Effect of Vanillin on the Miscibility of Poly(vinyl alcohol)/Gelatin Composite Hydrogel: Physical and Chemical Properties

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ABSTRACT: A composite based on poly(vinyl alcohol) (PVA) and gelatin (Gel) was prepared in the form of a miscible interphase by freezing-thawing method using vanillin as a new compatilizer. The chemical and physical properties of PVA/Gel/vanillin composite hydrogels with different reagent concentration, and reaction time were investigated using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), tensile testing, and scanning electron microscopy (SEM). The results elicit that the formation of C=N Schiff base between Gel and vanillin may break the intrahydrogen bonding of Gel. Proper reaction time and more interactive groups result in optimal interhydrogen bonding between modified Gel and PVA so that the miscibility between two polymers has been improved. In addition, the PVA/Gel/V composite with the vanillin concentration of 0.20 g/mL and 2 h stirring exhibits a better compatibility and a higher tensile strength, which can be tailored for potential use in cartilage tissue engineering.
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Key words: poly(vinyl alcohol)/gelatin composite; vanillin; miscibility; thermal behavior

INTRODUCTION

Adult cartilage tissue has poor capability of self-repair, especially in case of severe cartilage damage due to trauma or age-related degeneration.[1] Artificial materials provide an option for the repair of defects in adult cartilage tissue and have been successfully applied in joint prosthesis for many years. As one of the most attractive polymers, poly(vinyl alcohol) (PVA) hydrogel has been studied extensively as a potential cartilage replacement material in previous studies. Many excellent properties of PVA materials such as chemical properties, mechanical properties, and bio-tribological properties have been confirmed.[2] In addition, its viscoelastic character can be adjusted similar to human cartilage by water content in gel production process.[3–7]

It is pointed out that good biocompatibility is important for artificial biomaterials, such as for protein adsorption, cell adhesion, and tissue ingrowth. PVA shows the absence of toxicity, however, the high hydrophilicity of PVA is not favorable for the construction of cell/material composite.[8] To improve the biocompatibility of PVA hydrogels, blends of PVA with different biological macromolecules, such as hyaluronic acid, dextran, and gelatin have been prepared.[9] Among these candidates, gelatin (Gel) shows putative bioadhesive properties to promote cellular growth and attachment.[10] As a natural polymer, Gel is derived from collagen denaturation, of which similar peptide chains maintain functional groups suitable for cell adhesion.[11,12] Therefore, Gel with low immune response and pyrogen-free form was introduced into PVA hydrogels to promote cell adhesion on the material surface in this study.

Previous study on PVA and gelatin blending indicates that the blended films are macroscopically homogeneous. In physical–chemical terms, however, these films do not describe a homogeneous system that destroys the mechanical properties of the blended composite and cannot match for clinical need.[13] PVA is also found immiscible with gelatin because no interactive evidence of the components has been obtained.[14]

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To get homogenous composite with good mechanical properties for clinical applications, a lot of compatilizer have been investigated. For hydrogel materials, chemical crosslinkers, such as formaldehyde or glutaraldehyde, are commonly adopted in the procedure but their safety is still an open question.[10] The presence of residual crosslinkers or unwanted side reaction may create host reactions due to inflammation or toxicity. Vanillin (3-methoxy-4-hydroxy benzaldehyde) is an active reagent, which is widely used in food, pharmaceuticals, and daily chemicals.[15] From a chemical standpoint of view, vanillin has both aldehydic and phenolic groups, and it can create several types of reactions.[16] Study on chitosan crosslinked by vanillin has been reported in some researches,[17] but few studies have been reported on the PVA/Gel, which is reconstructed by vanillin.

In this work, we focused our attention on technical procedures to improve the miscibility of PVA/Gel composite by vanillin crosslinking (PVA/Gel/V). Herein, the effect of reagent concentration and reaction time was investigated to obtain an optimal composite hydrogel. Characterization studies were based on Fourier transform infrared spectroscopy, differential scanning electron microscopy, tensile testing, and scanning electron microscopy.

