Physically Crosslinked Composite Hydrogels of Hemicelluloses with Poly(vinyl alcohol phosphate) and Chitin Nanowhiskers

Ying Guan, Xian-Ming Qi, Bing Zhang, Ge-Gu Chen, Feng Peng,* and Run-Cang Sun*

Hydrogels have been widely studied as tissue engineering scaffolds due to their swelling capabilities in water and other physical properties. In this study, phosphatized PVAs of varying degrees were prepared by esterification of phosphoric acid, and a freeze-thaw technique was introduced to fabricate hydrogels from hemicelluloses, poly(vinyl alcohol phosphate) (P-PVA), and chitin nanowhiskers. The obtained hydrogels were characterized by Fourier transform infrared (FT-IR) spectrometry, scanning electron microscopy (SEM), X-ray diffraction (XRD), and CP/MAS ^{13}C nuclear magnetic resonance; their swelling properties and compressive strength were also determined. The repeated freeze-thaw cycles induced physically crosslinked chain packing among the three polymers by hydrogen bonds. The results showed that the hydrogels possessed higher swelling ratio and mechanical strength when the PVA was phosphatized by phosphoric acid and urea and more hydroxyl groups were introduced into the polymer matrices. This suggested that hydrogels with good mechanical properties were prepared successfully by the freeze-thaw technique.

Keywords: Hemicelluloses; Poly(vinyl alcohol phosphate); Chitin nanowhiskers; Hydrogel; Freeze-thaw

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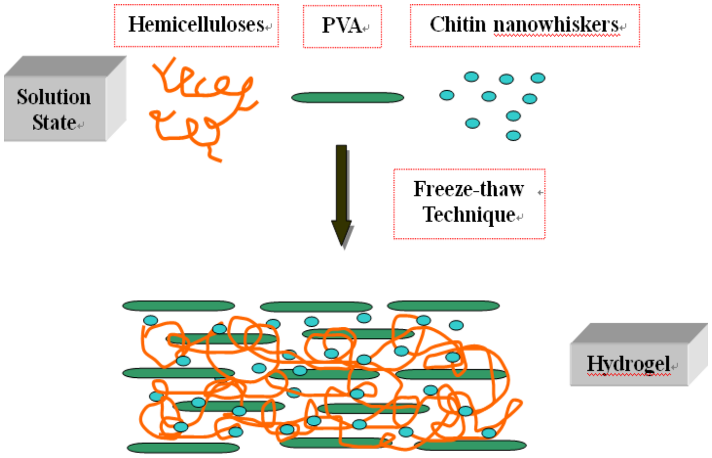
INTRODUCTION

Polymeric hydrogels consisting of three-dimensional polymer networks inundated with water have attracted much attention as functional soft materials (Haraguchi and Li 2006; Haraguchi *et al.* 2006; Zulfiqar *et al.* 2009; Rajeesh *et al.* 2010). These hydrogels can swell to equilibrium but do not dissolve in water (Han *et al.* 2008), and they have been considered promising biomedical materials because they are similar to human tissues and have eximious tissue compatibility (Smith *et al.* 2010). They have been studied for a wide range of biomedical, pharmaceutical, and daily-care applications, such as contact lenses, tissue engineering, drug delivery, and water retention in agriculture (Vermonden *et al.* 2012).

One method of producing hydrogels without chemical cross-links is freeze-thaw processing (Nugent and Higginbotham 2007). This method has the advantages of being experimentally straightforward, without the need for any chemical crosslinking agents. Gelation of polymers by the freeze-thaw method is driven by phase separation, which occurs as the solution freezes and the polymer is rejected from the growing ice crystallites. This process is refined with repeated cycling. The size of the ice crystallites increases with cycling, and the resultant gels are composed of water-filled pores, where the ice has melted, surrounded by a polymer skeleton. Physical crosslinking in the form of hydrogen bonds and crystalline polymeric regions reinforce the gel structure (Yokoyama *et al.* 1986; Peppas and Stauffer 1991). The mechanism of the freeze-thaw process is illustrated in Scheme 1.



Scheme 1. Gelation mechanism of hemicelluloses, PVA, and chitin nanowhiskers in a freeze/thaw process

Polysaccharides are a large source of biomass-based materials with various applications (Dumitriu 2004). They can be processed in different ways. Their ability to form gels under specific conditions, however, is particularly interesting. Polysaccharide hydrogels have been proposed for food, cosmetic, biomedical, tissue engineering, and pharmaceutical applications (Rinaudo 2008). Hemicelluloses, the rich, inexhaustible, and renewable polysaccharides, are second only to cellulose with respect to their presence in plant resources, accounting for one-fourth to one-third of plant resources (Lawther *et al.* 1995; Sun *et al.* 2005). They are non-cellulosic and short-branched chain hetero-polysaccharides consisting of various sugar units, arranged in different ratios and with different substituents. Generally, hemicelluloses contain a backbone of D-xylopyranosyl residues, linked together by β-(1→4)-glycosidic bonds (Gabrielii *et al.* 2000). Hemicelluloses have excellent hydrophilicity, biodegradability, and biocompatibility; therefore, materials based on them would have broad prospects.

