

STABILIZATION OF ELECTROSPUN POLY(VINYL ALCOHOL) NANOFIBROUS MATS IN AQUEOUS SOLUTIONS*

Sheng-guang Cao, Zhao-fang Liu, Bing-huan Hu and Hai-qing Liu**
College of Chemistry and Materials Science, Fujian Normal University, Fuzhou 350007, China
Fujian Key Laboratory of Polymer Materials, Fuzhou 350007, China

Abstract The stability of poly(vinyl alcohol) (PVA) nanofibrous mats in water media was improved by post-electrospinning treatments. Bifunctional glutaraldehyde (GA) in methanol was used as a crosslinking agent to stabilize PVA nanofiber, but fiber twinning was observed frequently, and the highly porous structure of PVA nanofibrous mats was destroyed when the crosslinked fiber was soaked in water. To overcome this shortcoming, chitosan (CS) was introduced into the PVA spinning solution to prepare PVA/CS composite nanofibers. Their treatment in GA/methanol solution could retain the fiber morphology of PVA/CS nanofibers and porous structure of PVA/CS nanofibrous mats even if they were soaked in aqueous solutions for 1 month. Scanning electron microscopy (SEM), X-ray diffraction (XRD), thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were applied to characterize the physicochemical structure and thermal properties of PVA nanofibers. It was found that the water resistance of PVA nanofibrous mats was enhanced because of the improvement of the degree of crosslinking and crystallinity in the electrospun PVA fibers after soaking in GA/methanol solution.

Keywords: Electrospinning; Polyvinyl alcohol; Chitosan; Nanofiber.

INTRODUCTION

Poly(vinyl alcohol) (PVA) has been a promising functional material due to its good chemical stability, hydrophilicity, biocompatibility and nontoxicity^[1–3]. PVA nanofibers, with diameter down to several hundred nanometers or even several tens of nanometer, have attracted many research interests for potential biomedical applications. However, the water solubility of PVA limits its application in the aqueous medium. Thus the first priority is to render water insolubility to PVA materials. The chemical crosslinking through acetal ring formation^[4, 5] or photo-induced crosslinking^[6] is successfully used in the preparation of water-insoluble PVA materials. The immersion in non-solvent such as methanol^[7] or ethanol^[8] was reported to be another effective crosslinking method in introducing more crystallites, which acted as physical cross-links for the PVA.

Due to the high specific surface areas and ultrafine diameter, PVA nanofibers swiftly swell to merge together and to lose their fibrous morphology in the post-electrospun treatment^[9]. Moreover, the highly porous structure of PVA nanofibrous mats, which is pivotal to the cell proliferation when it is utilized as a tissue engineering scaffold, is readily lost after crosslinking treatment^[7, 10]. As a consequence, the traditional crosslinking methods for PVA films can not serve well for PVA nanofibrous mats. From literatures and our own experience, PVA nanofibers hardly retain their fiber morphology and porous structure by treating with a single chemical or physical crosslinking method^[10, 11]. It is speculated that simultaneous chemical and physical

* This work was financially supported by the National Natural Science Foundation of China (Nos. 50973019, 50843030) and the Key Project of Natural Science Foundation of Fujian Province (No. E0620001).

** Corresponding author: Hai-qing Liu (刘海清), E-mail: haiqing.liu@gmail.com

Received September 28, 2009; Revised December 21, 2009; Accepted December 30, 2009
doi: 10.1007/s10118-010-9140-8

crosslinking reactions would more effectively stabilize PVA nanofibrous structure and porous character. In this work, glutaraldehyde (GA)/methanol was used as the treating medium for the PVA nanofibrous mats. GA acts as a chemical crosslinking agent for PVA, while methanol induces the formation of crystallites, which act as physical cross-links. To further enhance the dimensional stability of PVA nanofibrous mats in aqueous medium with no loss of biocompatibility, semi-rigid biocompatible chitosan (CS) was introduced to PVA to make PVA/CS composite nanofibers. The PVA/CS composite nanofibers were further treated in GA/methanol solution to make water stable nanofibers. The morphology of PVA and PVA/CS composite nanofibers was studied. Their stability against aqueous medium was examined.

