Medicated Wound Dressings Based on Poly(vinyl alcohol)/Poly(*N*-vinyl pyrrolidone)/Chitosan Hydrogels

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ABSTRACT: Poly(vinyl alcohol)/poly(*N*-vinyl pyrrolidone) (PVP)/chitosan hydrogels were prepared by a low-temperature treatment and subsequent ^{60}Co γ-ray irradiation and then were medicated with ciprofloxacin lactate (an antibiotic) and chitosan oligomer (molecular weight = 3000 g/mol). The gel content, swelling ratio, tensile strength, and crystallinity of the hydrogels were determined. The effects of the chitosan molecular weight, the low-temperature treatment procedure, and the radiation dosage on the hydrogel properties were examined. The molecular weight of chitosan was lowered by the irradiation, but its basic polysaccharide structure was not destroyed. Repeating the low-temperature treatment and γ-ray irradiation caused effective physical crosslinking and chemical crosslinking, respectively, and contributed to the mechanical strength of the final hydrogels. The incorporation of PVP and chitosan resulted in a significant improvement in the equilibrium swelling ratio and elongation ratio of the prepared hydrogels. The ciprofloxacin lactate and chitosan oligomer were soaked into the hydrogels. Their *in vitro* release behaviors were examined, and they were found to follow diffusion-controlled kinetics.
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Key words: chitosan; diffusion; hydrogels; radiation

INTRODUCTION

The principal function of a wound dressing is to provide an optimal healing environment, including isolation from the external environment, complete coverage of the wound surface to prevent further contamination or infection, and maintenance of a moist microenvironment next to the wound surface.[1] The most important features for burn wounds are the prophylaxis of wound sepsis and fluid loss control. Many kinds of wound dressing materials are available in the market,[2–4] but some of them cannot effectively prevent subsequent microbial invasion. Sometimes, they are easy to adhere to the wound surface; however, when the dressing is removed, considerable damage is inflicted on the newly formed epithelium. To overcome current limitations in wound dressings for burn injury treatment, antibiotic-containing hydrogels would be a better choice.

Many polymers can be used to form hydrogels, such as poly(vinyl alcohol) (PVA) and poly(*N*-vinyl pyrrolidone) (PVP). PVA is a well-known biologically friendly polymer because of its biocompatibility and appropriate mechanical properties. It was one of the first synthetic polymers to be tested as an artificial cartilage,[5] wound dressing, artificial skin, cardiovascular device, and drug vehicle.[6,7] PVP is one of the most widely used polymers in medicine because of its solubility in water and its extremely low cytotoxicity.[8,9] A recent work has described the use of PVP for topical applications onto the skin for the transdermal delivery of drugs.[10] Chitosan is a biodegradable polysaccharide composed of D-glucosamine and partial *N*-acetyl-D-glucosamine linked together by (1,4)-glycosidic bonds. Chitosan or chitosan oligomer is useful in various fields because it has specific biological activities such as biocompatibility, biodegradability, hemostatic activity, anti-infection activity, and an ability to accelerate wound healing.[11–14] The combination of the properties of PVA, PVP, and chitosan in PVA/PVP/chitosan blends may lead to the preparation of new biomaterials.[1]

Among the many methods used to produce hydrogels, irradiation has special advantages. Irradiation can produce hydrogels without noxious chemical crosslinkers such as glutaraldehyde,[15] and it is possible to control the physical properties of hydrogels by the adjustment of the radiation dosage and the polymer composition.[16] Several kinds of PVA- or PVP-containing hydrogels produced by radiation have

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been reported,[8,17] and they possess better water absorption and retention ability but have poor mechanical properties.

A hydrogel wound dressing provides a wet environment for the wound. At the same time, it provides an environment for the propagation of bacteria on the wound surface. Therefore, it is important to endow a hydrogel wound dressing with an antibacterial ability.

In this study, great attempts have been made to prepare PVA/PVP/chitosan hydrogels containing both an antibiotic agent and chitosan oligomer. They are expected to have the following superiorities: acceptable mechanical strength, excellent biocompatibility, reduction of the fluid and heat loss from the wound, and prophylaxis against wound sepsis. This article discusses the formulation and properties of this wound dressing as well as the *in vitro* release profiles of the antimicrobial agent and chitosan oligomer from the hydrogels.

EXPERIMENTAL

Materials

PVA with a hydrolysis degree of 99.0–99.8% (molecular weight = 7.3×10^4 to 7.7×10^4), dimethyl sulfoxide (DMSO), lithium acetate, and glacial acetic acid were all supplied by Shanghai Chemical Reagent Co. (Shanghai, China). PVP (molecular weight = 3.6×10^5) was purchased from BASF Chemical Co. (Ludwingshafen, Germany). These polymers were used without further purification. The chitosan samples were supplied by Yuhuan Ocean Biochemistry Co., Ltd. (Zhejiang, China). Their average viscosity-average molecular weight (M_η) values were 5.05×10^5 (Chi-1), 6.99×10^4 (Chi-2), 7.38×10^3 (Chi-3), and 3.00×10^3 g/mol (chitosan oligomer). They all had a degree of deacetylation (DD) of 90%. Ninhydrin and hydrindantin were purchased from Sigma (Dorset, United Kingdom). Ciprofloxacin lactate was purchased from Sichuan Qili Pharmaceutical Co., Ltd. (Sichuan, China). Distilled water was used as a solvent in all experiments. All other reagents were analytical-grade and were used without further purification.