Chitin whiskers have had extensive applications in many areas, including cosmetics, the food industry, drug delivery, tissue engineering, and in reinforcing polymer nanocomposite nanofibers (Zeng *et al.* 2012). Poly(vinyl alcohol) (PVA)/chitin whisker nanocomposite fiber mats with different amounts of chitin whiskers have been prepared by electrospinning, and the Young’s modulus of the nanocomposite fiber mat was four to eight times greater than that of pure PVA fiber mats (Junkasem *et al.* 2006). The storage modulus of PVA nanofiber mats increased with chitin whisker loading in the considered range (Junkasem *et al.* 2010). Alginate/chitin whisker nanocomposite fibers with 0.5 to 2.0 wt% chitin whiskers have been prepared by wet spinning. Incorporation of such a low amount of chitin whiskers in the nanocomposite fibers improved the mechanical properties significantly. This was possibly due to the specific hydrogen bonding and electrostatic interactions between the alginate molecules and the homogeneously dispersed chitin whiskers (Watthanaphanit *et al.* 2008). Poly(vinyl alcohol) (PVA) is a water-soluble, non-toxic, biodegradable, biocompatible synthetic polymer (Păduraru *et al.* 2007). Because of the hydroxyl groups present in each repeating unit, PVA exhibits a strong hydrophilic and hydrogen-bonding character; thus, it is able to form hydrogels. Physical hydrogels can be obtained by exposing PVA aqueous solutions to repeated cycles of freezing and thawing, which results in the formation of crystallites. Compared with chemically crosslinked hydrogels, physical hydrogels show higher elasticity and mechanical strength because of the crystalline regions, which are capable of better distributing a given mechanical load (Hassan and Peppas 2000; Bolto *et al.* 2009). The use of phosphate groups can improve hydrophilicity, anionic properties, compatibility, solution stability, drug uptake, and retention of hydrogels (Sreenivasan 2004; Pramanik *et al.* 2008a). Therefore, phosphorylated PVA has attracted considerable interest in the field of hydrogels.

In the present study, we report a novel hybrid hydrogel that can be easily prepared *via* physical crosslinking of hemicelluloses, poly(vinyl alcohol phosphate) (P-PVA), and chitin nanowhiskers. The study emphasizes the effect of the hydrogen bonds among polymers with a freeze-thaw technique by comparing the structural and morphological features, as well as the mechanical and swelling properties. The hydrogels were characterized by Fourier transform infrared (FT-IR) spectrometer, scanning electron microscopy (SEM), X-ray diffraction (XRD), and cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C-NMR); their swelling properties and compressive strength were also determined.

EXPERIMENTAL

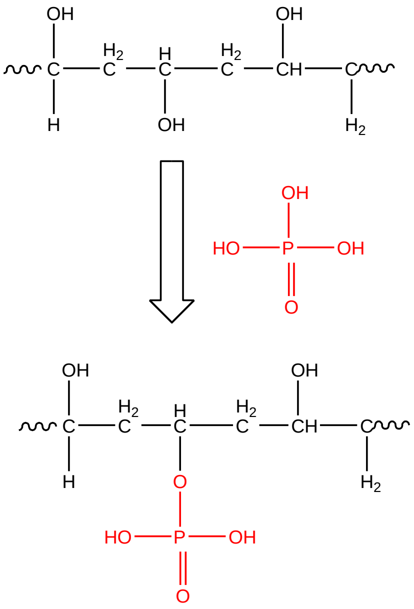
Materials

Poly(vinyl alcohol) (the degree of polymerization was 1750 ± 50) was purchased from Beijing Yili Fine Chemicals Co., Ltd. (China). Raw α-chitin powder was purchased from Yuhuan Chitin Co. Ltd. (Zhejiang, China). The weight-average molecular weight (M_w) was 5.0×10^5, and degree of acetylation (DA) was calculated to be 73% by Ren *et al.* (2008). After hydrochloric acid hydrolysis, chitin nanowhiskers were successfully obtained with an average length of 200 nm and average width of 40 nm, as reported in a previous study (Guan *et al.* 2014a). The hemicelluloses were prepared according to Guan *et al.* (2014a, b). All the reagents used were of analytical grade.

Methods

Synthesis of partially P-PVA and determination of phosphorylation

Poly(vinyl alcohol phosphate) (Scheme 2) was synthesized in accordance with a previous study, with minor modifications (Pramanik *et al.* 2008b). A mixture of 10 g of PVA powder, 50 mL of phosphoric acid, an amount of urea (from 0.5 to 4.5 g), and 50 mL of distilled water were put into a three-neck round bottom flask equipped with a vigorous mechanical stirrer, a condenser, and a thermometer. The mixture was heated up to 90 °C under constant stirring until the formation of a homogeneous melt mixture. The completely homogeneous melt mixture was refluxed at 90 °C for 3 h, cooled down to room temperature, and precipitated in excess ethanol. The product was washed thoroughly with ethanol to remove un-reacted molecules until the pH of the washed solution reached 5.0 to 6.0. The product was then freeze-dried. The determinations of phosphorylation degrees (DP) of P-PVAs were carried out in a previous study (Peng *et al.* 2014).



Scheme 2. Representation of the poly(vinyl alcohol phosphate) formation by phosphorylation of PVA

Preparation of hydrogels

The hemicelluloses, PVA or P-PVA, and chitin nanowhiskers at a proportion of 1:1:1 were dissolved in distilled water with a total concentration of 3.75 wt%. The three solutions were then mixed together and heated slowly up to 80 °C with vigorous stirring for 1 h to obtain a translucent paste mixture. After cooling to room temperature, the mixtures were poured into plastic tubes, frozen at -20 °C for 10 h, and subsequently thawed for 1 h at room temperature. This freeze-thaw cycle was repeated three times. The PVA hydrogel and P-PVA hydrogel were obtained by the same method but without the addition of hemicelluloses and chitin nanowhiskers. The hydrogels of hemicelluloses, PVA or P-PVA, and chitin nanowhiskers were named PVA-Gel and P-PVA-Gel, respectively. The hydrogels of P-PVA with dosages of urea from 0.5 to 4.5 g were named P0.5-Gel, P1.5-Gel, P2.5-Gel, P3.5-Gel, and P4.5-Gel. All these hydrogels were freeze-dried for analysis, and labeled in Table 1.

