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Synthesis and Properties of Hydrogels Based on Chitosan and Poly(Vinyl Alcohol) Crosslinked by Genipin

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Semi-interpenetrating polymeric networks of chitosan and poly(vinyl alcohol) [PVA] were prepared by varying the ratio of the constituents. The hydrogels were crosslinked using genipin, a naturally occurring nontoxic cross-linking agent. The swelling behavior of these hydrogels was studied by immersing the films in deionized water at various temperatures and in buffer solutions of different pH. The states of water in the hydrogels, swollen at 25°C and pH 7, were determined using Differential Scanning Calorimetry (DSC). The swelling behavior of the gels was found to be dependent on temperature and pH of the medium. The amount of freezing water in the swollen hydrogels increased, whereas the amount of nonfreezing bound water remained more or less the same with increasing PVA concentration.

Keywords hydrogels, crosslinking, hydrophilic polymers, thermal properties, genipin

Introduction

Hydrogels are three-dimensional polymeric networks, which can swell in aqueous medium and retain a significant amount of water within their structure without dissolving. Because of the presence of certain functional groups along the polymer chains, hydrogels are often sensitive to the conditions of the surrounding environment such as temperature, pH, solvent composition, ionic concentration, electric field, and magnetic field and ultraviolet light.

Hydrogels have become an important area of research due to the properties such as the degree of swelling, sorption kinetics, solute permeability, and their in vivo performance characteristics. A wide range of hydrophilic polymers have been used to prepare hydrogels which are being tried in the fields of medicine, pharmacy and biotechnology, particularly as candidates for wound healing (1) and carriers for the release of drugs (2, 3).

Chitosan is an amino functionalized polysaccharide produced from chitin, an abundant biomass in the world. Its potential applications range from biomedicine and

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pharmacy to water treatment. It is found to be non-toxic, biocompatible in animal tissues and enzymatically biodegradable (4). Because of their biodegradability, excellent swelling and solvent retaining properties, chitosan hydrogels are receiving a great deal of interest for medical and pharmaceutical applications (5). A number of hydrogels containing chitosan have been reported (6, 7) and tested for various applications. Since chitosan is soluble in acidic medium, chitosan based gels are often crosslinked to give stability to their structure.

Commonly used crosslinking agents are glutaraldehyde, formaldehyde, epoxy compounds, diisocyanate and dialdehyde starch. However, all these compounds are chemically synthesized and are not free from the problems of physiological toxicology.

Recently a new crosslinking agent, genipin (Figure 1), has been successfully used (8, 9) and its crosslinking reaction with chitosan has been reported (9). It is obtained from its parent compound, geniposide, via enzymatic hydrolysis with β-glucosidase (10). Geniposide is extracted from fruits of *Gardenia jasminoides Ellis* and *Genipa americana* which have long been used in the traditional Chinese medicine (9). It is an effective crosslinking agent for polymers containing amino groups (10) and has been found to be much less cytotoxic than glutaraldehyde (11).

Like chitosan, poly (vinyl alcohol) (PVA) is also non-toxic, non-carcinogenic and is biocompatible. Because of its other desirable properties such as elastic nature and good film forming ability (12), PVA has been used as a basic material for a variety of biomedical applications including contact lenses, skin replacement material, artificial muscle and vocal chord reconstruction (13).

Hydrogels based on chitosan and PVA have been prepared and crosslinked using gutyraldehyde (14), UV irradiation (15) and gamma irradiation (16). The swelling behavior of the hydrogels at different pH's and temperature, and the states of water present in the networks have been investigated. However, no study has been reported in the literature on chitosan and PVA hydrogels in which genipin has been used as the crosslinking agent.

In this study, we prepared semi-interpenetrating genipin crosslinked hydrogels of chitosan and PVA. Swelling behavior of the hydrogels were studied at various pH's and temperatures. Furthermore, the different states of water in the swollen hydrogels were investigated using DSC.

Experimental

Materials

Chitosan with 85% degree of deacetylation was obtained from Fluka, U.K. PVA (100% hydrolysed, Mw: 1.46×10^5) and genipin were purchased from Aldrich and Challenge

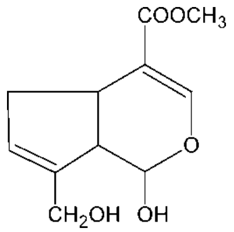

Figure 1. Chemical structure of genipin CAS number CAS: 6902-77-8.

