Carbohydrate Polymers 85 (2011) 221–227

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chitosan derivatives with dual-antibacterial functional groups for antimicrobial finishing of cotton fabrics

Xiaorong Fu [a], Yun Shen [a], Xue Jiang [a], Dan Huang [a,*], Yiqi Yan [a,b,**]

[a] Key Laboratory of Eco-Textile (Jiangnan University), Ministry of Education, Wuxi 214112, China
[b] Dept. of Textiles, Clothing and Design, University of Nebraska-Lincoln, Lincoln, NE 68583, United States

ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form 7 February 2011
Accepted 9 February 2011
Available online 15 February 2011

Keywords:
Chitosan
Double functional chitosan derivatives
Cotton fabric
Textile finishing
Antibacterial activity

ABSTRACT

The three water-soluble chitosan derivatives bearing double functional groups were synthesized with 2,3-epoxypropyltrimethylammonium chloride and benzaldehyde as modifiers through formation of Schiff base, reduction, N-methylation and O-quaternization. The synthesized chitosan derivatives were applied to cotton fabrics together with citric acid (CA) as the crosslinking agent to evaluate their use as durable antimicrobial textile finishing agents. The optimal finishing conditions were investigated and antibacterial activities of finished fabrics were tested with "Shake-Flask Test". The fabrics finished showed strong antimicrobial activities and fairly good durability. The antibacterial efficiency of *S. aureus* and *E. coli* was more than 99% and 96% respectively, when crosslinked with CA (14%, o.w.f) through a two-bath process. The order of antimicrobial activities of the chitosan derivatives was O-quaternized-N,N-biethyl-N-benzyl ammonium chitosans chloride (O-QCTS-DEBn) ≥ O-quaternized-N-chitosan Schiff bases (O-QCTSS) > O-quaternized-N-benzyl-chitosans (O-QCTS-Bn). The antibacterial activity of using O-QCTSS finishing fabric was still more than 75% after 20 consecutive home launderings.

1. Introduction

Nowadays, a safe, healthy and comfortable living environment turns more important and the protection from the infection of pathogenic microorganisms is strengthened. Hence, the demand for medical textiles and healthcare textiles is ever-increasing. SARS and H1N1 virus have spread all over the world and had a tremendous impact on people's daily life. Increasing attention has been paid to studying antimicrobial products since 2003.

Till now, a number of chemicals have been employed to impart antibacterial activity to textiles. These chemicals include inorganic salts, organometallics, iodo-phors (substances that slowly release iodine), phenols and thiophenols, onium salts, antibiotics, heterocyclics with anionic groups, nitro compounds, ureas and related compounds, formaldehyde derivatives, and amines (Lim & Hudson, 2004a; Lim & Hudson, 2004b). However, many of these chemicals are toxic to humans and cannot easily degrade in the nature.

Chitosan, the second most abundant polysaccharide in the nature (Ökem, 2003), is in a deacetylated form of the polysaccharide chitin, which is mainly found in the *exo*-skeleton of crustaceans and has received much attention due to its versatility, biodegradability, non-toxicity and antimicrobial properties (Diana, Miquel, & Keiko, 2009). The textile industry continues to look for eco-friendly process that can be carried out without toxic textile chemicals. In view of this point, chitosan is an excellent candidate for an eco-friendly textile industry. However, the major problems of chitosan as an antimicrobial agent are its loss of the antimicrobial activity under alkaline conditions due to its loss of the cationic nature (Shin & Min, 1996) and its poor durability on textile fabrics due to its poor adhesion to fabrics (Lim & Hudson, 2004b). Chitosan contains a primary amine and two hydroxyl groups in every monosaccharide residue and is able to undergo many reactions with amines and alcohols. Chemical modifications on chitosan can overcome the problems and enhance the properties of chitosan. It is acknowledged that, both quaternary ammonium salt group and Schiff base are antimicrobial. Quaternary chitosan derivatives can be applied to drug carriers (Thanou, Verhoef, & Junginger, 2001; Wan, Sun, & Li, 2009) and Schiff bases are described as promising antibacterial agents (Venugopala & Jayashree, 2008). The quaternized chitosans (Daly & Guerrini, 1998; Rúnarsson et al., 2007; Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2008; Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2009) and chitosan-Schiff bases (Jin, Wang, & Bai, 2009; Guo et al., 2007) have been respectively synthesized and their antimicrobial activ-

