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Preparation and characterization of collagen/hydroxypropyl methylcellulose (HPMC) blend film

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ABSTRACT

This study aimed to prepare and characterize the collagen/HPMC blend film (1/1). Thermogravimetric analysis and differential scanning calorimetry were used to investigate the thermal properties of the film. Both thermal decomposition temperature and denaturation temperature of the blend film were higher than those of the collagen film due to the intermolecular hydrogen bonding interaction between collagen and HPMC, which was demonstrated by Fourier transform infrared spectroscopy. Additionally, the morphologies, mechanical properties and hydrophilicity of films were examined. The blend film exhibited a more homogeneous and compact structure compared with that of the collagen film, as observed from scanning electron microscopy and atomic force microscopy. The tensile strength, ultimate elongation and hydrophilicity of the blend film were superior to those of the pure collagen film. Furthermore, the introduction of polyethylene glycol 1500 had almost no influence on the thermal properties of the blend film but obviously improved its stretch-ability and smoothness.

1. Introduction

Type I collagen, which is the major structural protein of connective tissues, is widely used in fields including medicines, cosmetics, foods and chemical industries owing to its weak antigenicity, biodegradability, biocompatibility and bioactivity (Kolodziejska, Sikorski, & Niecikowska, 1999).

Collagen has a good film-forming property and collagen film has been extensively used for preparing scaffolds as a dural substitute, sustained-release film, dressing as well as edible sausage casing (Collins, Christiansen, Zazanis, & Silver, 1991; Maeda, Kadota, Kajihara, Sano, & Fujioka, 2001; Minabe, Takeuchi, Tomomatsu, Hori, & Umemoto, 1989; Tharanathan, 2003). However, collagen film had some disadvantages in physicochemical properties such as rough surface and low thermostability which limit its applications.

Hence, suitable chemical or physical modifications are needed to improve the physicochemical properties of collagen-based films. Over the past years, many studies have focused on the blends of collagen and other natural and synthetic polymers, such as hyaluronate, chitosan, cellulose and polyvinyl alcohol (Gough, Scotchford, & Downes, 2002; Hart et al., 2002; Lima et al., 2006; Tsai et al., 2005). Among these polymers, cellulose was usually blended with collagen for producing composite materials used for wound healing (Veves, Sheehan, & Pham, 2002) and tissue culture (Leighton, Justh, Esper, & Kronenthal, 1967; Russo, Bradley, McGrath, & Russo, 1977).

Cellulose is the most plentiful natural polymer on earth, which is of particular interest owing to its abundant availability and biodegradability. Although it is a highly hydrophilic biopolymer, it is not soluble in water owing to its highly crystalline nature. Solubility can be achieved by altering its ordered crystalline regions by chemical substitution, generating cellulose derivatives (Pérez, Sánchez, Rodríguez Patino, & Pilosof, 2006). Cellulose derivatives are used in a wide variety of fields such as food, pharmaceutical, textile and adhesive (Clasen & Kulicke, 2001; Stephen & Phillips, 2006). Among the cellulose derivatives, hydroxypropyl methylcellulose (HPMC) has been extensively employed because of its ease of use, wide availability, superior film-forming capability, good biocompatibility and biodegradability. It is usually used in the pharmaceutical industry as a drug delivery matrix (film or gel) (Ford, 1999; McCrystal, Ford, & Rajabi-Siahboomi, 1997) and food industry as a film former, emulsifier, stabilizer or thickener (Barcenas & Rosell, 2005; Rosell, Rojas, & Benedito de Barber, 2001).

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As both collagen and HPMC possess good biological properties and film-forming ability, some researchers mixed collagen and HPMC for the preparation of collagen/HPMC composite film used in drug delivery system (Veeruraj, Arumugam, Ajithkumar, & Balasubramanian, 2012). They tested the composite film as the drug carrier against human pathogenic (bacteria and fungi) microorganisms and found that the standard commercial drugs such as ampicillin and tetracycline were successfully delivered by the composite film. However, there is little information on the comprehensive characterization of their binary blend films which have high potential to be used as drug delivery membrane, food packaging and artificial skin materials. Considering all these potentials, this present work focused on the preparation and characterization of the collagen/HPMC blend film. In our previous studies, the rheological properties of collagen/HPMC blend solutions with different HPMC content were investigated and the results indicated that the collagen/HPMC blend solution with a ratio of 1/1 by weight was more thermally stable compared with other ratios, and the compatibility between collagen and HPMC was good under such condition (Ding, Zhang, & Li, 2014). Since the properties of composite material were closely related to the compatibility among each single component, we investigated the collagen/HPMC composite film with the ratio of 1/1, expecting to obtain a blend film with better physicochemical properties.

