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Preparation and characterization of IPN hydrogels composed of chitosan and gelatin cross-linked by genipin

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ABSTRACT

The interpenetrating polymer networks (IPN) hydrogels based on chitosan and gelatin using genipin as the cross-linker were prepared and characterized. The IPN formation of the genipin-cross-linked chitosan/gelatin hydrogel was confirmed by means of the instrinsic viscosity measurement, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and the ninhydrin assays. The instrinsic viscosity measurement, FT-IR and SEM suggested that chitosan and gelatin were miscible in the molecular level. The miscibility leads to the formation of IPN after cross-linking. FT-IR also examined the cross-linking mechanism of genipin with primary amino groups. The degree of cross-linking increased with increase genipin concentration. Swelling results revealed that the IPN hydrogels are pH-sensitive, exhibiting reversibility and rather rapidly response in swelling to pH changes. It is expected this IPN hydrogel has potential as controlled drug delivery system or as alternative sorbents for biomedical and environmental use as pH altered.

1. Introduction

Hydrogels are three-dimensional networks of polymeric materials that can swell significantly without dissolving or losing their structural integrity (Dragan, Perju, & Dinu, 2012). Hydrogels prepared from natural polymers such as cellulose, gelatin and chitosan have widespread applications in biomaterials because of their high water content, which are similar to a variety of natural living tissues. They can be used as the scaffold for tissue engineering (Chiono et al., 2008), which also have found large applicability in drug delivery systems (Hoare & Kohane, 2008; Lin & Metters, 2006; Reis et al., 2008), pharmaceutics (Muzzarelli, 2009; Peppas, Hilt, Khademhosseini, & Langer, 2006), environment (Jeon, Lei, & Kim, 2008; Yilmaz, Kavakli Akkas, Sen, & Guven, 2006), agriculture (Abd El-Rehim, 2006; Zohuriaan-Mehr, Omidian, Doroudiani, & Kabiri, 2010), food, personal care products and electronics (Bingol, Strandberg, Szabo, & Wegner, 2008) because of the characteristics of biocompatibility, biodegrability, hydrophilicity and lack of toxicity.

To improve the mechanical properties as well as the biodegradability and to control the diffusion of solutes in hydrogels, multicomponent networks as semi- or interpenetrating polymer networks (IPN) have been projected (Liang, Liu, Huang, & Yam, 2009; Babu, Hosamani, & Aminabhavi, 2008; Rokhade, Patil, & Aminabhavi, 2007; Xia, Guo, Song, Zhang, & Zhang, 2005). Semi- or interpenetrating polymer networks technology is a simple and feasible method to fabricate hydrogels, in which hydrophilic polymer chain permeates another cross-linked polymeric network without any chemical bonds between them. It is reported that the formation of semi- or interpenetrating polymer networks can preserve the characteristics of each network structure and improve the stability of the materials because of the interlocked structure in the cross-linked networks, thus ensuring the mechanical strength (Zhao, Ma, & Zhang, 2006). A variety of synthetic polysaccharide-based semi-IPN hydrogel systems have been employed to investigate their swelling characteristics and evaluate drug delivery (Zhao et al., 2006; Zhao, Zhao, Li, Cao, & Xu, 2011). Among them, chitosan is one of the commonly used.

Chitosan, the linear and typically 20% acetylated (1-4)-2-amino-2-deoxy-<beta>-D-glucan, is isolated from marine chitin (Muzzarelli, 2011; Muzzarelli et al., 2012). Chitosan consists of both reactive amino and hydroxyl groups that can be modified chemically and physically easily. It has also demonstrated good pH-sensitive due to protonation of free amino groups in response to external pH changes. Nevertheless, chitosan dissolves readily in acidic conditions at pH below 6. Cross-linking is required to improve the mechanical resistance and reinforce the chemical stability in acidic media. Various cross-linking agents such as glutaraldehyde, glyoxal or epichlorohydrin have been used to crosslink chitosan (Gomez-Estaca, Gomez-Guillen, Fernandez-Martin, & Montero, 2011; Tual, Espuche, Escoubes, & Domard, 2000). Mixing of chitosan with other biopolymers has been proved as an effective way to enhance the water resistance (Fernandez-Saiz, Lagaron, Hemandez-Munoz, & Ocio, 2008) and result in an

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improvement of physico-chemical properties of chitosan (Garcia, Pinotti, Martino, & Zaritzky, 2004; Garcia, Pinotti, & Zaritzky, 2006).

Gelatin obtained from collagen has gained much attention for its abundance, biodegradability, relatively low cost, excellent functional and filmogenic properties. However, gelatin is weak in mechanical strength and rather rapidly dissolve in aqueous environments, which limit its practical applications. Chemical cross-linking or blending gelatin with a natural polymer may provide a simple method for increase the chemical stability and improving the mechanical properties of gelatin (Sarem, Moztarzadeh, & Mozafari, 2013). Commonly used cross-linkers include aldehydes, polyepoxy compounds and carbodiimides. The main limitation of chemical cross-linkers arises from the risk of toxic effects owing to the residual cross-linker. For this reason, much interest has been addressed toward naturally derived cross-linking agents.