EXPERIMENTAL

Materials

Vanillin (3-methoxy-4-hydroxy benzaldehyde) (Chengdu Kelong Chemical Reagent Corporation, China, analytical reagent (AR) grade) was dissolved in ethanol at the concentrations of 0.05, 0.10, and 0.20 g/mL. PVA with a polymerization degree of 1799 ± 50 was from Chengdu Kelong Chemical Reagent Corporation, China, AR grade. Gel was from bovine skin (Type B, Sigma). Other reagents used in this research were all AR grade.

Preparation of PVA/Gel/V composites

PVA powder was dissolved in deionized water and continuously stirred for 4 h at 90°C to form a homogeneous viscous solution. The concentration of aqueous PVA solution was 20 wt %. Then the temperature was set to 70°C, and gel was added in and stirred for another 2 h. The mixed viscous solution of PVA and Gel with a dry PVA/Gel weight ratio of 3 : 1 was obtained.

The process of PVA/Gel/V composite was described in Figure 1. Firstly, 1 mL ethanol solution of vanillin was added into 10 g PVA/Gel mixture and stirred at 70°C for 2 h. Subsequently, the modified mixture was poured into a glass container and placed in a commercial freezer regulated at about −20°C. The glass containers with PVA/Gel or PVA/Gel/V composites were repeated freezing-thawing for seven times. In each cycle, the samples were frozen in freezer at about −20°C for 20 h and thawed at room temperature for 4 h, then returned to the freezer for another cycle. Finally, the PVA/Gel or PVA/Gel/V composites were fully washed by deionized water. The final gels were stored in deionized water at 4°C until further use. Table I shows the reaction conditions of PVA/Gel/V with different concentration and reaction time.

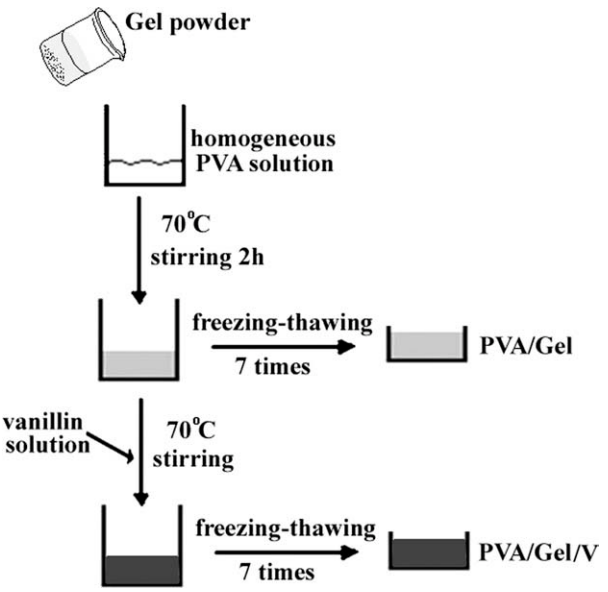

Figure 1 Scheme of preparation procedure of PVA/Gel and PVA/Gel/V composites.

Characterization of PVA/Gel/V composites

The composites were dried in an oven at 50°C for 48 h for chemical properties testing. The FTIR spectra were obtained over a wavenumber range of 500–4000 cm^{-1} using an infrared spectrometer (TENSOR 27, BRUKER Co. Germany). Differential scanning calorimetry (DSC, NETZSCH DSC 204 F1, Germany) was used to test the crystallization degree of PVA in the composites. The samples were sealed in aluminum pans under a nitrogen atmosphere at a heating rate of 10 °C/min. The samples were first heated up to 240°C and held for 2 min and subsequently cooled to −20°C. After that, the samples were reheated to 250°C to record stable thermograms. The melting enthalpy (ΔH_m) of PVA in composites was measured, and the degree of crystallinity (X_c) of PVA was calculated by the equation:

$$X_c = \Delta H_m / \Delta H_m^o$$

where ΔH_m^o is the melting enthalpy of PVA with 100% crystallinity, i.e., $\Delta H_{PVA}^o = 138.6$ J g^{-1}.[18]

TABLE I
Reaction Conditions of PVA/Gel/V

	Variable condition	
Invariable condition	Stirring time	Concentration of vanillin
Stirring time was fixed	2 h	0.05 g/mL
	2 h	0.10 g/mL
	2 h	0.20 g/mL
Concentration of vanillin was fixed	1 h	0.20 g/mL
	2 h	0.20 g/mL
	3 h	0.20 g/mL

The tensile testing of PVA/Gel composites were performed using a universal testing machine (AGIC Shimadzu, Japan) at a rate of 60 mm/min, and the swollen gel samples were cut with a dumb bell-shaped die at the length 11.5 cm, end width 2.5 cm, and neck width 0.6 cm. After freeze-dried, samples were observed with scanning electron microscopy (SEM, JEOL, JEM-100CX, Japan) using an accelerating voltage of 20 kV.

