

The rheological behavior of collagen dispersion/poly(vinyl alcohol) blends

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Abstract

Blends of collagen dispersion (COL) with poly(vinyl alcohol) (PVA) in different weight ratios were investigated by oscillatory rheometry, Fourier transform-infrared spectroscopy and scanning electron microscopy. It was found that even with 80% of PVA, the COL/PVA blends behaved more like collagen dispersion than pure PVA solution in the dynamic thermal and frequency processing, for instance, a dominant elastic appearance ($G' > G''$), a similar shear thinning behavior and the thermal denaturation below 40°C. However, influence on the blend behaviour by PVA was noticeable, for instance, an increase of dynamic denaturation temperature, the decreasing intensity of amide I, II and III bands as well as the diminishing irregular pores on the surface of blends. The interaction between collagen and PVA could be observed, especially at the regions with low content or high content of PVA.

Keywords : collagen dispersion, poly(vinyl alcohol), rheology

1. Introduction

As a biomaterial for industrial applications, collagen is used in wide fields including foods, medicines, cosmetics, and cell cultures (Yoshimura *et al.*, 2000). In most cases, aqueous collagen dispersion can be used directly, e.g. as injectable materials for tissue augmentation or as drug carriers for tumor treatment or tissue regeneration (Friess, 1998; De Paula *et al.*, 2005). On the other hand, solutions or dispersions may be transferred into solid implants, e.g. as sponges for tissue regeneration or drug delivery and as environmentally degradable films for agricultural applications (Friess and Schlapp, 2006; Chiellini *et al.*, 2003). Besides, it can also be fabricated to textile fibers as seam or blood vessel in surgery (Stitzel *et al.*, 2006; Thomas *et al.*, 2007). With respect to the fiber spinning procedure, it is necessary to investigate the rheological behaviors of the fiber-forming blend in shear and elongation flow to form the polyfibrillar structure.

The easiest way to obtain new materials based on a blend of two polymers is mixing their solutions at proper ratios (Sionkowska, 2006). Along these lines, the present work evaluated the properties of blends of collagen with poly(vinyl alcohol) (PVA). As a synthetic petrochemical thermoplastic, PVA has been studied intensively because of its attractive features, such as high hydrophilicity, recognized

biocompatibility and biodegradability, and good processability of film formation (Sionkowska *et al.*, 2004; You *et al.*, 2007; Chiellini *et al.*, 2003). It has been used in blends and composites with natural polymers since its hydrophilic and filming character allows for some degree of compatibility with functional natural polymeric materials (Alexy *et al.*, 2003).

Although the miscibility and the structure of collagen/PVA blends were studied previously (Sionkowska *et al.*, 2004; Alexy *et al.*, 2003; Lešinský *et al.*, 2005; Sarti and Scandola, 1995; Degirmenbasi *et al.*, 2006), there is a lack of information about the rheological behavior during the fiber spinning procedure. The present study aimed specifically on the rheological behavior of collagen dispersion and PVA blends in various ratios using oscillating rheometry. Additionally, the interaction of the two materials in the aqueous polymer blends was studied by Fourier transform-infrared spectroscopy (FT-IR) and the appearance of sponges made of collagen/PVA blends was observed by scanning electron microscopy (SEM).

2. Experimental

2.1. Materials

1000 g fresh bovine hide was unhaird with 15 g Na₂S and 50 g lime powder. Then the dermis of hide was delimited and neutralized in a process described by Zhang *et al.* (2006). After washing, they were cut into smaller pieces and pulverized with a mill (Fritsch Pulverisette 14, Ger-

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many). The powders were dispersed in 40 volumes of 0.5 M acetic acid at room temperature for 48hrs with stirring. Then the prepared collagen (as 2.1% dispersion) was stored at 4°C for no more than three months.

PVA sample with degree of polymerization of 1750 was commercially obtained from the Beijing Bailee Chemical R&D Plant. The degree of saponification was 99 mol%. The PVA solution was prepared by dissolving the polymer in 0.5 M acetic acid at 90°C for enough time to achieve complete dissolution.

2.2. Preparations of blends and sponges

Polymer blends were prepared by mixing suitable volumes of collagen dispersion (COL) and poly(vinyl alcohol) (PVA) acetum and stirring for 6h in an ice bath. The final weight ratio of COL and PVA was respectively 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90 according to the experimental design. The final polymer content was kept at 2.1% wt for all blends, which was limited by the concentration of collagen dispersion. All systems were centrifuged at 9,000×g for 10 min to remove entrapped air-bubbles.

Sponges of COL/PVA blends with different weight ratios were prepared by being dialyzed against 0.1 M acetic acid for three days and then lyophilized by a freeze dryer (Labconco Freeze Dryer FreeZone 6 Liter, USA) at -40°C for two days.