Bioproducts Company (Taiwan, R.O.C.), respectively. All chemicals were used without further purification.

Preparation of Hydrogels

Chitosan was dissolved in 1% aqueous acetic acid solution at room temperature, with continuous stirring for 24 h to obtain a 1.5% (w/v) solution. The viscous solution was passed through a filter to remove any undissolved matter. PVA was dissolved in deionized water at 90°C with continuous mechanical stirring for 1 hr to obtain a 5% (w/v) solution, which was used without filtering. Genipin was dissolved in deionized water to obtain 0.5% (w/v) solution.

PVA and chitosan solutions were mixed in varying amounts to obtain mixtures having chitosan: PVA weight ratios of 1 : 3, 1 : 1 and 3 : 1. Genipin solution was slowly added to the mixtures under constant stirring. The detailed composition and designations of the pregels are listed in Table 1.

The pregel solutions were poured into polystyrene petri dishes and allowed to undergo gelation at room temperature for 12 h. By this time, the color of the gels became bluish green. The hydrogels were dried at 50°C to obtain thin films of thickness of approximately 0.1 mm. Films were vacuum dried until further treatment.

Swelling Measurements

The swelling kinetics of the hydrogels in deionized water at 25, 35, and 45°C were investigated. Film samples (surface area approximately 1 cm^2) were weighed and placed in stainless steel wire mesh of mesh size 1 mm. The mesh containing the film sample was then submerged into a 250 cm^3 beaker containing the swelling medium and covered with parafilm to avoid evaporation. At the preset time intervals, the films were withdrawn, surface water was removed by blotting with a filter paper and immediately weighing the films to determine the wet weight. Swelling measurements were continued until a constant wet weight was observed. The experiments were repeated in triplicate. The swelling ratio was calculated using Equation (1)

$$E_{sr}(\%) = [(W_s - W_d)/W_d] \times 100 \qquad (1)$$

where E_{sr} is the percentage water absorption of the films, W_d and W_s are the weights of the samples in the dry and swollen states, respectively.

The swelling behavior of the hydrogel films was also measured by swelling the films in buffer solutions of different pH (2.2, 4.2, 7.0, 9.1 and 10.0) ± 0.1 at 25 ± 1°C.

Table 1

Compositions of genipin crosslinked chitosan: PVA hydrogels

Designation	Vol. of chitosan (cm^3)	Vol. of PVA (cm^3)	Vol. of genipin (cm^3)	Molar ratio Chs : PVA : genipin
CLChs	40	0	4	1 : 0 : 0.02
ChsPVA1	40	4	4	1 : 1.22 : 0.02
ChsPVA2	40	12	4	1 : 3.67 : 0.02
ChsPVA3	40	36	4	1 : 10.9 : 0.02

Equilibrium wet weight of the hydrogels were taken after 12 h. The equilibrium water content (EWC) was calculated using Equation (2)

$$\mathrm{EWC}(\%) = [(W_e - W_d)/W_e] \times 100 \tag{2}$$

where W_e represents the weight of the swollen state at equilibrium.

DSC Measurements

A Perkin-Elmer Pyris 6 DSC unit was used to investigate the different states of water in the hydrogels. The hydrogels samples were swollen to equilibrium in deionized water at 25°C and pH 7 for 12 h. Samples of the swollen gels of mass approximately 2–5 mg were sealed in weighed aluminum pans. Scanning was done from −50°C to 20°C with a heating rate of 5°C/min under nitrogen flow. The average of five measurements were taken.

Results and Discussion

Hydrogels of chitosan/PVA crosslinked by genipin were bluish green in color. The time dependent swelling behaviors of the chitosan/PVA hydrogels in deionized water at 25, 35 and 45°C are shown in Figures 2–4. All hydrogels revealed a rapid increase in water content and reached equilibrium state in approximately 40 min.

Swelling in hydrogels occurs when water molecules get absorbed by the gel. Initial swelling is due to water molecules forming hydrogen bonds with hydrophilic functional groups present in the polymer chains. More water then orientates around the bound water to form cage like structures or clusters. Finally, excess water enters freely into the gel network resulting in more swelling (17, 18).