EXPERIMENTAL

Materials

Chitosan (CS) (deacetylation > 90%) was bought from Jinan Haidebei Marine Bioengineer Co., Shangdong, China. Poly(vinyl alcohol) (PVA) ($M_w = 1750 \pm 50$, hydrolysis degree > 99%), was from commercial market in China. Glutaraldehyde (GA) (25% aq. solution), and glacial acetic acid were from Sinopharm Chemical Regent, Shanghai, China.

Preparation of PVA and PVA/CS Nanofibers through Electrospinning

A 9 wt% PVA solution was prepared by dissolving PVA in distilled water. Pre-calculated amount of PVA and CS were dissolved in 9 vol% acetic acid aqueous solution with a total polymer concentration of 9 wt%. The weight ratio of PVA/CS in the mixture was 9:1 and 8:2. The spinning solutions were transparent and degassed before electrospinning, and then filled into a syringe with a stainless needle of gauge 16. An electrode was clamped on the needle and connected to a power supply (DW-P303-IAC, Tianjin Dongwen High Voltage Plant, Tianjin, China). The grounded counter electrode was connected to a collector aluminum (Al) foil. The electrospinning conditions of voltage of 20–25 kV and tip-to-collector distance of 11.5–16 cm were applied. The nanofibrous mats on Al foil were dried in a vacuum oven at 100°C for 10 h. As-obtained nanofibrous mats were coded as PVA, PVA/CS91, and PVA/CS82.

Stabilization of PVA and PVA/CS Nanofibrous Mats

The PVA and PVA/CS nanofibrous mats were treated in two solutions, (1) 1.0 vol% GA/H₂O solution with 0.1 vol% HCl, and (2) 1.0% GA/methanol solution with 0.1 vol% HCl. After soaked in the solutions for 2 and 4 h, the mats were taken out and dried in a vacuum oven at 40°C for 4 h. They were then rinsed with methanol to remove residual GA and HCl.

Characterization

The Morphology of nanofibrous mats were observed on a scanning electron microscope (SEM) (JSM-7500F, JEOL). All samples were coated with platinum before observation. The nanofiber diameters were measured with image analyzer software Smileview. The average diameter was obtained by measuring at least 35 individual nanofibers. Fourier transform infrared (FT-IR) spectra of nanofibers were collected on a Nicolet Avatar 360 spectrometer (Nicolet, USA) in KBr pellet form. The crystalline structure was analyzed on a wide-angle X-ray diffractometer (X'pert MPD, Philip) using CuK α radiation ($\lambda = 0.154$ nm). The thermal stability was recorded on a thermal gravimetric analyzer (TGA) (TGA/SDTA 851e, Mettler Toledo) in N₂ atmosphere at a heating rate of 10 K/min in the range from 30°C to 600°C. The melting temperature and enthalpy of nanofibers were performed on a differential scanning calorimeter (DSC) (DSC822e, Mettler-Toledo). To eliminate the thermal history, samples were heated from 25°C to 130°C at a heating rate of 15 K/min, then cooled to 25°C. In the second scan, samples were heated from 25°C to 230°C at a heating rate of 10 K/min, and the thermograms were recorded. All the DSC measurements were run under N₂ atmosphere.

RESULTS AND DISCUSSION

Stabilization of PVA Nanofibers in the Presence of GA

The SEM images of the electrospun PVA nanofibrous mats before and after treatment are shown in Fig. 1. The original PVA nanofibers are round and smooth with average diameter of (377 ± 42) nm (Fig. 1a). After exposure in GA/H₂O solution, PVA fiber morphology disappeared completely and a film of rough topography was formed (Fig. 1b). Yao *et al.* found that the immersion of PVA nanofibers in water for 1 h turned them into a PVA film^[7]. With the high surface areas and ultrafine diameter, PVA nanofibers swiftly swell and stick together in aqueous solution even with the presence of crosslinking agent GA.