Preparation

Radiation degradation of chitosan

Three chitosan solutions were prepared. Chi-1 and Chi-2 were dissolved in 0.9 and 0.5 wt % acetic acid solutions, respectively, and Chi-3 was dissolved in distilled water. The solid concentration of the three solutions was 5.0 wt %. They were filtered through a G2 glass filter and then irradiated with ^{60}Co γ ray at a dosage of 25 kGy at room temperature (25°C). The radiation degradation products were obtained after a series of posttreatments.[18]

Preparation of the PVA/PVP hydrogels

PVA and PVP were dissolved in distilled water at 100°C. The PVA-to-PVP weight ratios were 100 : 0, 80 : 20, 75 : 25, 70 : 30, and 60 : 40. The total solid concentration was 15 wt %. The solutions were kept at room temperature for 24 h to remove air bubbles, and then they were put into polyethylene film bags and pressed between two glass plates with a gap of 3 mm. The solutions became physically crosslinked hydrogels after repeated low-temperature treatments (i.e., freeze–thaw cycling), that is, lowering the temperature to −30°C, standing at this temperature for 12 h, raising the temperature to 25°C, and standing at this temperature for 12 h. This treatment was repeated up to seven times.

Preparation of the PVA/PVP/chitosan hydrogels

PVA and PVP were dissolved in distilled water at 100°C with a PVA/PVP weight ratio of 70 : 30 and then mixed with a chitosan solution by mechanical stirring for 5 h at 60°C to give a PVA/PVP/chitosan solution. The weight ratio of PVA/PVP to chitosan was 5 : 1, and the total solid concentration was 15 wt %. With the same procedure presented in the previous section, the solution was converted into a physically crosslinked hydrogel first, and then the hydrogel plates were exposed to ^{60}Co γ rays to achieve chemical crosslinking. Three chitosan samples (Chi-1, Chi-2, and Chi-3) were used in this experiment. The radiation dosages were 15, 25, 35, and 45 kGy.

Preparation of the medicated PVA/PVP/chitosan hydrogels

The PVA/PVP/Chi-1 plate was prepared with three freeze–thaw treatments and then was crosslinked by radiation with a dosage of 25 kGy. It was freeze-dried and sterilized by ^{60}Co γ rays at a sterilizing dosage. The sterilized samples were immersed in phosphate-buffered saline [PBS; pH 7.4, 0.1 mol/L phosphate, 0.9 (w/v) % saline] containing ciprofloxacin lactate and chitosan oligomer in an aseptic environment for 24 h at 37°C. The mass ratio of the gel to the solution was about 1 : 50.

Characterization of the chitosan and hydrogels

Molecular weight determination of chitosan

A chitosan sample was dissolved in a buffer solution containing 0.1 mol/L CH_3COOH and 0.2 mol/L NaCl. The relative viscosity (η_r) and specific viscosity (η_{sp}) of this solution were measured with an Ubbelohde viscometer (Shanghai Experiment Reagent Co., Ltd., Shanghai, China) at 25.0 ± 0.1°C. The molecular

weight of the chitosan was calculated according to following equations :[19]

$$[\eta] = (\eta_{sp} + 3 \ln \eta_r)/4C$$

$$[\eta] = 1.81 \times 10^{-3} M_\eta^{0.93}$$

where $[\eta]$ is the intrinsic viscosity (dL/g) and C is the concentration (g/dL).

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of chitosan samples were recorded on a Bio-Rad FTS135 (Bio-Rad Laboratories, Inc., Hercules, CA) at a resolution of 4 cm^{-1} with 32 scans at room temperature by a KBr disc method.

Differential scanning calorimetry (DSC) measurements

DSC measurements (DSC-7, PerkinElmer, Norwalk, CT) were conducted over the temperature range of 50–240°C at a scanning rate of 10°C/min under nitrogen protection to detect the crystalline status in the PVA/PVP gels. The specimens weighed approximately 5–10 mg.

Gel content

The irradiated hydrogel samples were vacuum-dried at 40°C to a constant weight. The dried samples were extracted by distilled water in a Soxhlet apparatus (Beijing Glass Instruments Factory, Beijing, China) for 24 h and then dried *in vacuo* to a constant weight. The gel fraction was calculated gravimetrically with the following formula: Gel content (%) = $W_g/W_0 \times 100\%$, where W_g and W_0 are the dried sample weights after and before extraction, respectively.

Water take-up

The hydrogel plates were dried by a freeze drier (Beijing Glass Instruments Factory, Beijing, China). The dried samples were immersed in distilled water (200-fold mass) at 37°C. The swollen samples were taken out at a certain time interval and were weighed after the removal of free water on the gel surface with filter paper. The swelling was continued to achieve a constant weight. The equilibrium swelling ratio (ESR) was calculated with ESR (%) = $(W_e - W_d)/W_d \times 100\%$, where W_e is the weight of the swollen gel in the equilibrium state and W_d is the weight of the freeze-dried gel.

Mechanical properties

The tensile strength and elongation at break were determined on dumbbell-shaped specimens according to ASTM standard with an Instron 1121 tester (Instron, Danvers, MA) at a constant extension rate of 20 mm/min at room temperature (25°C).

Determination of the drug content

Three round discs 10 mm in diameter were cut from a PVA/PVP/chitosan hydrogel plate and were medicated with the antibiotic with the same procedure described in the section "Preparation of the Medicated PVA/PVP/Chitosan Hydrogels". After 24 h of immersion, the weights of these swollen discs were determined after the adsorbed drug on the disc surfaces was washed off with distilled water and the surface water was removed by filter paper. Then, each of the medicated hydrogel discs was put into a conical flask containing 100 mL of PBS. After the equilibrium was reached in 3 days, the ciprofloxacin lactate in the PBS was determined with an ultraviolet–visible (UV–vis) spectrophotometer (2100, Shimadzu, Kyoto, Japan) at 270 nm. A solution prepared with a blank hydrogel disc was used as a reference in the UV–vis determination. The final result was obtained via averaging over three disc specimens.

