The Novel Alginate/*N*-Succinyl-Chitosan Antibacterial Blend Fibers

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ABSTRACT: Alginate/*N*-Succinyl-chitosan (SCS) blend fibers, prepared by spinning their mixture solution through a viscose-type spinneret into a coagulating bath containing aqueous $CaCl_2$, were studied for structure and properties with the aid of infrared spectroscopy (IR) and X-ray diffraction (XRD). The results indicated a good miscibility between alginate and SCS, because of the strong interaction from the intermolecular hydrogen bonds. The best values of the dry tensile strength and breaking elongation were obtained when SCS content was 30 wt %. The wet tensile strength decreased with the increase of SCS content, and the wet breaking elongation achieved maximum value when the SCS content was 30 wt %. Introduction of SCS in the blend fiber improved water-retention properties of blend fiber compared to pure alginate fiber. Antibacterial fibers, obtained by treating the fibers with aqueous solution of silver nitrate, exhibited good antibacterial activity to *Staphylococcus aureus*. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2151–2156, 2010

Key words: alginate; *N*-succinyl-chitosan; blend fibers; antibacterial properties

INTRODUCTION

Alginate fibers have been extensively used in wound dressing applications due to their excellent biocompatibility, nontoxicity, and potential bioactivity, which can offer many advantages over traditional cotton and viscose gauzes. 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 exudates.[1] This eliminates fiber entrapment in the wound, which is a major cause of patient trauma at dressing change. Such gelation provides the wound with a moist environment, which promotes healing and leads to a better cosmetic repair of the wound.[2] This *in situ* generation of a moist healing environment and the consequent high absorbency of the alginate dressings are two of the outstanding properties which make the alginate dressing one of the most versatile wound dressings available today.

Calcium alginate dressings have been used successfully in the treatment of skin wounds of various origins such as split skin graft, donor sites, full-thickness excisions, leg ulcers and pressure sores.[3] Because of the release of calcium ions from alginate dressings in contact with body fluids, alginate dressings have also been reported to reduce bleeding during various surgical interventions.[4] Calcium alginate dressings have been successfully applied to cleanse a wide variety of secreting lesions. The high absorption is achieved via strong hydrophilic gel formation.[5] This limits wound secretions and minimizes bacterial contamination.[6] 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 of healing wounds.[7] Therefore, many commercially available wound dressings contain calcium alginate fibers.

Another type of natural polysaccharide used in wound management products is chitin, and its partially deacetylated derivative chitosan. Recent observations have suggested that chitin and chitosan can accelerate wound healing.[8] Chitin and chitosan have rigid crystalline structures formed by hydrogen bonding intra- and inter-molecularly and do not dissolve in water. Probably the wound healing acceleration effects of chitin and chitosan do not become fulfilled due to relatively low interaction between the

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wound sites and the healing agents. *N*-Succinyl-chitosan (SCS), a water-soluble chitosan derivative, has already been used in the field of drug carrier and wound dressings, due to its excellent biocompatibility and low toxicity.[9] SCS was initially was reported to be used as wound dressing materials.[10] The wound dressings composed of Suc-Chi and gelatin were also developed.[11]

Silver ions have been found to have antibacterial effects on some microbes. The silver salt is the most effective antimicrobial agent in the treatment of burn patients.[12] The antibacterial fibers were obtained by treatment with an aqueous solution of silver nitrate.

It is well known that blending is an effective and convenient method to improve the performance of polymer materials. Thus, in the present study, novel bicomponent fibers were prepared from alginate and SCS. SCS has a molecular structure similar to that of alginate in terms of its carboxyl groups. This similarity is expected to give high compatibility between these two polymers. For this reason blend fibers were made in the present work. In this article, therefore, attempts are made to treat blend fibers with silver nitrate. The blend fiber can become potential antibacterial wound dressing materials.

EXPERIMENTAL

Materials and methods

Sodium alginate was purchased from Shanghai Chemical Reagents Company, chemical grade. Chitosan was supplied by Yuhuan Ocean Biochemistry, in Zhejiang province in China. The degree of deacetylation (DD) as determined by elemental analysis was 0.93, and the molecular weight calculated from GPC was 2.9×10^5. Standard pullulans for GPC were purchased from Showa Deuko, Tokyo, Japan. All of other reagents used were of analytical grade.

