

Structure and Properties of Collagen-Konjac Glucomannan-Sodium Alginate Blend Films

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Received 17 November 2006; accepted 2 April 2007

DOI 10.1002/app.26670

Published online 15 June 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A new kind of blend film was made by mixing the solutions of collagen, konjac glucomannan (KGM), and sodium alginate. The structure of the blend film was characterized by FTIR, XRD, atomic absorption spectrophotometer, and SEM, respectively. The effects of weight ratio between the polymers on light transmittance, tensile strength, elongation at break, water permeability, and water absorption of the film were studied. The results showed that there

were strong interactions and good compatibility between collagen, KGM, and sodium alginate in the film. The film possesses good mechanical properties. Therefore, it can be applied in biomedicine as a new type of biomaterial. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 327–332, 2007

Key words: collagen; konjac glucomannan; sodium alginate; blend film; compatibility; mechanical property

INTRODUCTION

Because of its peculiar fibrous structure and biological functions, collagen has been widely used in biomedical material field. However, collagen film shows poor mechanical properties and low resistance to water.^{1–3} Konjac glucomannan (KGM) possesses good abilities to adsorb and hold water, to form gel and film,³ as well as some special biological activities, such as facilitating wound healing, stopping hemorrhage, and controlling the release of drugs, whereas its film has poor water resistance.^{4,5} Sodium alginate has been widely applied in food and medicine both as an additive and a raw material because of its rich source, lower price, excellent biodegradability, and biocompatibility.^{6–9} Recently, much attention has been drawn on sodium alginate in biomedical material field. When it is used as a wound-bound material, it can produce various effects, such as wound protection, hemostasis, preventing the wounded tissues from conglutination, and burn treatment by forming gel on wound surface.^{10,11} Moreover, sodium alginate has been widely used to make materials for the control of drug release and supporting materials for tissue engineering.^{12–14} But, sodium alginate film also shows higher hydrophilic and lower mechanical property.

Solution blend is one of the effective methods used to improve the properties of blend films. Incorporation of KGM to collagen film could greatly increase its mechanical property but had few effects on its resistance to water.¹⁵ Blend films made by gelatin and sodium alginate or sodium alginate and KGM showed a good resistance to water and poorer mechanical property in wet state.^{16,17} Maybe, collagen, KGM, and sodium alginate can be combined to make a composite film with much better properties. However, little work has been reported on this kind of composite film to date.

In the present study, collagen, KGM, and sodium alginate were used as film-forming agents and ion calcium as a crosslinking agent to make a new blend film with better physicochemical properties by solution blend method. FTIR, XRD, atomic absorption spectrophotometer, and SEM, respectively, were applied to determine the structure of the films. In addition, the effects of weight ratio between the polymers on light transmittance, mechanical property, water permeability, and absorption of the film were investigated. The objective of the present study was to determine the structure and properties of the blend film and explore its possible application in biomedical field.

EXPERIMENTAL

Materials

Sodium alginate of chemical purity was purchased from Shanghai Chemical Company (Shanghai, China). KGM from Chengdu Xieli Company (Chengdu, China) was purified according to the method of Li and Xie,¹⁸

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Contract grant sponsor: Department of Science and Technology, Sichuan Province, China; contract grant number: 04JY029-022.

its purity being over 98% and the average molecular weight 220 kDa. Collagen was made from fresh oxide by complex enzymes, and its purity was above 99% by Kjeldahl method and its average molecular weight 155–202 kDa by gel filtration.

Preparation of blend films

Solutions of 3% (w/v) collagen, 1% (w/v) KGM, and 3% (w/v) sodium alginate were prepared with distilled water, respectively, and were kept in a water bath at 45°C for use after filtration. The three solutions were completely mixed at a given ratio at 45°C. The mixtures were then deaerated under vacuum and poured on leveled glass plates for film forming. After the films were put into the solution of 3% (w/v) calcium chloride for 30 min followed by washing with distilled water, it was then soaked in the solution of 1% (v/v) hydrochloric acid for 20 min. The resulting blend films were washed with distilled water and air-dried at room temperature. The dried films were stored in a desiccator. The polymer composition and abbreviations of various blend films are listed in Table I.