In addition, it was known that plasticizers strongly affect the physical properties of biopolymer films (Zhang & Han, 2008). The plasticizers could help to decrease inherent brittleness of films by reducing intermolecular forces, increasing the mobility of polymer chains, decreasing the glass transition temperature of these materials and improving their flexibility (Galdeano, Mali, Grossmann, Yamashita, & García, 2009; Zhang & Han, 2008), thus a non-toxic plasticizer-polyethylene glycol 1500 (PEG 1500) was added to the collagen/HPMC blend film, considering improving the physical properties of the composite film.

The objective of the present work was to prepare the collagen/HPMC blend film and characterize its thermal stability, morphology, mechanical properties and hydrophilicity. Meanwhile, the effect of PEG on the properties of the composite film was also investigated. Furthermore, the relationship among the compatibility, interactions and properties of the composite film was discussed as well.

2. Experimental

2.1. Materials

Collagen was extracted in our laboratory from bovine skin by the method described by Zhang, Li, and Shi (2006). Hydroxypropyl methylcellulose (HPMC) (HF 4000) 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%. Polyethylene glycol (PEG) 1500 (>99.5% purity) was used as a plasticizer.

2.2. Extraction of collagen

The delimed and neutralized bovine splits were cut into smaller pieces and pulverized with a mill (Fritsch Pulverisette 14, Germany). The powders were extracted with 0.5 mol/L acetic acid containing 3% pepsin (1:3000, calculated on the dry weight of powders) at 4 °C for 3 days, and then the supernatants were collected by refrigerated centrifugation at 9000 × *g* and salted out by the addition of NaCl to a final concentration of 0.7 mol/L, the precipitate was again dissolved in 0.5 mol/L acetic acid, and then dialyzed against 0.1 mol/L acetic acid for 3 days, finally, lyophilized in a freeze dryer (Labconco Freeze Dryer FreeZone 6 Liter, USA) at −50 °C for 2 days and stored at 4 °C until used. In addition, the dialysis membrane tube with molecular weight cut off 14,000 was used and its diameter was 28 mm.

2.3. Sample preparation

Four solutions were prepared as follows: the collagen solution with concentration of 15 mg/mL was prepared by dissolving collagen sponge in 0.1 mol/L acetic acid; The HPMC solution with concentration of 15 mg/mL was prepared by dissolving HPMC powder in 0.1 mol/L acetic acid. The blend solution of collagen and HPMC was prepared by mixing collagen solution (15 mg/mL) with HPMC solution (15 mg/mL) at the collagen/HPMC ratio of 1/1 (by weight), and the solutions were mixed by gently stirring for 24 h at 4 °C; PEG 1500 (10%, based on polymer weight percentage) was added into collagen/HPMC blend solution to obtain solution with plasticizer.

All prepared solutions were then centrifuged at 9000 × *g* for 10 min at 4 °C to remove entrapped air-bubbles, and then poured into a silicon mold, left them to dry at room temperature (~20 °C). After this step, these air-dried films were peeled off carefully and stored in a glass desiccator equipped with silicone for 7 days before use (50 ± 2% RH, 20 ± 1 °C). The films derived from collagen solution, HPMC solution, collagen/HPMC blend solution as well as collagen/HPMC blend solution with PEG, were named as COL, HPMC, COL/HPMC and COL/HPMC(P), respectively.

2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the films were recorded using a FTIR spectrophotometer (Thermo Scientific Nicolet IS10, USA). The measurement was performed with a resolution of 4 cm^{-1} and in the range of 800–4000 cm^{-1}, 32 scans were performed for each film.

2.5. Thermogravimetric analysis (TGA)

TG analysis of the films was carried out on a thermal analyzer (Netzsch TG 209, Germany). Sample of approximately 2 mg was heated under N_2 atmosphere from 40 to 600 °C with a heating rate of 10 °C/min.

2.6. Differential scanning calorimetry (DSC)

The thermal stability of collagen in the prepared films was characterized using DSC (Netzsch DSC 200PC, Germany). The samples (2–3 mg) were weighted accurately into aluminium pans and sealed, and then scanned from 25 to 90 °C with a heating rate of 5 °C/min under N_2 atmosphere. Liquid nitrogen was used as a cooling medium and empty pans were used as a reference.

2.7. Dynamic mechanical analysis (DMA)

Storage modulus (E') and loss factor ($\tan\delta$) of the samples (10.0 × 5.0 mm^2) were measured as a function of temperature using a DMA analyzer (Netzsch DMA 242 C, Germany). The tests were conducted at a constant frequency of 1 Hz from 40 to 180 °C with a heating rate of 10 °C/min. The environmental chamber was purged with dry N_2.

2.8. Scanning electron microscopy (SEM)

The cross sectional morphologies of the prepared films were observed using SEM (JEOL, JSM-7500F, Japan). The treated films were fixed on the conductive adhesive, gold coated, and observed with an accelerating voltage of 5 kV.