N-Succinyl-chitosan (SCS) synthesis

Ten grams of chitosan was dissolved into 1000 mL of 1 wt % HAc solution and then transferred into a flask. Succinic anhydride (5 g) was dissolved in acetone (100 mL), and added into the flask by dropwise for 30 min at room temperature, and then the reaction was allowed for 4 h at 40°C. The reaction mixture was cooled to room temperature. The mixture precipitated in an excess of acetone, filtered to remove the solvent and then washed with 70, 80, and 100% acetone, respectively. Finally, the product was dried at 40°C under vacuum for 24 h. The obtained white powder *N*-succinyl-chitosan (SCS) was 10 g.

Preparation of blend Fibers

SCS was dissolved in distilled water to prepare a 2 wt % solution. Sodium alginate was dissolved in distilled water at room temperature to a concentration of 5 wt % and then mixed with SCS. The mixed solutions were vigorously stirred at room temperature for an hour, 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 an hour. 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 containing 5 wt % calcium chloride aqueous solutions. The volume ratio of calcium chloride aqueous solution to ethanol was 50/50. The as-spun fibers were washed and stretched (stretching ratio is 20%) in distilled water, then air dried to afford fibers. According to the SCS contents of 10, 30, 50, and 70 wt %, the blend fibers were labeled as ASu10, ASu30, ASu50, and ASu70, respectively. The pure alginate fiber and SCS were coded as AL and SCS, respectively.

Antibacterial treatment of the fibers

The pure alginate fiber and blend fibers (AL, ASu10, ASu30, ASu50, and ASu70) were placed in a treatment bath containing silver nitrate (0.01 wt %) for 10 min, rinsed, and dried. The antibacterial fibers were coded as AL_{Ag}, $ASu10_{Ag}$, $ASu30_{Ag}$, $ASu50_{Ag}$, and $ASu70_{Ag}$.

Characterization of fibers

Infrared spectra (IR) of the sample were recorded with a Nicolet-170SX FTIR (USA). The test specimens were cut into small pieces for preparation of KBr discs. The samples were made thin enough so to obey the Lambert-Beer Law.

X-ray diffraction (XRD) patterns were performed on a D/max-2500 X-ray diffractometer (Rigaku Denki, Japan) with Cu $K\alpha_1$radiation ($\lambda = 0.154$ nm) in a range of $2\theta = 3$–50° using a fixed time mode with a step interval of 0.02°. Additionally, the crystalline degree (χ_c) was calculated from the curve-fitting patterns by the accessory software of instrument.

Morphology of the cross-section of the fibers was observed by an optical polarizing microscope (Leica DMLP, Germany). A bundle of fibers was embedded in celloidin and allowed to harden for about 5 min at 25°C. Cross-sections normal to the fiber axis of about 2-mm thickness were prepared using a Struers Accutom. The cross-sections were ground and polished down to a thickness between 10 and 40 μm,