Genipin, a naturally occurring cross-linker, is obtained from geniposide, an iridoid glucoside isolated from the fruits of *Genipa americana* and *Gardenia jasminoides* Ellis. It has recently attracted much interest for its ability to crosslink chitosan and proteins containing residues with primary amine groups (Bigi, Cojazzi, Panzavolta, Roveri, & Rubini, 2002; Muzzarelli, 2009). Genipin has been used as a cross-linker in studies of tissue fixation, foodstuff and drug delivery (Kawadkar, Jain, Kishore, Pathak, & Chauhan, 2013). In all of these applications, genipin was chosen because of its markedly lower cytotoxicity and higher biocompatibility as compared with other cross-linkers, such as glutaraldehyde (Mi, Tan, Liang, Huang & Sung, 2001).

Both of chitosan and gelatin are natural and biocompatible. Mixing of the two together has been proved as an effective means to enhance the mechanical properties of the resulting materials (Tseng, Tsou, Wang, & Hsu, 2013). The objective of this present work was to develop a genipin-cross-linked interpenetrating polymeric network hydrogel based on chitosan and gelatin and investigate the structure feature and swelling characteristics. The structure feature was confirmed by means of intrinsic viscosity measurement, Fourier transform infrared and scanning electron microscopy. Swelling characteristics of these hydrogels as a function of weight ratios, cross-linking agent content and pH values were investigated in detail.

2. Experimental

2.1. Materials

Chitosan ($M_W \sim 5 \times 10^4$) with a degree of deacetylation of approximately 93% purchased from Qingdao Yunzhou Biochemistry Co. Ltd., Qingdao, China. Gelatin type B was supplied from Shanghai Chemical Reagent Co., Shanghai, China. Genipin was purchased from Linchuan Biological technology Co. Ltd., Fuzhou, China. All other reagents were analytical reagents and used as received.

2.2. Preparation of chitosan/gelatin hydrogels

The chitosan/gelatin hydrogels used in this study were prepared by a solvent evaporation technique. Chitosan/gelatin hydrogels with chitosan-to-gelatin weight ratios of 10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10 were fabricated as follows. Chitosan solution (3%, w/v) was prepared by dissolving 3 g chitosan in 100 mL deionized water containing 1% (v/v) acetic acid at room temperature. Gelatin was dissolved in deionized water at 50 °C, obtaining a 6% (w/v) solution. Subsequently, the two solutions were poured together as per the aforementioned weight ratios and stirred for 1 h at 50 °C to get a homogeneous blend solution. An aqueous genipin solution in a concentration of 0.50, 0.75 and 1.00 mM was added to the blend solution. After thoroughly stirring and sonicating, the air bubble-free solutions were poured into shallow disk and dried in air. To remove the residual acetic acid in the just obtained samples, the samples were immersed in NaOH solution for 2 h and then rinsed with deionized water until pH was neutral. Finally, the samples were dried in air and then dried under vacuum at room temperature for 24 h.

2.3. Intrinsic viscosity measurements

Varying weight ratios (10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) of chitosan-to-gelatin were dissolved in a 0.5 M acetic acid solution to get a final concentration of 0.5 g/l. The blend solution was diluted with 0.5 M acetic acid solution. Miscibility of chitosan and gelatin molecules were evaluated by intrinsic viscosity ($[\eta]_m$) as described by Garcia and Melad (1999). It was measured by using the Ubbelohde capillary viscometer in a constant temperature bath at 28.0 ± 0.1 °C. They suggested that the intrinsic viscosity of chitosan/gelatin solution can be expressed as the following equations:

$$\eta_{sp,m}/(c_1 + c_2) = [\eta]_m + b_m(c_1 + c_2) \quad (1)$$

$$b_m = x_1^2 b_{11} + 2x_1 x_2 b_{12} + x_2^2 b_{22} \quad (2)$$

where c_1, x_1 and c_2, x_2 are concentrations and weight fractions of the chitosan and gelatin, respectively. The b_{11}, b_{22} and b_{12} values reflect the binary interactions between chitosan, gelatin and chitosan and gelatin molecules. As shown in Eq. (1), the values of b_{11}, b_{22} and b_m can be obtained from the slope of the plots of $\eta_{sp}/(c_1)$ vs. c_1, $\eta_{sp}/(c_2)$ vs. c_2 and $\eta_{sp,m}/(c_1 + c_2)$ vs. $(c_1 + c_2)$, respectively. The b_{12} can be obtained after putting b_{11}, b_{22} and b_m into Eq. (2). Catstiff and Hewett (1962) further theorized the following equations:

$$b_{12}^i = \frac{b_{11} + b_{22}}{2} \quad (3)$$

The b_{12}^i reflect the binary interactions between chitosan and gelatin segments in the ideal chitosan/gelatin solution. As suggested by Catsiff and Hewett, chitosan and gelatin molecules are miscible or immiscible in the molecular level if $b_{12} > b_{12}^i$ or $b_{12} < b_{12}^i$.

2.4. FT-IR study

FT-IR spectra were performed using a Perkin-Elmer 6000 FT-IR spectrometer, resolution 2 cm^{-1}, in the wave number range of 4000–400 cm^{-1} by KBr pellet technique.

2.5. Morphological analysis

The cross-linked samples used as SEM were kept in deionized water with the bath temperature 50 °C for 2 h to remove not cross-linked gelatin, which may dissolve in hot water. The cross-section of the dried samples was studied by scanning electron microscope (SEM, JEOL JSM-5600LV) at an accelerated voltage of 10–20 kV.