* Corresponding author.
** Corresponding author at: Key Laboratory of Eco-Textile (Jiangnan University), Ministry of Education, Wuxi 214112, China.
E-mail addresses: huangdan6@163.com, huangdan@jiangnan.edu.cn (D. Huang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.02.019

ity has been investigated. Recently Zhao and Xia (2006) reported that the chitosan derivatives bearing double functional groups had higher growth suppression against *S. aureus* and *E. coli* compared to corresponded single functional group derivative.

In this investigation, we synthesized three water-soluble chitosan derivatives bearing double functional groups. The structures of the chitosan derivatives are depicted in Scheme 1. The quaternary ammonium salt group, which enhances the antimicrobial activity as well as the water solubility of chitosan over the entire pH range, is on the O-6. Schiff base is on the N, which also enhances the antimicrobial activity toward different microorganisms. The synthesized chitosan derivatives were applied to finish cotton fabrics using CA as the crosslinking agent, and the optimal application condition and antibacterial activities of finished fabrics were investigated.

2. Experimental

2.1. Materials

Chitosan is a commercial material supplied by Yuhuan Biochemical Co. Ltd., Zhejiang, China. The deacetylation degree is more than 90% and viscosity average molecular weight is 1.75×10^5. As a fabric for test, 100% cotton cloth, which was bleached, desized and mercerized, was purchased from Qing Feng Co. Ltd. (Wuxi, China). Benzaldehyde, GTMAc (glycidyl-trimethylammonium chloride), $NaBH_4$, NaI, C_2H_5I, isopropanol, citric acid monohydrate (CA), sodium hypophosphite (NaH_2PO_2) and nonionic surfactants (JFC) were purchased from Sinopharm Chemical Reagent Co. Ltd. and used without further purification. *S. aureus* and *E. coli* were supplied by Microbiology Laboratory of the Jiangnan University of China.

2.2. Instruments

Scanning electron microscope (SEM) Quanta 200 was used to examine the surface morphology of cotton fiber. The samples were coated with a thin layer of gold by sputtering before the SEM imaging. An accelerating voltage of 5 kV with accounting time of 100 s was applied. Fourier transform infrared spectroscopy (Nicolet Nexus 470, Thermo Electron Corporation Company) was used to recorded FTIR spectra. All samples were prepared as potassium bromide pellets.

2.3. Synthesis of chitosan derivatives

The synthesis route of chitosan derivatives is showed as Scheme 1.

2.3.1. Synthesis of O-quaternized-N-benzylidene-chitosan (O-QCTSS)

Chitosan Schiff base (N-benzylidene-chitosan) was prepared according to Kurita's method (Kurita, Mori, Nishiyama, & Harata, 2002). Chitosan (2.000 g, 12.4 mmol pyranose) was dissolved in 60 mL of 2% aqueous acetic acid. Then 40 mL of methanol was added and the mixture was stirred for an hour for swelling of chitosan. Then benzaldehyde (13.14 g, 124 mmol) dissolved in 40 mL methanol was added to the resulting viscous solution and the mixture was stirred at room temperature for 24 h. When the reaction ended, the resulting white gel-like mixture was broken with a spatula, washed with 200 mL of acetone, and filtered. The product was pulverized, washed with 100 mL of acetone for 30 min by stirring, and filtered. It was then treated with 400 mL of 5% aqueous sodium hydrogen carbonate for 10 min at room temperature, filtered, and washed with deionized water repeatedly until neutral. After subsequent immersing with 300 mL of methanol overnight, the resulting Schiff base of chitosan was filtered and dried under vacuum to give a white powder (2.5631 g). FTIR (KBr) (cm^{-1}): 3415 (ν_{O-H}), 2960 and 2870 (ν_{C-H}), 1641 ($\nu_{C=N}$; $\nu_{C=O}$), 1458 (ν_{Ar}), 1150–1000 (pyranose).

