Preparation and Characterization of Collagen–Chitosan Composites

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ABSTRACT: In this article, nature derived collagen was mixed with chitosan and crosslinked by formaldehyde to form a homogeneous composite membrane. The microstructure of the composite was characterized by transmission electron microscopy and differential scanning calorimetry. Mechanical and swelling properties of the composite were improved compared with pure collagen and can be modulated via changing the crosslinking conditions, such as pH, time, and concentration. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **64:** 2127–2130, 1997

Key words: collagen–chitosan composite; microstructure; crosslinking

INTRODUCTION

Collagen is a widely used natural biomaterial for applications such as surgery sutures,[1] guided tissue regeneration membranes,[2] artificial vitreous grafts,[3] etc. As a natural polymer, its mechanical properties are very weak, especially in aqueous media. It is found that the interactions between natural polymers of different chemical structures, whether hydrogen bonding or electrostatic in nature, considerably improve the mechanical properties of the material obtained from such mixtures.[4] Chitosan, also a natural polymer, has been extensively studied in the 1990s in the field of biomaterials since some of its biological properties are quite similar to those of collagen.

Crosslinking of biodegradable polymers is important because the degradation rate can be controlled by the crosslinking conditions. For collagen based material, present crosslinking procedures often use bifunctional reagents containing reactive groups that form bridges between two amino acid side chains of adjacent protein molecules, such as glutaraldehyde, formaldehyde, carbodiimides, etc.[5] In this article, we report on the properties of collagen and chitosan composites, using formaldehyde as the crosslinking reagent. In order to decide the optimum crosslinking condition, we also crosslinked the composites at different pHs, times, and concentrations. Microstructure and mechanical and swelling properties of the composites were characterized.

EXPERIMENTAL

Materials

Collagen was extracted from bovine tendon and was treated by the method of enzyme digestion described in Zhang et al.[1] The collagen solution with a concentration of 1.0% (w/v) was used in the following procedure for preparing collagen–chitosan composites. For chitosan, the molecular weight is about 20,000–40,000, and *N*-deacetylation degree is about 80–95%. All other reagents

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Table I Details of Synthesis of Collagen–Chitosan Composites

The ratio of collagen–chitosan composite (w/w)	1 : 0	9 : 1	4 : 1	2 : 1
Specimen	A	B	C	D

were of analytical grade and were used without further purification.

Synthesis of Collagen–Chitosan Composites

Collagen–chitosan composites were synthesized as follows (see Table I). The collagen solution in dilute acidic media was blended with chitosan solution (0.2*M* acetic acidic solution, with a concentration of 1.0% w/v). The mixture was homogenized in a high-speed stirrer and was defoamed for 10 min in a freeze centrifuge (2000 rpm, 5°C) to form a homogeneous solution. Then the solution was poured onto a Teflon™ plate (8 × 12 cm) and dried with filtered air to form the composite membrane.