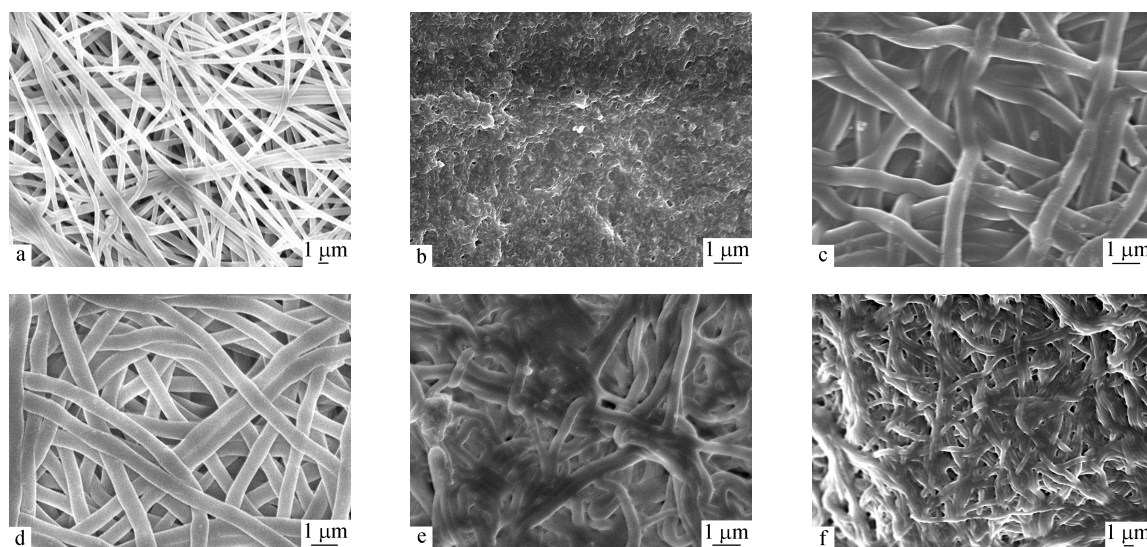


Fig. 1 SEM images of (a) untreated PVA nanofibers, (b) PVA nanofibers treated in 1.0% GA/water, (c) PVA nanofibers treated in 1.0% GA/methanol for 2 h, (d) PVA nanofibers treated in 1.0% GA/methanol for 4 h, (e) PVA nanofibers in (c) after soaked in water for 24 h, (f) PVA nanofibers in (d) after soaked in water for 24 h

After treatment in GA/methanol solution for 2 h, PVA fibrous morphology is retained to large extent, but its mean fiber diameter increases to (606 ± 48) nm. Fibers are more densely packed, and many adjacent fibers twine together (Fig. 1c). It is reported that the GA crosslinked PVA nanofibers without sufficient crosslinking density are still swellable in methanol^[12], resulting in the increasing of fiber diameter and fiber conglutination. For the PVA nanofibers treated in GA/methanol solution for 4 h, their round and smooth fiber structure is kept very well, with fiber diameter of (503 ± 41) nm. Fiber twining and adherence are much less frequent than that treated for 2 h. Porous structure among fibers is well retained (Fig. 1d). This is closely associated with the high crosslinking density in the PVA nanofibers. Prolonging the soaking time in GA/methanol solution is believed to allow more crosslinking reactions between the aldehyde groups of GA and the hydroxyl groups of PVA. The dense crosslinking helps stable the PVA fiber morphology and pore structure.

The crosslinked PVA nanofibrous mats shown in Figs. 1(c) and 1(d) were further soaked in water for 24 h at room temperature. Their morphologies are shown in Figs. 1(e) and 1(f). It can be seen that they still show fiber structure, indicating that effective crosslinking reaction through the formation of acetal ring took place between the hydroxyl groups of PVA and aldehyde groups of GA. However, PVA nanofibers were heavily twinned, and densely packed. Additionally, the porosity among fibers is substantially poorer than that of the PVA nanofibrous mats shown in Figs. 1(c) and 1(d). These results suggest that crosslinked PVA nanofibers are still readily swollen in water. This is possibly attributable to the flexible PVA molecular chain and to the insufficient crosslinking density in the PVA nanofiber.