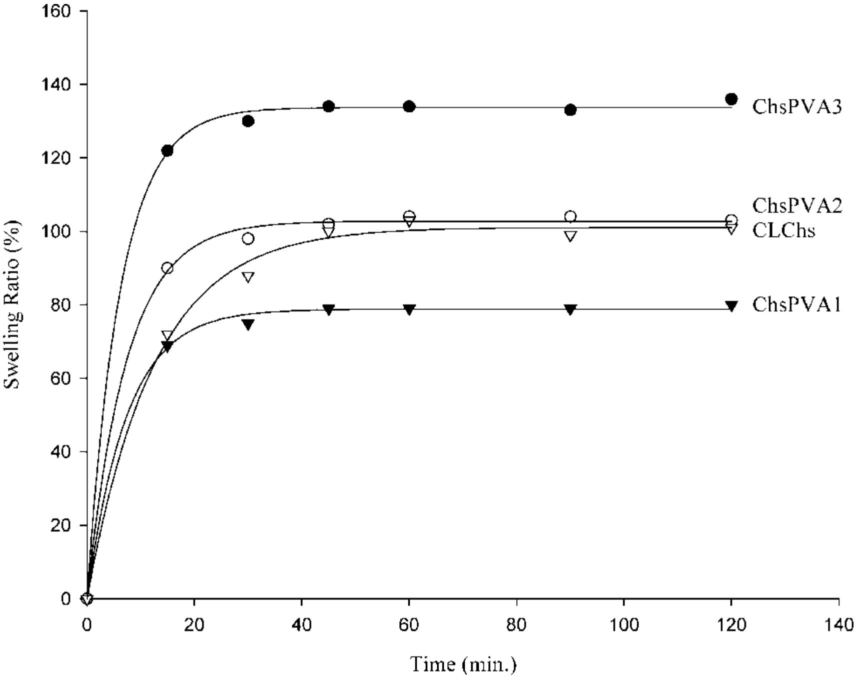

Figure 2. Swelling kinetics of the hydrogels at 25°C and pH 7.

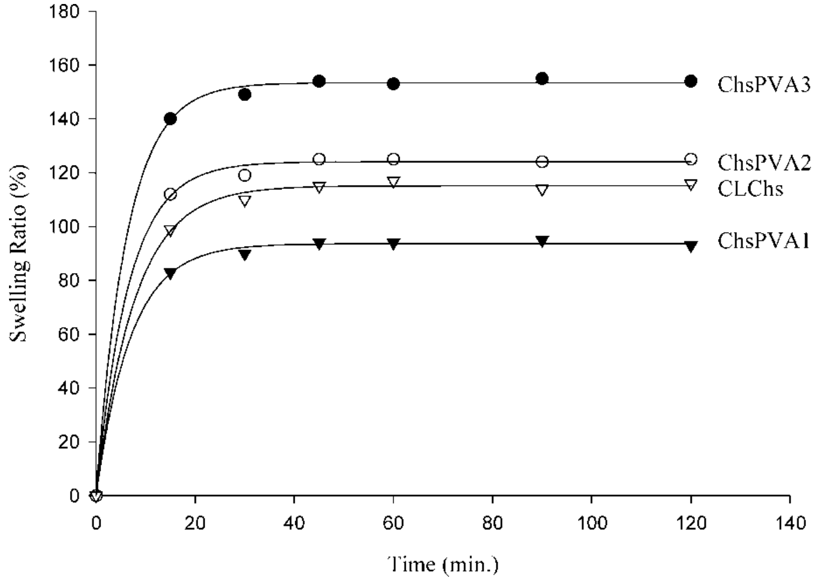

Figure 3. Swelling kinetics of the hydrogels at 35°C and pH 7.

ChsPVA3 showed the highest swelling while ChsPVA1 showed the lowest, whereas crosslinked chitosan, CLChs, showed almost the same swelling as ChsPVA2. The addition of PVA to chitosan decreased the swelling ratio. However, on further addition of PVA, the swelling ratio increased. The reason is that in the ChsPVA1 gel, the ratio of NH_2 groups of chitosan and OH groups of PVA is nearly the same. Hydrogen bond formation between