Structure characterization of blend films

MX-1E Fourier Transform Infrared (FTIR) spectrometer (Nicolet Corp) was applied to characterize the blend films.

The XRD spectra of the films were recorded by X'PertPro X-ray diffraction meter (Philips Corp., Netherlands) under the following conditions: voltage 40 KV, electric current 45 mA, scan angle 5–50°, and scan speed 8°/min. The relative crystallinity was calculated as follows:

$$\text{Relative crystallinity (\%)} = F_c / (F_c + F_a) \times 100$$

where F_c represents the crystalline area whereas F_a the noncrystalline area.

The morphological structure of the cross section of the blend films was illustrated applying JSM-5900LV scanning electron microscope (JEOL Corp., Japan) by breaking the films with liquid nitrogen followed by spraying with golden under vacuum.

TABLE I
Contents of Three Natural Polymers in Six Blend Films

Blend film	Content of polymer (% , g/g)		
	Collagen	KGM	Sodium alginate
CKA-1	50.0	25.0	25.0
CKA-2	33.3	33.3	33.3
CKA-3	25.0	50.0	25.0
CKA-4	25.0	25.0	50.0
CA	50.0	0.0	50.0
CK	50.0	50.0	0.0

Ion calcium content in blend films

Ion calcium content in blend films was determined by TAS986 graphite furnace atomic absorption spectrometer (Beijing Puxi Corp., China). Four milliliters of nitric acid was added to 0.1 g of each blend film. The mixture was then heated to 100°C for carbonation. Two milliliters of nitric acid was taken into this mixture after cooling. The resulting solution was heated to 100°C to a final volume of 0.5 mL. The cooled residue was then diluted with distilled water to 50 mL for Ca^{2+} determination.

Property assay of blend films

Mechanical property

Tensile strength (TS) and percentage elongation at break (%E) of the blend films were analyzed applying an electric pull meter according to China National Standard GB-4456-84 at a drawing speed of 10 mm/min.

Water absorption

Film's water absorption was determined according to the method described by Fan et al.¹⁶ Each film specimen 2.0 cm wide, 2.0 cm long, and 2–3 mm thick was soaked in distilled water for 48 h at room temperature and then taken out. After its surface water was fully wiped up by filter paper, the wet film was weighed, with its weight recorded as W_1 . The weighed wet film was then dried under vacuum for 48 h at 60°C until its weight was remained unchanged. The weight of dried film was recorded as W_2 . Water absorption (Q) of each blend film was calculated by the equation:

$$Q = W_1 - W_2 / W_2$$

Water permeability

Water permeability was determined according to ASTM.¹⁹ Film sample ($12 \pm 0.5 \mu\text{m}$ thick) was tightly covered on the neck of a weighed bottle ($25 \times 25 \text{ mm}^2$) with 3 g of anhydrous calcium chloride in it. The bottle was then placed into a desiccator with saturated sodium chloride solution in its bottom (RH 75%) at 25°C. The bottle was weighed per 24 h until its weight was unchanged. Film's water permeability was calculated as the following equation.

$$P_{\text{H}_2\text{O}} = (m_f - m_i) / (t \times S)$$

Where m_f and m_i represent the final and the first weight of the bottle, respectively; S is the effective area of the film strip; t represents time (d).