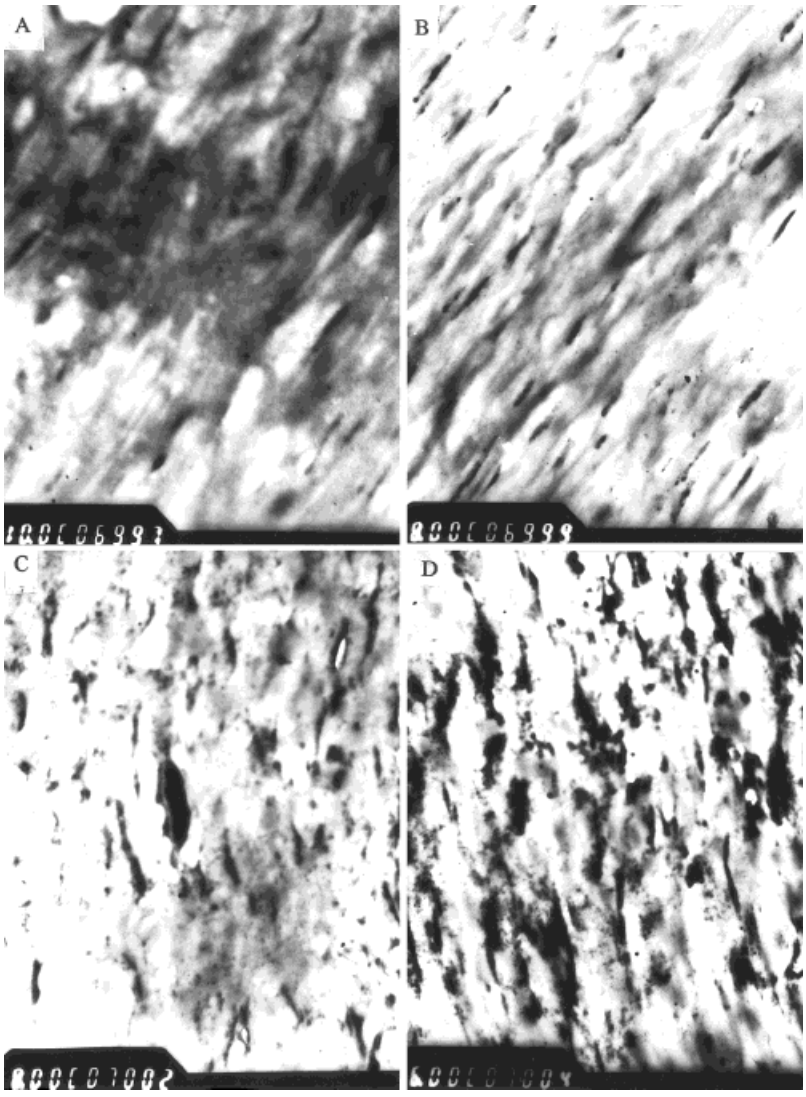

Figure 1 TEM photo of pure collagen.

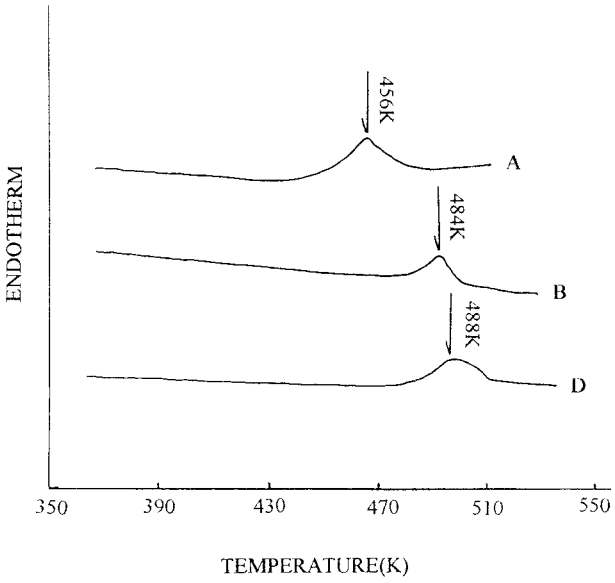

Figure 2 DSC curve of collagen–chitosan composites.

Crosslinking

The membrane were immersed in a crosslinking bath with formaldehyde concentration of 0.3%, pH 8.4, for 1 h. Specimen B membranes were selected to be cross-linked at different formaldehyde concentrations, pHs, and times.

Transmission Electron Microscopy

The osmium tetroxide fixed membranes were cut an by ultrathin slicer, stained with uranyl acetate and lead citrate. The treated specimens were observed by an H-600 transmission electron microscope.

Differential Scanning Calorimetry

The thermal properties of membranes were determined by a Perkin–Elmer EL2C differential scanning calorimetry (DSC) analyzer, DSC curves were recorded with a heating rate of 5°C/min.