2.6. Determination of cross-linking degree

The cross-linking degree, determined by ninhydrin assay (Mi, Tan, Liang, Huang & Sung, 2001), determines the percentage of amino groups reacting with genipin. Ninhydrin solution was prepared as follows. Solution I: 1.05 g citric acid, 10 mL (1.0 M) aqueous NaOH solution and 0.04 g $SnCl_2 \cdot 2H_2O$ were mixed, then deionized water was added until 25 mL; solution II: 1 g ninhydrin was added to 25 mL ethylene glycol monomethyl ether. The solutions I and II were blended and stirred for 45 min, then stored in dark bottle. For the assay, the test samples were lyophilized for 24 h and then

weighed. Subsequently, a 1.5 mg lyophilized sample was heated to 100 °C in water bath with 2 mL ninhydrin solution for 20 min. The solution was cooled down to room temperature, diluted with 15 mL 50% isopropanol and then the optical absorbance of the solution at 570 nm was recorded with a spectrophometer (Model UV-150-02; Shimadzu Corp., Kyoto, Japan). After heating with ninhydrin, the amount of free amino groups in the test sample is proportional to the optical absorbance of the solution. The concentration of free amino groups is determined from a standard curve of glycine concentration vs absorbance. The cross-linking degree of sample is calculated as follows:

$$\text{Cross-linking degree} = \frac{M_o - M_t}{M_o} \times 100\% \quad (4)$$

where M_o is the mole fraction of free amino groups in non-cross-linked samples and M_t is mole fraction of free amino groups remaining in cross-linked samples. Each sample consisted of three replicate measurements and the result was expressed as an average value.

2.7. Swelling studies

The swelling studies of the chitosan/gelatin hydrogels were carried out by the following method. All hydrogels were cut into 2 cm ×2 cm length (0.15 mm in thickness).

Swelling kinetics of the hydrogel was conducted in a buffer solution at pH 1.2 or 7.4 by determining the weight of the hydrogel at 37 °C at specific time intervals, after absorbing the excess liquid on the surface of the hydrogel by filter paper. The swelling ratio (*SR*) was calculated as follows:

$$SR\ (\%) = \frac{W_t - W_o}{W_o} \times 100\% \quad (5)$$

where W_t and W_o are the weights of swollen sample at time t and that of the original sample, respectively.

Swelling reversibility of the hydrogel was evaluated in the pH 2 and pH 8 buffers. The samples were first placed in the pH 8 buffer solution and transferred to that of pH 2 after they reached equilibrium. The weights were measured once in every 15 min and equilibrated samples in one buffer solution were transferred to the other buffer solution cyclically. The swelling ratio was calculated according to Eq. (5).

Equilibrium swelling ratio as a function of pH was measured by immersing dried samples in buffer solutions with pH values of 2.5, 5.0, 6.0, 7.0 and 8.0, the contact duration being 24 h. The swelling ratio was calculated from the following expression:

$$SR\ (\%) = \frac{W_w - W_d}{W_d} \times 100\% \quad (6)$$

where W_w and W_d are the sample weights after swelling for 24 h and dry state, respectively. Date from each sample was calculated using triplicate measurement.

3. Results and discussion

3.1. Intrinsic viscosity measurements

The plots of reduced viscosity vs. total concentrations of chitosan, gelatin and chitosan/gelatin mixture solution are summarized in Fig. 1. As shown in Fig. 1, nearly 1inear plots were observed for chitosan, gelatin and chitosan/gelatin solution for the whole compositions. The $[\eta]_m$, b_m, b_{12} and b^i_{12} values are summarized in Table 1. It was observed that the $[\eta]_m$ value of chitosan solution was high. This may be caused by its high deacetylation degree (DD = 93%). Presumably, higher molecular interactions (e.g. hydrogen bonding) between chitosan molecules or between chitosan and water molecules of chitosan solution are expected with

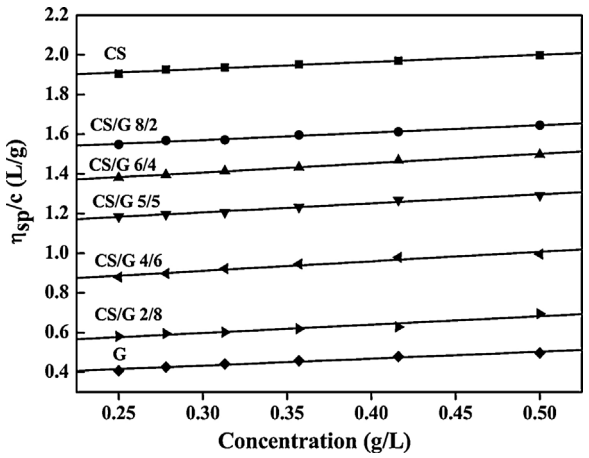

Fig. 1. Plots of reduced viscosity versus concentration of chitosan (CS), gelatin (G) and chitosan/gelatin (CS/G) solutions.

higher deacetylation degree. It was interesting to note that the $[\eta]_m$ value of the chitosan solution was significantly greater than gelatin solution. Moreover, the $[\eta]_m$ value of chitosan/gelatin solution increased consistently as the content of chitosan increase from 0 to 100 wt%. The beneficial chitosan effect on the $[\eta]_m$ value of chitosan/gelatin mixture solution was probably due to the higher viscosity of chitosan solution than gelatin solution. On the other hand, it was worth noting that the evaluated b_{12} of the chitosan/gelatin solution was significantly greater than the ideal b^i_{12}. As suggested by Catstiff and Hewett (1962), chitosan and gelatin molecules are miscible in the molecular level for all compositions of the chitosan/gelatin mixture solution prepared in this study.

