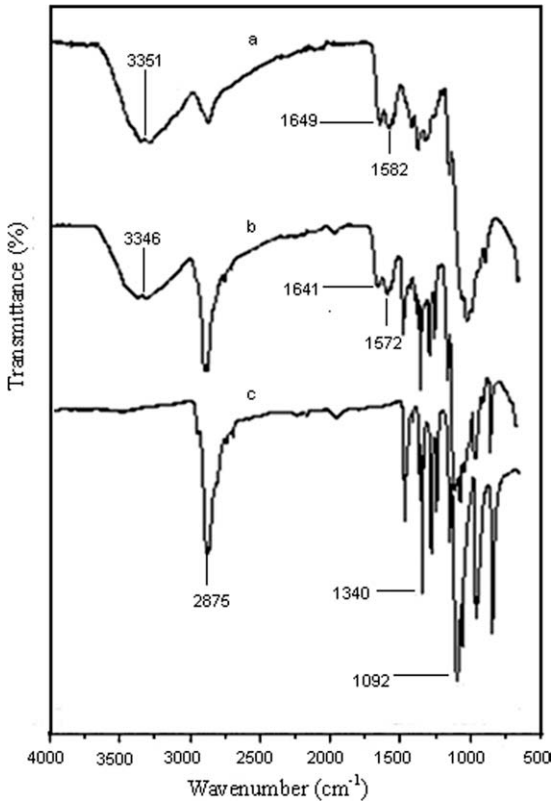

Figure 1 IR spectra of (a) chitosan, (b) GC/PEG (80/20) hydrogel crosslinked with genipin (0.6 wt %) and containing no rhodamine B, and (c) PEG.

RESULTS AND DISCUSSION

FTIR-ATR characterization

Figure 1 shows the FTIR-ATR spectra of chitosan, PEG, and GC/PEG hydrogels. Chitosan has two characteristic absorption bands, amide I and amide II, caused by C—N stretching vibrations at 1649 cm^{-1} and N—H in-plane bending vibrations at 1582 cm^{-1}, respectively. The bands due to N—H and O—H stretching vibrations are overlapped in the absorption peak at 3351 cm^{-1}. The characteristic absorption band of PEG at 1092 cm^{-1} is attributed to the bending vibration of C—O. Two absorption bands at 1340 and 2875 cm^{-1} are attributed to the bending and the stretching vibration of C—H, respectively. The wide absorption band around 3421 cm^{-1} is due to the stretching vibration of O—H. The characteristic absorption bands of chitosan at 1582 and 1648 cm^{-1}

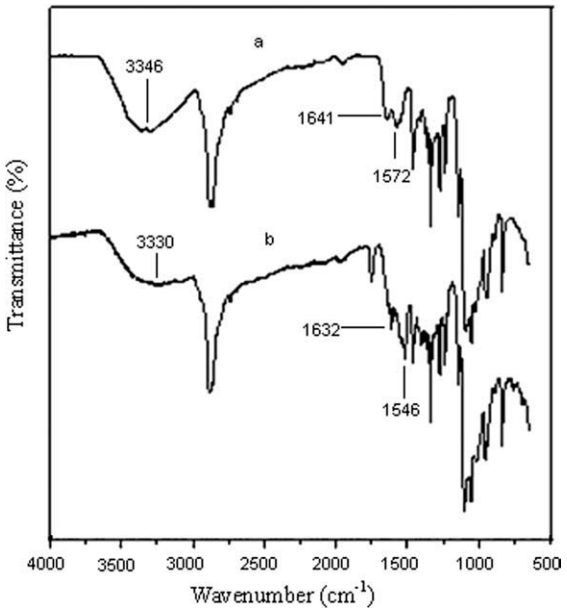

Figure 2 IR spectra of (a) GC/PEG (80/20) hydrogel crosslinked with genipin (0.6 wt %) and containing no rhodamine B and (b) GC/PEG (80/20) hydrogel containing rhodamine B (GC/PEG-2).

shifted to 1572 and 1641 cm^{-1} for the GC/PEG hydrogels. The absorption band at 3351 cm^{-1} also shifted to 3346 $cm^{-1}$and became wider due to the extensive formation of the favorable intermolecular interactions between chitosan and PEG.[30]

