Preparation and Characterization of Alginate/Hydroxypropyl Chitosan Blend Fibers

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ABSTRACT: Hydroxypropyl chitosan (HPCS) was synthesized from chitosan and propylene oxide under alkali conditions. It was characterized by IR spectroscopy and X-ray diffraction (XRD). We prepared alginate/HPCS blend fibers by spinning their solution through a viscose-type spinneret into a coagulating bath containing aqueous $CaCl_2$ and ethanol. The structure and properties of the blend fibers were studied with the aid of IR spectroscopy, scanning electron microscopy, and XRD. The results indicate a good miscibility between alginate and HPCS because of the strong interaction of the intermolecular hydrogen bonds. The mechanical properties and water-retention properties were also measured. The best values of the tensile strength and breaking elongation of the blend fibers were obtained when the HPCS content was 30 wt %. The water-retention values of the blend fibers increased as the amount of HPCS increased. Antibacterial fibers, obtained by the treatment of the fibers with an aqueous solution of silver nitrate, exhibited good antibacterial activity to *Staphylococcus aureus*. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 829–835, 2012

Key words: hydroxypropyl chitosan; alginate; blends fibers; miscibility; properties

INTRODUCTION

Alginates are linear copolymers of β-(1-4)-linked D-mannuronic acid (M) and α-(1-4)-linked L-guluronic acid (G) units and are composed of the homopolymeric blocks M–M or G–G and blocks with an alternating sequence of M–G.[1] Alginate, widely used in the food and pharmaceutical industries, is a water-soluble salt of alginic acid, a naturally occurring nontoxic polysaccharide found in all species of brown algae.[2,3]

Alginate fibers can be prepared by the extrusion of solutions of sodium alginate into a bath of calcium ions because of its unique property of crosslinking in the presence of multivalent cations (e.g., Ca^{2+}) in aqueous media, which complex with G–G sequences in the polymer chain to form egg-box junctions[4–6] and insoluble calcium alginate. Depending on the degree of crosslinking, alginate will significantly reduce its swelling in the presence of a solvent; this generally results in a reduction of the permeability of different solutes. As a result, the release of embodied drugs in alginate matrices will be delayed; this will allow these systems to be used in drug controlled release.[7,8] Alginate fiber is one kind of biodegradable fiber, and the products made of alginate fibers are biocompatible and friendly to the environment.[9] Alginate fibers have been used extensively in wound-dressing applications because of their excellent biocompatibility, nontoxicity, and potential bioactivity, which can offer many advantages over traditional cotton and viscose gauzes.[10] Alginate fibers, typically as a calcium salt, interact with the wound exudates to form a moist gel as a result of the ion exchange between the calcium ions in the fiber and the sodium ions in the exudates.[11] This eliminates fiber entrapment in the wound, which is a major cause of patient trauma during dressing changes; such gelation provides the wound with a moist environment, which promotes healing and leads to better cosmetic repair of the wound.[12]

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This *in situ* generation of a moist healing environment and the consequent high absorbency of the alginate dressing are two of the outstanding properties that make alginate dressings one of the most versatile wound dressings available today.[13–16] In addition, alginate-containing dressings have been demonstrated to activate macrophages within the chronic wound bed and generate a pro-inflammatory signal, which may initiate a resolving inflammation characteristic in healing wounds.[17] Therefore, many commercially available wound dressings contain calcium alginate fibers.

As a natural renewable resource, chitosan (CS) has a number of unique properties, including biocompatibility, biodegradability, nontoxicity, and antimicrobial activity, which have attracted much scientific and industrial interest in fields such as biotechnology, pharmaceutics, wastewater treatment, cosmetics, agricultures, food science, and textiles.[18] They have also been used as wound-management products. Recent observations have suggested that chitin and CS can accelerate wound healing.[19] However, although CS is soluble in aqueous dilute acids below pH 6.5, it is insoluble in water and most organic solvents. CS has poor solubility because of the rigid crystalline structures formed by intermolecular and intramolecular hydrogen bonding and does not dissolve in water; these properties have limited its applications and chemical modification, especially at neutral pH. Probably, the wound-healing acceleration effects of chitin and CS do not become fulfilled because of relatively low interaction between the wound sites and the healing agents. Therefore, special attention has been paid to its chemical modification and depolymerization to obtain derivatives that are soluble in water. Hydroxypropyl chitosan (HPCS), a kind of water-soluble functional derivative of CS, is obtained by means of etherification through propylene oxide at the C_6 position under alkali conditions. Liquid-crystal phases, foam performance, and emulsifying power have been observed in solutions of HPCS.[20,21] In addition, HPCS grafted with maleic acid was found to kill 99.9% of *Staphylococcus aureus* and *Escherichia coli* within 30 min at a concentration of 100 mg/mL.[22]

