Journal of Applied Polymer Science

Rheological Properties of Collagen/Hydroxypropyl Methylcellulose (COL/HPMC) Blended Solutions

Cuicui Ding,[1] Min Zhang,[2] Guoying Li[1]

[1]The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065, People's Republic of China

[2]College of Materials Engineering, Fujian Agriculture and Forestry University, Fuzhou 350002, People's Republic of China

Correspondence to: G. Li (E-mail: liguoyings@163.com)

ABSTRACT: Collagen/HPMC blends with different HPMC content (HC) were investigated by dynamic responses, creep recovery, thixotropy, and morphological observation. Storage modulus, loss modulus, complex viscosity, and activation energy decreased with the increased HC, while the flow behavior index increased with the increased HC and the dynamic denaturation temperature reached the maximum as HC increased to 50%, indicating that the flowability and thermal stability of collagen solution were improved by the addition of HPMC. However, the blends with higher HC tended to have a relatively lower recovery capacity, and the hysteresis loop areas of the blends were lower than that of collagen (especially when HC > 50%). Additionally, the morphology of collagen/HPMC was examined by both of atomic force microscopy and scanning electron microscopy. By combining with these results, it seemed that the rheological and structural properties of collagen/HPMC were related to the hydrogen-bond interaction and compatibility between collagen and HPMC molecules. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40042.

KEYWORDS: rheology; morphology; blends

Received 13 May 2013; accepted 7 October 2013

DOI: 10.1002/app.40042

INTRODUCTION

Collagen, which is the major structural protein of connective tissues, is widely used in fields including medicines, cosmetics, foods, and chemical industries due to its weak antigenicity, biodegradability, biocompatibility, and bioactivity.[1] However, as a natural polymer, collagen had some weakness in its properties, including the relatively lower thermal stability and a high vicidity, which are limiting factors for its application. The denaturation temperature (a temperature at which a transition of collagen from a rod-like molecule to a randomly coiled form occurs)[2] of collagen in solution was no more than 39°C and the complex viscosity of collagen with concentration of 1.5% reached up to 406.2 Pa s (25°C),[3,4] which means that collagen was easy to denature or was difficult to flow in pipe during processing. For improving the properties of collagen, blending of collagen with other polymers has been of a considerable interest in recent years. However, synthetic polymeric scaffolds may not interact with cells in a desired manner and may induce toxic and inflammatory reactions.[5,6] In addition, it is difficult to construct a three-dimensional synthetic polymeric scaffold that resembles the structure of natural tissues. Natural biodegradable polymers have received much attention due to the fact that they are biocompatible and the application of natural polymers reduces the risk of infection caused by the implants.

Cellulose, the most abundant natural polymer in plant kingdom, is of particular interest due to its abundant availability and biodegradability. While the pure cellulose is not soluble in water because of the intramolecular hydrogen bonding, which highly limits the utilization of cellulose in a large number of technical applications and products.[7] Alternatively, cellulose derivatives, particularly, cellulose ethers are widely studied and many of them were mixed with collagen to obtain new polymers with improved properties such as tensile strength, thermal, and enzymatic stability.[8,9] Hydroxypropyl methylcellulose (HPMC), which is a typical nonionic polysaccharide, is a hydrophilic cellulose ether derivative and has been extensively used as a matrix for drug delivery as well as used in adhesives and cosmetics.[10–12] Since blending polymers provides a very attractive technique to generate new materials, we have tried to mix the collagen and HPMC to obtain new materials for medical application such as controlled-release. In previous work, we have found that the interaction (hydrogen-bond interaction) and compatibility between collagen and HPMC became weak when the HPMC content in collagen/HPMC was above 50%.[13]

Besides, we studied the influence of hydrogen-bond interaction on the self-assembly behavior of collagen under physiological conditions, noting the properties of resultant collagen fibril hydrogels, including the thermal stability and viscoelastic properties, could be adjusted by the addition of HPMC.[14]

In most cases, new materials based on collagen processing involve liquid aqueous preparations. These solutions can on the one hand be used directly such as injectable materials for tissue augmentation or as tissue regeneration.[15,16] On the other hand, solutions may also be transferred into solid implants such as fibers, films, sponges, and membranes.[17–19] From processing and application points of view, the rheological properties of COL/HPMC blends are of vital importance, as the knowledge of their rheological properties such as the viscosity, relating to the process of pumping into molds, would help the manufacturer to control or manipulate the physical properties for producing good and stable products.[20] To provide the information and the theoretical guidance for practical applications, the rheological properties of collagen/HPMC blended solutions under both dynamic and shear conditions, including the dynamic responses, creep recovery, and thixotropy, were characterized in the present article. Additionally, the morphology of collagen/HPMC blends was studied by atomic force microscopy (AFM) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials

Type I collagen was extracted from calf skin by the method described by our previous article.[21] After dialyzing against 0.1 mol/L acetic acid for three days, the collagen solution was lyophilized by a freeze dryer (Labconco Freeze Dryer FreeZone 6 L, USA) at −40°C for two days. HPMC was purchased from Hercules (U.S) with a methoxy group content of 27.0–30.0% and with a hydroxypropoxy group content of 4.0–7.5%.

Sample Preparation

Both freeze-dried collagen and HPMC were dissolved in 0.1*M* acetic acid to obtain the final concentration of 8 or 15 mg/mL. The collagen and HPMC solutions (8 mg/mL) were mixed by gently stirring for 7 h at 4°C, using various collagen/hydroxypropyl methylcellulose (COL/HPMC) ratios of 7/3, 5/5, and 3/7 (by weight). Hereafter the sample of collagen was shorted as COL. Similarly, the COL and HPMC solutions with the concentration of 15 mg/mL were mixed according to the method mentioned earlier. These blends were named as C/H (7/3), C/H (5/5), and C/H (3/7), respectively. All samples, including COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC, were centrifuged at 9000 × *g* for 10 min at 4°C to remove entrapped air-bubbles and stored after centrifugation for at least 24 h before testing so that any stresses in these samples were able to relax.

The samples including collagen solution (8 mg/mL), HPMC solution (8 mg/mL) and their blends were used for oscillatory rheological tests. Meanwhile, the creep and recovery tests and thixotropic property tests used the samples including collagen solution (15 mg/mL), HPMC solution (15 mg/mL) and their blends for detecting the sample's elasticity and the ability to build up its structure after shearing. The higher concentration was useful to obtain more accurate results for steady rheological tests.

Oscillatory Rheological Measurements

All the rheological measurements were performed on a Rheometer System Gemini 200 (Malvern Instruments, UK) equipped with a cone-and-plate geometry (diameter 40 mm; angle 4°) or a parallel plate geometry (40 mm plate and 1 mm gap). For precise control of sample temperature, the temperature was controlled by a Peltier temperature controller, with an accuracy of ±0.1°C.

The samples for oscillatory rheological tests were measured after each equilibration time for 3 min. Dynamic stress sweeps were conducted prior to the frequency sweeps to ensure operation within the linear viscoelastic region.

