

Composite Films of N,O-Carboxymethyl Chitosan and Bamboo Fiber

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ABSTRACT: To obtain an antibacterial chitosan derivative, composite films of N,O-Carboxymethyl Chitosan (N,O-CMCS) and bamboo fiber were prepared. A water-soluble chitosan derivative-N,O-CMCS was synthesized from chitosan with chloroacetic acid in alkaline solution. Composite films with 1–5 wt % N,O-CMCS content were prepared in NaOH/urea/thiourea solution. The DS of N,O-CMCS reached 1.70 and the water solubility increased with the increasing of DS. The carboxymethyl group was introduced into chitosan, which led to the decrease of thermal stability and crystallinity. The structural characterization confirmed that N,O-CMCS was adsorbed on the surface of bamboo fiber. The antibacterial performance of the composite films were enhanced with the increasing of N,O-CMCS content. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 39851.

KEYWORDS: N, O-carboxymethyl chitosan; bamboo fiber; antibacterial activity

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INTRODUCTION

Bamboo fiber, known as “Chinese fiber” in the Canton fair, is a textile fiber material. It has very wide applications and has been processed into many kinds of textile products. The overall mechanical properties of bamboo fiber are comparable to or surpass those of wood fibers.¹ Recently, bamboo fiber has been highlighted because of its unique characteristics. For example, bamboo fiber reinforced biocomposites^{2–4} has already been prepared owing to the rapid growth rate of bamboo⁵ and specific properties of bamboo fiber, like antibacterial activity, environmental friendliness, and outstanding hygroscopicity.⁶

Chitosan is extracted from chitin which is the second most ubiquitous natural polysaccharide after cellulose on earth and is often considered as the cellulose derivative.^{7,8} As the naturally plentiful and renewable polymer, chitosan has attracted considerable interests due to its excellent properties such as antibacterial activity, biocompatibility, biodegradability, film forming property, nontoxicity, and nonpollution.^{9,10} Although soluble in some dilute acid solution, chitosan has poor solubility in water, alkaline solutions, and most organic solvents. To overcome this problem, numerous studies have been carried out to obtain water-soluble chitosan derivatives by chemical modification.^{11–14} Among the derivatives, carboxymethyl chitosan (CMCS) is the most common kind because of its better solubility in water with wide pH value.^{15–18}

Having a molecular structure similar to that of cellulose, chitosan with its derivatives has been mixed with cellulose to

prepare antibacterial materials. Such writers as Zhuang and Liu¹⁹ combined CMCS and cotton fiber to prepare CMCS/cellulose blend films and discussed the antibacterial activity, compatibility, and mechanical properties. Teli and Sheikh²⁰ applied chitosan extracted from shrimp shells waste to the antibacterial finishing of bamboo rayon. Nitayaphat et al.²¹ prepared chitosan/bamboo charcoal composite films and discussed the mechanical properties. However, there has been no research about preparing composite films of CMCS and bamboo fiber.

Considering that the antibacterial activity of CMCS is related to the content of NH_2 ²² and the preparation process of O-CMCS is relatively more complicated than other derivatives, N,O-CMCS with high total degree of substitution (DS) and low substitution on NH_2 (DS_N) was synthesized from chitosan and chloroacetic acid with light concentration of alkaline. N,O-CMCS/bamboo fiber composite films with different content of N,O-CMCS were synthesized. The structures of chitosan, N,O-CMCS, and composite films were characterized. Besides, the antibacterial activity of composite films against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was examined in this study.

EXPERIMENTAL

Materials

Chitosan, whose molecular weight measured by viscometry was 4.5×10^5 and the degree of deacetylation was 0.85, was supplied by Sinopharm Chemical Reagent (Shanghai, China). Bamboo fiber was prepared by unbleached sulfate sinocalamus

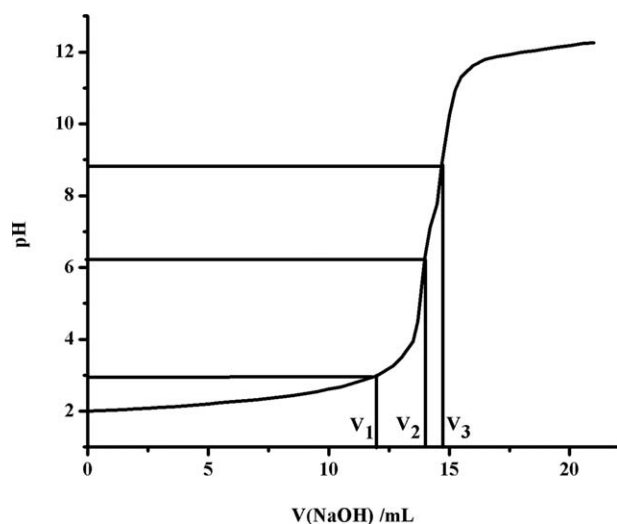


Figure 1. Potentiometric titration curve of N,O-CMCS.

affinis pulp with HQPA treatment. All other chemicals were analytical grade.