It is well know that blending is an effective and convenient method for improving the performance of polymer materials. Thus, in this study, novel bicomponent fibers were prepared from alginate and HPCS. HPCS has amide groups, which can produce a polyelectrolyte effect with that of alginate in terms of its carboxyl groups. This affection is expected to give high compatibility between these two polymers. For this reason, blend fibers were made in this work. Silver ions have been found to have antibacterial effects on some microbes. Silver salt is the most effective antimicrobial agent in the treatment of burn patients.[23] In this study, therefore, attempts were made to treat the blend fibers with silver nitrate ($AgNO_3$). The blend fibers could become potential antibacterial wound-dressing materials.

EXPERIMENTAL

Materials

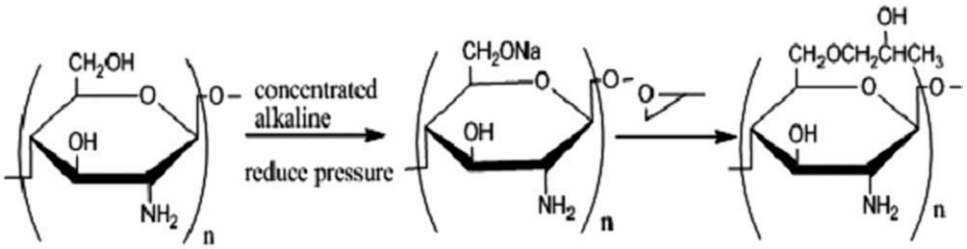
Sodium alginate was purchased from Sinopharm Chemical Reagents Co. (Zhejiang, China.) and was chemical grade. CS was supplied by Yunhuan Ocean Biochemistry (Zhejiang Province, China). The degree of deacetylation, as determined by elemental analysis, was 0.92, and the molecular weight calculated from gel permeation chromatography was 5.2×10^5. All of the other reagents used were analytical grade.

Preparation of HPCS

Purified CS (20 g) was added to 20 mL of a 50 wt % NaOH aqueous solution, completely mixed, swollen enough at room temperature, and then put into a refrigerator at −19°C for alkalization. The mixture was then thawed and transferred to a three-necked, round-bottom flask that contained 200 mL of isopropyl alcohol. After vigorous stirring for 30 min at room temperature, 4 mL of a 10% tetramethyl ammonium hydroxide solution and 200 mL of propylene oxide were added, stirred for 1 h at room temperature, and then refluxed for 5–6 h at 45°C with continuous stirring. The reaction mixture was cooled to room temperature, filtered, and then redissolved by distilled water. The resulting solution was subsequently filtered and then dialyzed with a regenerated cellulose tube (molecular weight cutoff = 8000, Union Carbide Co. Bhopal, India) against distilled water for 3 days. Next, the solution was concentrated by a rotary evaporation at reduced pressure below 60°C. Finally, the product was dried at 40°C *in vacuo* for 24 h. Refined HPCS derivatives were obtained (Scheme 1).