Determination of the chitosan oligomer content

The determination of the chitosan oligomer content in the hydrogel was based on the quantitative reaction of primary amino groups with a ninhydrin reagent.[20] Hydrogel discs 10 mm in diameter containing chitosan oligomer were soaked in 50 mL of PBS for 3 days to get the abstract of the chitosan oligomer. A lithium acetate buffer (10 mL) was prepared by the dissolution of 4.08 g of lithium acetate dihydrate in approximately 6 mL of deionized water. The pH of the resulting solution was adjusted to 5.2 with glacial acetic acid, and the volume was adjusted to 10 mL with deionized water. The ninhydrin reagent was freshly prepared on the day of the assay by the addition of the aforementioned lithium acetate buffer (10 mL) to a solution of 0.8 g of ninhydrin and 0.12 g of hydrindantin in 30 mL of DMSO. Then, 0.5 mL of the ninhydrin reagent was added to 0.5 mL of the abstract solution in a glass vial. The vial was immediately capped, gently shaken by hand, and heated in boiling water for 30 min to allow the reaction to proceed. After cooling, 15 mL of a 50 : 50 mixture of ethanol and water was added to each vial. The vial was then agitated for 15 s to oxidize the excess hydrindantin. The absorbance of each solution was measured on a UV–vis spectrophotometer at 570 nm, and the concentration of chitosan in the abstract was read from the calibration curve and converted into the chitosan content in the hydrogel sample.

In vitro release behaviors of the drug and chitosan oligomer

The *in vitro* drug and chitosan oligomer release behaviors were studied at 37°C in a modified Franz diffusion cell, which consisted of two cylindrical poly(methyl methacrylate) half-cells with their common central axis in the horizontal direction.[21] Its effective area was 7.06 cm^2. Each half-cell had a volume of 30 mL and had an outlet at the upper top. One side of the hydrogel plate was covered with a dialytic membrane of a cutoff of about 10,000 g/mol and was placed facing the releasing medium; the other side of the hydrogel plate was left empty to simulate the working condition of a wound dressing. The half-cell in the dialytic membrane side was equipped with a magnetic stirrer and filled with PBS. The connection part of the half-cells and the outlets were sealed with parafilm to reduce water evaporation. Half of the PBS was withdrawn for UV–vis measurement, and the same amount of fresh PBS was refilled at certain times. The concentration of ciprofloxacin lactate released into the PBS was determined with a UV–vis spectrophotometer and was converted into the accumulated release amount. The chitosan oligomer released into the PBS solution was determined by the method described previously and then was converted into the accumulated release amount.

RESULTS AND DISCUSSION

Radiation effect on the molecular weight and chemical structure of chitosan

Chitosan has many important characteristics as a component of hydrogels for wound dressings.[22,23] The radiation effect on chitosan was considered first because it was added before irradiation. Three chitosan samples (Chi-1, Chi-2, and Chi-3) of different molecular weights were used for this purpose. The γ-ray dosage of 25 kGy was chosen because it was a suitable dosage for the preparation of PVA/PVP/chitosan hydrogels, as shown in the following paragraphs.

The γ irradiation was carried out on 5 wt % chitosan solutions. To ensure complete dissolution of the chitosan samples, dilute aqueous acetic acid solutions were used as the solvents. Their concentrations were 0.9 wt % for Chi-1, 0.5 wt % for Chi-2, and 0 wt % for Chi-3.

TABLE I
Molecular Weight (g/mol) Changes in Chitosan

	Sample		
	Chi-1	Chi-2	Chi-3
Before irradiation	5.05×10^5	6.99×10^4	7.38×10^3
After irradiation	1.45×10^4	1.82×10^4	2.82×10^3

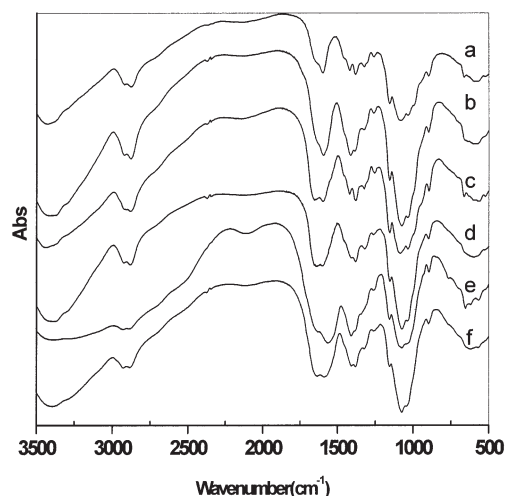


Figure 1 FTIR spectra of chitosans before and after irradiation: (a,c,e) Chi-1, Chi-2, and Chi-3 before irradiation, respectively, and (b,d,f) Chi-1, Chi-2, and Chi-3 after irradiation, respectively.

The molecular weight changes after γ irradiation are listed in Table I. As shown in Table I, the weight-average molecular weight (M_w) of chitosan was reduced by factors of 35, 3.8, and 2.6 for Chi-1, Chi-2, and Chi-3, respectively. This was different from the results reported by Ulanski et al.,[24] that the irradiation of solid chitosan (DD = 91.0%, $M_w = 1.4 \times 10^6$) in air gave a molecular weight reduction of one-third at 25 kGy. This meant that the radiation degradation was more effective for solution samples than for solid samples if their M_w was comparable, and it was even much more effective for higher M_w samples than for lower M_w ones. This is because high-M_w molecules have a high probability of being attached by the γ rays and the chitosan molecular chains were all in a coil configuration in an aqueous solution, whereas there was a certain amount of crystalline segments in the solid state that were more resistant to the γ-ray radiation degradation than the amorphous ones. Furthermore, the H^+ ions present in the chitosan solution also acted as an accelerator for the radiation degradation of chitosan.[17] After the irradiation, the solution remained transparent, and there was no evidence for gel formation, in agreement with Yoksan et al.'s[25] observation that chitosan is not radiation-crosslinkable is but radiation-scissile.

An FTIR technique was employed to follow the structure changes in chitosan after γ irradiation. As shown in Figure 1, all characteristic features of Chi-1 and Chi-2 remained unchanged, especially those in the region of 895–1200 cm^{-1}. This implied that although the irradiation caused a significant M_w decrease because of the scissions of 1–4-glycosidic bonds in chitosan, the basic saccharide subunits still remained,[26,27] so the use of chitosan in the PVA/PVP/chitosan hydrogel should be effective.