As shown in the FT-IR spectra of GC/PEG-2 film (Fig. 2), it was seen that the characteristic absorption bands at 1641 and 1572 cm^{-1} of GC/PEG (no drug loading) shifted to lower wave number at 1632 and 1546 cm^{-1}, respectively, and the characteristic absorption band at 3346 cm^{-1} also shifted to a lower wave number at 3330 cm^{-1}. All these results indicated that the rhodamine B used in this work had strong interactions with the matrices of the hydrogel films, and the existence of band at 1749 cm^{-1} indicated the deposition of rhodamine B on GC/PEG films.

DSC analysis

The thermal transitions of chitosan, PEG, and their blends before and after water dissolution were determined by DSC analyses as shown in Table II. It was shown that the melting point decreased with increasing chitosan content. The melting point of PEG was depressed for all the blends implying the intermolecular interactions, which govern the compatibility of the blends to some extent.

The melting point of PEG in each GC/PEG residue after the partial dissolution in water showed the little variation with the melting point of PEG in GC/PEG before dissolution. However, the heat of melting of GC/PEG residue after the partial dissolution in water decreased distinctly compared to that of GC/PEG before the water dissolution, because some of PEG was dissolved out from GC/PEG hydrogel films. Therefore, partial dissolution of PEG from GC/PEG film did not give any big difference in the thermal characteristics of GC/PEG hydrogel films.

Reaction mechanism of chitosan crosslinking with genipin

According to Mi et al.,[31] the crosslinking reaction mechanisms for chitosan with genipin are different depending on pH values. This study was carried out under neutral conditions. Nucleophilic attack by the amino groups of chitiosan on the olefinic carbon atom at C-3 of deoxyloganin aglycon occurs followed by opening the dihydropyran ring to form heterocyclic amine. The intermediate compounds could further associate to form cross-linked networks with short chains of cross-linking bridges. The nucleophilic substitution of the ester group on genipin by the primary amine group on chitosan is the main mechanism of crosslinking.

Swelling behavior of GC/PEG hydrogels

As the hydrogels swell, the elastically retractile force of the chain segments between the crosslinks in the hydrogels restricts the swelling due to the decreased configurational entropy resulting in the thermodynamic equilibrium of the swelling. The swelling behavior of the carboxylic acid-containing hydrogels was greatly influenced by the pH of swelling medium, the content of crosslinker, and the composition of hydrogels.

The pH-sensitive swelling behavior of GC/PEG hydrogels is shown in Figure 3. The GC/PEG hydrogel crosslinked with lower content of genipin (0.1%) was dissolved in the buffers of lower pH. GC/PEG hydrogels showed the higher swelling as the pH of media decreased to the acidic condition. The swelling ratio of GC/PEG hydrogels decreased significantly as the content of crosslinker increased.

TABLE II
Thermal Transitions of GC/PEG (PEG = 4000) Hydrogel Films Before and After Dissolution in Water

Composition of GC/PEG	Before dissolution		After dissolution	
	T_m (°C)	ΔH_m (J/g)	T_m (°C)	ΔH_m (J/g)
100/0	–	–	–	–
80/20	51.6	8.2	51.2	5.6
70/30	52.8	20.6	52.4	16.7
60/40	54.3	45.3	53.5	32.2
0/100	61.5	181.2	–	–

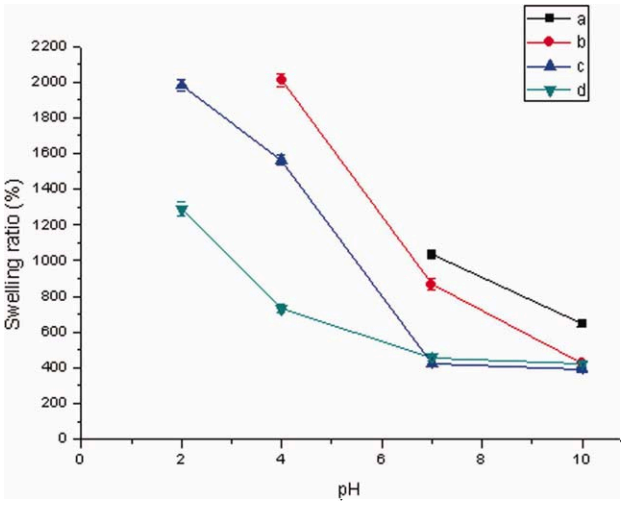

Figure 3 pH-sensitive swelling behavior of GC/PEG (80/20) hydrogels (PEG: Mw = 4000) with various contents of crosslinker (a) 0%, (b) 0.1%, (c) 0.6%, and (d) 1.2%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