3.2. Characterization of cross-linking mechanism by FT-IR

Fig. 2 shows the FT-IR spectra of chitosan, gelatin, chitosan/gelatin (5/5), together with that of genipin-cross-linked chitosan/gelatin (5/5) samples, respectivey. FT-IR spectra of chitosan showed adsorption band at 3445 cm^{-1} due to the partially overlapped of amine and hydroxyl group stretching vibrations and a strong protonated amino characteristic peak at around 1576 cm^{-1}. The absorption band at 1629 cm^{-1} was characteristic of amide absorption. The peaks at 926 cm^{-1} and 1151 cm^{-1} were assigned to vibrational modes associated with saccharide units of chitosan, at 1281 cm^{-1} could be attributed to the hydroxyl group and at 1026 and 1078 cm^{-1} resulted from C—O and C—N stretching, respectively. The gelatin was characterized by its amide I and amide II, respectively arose from carbonyl peak at 1623 cm^{-1} and amino band at 1573 cm^{-1}, and that of amide III at 1274 cm^{-1}, caused by C—N stretching vibrations, amide N—H in-plane bending vibrations and CH_2 wagging vibrations. The shifting of carbonyl band to 1645 cm^{-1} indicated an interaction between gelatin and chitosan, which further identifies the result of intrinsic viscosity measurement, suggesting that the chitosan and gelatin molecules are miscible. Comparison of the IR spectrum of

Table 1
Miscibility parameters of CS, G and CS/G mixture solutions.

CS/G	$[\eta]_m$	b_m	b_{12}	b^i_{12}
10/0	1.823	0.355	–	–
8/2	1.459	0.373	0.410	0.353
6/4	1.266	0.471	0.598	0.353
5/5	1.070	0.454	0.555	0.353
4/6	0.767	0.482	0.622	0.353
2/8	0.472	0.421	0.570	0.353
0/10	0.328	0.351	–	–

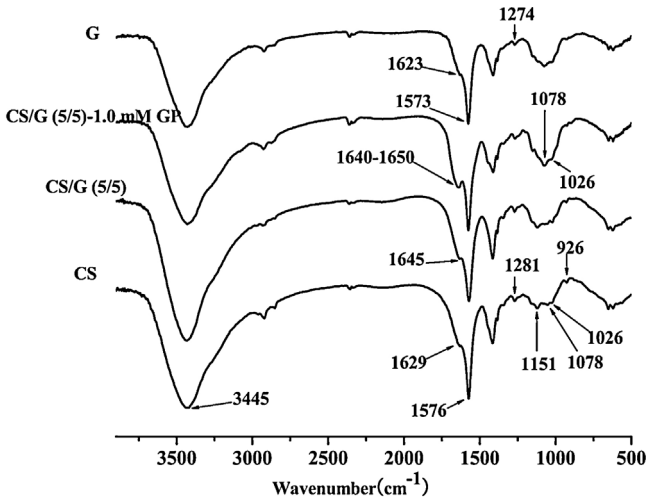

Fig. 2. FT-IR spectra of chitosan (CS), gelatin (G), chitosan/gelatin (CS/G) and chitosan/gelatin (CS/G) cross-linked with 1.0 mM genipin (GP).

chitosan/gelatin (5/5) to genipin-cross-linked chitosan/gelatin (5/5) showed a pronounced difference. For genipin-cross-linked samples, the significantly increased adsorption at 1078 cm^{-1} at the expense of 1026 cm^{-1}, combined with the decrease in intensity of the protonated amine band at 1576 cm^{-1}, is interpreted as the formation of C—N bonds at the expense of C—O bonds during the formation of the heterocyclic genipin-cross-linked compound. An increase in the intensity of the ratio between the adsorption bands at 1640–1650 cm^{-1} and 1570–1580 cm^{-1} could be attributed to the formation of secondary amide and the absorption of protonated amine as a result of the reaction between primary amine groups on chitosan/gelatin and ester groups of genipin. The probable reaction mechanisms between primary amine groups and genipin include two reaction processes (Butler, Ng, & Pudney, 2003).

The fastest, and therefore the first reaction begins with an nucleophilic attack on the genipin olefinic carbon atom at C3 from a primary amine group to form an intermediate aldehyde group, followed by the opening of the dihydropyran ring and attacked by the secondary amine group formed in the first step of the reaction on the resulting aldehyde group (Chen et al., 2004). A heterocyclic compound of genipin-cross-linked to the polymers containing primary amine groups, is thereby formed. In this study, the evidence for the formation of the heterocyclic genipin-cross-linked compound was the increase in the intensity of C—N band at 1078 cm^{-1} at the expense of the C—O band at 1026 cm^{-1}. The slower and second reaction is a nucleophilic substitution reaction that converts the primary amine groups to secondary amide linkages, involving the replacement of the ester group on genipin. Its occurrence was identified by the increase in the intensity of the ratio between the adsorption bands at 1640–1650 cm^{-1} and 1570–1580 cm^{-1}. Furthermore, the aforementioned studies suggested that genipin may form intramolecularly or intermolecularly cross-linked products with a heterocyclic structure among nucleophilic reagents (Fig. 3) (Chen et al., 2004; Mu, Zhang, Lin, & Li, 2013). In this study, it was observed that the color of chitosan/gelatin hydrogel changed from transparent to dark bluish on treatment with genipin, which suggested that in addition to the cross-linking reactions, other more complex reactions occurred. Previous studies (Butler et al., 2003) of the dark bluish color obtained in the reaction of genipin with primary amino groups found that they were formed through the oxygen radical-induced polymerization and dehydrogenation of intermediate compounds. Moreover, it was clearly observed that the blue coloration was initially more pronounced at the interface of the hydrogel and gradually moved down with time, which also suggest that the polymerization reactions are induced by the presence of oxygen radicals.