Statistical analysis

Data were presented as means and standard deviations (SD) of at least four samples. The results were analyzed using GraphPad Instat software. Differences between groups of $p < 0.05$ were considered statistically significant.

RESULTS

FTIR analysis

Figure 2 A(a) is the IR spectrum of gelatin. The peak at 1624.76 cm^{-1} represents carbonyl (C=O) stretching vibration of amide I band. The peaks at 1532.08 and 1232.23 cm^{-1} are amide II band and amide III band, respectively. Both of them could be assigned to carbon-nitrogen (C—N) stretching vibration and nitrogen-hydrogen (N—H) bending vibration, which imply the characteristic multi-peak absorption pattern typical of the protein backbone. Figure 2 A(b) is the IR spectrum of PVA. In PVA/Gel [Fig. 2A(c)], the characteristic peaks of amide I band and amide II band shift slightly, moving to 1635.66 and 1542.98 cm^{-1}.

Figure 2(B) shows spectra of PVA/Gel/V composites with different vanillin concentration. Figure 2B(a) is the spectrum of PVA/Gel. It can be seen from Figure 2(B) that some peaks shift apparently. After addition of vanillin, the peak at 1542.98 cm^{-1} of PVA/Gel disappears, and the newly appeared band at 1586.26 cm^{-1} of PVA/Gel/V composites in Figure 2(B)(c,d) suggests the vibration of C=N on Schiff base. With the increase of vanillin, the characteristic peak of amide I band at 1635.66 cm^{-1} in Figure 2(B)(a) shifts to higher wavenumber in Figure 2(B). The benzene ring absorption peaks at 1508.62 cm^{-1} of vanillin appear in composite as shown in Figure 2(B)(c,d), and the peaks are strengthened with the increase of vanillin addition. The hydroxyl band of vanillin can be observed at 1283.60 cm^{-1} in Figure 2(B)(c) and at 1287.34 cm^{-1} in Figure 2(B)(d).

When the concentration of vanillin is fixed at 0.20 g/mL, some characteristic peaks of PVA/Gel/V composite change with the stirring time [Fig. 2(C)]. The characteristic peak of amide I band at 1635.66 cm^{-1} in Figure 2A(c) shifts to higher wavenumber of 1660.99 cm^{-1} in Figure 2(C)(a,b) and to 1657.25 cm^{-1} in Figure 2(C)(c). The characteristic absorption bands of benzene ring are present in all spectra of Figure 2(C). The C=N vibration of Schiff base at 1582.52 cm^{-1} in Figure 2(C)(a) moves to 1586.26 cm^{-1} in Figure 2(C)(b,c) with the time increase.

DSC measurements

Figure 3 is the DSC curves of PVA/Gel and PVA/Gel/V composites. It can be seen in Figure 3A(a) that there is a melting peak at 229.6°C in the curve of PVA/Gel, and the melting peaks are associated with the crystalline fraction of PVA according to previous research.[13] In Figure 3(A)(b–d), only one melting peak can be observed, and the melting temperature decreases with the increase of vanillin concentration.

Figure 3B shows that when the concentration of vanillin is fixed at 0.20 mg/mL, the stirring time affects the thermal behavior of PVA/Gel/V composite slightly. The melting peak at 195.1°C drops to 192.0°C when stirring for 2 h and then rises to 194.9°C when stirring for 3 h.