The variation in swelling ratios of the various GC/PEG hydrogels depending on the composition of hydrogel and the pH of medium is shown in Figure 4. The swelling ratio of GC/PEG hydrogel increased as the content of PEG increased due to the increased hydrophilicity of the hydrogels. The higher swelling of GC/PEG hydrogels at lower pH is ascribed to the hydrolysis of amide linkages in the crosslinked chitosan networks by acid and the regeneration of amine groups in the networks.[32] The equilibrium swelling ratio of GC/PEG hydrogel in the acidic medium was larger due to the amino groups reformed in the network. The electrostatic

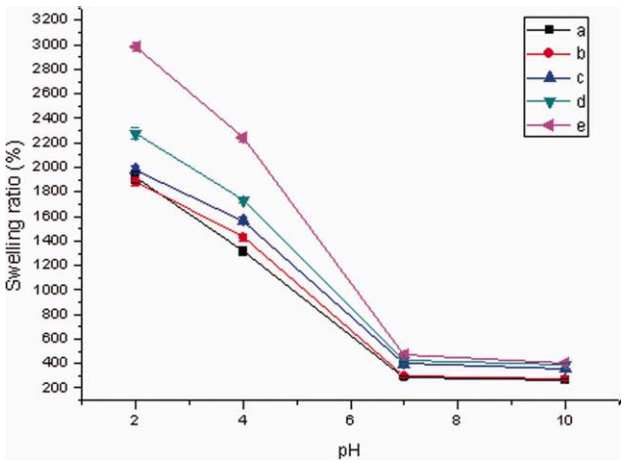

Figure 4 Variation in swelling ratios of GC/PEG hydrogels (genipin: 0.6%, PEG: Mw = 4000) with various chitosan/PEG compositions (a) 100/0, (b) 90/10 (GC/PEG-1), (c) 80/20 (GC/PEG-2), (d) 70/30 (GC/PEG-3), and (e) 60/40 (GC/PEG-4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

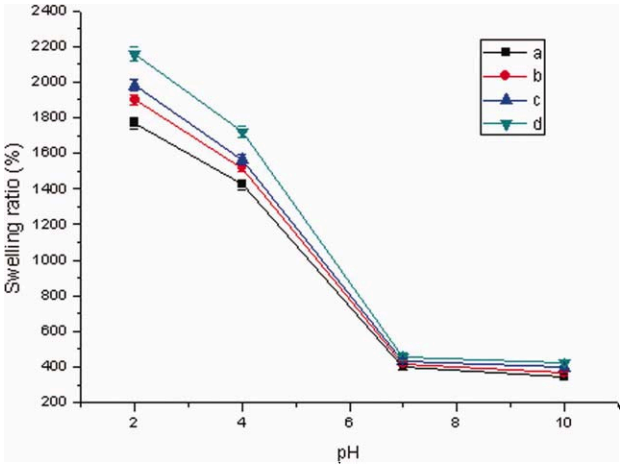

Figure 5 Swelling behavior of GC/PEG (80/20) hydrogels (genipin: 0.6%) with various molecular weights of PEG (a) 750, (b) 2000, (c) 4000, and (d) 8000. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

repulsion of the protonated $-NH_3^+$ groups along the chitosan chain could also lead to the expansion of networks and hence a higher swelling.