2.9. Atomic force microscopy (AFM)

The microstructure of the films was observed by AFM (SHIMADZU SPM 9600, Japan) in dynamic mode at room temperature (~20 °C). Each sample was scanned with a scanning rate of 1 Hz.

2.10. Tensile tests

Film thickness was measured using a 0–25 mm manual micrometer, with a resolution of 0.01 mm. The reported values were the average of 6 readings taken randomly on each film sample.

The mechanical characteristics of films were evaluated by tensile strength (TS, MPa) and ultimate elongation (UE, %) using an electronic universal material tensile machine (UTM 6203, China) according to standard NF T 54-102 (1971) on five specimens previously stored for 7 days at 20 ± 1 °C and 50 ± 2% RH. Films of 50 mm × 5 mm (analysed area = 25 mm × 5 mm) uniaxially stretched in the vertical direction at a constant speed of 10 mm/min. Maximum TS is the largest stress that a film is able to sustain, and UE is the maximum percentage change in the length of a film before breaking.

2.11. Water contact angle tests

The hydrophilicity of the films was tested with a goniometer (dataphysics OCA-H200, Germany) by measuring the surface water contact angle (WCA) at room temperature (20 ± 1 °C). In brief, distilled water droplet of 6 μL was carefully deposited onto the surface of a sample, and angles were measured on six different regions of each surface and averaged.

3. Results and discussion

3.1. FTIR of the films

The FTIR spectra of the COL film, the HPMC film and the blend films in the wavenumber range of 800–4000 cm^{-1} are shown in Fig. 1. The corresponded assignments of the absorption peaks are summarized in Table 1. The assignments of absorption peaks associated with major functional groups of collagen were based upon Sionkowska's works (Sionkowska, Wisniewski, Skopinska, Kennedy, & Wess, 2004); the assignments of absorption bands of HPMC were based on Sadtler standard IR spectra handbook (Bio-Rad Laboratories, Inc. U.S.) and some literatures (Wang, Dong, & Xu, 2007; Yin, Luo, Chen, & Khutoryanskiy, 2006).

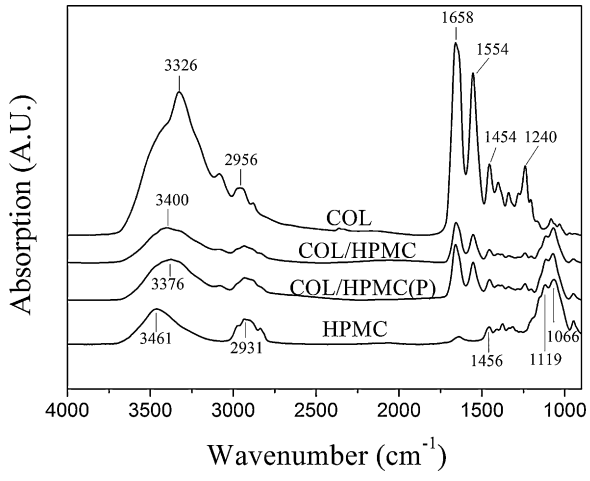

Fig. 1. FTIR spectra of films of COL, COL/HPMC, COL/HPMC(P) and HPMC.

Table 1
Assignments of the bands in the FTIR spectra of the films.

	Wavenumber (cm^{-1})	*Assignment*
COL	3326	N—H stretching vibration, coupled with hydrogen bonding.
	2958	—CH_2 asymmetric stretching
	1658	C=O stretching + C—N stretching + N—H bending vibrations.
	1554	N—H bending + C—N stretching vibrations.
	1240	C—N stretching + in-plane N—H bending + CH_3—C stretching vibrations.
COL/HPMC	3400	O—H stretching vibrations.
	1662	C=O stretching + C—N stretching + N—H bending vibrations.
	1552	N—H bending + C—N stretching vibrations.
	1240	C—N stretching + in-plane N—H bending + CH_3—C stretching vibrations.
	1068	C—O stretching vibration.
HPMC	3461	O—H stretching vibrations.
	2931	C—H stretching.
	1456	CH_3 asymmetric bending vibration.
	1119	C—O—C asymmetric stretching vibration.
	1066	C—O stretching vibration.