It has been reported that the immersion of PVA nanofibrous mats in non-solvent such as methanol and

ethanol can render them water insolubility^[7, 8]. This is because the alcohol treatment increases the degree of crystallinity, which acts as physical cross-links in PVA nanofibers. In our lab, PVA nanofibrous mats were also treated with absolute methanol for 10 h, then immersed in water. We found that as-obtained PVA nanofibrous mats could not retain the fiber structure after 2-hour-immersion. Yi *et al.*^[11] also confirm this phenomenon in their research. Comparing to the single physical crosslinking method, the combination of physical and chemical crosslinking of PVA nanofibers treated in GA/methanol shown in this work is obviously more effective in stabilizing them against disintegration in water.

Stabilization of PVA Nanofibers by Compositing with Chitosan

Though PVA nanofiber morphology can be retained to large extent after being treated with 1.0% GA/methanol solution, the porous structure of PVA nanofibrous mats was destroyed significantly upon immersion in water (Figs. 1e, 1f). Many researches have pointed out that the porous structure of tissue engineering scaffold is of great importance to the cell proliferation^[13]. In order to keep the fiber morphology and pore structure of PVA nanofibrous mat, PVA/CS composite nanofibers were developed.

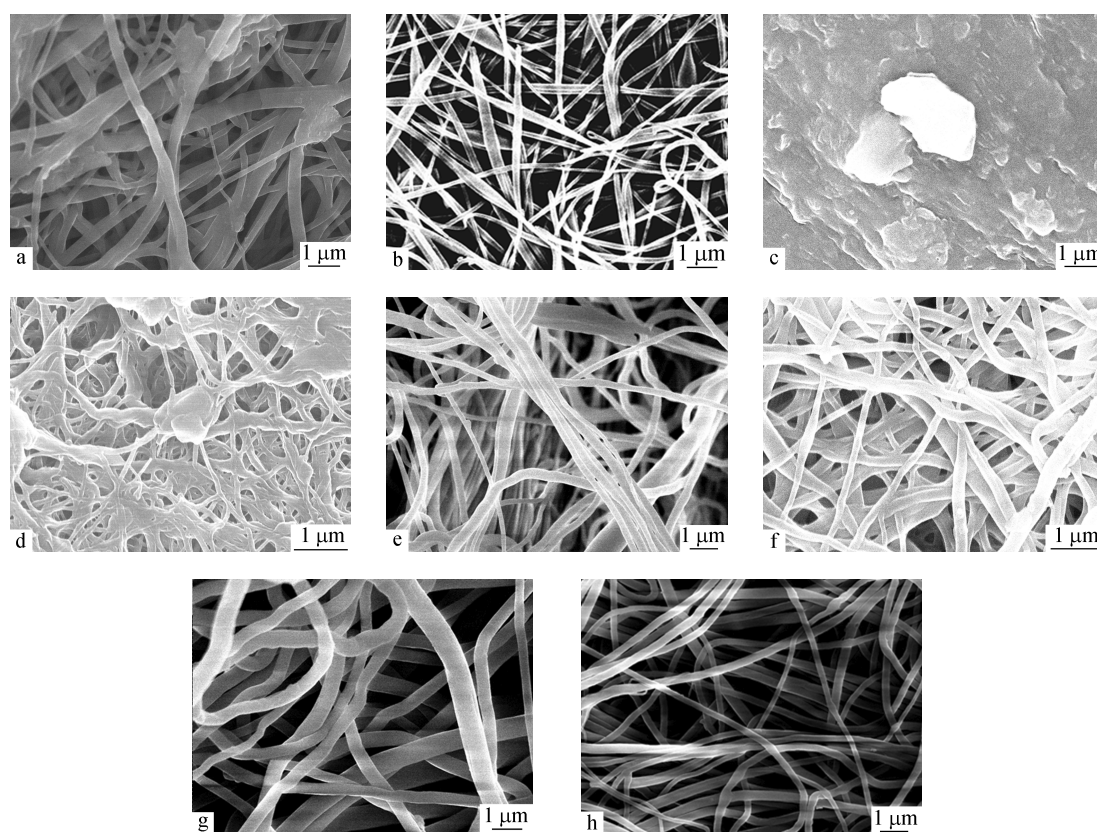


Fig. 2 SEM images of (a) untreated PVA/CS91 nanofibers, (b) untreated PVA/CS82 nanofibers, (c) PVA/CS91 nanofibers treated in 1.0% GA/H₂O for 24 h, (d) PVA/CS82 nanofibers treated in 1.0% GA/H₂O for 24 h, (e) PVA/CS82 nanofibers treated in 1.0% GA/methanol for 24 h, PVA/CS82 nanofibers in (e) soaked in buffer solutions with (f) pH 4, (g) pH 7, (h) pH 9 for 4 weeks

The morphology of original PVA/CS91 and PVA/CS82 composite nanofibers is shown in Figs. 2(a) and 2(b), respectively. Both fibers present irregular morphology and broad fiber size distribution. The immersion of PVA/CS91 nanofibers in 1.0% GA aqueous solution caused the loss of fiber structure due largely to the readily swelling of PVA (Fig. 2c). This phenomenon is similar to that of PVA nanofibrous mat treated in the same solution (Fig. 1b). However, by increasing the content of CS in the PVA/CS composite nanofibers to 20%, the