Preparation of the blend fibers

A 5 wt % HPCS aqueous solution was obtained by the addition of the required amount of HPCS with stirring to a known volume of distilled water at room temperature. Sodium alginate was also dissolved in distilled water at the same temperature to a concentration of 4 wt % and then mixed with HPCS. The mixed solutions were stirred vigorously at room temperature for 1 h and filtered through a 200-mesh filter cloth under pressure. The clear filtrate as a spinning solution was poured into the spinning tank and degassed under diminished pressure for 1 h. After that, the spinning solution was extruded at 25°C from a 30-hole (0.08-mm-diameter) viscose-type spinneret into a coagulating bath



Scheme 1 Synthesis of HPCS.

containing a 5 wt % calcium chloride aqueous solution and ethanol to form fibers. The volume ratio of the calcium chloride aqueous solution to ethanol was 50/50. The as-spun fibers were washed and stretched (stretching ratio = 20%) in distilled water and then air-dried to provide fibers. According to HPCS contents of 10, 30, 50, and 70 wt %, the blend fibers were labeled as AHC10, AHC30, AHC50, and AHC70, respectively. The pure alginate fiber and HPCS were coded as AL and HC, respectively.

Antibacterial treatment of the fibers

AL and the blend fibers (AHC10, AHC30, AHC50, and AHC70) were placed in a treatment bath containing silver nitrate (0.01 wt %) for 10 min, respectively, rinsed in water, and dried at 25°C. The antibacterial fibers were coded as AL_{Ag}, $AHC10_{Ag}$, $AHC30_{Ag}$, $AHC50_{Ag}$, and $AHC70_{Ag}$.

Characterization of the fibers

IR analysis

IR spectra of the samples were recorded with a Nicolet-170SX Fourier transform infrared spectrometer (Nicolet Instrument Co., USA). The test specimens were cut into small pieces for the preparation of KBr discs. The samples were made thin enough to obey the Lambert–Beer law.

X-ray diffraction (XRD) studies

XRD patterns of the sample were measured with a D/max-2500 X-ray diffract meter (Rigaku Denki, Tokyo, Japan) with a Cu Kα target at 40 kV and 50 mA. The diffraction angle ranged from 5 to 45°.

Morphology observations

The morphological structures of the blend fiber samples were studied with a Hitachi XZ-650 scanning electron microscope (Tokyo, Japan).

Mechanical properties

The tensile strength and breaking elongation of the fibers were determined on a fiber electron tensile tester (CMT8502, Shenzhen SANS Test Machine Co. TD, Shenzhen, China). The gauge length was 90 mm, and the crosshead speed was 50 mm/min. All samples were preconditioned at 20°C and 65% relative humidity for 24 h before mechanical testing. All of the experiments were done five times.

Water-retention values (WRVs)

The WRVs of the fibers were calculated as follows:

$$\mathrm{WRV} = (W_1 - W_0)/W_0 \times 100\%$$

where W_0 denotes the original weight of fiber (g), which was dried at 80°C until a constant weight was achieved, and W_1 is the weight of the fully swollen fiber, which was centrifuged at 4000 rpm for 10 min. All of the experiments were done in triplicate.

Antibacterial testing

A shake-flask method was used to evaluate the antibacterial activity of the fibers against *S. aureus* (a Gram-positive bacterial inhabitant of colonized infected wounds) in terms of the bacterial reduction rate (BRR). A 0.5-mL aliquot of a known, fresh culture was added to 0.03*M* sodium phosphate buffer at pH 7.3 (70 mL) containing fibers (0.75 g). After the cultivation was shaken (300 rpm) at 37°C for 1 h, an aliquot (0.5 mL) was diluted with sodium phosphate buffer and spread onto nutrient agar (made up of 15 g of agar, 10 g of peptone, 3 g of beef extract, and 3 g of NaCl in 1000 mL of distilled water at pH 7.0) plates to give the single colonies. After incubation at 37°C for 24 h, the number of survivors was counted. The number of bacteria in 0.5 mL of fresh culture was also determined by means of this plate-counting method. The BRR of each fiber was calculated as follows:

$$\mathrm{BRR} = (N_1 - N_2)/N_1 \times 100\%$$

where N_1 is the average number of colonies before shaken incubation and N_2 denotes the average number of colonies after shaken incubation.