Preparation of N,O-CMCS

Chitosan (5 g) and a certain amount of chloroacetic acid were dissolved in 200 mL NaOH solution (6.25 wt %). The reaction mixture was heated to a certain temperature with continuously stirring and maintained for a period of time. During the reaction, the reactant system should always be kept weak alkali. The resultant mixture was adjusted to pH 7.0 using HCl solution. After filtration for removal of the unreacted chitosan, the filtrate was diluted with 95% ethanol (v/v 1 : 3). The precipitation was filtered off, washed repeatedly with absolute ethanol until the color of solid was light yellow or white, and freeze-dried under vacuum to obtain the final desired product N,O-CMCS. Eight groups of N,O-CMCS were synthesized.

Water Solubility and DS Measurement

The solubilities of N,O-CMCS with different DS were measured in water at room temperature. The sample was slowly added into 10 mL stirring water until the solution was saturated. The DS of N,O-CMCS was measured using potentiometric titration method. N,O-CMCS was dissolved in HCl standard solution and titrated by NaOH standard solution. Potentiometric titration curve as is shown in Figure 1 was drawn based on the pH and the volume of NaOH solution. The second order micro commercial law was used to get the sudden-change point to calculate the DS of N,O-CMCS according to the following formulas:

$$A = \frac{(V_2 - V_1)C}{m}; B = \frac{(V_3 - V_2)C}{m}; \text{NH}_2\% = \frac{0.203B}{1 - 0.058B} \times 100\%$$

$$\text{DS} = \frac{0.203A}{1 - 0.058A}; \text{DS}_N = \text{DD} - \text{NH}_2\%; \text{DS}_O = \text{DS} - \text{DS}_N$$

where, V_1 : the potentiometric titration end-point of excessive HCl (mL); V_2 : the potentiometric titration end-point of $-\text{COOH}$ (mL); V_3 : the potentiometric titration end-point of

$-\text{NH}^+$ (mL); C: the molar concentration of NaOH solution (mol/L); m : the weight of N,O-CMCS (g); DD: the degree of deacetylation of chitosan (85%); DS_N : the DS on NH_2 ; DS_O : the DS on OH.

Preparation of N,O-CMCS/Bamboo Fiber Composite Films

The optimal group of N,O-CMCS was used to prepare composite films with bamboo fiber. Bamboo fiber (2 g) and N,O-CMCS were combined in NaOH/urea/thiourea (8 : 8 : 6.5 wt %) solution with constantly stirring at -12°C for 20–30 min. The films were fabricated by casting the mixtures on a glass plate, followed by soaking in water and drying. The resultant films were washed three times to ensure complete removal of NaOH/urea/thiourea solution. In view of its following application and cost, composite films contained 1–5 wt % N,O-CMCS in relation to the content of bamboo fiber. The controlled sample of pure bamboo fiber film was also prepared without the addition of N,O-CMCS.

Thermal Analysis

The thermogravimetric analysis (TGA) and differential thermal analysis (DTG) of N,O-CMCS and chitosan were performed using a TGA Q5000 V3.15 Build 263 thermo gravimetric analyzer. The samples weighted around 10 mg were heated from 50 to 500°C at a heating rate of $10^\circ\text{C}/\text{min}$ under an inert atmosphere of N_2 .

Fourier Transform Infrared Analysis

Fourier transform infrared (FTIR) spectra of N,O-CMCS, chitosan and composite films were recorded on a Tensor 27 spectrometer (Bruker) with KBr pellets in the range of $4000\text{--}400\text{ cm}^{-1}$.

X-ray Diffraction

Crystallinity of N,O-CMCS, chitosan, and composite films was measured by X-ray diffraction method using an XRD-6000X diffractometer with $\text{Cu K}\alpha$ X-radiation between 2θ angles of $5^\circ\text{--}40^\circ$.