treatment of PVA/CS82 in 1.0% GA aqueous solution did not disintegrate the fiber structure, though large amount of fibers adhere together (Fig. 2d). Therefore PVA/CS82 showed better water resistant than PVA/CS91.

The water-soaking of PVA/CS82 nanofibers treated by GA/methanol technique showed little effect on their fiber morphology (Fig. 2e). Round and smooth fiber morphology was kept intact, fiber twinning was hardly observed, and the pore structure among fibers remained. Furthermore, such obtained PVA/CS82 nanofibers are stable in buffer solutions with a broad range of pH 4–9 for 4 weeks (Figs. 2f–2h). Comparing to pure PVA nanofibers, the containing of 20% CS in the PVA/CS composite nanofibers obviously enhances their water-resistance.

Chitosan (CS) is a biocompatible, biodegradable and water insoluble natural polymer^[14]. Its application as one component to modify PVA nanofibers does not lower its biocompatibility, and can substantially retain fiber morphology and pore structure in aqueous media. Therefore, the compositing of PVA with CS, in addition to GA/methanol thermal crosslinking method, is a very effective technique to prepare PVA based scaffolds for tissue engineering.

The degree of crystallinity of PVA/CS82 nanofibrous mats before and after immersion in the GA/methanol solution changed significantly as shown in their XRD patterns Fig. 3. The reflection peak at $2\theta = 20^\circ$ is attributed to the crystal structure of PVA (19.8°) and CS (20.1°)^[15]. The intensity for the treated PVA/CS82 increased greatly. The area under the peak at *ca.* 20.1° of PVA/CS82 was 2.6 times more than that of PVA, indicating that the crystallinity was enhanced. It is reported that methanol or ethanol treatment can effectively increase the degree of crystallinity of electrospun PVA nanofibers^[2, 8]. It is thought that this phenomenon is induced by the replacement of PVA-water hydrogen bonding by intermolecular polymer hydrogen bonding following the removal of residual water within the fibers by methanol or ethanol. These crystallites, acting as physical cross-links, make the methanol treated PVA nanofibrous mats more stable in water. In view of the simultaneous chemical crosslinking reaction during the treatment, thus it is speculated that the crosslinking and improvement of crystallization of PVA/CS82 contributed to the stability in an aqueous environment.