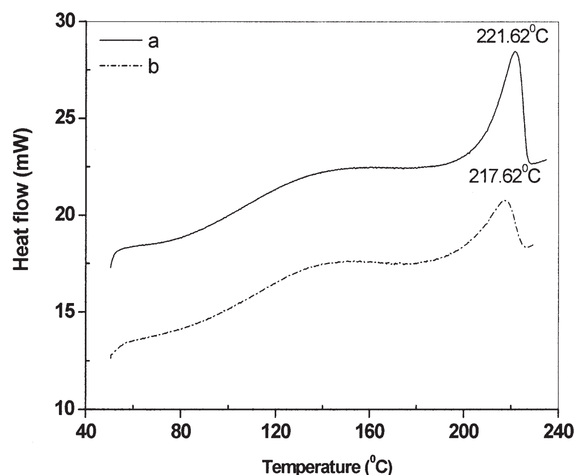


Figure 2 Typical DSC traces of PVA/PVP blends after three treatments of −30°C for 12 h and 25°C for 12 h: (a) PVA 100 and (b) PVA70/PVP 30.

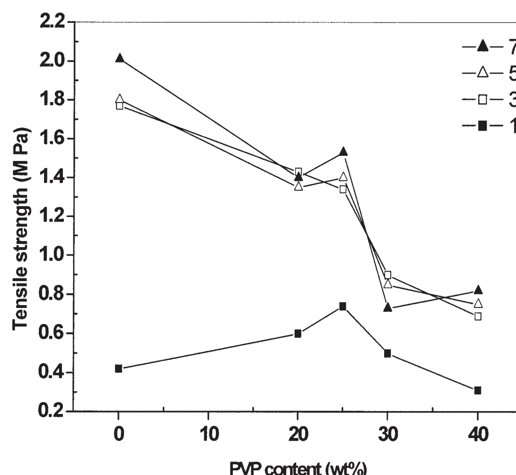


Figure 4 Tensile strength versus the PVP content for physically crosslinked PVA/PVP hydrogels. The number of freeze–thaw cycling treatments is shown in the figure.

PVA/PVP hydrogels

A series of physically crosslinked PVA/PVP hydrogels with different weight ratios were prepared by a freeze–thaw cycling method. Their crystallinity (X_t), mechanical properties, and ESRs are shown in Figures 2–6. Among the synthetic polymers, PVA is special in that its aqueous solution can form a hydrogel when subjected to repeated freeze–thaw treatments.[28] This treatment results in the formation of a porous network in which polymer crystallites act as junction points, which subsequently contribute to the improved mechanical strength of the hydrogel.[28] Taking advantage of this feature of PVA, we chose PVA as one of the main components of the medicated hydrogels to be prepared. The other component was PVP because of its high solubility in water, its biocompatibility, and especially its compatibility with PVA.[29] A natural issue arose of whether the PVA could crystallize in the presence of PVP in an aqueous solution. Fortunately, after the freeze–thaw cycling, physically crosslinked hydrogels were obtained from the PVA/PVP solutions, and the DSC measurement revealed the presence of PVA crystallites in the hydrogels (Fig. 2). After corrections for PVP and water present in the sample, X_t of PVA could be calculated from the corrected enthalpy (ΔH) and melting enthalpy (ΔH_c) of 100% crystalline PVA (138.6 J/g):[29]

$$X_t = \Delta H/\Delta H_c \times 100\%$$

In Figure 3, X_t of PVA is examined as a function of the PVA weight content and number of freeze–thaw cy-

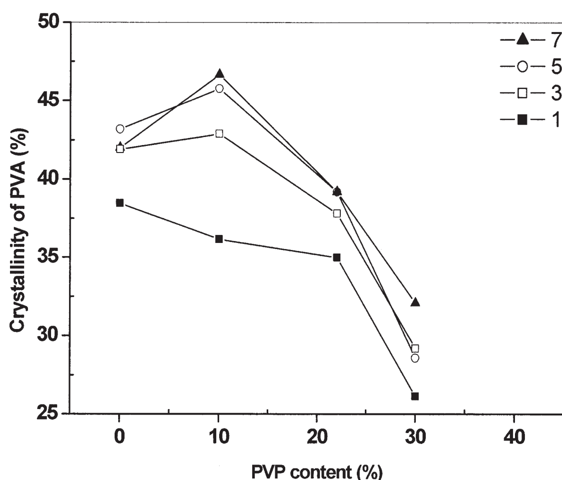


Figure 3 X_t of PVA versus the PVP content in physically crosslinked PVA/PVP hydrogels with different freeze–thaw cycles, as indicated in the figure.

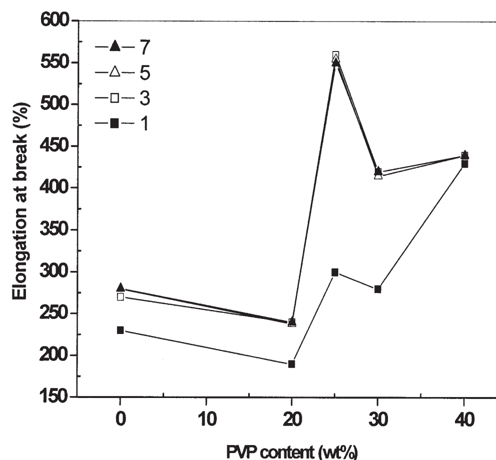


Figure 5 Elongation at break versus the PVP content for physically crosslinked PVA/PVP hydrogels. The number of freeze–thaw cycling treatments is shown in the figure.