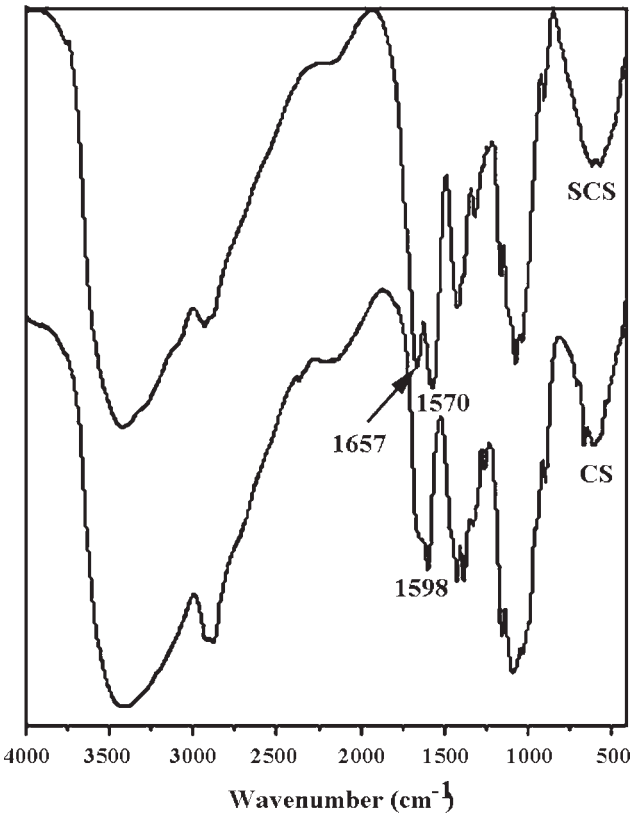

Figure 1 IR spectra of chitosan (CS) and *N*-succinyl-chitosan (SCS).

and cemented to a microscope glass side with glycerol.

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

The water-retention values (WRV) of fibers were calculated as follows:

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

Where W_0 denote the original weight (g) of fiber which was dried at 80°C until a constant weight achieved, W_1 is the weight of fully swollen fiber that was centrifuged at 4000 rev/min for 10 min.

A shake-flask method was used to evaluate the antibacterial activity of the fibers against *Staphylococcus aureus* (a gram-positive bacterial inhabitant of colonised or infected wounds) in term of bacterial reduction rate. Aliquots (0.5 mL) of fresh culture were added to 0.03*M* sodium phosphate buffer 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 the sodium phosphate buffer, and spread on nutrient agar (made up from agar, 15 g; peptone, 10 g; beef extract, 3 g; NaCl, 3 g in 1000 mL distilled water, pH 7.0) plates to give the single colonies. After being incubated 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 bacteria reduction rate (BRR) of each fiber was calculated as follows:

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

Where N_1 and N_2 are is the average number of colonies arising from preincubation and postincubation cultured samples, requisitely.

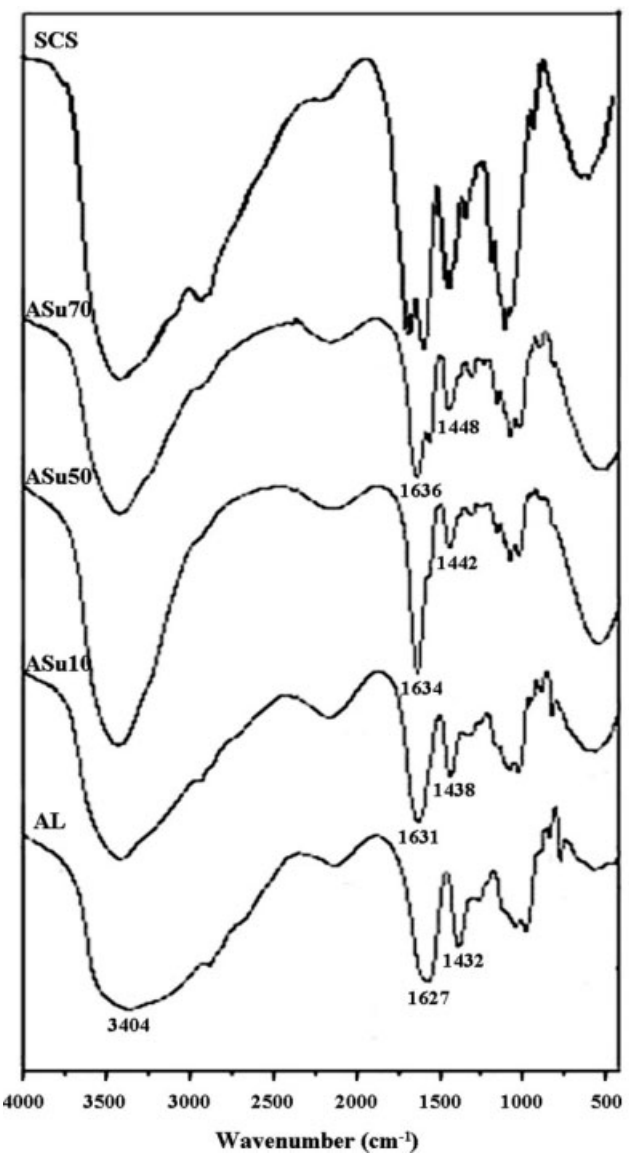

Figure 2 IR spectra of pure alginate (AL), alginate/ *N*-succinyl-chitosan (w/w), 90/10 (ASu10), 50/50 (ASu50), 30/70 (ASu70), and pure *N*-succinyl-chitosan (SCS).