As shown in Fig. 1, the FTIR spectrum of the COL film exhibited four characteristic absorption bands at 3326, 1658, 1554 and 1240 cm^{-1}, which corresponded to amide A band, amide I band, amide II band and amide III band, respectively. From the spectrum of HPMC, the characteristic peaks were at 3461 and 1066 cm^{-1} attributed to the stretching vibration of O—H and C—O groups, respectively. The band at 1643 cm^{-1} was assigned to water in the amorphous region (Liang & Marchessault, 1959). It was interesting to note that the COL/HPMC film show characteristic bands of both collagen (1662, 1552 and 1240 cm^{-1}) and HPMC (3400 and 1068 cm^{-1}). Compared with the characteristic peaks of COL and HPMC, the absorption peaks of the COL/HPMC film assigned to amide I band and amide II band from collagen as well as O—H and C—O groups from HPMC moved from 1658, 1554, 3461 and 1066 cm^{-1} to 1662, 1552, 3400 and 1068 cm^{-1}, respectively. This indicated that the intermolecular hydrogen bonds formed between collagen and HPMC in the composite film. Moreover, it was known that the absorption band of OH would shift to a lower frequency due to stretching vibration if the intermolecular and intramolecular hydrogen bonds are formed (Wang, Ren, Li, Sun, & Liu, 2014). As seen in Fig. 1, O—H stretching vibration absorption band in the blend film shifted from 3400 to 3376 cm^{-1} caused by the introduction of PEG, indicating the formation of hydrogen bonds between PEG and collagen or HPMC molecules.

3.2. Thermal properties of the films

TGA determines mass change as a function of temperature or time and is used to determine residual solvents in a sample as well as to evaluate thermal stability. TGA curves which characterized the thermal destruction of all films in nitrogen are presented in Fig. 2(a). The derivative thermogravimetric (DTG) curves at the bottom left shows the temperature of the starting point of decomposition (T_0) and the maximum speed of the process (T_m).

The thermal destruction of the COL film consisted of two stages and the first stage was the evaporation of water present in collagen. Usually, the first peak related to the evaporation of unbound water is very wide and therefore it is not possible to determine the temperature of the process, so we did not analyze the first stage in the TGA curves. The second thermal event of the COL film started at 276 °C (T_0) with a maximum value at 328 °C (T_m) and in

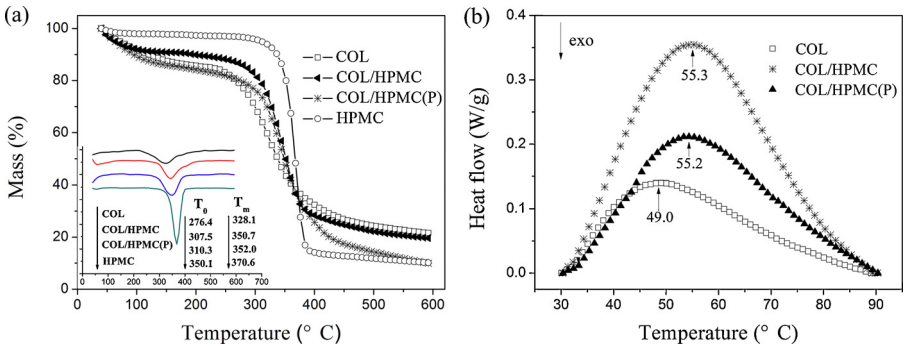

Fig. 2. TGA curves of COL, COL/HPMC, COL/HPMC(P) and HPMC obtained at a heating rate of 10 °C/min under dynamic N_2 atmosphere (a) (DTG curves at the bottom left); DSC curves of COL, COL/HPMC and COL/HPMC(P) obtained at a heating rate of 5 °C/min under dynamic N_2 atmosphere (b).

the second stage water bound to collagen was released and small molecular products of thermal degradation of collagen were liberated. This degradation profile of the COL film was in good agreement with previous study (Kaminśka & Sionkowska, 1996). The starting point of decomposition (T_0) in the second stage of the HPMC film was observed at about 350 °C with the maximal rate at 370 °C and was related to the cellulose ethers degradation, which included the parallel processes of dehydration and demethoxylation (Yin et al., 2006).

Notably, the second stage of the COL/HPMC film started at 307 °C (T_0) with T_m of 351 °C, both of which were higher than those of the COL film (276 and 328 °C, respectively). Hence, it was indicated that the COL/HPMC film had a higher stability toward thermal degradation compared with the COL film. There was no other peak in the second thermal event, which suggested well compatibility between collagen and HPMC at the ratio of 1/1. Furthermore, the addition of PEG (10%) had little influence on the degradation profile for the composite film (T_0 and T_m of COL/HPMC(P) were 310 and 350 °C, respectively). Based on the results of FTIR, the improved thermal stability of the composite film was probably due to the formation of intermolecular hydrogen bonds between collagen and HPMC.

Another temperature-related test is DSC, which is often conducted on collagen-based materials. In general, DSC is now accepted as the technique to determine the energetics of protein folding/unfolding transitions (Freire, 1995). One of the main indicators of thermal denaturation behavior of collagen is thermal denaturation temperature (T_d). Fig. 2(b) shows the DSC profiles of the films containing collagen component. The endothermic peak of the COL/HPMC film was about 55 °C, which was higher than that of the COL film (49.0 °C), indicating the addition of HPMC improved the thermal stability of collagen owing to the interactions between collagen and HPMC molecules. Furthermore, the addition of PEG (10%) almost had no influence on T_d of collagen in the composite film.