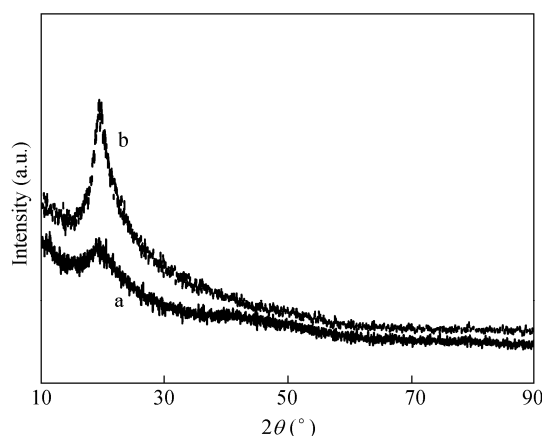


Fig. 3 X-ray diffraction patterns of PVA/CS82 (a) before and (b) after treatment in GA/methanol solution

The DSC thermograms of PVA and PVA/CS nanofibers are shown in Fig. 4. The $T_{m, \text{peak}}$, melting enthalpy (ΔH_m) were summarized in Table 1. For the PVA nanofibers treated in GA/methanol, the $T_{m, \text{peak}}$ increased by 2°C to 192.9°C , as compared to 190.9°C for the untreated PVA nanofibers. Moreover, the melting enthalpy of the treated PVA nanofibers substantially increased to 51.20 J/g from 39.02 J/g of the untreated ones. This indicates that the crystallinity of the treated PVA nanofibers was increased by 31.2%. Similar phenomenon was found for the PVA/CS composite nanofibers. For the treated PVA/CS82 composite nanofibers, its $T_{m, \text{peak}}$ was increased to 193.5°C from 191.6°C of the original PVA/CS82 nanofibers, and its ΔH_m increased to 45.38 J/g from 26.33 J/g of the original PVA/CS82 nanofibers. These findings confirm that immersion of PVA nanofibers

in methanol media can induce the formation of crystallites. This is in accordance with the XRD result shown in Fig. 3. It should be pointed out that the ΔH_m of PVA/CS82 is less than that of PVA nanofibers. This is mainly because the component CS in the PVA/CS composite disturbs the hydrogen bonding interactions among PVA molecular chains, while these interactions are the driving force for the formation of PVA crystallites.

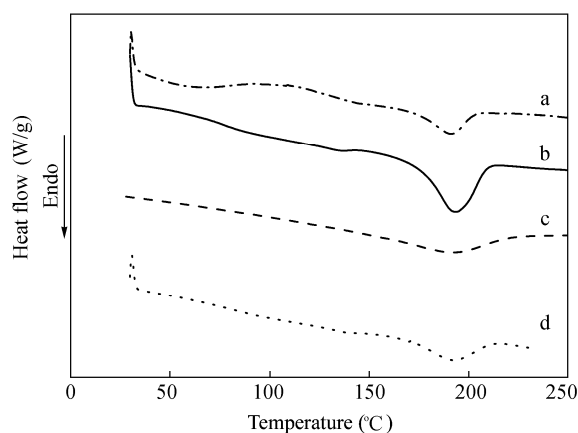


Fig. 4 DSC thermograms of nanofibers

a) PVA; b) Treated PVA in GA/methanol; c) PVA/CS82; d) PVA/CS82 treated in GA/methanol

Table 1. T_m at peak, melting enthalpy (ΔH_m) of PVA and PVA/CS82 nanofibers

Nanofiber	$T_{m, peak}$ (°C)	ΔH_m (J/g)
PVA fiber	190.9	39.02
PVA fiber treated in GA/methanol	192.9	51.20
PVA/CS82 fiber	191.6	26.33
PVA/CS82 fiber treated in GA/methanol	193.5	45.38