Scanning Electron Microscopy (SEM) Analysis

SEM was used to investigate the surface smoothness of composite films and the connection between N,O-CMCS and bamboo fiber. Pieces were cut from films and coated with gold, and then observed with a SEM (S-3000N) at acceleration voltages of 10 kV.

Antibacterial Activity Determination

GB/T 20944.3-2008 test method was applied for determining the antibacterial activity. *E. coli* and *S. aureus* were selected as test bacteria.

RESULTS AND DISCUSSION

Single-Factor Experiments of N,O-CMCS Preparation

The synthesis of N,O-CMCS included two steps: (1) chitosan reacted with alkali to generate alkaline activation center;

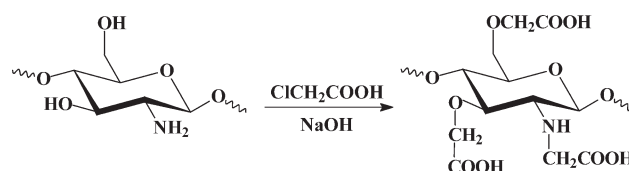
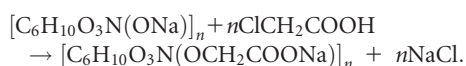
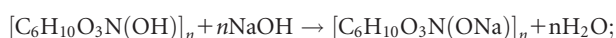


Figure 2. Reaction schematic of N,O-CMCS preparation.

Table I. Single-Factor Experiments and Water Solubility

	Chitosan : Chloroacetic acid (mass ratio)	Temp (°C)	Time (h)	DS	DS _O	DS _O /DS (%)	Water solubility (g/10 mL)
1	1 : 5	90	5.5	1.53	1.04	67.52	0.90
2	1 : 4	90	5.5	1.57	1.01	64.46	0.97
3	1 : 5	85	5.5	1.68	1.35	80.34	1.02
4	1 : 4	85	5.5	1.70	1.38	80.97	1.03
5	1 : 5	80	5.5	1.46	1.06	72.74	0.73
6	1 : 5	80	5	1.25	0.85	68.24	0.43
7	1 : 4	80	5	1.35	0.88	65.15	0.53
8	1 : 5	70	5.5	1.22	0.75	61.28	0.43

(2) nucleophilic substitution reaction took place between the generated alkaline activation center and chloroacetic acid to obtain the structure of carboxylic acid salt. The schematic of the reaction is shown in Figure 2. The chemical equations are as follows:



According to the above reaction kinetics, and experiments Song et al.⁸ and our research group did before, the mass ratio of chitosan to chloroacetic acid, reaction temperature and reaction time were chosen to discuss the influence of DS on N,O-CMCS preparation. The results are listed in Table I. The DS of N,O-CMCS were all higher than 1.0, of which the highest one is up to 1.70. The carboxymethyl group was mostly introduced onto OH at C6 and C3 with a small amount onto NH₂ at C2 (DS_O/DS > 50%). So the aim to synthesize N,O-CMCS with high total DS and low DS_N was realized and the antibacterial activity of chitosan was not influenced seriously.²³ The reason is that nucleophilic substitution reaction of NH₂ at C2 and OH at C6 and C3 can both take place. The electronegativity of O is bigger than N, and the steric hindrance of OH at C6 is obviously the smallest. Thus, the sequence of substitution site is: OH-6 > OH-3 > NH₂-2.²⁴

As shown in Table I, the DS of the product increased with the reaction temperature. The reaction proceeded very slowly below 80°C, and little product was obtained. However, when the temperature reached 90°C, the product turned into yellow. Therefore, the temperature 85°C was the most appropriate. It was also observed that with the increase of the amount of chloroacetic acid, the DS decreased accordingly. In addition, longer reaction time contributed to higher DS. Therefore, the optimal reaction conditions were as follows: the mass ratio of chitosan to chloroacetic acid was 1 : 4, the reaction temperature was 85°C, and the reaction time was 5.5 h. Therefore, the fourth group of N,O-CMCS was chosen to prepare composite films with bamboo fiber.

Water Solubility

The solubilities of N,O-CMCS were measured in diluted water. The results are listed in Table I. It was found that all the products were well soluble in water. The solubilities of the products increased with the DS. When the DS was 1.70, the solubility of product reached 1.03 g/10 mL.