The melting enthalpy and crystallinity of PVA in different composites are shown in Table II. The data show that when the concentration of vanillin increases from 0.05 to 0.10 mg/mL and 0.20 mg/mL, both the melting enthalpy and the crystallinity of PVA in different composites represent a decrease trend. That is, the melting enthalpy ΔH_{PVA} decreases from 26.33 to 18.84 J g^{-1} and 11.17 J $g^{-1}$and the crystallinity (X_c value) changes from 19.00 to 13.59% and 8.06%, respectively. When the concentration of vanillin is kept at 0.20 mg/mL, the melting enthalpy ΔH_{PVA} first decreases from 14.01 to 11.17 J g^{-1} then increases to 14.04 J g^{-1} with the increase of stirring time from 1 to 3 h. Meanwhile, the X_c value shows a similar trend, from 10.11% drops to 8.06% then increases to 10.13%.

The results indicate that the concentration of vanillin significantly influences the property of PVA/Gel/V composite.

Tensile strength

Effects of vanillin concentration and the reaction time on tensile strength of composites are shown in

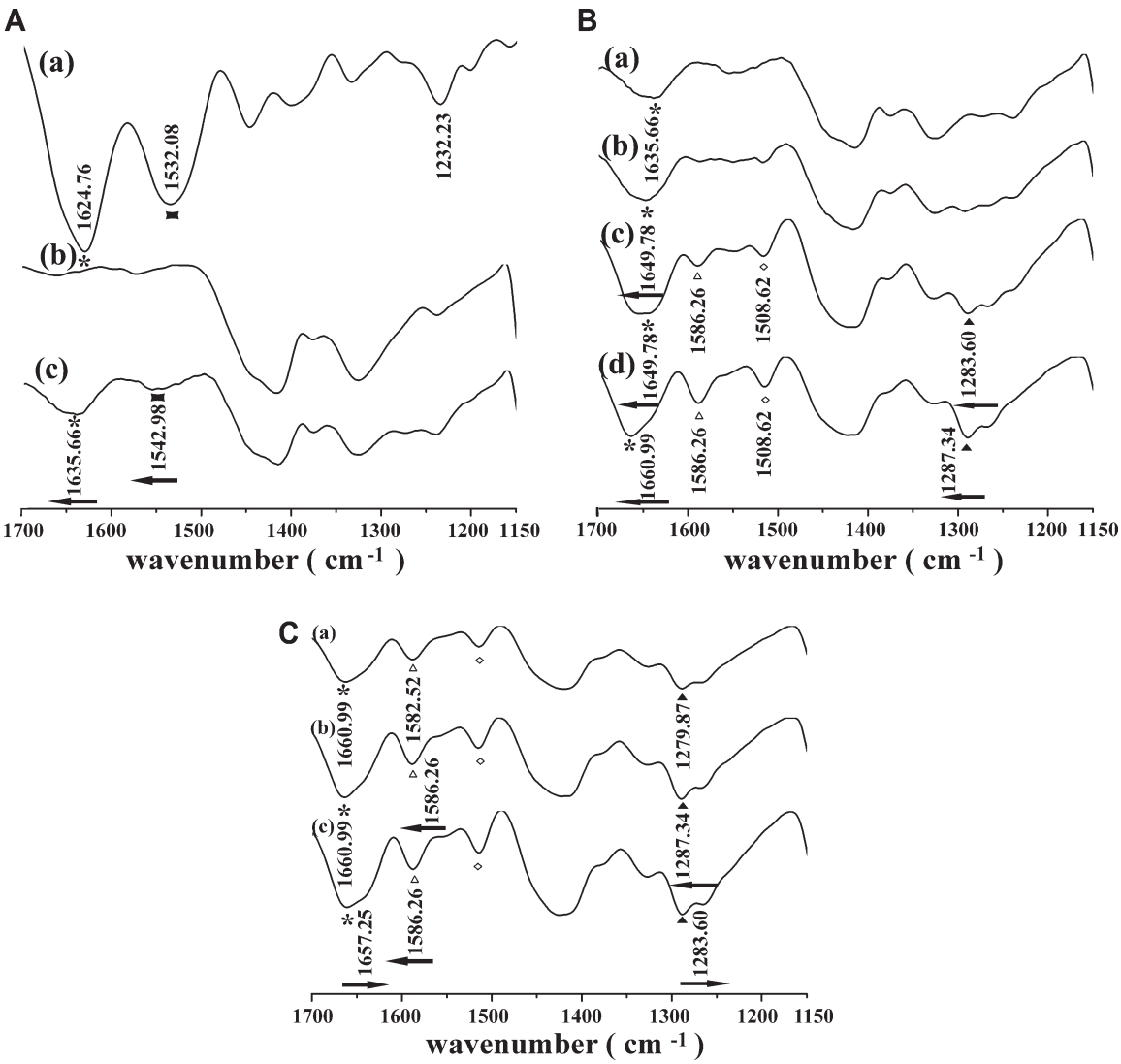

Figure 2 IR spectra of PVA/Gel and PVA/Gel/V composites. **A:** IR spectra of (a) Gel, (b) PVA, (c) PVA/Gel; **B**: IR spectra of PVA/Gel/V with different vanillin concentration (a) 0 g/mL, (b) 0.05 g/mL, (c) 0.10 g/mL, (d) 0.20 g/mL; **C:** IR spectra of PVA/Gel/V stirred with different time, (a) 1 h, (b) 2 h, (c) 3 h *: amide I band, ■: amide II band, △: C=N vibration of Schiff base, ▲: hydroxyl peak of vanillin, ◇: peaks of benzene ring.