3.3. DMA of the films

DMA experiments were conducted to measure the thermomechanical strength of the films by monitoring temperature-modulus curves. Storage modulus (E') and loss factor ($\tan\delta$) (ratio of loss modulus to storage modulus) are determined. Fig. 3(a) and (b) respectively show the E' and $\tan\delta$ curves of the films as a function of temperature in the range of 40–180 °C.

It could be seen from Fig. 3(a) that the E' values of the COL/HPMC film were lower than those of the COL film till E' dropped sharply. Since E' reflected the stiffness (rigidity) of material (Jiang et al., 2012), the decrease of E' meant that the COL/HPMC film became softer. Furthermore, the E' values of the blend film decreased with the addition of PEG, similarly indicating the stiffness of the blend film decreased by the introduction of PEG. Tan δ reached the maximum at the temperature where E' decreased sharply, as shown in Fig. 3(b). As for the COL film, the maximum tan δ value was at 153 °C, basically consistent with the results of Witnauer and Fee (1957) as well as Flory and Garrett (1958). Meanwhile, glass transition temperature (T_g) for HPMC film was 166 °C, also in accordance with the values in other literatures, in which T_g varied from 157 to 180 °C (Kararli, Hurlbut, & Needham, 1990). It was worthwhile to mention that the tan δ value of COL/HPMC (171 °C) was obviously higher than that of COL and HPMC, which might be due to the intermolecular hydrogen bonds and entanglement between collagen and HPMC molecules. The interactions brought about some difficulties in the cooperative micro-Brownian motion of the molecular chains. Moreover, the thermal transition temperature of COL/HPMC(P) (161 °C) was lower than that of COL/HPMC, since PEG gradually diffused into the polymer chains, reduced the intermolecular forces of attraction along the polymer chains, allowed the polymer chains to move more easily, and consequently, lowered the storage modulus and the glass transition temperature (Bhushan, 2010).In addition, in DMA studies the peak temperature of the tan δ curve is often used as a criterion to assess the miscibility of the polymer blend: a single transition of the blend indicates a good miscibility, whereas separate transitions of the mixture indicates immiscibility of their components (Wang & Xiao et al. 2014). Under the present conditions, only one transition appeared in the curves of the blend films (COL/HPMC and COL/HPMC(P)), indicating the thermodynamic miscibility among collagen, HPMC and PEG molecules.

3.4. SEM

The morphologies of the cross-section of films observed by SEM are displayed in Fig. 4. Both of the COL (Fig. 4(a)) and COL/HPMC (Fig. 4(b)) films showed layered structures across the cross-section (the red dashed outline). The cross-section of COL/HPMC presented a more homogeneous and compact structure compared with that of COL, which confirmed the compatibility of the two polymers. The pure HPMC film showed a quite homogeneous structure exhibiting good properties of film-forming (Fig. 4(c)). In addition, the layered structure disappeared in the COL/HPMC(P) film and exhibited a dense structure (Fig. 4(b′)), which was probably due to the hydrogen bonding between collagen or HPMC and PEG molecules, confirming the good compatibility among them.

Meanwhile, the inserts at the bottom left with a magnification of 10,000 showed the morphologies of films more clearly (the blue dashed outline). The cross-section of the COL/HPMC film became more homogeneous compared with that of the COL film; what is more, after adding PEG, the interspace disappeared in the

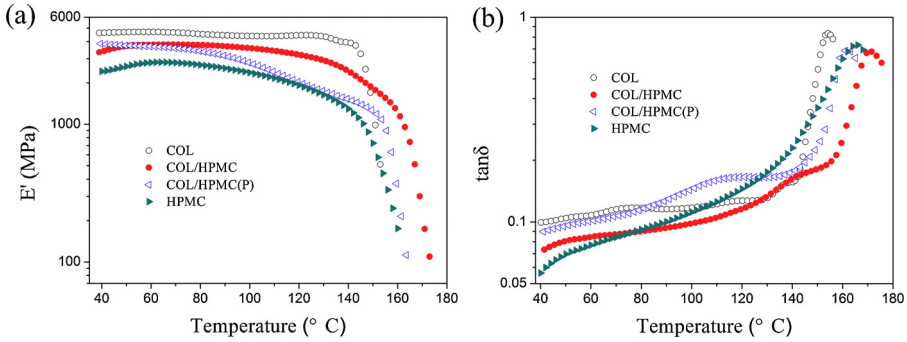

Fig. 3. Storage modulus (E') and loss factor (tan δ) of COL, COL/HPMC, COL/HPMC(P) and HPMC measured by DMA (scanning rate: 10 k min^{-1}; 1 Hz).

cross-section of the COL/HPMC film and a quite compact structure could be observed.