TG Analysis of N,O-CMCS and Chitosan

TGA of N,O-CMCS and chitosan was carried out to evaluate their degradation profiles and thermal stability. The TGA and DTG curves are presented in Figure 3. As shown from the thermogram of chitosan, the TGA curve of the pyrolysis process could be divided into two weight loss stages, corresponding to the slow pyrolysis (50–100°C) and first pyrolysis (255–400°C) stages, respectively. At the first stage, the mass loss was associated with the volatilization of water. The maximum rate of weight loss was observed at the second stage, in which over 45% weight was pyrolyzed between 255 and 400°C. In comparison, the TGA curve of N,O-CMCS could also be divided into two stages, but the thermal stability of N,O-CMCS was poorer than that of chitosan since the onset of thermal degradation of N,O-CMCS

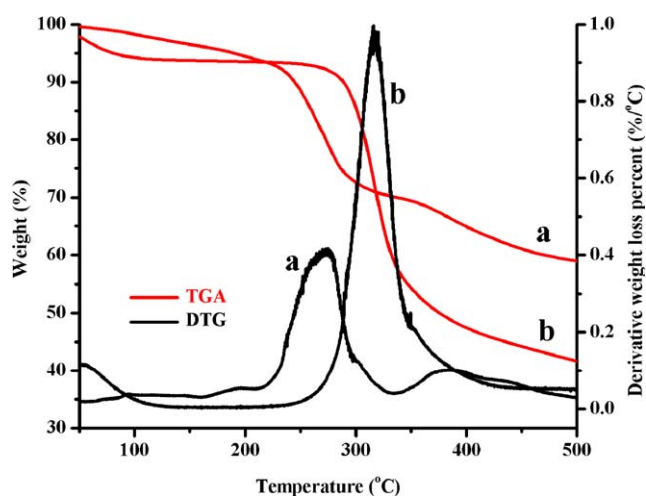


Figure 3. TGA and DTG curves of (a) N,O-CMCS and (b) chitosan. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

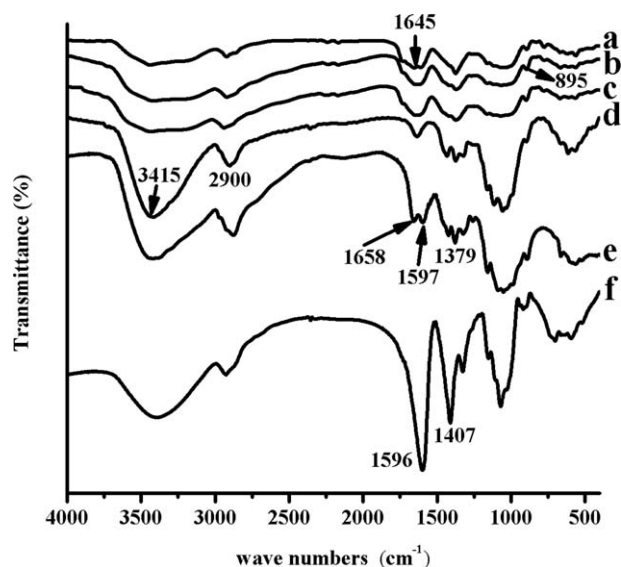


Figure 4. FTIR spectra of (e) chitosan, (f) N,O-CMCS, (d) pure bamboo fiber film and composite films with different N,O-CMCS content: (a) 1, (b) 3, and (c) 5%.

was about 220°C.²⁵ And the maximum rate of weight loss of N,O-CMCS was only about 23% between 220 and 340°C. This is mainly because during the process of carboxymethylation, the macromolecule chains of chitosan were broken by the high temperature and alkali. In addition, this contributed to the decrease and even damage of polymerization degree of chitosan. Therefore, the thermostability of chitosan decreased.