Chitosan Schiff base (1.500 g, 6.15 mmol pyranose) and GTMAc (4.650 g, 30.6 mmol) were distracted in 75 mL of isopropanol and the mixture was stirred at 70 °C for 30 h. Then the solid was filtered, washed with acetone and ethanol solution (95%), filtered and dried under vacuum yielding 1.722 g of O-QCTSS as a buff powder. It can dissolve in hot water. The DS (degree of substitution) of GTMAc on chitosan was measured by titration of Cl^- with standard aqueous silver nitrate ($AgNO_3$) solution (Hamman & Katze, 2001) and was 0.9. FTIR (KBr) (cm^{-1}): 3449 (ν_{O-H}), 2927 and 2860 (ν_{C-H}), 1648 ($\nu_{C=N}$; $\nu_{C=O}$), 1479 (ν_{Ar}; δ_{C-H} of $(CH_3)_3N^+R$), 1161–1027 (pyranose).

2.3.2. Synthesis of O-quaternized-N-benzyl-chitosans (O-QCTS-Bn)

O-QCTSS (1.000 g, 5.00 mmol pyranose) was dispersed in 100 mL of methanol, and the dispersion was stirred for 30 min. $NaBH_4$ solution (10%, 2 mL) was added, and the mixture was stirred at room temperature for 24 h. The solid was obtained after solvent was removed via rotary evaporator, washed thoroughly and dried to yield 693.5 mg (63%) of O-QCTS-Bn. It can dissolve in warm water. Degree of quaternization (DQ) of O-QCTS-Bn was measured by conductometric titration (Lim & Hudson, 2004a; Lim & Hudson, 2004b) at 20 ± 0.5 °C. The calculated value of DQ was 92.7. FTIR (KBr) (cm^{-1}): 3448 (ν_{O-H} and ν_{N-H}), 2960, 2930, and 2870 (ν_{C-H}), 1653($\nu_{C=O}$, δ_{N-H}), 1478 (ν_{Ar}, δ_{C-H} of $(CH_3)_3N^+R$), 1424 (ν_{Ar}), 1383 (δ_{C-H}) and 1150–1020 (pyranose).

2.3.3. Synthesis of O-quaternized-N,N-bimethyl-N-benzyl ammonium chitosans chloride (O-QCTS-DEBn)

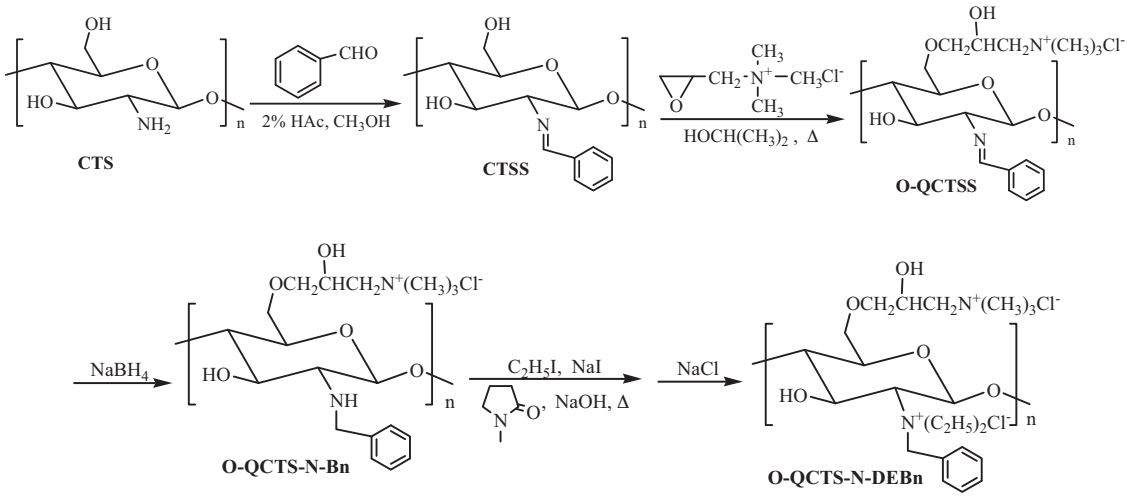
O-quaternized-N-benzyl-chitosans (about 2.000 g, 5.0 mmol) was dispersed in 75 mL of *N*-methyl pyrrolidone at room temperature and the solution was stirred. Then sodium hydroxide (5 mL, 3 mol/L), sodium iodide (1.500 g, 10.0 mmol) and ethyl iodide (4.0 mL, 49.5 mmol) were added and the mixture was stirred for 8 h at 36 °C. Finally acetone was added and the precipitate of bisquaternary ammonium salt of chitosan was collected. For exchanging I^- with Cl^-, the polymer was dissolved in 20 mL of 10% sodium chloride solution. The polymer was precipitated with acetone, centrifuged and dried to obtain O-QCTS-DEBn as a white water-soluble powder (1.058 g, yield: 43%). DQ of O-QCTS-DEBn was 120.4 (Lim & Hudson, 2004a; Lim & Hudson, 2004b). FTIR (KBr) (cm^{-1}): 3427 (ν_{O-H}), 2960, 2937, and 2829 (ν_{C-H}), 1649 ($\nu_{C=O}$, δ_{N-H}), 1483 (ν_{Ar}, δ_{C-H} of $(CH_3)_3N^+R$), 1383 (δ_{C-H}) and 1156–1066 (pyranose). (ν_{C-O}).