On the other hand, the deprotonation of amino groups took place in the higher pH media and the repulsion in polymer chains was reduced resulting in the shrinkage of hydrogels. The genipin-crosslinked chitosan networks showed the pH-sensitive swelling characteristics that would be desirable for the biomedical applications such as artificial muscles or switches, biomedical separation systems, and controlled release systems.[33,34]

The variation in swelling ratios of GC/PEG hydrogels depending on the molecular weight of PEG is shown in Figure 5. The higher the molecular weight

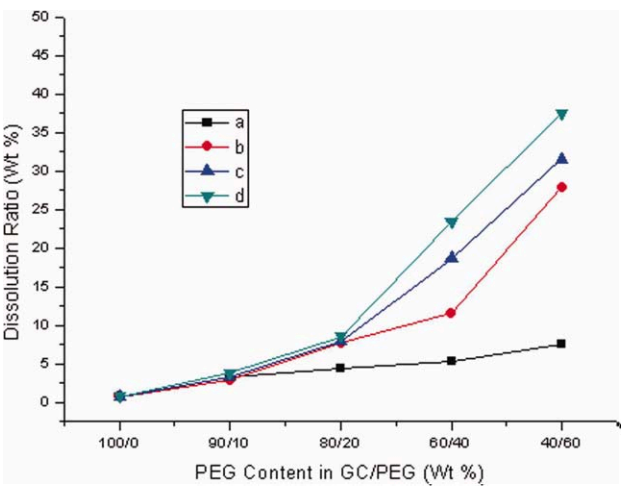

Figure 6 Dissolution ratios of GC/PEG (80/20) hydrogels (genipin: 0.6%) at pH 2 depending on composition with various molecular weights of PEG (a) 750, (b)2000, (c) 4000, and (d) 8000. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

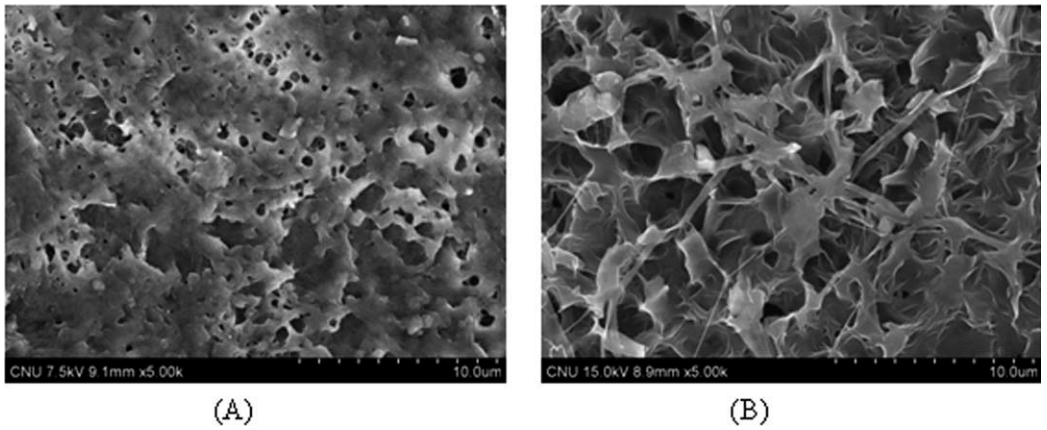

Figure 7 Surface morphology of GC/PEG-2 hydrogel swollen at various pHs (A) pH 7 and (B) pH 2.

of PEG is, the higher is the swelling ratio of GC/PEG hydrogel due to the formation of larger pores and the higher porosity in the networks of chitosan.

Dissolution of PEG from GC/PEG hydrogels in water

The dissolution ratios of GC/PEG hydrogel films are shown in Figure 6. The dissolution of PEG from GC/PEG hydrogels increased as either the molecular weight or the content of PEG increased. GC/PEG hydrogels containing PEG of lower molecular weight showed the least dissolution, because the miscibility of lower molecular weight PEG with chitosan was effective in preventing PEG from dissolution in water. The controlled dissolution of biodegradable films offers advantages for use in the food, pharmaceutical, and agricultural industries. It has been reported that microporous GC/PEG films can control the rate of drug release by stepwise dissolution of PEG in water.[35] The partial dissolution of PEG from GC/PEG hydrogels was beneficial to the formation of various morphologies without inducing any noticeable variation in the characteristics of hydrogels.