2.3. Dynamic rheometry

Oscillatory rheological experiments were performed on a Rheometer System Gemini 200 (Molven Instruments, UK) using a 4° cone/plate system (40 mm diameter) operating in the small-amplitude oscillatory mode.

Dynamic temperature sweep tests were induced after equilibration at the initial temperature (20°C). The blend samples were heated from 20°C to 40°C at 0.5°C/min at a constant frequency of 1 Hz (6.28 rad/s) and a constant strain of 5%. Dynamic frequency sweep tests were also conducted.

The gap between the plates was set at 150 μm in all sweeps. Heating and cooling were done by circulating water bath. The storage modulus (G'), loss modulus (G''), complex viscosity (η^*) and phase angle ($\delta = \arctan G''/G'$) of blends were recorded as function of temperature or frequency in the linear viscoelastic region.

2.4. Fourier transform-infrared spectroscopy

FT-IR spectra of the COL/PVA blended sponges were recorded with a Spectrum One FT-IR spectrophotometer (PerkinElmer instruments, USA) by making potassium bromide (KBr) pellets. All spectra were recorded by absorption mode in the wavelength range of 450-4000 cm^{-1} wavenumber.

2.5. Scanning electron microscopy

The surfaces of the blended sponges were observed after coating with gold-palladium by means of a scanning electron microscopy (JSE-5900LV SEM, JEOL LTD, Japan) at an accelerating voltage of 20 kV.

3. Results and discussion

3.1. Dynamic temperature sweep tests

The storage modulus (G' , elastic modulus) describes the elasticity of the material whereas the loss modulus (G'' , viscous modulus) reflects the dissipated energy as a characteristic of the viscous properties (Friess and Schlapp, 2001). Using oscillatory rheometry, the viscoelastic behavior of blends was measured as a function of temperature.

It seemed that the dynamic thermal curves of COL/PVA blends were similar to that of the pure collagen dispersion (Fig. 1). Corresponding to the pure PVA solution, G' and G'' of pure collagen dispersion and COL/PVA blends slightly decreased with rising temperature and fell suddenly at 32°C-38°C reflecting the typical denaturation process of pure collagen dispersion and the blends (Fig. 1a and b). It was reported that the denaturation temperature was defined as the peak of derivative curves of G' (Yoshimura *et al.*, 1999). When PVA was added to the collagen dispersion matrix, the denaturation temperatures of the blends shifted toward higher temperatures (shown in Fig. 2). It was worth noticing that the denaturation temperature for 20/80 (COL/PVA) blend was 35.6°C which was about 2°C higher than that for pure collagen dispersion alone (33.9°C).

Loss tangent ($\tan\delta$) is the ratio of G'' and G' . The smaller the value of $\tan\delta$, the more elastomeric was the behaviour (Korhonen *et al.*, 2001). Fig. 1c shows the changes of $\tan\delta$ as a function of temperature. $\tan\delta$ values were less than 1 for each blend, which indicated a predominant elastic behaviour. Besides, the concomitant rapid increase in $\tan\delta$ at 32°C-38°C also reflected the denaturation for all the COL/PVA blends measured, while a gradual drop in $\tan\delta$ was observed in the pure PVA as temperature increased. In addition, compared to the pure collagen dispersion, more elastic solid-like behavior was observed for the blend with 20% PVA in the higher temperature (>32°C), namely a higher thermal stability than the pure collagen due to the addition of PVA.

It was interesting to note that COL/PVA blends and pure collagen dispersion showed similar behaviors in the dynamic temperature sweep tests. Even with 80% of PVA, the COL/PVA blends exhibited more like collagen dispersion than pure PVA solution in the dynamic thermal processing. Subsequently, the dynamic frequency sweep tests were operated at the constant temperature 25°C in order to avoid the denaturation of blends.