Table 1. Different Proportions of Hemicelluloses, PVA, and Chitin Nanowhiskers in Hydrogels

Sample codes	DP of PVA	V (hemicelluloses) : V(chitin)
P0.5-Gel	1.8	1:1
P1.5-Gel	3.7	1:1
P2.5-Gel	4.4	1:1
P3.5-Gel	7.7	1:1
P4.5-Gel	4.0	1:1
P-PVA(2.5) hydrogel	4.4	-
P-PVA(3.5) hydrogel	7.7	-

Scanning electron microscopy analysis

Scanning electron microscopy of the hydrogel samples was carried out with a Hitachi S-3400N II (Hitachi, Japan) instrument operating at an accelerating voltage of 15 kV. Prior to taking pictures, the freeze-dried samples were sputter-coated with a thin layer of gold. Images were obtained at magnifications ranging from 200× to 5000×, depending on the feature to be traced.

X-ray diffraction

The crystallinities of the PVA, chitin nanowhiskers, and hydrogels were measured using an XRD-6000 instrument (Shimadzu, Japan) with a Cu Kα radiation source (λ = 0.154 nm) at 40 kV and 30 mA. Samples were scanned from 5° to 40° (2θ) at a speed of 2°/min.

FT-IR spectroscopy

The FT-IR spectra of the hydrogels were recorded using a Thermo Scientific Nicolet iN 10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen-cooled MCT detector. Dried samples were ground and pelletized using BaF_2, and their spectra were recorded from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1} and 128 scans per sample.

^1H and ^{13}C NMR spectroscopy

The CP/MAS ^{13}C-NMR spectra of samples were obtained at 100 MHz using a Bruker AV-III 400 M spectrometer (Germany). Samples were packed in 4-mm zirconia (ZrO_2) rotors, and the measurements were performed using a CP pulse program with a match time of 1 ms and a 2-s delay between transients. The spinning rate was 5 kHz. Calibration was conducted externally to the carbonyl carbon of glycine at 176 ppm.

Mechanical property evaluation

The undried hydrogel samples were 5 × 10 mm (diameter × height) in dimension and incubated in distilled water for 24 h at 25 °C before testing. The compression test of the hydrogels was performed on a CMT6503 test machine (ShenZhen SANS, China), with a speed of 5 mm/min, according to ISO 527 (1995) (E).

Swelling measurements

After lyophilization, the dry hydrogels were immersed into excessive distilled water to reach a state of equilibrium swelling. The swollen superabsorbent was filtered using a 100-mesh sieve and drained for 20 min until no free water remained. After weighing the swollen hydrogels, the equilibrium water absorption was calculated using the following equation,

$$Q_{\mathrm{eq}} = \frac{W_2 - W_1}{W_1} \tag{1}$$

where Q_{eq} is the equilibrium water absorption, defined as grams of water per gram of sample; W_1 and W_2 are the mass of the sample before and after swelling, respectively.

RESULTS AND DISCUSSION

Phosphorylation of PVA

The different dosages of urea and the corresponding substitution degree of P-PVA are listed in Table 2. With an increase in urea dosage from 0.5 to 3.5 g, the degree of phosphorylation of PVA increased from 1.8 to 7.7. The highest DP of P-PVA was obtained when 3.5g urea was added in this synthetic pathway. This phenomenon occurred because ammonium biphosphate can be formed by the reaction between the NH_3 that originated from urea and aqueous H_3PO_4 and because of the polarization and esterification that occurred in the presence of the hydroxyl groups of PVA (Pramanik *et al*. 2008). Although the DP of P-PVA was not high, there are three hydroxyl groups for every vinylalcohol unit added into PVA chains. However, when the urea dosage increased to 4.5 g, the degree of phosphorylation of PVA decreased to 4.0, which was probably because the superfluous urea was neutralized with H_3PO_4.

Table 2. Urea Dosage and the Corresponding Degree of Phosphorylation of PVA

m_{urea} (g)	0.5	1.5	2.5	3.5	4.5
DP	1.8	3.7	4.4	7.7	4.0

Morphological Analysis of Hydrogels

SEM is a useful tool for studying the three-dimensional network structure and morphology of hydrogels (Fig. 1).