Morphology of GC/PEG hydrogels

Figure 7 shows the SEM images of the freeze-dried GC/PEG hydrogels swollen at pH 7 and 2, respectively. The smaller pores were found in the GC/PEG hydrogels swollen at pH 7. However, the larger pores were developed extensively in the GC/PEG hydrogels swollen at pH 2 indicating the correspondence of morphology to the swelling behavior of GC/PEG hydrogel films.

Drug release behavior of GC/PEG hydrogels

The effect of composition on the release behavior of GC/PEG hydrogels is shown in Figure 8. The release of rhodamine B from GC/PEG hydrogels increased as the content of PEG increased due to the formation of extensive pores that accelerated the release of drug from the hydrogel matrix. The release of

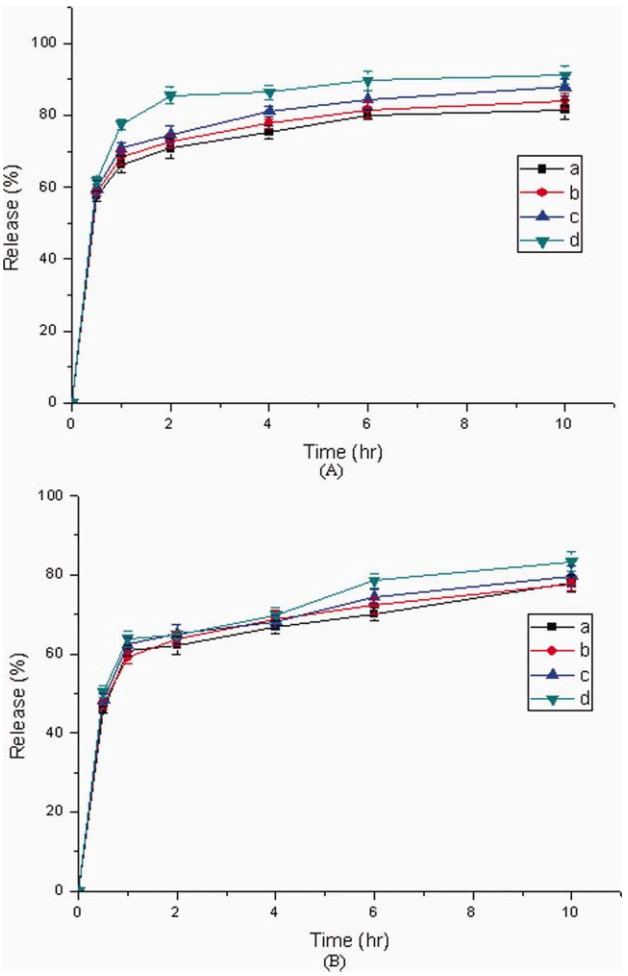

Figure 8 Drug release behavior of GC/PEG hydrogels at (A) pH 2 and (B) pH 7 with various contents of PEG (a) GC/PEG-1, (b) GC/PEG-2, (c) GC/PEG-4, and (d) GC/PEG-5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