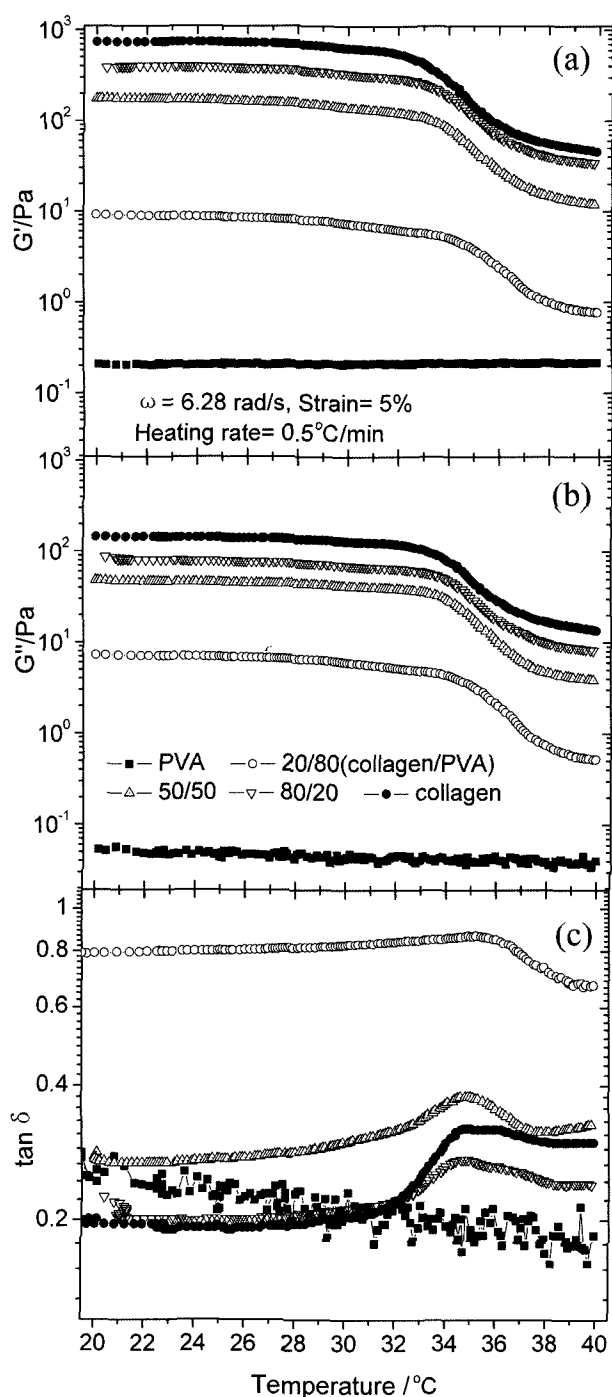


Fig. 1. Temperature dependence of G' , G'' and $\tan \delta$ of pure collagen dispersion, PVA and COL/PVA blends.

3.2. Dynamic frequency sweep tests

Fig. 3 shows the linear viscoelastic behavior of the elastic modulus, viscous modulus and complex viscosity of the COL/PVA blends as a function of oscillation frequency (0.1-100 rad/s). With the increase in the content of PVA in blends, G' and G'' decreased at the frequency of 0.1-20 rad/s (shown in Fig. 3a, b, c, d). Besides, the increase of fre-

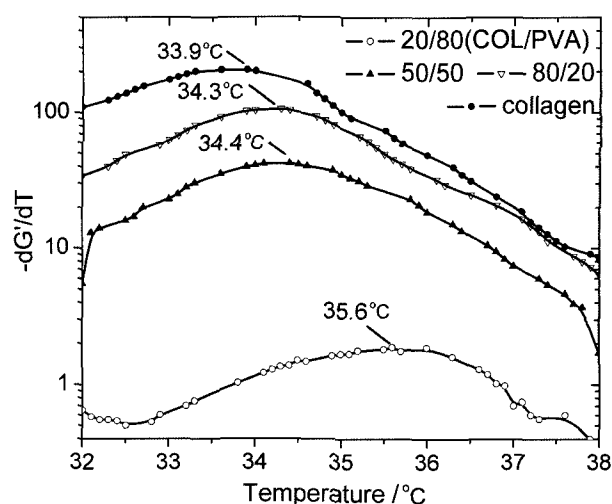


Fig. 2. Dynamic denaturation temperature of the COL/PVA blends.

quency brought about the increase of both G' and G'' significantly. The G' and G'' values of samples (with 0%-50% of PVA in blends) increased from 75-442 Pa for G' and 30-125 Pa for G'' to about 280-1020 Pa for G' and 60-200 Pa for G'' at the 0.1-100 rad/s frequency range (shown in Fig. 3a and c). The storage modulus was higher than the loss modulus suggesting a greater contribution from the elasticity than the viscosity for the blends with 0%-50% of PVA. The elastic modulus increased monotonously, while the viscosity modulus did not. As shown in Fig. 3c, the small peaks of G'' at the fixed frequency, 7.36 rad/s, were observed for the blends with 0%-50% of PVA. The higher content of collagen in blends, the more obvious was the peak. Thus, it seemed that the viscous property of collagen dispersion had certain sensitivity to this special frequency. Fig. 3e and f present the results of the complex viscosity of blended samples at different frequency. All blends containing collagen showed a shear thinning behavior as η^* decreased with rising frequency. It indicated that the structure of blends was quite different from that of the pure PVA.