3.3. Scanning electron microscopy study (SEM)

Fig. 4 shows SEM photographs of cross-section of the chitosan/gelatin (5/5) hydrogels with or without cross-linking. It was found that the hydrogel without cross-linking was characterized by a compact, uniform, smooth structure and homogenous appearance, which indicates a good compatibility between chitosan and gelatin. The instrinsic viscosity measurement and FT-IR study also support the result. It showed no difference of genipin-cross-linked chitosan/gelatin samples before and after washing, which indicates that the free amino groups both on chitosan and gelatin react with genipin, forming a network structure and limiting the mobility of the polymer chains. Thus, in the formation of genipin-cross-linked chitosan/gelatin hydrogels, cross-linking reaction took place both on chitosan and gelatin, resulting in the formation of IPN The IPN formation can be schematically illustrated by Fig. 3. Furthermore, at lower genipin content (Fig. 4a and a′), the cross-section of the samples appeared smooth compared with those prepared at higher genipin content (Fig. 4b, b′, c and c′). The cross-section of the samples appeared rougher as the genipin content increased.

3.4. Cross-linking degree

As determined by the ninhydrin assay, the cross-linking degree of experimental values (theoretical values) of the chitosan/gelatin (5/5) hydrogels cross-linked with 0.50, 0.75 and 1.00 mM genipin were 14.8% (12.9%), 25.2% (22.2%) and 28.9% (24.7%), respectively. It was worth noting that under the same condition, the experimental values were different from the theoretical values. This may be due to some free amino groups take part in the interactions between chitosan and gelatin, such as amidization. On the other hand, at 0.50, 0.75, and 1.00 mM genipin concentration, the cross-linking degrees of chitosan (gelatin) were 15.2% (9.7%), 26.3% (15.9%) and 29.4% (18.1%), respectively. The degree of cross-linking of chitosan was far greater than gelatin, which was an evidence for chitosan molecule has a more average number of primary amine groups than gelatin molecule (Mi, 2005).

3.5. Swelling characteristics

The swelling ability of the hydrogel is an important aspect to evaluate its property, used as scaffold materials for tissue engineering, adsorbents for dyes, controlled drug release system, et al. The swelling kinetics of chitosan/gelatin IPN hydrogel was performed in a pH 1.2 or 7.4 buffer solution at 37 °C for 3 h. The effect of the weight ratios on swelling ratio were also analyzed and the results are presented in Fig. 5.All samples were characterized by a superfast swelling at both pH 1.2 and 7.4. At pH 1.2, the swelling ratio was much greater than at pH 7.4 and increased with increase chitosan content. However, at pH 7.4, the higher the percentage of chitosan, the lower was the swelling ratio. This may be attributed to the protonation of amino groups ($—NH_3^+$) on chitosan at pH 1.2. At acidic condition (pH 1.2), the protonation of amino groups on chitosan ensure chain relaxation, leading to efficient solvent diffusion (Zeng & Fang, 2004). With increase the chitosan content in the IPN hydrogels, more positively charged amino groups lead to much more expanding hydrogel network (Zhao et al., 2011). At pH 7.4, chitosan' amino groups are kept in the form of $—NH_2$, which makes it insoluble, resulting in the shrinkage of the hydrogel network. The swelling behavior of the IPN hydrogel mainly results from the electrostatic repulsion between the negatively charged carboxyl groups on gelatin. Moreover, it is known that only primary amine groups of lysine and arginine residues on gelatin could react with