Oscillatory rheological measurements were performed because they are very suitable to give an overview of the rheological response of polymers. The dynamic viscoelasticity tests were performed on the shear-rate controlled rheometer under the oscillatory mode with a cone-and-plate geometry (diameter 40 mm; angle 4°) for its advantage of a homogeneous shear rate across the sample. Rheological properties were measured by dynamic frequency sweeps and by dynamic temperature sweeps at a constant strain of 5% within the linear range. Dynamic frequency sweep tests for all the samples were performed from 0.01 to 10 Hz at constant temperature (15, 20, 25, 30, and 35°C). Dynamic temperature sweep tests for all the samples were conducted at a given frequency of 0.1 Hz and were heated from 25 to 45°C at a rate of 1.5°C/min. The storage modulus (G'), loss modulus (G''), complex viscosity (η^*), and loss tangent ($\tan \delta = G''/G'$) were recorded.

Creep and Recovery Tests

Creep and recovery tests of the samples were performed under the CS mode with a parallel plate geometry, which is suitable for high viscosity samples at a constant shear stress of 1 Pa for 100 s, after which they returned to zero holding for 300 s to observe recovery capability. We investigated the influence of temperature on the creep and recovery capability of samples. The creep and recovery capability of samples were tested at a constant shear stress of 1 Pa at 25°C and 34°C, respectively.

Thixotropic Tests

For the investigation of the thixotropic property of the samples, the flow curves were measured under the CS mode with a parallel plate geometry at 25°C by increasing the shear rate from a minimum of 0.1 s^{-1} to a maximum of 50 s^{-1} and then decreasing the shear rate in the same equal steps. The duration of shear at each step was about 75 s. The thixotropic behavior was investigated by evaluating the hysteresis loop areas obtained through a two-step experiment (upward curve and downward curve), and it is generally admitted that the greater the hysteresis area, the stronger the thixotropic properties.[22]

Differential Scanning Calorimetry (DSC)

The thermal transition of collagen was determined by differential scanning calorimetry (Netzsch DSC 200PC, Germany). The native collagen (8 mg/mL) and COL/HPMC blends of 10 mg were weighed accurately into aluminum pans and sealed, and

scanned at a heating rate of 5°C/min over the range from 5 to 60°C in a nitrogen atmosphere. Liquid nitrogen was used as a cooling medium and pan equipped with 0.1*M* HAc (10 mg) was used as the reference. The denaturation temperature (Td) was determined from the thermograms.

Atomic Force Microscopy

The solutions including collagen solution (8 mg/mL), HPMC solution (8 mg/mL), and their blends were diluted 16 times by 0.1*M* acetic acid, 10 μL of which was dropped quickly and evaporated on mica, and then dried at room temperature (~20°C) for 2 days. The surface morphology of the dried samples was observed by AFM (SHIMADZU SPM 9600, Japan) in the dynamic mode at room temperature (~20°C). Each sample was scanned with a scan rate of 1 Hz.

Scanning Electron Microscopy

The samples including collagen solution (8 mg/mL), HPMC solution (8 mg/mL), and their blends were lyophilized by a freeze dryer (Labconco Freeze Dryer FreeZone 6 L, USA) at −40°C for three days and stored in a desiccator, which was prepared for SEM.

The surfaces of the lyophilized COL/HPMC blends were observed after coating with gold-palladium by means of a scanning electron microscopy (JSM-7500F SEM, JEOL, Japan) at an accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

Dynamic Frequency Sweep Tests

The frequency dependence of G', G'', tanδ, and η^* for all the samples over the frequency range of 0.01–10 Hz at 25°C was depicted in Figure 1.

The storage modulus (G') describes the elasticity of polymers whereas the loss modulus (G'') reflects the dissipated energy as a characteristic of the viscous properties.[23] Comparison of the linear viscoelastic response of the blends showed a significant effect of the HPMC, particularly at a low frequency [Figure 1(a,b)]. That is, at low and intermediate frequency zone (~0.01–1 Hz), collagen had higher values of G' and G'' as compared with the