Table II Swelling and Mechanical Properties of Composites

Specimen	A	B	C	D
S_w (%)	1.94	1.32	1.12	1.05
Tensile strength $(N/m^2) \times 10^{-7}$	5.608	7.558	10.45	6.530

Table III Swelling and Mechanical Properties of Composites Crosslinked at Different Times (pH = 8.4, c = 0.3%)

Time (h)	0	0.5	1	2
S_w (%)	62.23	2.32	1.46	1.36
Tensile strength $(N/m^2) \times 10^{-7}$	—	7.546	8.322	7.142

Swelling Properties

Dried, constant weight samples (about 1 × 1 cm) were weighed (W_d), immersed in distilled water for five hours, then reweighed (W_h). The swelling degree (S_w) was calculated using the following formula:

$$S_w = (W_h - W_d)/W_d \times 100\%$$

Mechanical Properties

The specimens were cut to 1 × 4 cm, and the thickness was measured with an electronic digital caliper. The mechanical properties of the samples were determined using a Shimadzu AGS-100A Tester with a drawing rate of 10 mm/min.

RESULTS AND DISCUSSION

Transmission Electron Microscopy

The transmission electron microscopy (TEM) photo of pure collagen shows that it is homogeneous (see Fig. 1). The photo of collagen–chitosan composites indicates that the chitosan phase is wrapped in the collagen phase and is denser (see Fig. 1). With the content of chitosan increasing, the amount of chitosan phase grows. Therefore, it may be concluded that the chitosan network can interpenetrate into the collagen network, and the former is denser than the later.

Table IV Swelling and Mechanical Properties of Composites Crosslinked at Different Times (pH = 8.4, t = 1 h)

Concentration (%)	0	0.1	0.3	0.6
S_w (%)	62.23	2.49	1.46	1.25
Tensile strength $(N/m^2) \times 10^{-7}$	—	11.80	8.322	7.142

DSC Analysis

In Figure 2, the main feature in the DSC curve of collagen is the denaturation endotherm at T_d = 456 K. The DSC curves of collagen–chitosan composites show that the denaturation endotherm shifts to higher temperature with increasing chitosan content. This is attributed to the rigidity of the chitosan molecular chain because of its glucopytanose rings.

Swelling Properties

In Table II, the swelling degree of the composite membranes decreases with the increase of the concentration of chitosan in the composites. As is known, the microstructure of crosslinked material determine its macroproperties, such as swelling degree and tensile strength. The microstructure of the material can be revealed through its swelling and mechanical properties. Due to chitosan, the network of the composites becomes denser compared with pure collagen; this may lead to a decrease of the swelling degree. In Table III, the swelling degree of composite B becomes small when the crosslinking time lengthens. In Table IV, the swelling degree declines with the increase of concentration. In Table V, by increasing pH, the swelling degree decreases, which may be attributed to growth in the number of the reactive —NH_2 groups with pH increase. In general, the swelling degree is inversely proportional to the crosslinking density.[5] So, it may be concluded that the crosslinking density of the composites can be controlled by crosslinking time, concentration, and pH.

Mechanical Properties

From Table II, we can see the improvement of tensile strength of composites: the extent of improvement is dependent on the chitosan concentration. The tensile strength of the composites does not change obviously as the crosslinking time is lengthened (see Table III). With increasing concentration, the tensile strength decreases (see Table IV), but not very notably. In Table V, the tensile strength is remarkably influenced by pH; at ca. 8.4, the tensile strength is the highest. This phenomenon can be explained as follows. For a crosslinked polymer, the quantity of network chain per area that bears the load becomes larger with an increase in the crosslinking density. When the crosslinking density is rather small, and every network chain carry the load is quite well distributed, the tensile strength increases. Then as the crosslinking density increases, network chains do not bear the load evenly, the stress distribution is not uniform, and tensile strength decreases.

Table V Swelling and Mechanical Properties of Composites Crosslinked at Different pHs (c = 0.3%, t = 1 h)

pH	6.77	7.36	7.94	8.41	8.98	9.24
S_w (%)	16.8	3.81	2.29	2.19	1.78	1.76
Tensile strength (N/m^2) $\times 10^{-7}$	9.224	9.742	12.21	12.85	7.686	6.490

CONCLUSION

We prepared collagen–chitosan composites. The TEM photo of composites shows that the chitosan network can interpenetrate into the collagen network. The denaturation endotherm in the composite curve changes to higher temperature compared to pure collagen. The mechanical and swelling properties of composites were enhanced. The crosslinking conditions, such as time, concentration, and pH, can effect the swelling degree and tensile strength of composites.

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