Figure 4. The tensile strength of PVA/Gel is 1.28 ± 0.19 MPa [Fig. 4A(a)]. With the increase of vanillin concentration, the tensile strength increases as shown in Figure 4A(b, c, d). Until vanillin concentration is 0.20 mg/mL, the strength data (1.63 ± 0.21 MPa) has a significant difference with the composite without vanillin. When the concentration of vanillin is stable (0.20 g/mL), the tensile strength of composites first increase, then decrease with the stirring time as indicated in Figure 4(B).

SEM observation

The morphologies of PVA/Gel and PVA/Gel/V composites are presented in Figure 5. The photographs show that PVA forms a continuous matrix in which Gel disperses. In Figure 5(a), Gel granules disperse in PVA matrix with different size ranging from 100 to 500 μm. The distinct loose interface between PVA and Gel exhibits a poor compatibility of them [Fig. 5(c)]. However, the addition of vanillin has apparently changed the Gel dispersion in PVA matrix [Fig. 5(b)]. The Gel particles with similar small size embed uniformly in PVA hydrogel matrix, as shown in Figure 5(d).

DISCUSSION

In the PVA/Gel system, a competitive relationship exists between the break of self-bonding in single polymer and the formation of interbonding between the two polymers.[19] When the interbonding of two polymers predominates over the self-bonding of each polymer, a miscible system can be achieved.

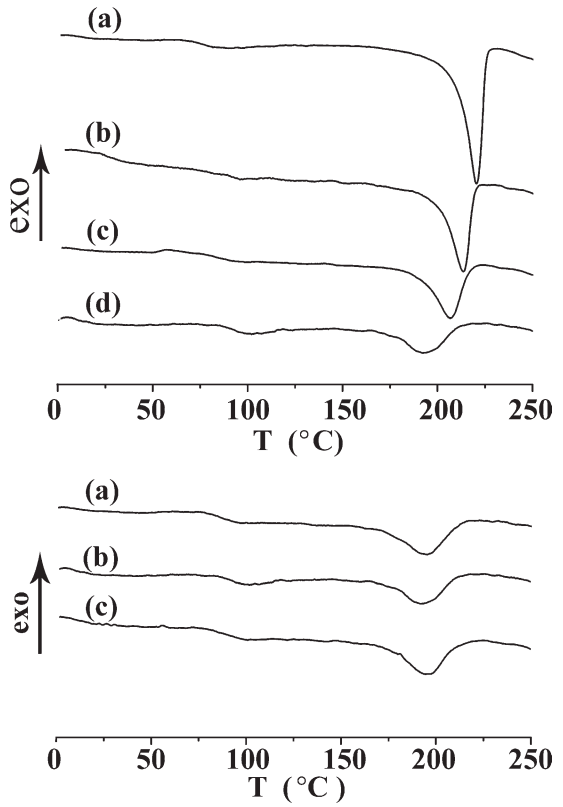

Figure 3 DSC melting curves of PVA/Gel and PVA/Gel/V composites. **A:** DSC curves of PVA/Gel and PVA/Gel/V composites with different concentration of vanillin stirred for 2 h (a) 0; (b) 0.05 g/mL; (c) 0.10 g/mL and (d) 0.20 g/mL; **B:** DSC curves of PVA/Gel/V composites (V: 0.20 g/mL) stirred at different time: (a) 1 h, (b) 2 h, (c) 3 h.