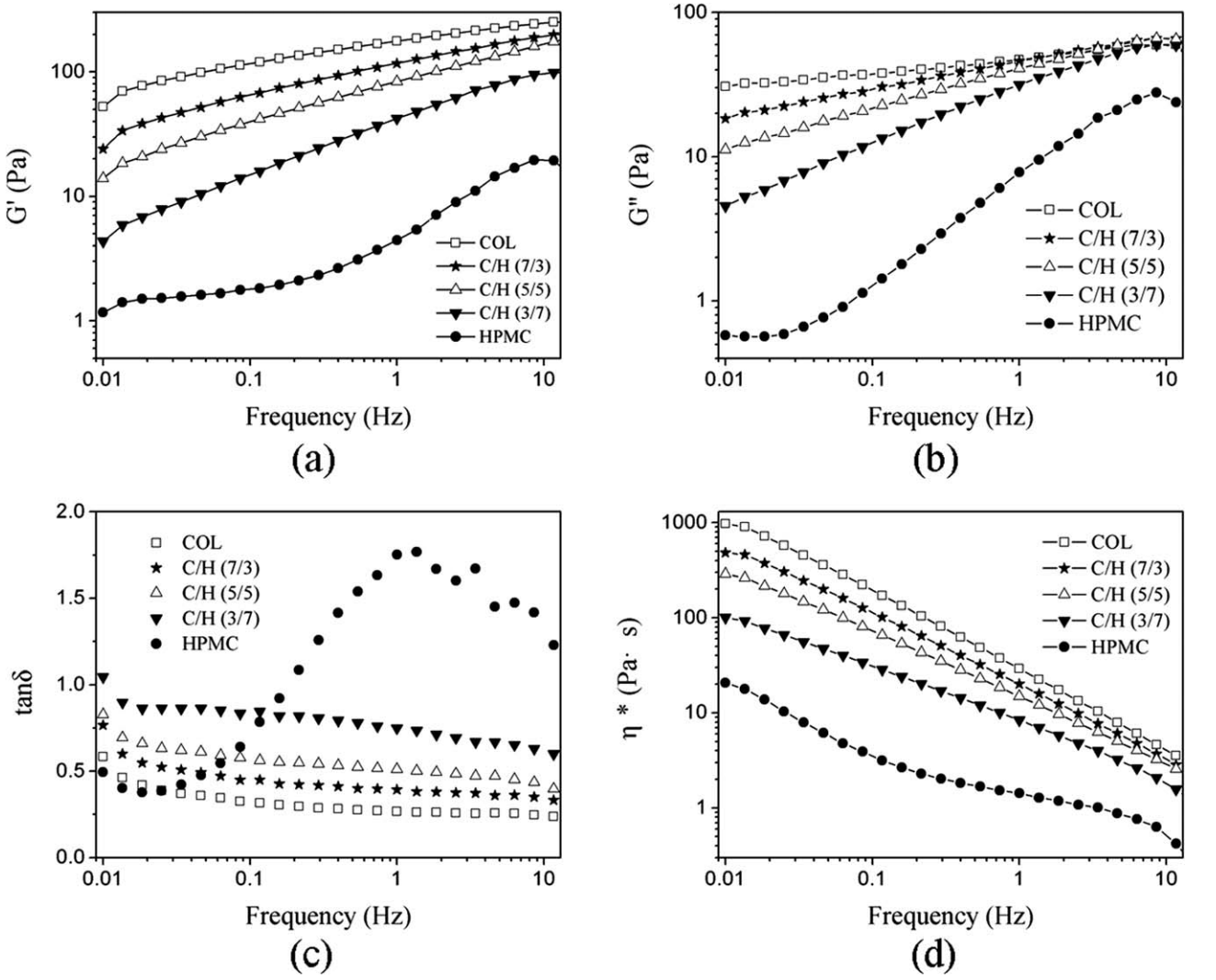

Figure 1. The dynamic modulus (a) G', (*b*) G'', (c) loss tangent (tanδ), and (d) complex viscosity η^* of COL/HPMC blends and their pure component at 25°C.

COL/HPMC blends and showed a monotonic decrease with HPMC content.

At high frequencies (~1–10 Hz), the time of strain response was short and then the sample response is not able to follow the stress variation, which would certainly cause an additional reduction in the strain response of the sample. So the modulus values would be approaching with the increase of frequency. The storage modulus and loss modulus in Figure 1 presented the trend of approaching at high frequencies. Therefore the modulus variation at low and intermediate frequencies could be better to show the effect of HPMC on the COL/HPMC blends.

Furthermore, on the log scale, all G', G'', and η^* curves [Figure 1(a,b,d)] of COL/HPMC blends showed a linear trend being similar to that of COL, even up to 70% of the HPMC content.

The ratio of G''/G', which is defined as tanδ, crossed the threshold (tan$\delta = 1$) from solid-like to liquid-like behavior.[24] As can be seen from Figure 1(c), the tanδ values of samples increased with the increase of HPMC content, indicating that the COL/HPMC blends with higher content of HPMC tended to exhibit a liquid-like behavior. Furthermore, it should be noted that HPMC exhibited an irregular tanδ curve, which was different from collagen and COL/HPMC blends, reflecting that collagen and COL/HPMC blends possessed a different viscoelastic behavior from that of HPMC. Moreover, the tanδ values of collagen and COL/HPMC blends were smaller than 1, implying that the majority of the energy was dissipated by elastic deformation. At lower deformation rate, the polymer chains had more time to relax to a more favorable state by slippage of the entanglement point of chains. However, as the sweeping frequency increased, the available time for the polymer chains to relax declined. Here, the blend chains could no longer slip past one another as readily and the entanglement points acted more like fixed network anchors. Consequently, the ability of this entanglement blend network to temporarily store the imposed energy increased, and the blend network behaved more like elastic solid.[25,26]

Figure 1(d) suggested that all samples showed a shear thinning behavior. In addition, the η^* values decreased with the increase of HPMC content in blends, especially when the HPMC content was over 50%. For instance, at 0.1 Hz, the η^* values of COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC were 171.6, 101.4, 65.6, 28.4, and 3.2 Pa s, respectively. The decrease in viscosity, due to the addition of HPMC, was in favor of processing for collagen solution.

Dynamic Temperature Sweep Tests

Figure 2 shows the changes in η^* and tanδ of all the samples as a function of temperature at the frequency of 0.1 Hz. The dynamic thermal curves of η^* and tanδ for COL/HPMC blends were similar to that of COL, while were completely different from that of HPMC, which was consistent with the results from Figure 1.

In the range of 25–45°C, the η^* of COL was firstly decreased slightly and then decreased dramatically, in contrast, the η^* of HPMC was increased slowly and continuously. The sudden decrease of η^* in magnitudes and the concomitant rapid increase in tanδ at 37.5–42.5°C reflected the collapse of the collagen triple helix to a random coil. The dynamic denaturation temperature (T_{dd}) under dynamic rheological measurement of collagen solution could be determined as the temperature where the increase of tanδ attained a peak value.[27] There was no peak in the tanδ curve of HPMC, that is, a continuous drop was observed in HPMC as the temperature increased, which might be caused by the thermal gelation properties of HPMC. Nevertheless, when HPMC was added to COL, the denaturation temperature of the blends gradually shifted toward higher temperatures. For instance, the T_{dd} for C/H (5/5) was 41.2°C, which was nearly 2°C higher than that for COL (39.3°C). The increase in thermal stability might be due to the hydrogen-bond interaction between COL and HPMC molecules. The improvement of thermal stability was helpful for collagen to keep its native structure during processing.

The Time–Temperature Superposition (TTS)

According to the principle of TTS, the curve of dynamic modulus versus logarithmic loading frequency at one temperature T, can be horizontally shifted along the frequency (or the response

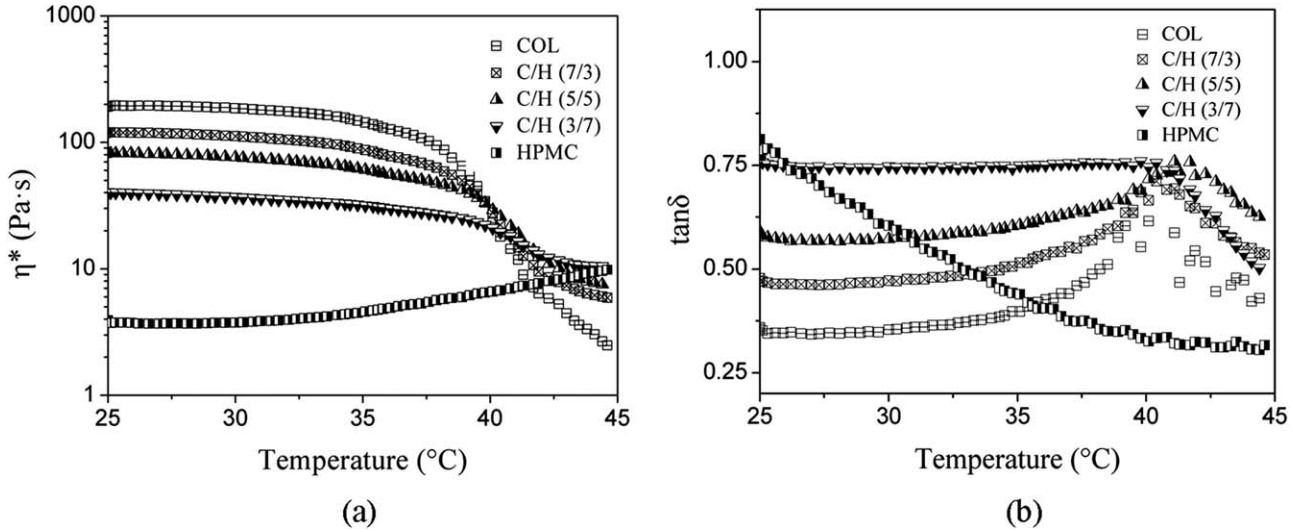

Figure 2. Temperature dependence of η^* and tanδ of COL, HPMC, and COL/HPMC blends.

time) axis then overlapped on the curve at the reference temperature T_0. The shift distance along the logarithmic frequency axis is called the frequency-temperature shift factor α_T.[28] Figure 3 shows the master curves at 25°C (T_0) by horizontal shifting to make all the modulus data at different temperatures superimposed. The master curve was assumed to represent polymer behavior over a wide frequency or time range, allowing prediction of long-term behavior from short-term tests. The modulus data at lower frequencies did not superimpose well and showed a little bit of mismatch.