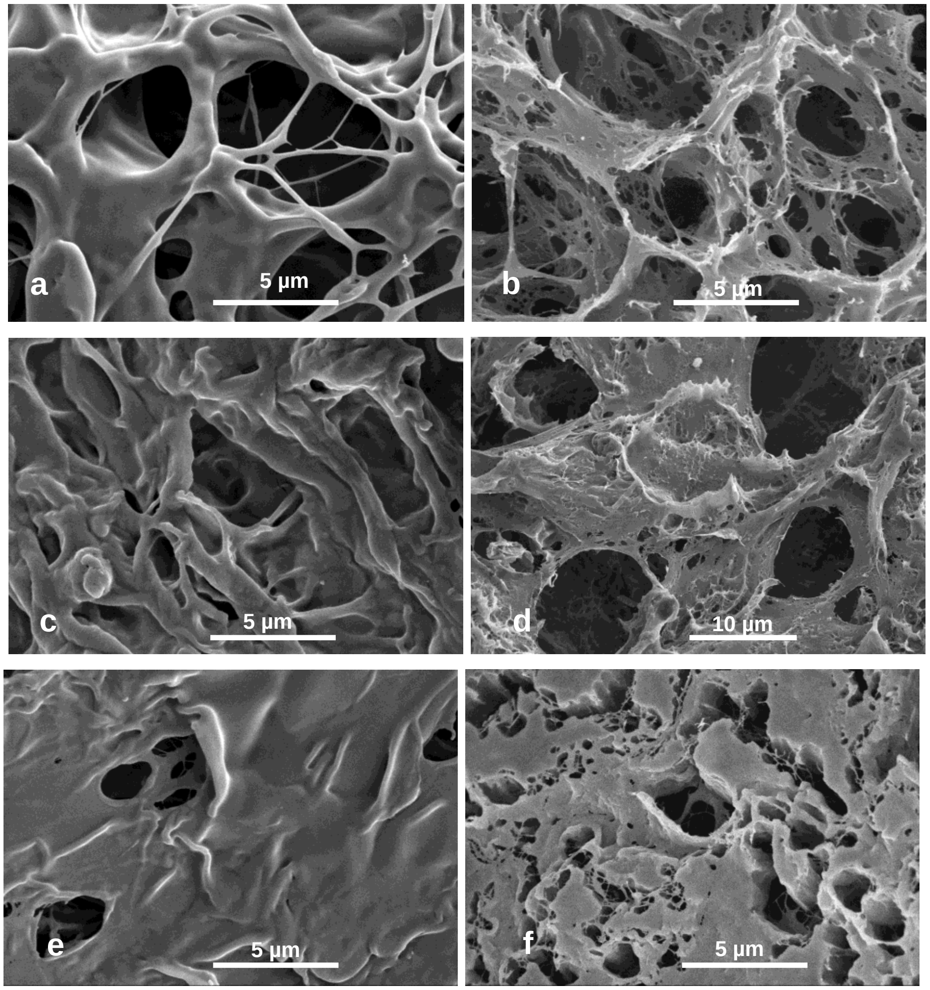


Fig. 1. SEM images of (a) PVA hydrogel, (b) PVA-Gel, (c) P-PVA(3.5) hydrogel, (d) P3.5-Gel, (e) P-PVA(2.5) hydrogel, and (f) P2.5-Gel

The SEM cross-section images of different hydrogel, PVA-Gel, P-PVA(3.5) hydrogel, P3.5-Gel, P-PVA(2.5) hydrogel, and P2.5-Gel are shown in Fig. 1. The microstructural changes on the hydrogel surface caused by freeze-thaw treatment can be clearly observed. The surfaces of the PVA hydrogels, P-PVA(3.5) hydrogel, and P-PVA(2.5) hydrogel were intact and condensed with several observable pores (Figs. 1a, c, e). However, the hydrogels, which were obtained by PVA, P-PVA(3.5) and P-PVA(2.5) with the addition of hemicelluloses and chitin nanowhiskers (Figs. 1b, d, f), exhibited similar honeycomb-like network structures after freeze-thaw treatment. The pores present in the hydrogel network were mainly due to a varied process. The hydrogen-bonded network was easily formed among the polymers under the frozen state; the water disappeared, but pores were retained when the temperature was raised. This suggested that these polymers had abundant hydroxyl bonds and that hydrogen-bonded network structures were at a highly stable state at low temperatures, similar to the case in the previous study (Păduraru *et al.* 2012). Compared to the pore size of PVA-Gel from Fig. 1b, the pore size of P-PVA-Gel (Fig. 1d) was larger, and tighter structures appeared as a result of the hydrogen bonds formed among polymers. The additional hydroxyl groups, which were due to the presence of phosphate groups, presumably could engage in the formation of hydrogen bonds, resulting in the bigger pore size. The linkages among compact structures of polymers were ruptured and appeared irregular, which may have been as a result of the intrinsic pressure of ice crystals after freezing.

As shown in Fig. 1c-f, the hydrogels obtained from different P-PVAs presented different configurations. Since the DP of PVA was highest when the urea dosage was 3.5 g (Table 2), the P3.5-Gel was selected as the typical sample to analyze. When P3.5-Gel was compared with P2.5-Gel, it was found that perfect pores were formed in the network of P3.5-Gel, while smaller pores were beginning to sprout in P2.5-Gel (Fig. 1f). Evidently, the pores of P3.5-Gel were bigger than those of P2.5-Gel; and more condensed structures were observed in P-PVA(2.5) hydrogel than that of P-PVA(3.5) hydrogel. These results indicated that the different phosphorylation of PVA played an important role in the morphology of hydrogels, and could affect the properties of hydrogels.

FT-IR

Fourier transform IR spectroscopy was used to characterize the interaction among phases. The IR spectra of PVA and phosphatized PVA are shown in Fig. 2a. For both PVA and P-PVA, the bands around 1090 and 1426 cm^{-1} are assigned to the stretching vibration of C-O-C and -CH_2 bending, respectively (Yang *et al.* 2010). After the phosphorylation, P-PVA had a new signal at 1650 cm^{-1}, which was attributed to C=O stretching in carboxamide groups of residual urea. The characteristic bands at 1236, 989, and 849 cm^{-1} can be assigned to the introduction of P=O, P-O, and P-OH groups of P-PVA, respectively (Mohapatra *et al.* 2006; Pramanik *et al.* 2008). The absorption band near 3314 cm^{-1} can be attributed to OH stretching vibration. This band became broader after phosphorylation because of the presence of an increased amount of hydroxyl groups with the addition of polyphosphonate.