2.4. Fabric treatment

As there are no reactive groups between chitosan derivative and cellulose fiber, CA was used as the crosslinking agent to anchorage the chitosan derivative to the fabric so as to improve the durability and efficiency of the resultant antimicrobial textiles.

2.4.1. One-bath process

A pad-dry-cure method was used and crosslinking agent is CA in the presence of NaH_2PO_2 as catalyst. Cotton samples were dipped in an aqueous solution containing O-QCTSS (0–5%, o.w.f = on weight of fabric), crosslinking agents (0–20%, o.w.f), penetrant JFC (1%, o.w.f) and catalyst (6%, o.w.f) at 80 °C for 30 min using material-to-liquor ratio 1:20, then double-dip-double-nip at 100% wet pick-up (WPU) using a laboratory padder (Rapid P-AO). The padded samples were dried at 80 °C for 3 min and cured in a laboratory oven (Rapid R-3) for 1–5 min at 120–160 °C, respectively. The treated fabrics were washed thoroughly to remove unfixed O-QCTSS, crosslinker, and catalysts, and placed in a desiccator till further study after drying at 80 °C.



Scheme 1. Synthetic route of chitosan derivatives.

2.4.2. Two-bath process

Cotton samples were first dipped in aqueous solutions containing CA (0-20%, o.w.f) in the presence of NaH_2PO_2 as catalyst (6%, o.w.f) at 80 °C for 30 min using material-to-liquor ratio 1:20 and double-dip-double-nip at 100% wet pick-up (WPU), dried at 80 °C for 3 min. The samples were subsequently dipped in the aqueous solutions containing O-QCTSS (0–5% o.w.f) and JFC (1%, o.w.f) at 80 °C for 30 min using material-to-liquor ratio 1:20, followed by pad-dry-cure method and cured at 120–160 °C for 1–5 min. Finally the treated fabrics were washed thoroughly to remove unfixed O-QCTSS, crosslinker and catalysts, and placed in a desiccator till further study after drying at 80 °C.

2.5. Quantitative analysis of the amount of chitosan derivatives on fabrics

The amount of chitosan derivatives was measured by a stoichiometric dye adsorption method (Roberts, 1997) using C.I. Acid Red 42 (CAS: 6245-60-9) as a dye indicator. Each chitosan derivative treated cotton fabric swatch (0.4 g) dye in 20 mL aq. Dye solution (0.5 g/L) at 50 °C for 24 h. The dyed fabric swatches were thoroughly washed in deionized water until the water did not show any color. The dyed solution and the washed water were collected, transferred to a 250 mL volumetric flask and make up to volume. The absorbance of the solution was measured at λ_{max} (=512 nm) using a UNIC UV-2100 spectrophotometer. Based on the absorbance, the concentration of the solution was calculated by using the Beer-Lambert Law. The calculated concentration was converted to the amount of dye (mmol/kg fiber) adsorbed on the chitosan derivatives treated fabric. The amount of dye on fabrics (*Ad*) was calculated using Eq. (1):

$$Ad(\text{mmol/kg fiber}) = \frac{(A_1 - A_2)V}{KbM} \times 10^3 \quad (1)$$

where A_1 and A_2 are the absorbance of dye solution for the fore and after dyeing respectively; V = 500 mL; K = 8495 (calculated from Calibration curve of C.I. Acid Red 42 in deionized water); b = 1 cm; M (g) stands for the weight of fabrics.