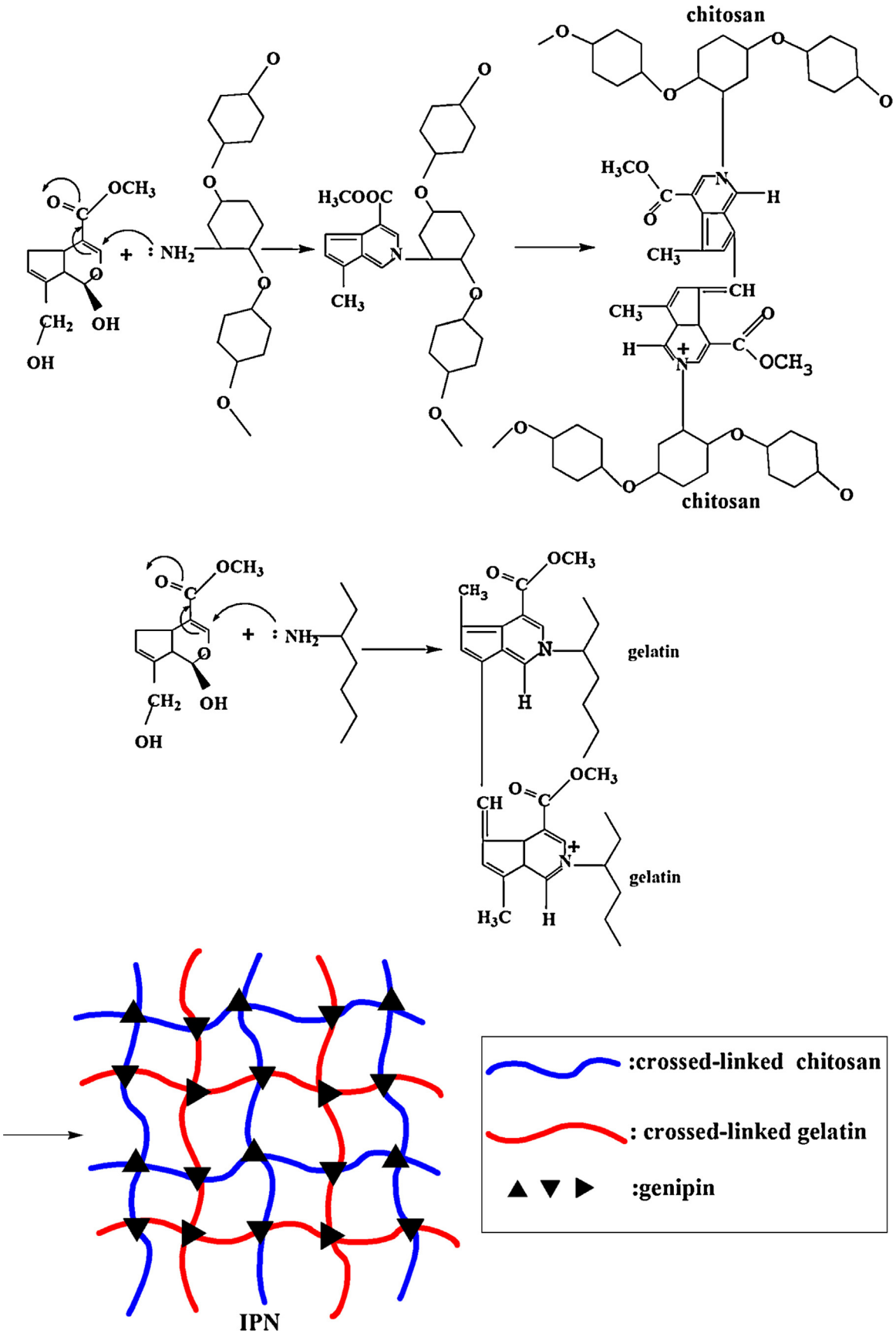

Fig. 3. Presumable reaction mechanism of genipin with chitosan and gelatin and schematic illustration of the IPN formation of chitosan and gelatin cross-linked with genipin.