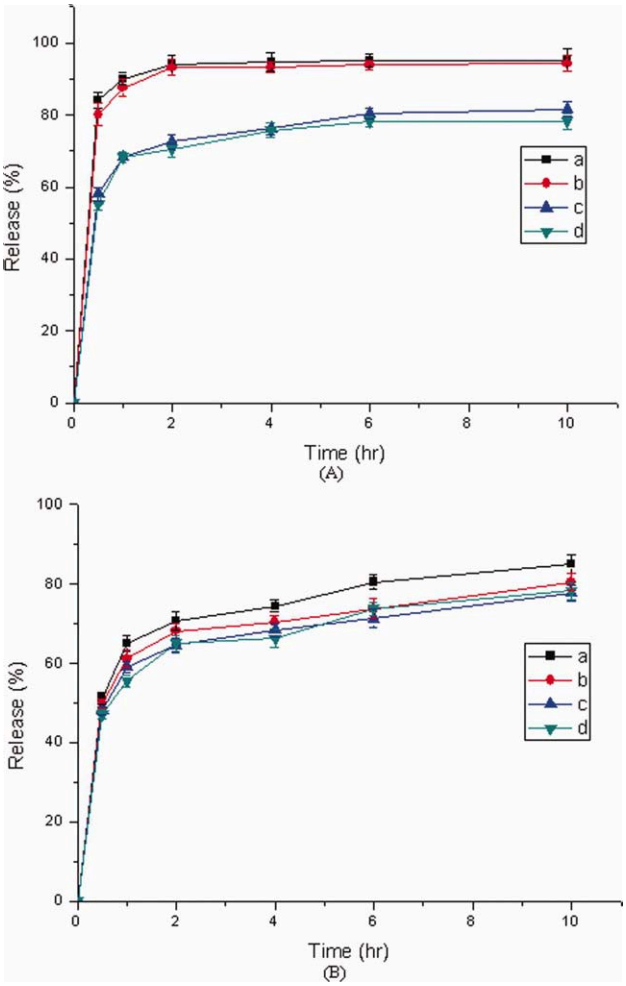

Figure 9 Drug release behavior of GC/PEG hydrogels at (A) pH 2 and (B) pH 7 with various contents of crosslinker (a) GC/PEG-6, (b) GC/PEG-7, (c) GC/PEG-8, and (d) GC/PEG-9. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

rhodamine B from GC/PEG hydrogels also increased as the pH of medium decreased to the acidic condition.

The effect of the content of crosslinker on the release behavior of GC/PEG hydrogels is shown in Figure 9. The release of rhodamine B from GC/PEG hydrogels decreased as the content of crosslinker increased, because higher degree of crosslinking obstructed the passage of drug loaded inside the hydrogel by decreasing the diffusion efficiency of polymer matrix.

The release of rhodamine B from GC/PEG hydrogels increased as the molecular weight of PEG increased as shown in Figure 10. PEG with higher molecular weight gave the larger pore size in the hydrogel matrix due to the limited miscibility with the genipin-crosslinked chitosan. The highly porous hydrogels were more effective in both swelling the matrix and releasing the drug loaded in the matrix.

The drug release characteristics of GC/PEG hydrogels were very sensitive to the pH of medium as shown in Figures 8 and 9. The release of drug was generally accelerated with decreasing the pH to the acidic condition, because the electrostatic repulsion of the protonated $-NH_3^+$ groups along the chitosan segments led to the expansion of networks and hence a higher swelling of hydrogels.

Effect of pH on drug release behavior of GC/PEG hydrogels

The drug release behavior from GC/PEG-7 hydrogel film was studied in four different buffer solutions of pH 2.0, 4.0, 7.0, and 10.0. Figure 11 shows that the drug release from GC/PEG is very sensitive to the pH of the medium. The release was accelerated with the decrease of pH, because the electrostatic repulsion of the protonated $-NH_3^+$ groups along the