On the log scale, all G' , G'' and η^* curves of COL/PVA blends showed a strong linear trend being similar to that of the pure collagen dispersion, even up to 90% of the PVA content (Table 1). For the blends with 0%-50% of PVA, the slopes of $\log G'$, G'' and η^* with respect to \log frequency were similar respectively indicating the analogous structure of the blends. Then a substantial decrease in η^* by several orders of magnitude, an increase in the slope of η^* to 0.70 and an increase in the slope of G' and G'' were observed as the PVA content in blends reached to 100%. Therefore, it exhibited a remarkable structural change of blends due to the dilution and isolation of collagen fibers.

As the proportion of PVA in blends increased, it was possible to observe a drop in G' and G'' curves as well as a

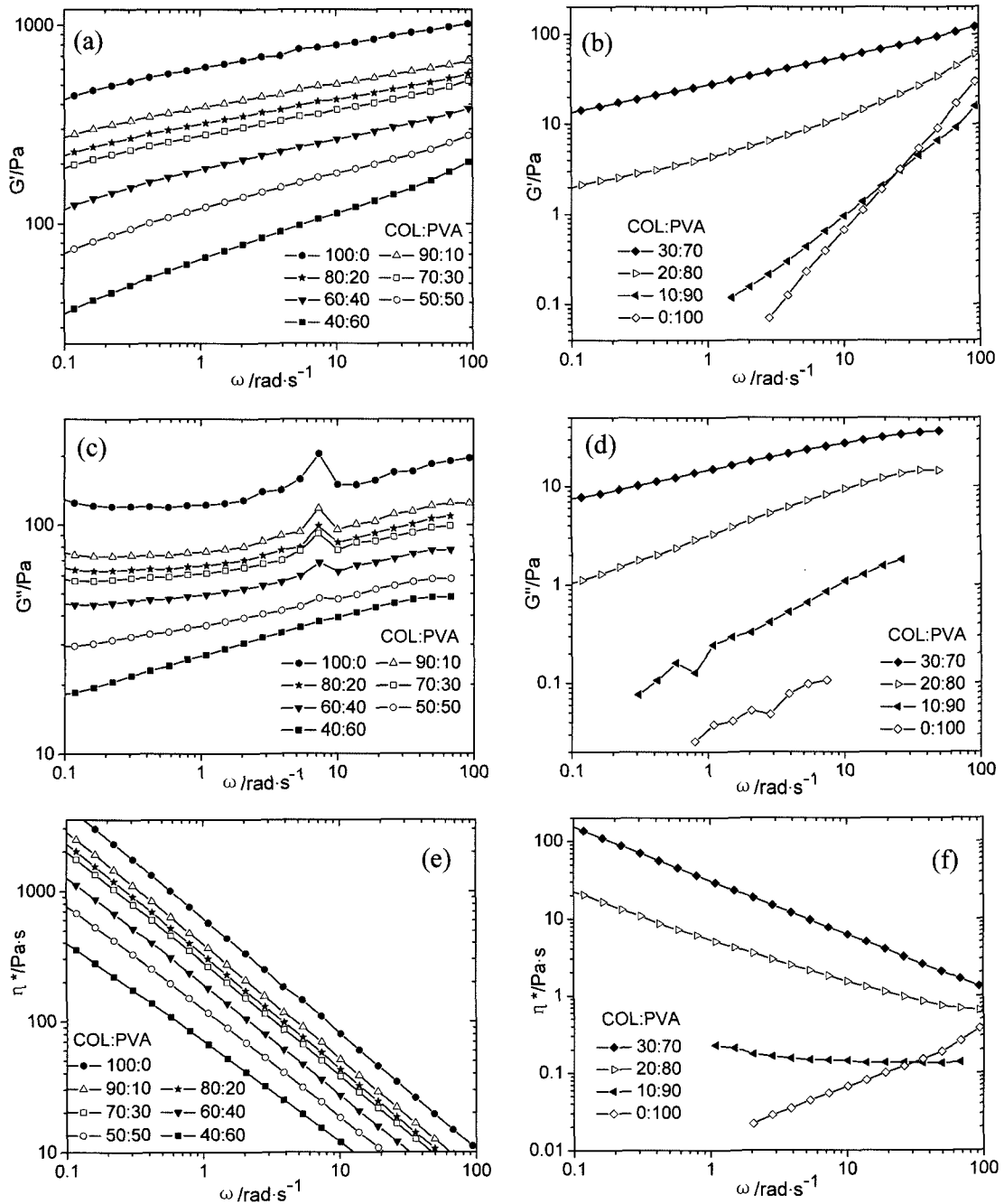


Fig. 3. Oscillatory rheometry: (a) and (b) the elastic modulus of COL/PVA blends in different blend ratios; (c) and (d) the viscous modulus of COL/PVA blends in different blend ratios; (e) and (f) the complex viscosity of COL/PVA blends in different blend ratios.