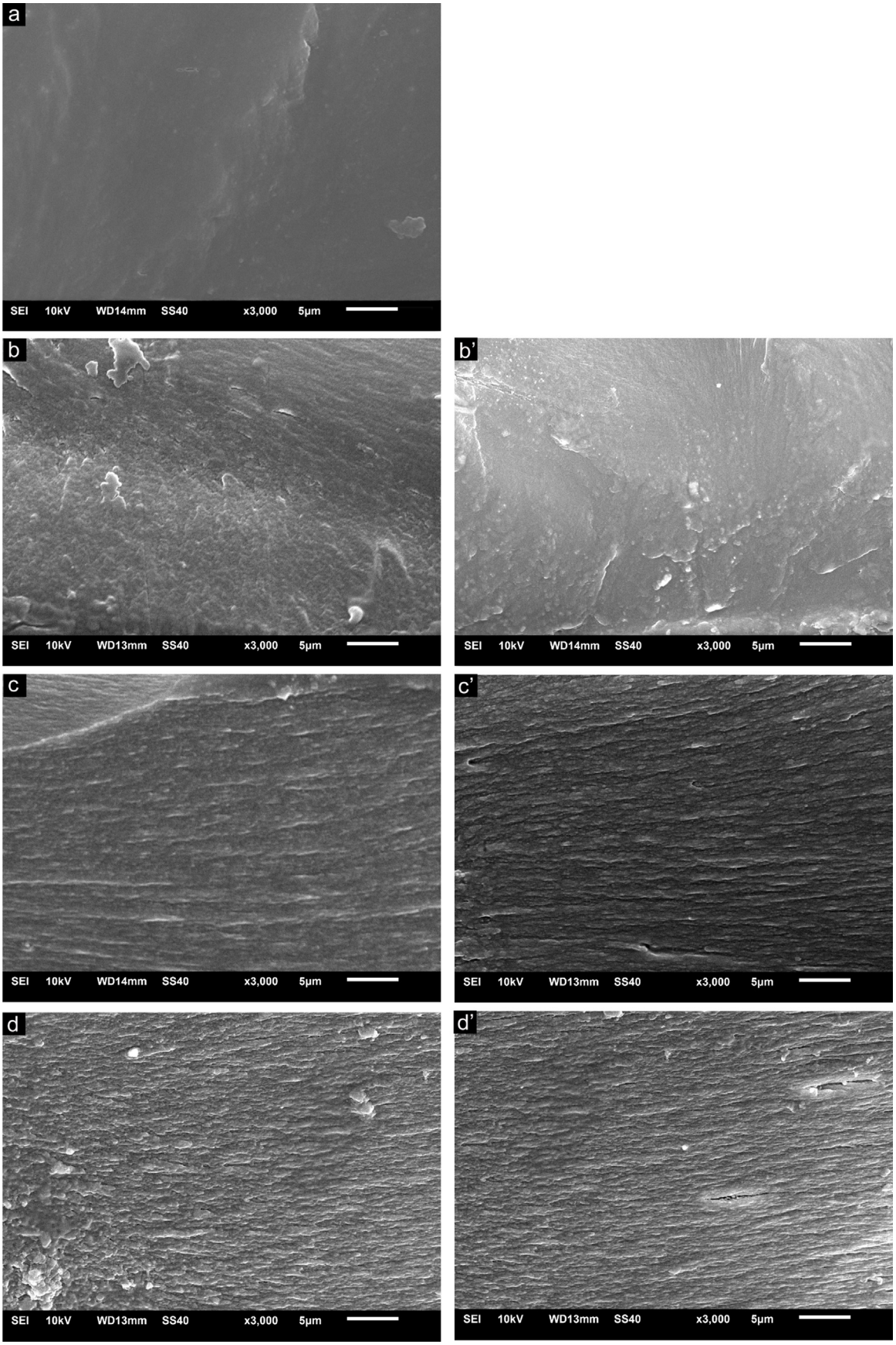

Fig. 4. SEM images of cross-section morphology of chitosan/gelatin hydrogels with different genipin content: (a) 0 mM; (b) 0.5 mM, before washing; (b′) 0.5 mM, after washing; (c) 0.75 mM, before washing; (c′) 0.75 mM, after washing; (d) 1.0 mM, before washing; (d′) 1.0 mM, after washing.

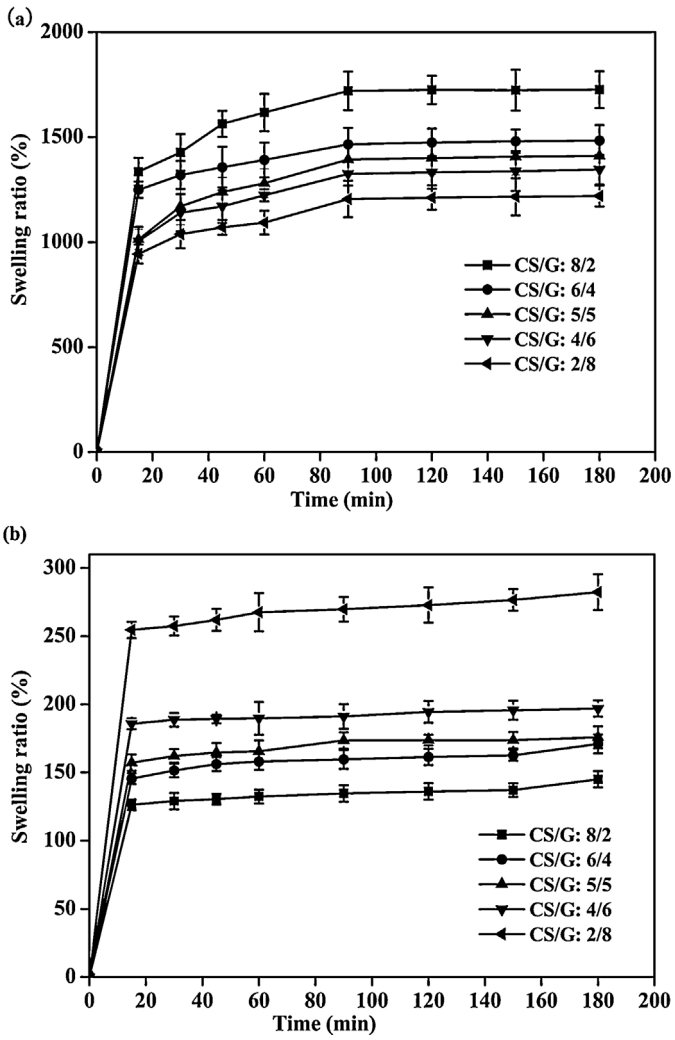

Fig. 5. Swelling ratio of the chitosan/gelatin (CS/G) IPN hydrogels with distinct weight ratios cross-linked with 0.75 mM genipin (GP) at (a) pH 1.2, (b) pH 7.4.

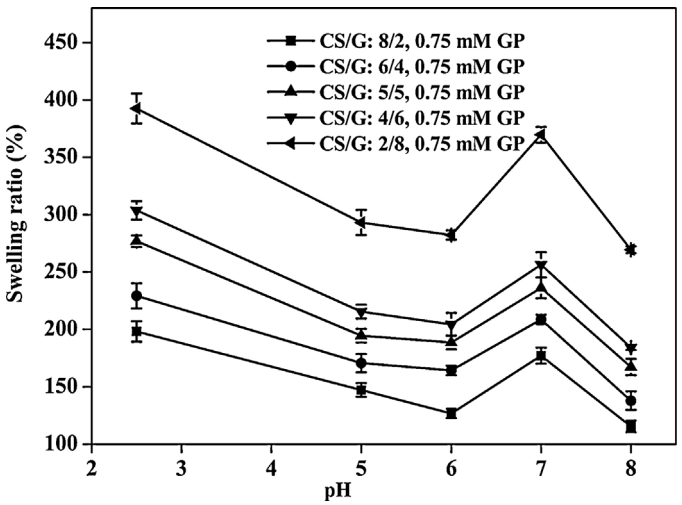

Fig. 6. Swelling ratio of the chitosan/gelatin (CS/G) IPN hydrogels with distinct weight ratios cross-linked with 0.75 mM genipin (GP) as a function of pH.