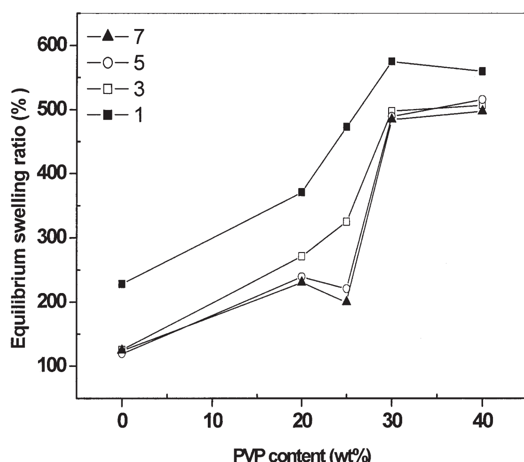


Figure 6 ESR versus the PVP content for physically crosslinked PVA/PVA hydrogels. The number of freeze–thaw cycling treatments is shown in the figure.

cling treatments. X_t increased after the first to fifth freeze–thaw cycling treatments and increased slowly or did not do so after five cycles. This is in agreement with the results obtained by Auriemma et al.,[31] who suggested that under the freeze–thaw conditions employed, the PVA crystallites were formed in the first to fifth cycles and further cycling made few contributions to it. Although these crystallinities were detected in the heated samples and after some extent of water evaporation, they were attributed to the crystallite formation in the hydrogel state due to the aggregation of PVA chains. As shown in Figure 3, X_t of PVA in the hydrogels decreased with increasing PVP content; the decrement was approximately proportional to the PVP content. In the hydrogels after the third freeze–thaw cycling, for example, X_t of pure PVA was 41.9%, whereas that of PVA70/PVP30 was only 29.2%. This phenomenon indicated that the PVP interfered with but did not prevent the crystallization of PVA. When a proper ratio of PVA to PVP was chosen, the crystallization of PVA could improve the mechanical properties of the target hydrogels.

As shown in Figure 4, the tensile strength of the hydrogels increased with an increasing number of freeze–thaw cycles and decreased with increasing PVP content. The former was caused by the crystallization of PVA segments after the cycling treatment, and the latter was due to the interference of PVP with the crystallization of PVA. When the PVP concentration was below 30 wt %, the strength of the hydrogels was acceptable.

Because of the crystallization of PVA segments, PVA hydrogels always exhibit low swelling ratios and tensile elongation. This is a disadvantage for PVA hydrogels used for wound dressings and has to be improved. As shown in Figure 5 and 6, the elongation at break and swelling ratio of the PVA/PVP hydrogels increased with increasing PVP content. This is because the addition of PVP inhibited to some extent the crystallization of PVA segments, and the strong interaction between molecules of PVA and PVP thus caused significant improvements in these two properties. When the PVP concentration was 25–30 wt % in the PVA/PVP hydrogel, the elongation at break increased 1.5–2-fold and the swelling ratio increased 2.5–3.8-fold in comparison with PVA hydrogels after three freeze–thaw cycling treatments.

On the basis of these analyses, the composition of PVA/PVP = 70 : 30 and three freeze–thaw cycles were considered the optimum conditions for the preparation of PVA/PVP hydrogels and were used for preparing PVA/PVP/chitosan hydrogels.

PVA/PVP/chitosan hydrogels

Chitosan was chosen as the third component of the hydrogels to be prepared because of its excellent biocompatibility and affinity to many drug molecules. We have discussed the radiation effect on chitosan in the previous section. After γ-ray irradiation, its basic saccharide subunits remained unchanged, but the 1,4-glycotide linkages were broken, and its molecular weight decreased significantly. Therefore, the influence of the molecular weight of chitosan on the hydrogel properties should be examined first. For this purpose, three chitosan samples were used. Their M_η values were 5.05×10^5, 6.99×10^4, and 7.38×10^3 g/mol. In all the hydrogels, the weight ratio of PVA to PVP was 70 : 30, the weight ratio of chitosan to PVA/PVP was 1 : 5, and the solid concentration was 15 wt %. To find the optimal conditions for preparing PVA/PVP/chitosan hydrogels, the gel content, ESR, elongation at break, and force at break were examined as a function of the chitosan molecular weight, freeze–thaw cycling times, and radiation dosage. As shown in Figure 7, for each chitosan sample, the gel content increased rapidly when the radiation dosage was increased from 15 to 25 kGy but did not increase any more afterwards. At a given γ-ray dosage, such as 25 kGy, the PVA/PVP hydrogel had the highest gel content, the PVA/PVP/Chi-1 and PVA/PVP/Chi-2 hydrogels had lower gel contents, and the PVA/PVP/Chi-3 hydrogel had the lowest gel content. The gel content difference between PVA/PVP and PVA/PVP/Chi-1 or PVA/PVP/Chi-2 was about 15 wt %, corresponding to the chitosan content (~17 wt %) in the hydrogels. Although the chitosan content in PVA/PVP/Chi-3 was about 17 wt % too, its gel content was 15 wt % lower than that in the PVA/PVP/Chi-1 or PVA/PVP/Chi-2 hydrogel. This was because chitosan was radiation-degradable and not crosslinkable also; it existed in the hydrogel as linear molecules after irradiation. At the same time, chitosan was a kind of multihydroxy polysaccharide and might act as a rad-

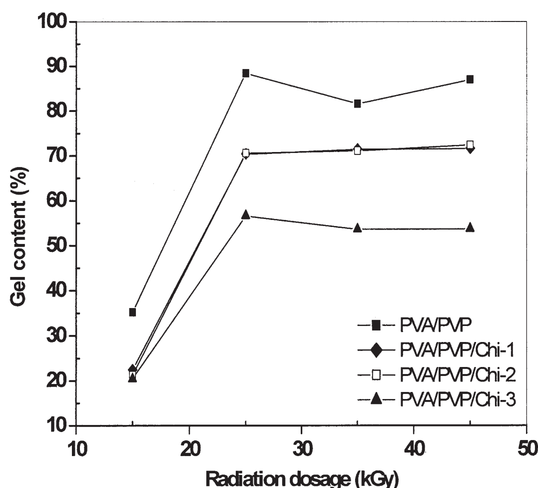


Figure 7 Gel content versus the radiation dosage for PVA/PVP/chitosan hydrogels after three freeze–thaw cycling treatments.