RESULTS AND DISCUSSION

Structure characterization

FTIR analysis

Figure 1 shows the FTIR spectra of collagen, KGM, sodium alginate, and the blend films. In the FTIR spectrum of collagen, the fundamental stretching vibration of N—H bond was observed around 3200–3500 cm^{-1} . The strong absorption peak at 1652 cm^{-1} was stretching vibration of amide band I, whereas the peak at 1533 cm^{-1} was bending of N—H bond of amide band II. Absorption peak at 1241 cm^{-1} was N—H twisting and C—N stretching of amide band III. The peaks at 1337, 633.5, and 1451.2 cm^{-1} correspond to C—N or N—H stretching or N—H twisting of amide band IV, amide band V and N—H stretching or twisting, respectively. In the FTIR spectrum of KGM, the peaks at 3408 and 1730 cm^{-1} were the absorption of hydroxyl group (—OH) and the characteristic absorption of carbonyl group (C=O) in acetyl group, respectively, whereas 1375 and 1305.6 cm^{-1} were the absorption wavenumbers of the coupling of C=O stretching and —OH bending. Three peaks around 1153–1031 cm^{-1} indicated the presence of β -glucosidic bond. The characteristic absorption wavenumbers of mannose in KGM molecule were 880.5 and 807.1 cm^{-1} . In the FTIR spectrum of sodium alginate, the peak at 3260 cm^{-1} was —OH stretching. The peaks at 1607 and 1411 cm^{-1} belonged to the symmetric and asymmetric stretching vibration of carboxyl group (COO^-) in alginic acid, respectively.

Analysis on the FTIR spectra of the films shows that the spectra of the films treated by CaCl_2 and hydrochloric acid have changed in the following aspects. First, the peak wavenumber around amide band I (1652 cm^{-1}) of collagen molecule in all blend films decreased to different degrees, indicating there

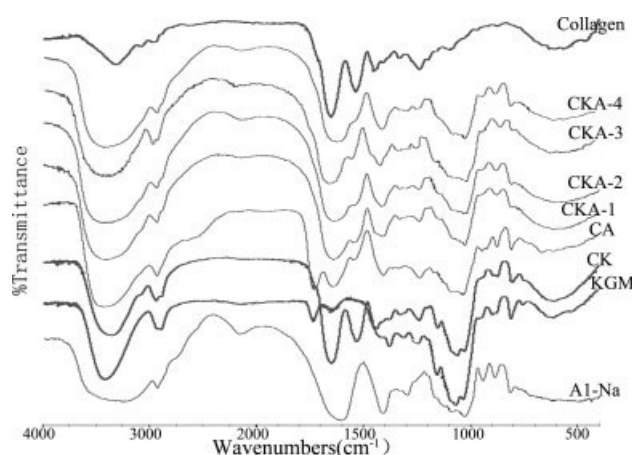


Figure 1 FTIR spectra of three polymers and their blend films.

would be a strong hydrogen bond between the polymers in blend films. Because crosslinking by ion calcium can increase the electrostatic force between the polymers, the peak wavenumbers around amide bands II (1533 cm^{-1}) and III (1241 cm^{-1}) of collagen molecule in all blend films increased to various extents. Moreover, the strength of absorption peak around 1652 and 1541 cm^{-1} weakened with the decrease of collagen content in blend films CKA. Except film CKA-1 containing 50% collagen, other three blend films had weaker absorption peaks around 1652 and 1541 cm^{-1} than that around 3400–3300 cm^{-1} , and the absorption of amide band II of collagen molecule in blend films was weakened. However, blend film CK with the same collagen content as film CKA-1 possessed the strongest absorption in this area. Second, the peaks of amide band IV in collagen molecule at 1337 cm^{-1} , C—O stretching vibration at 1375 cm^{-1} , and O—H bending vibration at 1305 cm^{-1} in KGM molecule overlapped and changed into an impartible shoulder peak at 1334–1300 cm^{-1} . Compared with FTIR spectrum of collagen, KGM or sodium alginate, the peaks of amide band IV in collagen molecule and C—O stretching vibration in KGM molecule were red-shifted in all blend films CKA. Blend film CA showed stronger absorption at 1734 and 1650 cm^{-1} , respectively, whereas film CK at 1647 cm^{-1} . However, the corresponding absorption peaks of blend films CKA appeared around 1640–1630 cm^{-1} . All these revealed that there was a stronger hydrogen bond between the polymers. Third, the absorption peak of KGM at 1730 cm^{-1} disappeared in blend films CKA. Fourth, stretching vibrations of O—H and N—H bonds appeared around 3445–3400 cm^{-1} in blend films CKA. The wavenumbers at 1607 and 1411 cm^{-1} from the symmetric and asymmetric stretching vibrations of carboxyl group (COO^-) in alginic acid in sodium alginate molecule were blue-shifted to 1648–1632 cm^{-1} and 1418–1412 cm^{-1} , respectively, after solution blend, which showed that ion calcium crosslinking enhanced intermolecular electrostatic force. The above results exhibit that the solution blend of the polymers may produce calcium-crosslink, hydrogen bond, and electrostatic force, resulting in good compatibility between collagen, KGM, and sodium alginate.