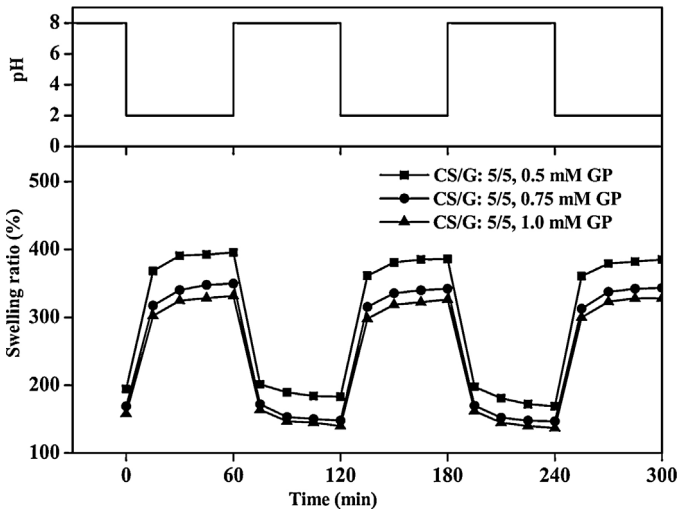

Fig. 7. Oscillated swelling behavior of the chitosan/gelatin (CS/G) IPN hydrogels with different genipin (GP) content.

genipin, whereas every glucosamine unit of chitosan could react with genipin and the lysine and arginine residues in gelatin are much less. On the other hand, chitosan has a more average number of primary amine groups than gelatin for the reaction with genipin (Mi, 2005). The more the percentage of chitosan, the higher the cross-linking reaction would take place. As a result, chitosan could react with genipin to form more sufficient cross-linking bridges than gelatin. This would in turn form a more compact wall, resulting in the decrease of the swelling ratio.

To investigate the swelling behavior with various pH levels, the IPN hydrogels were swollen in buffer solutions of pH 2.5, 5.0, 6.0, 7.0, and 8.0, respectively. Fig. 6 shows the effect of pH on swelling behavior of chitosan/gelatin IPN hydrogels with different weight ratios cross-linked with 0.75 mM genipin. The swelling ratio was higher in acidic media compared to the neutral and alkaline media. It decreased when pH increased from 2.5 to 6, while from 6 to 7, a slightly rising was found. This may be because at pH < 2.5, the swelling feature is dominated by the cationic network based on chitosan. When pH increases from 2.5 to 6, the deprotonation of the amine groups in chitosan lead to the hydrophilicity of the hydrogel decrease. On the other hand, the swelling ratio is limited due to the formation of hydrogen bonds between —COOH, —OH and $—NH_2$. At pH > 6, the carboxylic acid groups on the IPN hydrogels become progressively ionized ($—COO^-$). In this case, the hydrogels swell due to a large swelling force created by the electrostatic repulsion between the negatively charged carboxyl groups. At pH > 7, the electrostatic screening effect of the $—OH^-$ becomes effective, causing the decrease of the swelling ratio.

Oscillated swelling behavior with pH alternating between 2 and 8 was investigated to confirm the reversibility and response rate of the swelling process. The oscillatory swelling data produced from chitosan/gelatin (5/5) IPN hydrogels with various cross-linker content are shown in Fig. 7. It was observed that all samples showed strong swelling reversibility and rapid response rate, regardless of the pH was from 8 to 2 or from 2 to 8. The IPN hydrogels produced from other weight ratios gave the similar oscillated swelling behavior. In addition, with increase genipin concentration, the swelling ratio of the IPN hydrogel decreased appreciably. The result revealed a strong influence of genipin concentration on swelling ratio. This could be explained by the fact that the lower the degree of the cross-linking, the lower is the polymer network density. Consequently, the polymer network has a higher available hydrodynamic free volume to accommodate more of the solvent molecules, thereby increase matrix swelling. On the contrary, an increase of the cross-linking degree increases polymer network density. The mobility and relaxation of the polymer chains are hindered and the available

free space decreases, which in turn impede the diffusion of solvent molecules, lowering the swelling ratio (Rokhade et al., 2007).

4. Conclusions

Genipin-cross-linked chitosan/gelatin IPN hydrogel was successfully obtained by solvent evaporation. Judging from the results of intrinsic viscosity measurement $b_{12} > b_{12}^{i}$, FT-IR (the shifting of carbonyl band from 1623 cm^{-1} to 1645 cm^{-1}) and SEM (smooth and homogenous morphology), it is reasonable to conclude that the polymer mixtures of chitosan/gelatin form a molecular miscibility in their blends. The cross-linking mechanism examined by FT-IR suggests that the cross-linking primary amino groups by genipin leads to the formation of secondary amide and heterocyclic amino linkage. The cross-linking degree of chitosan/gelatin hydrogel increased with increase genipin concentration. The swelling ratio decreased with increase genipin concentration. The chitosan/gelatin IPN hydrogel is pH-sensitive, exhibiting reversibility and rather rapidly response in swelling to pH changes. The results clearly suggested that the genipin-cross-linked chitosan/gelatin hydrogel could be used as a polymeric carrier for drug delivery or as an alternative sorbent in the removal or separation of dyes as environmental pH condition altered.

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