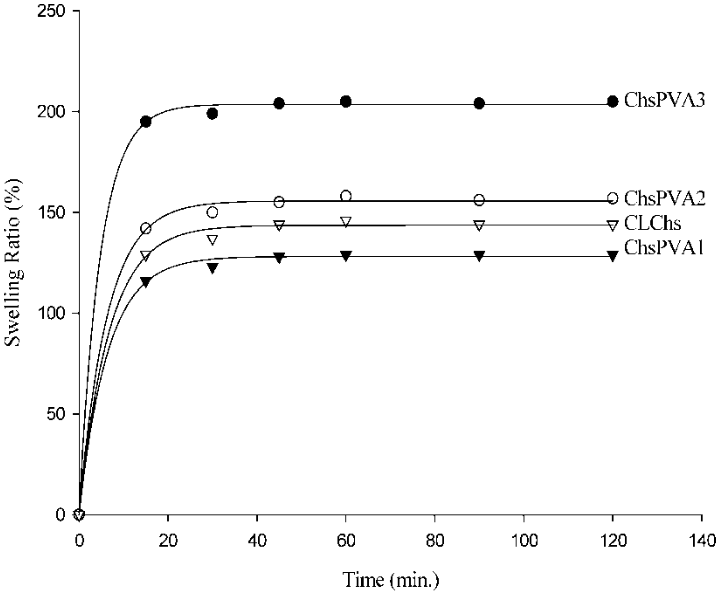

Figure 4. Swelling kinetics of the hydrogels at 45°C and pH 7.

NH_2 and OH groups results in a compact structure, which shows less swelling. In the other two hydrogels, the ratio of OH groups is much higher than the NH_2 groups and some OH groups not involved in the hydrogen bonding are free to attract more water to show higher swelling.

In a similar swelling study carried out by Kim et al. (15) on PVA/chitosan hydrogels crosslinked by UV radiation, an opposite trend in swelling behavior was observed. In their work, higher swelling was observed in the hydrogels containing more chitosan. This difference in the swelling trend may be due to the way in which the crosslinking of the hydrogels was achieved. Kim's group used UV radiation, which results in a full interpenetrating network involving both chitosan and PVA. However, genipin only crosslinks the amino groups of the chitosan chains and PVA is physically entangled in the chitosan network and its -OH groups are free to attract water molecules. Thus, more PVA result in higher swelling. Another reason may be the percentage hydrolysis of PVA. In the present study 100% hydrolyzed PVA was used whereas Kim's group did not indicate the percent hydrolysis of their sample.

The temperature dependent equilibrium swelling behavior of the hydrogels at pH 7 and temperature range of 25 to 45°C are summarized in Figure 5. All hydrogels exhibited a temperature responsive behavior and swelling of the hydrogels increased with increase in temperature.

An increase in temperature leads to dissociation of hydrogen bonds and secondary interactions between the chitosan and PVA chains, thus allowing more water within the hydrogel network. Kim et al. (15) observed similar temperature dependent swelling behavior for their chitosan/PVA hydrogels.

To study the affect of pH on the swelling behavior, the hydrogels were allowed to swell to equilibrium at 25°C in an aqueous medium of pH 2.2, 4.2, 7.0, 9.1 and 10.0 ± 0.1. The swelling behavior is shown in Figure 6.

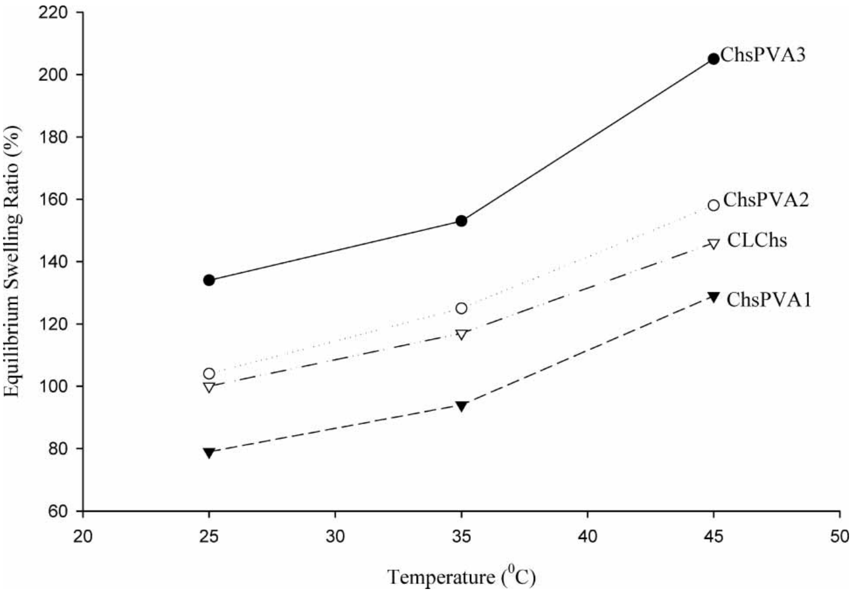

Figure 5. Equilibrium swelling kinetics of the hydrogels at pH 7 as a function of temperature.