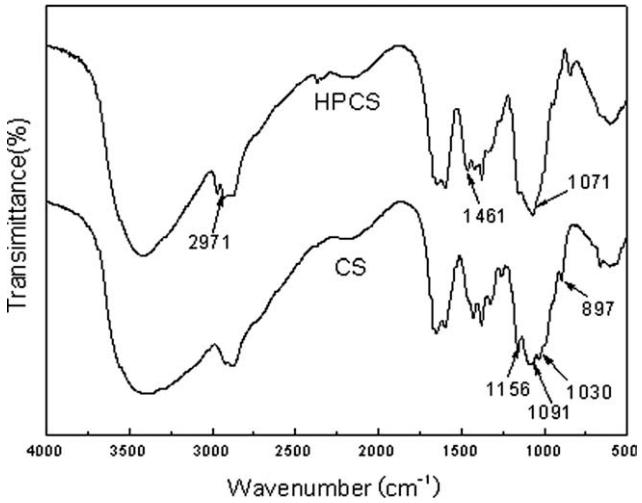


Figure 1 IR spectra of CS and HPCS.

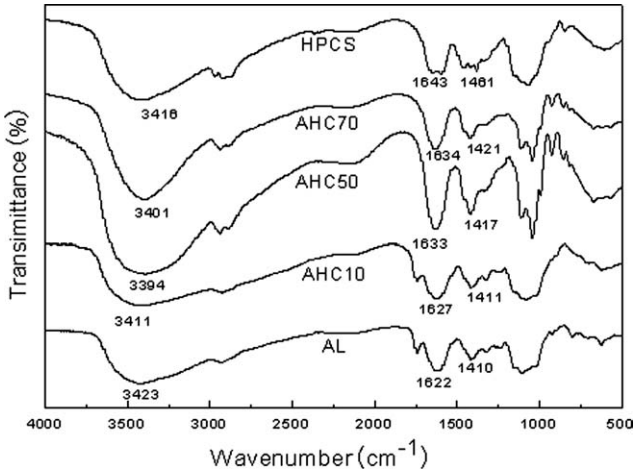


Figure 2 IR spectra of AL, alginate/HPCS (w/w) (AHC10, AHC50, AHC50, and AHC70), and pure HPCS.

RESULTS AND DISCUSSION

Structure and morphology

IR analysis

The IR spectra of the samples of CS and HPCS are shown in Figure 1. The IR spectrum of CS showed four peaks at 1156, 1091, 1030, and 897 cm^{-1}, which could be assigned to the saccharide moiety. Strong amino characteristic peaks around 3377, 1655, and 1324 cm^{-1} were assigned to the amide I and III bands. The peak at 1423 cm^{-1} was the joint contribution of bend vibrations of OH and CH. In the IR spectrum of HPCS, the strong peak at 1461 cm^{-1} could be assigned to the asymmetry deformation of CH_3. The C—O adsorption peak of secondary hydroxyl groups became stronger and moved to 1071 cm^{-1}. The new peak that appeared at 2971 cm^{-1} indicated the incorporation of the hydroxypropyl moiety. The results indicate that the substitution primarily occurred at the C_6 position.

The IR spectra of the samples AL, AHC10, AHC50, AHC70, and HPCS are shown in Figure 2. The IR spectrum of alginate showed absorption bands at 3423 cm^{-1} (OH stretching), 1622 cm^{-1} (COO^- asymmetric stretching), and 1410 cm^{-1} (COO^- symmetric stretching). For the blend fibers, the absorption band around 3423 cm^{-1} slightly broadened and shifted to a lower wave number with increasing HPCS; this suggested the formation of an intermolecular hydrogen bond. The strong absorption band at 1622 cm^{-1} for AL fibers, assigned to the asymmetric stretching vibrations of COO^-, was coupled with the absorption at 1643 cm^{-1} in HPCS and shifted to a higher wave number. At the same time, the absorption at 1410 cm^{-1} assigned to the symmetric stretching vibration of COO^- shifted to absorptions at 1411, 1417, and 1421 cm^{-1} with the absorption at 1461 cm^{-1} in AHC10, AHC50, and AHC70, respectively. On the basis of this evidence, we concluded that a certain degree of the interaction between the alginate and HPCS molecules was due to the formation of intermolecular hydrogen bands.