It was found that the temperature dependence of the shift factor could be well described by the Arrhenius equation, which was generally acknowledged as a suitable formula to describe the relationship between α_T and T_0 for the temperature range below the glass transition temperature. The activation energies were calculated on the basis of the Arrhenius equation given below:

$$\ln \alpha_T = \frac{E}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \quad (1)$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), α_T is the shift factor and E is identified as the activation energy for the jump of a molecule from one equilibrium position in the solution to the next, reflecting the need to overcome an energetic barrier of local rearrangements from one state to another.[29] The linear regression plotting of the shift factor α_T versus $1/T$ according to Eq. (1) yielded the activation energy of 79.3, 54.5, 50.3, and 34.2 KJ mol^{-1}, respectively for samples of COL, C/H (7/3), C/H (5/5), and C/H (3/7). It seemed that the activation energies decreased obviously when the HPMC content >50%. The lower flow activation energy caused a smaller energetic barrier for segmented motions in the confined space and thus caused the decrease in values of G', G'', and η^*.

Creep and Recovery Tests

Besides the dynamic viscoelasticity, the creep-recovery property was also important in rheological characterization. Creep was defined as a slow development of deformations at the applied constant stress. The recovery part of the test is a measure of the decline of the material deformation when the stress is removed. A slower recovery is indicative of a stronger ability for the material to store the residual stress. Especially, the absence of recovery for a sample indicated that the sample is in a viscous flow and no elastic effects remain active.[30]

Figure 4 shows the viscoelastic properties of samples in terms of creep recovery response. The blends tested at 25°C and 34°C showed similar behaviors, that is, larger strain values in the creep part as the amount of HPMC in the blends increased at a constant shear stress of 1 Pa, while the strain values of samples at 34°C were larger than those of corresponding samples at 25°C except for HPMC. As shown in Table I, the recovery capacity was reduced with the increased content of HPMC, and the recovery percentage of samples at 34°C was smaller than those at 25°C except for HPMC, which exhibited different changes from samples containing the component of collagen. The creep-recovery response of HPMC at 34°C might be related to its thermal-gelation property, which improved the elasticity of HPMC. In general, the variation of strain and recovery capacity for COL/HPMC blends exhibited negative correlation, and a larger strain value indicated the molecular chains of HPMC were easier to slide than those of COL when shearing.

As indicated by the oscillatory rheological tests, COL/HPMC blends showed similar linear viscoelastic behaviors to those of COL even when the content of HPMC reached 70%. Here, the creep recovery tests indicated that a distinct increase in the strain response of COL/HPMC blends was observed as the HPMC content was greater than 50% both at 25 and 34°C, while the recovery capability decreased substantially as the HPMC content was over 70% at 25°C. Thus, it seemed that collagen might have a greater contribution on the viscoelastic behavior in blends when the HPMC content in blends was below 70%. In addition, the recovery capability was closely

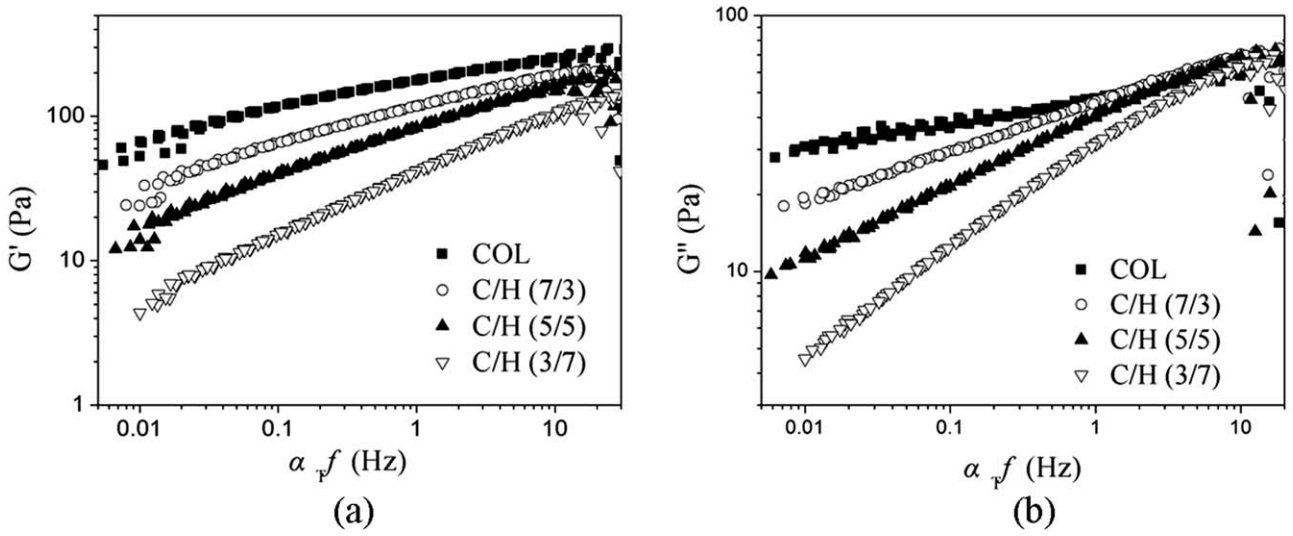

Figure 3. Master curves of shear modulus (G' and G'') for COL and COL/HPMC blends as a function of shifted frequency $\alpha_T f$ at the reference temperature of 25°C.