3.5. AFM

To further explore the surface microstructure of the films, the surface roughness was analyzed by AFM. Fig. 5(I) and (II) shows the AFM phase images and three dimensional (3D) images, respectively. The corresponding roughness parameters calculated by the system-provided software are listed in the supplementary Table 1S. It could be seen from Fig. 5(I)a that the COL film exhibited the typical fibrillar structure of collagen, that is, many curved molecules or microfibrils lied on the mica substrate and overlapped with one another, whereas the COL/HPMC film (Fig. 5(I)b) just showed bits of microfibrils and the HPMC film (Fig. 5(I)c) exhibited a relatively flat surface. Furthermore, the blend film with PEG became quite uniform and smooth (Fig. 5(I)b′) just as observed by SEM.

Surface roughness of the films was clearly exhibited by 3D images. As shown in Fig. 5(II), the surface roughness of the COL/HPMC film reduced obviously compared to that of the COL film. Meanwhile the roughness of the blend film further decreased by the addition of PEG (Fig. 5(II)B′). The detailed difference in surface roughness was easily found out by the roughness parameters *Ra* and *Rq* in Table 1S. That is, the surface roughness of the COL/HPMC film reduced to about one fifth of the COL film, with the addition of PEG, the surface roughness continued to decline to a level even slightly lower than that of the pure HPMC film.

Therefore, the surface and cross section morphologies of the COL/HPMC film were more compact and homogeneous than those of the COL film, suggesting the good miscibility of collagen and

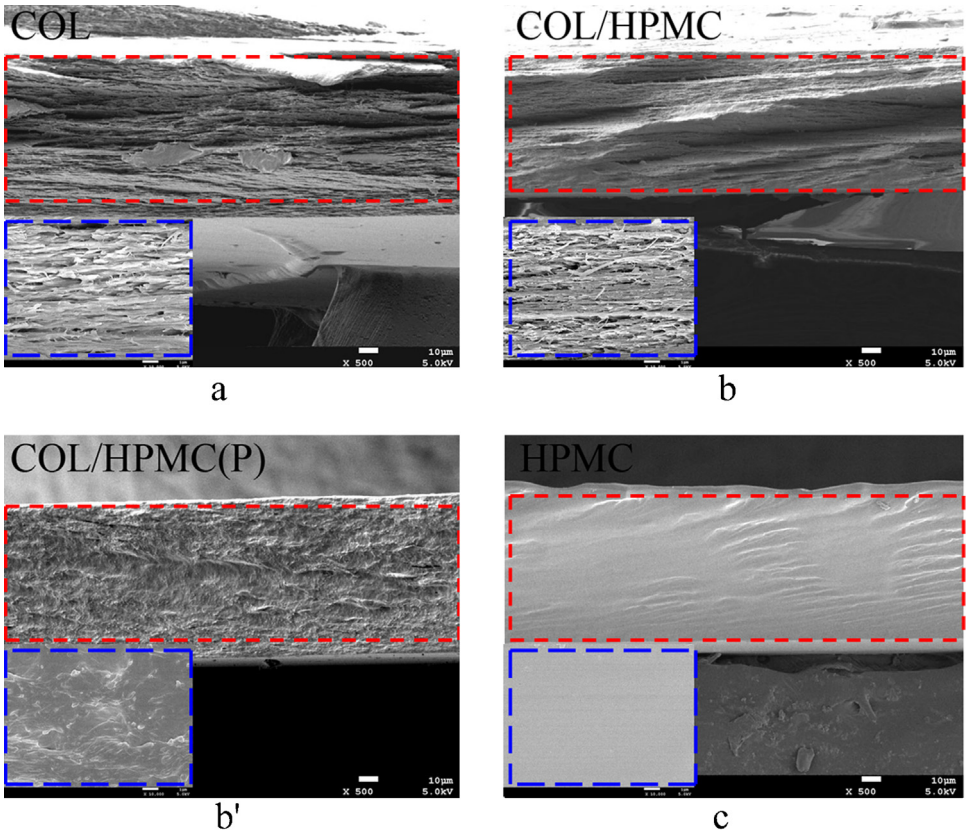

Fig. 4. SEM cross-sections of films of COL (a), COL/HPMC (b), COL/HPMC(P) (b′) and HPMC (c) with a magnification of 500 (the red dashed outline). Bars, 10 μm. Small figures at the bottom left with a magnification of 10,000 (the blue dashed outline). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