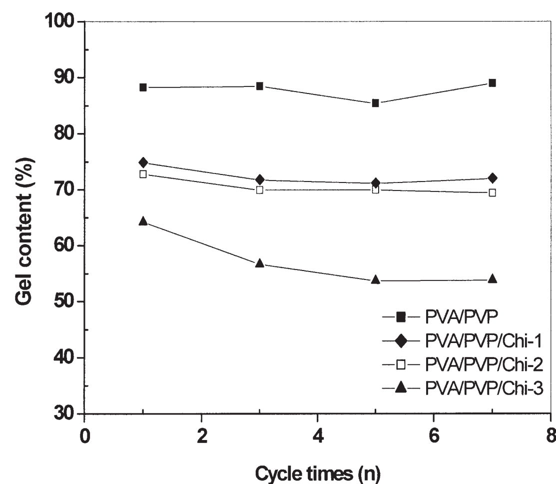


Figure 8 Gel content versus the number of freeze–thaw cycling treatments for PVA/PVP/chitosan hydrogels with a fixed radiation dosage of 25 kGy.

ical scavenger during the irradiation, reducing the crosslinking reactions of PVA and PVP. Therefore, the gel content of the PVA–PVP in PVA/PVP/chitosan hydrogels would be suppressed. Furthermore, the irradiation products of Chi-1 and Chi-2 were not soluble in neutral water, and they could not be extracted completely by distilled water; however, for Chi-3, both its radiation degradation product and Chi-3 itself were completely soluble in water, and they both could be extracted entirely under the experimental conditions. That is why the gel content in PVA/PVP/Chi-3 was 15 wt % lower than that in PVA/PVP/Chi-1 or PVA/PVP/Chi-2. The effect of the freeze–thaw treatment on the gel content was also investigated. As shown in Figure 8, the cycling times did not influence the gel content significantly.

The water absorption ability of the PVA/PVP/chitosan hydrogels is shown in Figure 9. With increasing radiation dosage, the swelling ratio of all the hydrogels decreased, especially in the dosage range from 0 to 25 kGy. This corresponded to the increase in the gel content (see Fig. 7). The swelling ratios of the three PVA/PVP/chitosan samples were much higher than that of the PVA/PVP hydrogel over this dosage range because of the water absorption and retention ability of chitosan. With the radiation dosage increased continually, for example, from 25 to 45 kGy, the change in the swelling ratio became negligible for all samples. This can be explained as the formation of a relatively stable network at a radiation dosage near 25 kGy. The difference in the swelling ratio between the PVA/PVP hydrogel and the PVA/PVP/chitosan became less and less (Fig. 9) because of the irradiation degradation of the chitosan.

The mechanical properties of the PVA/PVP/chitosan hydrogels were examined. As shown in Figure 10 and in Table II, after three freeze–thaw treatments and at the radiation dosage of 25 kGy, both the elongation at break and tensile strength of the PVA/PVP/Chi-1 hydrogel were higher than those of the PVA/PVP hydrogel, whereas the PVA/PVP/Chi-2 and PVA/PVP/Chi-3 hydrogels did not show an improvement in the mechanical properties in comparison with the PVA/PVP hydrogel. In Figure 11, the influence of radiation dosage on the mechanical properties of the PVA/PVP/Chi-1 hydrogel is shown. For three freeze–thaw cycling treatments, the hydrogel strength of all the irradiated samples was improved, except that irradiated at 45 kGy, whereas the elongation at break got worse after irradiation for all samples except for the one irradiated at 15 kGy (Table III).

In summary, the incorporation of chitosan into the PVA/PVP system can improve its water absorption

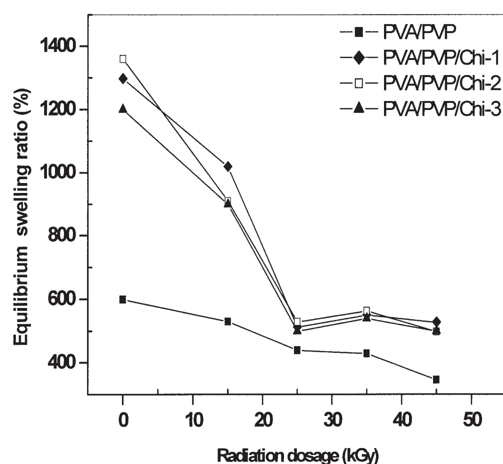


Figure 9 ESR versus the radiation dosage for PVA/PVP/chitosan hydrogels after three freeze–thaw cycles.

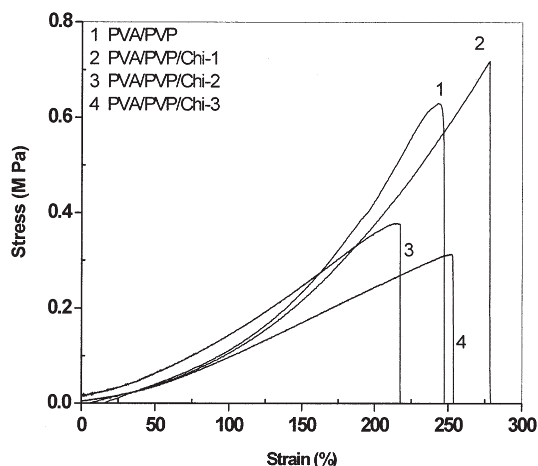


Figure 10 Stress–strain curves of the chitosan-containing hydrogels with a radiation dosage of 25 kGy.

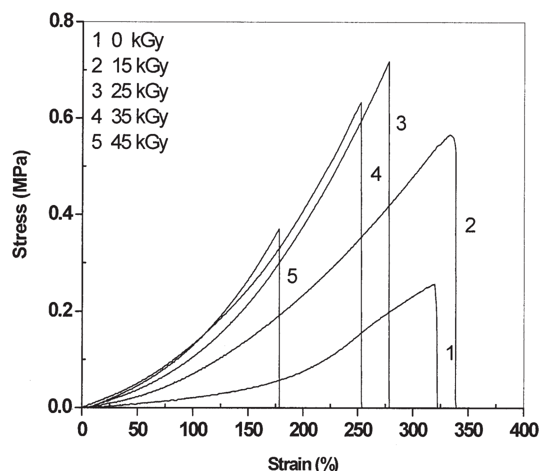


Figure 11 Stress–strain curves of PVA/PVP/Chi-1 hydrogels as a function of the radiation dosage after three freeze–thaw cycling treatments.

ability, flexibility, and biocompatibility, which are the most important properties for wound dressing; the tensile strength of the PVA/PVP hydrogel can also be improved a little when Chi-1 is incorporated. A chitosan with a high molecular weight, such as Chi-1, should be chosen as the third component of the hydrogels.