crossing of the two curves (Fig. 4a). When the percentage of PVA (P_{PVA}) was below 80%, the elastic modulus was higher than the viscous modulus ($G' > G''$) and the dispersion blends took an elastic appearance (Moresi *et al.*, 2001). However, a crossing of G' and G'' curves occurred at the region of $P_{PVA} > 80\%$ demonstrating a transition from solid-like to liquid-like behavior. Then the blend had a liquid-like appearance and was thought to receive a greater contribution from the viscosity than elasticity ($G'' > G'$).

The complex viscosity values of all blends were between

those of the pure collagen dispersion and pure PVA solution (Fig. 4b). The values decreased gradually with the increase of PVA content in blends. Especially, the composition dependency of viscosity reduction was relatively insensitive when the amount of PVA was in the range of 20%-30% in the blend. The slope value, -3.68 , in this range was higher than that, -6.23 , in the range of 10%-20% PVA. Similarly, it was higher than that, -8.32 , in the range of 30%-40% PVA (Fig. 4b). In addition, similar behaviors could be observed under other selected frequen-

Table 1. The effect of PVA content on the slopes of curves $\lg\omega$ - $\lg\eta^*$, $\lg\omega$ - $\lg G'$ and $\lg\omega$ - $\lg G''$

PVA content/%	Slope 1	Slope 2	Slope 3
0	-0.87	0.13	0.06
10	-0.87	0.13	0.08
20	-0.86	0.14	0.08
30	-0.86	0.15	0.09
40	-0.84	0.17	0.09
50	-0.81	0.20	0.11
60	-0.76	0.26	0.16
70	-0.69	0.33	0.27
80	-0.54	0.47	0.45
90	-0.11	1.19	0.71
100	0.70	1.70	0.62

Slope 1: slope of the curve $\lg\omega$ - $\lg\eta^*$; Slope 2: slope of the curve $\lg\omega$ - $\lg G'$; Slope 3: slope of the curve $\lg\omega$ - $\lg G''$.

cies, for instance, 0.12 rad/s, 5.36 rad/s and 10.11 rad/s (not shown). This result was consistent with the result of dynamic thermal measurement in which the blend with 20% PVA was observed a higher thermal stability than that of the pure collagen dispersion. Thus, stronger interaction between the two raw materials might occur at those blended ratios.

3.3. FT-IR spectra

FT-IR studies were performed to further elucidate the interaction between collagen and PVA in blends (Fig. 5). Fig. 5(1) shows the characteristic peaks of collagen containing 3342 cm^{-1} (amide A, N-H stretching and O-H stretching), 3082 cm^{-1} (amide B, C-H stretching), 1662 cm^{-1} (amide I, C=O stretching), 1556 cm^{-1} (amide II, N-H bending and C-N stretching) and 1240 cm^{-1} (amide III, C-N stretching and N-H bending) (Sionkowska *et al.*, 2004; Friess and Lee, 1996). For the PVA spectrum (shown in Fig. 5(6)), a strong absorption band at 3426 cm^{-1} from the O-H stretching and no obvious amide A, B and II bands was observed. Besides, it displayed a weak peak at 1647

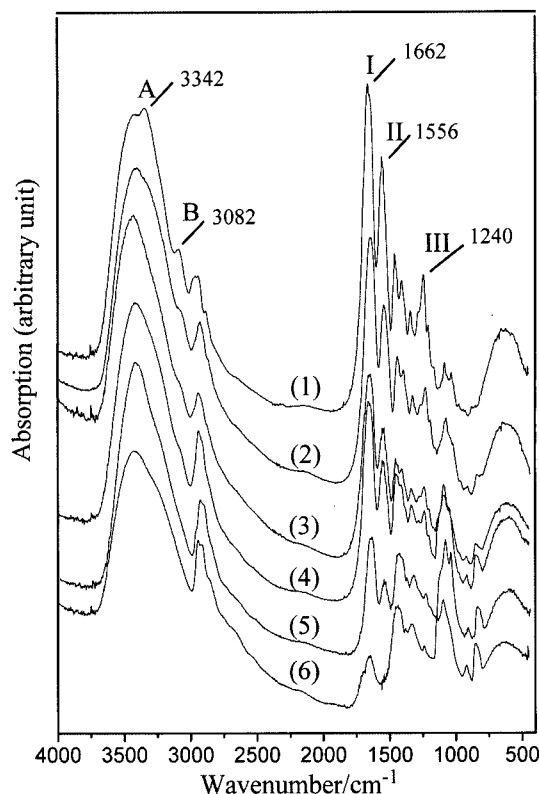


Fig. 5. Wide scan FT-IR for (1) collagen, (2) collagen/PVA=80:20, (3) collagen/PVA=70:30, (4) collagen/PVA=50:50, (5) collagen/PVA=20:80 and (6) PVA.

cm^{-1} resulting from the acetic acid solvent and a greater absorption band at 2943 cm^{-1} representing the $-\text{CH}_2$ aliphatic group comparing with the collagen spectrum (Sionkowska *et al.*, 2004).