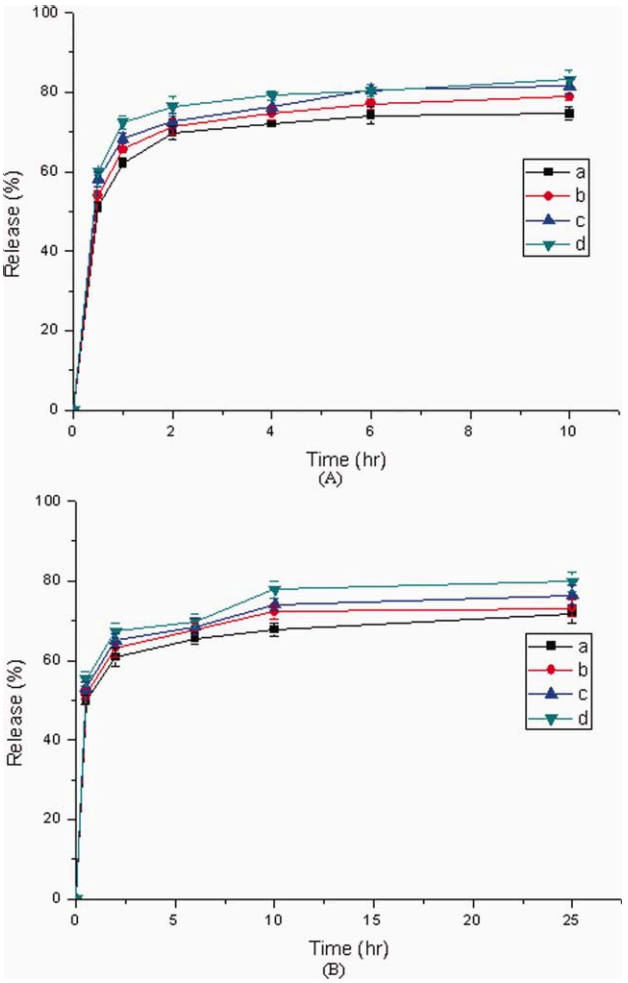

Figure 10 Drug release behavior of GC/PEG (80/20) hydrogels (genipin: 0.6%) at (A) pH 2 and (B) pH 7 with various molecular weights of PEG (a) 750, (b) 2000, (c) 4000, and (d) 8000. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

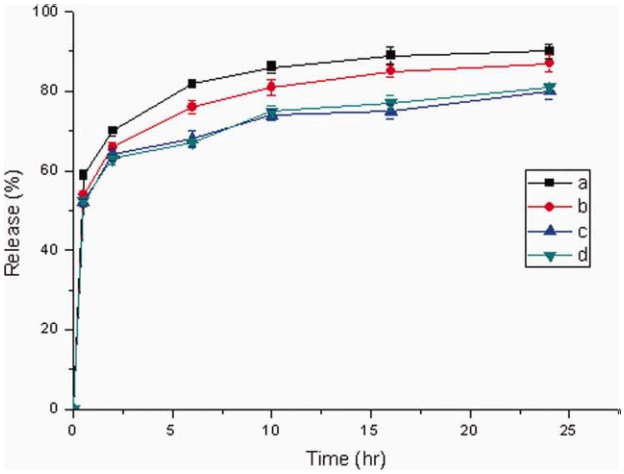

Figure 11 Drug release behavior of GC/PEG-7 hydrogels (genipin: 0.6%) at various pHs of the releasing medium (a) pH 2, (b) pH 4, (c) pH 7, and (d) pH 10. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

chitosan polymer chain lead to an expansion of the network and hence a higher swelling.

Mathematical models have been used to describe the drug release mode of GC/PEG hydrogel films. Ritger and Peppas[36] introduced an exponential model to analyze the drug release from polymeric devices with various geometrical shapes.

$$M_t/M_\infty = Kt^n$$
$$\log(M_t/M_\infty) = \log K + n\log t$$

where M_t/M_8 is the fractional solute release, t is the release time, K is a constant incorporating structural and geometric characteristics of the device, and n is the diffusion exponent characteristic of the release mechanism. When n is equal or less than 0.5, the diffusion mechanism is Fickian. It is case II when n is equal to 1, and the diffusion is very fast contrary to the rate of relaxation. The third case corresponds to an anomalous diffusion, non-Fickian release mode, with n values lying between 0.5 and 1.

For the drug release from GC/PEG-7 as shown in Figure 11, the n values at pH 2 and 7 were determined as 0.68 and 0.46, respectively. Drug release from GC/PEG showed the Fickian diffusion mechanism at pH 7. However, drug release from GC/PEG showed the non-Fickian diffusion mechanism at pH 2 where higher swelling of GC/PEG hydrogel occurred.

CONCLUSIONS

The pH-sensitive chitosan/PEG hydrogels were synthesized using the nontoxic genipin as a crosslinker. Various genipin-crosslinked chitosan/PEG hydrogels were prepared by varying the contents of PEG and genipin, and the molecular weights of PEG. Both the swelling ratio and the drug release increased as the pH of medium decreased, because the electrostatic repulsion occurred in the hydrogels by the protonation of amino acids along the chitosan chains in the acidic medium. The release of drug from GC/PEG hydrogels increased as the content of PEG increased due to the formation of larger pores in the hydrogel and the higher swelling of matrix. The release of drug from GC/PEG hydrogels decreased as the genipin contents increased due to the higher degree of crosslinking.

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