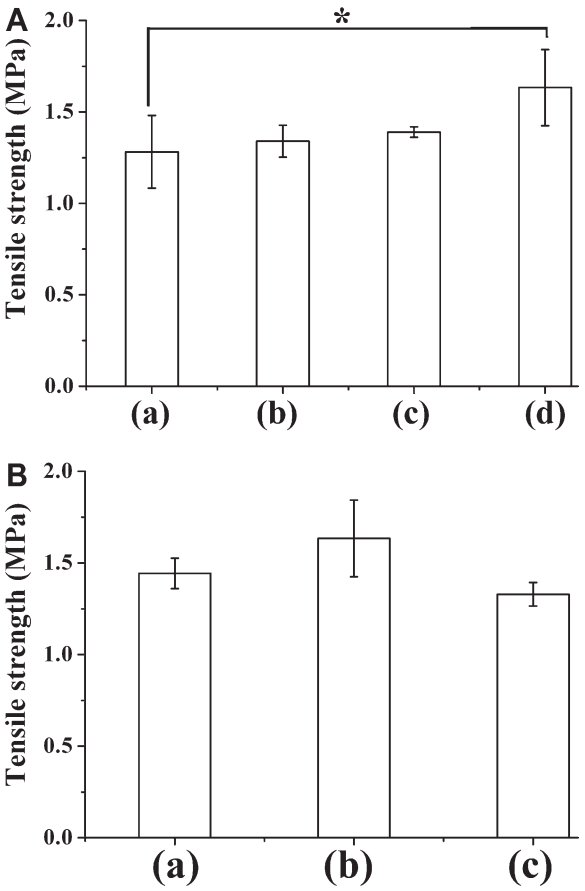

Figure 4 Tensile strength of PVA/Gel/V composites. Error bars represent means ± SD for $n = 4$, $^*P < 0.05$. **A:** PVA/Gel/V composites with different concentration of vanillin stirred for 2 h: (a) 0 g/mL; (b) 0.05 g/mL; (c) 0.10 g/mL; and (d) 0.20 g/mL; **B:** PVA/Gel/V composites stirred at different time with vanillin concentration of 0.20 g/mL: (a) 1 h, (b) 2 h, (c) 3 h.

However, Gel is a kind of protein, in which amino acids with different polarity are connected by peptide bonding. In aqueous solution, proportional amino-acid residues of Gel have a lot of apolar side chains and do not exhibit the ability and preference to react with water or other polar groups. From thermodynamic point of view, the hydrophobic interaction caused by apolar groups is an endothermic process. In our study, the mixing temperature was set at 70°C. Under such a high temperature for protein, these apolar side chains bring on molecular conglomeration and then dominate the tertiary structure of protein and its solubility in hydrogel. It is hard to

TABLE II
The Melting Enthalpy and Crystallinity of PVA in Different Composites

Sample		T_m [°C]	ΔH_{PVA} [J· g^{-1}]	X_c [%]	T_g
PVA		229.6	54.66	39.44	
Gel		—	—	—	
Concentration of vanillin (mg/mL)					
A					
(a)	0	220.1	37.95	27.38	70.3
(b)	0.05	213.2	26.33	19.00	—
(c)	0.10	206.2	18.84	13.59	83.0
(d)	0.20	192.0	11.17	8.06	83.8
Stirring time (h)					
B					
(a)	1	195.1	14.01	10.11	82.0
(b)	2	192.0	11.17	8.06	83.8
(c)	3	194.9	14.04	10.13	83.0