XRD studies

The diffraction of CS (in Fig. 3) showed typical peaks around 12 and 20°, which were assigned to the abundant —OH groups and —NH_2 groups existing in CS, and their existence improved the ordered structure of the molecule. However, the XRD intensities of the modified CS at 12° almost disappeared, and the peak at $2\theta = 20°$ turned more wide in HPCS. All of this illustrated that the peaks of amorphous diffusion scattering receded, and the degree of crystallization was broken down to a certain extent, which made the modified products possess many amorphous regions, and the water solubility was improved.

The diffraction of alginate (in Fig. 4) showed typical peaks around 14 and 23°.[24] The diffraction of

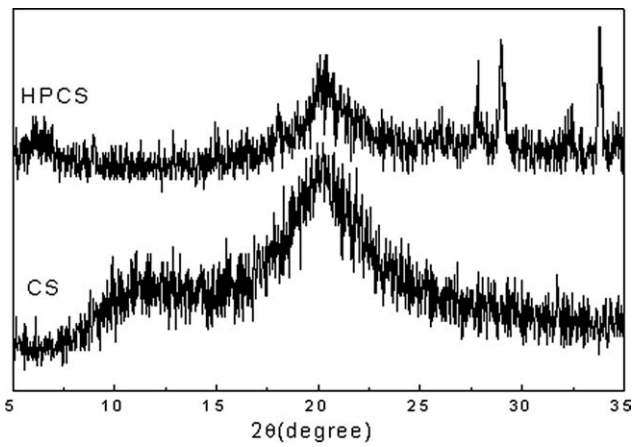


Figure 3 XRD patterns of CS and HPCS.

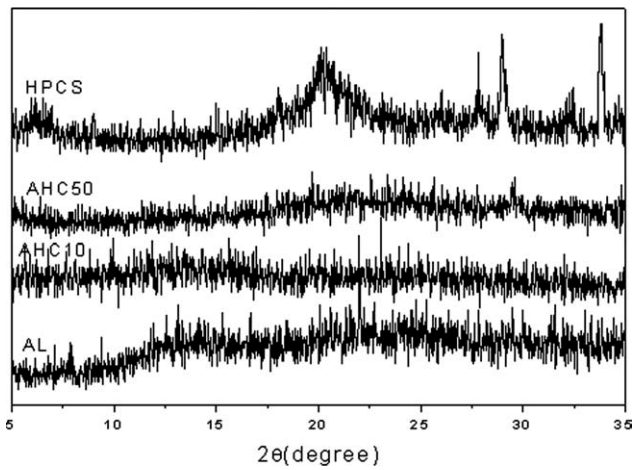


Figure 4 XRD patterns of AL, HPCS, and their blend fibers.

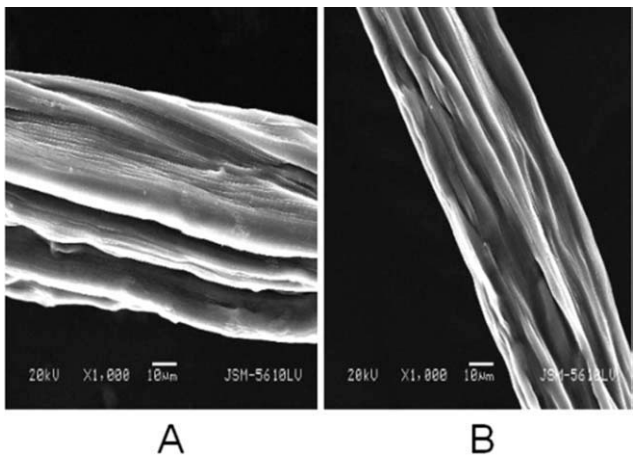


Figure 5 SEM photographs of the HPCS/alginate (w/w) blend fibers: (A) ACH10 and (B) ACH30.

alginate rapidly weakened with an increase in the content of HPCS. On the other hand, for the crystalline regularity of HPCS, the peaks located at 7 and 29° obviously disappeared with an increase in the AL content, for example. This indicated that there occurred some interaction between HPCS and AL. These results proved good miscibility between two components in the blend fibers. The results also supported the conclusion drawn from IR and scanning electron microscopy (SEM) that the good miscibility existing between alginate and HPCS was due the strong interaction from the intermolecular hydrogen bonds or ionic interactions.