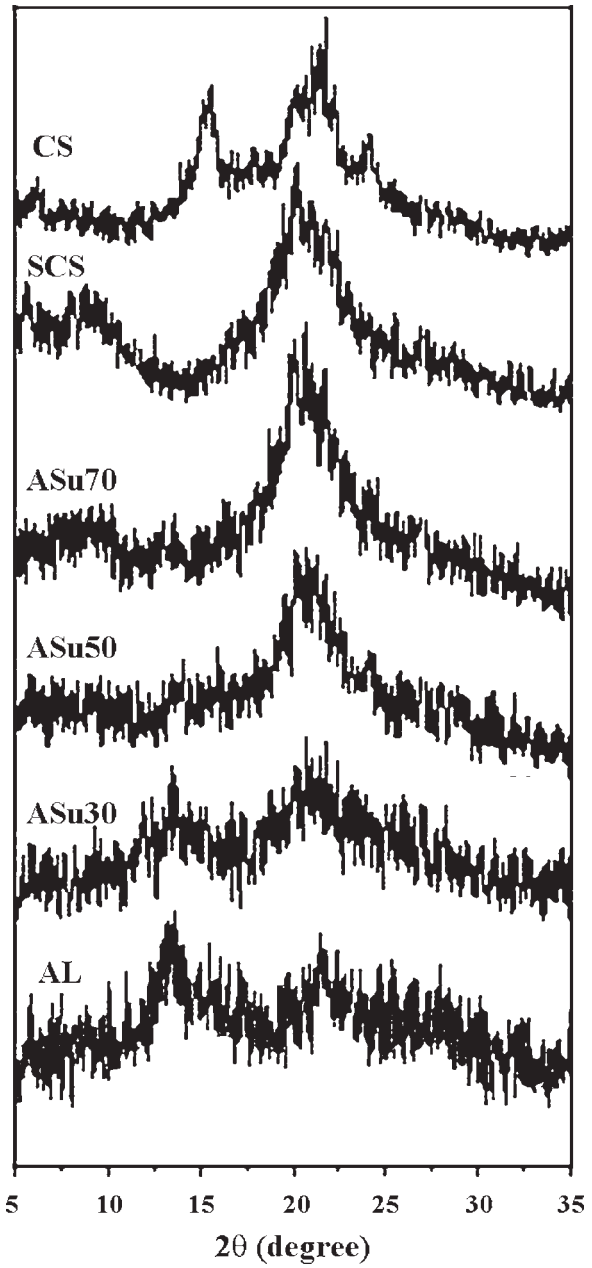

Figure 3 The X-ray diffraction patterns of pure alginate (AL), ASu30, ASu50, ASu70, SCS, and CS.

RESULTS AND DISCUSSION

Structure and morphology

The IR spectra of the samples of chitosan and SCS are shown in Figure 1. In the infrared spectrum of chitosan, the 1598 cm^{-1} peak corresponds to the amino group. The absorption band of amide I at 1656 cm^{-1} was very weak, and this was commensurate with a high DD.[13] Compared with the IR spectrum of chitosan (CS), the amino peak disappear in SCS. SCS have new absorption band at 1657 cm^{-1}, 1570 cm^{-1} and 1420 cm^{-1}, corresponding to amide I peak (1657 cm^{-1}), the amide II peak (1570 cm^{-1}) and the carboxyl group (symmetric stretching) of the succinyl. The changes indicated the amidation by reacting chitosan with succinyl.