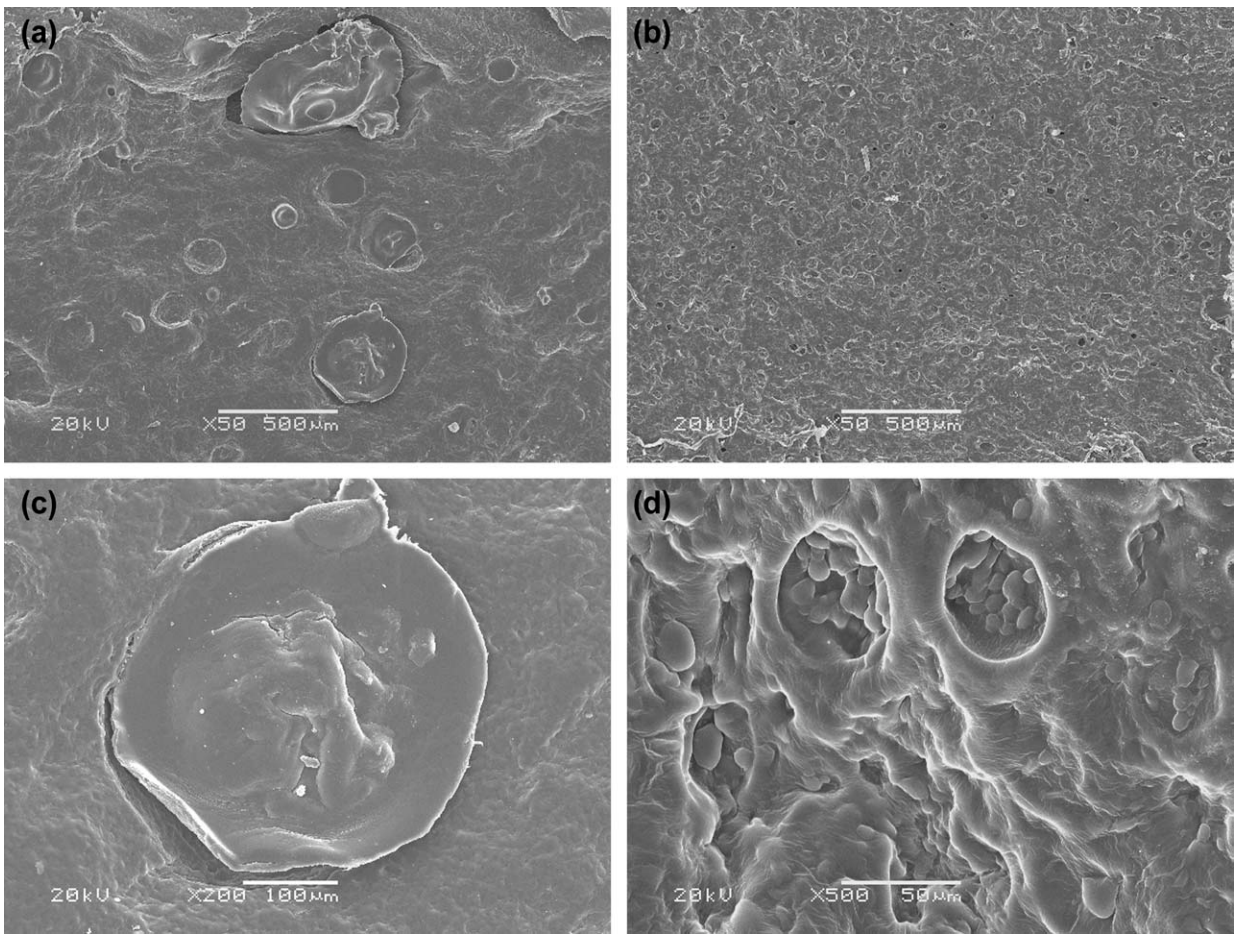

Figure 5 SEM photos of PVA/Gel (a, c) and PVA/Gel/V (0.20 g/mL, 2 h) (b, d).

break the self-bonding by simple mixing. Therefore, agglomerated large Gel granules have been observed in SEM micrographs.