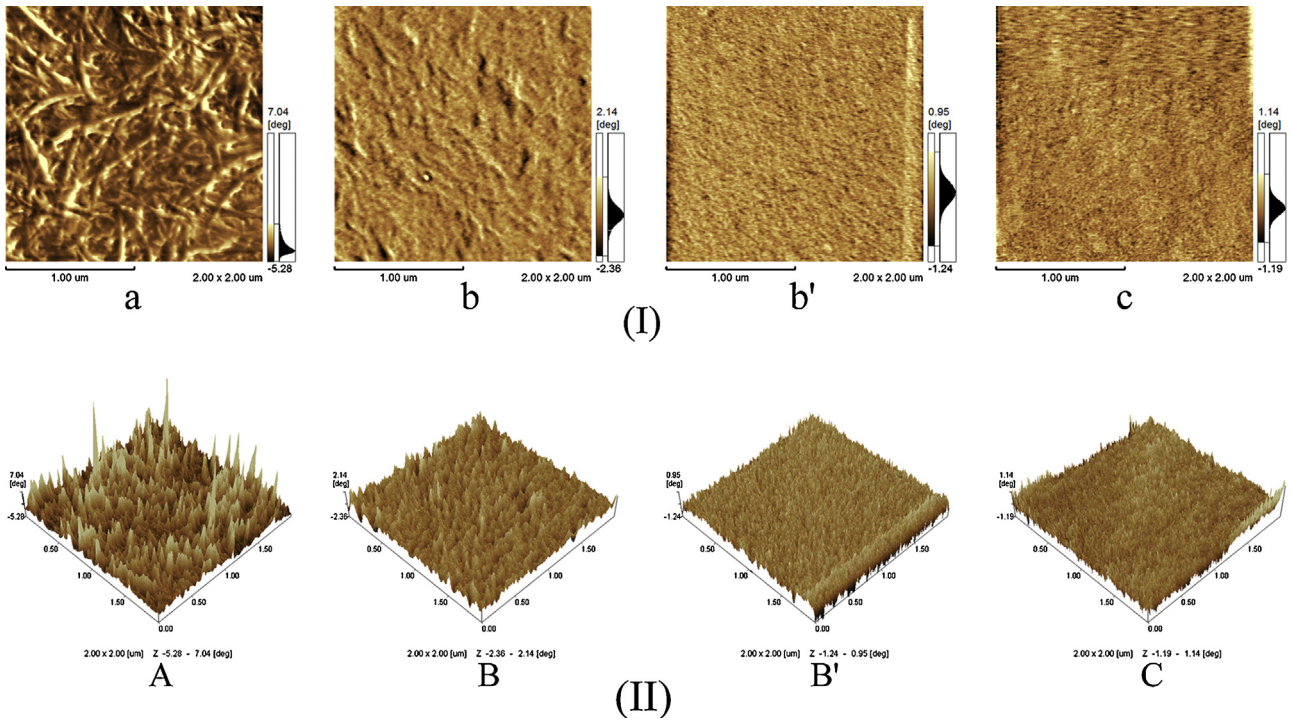

Fig. 5. AFM phase images (I) of COL (a), COL/HPMC (b), COL/HPMC(P) (b′) and HPMC (c) ($2 \times 2\,\mu m^2$); AFM 3D images (II) of COL (A), COL/HPMC (B), COL/HPMC(P) (B′), and HPMC (C) ($2 \times 2\,\mu m^2$).

HPMC blend at the composite ratio of 1/1 as demonstrated by TGA and DMA.

3.6. Mechanical properties

Mechanical properties of film materials are important for their practical applications. It was reported that the interaction among polymers will influence the mechanical properties of the blended polymer (Fan, Zhu, Zheng, Xu, & Zhang, 2007). Therefore, tensile strength (TS) and ultimate elongation (UE) of the films were evaluated from the load versus extension curves. The results of the relative mechanical parameters and the thickness of the films are presented in Table 2. It could be seen that both TS and UE of the COL/HPMC film were higher than those of the COL film, but the improvement was not significant, particularly for its stretch-ability. However, the introduction of PEG into the COL/HPMC film effectively improved the stretch-ability and just slightly decreased the mechanical stability. This was due to the fact that PEG as a plasticizer could help to decrease inherent brittleness of films by reducing intermolecular forces, increasing the mobility of polymer chains and improving their flexibility (Imran, El-Fahmy, Revol-Junelles, & Desobry, 2010), which was in agreement with the results of DMA. Hence, the increase of the elongation at break and the decreases of TS and storage modulus proved that PEG 1500 has the plasticizing effect on the COL/HPMC film.