The FT-IR spectra of hydrogels prepared from hemicelluloses, chitin nanowhiskers, and phosphatized PVA of different degrees are displayed in Fig. 2b. For the five P-PVA-Gels, the hydroxyl groups stretching vibration exhibited an absorption band centered at 3360 cm^{-1}; the peaks at 1660 and 1558 cm^{-1} can be assigned to the amide I and amide II regions of α-chitin, respectively (Goodrich and Winter 2007); the signals at 1039 and 893 cm^{-1} are due to C–O bond stretching and β-glycosidic linkages between sugar units of hemicelluloses (Ebringerová *et al.* 1992); and the absorption bands at 1418 and 850 cm^{-1} can be attributed to the P-PVA. Interactions were formed among hemicelluloses and chitin nanowhiskers, while P-PVA and hydrogen bonds were created during the freeze-thaw process. In addition, more hydroxyl groups in the hydrogels were obtained with the addition of P-PVA. The peak of the hydroxyl groups became broader as the degree of phosphorylation of P-PVA hydrogels increased from 1.8 (urea dosage of 0.5) to 7.7 (urea dosage of 3.5). This result suggested that stronger hydrogen-bonding interactions were formed among the hydroxyl groups because of the higher degree of phosphorylation of PVA due to the repeated freeze-thaw cycles.

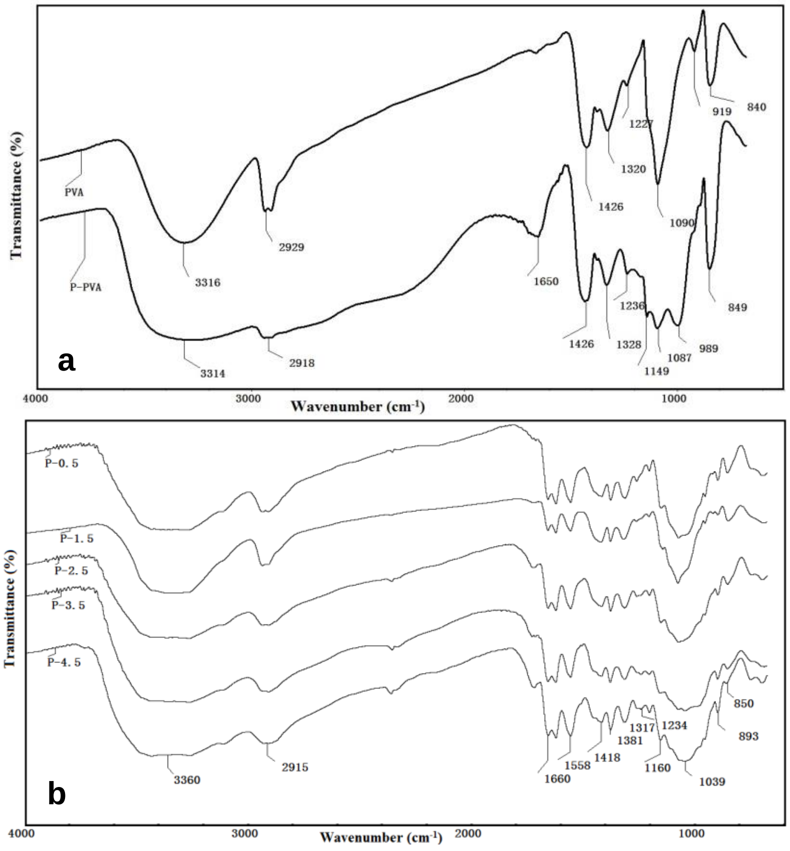


Fig. 2. FT-IR spectra of (a) PVA and P-PVA and (b) P0.5-Gel, P1.5-Gel, P2.5-Gel, P3.5-Gel, and P4.5-Gel

Cross-polarization magic angle spinning ^{13}C NMR has been widely applied for investigating the composition of hydrogels. The solid-state ^{13}C NMR spectra of hemicelluloses, PVA, chitin nanowhiskers, and the hydrogel formed by the three polymers are presented in Fig. 3a. For chitin nanowhiskers (Fig. 3a-a), the peaks from 172.5 to 175.0 ppm can be assigned to the carboxyl carbons of the acetamido group, whereas the characteristic peak of CH_3 carbons was observed at 22.1 ppm. The peaks at 103.6, 82.3 to 83.0, 75.1, 72.7, 60.2, and 54.5 ppm can be attributed to the C-1, C-4, C-5, C-3, C-6, and C-2 of glucopyranose units, respectively (Goodrich and Winter 2007). From the spectrum of chitin nanowhiskers, it was evident that the isolated chitin nanowhiskers were pure and residual protein and minerals were absent. The peaks at 76.1, 70.0, and 64.1 to 64.8 ppm are assigned to the three ^{13}C resonance lines (lines I, II, and

III) of CH carbons of PVA (Fig. 3a-b), respectively. This split was mostly thought to be due to the formation of two, one, and no intramolecular hydrogen bond in the triad sequences [CH(OH)-CH_2-CH(OH)-CH_2-CH(OH)] (Ketels *et al.* 1990; Imashiro and Obara 1995). The sharp peaks from 43.0 to 44.7 ppm can be assigned to CH_2 carbons in PVA (Horii *et al.* 1997). There are only a few peaks of hemicelluloses observed clearly in Fig. 3a-c. The signal at 101.8 ppm can be assigned to C-1 of the β-D-xylan units linking by 1→4 bonds, whereas C-4, C-2, and C-3 have characteristic peaks at 80.9, 74.3, and 72.8 ppm, respectively. The signal at 63.2 ppm can be attributed to C-5 for β-D-xylan units of hemicelluloses (Peng *et al.* 2009).