With the increase of PVA in blends, the amide A band shifted to higher wavenumber gradually compared to that of the pure collagen dispersion. Meanwhile, the amide B band was weakened and persisted in the blends until 30% content of blend was PVA (shown in Fig. 5(1), (2), (3)). This content range was much broader than that in the pre-

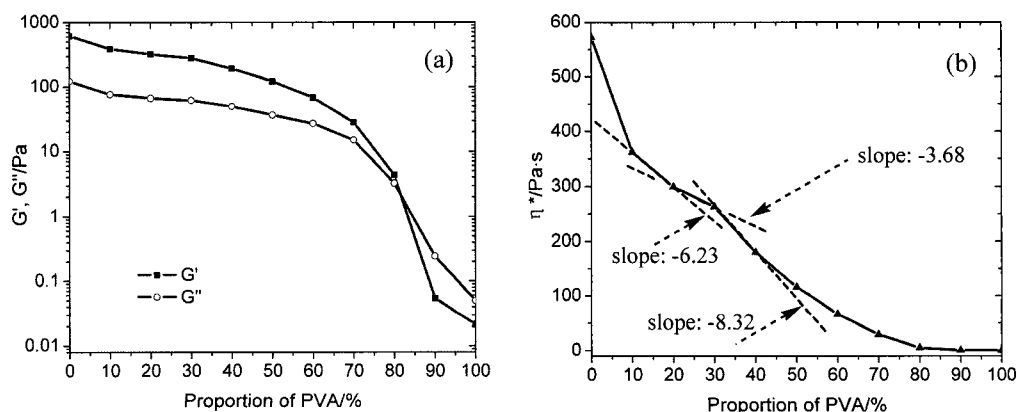


Fig. 4. Blended ratio dependence of the G' , G'' and η^* recorded at 25°C with frequency of 1.09 rad/s.