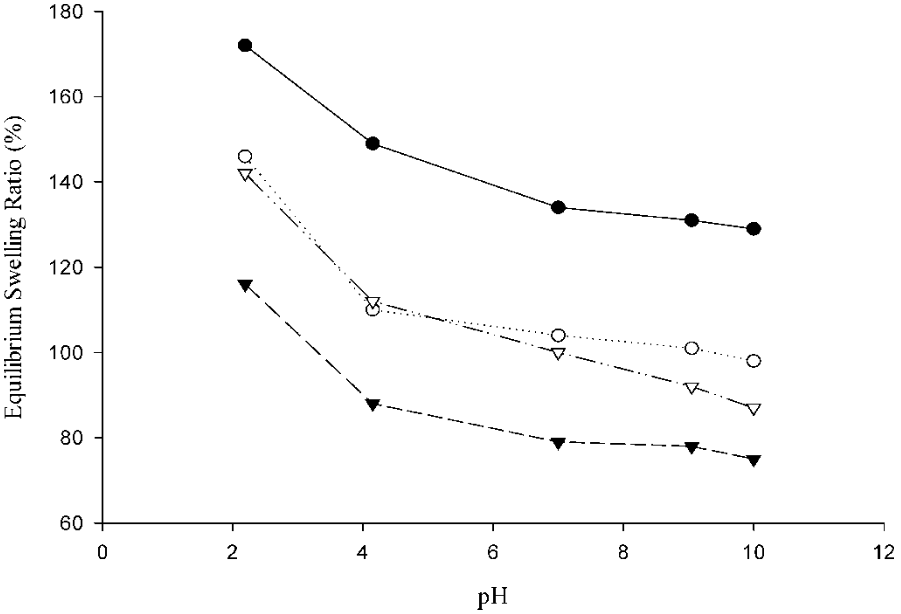

Figure 6. Equilibrium swelling kinetics of the hydrogels at 25°C as a function of pH.

The swelling ratio of all the hydrogels increased with the decrease in pH and maximum swelling was observed at the lowest pH. ChsPVA3 showed the highest swelling, while ChsPVA1 showed the lowest at all pH's. The high swelling observed at low pH is due to the protonation of the amino groups of chitosan. The protonation leads to polymer chain repulsion and dissociation of secondary interactions allowing more water into the gel network. At high pH, repulsion in the polymer chains recedes due to deprotonation of the amino groups and swelling decreases. In neutral and basic media, swelling was mainly driven by solvent diffusion and the swellability of the hydrogels remained relatively constant.

The states of water in the hydrogels were determined using DSC. Three types of water are known to be present in the hydrogels and are referred to as nonfreezing bound water, intermediate freezing water and free freezing water (18). Nonfreezing bound water refers to immobilized water molecules, which are bound to the hydrophilic sites of the polymer chains and shows no melting peak. Intermediate freezing water molecules preferentially orient around the bound water and have a melting endotherm below 0°C. Free freezing water molecules shows greater degree of mobility and show a melting endotherm around 0°C.

Figure 7 shows the DSC heating scans for the hydrogels. CLChs and ChsPVA1 hydrogels showed only one broad endothermic peak around 0°C, whereas ChsPVA2 and ChsPVA3 hydrogels clearly showed two well resolved peaks around −8°C (Peak 1) and 0°C (Peak 2).

The ΔH_{endo} for intermediate freezing water peak was found to increase with increasing PVA content in the hydrogels while ΔH_{endo} for free water peak was found to decrease. The enthalpy values associated with the melting peaks for the hydrogels are given in Table 2.