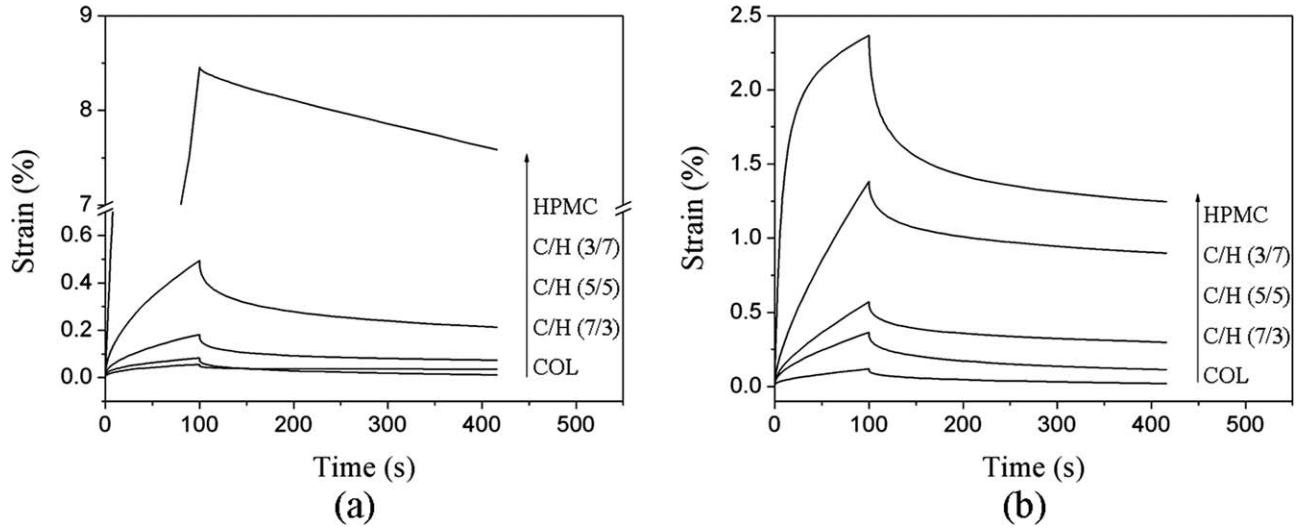

Figure 4. Creep-recovery results for COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC, obtained after the application of a shear stress of 1 Pa: (a) 25°C; and (b) 34°C.

related to the processing, like collagen injectable gels for plastic surgery applications. So the ratio of COL/HPMC should be adjusted according to the practical need.

Thixotropy Behavior

Thixotropy is generally used to describe the rheological behavior of a polymer solution exhibiting a reversible time-dependent decrease in apparent viscosity when subjected to a particular shear rate.[31] Figure 5 shows the flow curves of all samples in terms of apparent viscosity–shear rate relationships. The apparent viscosity was observed to decrease with the increase of shear rate, which could be expected as the shearing caused the orientation or deformation of macromolecular network in the direction of flow. With an increasing shear rate this effect would become more pronounced and would cause a decrease of the internal friction because of a smaller effective interaction amongst the molecules.[32]

The viscosity decrease was approximately linear on the log–log scale, independent of the content of HPMC, indicating that the relationship between steady shear viscosity and shear rate could be modeled by Ostwald-de Waele or power law:

$$\eta = K\dot{\gamma}^{n-1} \quad (2)$$

where coefficient K is a measure of consistency of the system and n $(0 < n < 1)$ is the flow behavior index. In this way, the parameters K and n can be obtained by linear regression analysis. In the case of Newtonian behavior, eq. (2) becomes Newton's law of viscosity, with viscosity equal to K. But for non-Newtonian behavior, the flow characteristic is termed as the shear-thickening behavior if $n > 1$ and the shear-thinning behavior if $n < 1$. The lower n means the more pronounced non-Newtonian behavior.

Table II gives the values of parameters K and n and the corresponding regression coefficients (R^2). The regression coefficients showed that the power law model was a basically suitable to fit for the experimental data of all the examined samples. It was found that all n values obtained are not excess 1, showing that these solutions behaved as pseudoplastic shear-thinning fluids. Moreover, the n values had a continuous increase with the increased content of HPMC, which indicated the more content of HPMC, the lower degree of shear thinning. Meanwhile, the n values of samples with ratios from 5/5 to 3/7 exhibited an obvious increase from 0.28 to 0.42, indicating the flow behavior of blends was distinctly different when the HPMC content was greater than 50%. In addition, the degree of thickening could

Table I. Percentage of Recovery Capacity of COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC When Subjected to a Shear Stress of 1 Pa

	Recovery (%)	
Samples	25°C	34°C
COL	94.89	84.03
C/H (7/3)	85.17	69.33
C/H (5/5)	60.24	47.87
C/H (3/7)	55.01	35.14
HPMC	10.03	47.42

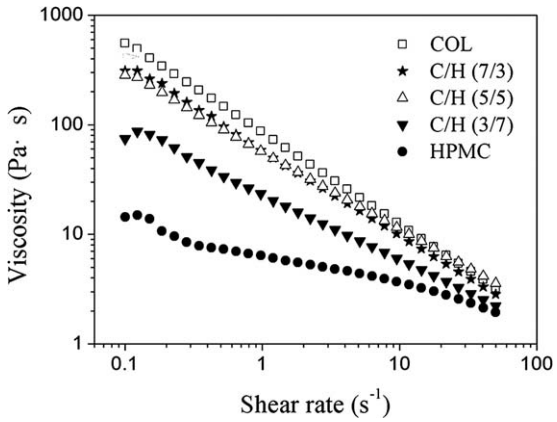

Figure 5. Apparent viscosity versus shear rate for COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC at 25°C.

Table II. Values of the Parameters in Power Law Model Fitting of COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC

Samples	*K*	*n*	R^2
COL	86.72	0.18	0.99
C/H (7/3)	62.32	0.26	0.99
C/H (5/5)	57.70	0.28	0.99
C/H (3/7)	24.26	0.42	0.98
HPMC	6.62	0.68	0.96

be quantified by the *K* values. As seen in Table II, the *K* values had an obvious decrease as the addition of HPMC, and there exhibited a dramatical decrease when the HPMC content was beyond 50%. These results demonstrated that these solutions became less pseudoplastic and less viscous as the HPMC content increased. It was evident that HPMC had a considerable effect on the flow behavior of collagen solution as the content of HPMC >50%.

To analyze the possible thixotropic character of the COL/HPMC blends and their component solutions, the "upward" and "downward" shear stress/shear rate curves were presented in Figure 6, and the areas of the corresponding hysteresis loops are displayed in Table III. The hysteresis areas decreased with the increased HPMC content, indicating collagen solution had a stronger thixotropic property than the others. It was noteworthy that the hysteresis areas of all samples were not distinct, especially when the HPMC content was greater than 50%, showing a weaker thixotropic property for all samples.

It is known that the thixotropy of a polymer solution is quantified by the solution ability to regain its gel structure when it is allowed to rest for a period of time, which is usually characterized by its capacity to rethicken when the solution is left at rest.[30] Therefore, collagen solution had a stronger ability to build-up its structure after shearing as compared with COL/HPMC blends and HPMC solution. Furthermore, the thixotropic areas of COL/HPMC blends were decreased rapidly especially when HPMC content was above 50%.

Table III. Values of the Thixotropic Areas of COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC

	COL	C/H (7/3)	C/H (5/5)	C/H (3/7)	HPMC
Thixotropic areas (Pa s)	82.6	80.2	74.5	26.8	19.8

DSC Thermograms

The influence of HPMC on the thermal stability of collagen was investigated by DSC measurement and the thermograms of COL and COL/HPMC blends are given in Figure 7. All the denaturation temperature (Td) of COL/HPMC blends was higher than that of COL, indicating an increase in thermal stability for the COL/HPMC blends, and the maximum Td was 41.6°C for C/H (5/5), which was 1.5°C higher than that of COL (40.1°C). In addition, the peak became weaker as the content of HPMC increasing, which was due to the collagen content in COL/HPMC blends. In general, the trends of Td observed from DSC were in keeping with those of rheological behavior in dynamic temperature sweeps.