The IR spectrum of alginate (Fig. 2) showed absorption bands at 3404 cm^{-1} (OH stretching), 1627 cm^{-1} (COO^- asymmetric stretching), and 1432 cm^{-1} (COO^- symmetric stretching). For the blend fibers,

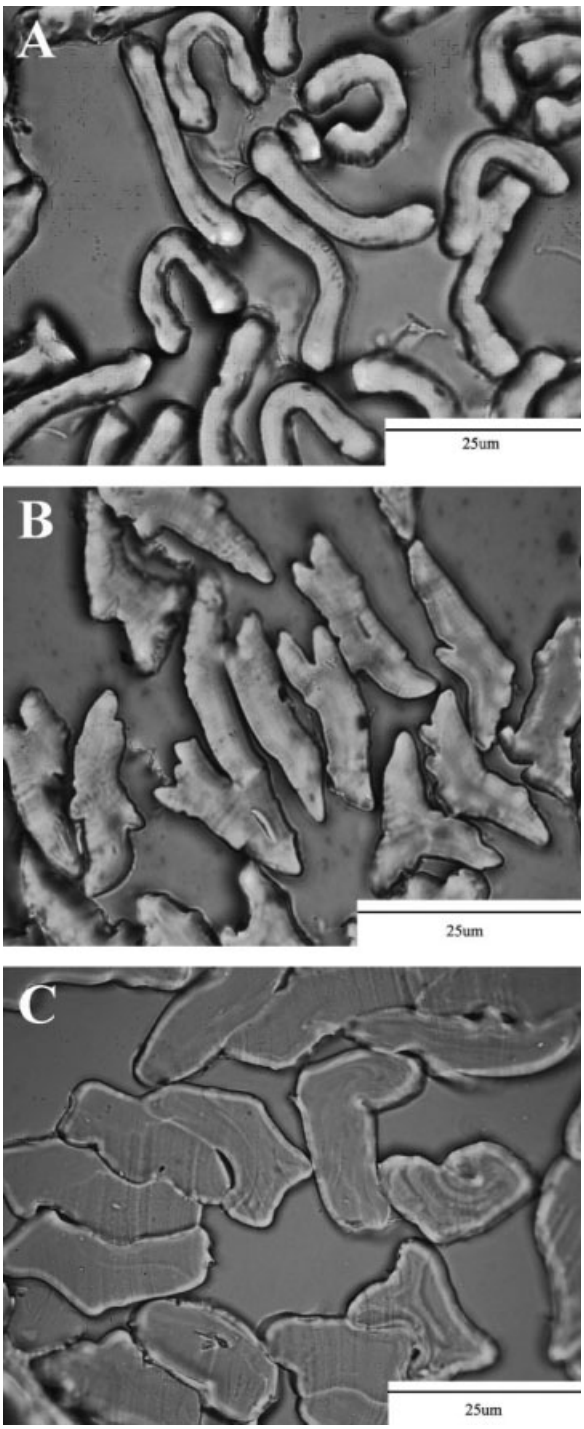

Figure 4 Morphology of the fibers: cross-sections of pure alginate (A), ASu30 (B), and ASu50 (C) obtained by optical microscopy.

TABLE I
The Properties of the Blend Fibers

Fiber	The bacteria reduction rate (BRR)	Tensile strength (cN/tex) (Dry/Wet)	Breaking elongation (%) (Dry/Wet)	WRV (%)
AL	0	10.21/2.51	18.2/42.5	91
ASu10	0	12.75/2.10	31.4/50.5	138
ASu30	0	14.32/1.65	43.5/69.3	306
ASu50	0	11.50/0.92	37.4/55.3	414
ASu70	0	10.32/0.85	20.4/50.1	610
AL_{Ag}	>99.99	10.14/2.23	19.4/41.5	93
$ASu10_{Ag}$	>99.99	11.53/2.08	32.4/51.7	140
$ASu30_{Ag}$	>99.99	14.43/1.84	45.7/70.4	310
$ASu50_{Ag}$	>99.99	11.56/0.88	35.8/52.3	413
$ASu70_{Ag}$	>99.99	10.40/0.76	20.7/49.9	615

the absorption band at around 3404 cm^{-1} concerned with OH stretching vibration for pure alginate narrow and shifted to a high wave number with the increase of SCS content. The absorption band at 1627 and 1432 cm^{-1} for pure alginate fiber assigned to the asymmetric and symmetric stretching vibration of COO^- shifted to a higher wave number with the increase of SCS content. The amide I peak (1657 cm^{-1}), the amide II peak (1570 cm^{-1}) of SCS disappeared in blend fibers. Based upon this evidence, it can be concluded that a certain degree of interaction between alginate and SCS molecules is due to the formation of intermolecular hydrogen bands and Ca^{2+} crosslinking between COO^- of alginate and SCS.