Medicated PVA/PVP/chitosan hydrogels

According to the discussion in the previous section, PVA/PVP/Chi-1 was chosen for preparing medicated PVA/PVP/chitosan hydrogels. Compared with PVA/PVP hydrogels, PVA/PVP/Chi-1 hydrogels exhibited improved water-take-up ability, flexibility, and biocompatibility. Because Chi-1 was not soluble in neutral water even after irradiation, its bioactivity was still not as good as that of water-soluble chitosan or chitosan oligomer. Therefore, chitosan oligomer was further incorporated into the hydrogel system together with the antibiotic drugs.

Ciprofloxacin lactate was chosen as the model antibiotic agent because of its broad-spectrum antimicrobial activity with little risk of primary bacteria resistance, minimal reported cytotoxicity, and minimal systemic absorption and toxicity.[2] Its stability to blood or wound exudates and wide use for surgical infection were among other critical considerations. Chitosan-3000 (M_w = 3000 g/mol) was selected as a model chitosan oligomer because it is commercially available and a molecule of 3000 g/mol is small enough to penetrate PVA/PVP/chitosan hydrogels.

The ciprofloxacin lactate and chitosan-3000 were loaded into the hydrogels via the soaking of the freeze-dried gels in a solution containing ciprofloxacin lactate and chitosan-3000 for 72 h. The contents of ciprofloxacin lactate and chitosan-3000 incorporated into the PVA/PVP/Chi-1 hydrogel from different loading solutions are shown in Figure 12(a,b). A linear relationship between the loaded concentrations and the loading concentrations was obtained.

In vitro drug and chitosan oligomer release

The drug and chitosan oligomer release behaviors were studied at 37°C in a modified Franz diffusion cell, which could simulate actual wound conditions. Although the medical practice for handling burn wounds required daily changing of the dressing and daily cleaning of the wound area, the duration of 90 h was selected for the release experiment to study the whole release process of the drug. Two initial loading concentrations of ciprofloxacin lactate and chitosan-3000 were examined. They were 1.0 and 2.0 mg/mL

TABLE II
Mechanical Properties of Chitosan-Containing Hydrogels with a Radiation Dosage of 25 kGy

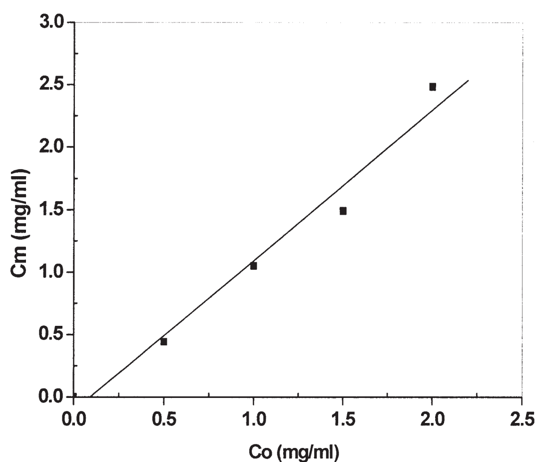
	Sample			
	PVA/PVP	PVA/PVP/Chi-1	PVA/PVP/Chi-2	PVA/PVP/Chi-3
Tensile strength (MPa)	0.63	0.72	0.31	0.38
Elongation at break (%)	244	277	252	217

TABLE III
Mechanical Properties of PVA/PVP/Chi-1 Hydrogels as a Function of the Radiation Dosage with Three Freeze–Thaw Cycling Treatments

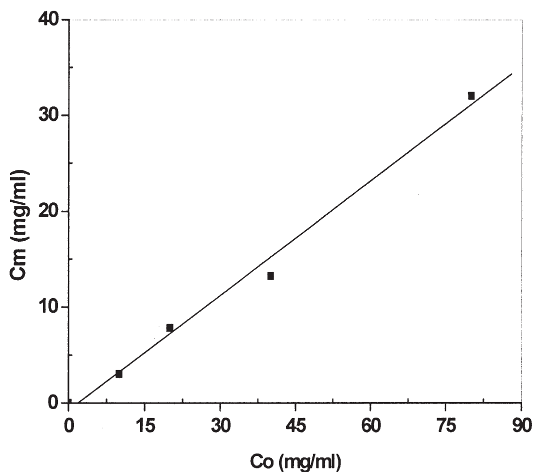
	Radiation dosage (kGy)				
	0	15	25	35	45
Tensile strength (MPa)	0.26	0.56	0.72	0.63	0.37
Elongation at break (%)	320	336	277	252	178

for ciprofloxacin lactate and 40.0 and 80.0 mg/mL for chitosan-3000.

The *in vitro* drug and chitosan-3000 release behaviors are shown in Figure 13(a,b). As expected, the drug release was very quick at the beginning and then became slower and slower, as shown in Figure 13(a). In the first 20 h, about 60% of the drug was released. At the end of the 90-h release experiment, the total amounts of the drug released were 85 and 65% for initial drug contents of 2.0 and 1.0 mg/mL, respectively. As shown in Figure 13(b), chitosan-3000 was released similarly, and the total amount released in 90 h was also dependent on the initial content of the

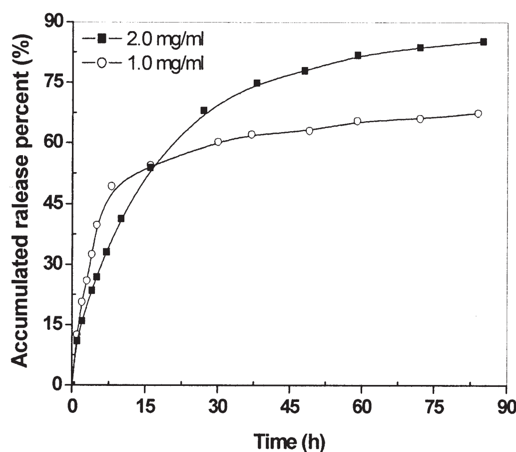


(a)