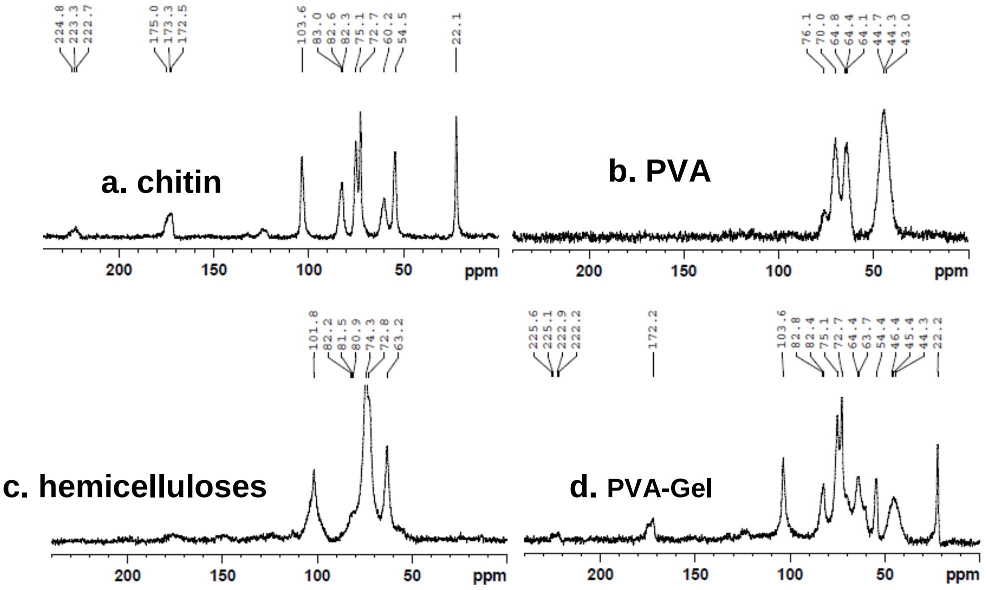


Fig. 3a. ^{13}C-NMR spectra of (a) chitin, (b) PVA, (c) hemicelluloses, and (d) PVA-Gel

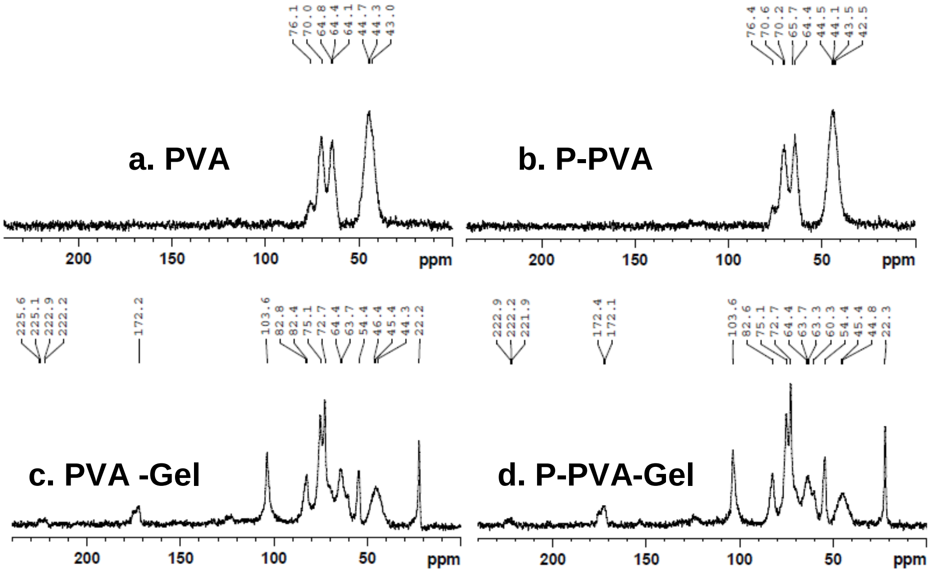


Fig. 3b. ^{13}C-NMR spectra of (a) PVA, (b) P-PVA, (c) PVA-Gel, and (d) P-PVA-Gel

The NMR results of the hydrogels indicated that the characteristic signal (60.2 ppm) of chitin nanowhiskers, the two lines (lines I and II, 76.1 and 70.0 ppm) of CH carbons of PVA, and the peak at 101.8 ppm of hemicelluloses disappeared (Fig. 3a-d). These signals could shift to other positions after freeze-thaw cycles. These results were consistent with FT-IR, confirming that a hydrogel of the three polymers formed successfully.

The solid-state ^{13}C NMR spectra of the PVA and P-PVA are shown in Fig. 3b (top). The two spectral profiles of characteristic bands of PVA and P-PVA were similar (Figs. 3b-a and 3b-b), and the only difference was that the intensity of line II (70.0 ppm) of PVA was markedly decreased after being phosphatized. Moreover, the resonance line ascribed to line I (76.1 ppm) changed only slightly in the case of the P-PVA. These phenomena suggested that the upfield shifted because of the γ-gauche effect (Tonelli 1989; Born and Spiess 1997) and the downfield shifted because of the formation of intramolecular hydrogen bonds (Masuda and Horii 1998). The PVA-Gel and P-PVA-Gel can be seen in (Figs. 3b-c and 3b-d). The peak intensity of PVA-Gel was higher than that of P-PVA-Gel, primarily because more hydroxyl groups of hydrogels were obtained by the introduction of the phosphate group, resulting in greater chances for polymer hydrogen bond formation.

The X-ray diffraction profiles of PVA, PVA hydrogel, and hydrogel of hemicelluloses, PVA, and chitin nanowhiskers are presented in Fig. 4a. The characteristic peaks of PVA are near $2\theta = 19.6°$ and 26.6°, and the intensities were decreased when hemicelluloses and chitin nanowhiskers were added into the matrix.