Morphology observations

The surfaces were examined by SEM to verify the compatibility between alginate and HPCS molecules, as shown in Figure 5. The surfaces of AHC10 and AHC30 showed a smooth and homogeneous morphology; this suggested a high miscibility and blend homogeneity between alginate and HPCS. The fibers displayed striation along the fiber length, and this agreed with the literature.[25]

Mechanical properties of fibers

It is known that the interaction between polymers should influence the mechanical properties of the blend polymer. The effect of the HPCS content on the tensile strength of the fibers in the dry and wet states is shown in Figure 6 and Table I. The dry tensile strengths of the blend fibers were higher than that of AL, and the maximum value was observed at 30% HPCS content: 12.29 cN/tex in the dry state and 2.51 cN/tex in wet state, 20.4 and 16.2% increases over AL, respectively. The addition of HPCS was effective in inducing an improvement in the dry tensile strength of the blend fibers. Figure 7 and Table I show the breaking elongation of the fibers in the dry and wet states. The alteration of breaking elongation expressed a tendency similar to that of the tensile strength, and the maximum values were 24.1% in the dry state and 42.5% in wet state, respectively, which were achieved when the HPCS content was 30 wt %. It all added up to the conclusion that there existed compatibility to a certain extent between AL and HPCS, and stronger intermolecular interaction existed between their molecules; this was also in accordance with the structural

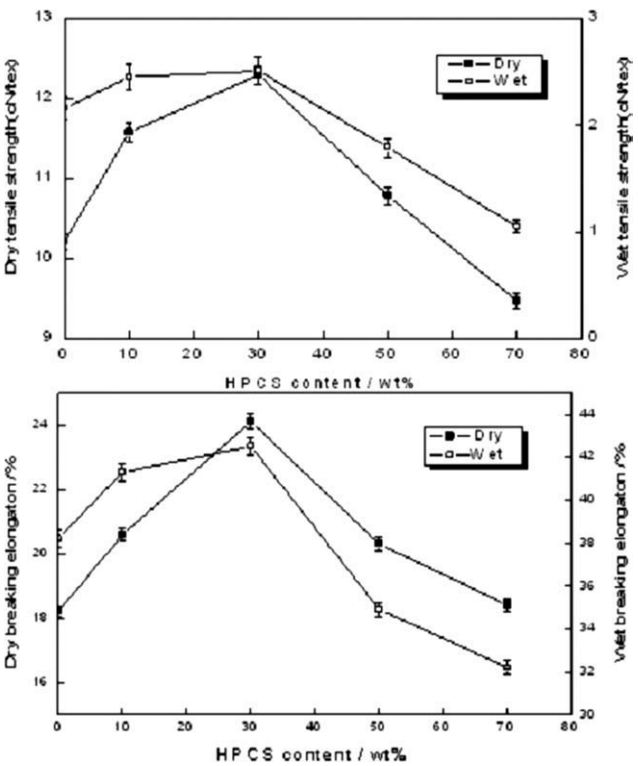


Figure 6 Effect of the HPCS content (wt %) on the tensile strength of the blend fibers and breaking elongation of the blend fibers.

TABLE I
Properties of the Blend Fibers

Fiber	Tensile strength (cN/tex; dry/wet)	Breaking elongation (%; dry/wet)	WRV (%)	BRR
AL	10.21/2.16	18.2/38.2	88	0
AHC10	11.58/2.45	20.6/41.3	131	0
AHC30	12.29/2.51	24.1/42.5	288	0
AHC50	10.79/1.79	20.3/34.9	396	0
AHC70	9.47/1.05	18.4/32.2	566	0
AL_{Ag}	10.01/1.96	18.5/39.0	90	>99.99
$AHC10_{Ag}$	11.32/2.11	20.3/41.0	129	>99.99
$AHC30_{Ag}$	12.41/2.36	24.0/42.4	290	>99.99
$AHC50_{Ag}$	11.65/1.84	20.6/35.3	397	>99.99
$AHC70_{Ag}$	10.11/1.34	19.1/33.4	575	>99.99

analysis. So, through the control of the blend conditions, fibers with better mechanical properties than AL were achieved. The mechanical properties of the $AgNO_3$-treated fibers were not significantly different from the untreated fibers (Table I). The latter implies that the silver ion was more like a coating and did not penetrate the alginate-based fibers.