AFM Images

Figure 8 is the AFM images of collagen, HPMC and their blends. There were obvious differences of images with the addition of HPMC. When compared to COL [Figure 8(a)], the fibers of C/H (7/3) and C/H (5/5) were observed to be thicker

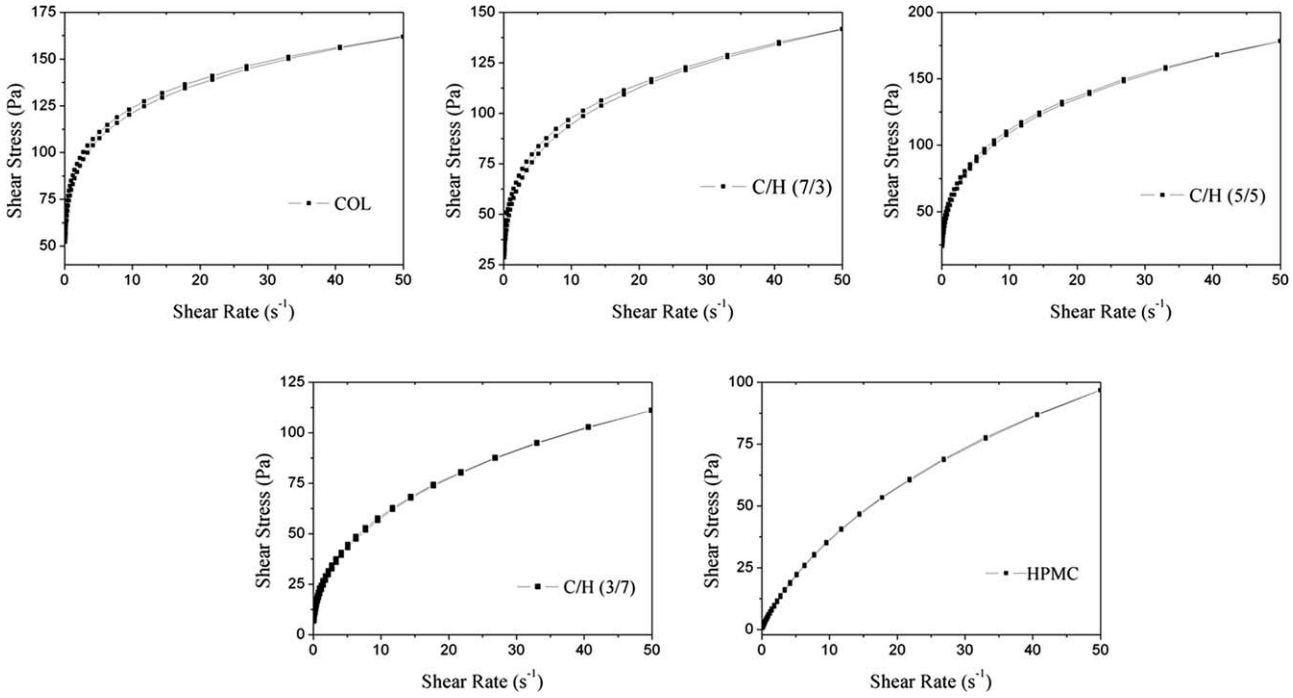

Figure 6. Thixotropic response of COL, C/H (7/3), C/H (5/5), C/H (3/7), and HPMC at 25°C.

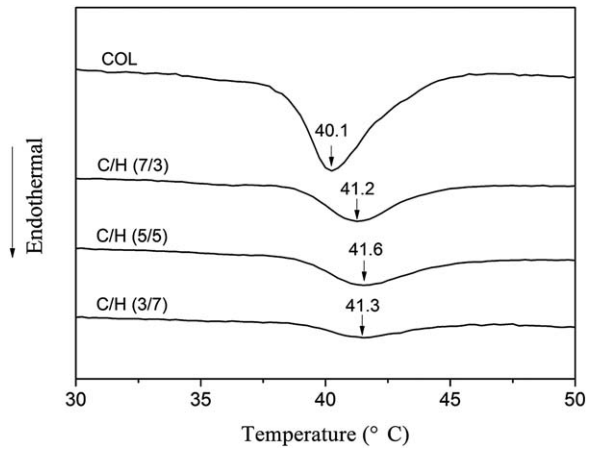

Figure 7. DSC thermograms of COL, C/H (7/3), C/H (5/5), and C/H (3/7).

and looser [Figure 8(b,c)], especially for the sample C/H (5/5), which exhibited a homogenous morphology of fibers with a larger diameter than other samples. The image of C/H (3/7) appeared relatively inhomogenous, which might be due to the relatively weak compatibility between collagen and HPMC molecules. Additionally, the HPMC showed the microstructure more like film with many pores [Figure 8(e)]. The changes in microstructure of blends reflected the aggregation behavior of molecules in solutions, which might be related with the hydrogen bonds and compatibility between collagen and HPMC.[13]

SEM Images

Figure 9 represents the SEM micrographs of COL/HPMC sponges with the magnification of 50. Both of COL and HPMC showed microporous structure [Figure 9(a,e)]. The morphology of COL/HPMC blends changed with the variation of HPMC content [Figure 9(b,c,d)]. The sample of C/H (7/3) still showed microporous structure, which was similar with COL but the pores were smaller than those of COL. More interestingly, the sample of C/H (5/5) showed regular sheet structure without any pores and the sheets were connected by little fibers, meanwhile the Td of C/H (5/5) reached the maximum. Thus, the interaction between collagen and HPMC molecules was probably strong when the HPMC content was 50% in blends. As the sample of C/H (3/7), the structure was inhomogenous, which was consistent with our previous work that the poor compatibility between collagen and HPMC molecules as the HPMC content reached 70% in blends. Although AFM and SEM exhibited the different state of collagen, the structure and the conformation of molecules in solution could be reflected by both the