X-ray diffraction analysis

Figure 2 illustrates the XRD spectra of the polymers and their blend films. It can be seen that there are diffraction peaks at $2\theta = 6.86^\circ$ for collagen whereas there is no diffraction peak for KGM. Calcium alginate film (Al-Ca) exhibited a strong diffraction peak at $2\theta = 10.1^\circ$ and diffusion peaks at $2\theta = 27^\circ$ and $2\theta = 41^\circ$, respectively. If there is no or weak interac-

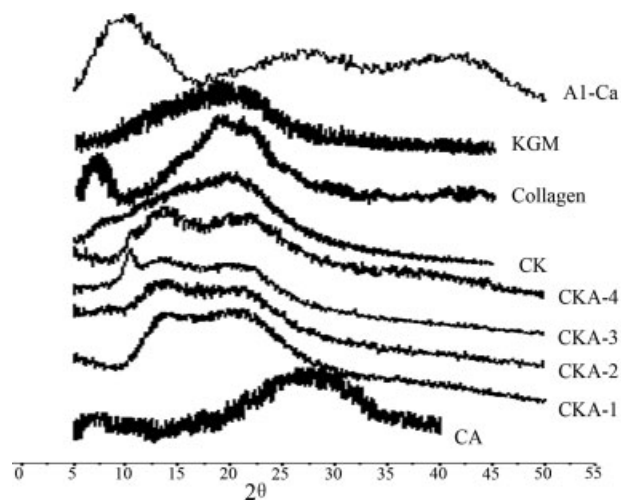


Figure 2 XRD photographs of three polymers and their blend films.

tions between collagen, KGM, and alginic acid, and if the blend films have their own crystal and amorphous areas, the XRD spectra of the blend films will be the superimposition of the components' spectra according to blend weight ratio. In fact, there was a small diffraction peak at $2\theta = 10.6^\circ$ in the XRD spectrum of blend film CKA-3 whereas no diffraction peaks at $2\theta = 6.86^\circ$ for collagen and at $2\theta = 10.1^\circ$ for calcium alginate were found in the XRD spectra of blend films including CKA-1, CKA-2, and CKA-4. Blend films CKA had different diffraction curves compared with blend films CA and CK. The order of crystallinities of all films was as follows: collagen film > CKA-3 > CKA-4, CA, CKA-2, CKA-1, CK, KGM film. Thus, the addition of amorphous KGM could significantly change the molecule arrangement and conformation of collagen and sodium alginate, resulting in decreased molecule arrangement order, increased amorphous area and significantly lowered crystallinity. Because of strong intermolecular interactions between the polymers, the solution blend of

the polymers influenced the structure of blend films CKA, changed, or destroyed the original crystalline structure of collagen and sodium alginate, thus bringing about lowered crystallinity, significantly weakened or disappeared diffraction peaks in the blend film system.