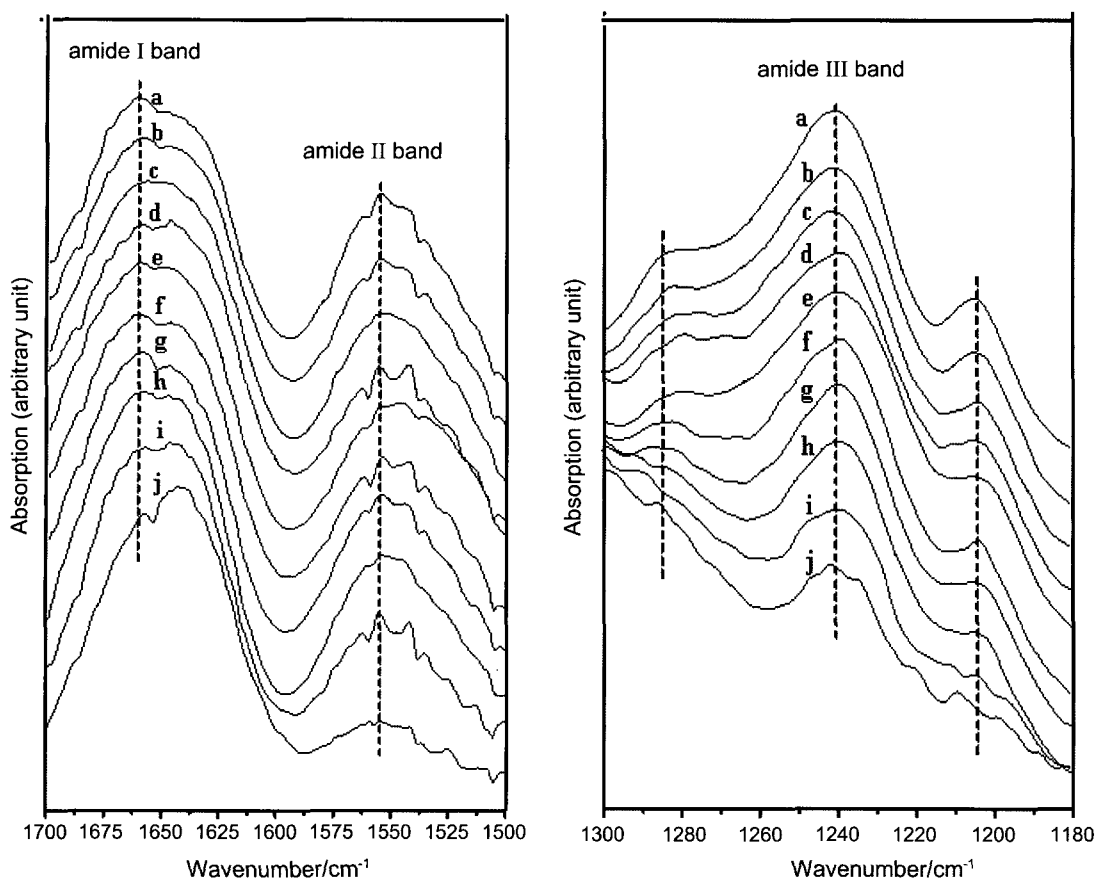


Fig. 6. FT-IR spectra for (a) pure collagen, and COL/PVA blended sponges prepared from PVA-collagen ratios of (b) 10:90, (c) 20:80, (d) 30:70, (e) 40:60, (f) 50:50, (g) 60:40, (h) 70:30, (i) 80:20, (j) 90:10.

vious report, in which amide B band was observed only for collagen/PVA blend weight ratio 99:1, and it suggested some interaction leading to miscibility could be observed only for a very low content of PVA in collagen (Sionkowska *et al.*, 2004).

The amide I, II, and III bands of collagen are directly related to polypeptide conformation (Thomas *et al.*, 2007). The peaks corresponding to the three bands displayed a loss of intensity with the increase of PVA. More details are shown in Fig. 6.

The peak of amide I band shifted gradually to lower wavenumber with the increase of PVA from 0% to 30%. Then it fluctuated at the location that was slightly lower than that of pure collagen dispersion while the proportion of PVA was from 40% to 70%. Finally it shifted to low wavenumber being about 15 cm^{-1} lower than the initial value with high content of PVA in blends (70%-90%). As the amide I absorption is sensitive to the secondary structure of collagen, a slight shift of the position of bands suggests conformational changes in collagen molecule (Sionkowska, 2006; Sionkowska *et al.*, 2004). In the COL/PVA blends, the shifts were reported to occur specifically on the basis of change in the hydrogen bonding (Sion-

kowska *et al.*, 2004). Since collagen, which is a hydrogen donor, should form hydrogen bonds with the hydroxyl group of PVA, the interaction occurred for low content of PVA in collagen or low content of collagen in PVA. When the weight ratio of collagen and PVA was over an appropriate level, weak interaction occurred as a result of the severe competition of forming hydrogen bonds among collagen-PVA, collagen-collagen and PVA-PVA.

Additional information could be obtained from the amide II and III band. No other alteration of amide II band positions was observed except for a shift nearly 10 cm^{-1} to low wavenumber for the blends with composition 30%-40% of PVA. For the amide III band of pure collagen, the main peak was located at 1240 cm^{-1} , with smaller peaks seen at 1204 cm^{-1} and 1279 cm^{-1} . It was found the main peak was in the same position for all blended samples. However, the increase of PVA in the blends beyond 70% brought about a loss of these two smaller peaks.

3.4. SEM images

Fig. 7 represents the SEM micrographs of collagen/PVA sponges. The pure collagen dispersion had micro-porous structure as shown in Fig. 7a₀. Because the pores possessed