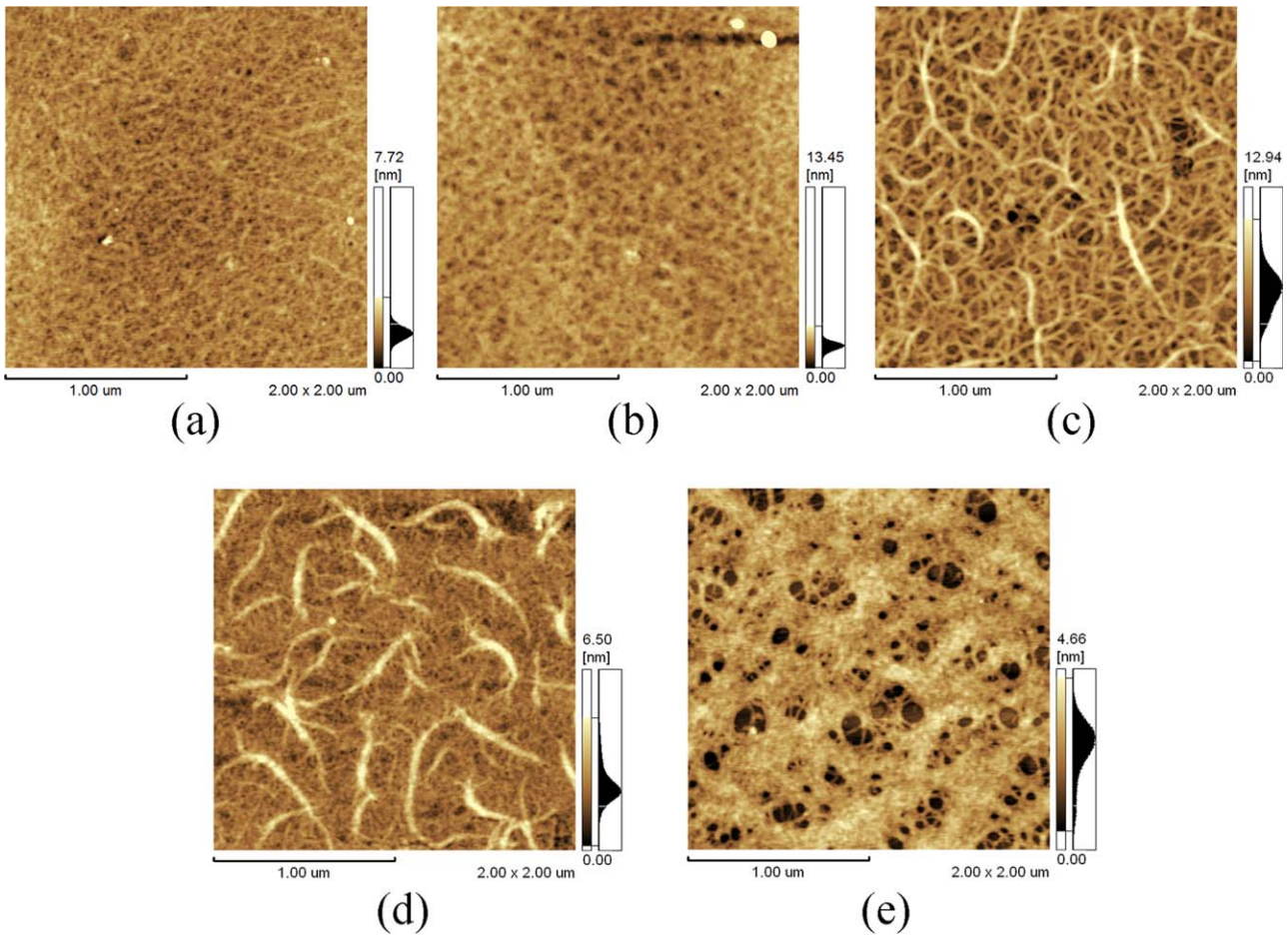

Figure 8. AFM images for (a) COL, (b) C/H (7/3), (c) C/H (5/5), (d) C/H (3/7), and (e) HPMC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

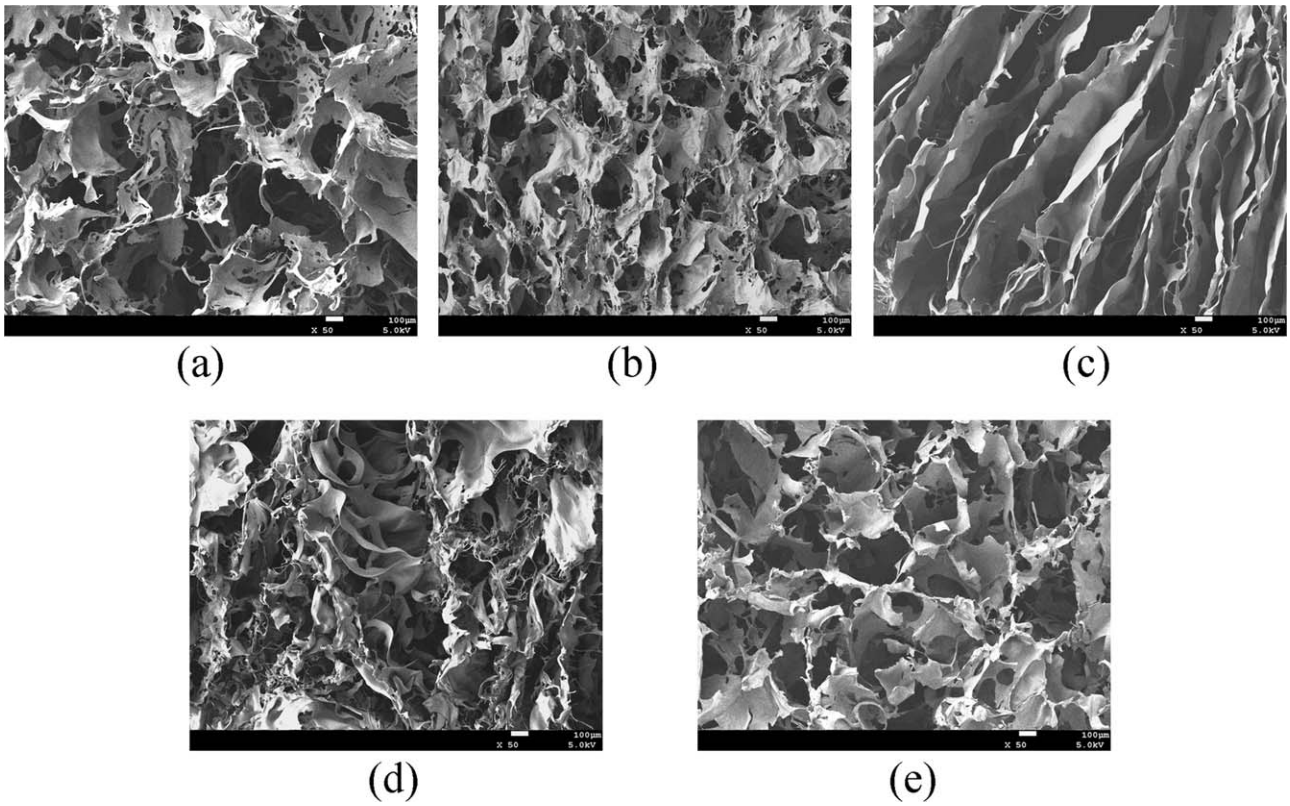

Figure 9. SEM images for (a) COL, (b) C/H (7/3), (c) C/H (5/5), (d) C/H (3/7), and (e) HPMC with a magnification of 50. Bars: 100 μm.

two methods. That is, as HPMC content was no more than 50%, samples with a rule morphology would be observed and these samples would become more compact with the increase of HPMC content, while a inhomogenous morphology of blend would be observed as HPMC content >50%.