Figure 3 presents the X-ray diffraction pattern of chitosan(CS), *N*-succinyl -chitosan(SCS), alginate, and their blend fibers. Compared to the chitosan, two typical peaks in 15° and 24° vanished, and only two peaks of SCS located at about 9° and 20°. At the same time, the diffraction of alginate shows typical peaks around 14° and 23°. The diffraction peak of alginate rapidly weakened with an increase in the content of SCS. On the other hand, the crystalline character of SCS, such as the peak located at 9°, also gradually disappeared with an increase of the AL content. It indicated that there occurred some interactions between CSC and AL. In addition, the crystallinity values were obtained in the order of SCS >ASu70 >ASu50 >ASu30 >ASu10 >AL. Obviously, that the crystallinities of blend fibers decreased due to the addition of alginate. These results proved a good miscibility between two components in the blend fibers. The results also supported the conclusion drawn from IR that the good miscibility existed between alginate and *N*-succinyl-chitosan was due to the strong interaction from the intermolecular hydrogen bonds.

The cross-sections of the alginate and blend fibers by optical polarizing microscope are shown in Figure 4. The cross-sections of samples showed a smooth and homogeneous morphology. Despite circular holes in the spinneret, extruded alginate fiber appeared flat and ribbon like.[14] The cross-sections of blend fibers expanded with the increase of SCS content, and appeared irregular.

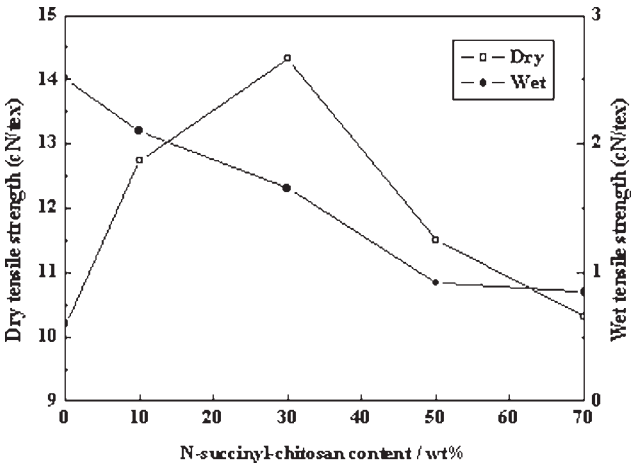

Figure 5 The effect of *N*-succinyl-chitosan content (wt %) on tensile strength of blend fibers.

Mechanical properties of fibers

It is known that the interaction between polymers should influence the mechanical properties of the blend polymer. The effect of SCS content on the tensile strength of fibers in dry and wet states is shown in Table I and Figure 5. The dry tensile strengths of blend fiber were higher than that of pure alginate, and the maximum value was observed at 30% SCS content which achieved 14.32 cN/tex. The wet tensile strength of fibers decreased with increase of SCS content. The addition of SCS was effective in inducing an improvement of the dry tensile strength of the blend fibers. Table I and Figure 6 show the