Morphological structures of blend films

Figures 3 and 4 are SEM photographs of film's section. It can be seen that blend films CKA-2 and CKA-4 with different compositions show smooth and compact section. This further proves the results from FTIR and XRD analyses on the blend films. All these indicate that there is good compatibility between collagen, KGM, and sodium alginate.

Light transmittance of blend films

Light transmittance can also be used to judge compatibility between the components in blend films. If there is poor compatibility between the polymers, there is lower light transmittance caused by light scattering or reflection on the surface between two phases. Blend films CKA with different compositions were transparent in appearance in the present study. The determination of percentage light transmittance in the visible region showed that it was related with weight ratio between the polymers. At fixed weight ratio of KGM to sodium alginate, the light transmittance of blend films increased with increasing collagen content. However, when the weight ratio of collagen to sodium alginate was fixed, the higher KGM content was, the higher the light transmittance of blend films CKA. Compared with blend films CA and CK, blend films CKA had slightly lower light transmittance. But, the light transmittance of blend films CKA was over 82%, further indicating that there was good compatibility between collagen, KGM, and sodium alginate.

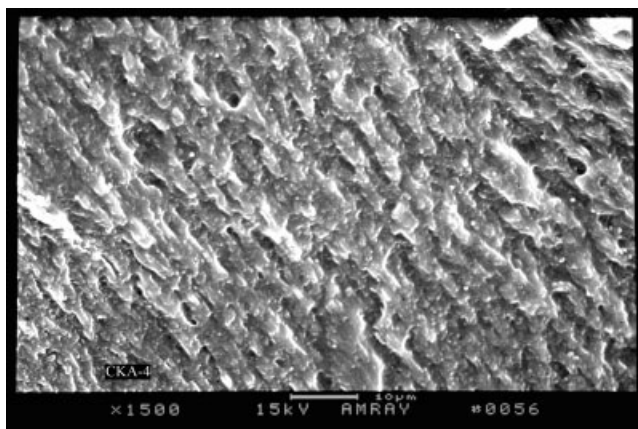


Figure 3 SEM photograph of blend film CKA-4 ($\times 1500$).

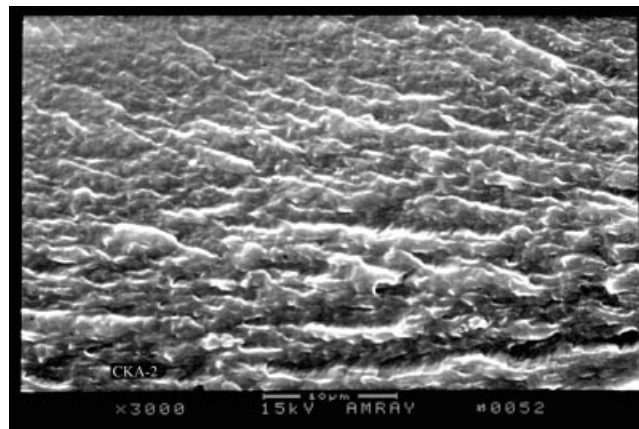


Figure 4 SEM photograph of blend film CKA-2 ($\times 3000$).

TABLE II
Physicochemical Properties of and Ion Calcium Content in the films

Film sample	Sodium alginate (%)	TS (MPa)	%E (%)	P_{H_2O} ($\text{mg cm}^{-2} \text{d}^{-1}$)	Q (%)	Percentage light transmittance (%)	Ca ²⁺ content (mg/g)
Al-Na		28.5	9.8				
Al-Ca	100	33.7	8.2	9.78	2.01		56.33
CA	50	90	13.2	13.65	3.99	88.0	46.02
CKA-1	25	104	10.9	11.78	4.39	87.8	43.93
CKA-2	33.3	100	10.5	10.86	4.16	84.6	46.41
CKA-3	25	94	10.2	12.32	5.07	84.3	44.90
CKA-4	50	97	10.4	10.05	2.74	82.7	49.92
CK	0	37	14.8			86.8	