The thermal gravimetric analyses of PVA and PVA/CS82 nanofibers are shown in Fig. 5. The initial 5% weight loss below 100°C was caused by the evaporation of moisture absorbed in the nanofibrous mats. Comparing to the thermogram of PVA nanofibers (Fig. 5a), the thermal stability of untreated PVA/CS82 nanofibers was obviously enhanced (Fig. 5b), and it was further increased for the treated PVA/CS82 nanofibers in GA/methanol solution (Fig. 5c). For instance, the decomposition setpoint was 228°C for PVA, 250°C for PVA/CS82 before treatment and increased by 40°C to 290°C for the treated PVA/CS82. The temperature for 50% weight loss was respectively 233°C, 320°C and 350°C for PVA, PVA/CS82 before and after treatment. In the PVA/CS82 composite nanofibers, the component CS possesses a higher thermal decomposition temperature at 280°C^[16] than that of PVA, resulting in the stronger thermal stability of PVA/CS82 nanofibers. As for the further enhanced thermal stability of the treated PVA/CS82, the key factor should be the formation of crosslinking networks among the macromolecular chains.

Chemical Crosslinking Mechanism for the Stabilization of PVA Nanofibers

The PVA, especially PVA/CS nanofibrous mats treated in GA/methanol can retain their fiber morphology and porous structure upon immersion in water for relatively long period. This is largely due to the dense chemical and physical cross-links in the PVA nanofibers. As for the chemical crosslinking, acetal rings^[17] and Schiff base^[18] are formed as a result of the readily reaction between hydroxyl groups of PVA, amino groups of chitosan and aldehyde groups of GA, respectively (Fig. 6). Crosslinked PVA molecular chains are still flexible^[12], so they can swell in water and form densely packed nanofibrous mats (Figs. 1e, 1f), whose porosity is substantially lower than the original PVA nanofibrous mats. In contrast, the PVA/CS82 composite nanofibrous mats show better capability in preserving their fiber morphology and porous structure (Figs. 3e–3h). This is because the component chitosan has semi-rigid molecular chains. Upon acetal ring and Schiff base crosslinking reactions

shown in Fig. 6, the swelling of PVA chains are constrained by the chitosan chains. Consequently, the highly porous structure of PVA/CS82 is retained after it was soaked in aqueous mediums for 1 month.

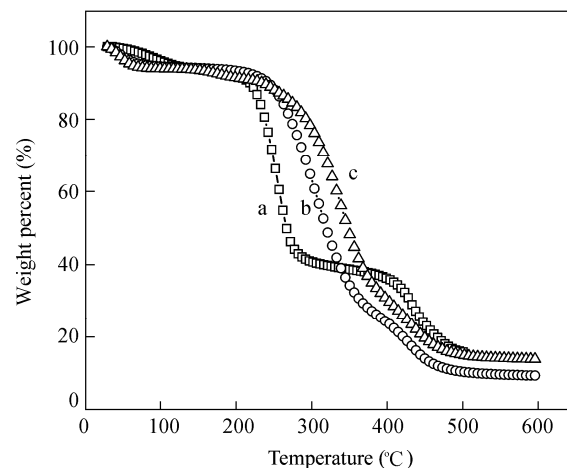


Fig. 5 TGA curves of (a) PVA nanofibers, and PVA/CS82 nanofibers (b) before and (c) after treatment in GA/methanol solution

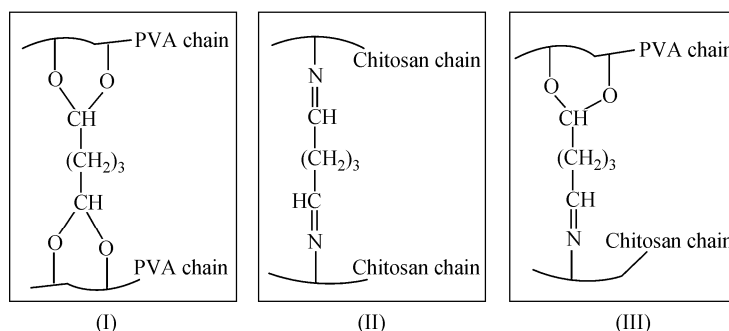


Fig. 6 Typical crosslinking reactions between PVA, CS and GA in the nanofibers in the course of GA/methanol immersion