C.I. Acid Red 42 is an anionic dye having ionic interaction with the quaternary ammonium salt groups on the chitosan derivatives. We assume that there existed a 1:1 stoichiometry between the dye and the quaternary ammonium group of the chitosan derivatives. Under the dyeing condition (50 °C, 24 h), the quaternary ammonium groups on the chitosan derivatives treated fabric were completely saturated with the dye molecules. The amount of dye on a fabric treated with chitosan derivatives was used as a measure of the amount of quaternary ammonium groups on the chitosan derivatives treated fabric. The amount of quaternary ammonium group for each sample was corrected by subtraction of that of a control (2.3 mmol/kg), which is a cotton not treated with the chitosan derivatives.

2.6. Whiteness measurement

The Hunter Whiteness Index (WI) was measured on each fabric using an Automatic WSB (Kangguang® WSD-III). Each fabric was folded thrice and the whiteness was measured for four times at different portions of the fabric surface. The final Hunter Whiteness was the average of the four values.

2.7. Antimicrobial test

The antimicrobial properties of the chitosan derivatives-treated cotton fabrics were evaluated by GB/T 20944.3-2008 (National Standards of the People's Republic of China, Textiles – Evaluation for antibacterial activity). This method was used for antimicrobial fabric. Antimicrobial percentage (*AP*) was calculated using Eq. (2):

$$AP\,\% = \frac{B - A}{B} \times 100\% \quad (2)$$

where *A* and *B* are the surviving cells (CFU/mL) for the flasks containing test samples (chitosan derivatives treated cotton) and the control sample (blank cotton), respectively.

2.8. Antimicrobial durability test

To evaluate the durability of chitosan derivatives on fabrics against repeated launderings, treated fabrics were washed according to GB/T 8629-2001 (National Standards of the People's Republic of China for textile, Experiment with the family washing and drying procedures). One cycle of laundering by this method is considered equivalent to five home machine launderings.

3. Results and discussion

Effect of finishing method, conditions, concentrations of crosslinking agent and chitosan derivative on the fixation amount of chitosan derivative on the treated fabrics and the whiteness of

the treated cotton fabrics were studied with O-QCTSS as representation.

3.1. The effect of finishing method

Two application methods, one-bath and two-bath, were employed to apply the O-QCTSS to cotton fabric and were compared in terms of the efficiency of the O-QCTSS adhering on the fabrics. The fixation amount of O-QCTSS on the fabrics with two-bath process and one-bath method was respectively 12.75 ± 0.11 mmol/kg fiber and 11.13 ± 0.10 mmol/kg fiber using CA as crosslinker under the same finishing conditions. The possible reason of lower fixation amount of O-QCTSS using one-bath process is that interaction of quaternary ammonium cations in O-QCTSS with cross-linker CA bearing negative charge formed precipitates, thus the concentration of O-QCTSS in finishing bath was decreased, which leads to reduction of the fixation amount on the fabrics.

3.2. The effect of concentration of cross-linker

Fig. 1(a) shows the influence of crosslinking agent concentration (CA) on the fixation amount of O-QCTSS on the treated cotton fabrics. The amount of O-QCTSS of the fabrics increased with increasing the crosslinking agent concentration and leveled off when concentration of the cross-linker reached 14% in two-bath method and 10% in one-bath process. The reason for the enhancement in the fixation amount is that increasing the crosslinking agent concentration makes crosslinking molecules more available and consequently increases its accessibility to crosslink the cellulosic and O-QCTSS's hydroxyls. The reason for level off in the fixation amount is that crosslink of the linker with cellulosic and O-QCTSS's hydroxyls has achieved equilibrium in the amount of CA over a certain point.