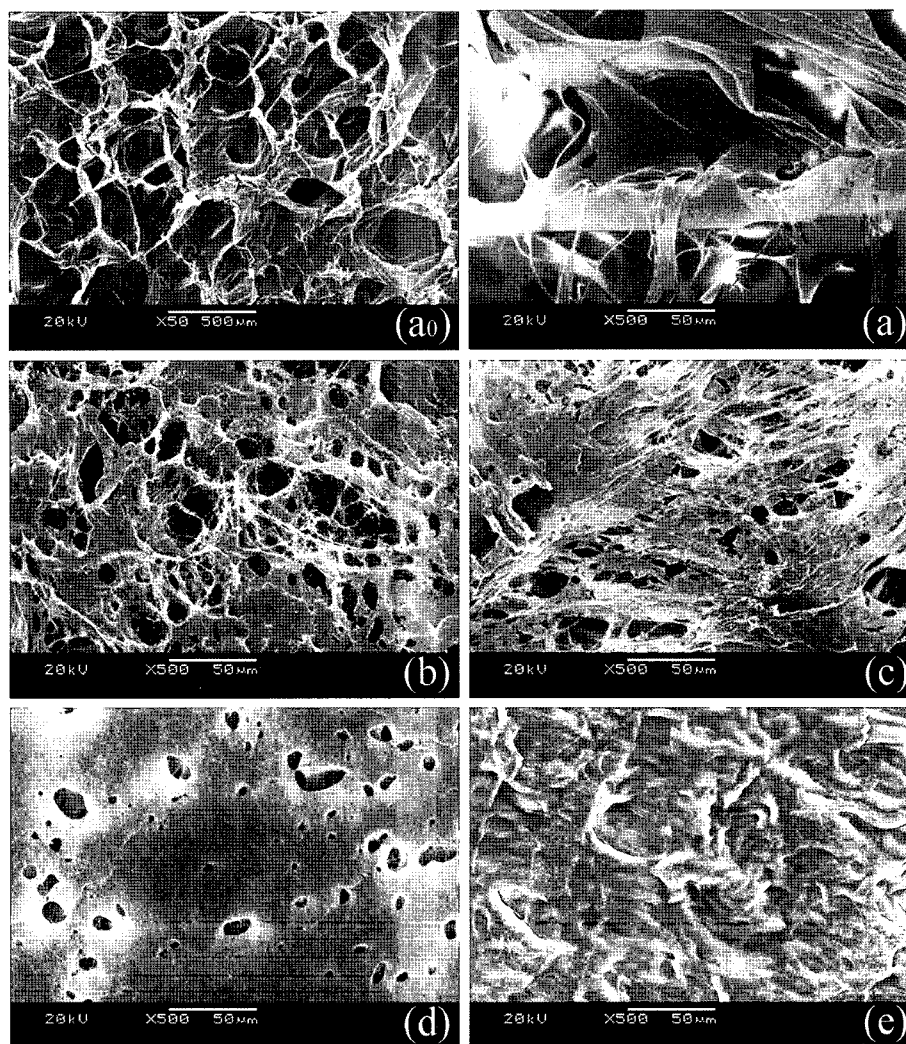


Fig. 7. SEM images for (a₀) collagen (50×), (a) collagen (500×), (b) collagen/PVA=80:20 (500×), (c) collagen/PVA=50:50 (500×), (d) collagen/PVA=20:80 (500×) and (e) PVA (500×).

large diameter (about 300 μm), it seemed to be film-like when the magnification was 500 in Fig. 7a. Comparing with pure collagen dispersion, pure PVA showed film-like structure without any pores (Fig. 7e).

All the sponges of COL/PVA blends showed micro-porous structure with irregular pores of 5-30 μm diameters (shown in Fig. 7b, c and d), which were much smaller than those of pure collagen dispersion (about 300 μm diameters). Besides, the diminishing pores with increasing intervals were observed when the content of PVA in blends increased. Thus, it seemed that the micro-porous structures of different COL/PVA blended sponges were induced by collagen and the increasing content of PVA showed the effect of dilution on collagen dispersion.

4. Conclusions

Gradual changes of the rheological behaviors and struc-

tures for COL/PVA blends, containing the shifts of flow behaviors in dynamic thermal and frequency processing, absorption density and peak position in FT-IR spectra as well as the decrease of the pores on the surface of sponges, were observed with the increasing content of PVA.

The dispersion blends revealed an elastic appearance when the content of PVA was lower than 30 wt% in the blend. Some interaction between PVA and collagen dispersion was shown, especially for 20%-30% content of PVA. The higher thermal stability than the pure collagen dispersion by dynamic thermal measurement and a slow-down in decrease of complex viscosity was observed in this region. Besides, the disappearance of amide B band and the shifts of amide I and II band to lower wavenumber by FT-IR analyses highlighted a result of reinforcement by increasing hydrogen bonds between collagen and low content of PVA.

Although both the predomination of elasticity in solution

and the appearance with irregular pores for the blends with 40%-70% of PVA were similar to the blends with less PVA (below 30%), unique properties were displayed in this blend-ratio region. The fluctuation of the amide I band at a high wavenumber showed a state lack of adequate stability. It seemed that the severe competition of forming hydrogen bonds among collagen-PVA, collagen-collagen and PVA-PVA resulted in the weak interaction when the weight ratio of collagen and PVA was over an appropriate level.

A liquid-like appearance with low values of G' , G'' and η^* in solution and a dense structure with small pores in sponges were exhibited if the content of PVA was in a high level (e.g. 80-100%). The higher denaturation temperatures than pure collagen dispersion (about 2°C) and the peak shift of amide I band to lower wavenumber again demonstrated the presence of interaction between low content of collagen and abundant PVA.

These fundamental data should provide a useful guideline when new polymeric materials, "biopolymer hybrids", were desired by blending between biopolymers and synthetic polymers, corresponding to collagen and PVA.

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