Ion calcium content in blend films

Ion calcium content in blend films is listed in Table II. It can be seen that ion calcium content in blend films slightly increases as sodium alginate content increases. During preparation, blend films CKA were soaked in the solution of 1% hydrochloric acid for 20 min after being treated with CaCl₂ solution, but they still contained substantial amount of ion calcium, suggesting that there might be present ion calcium crosslinking in blend films. Blend films CKA-1 and CA contained 25% of sodium alginate, but the former had higher ion calcium content than the latter, indicating there was stronger calcium crosslinking between collagen, KGM and sodium alginate.

Physical properties of blend films

Mechanical property

TS and percentage elongation at break of blend films are illustrated in Table II. It can be seen that blend film CKA-1 possesses the highest mechanical property, whereas blend film CKA-4 has the poorest TS, and blend film CKA-3 has the lowest elongation at break. When the content of sodium alginate was fixed, an increase in the weight ratio of KGM to collagen led to a decrease in both TS and elongation at break of films. At unchanged KGM content, TS and elongation at break of the blend films decreased with increasing weight ratio of sodium alginate to collagen. However, when collagen content was fixed, the film's TS and elongation at break slightly increased with an increase in weight ratio of sodium alginate to KGM. All the above results reveal that the content of sodium alginate can influence the TS and elongation at break of films. It can also be seen from Table II that blend film CKA-4 has much higher TS than blend films CA and CK, which all contain 50% collagen, indicating there are stronger interactions between the polymers.

Water absorption

The water absorption of film Al-Ca and blend films CKA-1, CKA-2, CKA-3, and CKA-4 was determined.

When the weight ratio of KGM to sodium alginate was fixed, the water absorption of blend films CKA increased as collagen content increased, which was different from that reported by Fan et al.¹⁶ At fixed weight ratio of collagen to sodium alginate, water absorption of blend films CKA increased with increasing KGM content. However, the water absorption of the blend films greatly decreased with increasing content of sodium alginate. This may be related with the higher hydrophilic property of collagen and KGM. Besides, ion calcium-crosslinking in the blend films increased with the increase of sodium alginate content. Films made by collagen, KGM, or collagen together with KGM completely dissolved after being kept in water for 24 h. But, the water absorption of sodium alginate film treated by calcium chloride solution and blend films CA, CKA-1, CKA-2, CKA-3, and CKA-4 were determined to be 2.01, 3.99, 4.38, 4.13, 5.04, and 2.84%, respectively. In general, the higher the water absorption of the film, the poorer water resistance. Compared with film Al-Ca, blend films CKA had a higher water absorption and lower water resistance. Compared with the films made by collagen or KGM or their combination, blend films CKA exhibited greater water resistance. Blend film CKA-4 had lower water absorption compared with blend film CA, which contained 50% sodium alginate. This further confirmed that there were stronger interactions between collagen, KGM and sodium alginate.

Water permeability

The water permeability of polymer blend films is highly positively related with their thickness, structure compactness, and crystallinity. In the present study, the thickness of the films was within 35 ± 3 μm . The structure compactness and crystallinity of the films (Al-Ca, CA, CKA-1, CKA-2, CKA-3, and CKA-4) changed with the contents of collagen, KGM, and sodium alginate, thus resulting in the changed water permeability. Compared with blend films CA and CK, blend films CKA showed much lower crystallinity. This may be caused by the stron-

ger interactions between the polymers and higher structure compactness. Blend films CKA exhibited lower water permeability than blend film CA.

CONCLUSION

From the above, it can be concluded that there is a good compatibility between collagen, KGM, and sodium alginate. Ion calcium-crosslinking can enhance the interactions between the polymers. It is the interactions that give the blend film a smooth and compact section structure, higher light transparency, and better mechanical properties. As a novel material, the blend film is hoped to be applied in biomedicine.

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