The amounts of free and bound water are related by Equation (3)

$$W_b(\%) = W_e - (W_f + W_{if}) \tag{3}$$

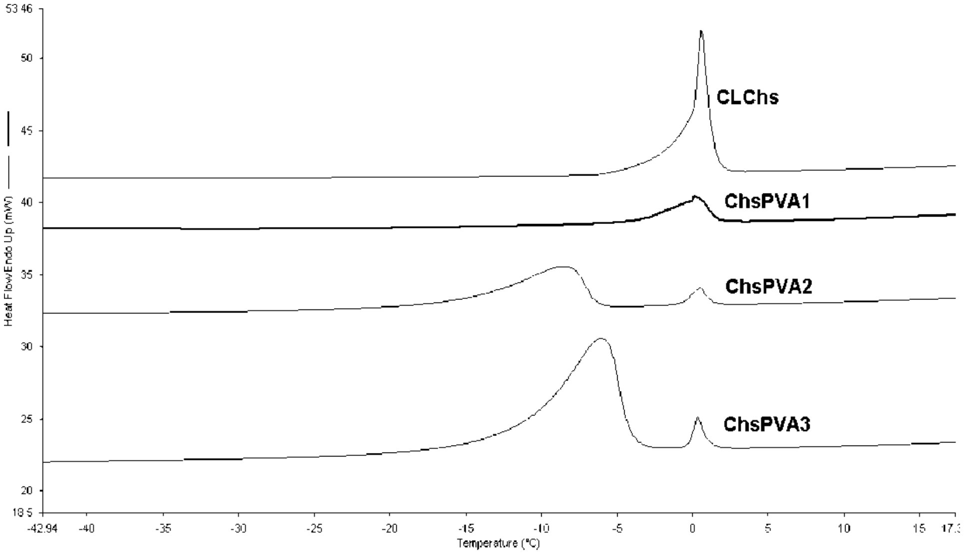

Figure 7. DSC first heating scans of the hydrogels.

where W_b is the amount of nonfreezing bound water, W_f and W_{if} are the amounts of free water and intermediate freezing water respectively; and W_e is the equilibrium water content (EWC).

The amount of total freezing water is estimated by taking the ratio of the enthalpy change associated with the endothermic peak (Total ΔH_{endo}) for intermediate and free water to the enthalpy of fusion (ΔH_{fus}) for pure water (333.7 J/g) as shown in Equation (4).

$$(W_f + W_{if}) = (\text{Total } \Delta H_{endo}/\Delta H_{fus}) \times 100 \tag{4}$$

EWC, total freezing and bound water contents of various hydrogels are listed in Table 3.

The results show that as the concentration of PVA in the hydrogels increases, the nonfreezing bound water remains relatively the same, but ECW and total freezing water increases, indicating the hydrogels becoming more hydrophilic. ChsPVA1 shows the lowest ECW and total freezing water. This supports our earlier view that due to hydrogen bonding, this hydrogel has a more compact structure. As the ratio of amino groups of chitosan and the hydroxyl groups of PVA is almost the same in this hydrogel,

Table 2

ΔH_{endo} of the different peaks calculated from DSC scans

Sample	ΔH_{endo} (J/g) (peak 1)	ΔH_{endo} (J/g) (peak 2)	Total ΔH_{endo} (J/g)
CLChs	—	100.3	100.3
ChsPVA1	—	62.9	62.9
ChsPVA2	65.4	10.2	75.6
ChsPVA3	94.7	6.5	101.2

Table 3
Percentage of different types of water calculated from DSC scans

Sample	EWC (%)	Total freezing water (%)	Bound water (%)
CLChs	49.3	30.1	19.2
ChsPVA1	44.8	18.9	25.9
ChsPVA2	51.0	22.7	28.3
ChsPVA3	56.9	30.3	26.5

most of these groups are involved in hydrogen bonding. In ChsPVA2 and ChsPVA3, the ratio of OH groups is much higher than the NH_2 groups; hence, some OH groups are free to attract water molecules.

Conclusions

Hydrogels of chitosan and of 100% hydrolyzed PVA were prepared and crosslinked using a naturally occurring nontoxic crosslinking agent, genipin. The hydrogels swelled rapidly and exhibited a swelling response to temperature and pH. The swelling of the gels increased with the decrease in the pH and increase in the temperature due to the repulsion and disassociation of secondary interactions of the polymer chains. PVA rich hydrogels swelled more in both stimuli. The amount of freezing water and nonfreezing bound water was investigated by DSC. The results revealed that as the percentage of PVA in the swollen hydrogels increased, the amount nonfreezing bound water remained more or less the same but the amount of freezing water increased. The increase in the EWC and the amount of freezing water is due to the presence of more PVA, which increases the hydrophilic nature of the hydrogels.

Acknowledgements

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