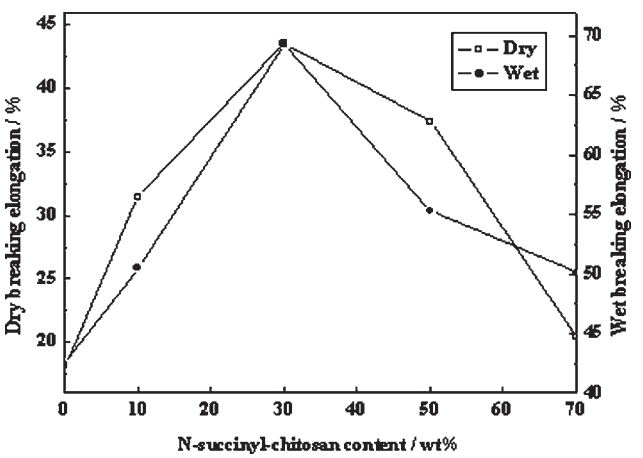

Figure 6 The effect of *N*-succinyl-chitosan content (wt %) on breaking elongation of blend fibers.

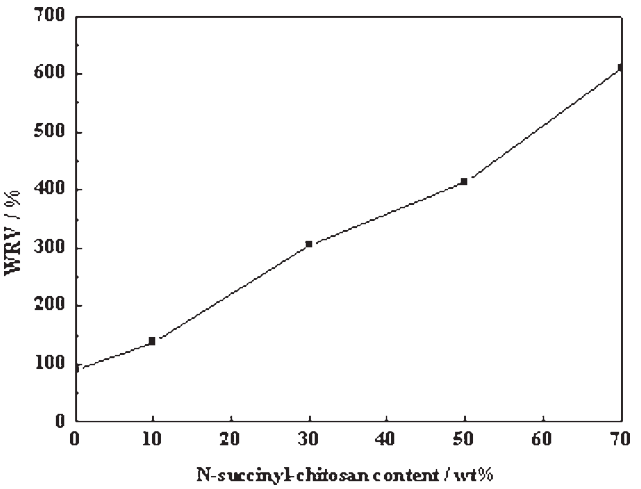

Figure 7 The effect of *N*-succinyl-chitosan content (wt %) on the water retention volume of blend fibers.

breaking elongation of the fibers in dry and wet states. The maximum value of 43.5% (in the dry state) was achieved when the SCS content was 30 wt %. The wet breaking elongation achieved maximum value (69.3%) when the SCS content was 30 wt %. So through controlling the blend condition, fiber with better mechanical property than pure alginate can be achieved. The mechanical properties and water-retention properties of $AgNO_3$-treated fibers were not significantly different from the untreated fibers (Table I). The latter implies that the silver ion was more like a coating, not penetrating the alginate-based fibers.

Water-retention properties

The water-retention values (WRV) of alginate/SCS blend fibers increase dramatically as the SCS content is raised (Fig. 7). The water-retention values of the blend fibers were in the range 138–610%, obviously higher than that of pure alginate fiber, which has the lowest values (91%) (Table I). The improvement in water-retention is due to the excellent water-retention ability of SCS. The good hydrophilicity is important for application as a good wound dressing fiber.

Antibacterial testing

The antibacterial properties of the fibers were investigated (Table I) showing that untreated fibers didn't have antibacterial activity and the fibers treated with $AgNO_3$ have good antibacterial activity toward *Staphylococcus aureus*. It is well known that silver ion has good antibacterial properties. The alginate-base fibers were immersed in silver nitrate solution, and the calcium alginate fiber converted into calcium/silver alginate fiber. Thus, the treated fiber has good antibacterial activity compared with the untreated fiber.

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

CONCLUSIONS

Alginate and SCS blend fiber can be obtained by spinning their solution through a viscose-type spinnet into a coagulation bath containing aqueous $CaCl_2$ and ethanol. The strong intermolecular interaction between alginate and SCS molecule occurred in the blend fibers. There was good miscibility between alginate and SCS molecules due to the strong intermolecular interaction. The best values of the dry tensile strength and breaking elongation were obtained when SCS content was 30 wt %. The wet tensile strength decreased with the increase of SCS content, and the wet breaking elongation achieved maximum value when the SCS content was 30 wt %. The most obvious change is the introduction of SCS in the blend fiber can dramatically improve water-retention properties of blend fiber compared to pure alginate fiber. The $AgNO_3$ treated fibers have good antibacterial activity toward *Staphylococcus aureus*. This novel alginate and SCS blend fiber is a kind of promising fiber in application of wound dressing.

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