The changes in the morphologies of COL/HPMC blends were consistent with the changes in their rheological properties. That is, when HPMC content was only 30%, both of the rheological properties and morphology of blends was similar to those of collagen. As HPMC content reached 50%, there might be a strong interaction between collagen and HPMC molecules, which brought about a higher thermal stability and a compact morphology for the blends. When HPMC content reached 70%, the viscoelastic behaviors of COL/HPMC blend still tend to be like those of collagen, indicating that collagen played a dominant role in the viscoelasticity of COL/HPMC even as HPMC content reached 70%. However, some of the rheological and structural properties such as the ability to build-up its structure after shearing became weaker, accompanied by the heterogeneous morphology as observed from both of AFM and SEM, which probably due to the weak interaction and the poor compatibility between collagen and HPMC molecules in solutions.

CONCLUSIONS

Rheological properties of collagen, HPMC and their blends were studied in the present work. In the blends, collagen played a dominant role in viscoelasticity even as HPMC content reached 70%, that is, the blends remained the viscoelastic behaviors similar to those of collagen but not HPMC. Meanwhile, the flowability and thermal stabilities of collagen solutions, which were important for the processing, could be improved effectively by the addition of HPMC. Besides, other properties of blends such as recovery capacity and thixotropic behavior also exhibited dependences on HPMC content. The changes in morphology observed from AFM and SEM indicated that the interaction and compatibility between collagen and HPMC molecules had an effect on the aggregation behaviors of collagen and HPMC molecules, which might be related to the changes in the rheological properties of the blends. The dependence of rheological parameters on HPMC content would provide a useful guideline for practice processing, especially in biomedical field.

ACKNOWLEDGMENTS

Thanks to Dr. Hui Wang in Analytical Testing Center of Sichuan University for her help. This research was supported by the National Natural Science Foundation of China (Nos. 21076129 and 21276167).

REFERENCES

1. Kolodziejska, I.; Sikorski, Z. E.; Niecikowska, C. *Food Chem.* **1999**, *66*, 153.
2. Nishihara, T.; Doty, P. *Proc. Natl. Acad. Sci. U S A* **1958**, *44*, 411.

3. Li, D.; Yang, W.; Li, G. Y. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 1041.
4. Lai, G. L.; Li, Y.; Li, G. Y. *Int. J. Biol. Macromol.* **2008**, *42*, 285.
5. Langer, R. *Pharm. Res.* **1997**, *14*, 840.
6. Ye, Q.; Zund, G.; Jockenhoevel, S.; Hoerstrup, S. P.; Schoeberlein, A.; Grunenfelder, J.; Turina, M. *Eur. J. Cardio. Surg.* **2000**, *17*, 449.
7. Clasen, C.; Kulicke, W. M. *Prog. Polym. Sci.* **2001**, *26*, 1839.
8. Kanth, S. V.; Ramaraj, A.; Rao, J. R.; Nair, B. U. *Process Biochem.* **2009**, *44*, 869.
9. Anumary, A.; Thanikaivelan, P.; Ashokkumar, M.; Kumar, R.; Sehgal, P. K.; Chandrasekaran, B. *Soft Mater.* **2013**, *11*, 181.
10. Karavas, E.; Georgarakis, E.; Bikiaris, D. *Eur. J. Pharm. Biopharm.* **2006**, *64*, 115.
11. Li, S. F.; Lin, S. S.; Daggy, B. P.; Mirchandani, H. L.; Chien, Y. W. *Int. J. Pharm.* **2003**, *253*, 13.
12. Lim, W. S.; Choi, J. W.; Iwata, Y.; Koseki, H. J. *Loss Prevent. Proc.* **2009**, *22*, 182.
13. Ding, C. C.; Tian, H. L.; Li, G. Y. *Funct. Mater.* **2012**, *43*, 992.
14. Ding, C. C.; Zhang, M.; Tian, H. L.; Li, G. Y. *Int. J. Biol. Macromol.* **2013**, *52*, 319.
15. McPherson, J. M.; Sawamura, S.; Armstrong, R. *J. Biomed. Mater. Res.* **1986**, *20*, 93.
16. Toung, J. S.; Ogle, R. C.; Morgan, R. F.; Lindsey, W. H. *Arch. Otolaryngol. Head Neck Surg.* **1999**, *125*, 451.
17. Buttafoco, L.; Kolkman, N. G.; Engbers-Buijtenhuijs, P.; Poot, A. A.; Dijkstra, P. J.; Vermes, I.; Feijen, J. *Biomaterials* **2006**, *27*, 724.
18. Li, Y. H.; Huang, Y. D. *J. Appl. Polym. Sci.* **2006**, *99*, 1832.
19. Sumita, Y.; Honda, M. J.; Ohara, T.; Tsuchiya, S.; Sagara, H.; Kagami, H.; Ueda, M. *Biomaterials* **2006**, *27*, 3238.
20. Kao, N.; Bhattacharya, S. N.; Shanks, R.; Coopes, I. H. *J. Rheol.* **1998**, *42*, 493.
21. Zhang, Z. K.; Li, G. Y.; Shi, B. *J. Soc. Leather Technol. Chem.* **2006**, *90*, 23.
22. Li, M.; Zhang, T. K. *Colloid Polym. Sci.* **2006**, *285*, 145.
23. Ferry, J. D. Viscoelastic Properties of Polymers, 3rd ed.; Wiley: New York, **1980**; Chapter *2*, pp 41–43.
24. Al-Ruqaie, I. M.; Kasapis, S.; Abeysekera, R. *Carbohydr. Polym.* **1997**, *34*, 309.
25. Doi, M.; Takimoto, J. I. *Phil. Trans. R. Soc. Lond. A* **2003**, *361*, 641.
26. Clasen, C.; Kulicke, W. M. *Prog. Polym. Sci.* **2001**, *26*, 1839.
27. Zhang, M.; Chen, Y. H.; Li, G. Y.; Du, Z. L. *Korea-Aust. Rheol. J.* **2010**, *22*, 119.
28. Schramm, G. A Practical Approach to Rheology and Rheometry, 2nd ed.; Gebrueder HAAKE GmbH, Karlsruhe, Federal Republic of Germany, **1998**; Chapter *9*, pp 235–239.
29. Monkos, K. *Int. J. Biol. Macromol.* **1996**, *18*, 61.
30. Edali, M.; Esmail, M. N.; Vatistas, G. H. *J. Appl. Polym. Sci.* **2001**, *79*, 1787.
31. Coussot, P.; Nguyen, Q. D.; Huynh, H. T.; Bonn, D. *J. Rheol.* **2002**, *46*, 573.
32. Salomé Machado, A. A.; Martins, V. C. A.; Plepis, A. M. G. *J. Therm. Anal. Calorim.* **2002**, *67*, 491.