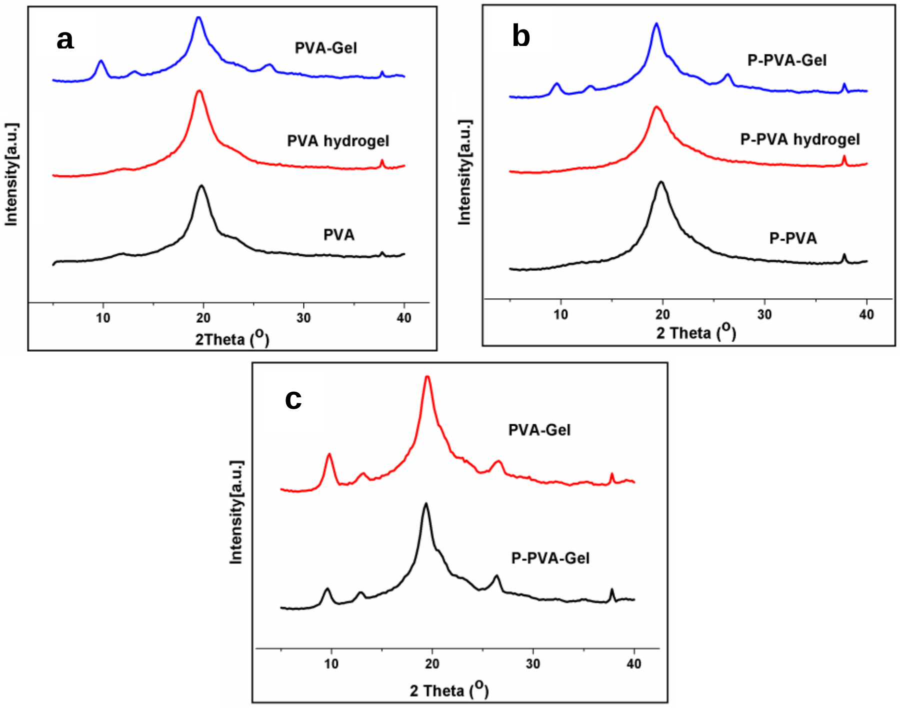


Fig. 4. X-ray diffraction patterns of (a) PVA, PVA hydrogel, and PVA-Gel, (b) P-PVA, P-PVA hydrogel, and P-PVA-Gel, and (c) PVA-Gel and P-PVA-Gel

The signal at $2\theta = 9.8°$ is the characteristic crystallinity of chitin nanowhiskers. From the curves in Fig. 4a, the crystalline intensity of PVA hydrogel increased because of the freeze-thaw treatment. This phenomenon could be explained by the creation of polymer regions in the matrices and the formation of ice crystals during freezing, where water remained partially unfrozen. Water also acted as a swelling agent in the disordered zones of the polymer matrices, forming hydrogen bonds with the hydroxyl groups of the three materials (Yokoyama *et al.* 1986; Willcox *et al.* 1999). Simultaneously, the repeated freeze-thaw treatments drove the aggregation of polymer molecules, leading to the easy separation of water from the polymer matrices. The XRD results of P-PVA (Fig. 4b) and PVA (Fig. 4a) were in full agreement.

For comparison, X-ray diffraction patterns of PVA-Gel and P-PVA-Gel are shown in Fig. 4c. Both of the hydrogels exhibited crystalline structures with peak angles near 2θ = 9.8°, 19.6°, and 26.6°. The pattern of PVA-Gel presented two sharp diffraction peaks, at approximately 2θ = 19.6° and 26.6°, which are typical fingerprints of PVA, and the chitin nanowhiskers had a major crystalline peak at 2θ = 9.8°. The intensity and area of the PVA diffraction decreased significantly after phosphatization treatment. The shift and decrease in intensity of each peak of PVA and the polymers after composite formation clearly indicated that the hydrogen bonds were present among polymer matrices. These results suggest that PVA and P-PVA with hemicelluloses and chitin nanowhiskers had good compatibility, which led to the formation of a porous network of hydrogel. The XRD patterns of PVA-Gel and P-PVA-Gel illustrated that the networks of hydrogels were successfully prepared from the polymers.

The Mechanical Properties of Hydrogels

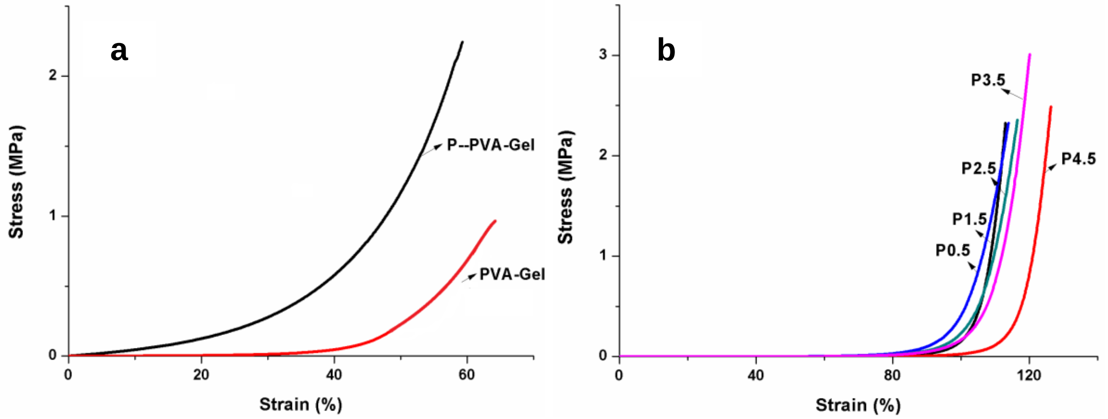


Fig. 5. The compressive stress-strain curves of (a) PVA-Gel and P-PVA-Gel and (b) P0.5-Gel, P1.5-Gel, P2.5-Gel, P3.5-Gel, and P4.5-Gel