Optimal intermolecular bonding could improve the miscibility of two incompatible polymers.[20] Vanillin, a new-style and safe compatilizer, was used in this study to ameliorate the miscibility of PVA and Gel components. As can be seen from the IR spectra of composites after vanillin added, new chemically bonding bands emerge and some polar bands change apparently. The emergence of benzene ring bands in PVA/Gel/V composite means the vanillin was compounded into the PVA/Gel composite. Newly formed C=N vibration of Schiff base at 1586.26 cm^{-1} provides an evidence for the compatibilization of Gel interaction to vanillin. The possible reaction is that the aldehyde groups of vanillin react with the free amino groups of Gel, resulting in the formation of Schiff base, as shown in Figure 6. The reactive process grafted hydroxyl group as a polar side chain into Gel molecule so that intramolecular apolar conglomeration became difficult. Besides, when vanillin is added, the peaks of amid I of Gel and hydroxyl of vanillin shift to higher wavenumber, indicating that the addition of vanillin influences the carbonyl (C=O) stretching vibration of Gel and disrupts the self-hydrogen-bond of amide group of the Gel. So the introduction of hydroxyl to

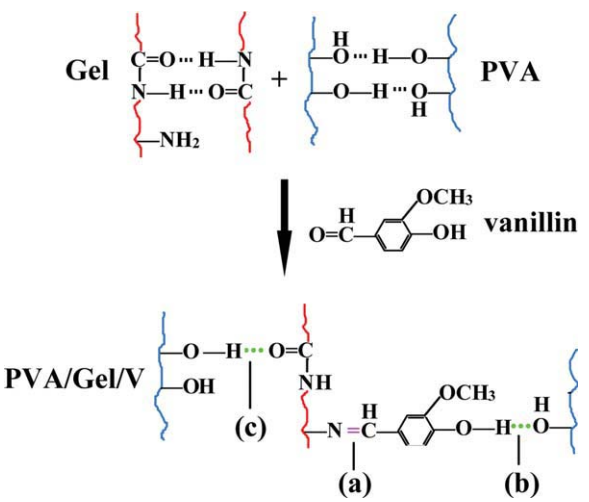

Figure 6 Scheme of interaction in PVA/Gel/V. "..." denoting hydrogen bond. (a) formation of Shiff base, (b) hydrogen bond between hydroxyls of PVA and vanillin, (c) hydrogen bond between carbonyl of Gel and hydroxyl of PVA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

protein accelerates the potential formation of hydrogen bond with PVA chains too. The presence of more than one T_g observed in the thermogram is an evidence of phase separation, whereas the detection of one T_g is not sufficient to conclude the presence of a single homogeneous phase.[21] In this study, only one T_g exist. The SEM photos in Figure 5(b,d) exhibit a uniform microstructure of PVA/Gel/V composite. Small size granules disperse in PVA matrix uniformly and the interphase between PVA and Gel has been largely improved.

According to the principle of structure reconstruction, the low temperature peak of DSC curve is the melting peak of imperfect crystallization in annealing process, and the high temperature peak is the peak of perfect crystallization during further temperature-rise period.[22] The introduction of Gel destroyed the intrahydrogen bonding of PVA crystal molecular chain and the crystallinity (X_c) decreased from 39.44 to 27.38%. With the addition of vanillin more inter and/or intrabondings are produced. Increasing vanillin concentration would also lead to more crosslinking and more disruption of self-association. As a result, the crystallinity of PVA decreased progressively, and the tensile strength of composite increased with the vanillin concentration.

Stirring time reflects reaction or mixing time. The influence of stirring time on PVA/Gel/V composite is also achieved by the formation of chemical bonding. Both the strengthening of C=N vibration of Schiff base in IR spectra, and the appearance of melting peak in DSC curves visualize the influence. A relative long time brings more chemical reactions in mixing process. As expected, the miscibility of composite has been improved by prolonged time. But the formation of interbonding and intrabonding is a competitive dynamic process, prolonged stirring/reaction time will lead the formation of intrabonding and also reduce the crosslinking. As a result, the crystallinity increased and the tensile strength of the composite decreased when stirring for 3 h compared with other composites of stirring for 1 or 2 h.

CONCLUSION

This study introduced vanillin as the compatilizer to improve the miscibility of PVA/Gel composite. The formation of C=N Schiff base between Gel and vanillin and interhydrogen bonding of PVA and modified Gel produce a miscible interphase to affect the microstructure and properties of the composite. The compounded PVA/Gel/V hydrogel with 0.20 g/mL vanillin compatilizer and stirring for 2 h exhibits a better compatibility and a higher tensile strength compared with other process conditions. More vanillin could make a miscible structure but overlong mixing time would damage the crystallinity and tensile strength of composite. The biological properties of PVA/Gel/V composite hydrogel are expected to be investigated in the future.

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