FTIR of N,O-CMCS, Chitosan, and Composite Films

The structure of N,O-CMCS, chitosan, and composite films was studied by FTIR spectroscopy (Figure 4). The spectrum of chitosan showed its unique characteristic peaks at 1658, 1597, and 1379 cm^{-1} , which were attributed to the stretch and vibration of amide I, II, and III separately. In the IR spectrum of N,O-CMCS, the bands at 1596 and 1407 cm^{-1} corresponded to the C=O stretching vibration and $-\text{CH}_2\text{COOH}$ vibration, respectively, which indicated the existence of carboxymethyl group.²⁶ The IR spectra of composite films with different N,O-CMCS content were similar. The peak at 3415 cm^{-1} contributing to OH apparently became wider and weaker in composite films than that in pure bamboo fiber film owing to the substitution of carboxymethyl group. The existence of N-H stretching vibration band at 895 cm^{-1} and the appearance of 1645 cm^{-1} in composite films assigned to $-\text{NH}_3^+$ indicated the carboxymethyl group to be mostly on OH.¹⁹ Compared with the spectrum of pure bamboo fiber, O-H and N-H stretching peaks at 3415 and 2900 cm^{-1} in composite films became wider and shifted to a higher wave number, which showed the presence of the interaction between N,O-CMCS and bamboo fiber. The presence of the adsorptions of NH_3^+ ensured the antibacterial activity of composite films.

XRD Analysis of N,O-CMCS, Chitosan, and Composite Films

The X-ray diffractograms of N,O-CMCS, chitosan, and composite films are shown in Figure 5. It was shown that the XRD pro-

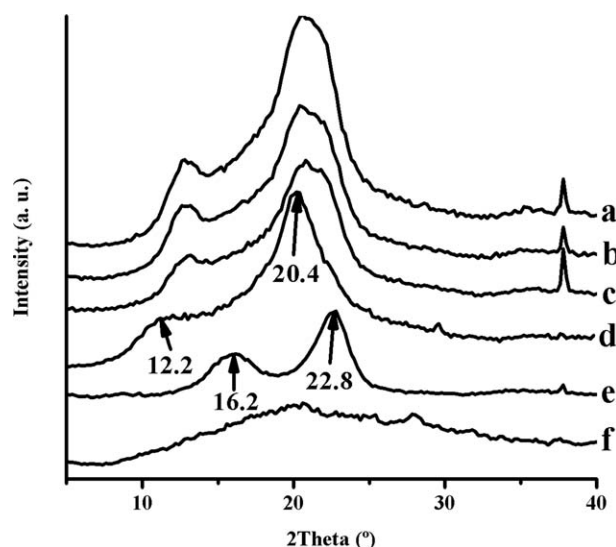


Figure 5. XRD patterns of (d) chitosan, (f) N,O-CMCS, (e) pure bamboo fiber film and composite films with different N,O-CMCS content: (a) 1, (b) 3, and (c) 5%.

files of chitosan exhibited typical diffraction angles (2θ) around at 12.2° and 20.4°, while N,O-CMCS was almost amorphous. This is because the introduction of carboxymethyl group increased the distance between molecular chains and destroyed the original crystal structure. The XRD diffraction pattern of pure bamboo fiber film showed two cellulose II angles (2θ) around at 16.2° and 22.8°, which are assigned to the diffraction planes of (110) and (200), respectively. These two peaks moved in the direction of lower diffraction angle apparently in composite films²⁷ and the intensity of diffraction peaks weakened gradually with the increasing of N,O-CMCS content, which illustrated that there presented interaction between N,O-CMCS and bamboo fiber.

SEM Analysis of Composite Films

SEM pictures of composite films with various N,O-CMCS content are presented in Figure 6. Random dispersion of N,O-CMCS in bamboo fiber matrix was observed. As is seen from the picture of pure bamboo fiber film, bamboo fiber was dissolved successfully and the surface was smooth. While in the composite films, erosion happened and some flake material was adsorbed on the surface of bamboo fiber, which meant N,O-CMCS was connected to bamboo fiber. Therefore, the roughness increased with N,O-CMCS concentration.

Antibacterial Activity Study of Composite Films

Antibacterial activity of pure bamboo fiber film and composite films were tested against *S. aureus* and *E. coli* and the results are shown in Table II. The antibacterial rate against *S. aureus* and *E. coli* apparently increased with the increasing of N,O-CMCS content. This is because the NH_2 in N,O-CMCS and the H in solution were combined into antibacterial factor $-\text{NH}_3^+$, which was responsible for the antibacterial activity of composite films. The content of antibacterial factor $-\text{NH}_3^+$ increased with the increasing of N,O-CMCS content.²⁸ And the antibacterial rate against *S. aureus* was higher than *E. coli* with the same N,O-CMCS content.