To analyze the mechanical properties of the hydrogels, the strain-stress curves in compression were evaluated, as shown in Fig. 5. All hydrogels showed typical “J” shape curves, indicating that they possessed high compressive strength. Compressive strength of PVA hydrogel prepared by freeze-thaw cycles was about 3.5 KPa according to the report by Stauffer (1992). When the same method was used in this study with addition of hemicelluloses and chitin, the strength of hydrogels were about 1.0 to 2.0 MPa, which indicated that the hydrogel with higher strength was obtained when the macromolecules were added. The compressive strength of the hydrogel samples increased incrementally with the applied load at first, and then rose abruptly, indicating that the hydrogels were soft and ductile. The compressive stress of P-PVA-Gel was higher than that of PVA-Gel (Fig. 5a). This result indicated that the P-PVA-Gel possessed a relatively denser and stronger network due to the addition of the PVA with phosphorylation during the freeze-thaw process, and the additional hydroxyl groups, which were due to the presence of phosphates groups, presumably could engage in the hydrogen-bonding formation, resulting in higher compressive strength.

As can be seen in Fig. 5b, the five hydrogel samples exhibited high compression stress of about 2 to 3 MPa. The P3.5-Gel presented the highest compressive stress (3.0 MPa), which was mainly due to the fact that the highest phosphorylation degree of PVA was added into the hydrogel matrix. The more rigid hydrogels were obtained by the formation of hydrogen bonds when the phosphorylation degree of PVA was at 7.7. This was consistent with the result of SEM. There were no significant differences among the other four hydrogels, and all these hydrogels exhibited good deformability and flexibility because of the numerous entanglements and strong physical interactions among the chains of mixed polymers (Zu *et al.* 2012). Because many load-bearing tissues need to frequently exhibit high strength and toughness, tissue engineering scaffolds in these fields require considerable mechanical strength. In this study, all the hydrogels presented high compressive strength; therefore, these hydrogels could be promising biomaterials for load-bearing tissue engineering (Geng *et al.* 2012).

Swelling Properties of Hydrogels

It is well known that compressive strength is consistent with swelling properties. Water absorption of hydrogels relates to their swelling ability, surface area, particle size, and crosslink density (Mohana *et al.* 2002). The swelling degree was determined by a gravimetric method. The equilibrium swelling ratios of the hydrogels with various P-PVA contents are shown in Fig. 6. The equilibrium swelling ratio increased from 15.5 to 22.3 g/g with increasing phosphorylation degree of PVA. The highest equilibrium swelling ratio among the five hydrogels (up to 22.3 g/g) was for P2.5-Gel. The more yielding structure of hydrogel was obtained with a lower degree of phosphorylation of PVA, which resulted in a higher swelling ratio. These results indicated directly that the P-PVA was hydrophilic and could improve the hydrophilicity of the hydrogels.

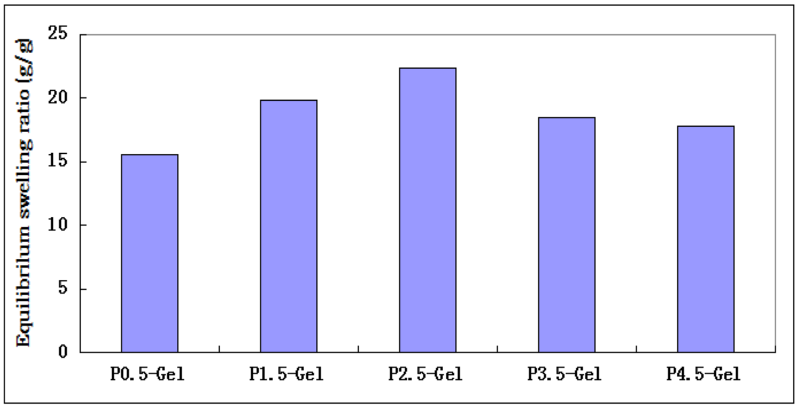


Fig. 6. Equilibrium swelling ratios of P0.5-Gel, P1.5-Gel, P2.5-Gel, P3.5-Gel, and P4.5-Gel

As can be seen from Fig. 1d, f, a honeycomb structure was observed in the P2.5-Gel, but a plate-like structure was present in the P3.5-Gel, which resulted in P2.5-Gel not being able to hold a large quantity of water. The degree of phosphorylation of PVA increased from 4.4 (the dosage of urea was 2.5) to 7.7 (the dosage of urea was 3.5) and led to more chances for the formation of hydrogen bonds with hemicelluloses and chitin nanowhiskers, the building of a stronger structure, and fewer pores among the hydrogel network. Therefore, stronger three-dimensional networks and lower hydrogel swelling ratios were obtained (Kabiri *et al.* 2003).

CONCLUSIONS

1. Phosphatized PVAs of varying degrees were obtained by esterification of phosphoric acid.
2. Hydrogels were successfully prepared with hemicelluloses, PVA or P-PVA, and chitin nanowhiskers by repeated freeze-thaw cycles.
3. The results of SEM analysis confirmed that the addition of hydroxyl groups due to the presence of phosphate groups could engage in hydrogen-bonding formations, resulting in larger pore size. Moreover, the FT-IR results of the hydrogels were consistent with the CP/MAS ^{13}C NMR data, suggesting the formation of hydrogen bonds among the polymers.
4. The presence of more hydroxyl groups significantly enhanced the mechanical properties of the hydrogels but reduced their swelling ratios.

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