Water-retention properties

The water-retention properties of the blend fibers are also plotted in Figure 7 as a function of the weight content HPCS. They showed that WRV of the AL/HPCS blend fibers increased dramatically as the HPCS content was raised. The WRVs of the blend fibers were in the range 88–566% and were obviously higher than that of the AL fiber, which had the lowest value (88%, Table I). The improvement in water retention could be explained by the excellent water-retention ability in HPCS, which was consistent with results reported in the literature.[26] On the other hand, the addition of HPCS weakened the closeness of macromolecular sodium alginate, reduced the crystalline regions of the fibers, and favored the penetration of water molecules into the fibers sequentially. Good hydrophilicity is important for application in good wound-dressing fibers. The water-retention properties of the $AgNO_3$-treated fibers were not significantly different from those of the untreated fibers (Table I).

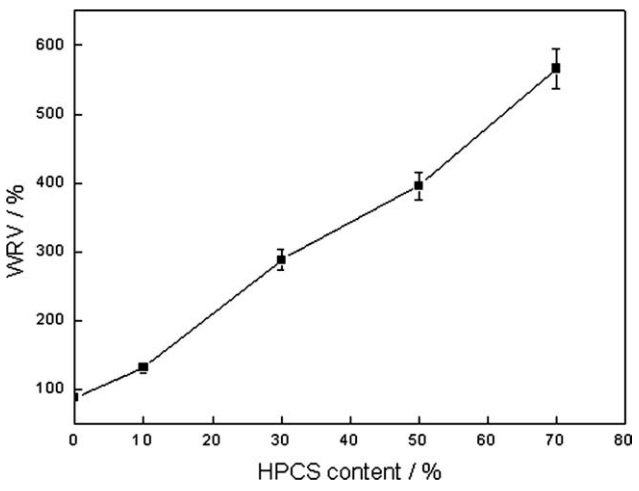


Figure 7 Effect of the HPCS content (wt %) on the WRVs of the blend fibers.

Antibacterial testing

The antibacterial properties of the fibers were investigated (Table I); this showed that the untreated fibers did not have antibacterial activity and that the fibers treated with $AgNO_3$ had good antibacterial activity toward *S. aureus*. It is well known that CS and its derivatives present significant bactericidal effects against both Gram-positive and Gram-negative bacteria below pH 6 because of their positively charged amino groups, which can interact with negatively charged bacterial cell membranes. Therefore, the poor inhibitory effect of HPCS on *S. aureus* could have possibly been due to the relatively small number of charged amino groups in the molecules.[27] We also all know that silver ions have good antibacterial properties. The alginate-base fibers were immersed in silver nitrate solution, and the calcium alginate fiber was converted into a calcium/silver alginate fiber. Thus, the treated fiber had good antibacterial activity compared with the untreated fiber.

The best values of the dry tensile strength and breaking elongation were obtained when the HPCS content was 30 wt %. The wet tensile strength decreased with the increase of HPCS content, and the wet breaking elongation achieved a maximum value when the HPCS content was 30 wt %.

CONCLUSIONS

We obtained alginate and HPCS blend fibers by spinning their solution through a viscose-type spinneret into a coagulation bath containing aqueous $CaCl_2$ and ethanol. The strong intermolecular interaction between the alginate and HPCS molecules occurred in the blend fibers. There was good

miscibility between the alginate and HPCS molecules. The best values of the tensile strength and breaking elongation were obtained when the HPCS content was 30 wt %. The wet tensile strength decreased with the increase of HPCS content, and the wet breaking elongation achieved a maximum value when the HPCS content was 30 wt %. The most obvious change was that the introduction of HPCS into the blend fiber dramatically improved the water-retention properties of blend fiber compared to that of the AL fiber. The $AgNO_3$-treated fibers had good antibacterial activity toward *S. aureus*. This novel alginate and HPCS blend fiber is a promising kind of fiber for application in wound dressings.

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