It was worthy to note that the COL film was worse in smoothness than the films of both HPMC and COL/HPMC. As reported by our previous paper (Ding et al., 2014), the complex viscosity (η^*) values at 0.1 Hz of collagen solution (8 mg/mL), COL/HPMC (1/1, w/w) blend solution and HPMC solution (8 mg/mL) were 171.6, 65.6 and 3.2 Pa s, respectively. Hence, collagen solution possessed a quite high η^*, which caused a poor flowability in the casting process, as evidenced by the inferior cohesive cross-section (Fig. 4) and the high roughness of the COL film (Table 1S). While the η^* value of HPMC solution was much lower compared with that of collagen solution and COL/HPMC (1/1) blend solution had a moderate η^*. So it could be speculated that the smoothness and mechanical properties of film were related to the flowability of solution for film-forming.

Table 2
Parameters of mechanical property of the films.

	Thickness (μm)	*Tensile strength (TS) (MPa)*	*Ultimate elongation (UE) (%)*
COL	75 ± 10	49.2 ± 7.1	13.7 ± 2.8
COL/HPMC	67 ± 3	56.3 ± 7.6	15.6 ± 2.6
COL/HPMC(P)	68 ± 1	48.6 ± 6.7	23.8 ± 3.7
HPMC	65 ± 1	70.2 ± 11.0	20.3 ± 2.2

3.7. Water contact angle tests

To explore the correlation between the microstructure of the films and their surface property, the hydrophilicity was evaluated by water contact angle (WCA) measurement. Fig. 6 shows WCA of the films after equilibrating for 4 min. WCA of the COL film and HPMC film was about 90° and 60°, respectively, indicating the hydrophilicity of HPMC was much better than that of collagen. As expected, the hydrophilicity of the COL/HPMC film was obviously improved owing to the addition of hydrophilic groups (hydroxyl and hydroxypropoxy) from HPMC. Meanwhile, the hydrophilicity of the blend film further increased with the addition of hydrophilic PEG. Fratzl (2008) found that a good hydrophilic property of collagen-based scaffolds was favorable for cell's migration, attachment and proliferation. Thus, the changes of hydrophilic property of collagen/HPMC blends might have a potential application to cell culture.

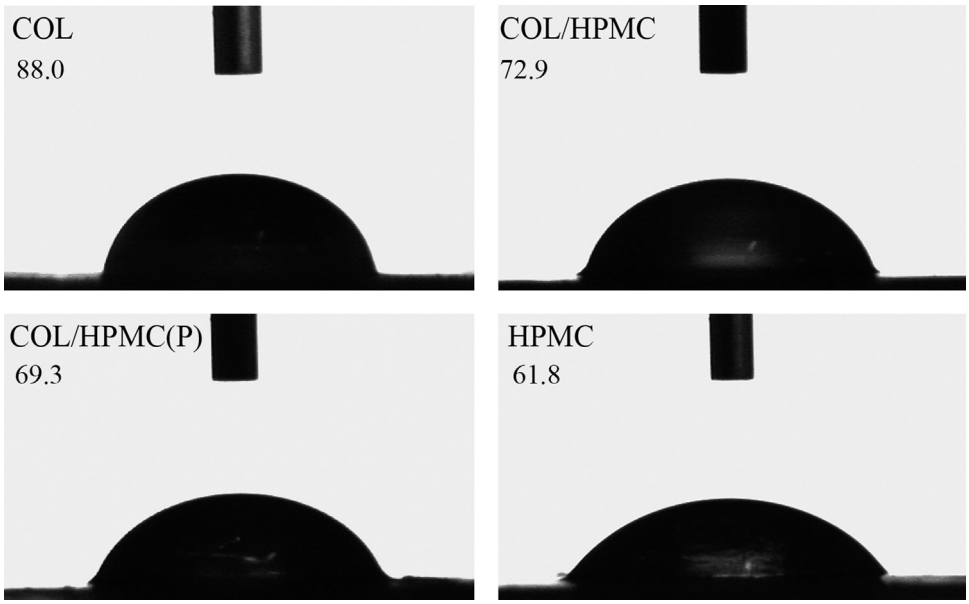

Fig. 6. WCA of the COL, COL/HPMC, COL/HPMC(P) and HPMC films after equilibrating for 4 min.

4. Conclusions

In this paper we studied the collagen/HPMC blend film, considering the development of new materials which have potential applications in food and biomedical fields. It was demonstrated that the collagen/HPMC blend film (1/1) had improved thermal and mechanical properties as well as hydrophilicity compared to those of the pure collagen film, which was due to the hydrogen bonding interaction and entanglement between collagen and HPMC molecules. Meanwhile, the surface and cross section morphologies of the collagen/HPMC blend film were more compact and homogeneous than those of the pure collagen film, suggesting a good miscibility between collagen and HPMC. In addition, the presence of PEG 1500 (10%, w/w) obviously improved the stretch-ability and smoothness of the blend film. These results are expected to have a meaning for the wide developing and application of collagen/HPMC composite films.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.11.057.

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