CONCLUSIONS

In summary, water resistant PVA electrospun nanofibrous mats were obtained through the combination of chemical and physical crosslinking reactions. PVA nanofibrous mats treated in glutaraldehyde/methanol solution retained their fiber structure, but significant fiber twinning and dense fiber packing took place, with the loss of most pores among fibers upon immersed in water. Crosslinked PVA/chitosan (weight ratio 8:2) composite nanofibers were highly stable, with little loss of fiber morphology and pore structure after soaking in an aqueous medium with varied pH values for 1 month. On the one hand, the crosslinking agent GA reacted with the hydroxyl groups of PVA and amino groups of chitosan, to form many chemical cross-links. On the other hand, the solvent methanol induced many crystallites, acting as physical cross-links in the PVA nanofibers. Both chemical and physical cross-links, in addition to the semi-rigid molecular chains of chitosan, make PVA/CS82 composite nanofibers a very promising tissue scaffold material applicable in the aqueous media.

REFERENCES

- 1 Ramires, P.A. and Milella, E., *J. Mater. Sci. Mater. Med.*, 2005, 13: 119
- 2 You, Y., Park, W.H., Ko, B.M. and Min, B.M., *J. Mater. Sci. Mater. Med.*, 2004, 15: 297
- 3 Burczak, K., Gamian, E. and Kochman, A., *Biomaterials*, 1996, 17: 2351
- 4 Yeom, C.K. and Lee, K.L., *J. Membr. Sci.*, 1996, 109: 257
- 5 Wang, Y. and Hsieh, Y.L., *J. Membr. Sci.*, 2008, 309: 73
- 6 Zeng, J., Hou, H.Q., Wendorff, J.H. and Greiner, A., *Macromol. Rapid Commun.*, 2005, 26: 1557
- 7 Yao, L., Haas, T.W., Guiseppi-Elie, A., Bowlin, G.L., Simpson, D.G. and Wnek, G.E., *Chem. Mater.*, 2003, 15: 1860
- 8 Hong, Y.L., Shang, T.C., Li, Y.Y., Wang, L.Z., Wang, C., Chen, X.S. and Jing, X.B., *J. Membr. Sci.*, 2006, 1-2: 1
- 9 Zheng, H.P., Nie, J., Sun, J.F., Guo, S. and Yang, D.Z., *Acta Polymerica Sinica*(in Chinese), 2007, (3): 230
- 10 Wu, L.L., Yuan, X.Y., Sheng, J., *J. Membr. Sci.*, 2005, 250: 167
- 11 Yi, F., Guo, Z.X., Hu, P., Fang, Z.X., Yu, J. and Li, Q., *Macromol. Rapid Commun.*, 2004, 25: 1038
- 12 Wang, Y.H. and Hsieh, Y.L., *J. Membr. Sci.*, 2008, 309: 73
- 13 Noh, H.K., Lee, S.W., Kim, J.M., Oh, J.E., Kim, K.H., Chung, C.P., Choi, S.C., Park, W.H. and Min, B.M., *Biomaterials*, 2006, 27: 3934
- 14 Qi, Q.L., Li, Q., Lu, J.W., Guo, Z.X. and Yu, J., *Chinese J. Polym. Sci.*, 2009, 27(3): 387
- 15 Ding, B., Kim, H.Y., Lee, S.C., Lee, D.R. and Choi, K.J., *Fibers Polym.*, 2002, 3: 73
- 16 Lu, J., Hu, Q.L. and Shen, J.C., *Acta Polymerica Sinica*(in Chinese), 2006, (8): 1019
- 17 Lu, H., Wei, D.F., Xiao, H.N., Zheng, A.N. and Hu, F.Z., *Acta Polymerica Sinica*(in Chinese), 2007, (12): 1161
- 18 Agnihotri, S.A. and Aminabhavi, T.M., *Int. J. Pharm.*, 2006, 324: 103