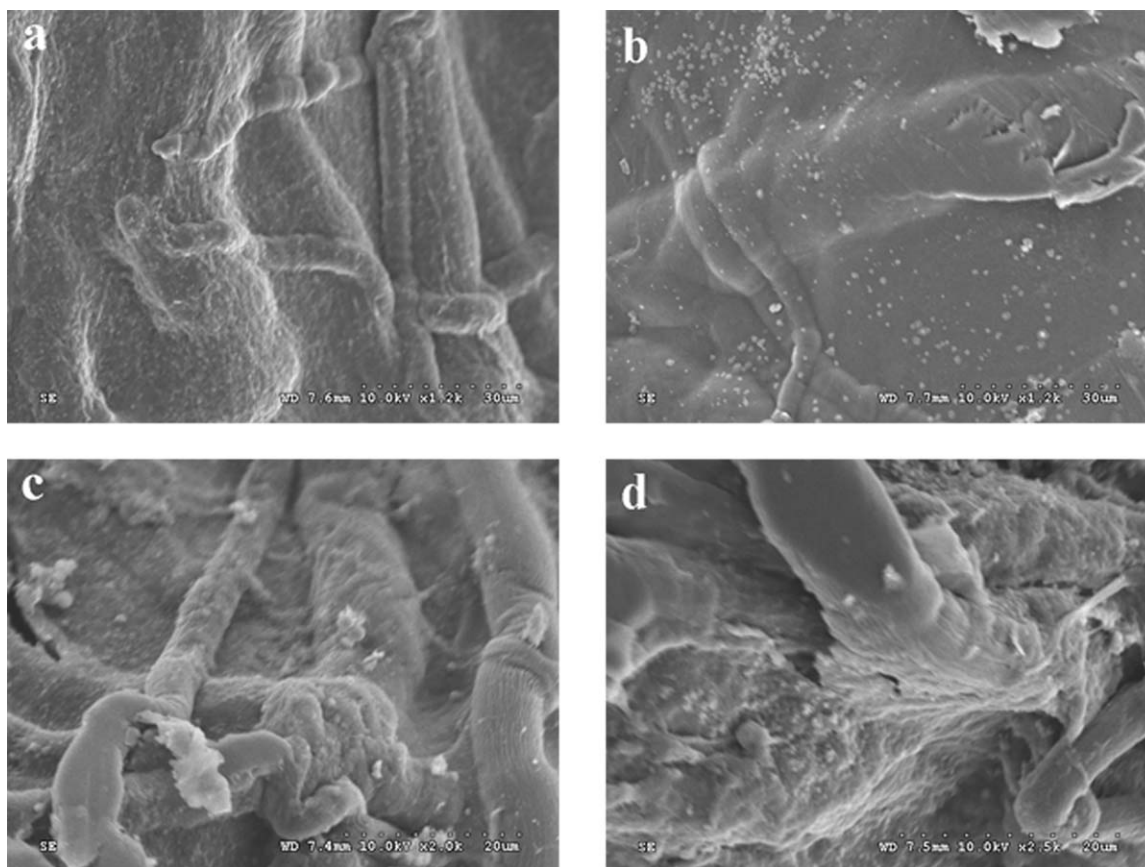


Figure 6. SEM pictures of (a) pure bamboo fiber film and composite films with different N,O-CMCS content: (b) 1, (c) 3, and (d) 5%.

Table II. Antibacterial Activity of Composite Films

Sample	Antibacterial rate against <i>Escherichia coli</i> (%)	Antibacterial rate against <i>Staphylococcus aureus</i> (%)
Pure bamboo fiber film	84.54	94.39
Blend film with 1% N,O-CMCS content	85.66	95.76
Blend film with 3% N,O-CMCS content	87.37	97.23
Blend film with 5% N,O-CMCS content	89.64	99.99

CONCLUSIONS

Water-soluble N,O-CMCS with high total DS and low DS_N was successfully prepared by modifying chitosan with chloroacetic acid in alkaline solution. The optimal reaction conditions were as follows: the mass ratio of chitosan to chloroacetic acid was 1 : 4, the reaction temperature was 85°C and the reaction time was 5.5 h. Using this method, the DS of synthesized N,O-CMCS was up to 1.70 and thus this is a valuable preparative method.

A functional composite film with antibacterial activity could be prepared by introducing N,O-CMCS to bamboo fiber. Composite films containing 1–5 wt % N,O-CMCS showed satisfying characterization and high antibacterial activity. Antibacterial activity increased with the increasing of N,O-CMCS content. The notable properties of composite films are promising for applications in biodegradable and antibacterial material.

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