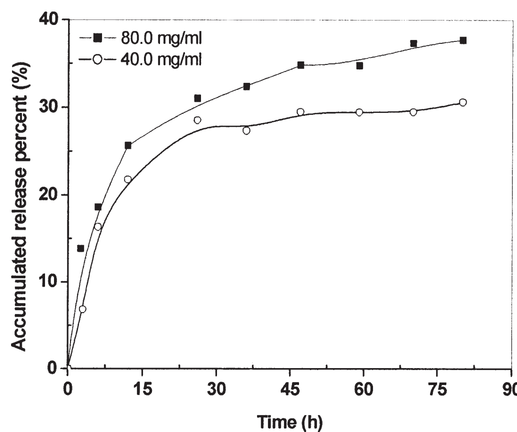


(b)

Figure 12 (a) Loaded concentration (C_m) of ciprofloxacin lactate in the hydrogel versus the loading concentration (C_0) and (b) C_m of the chitosan oligomer in the hydrogel versus C_0.

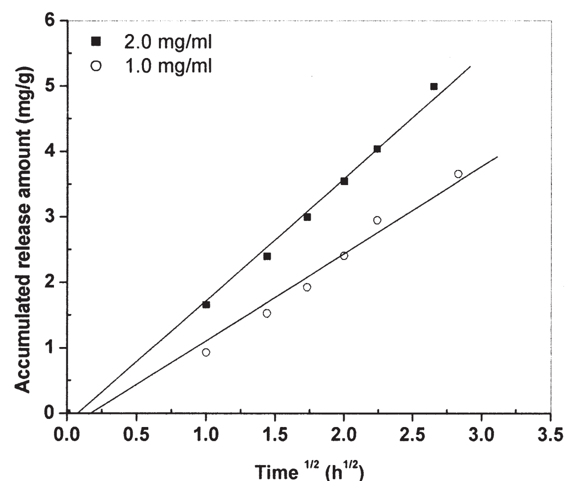


(a)

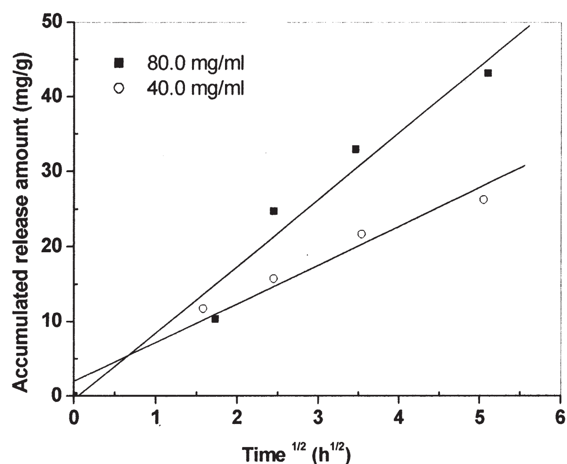


(b)

Figure 13 (a) Release behavior of ciprofloxacin lactate loaded in the PVA/PVP/Chi-1 hydrogel with an initial drug loading concentration of (■) 2.0 or (○) 1.0 mg/mL and (b) release behavior of chitosan-3000 loaded in the PVA/PVP/Chi-1 hydrogel with an initial chitosan oligomer loading concentration of (■) 80.0 or (○) 40.0 mg/mL.



(a)



(b)

Figure 14 (a) Release kinetics of ciprofloxacin lactate from the PVA/PVP/Chi-1 hydrogel with an initial drug loading concentration of (■) 2.0 or (○) 1.0 mg/mL and (b) release kinetics of chitosan-3000 from the PVA/PVP/Chi-1 hydrogel with an initial chitosan oligomer loading concentration of (■) 80.0 or (○) 40.0 mg/mL. The accumulated release amount (mg/g) of the drug and chitosan oligomer was denoted as the accumulated release amount of the drug and chitosan oligomer (mg) divided by the amount of the hydrogel (g).

chitosan oligomer. On the other hand, the total percentage of the oligomer released from the hydrogel was lower than that of the drug. A reasonable explanation may be its stronger interaction with the hydrogel and its higher molecular weight.

To analyze the kinetics of drug and chitosan oligomer release from the hydrogels, the accumulated release amount was plotted against the square root of the releasing time ($t^{1/2}$). A linear relationship was obtained, as shown in Figure 14(a,b), for ciprofloxacin lactate and chitosan-3000. This indicated that a pore-diffusion process controlled the release of the drug and oligo-chitosan from the hydrogel.[32]

CONCLUSIONS

PVA/PVP/chitosan hydrogels containing an antibiotic drug and chitosan oligomers were prepared in three steps: (1) the formation of physically crosslinked hydrogels by repeated freeze–thaw treatments, (2) the γ-ray irradiation of the physically crosslinked hydrogels to realize chemical crosslinking, and (3) the incorporation of ciprofloxacin lactate and chitosan-3000 into the hydrogel by the soaking of the dried gel in a solution containing the drug and chitosan oligomer. The optimum hydrogel composition was PVA/PVP/Chi-1 = 70:30:20. The selected radiation dosage was 25 kGy. The freeze–thaw cycle consisted of freezing at −30°C for 12 h and thawing at 25°C for 12 h and was repeated at least three times.

The prepared PVA/PVP/Chi-1 hydrogels exhibited comprehensive properties suitable for wound dressings, such as a high gel content, a reasonable ESR, and an acceptable tensile strength and elongation at break.

The ciprofloxacin lactate and chitosan-3000 were loaded into the PVA/PVP/chitosan hydrogels. Their release from the hydrogels followed kinetics controlled by pore diffusion. Further work about the morphological structure, antibiotic ability, and other biological activities of the hydrogels is in process.

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