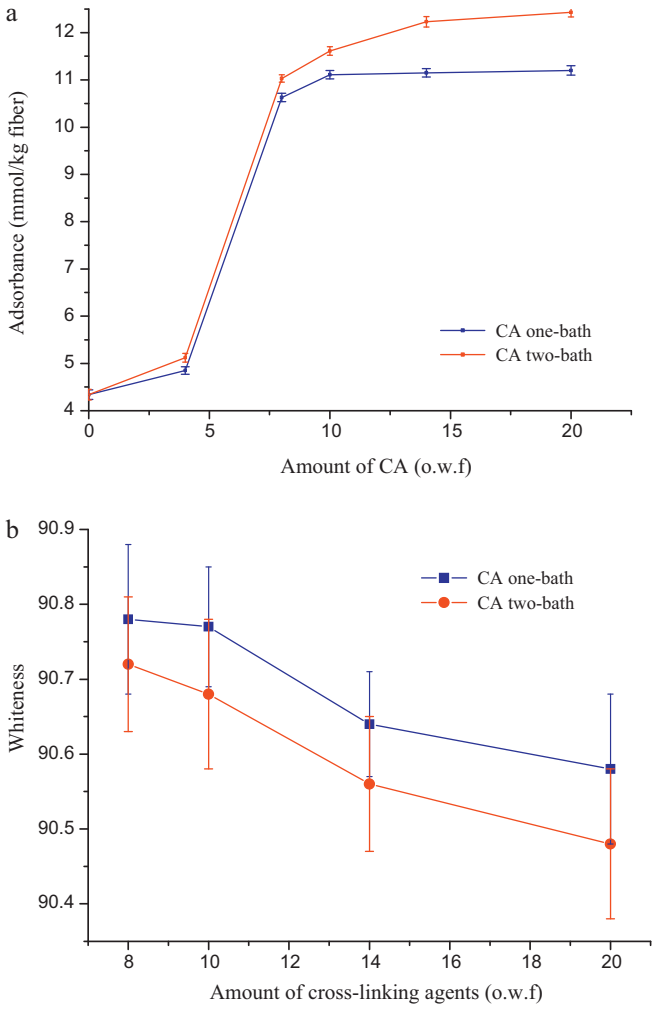


Fig. 1. (a) The effect of concentration of crosslinker on the amount of fixation of O-HTCC. (b) The effect of concentration of crosslinker on the whiteness. O-QCTSS (2% o.w.f.); catalyst NaH_2PO_2 (6%, o.w.f); penetrant JFC (1%, o.w.f); cure conditions (140 °C × 3 min).

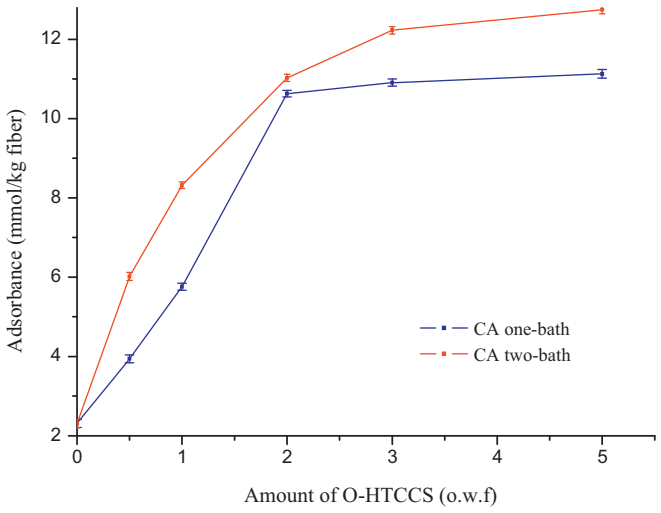


Fig. 2. The effect of O-QCTSS amount on the amount of fixation of O-QCTSS on the fabric crosslinking agent (14%, o.w.f); catalyst NaH_2PO_2 (6%, o.w.f); penetrant JFC (1%, o.w.f); cure conditions (140 °C × 3 min).

On the other hand, the values of whiteness decreased by increasing the cross-linker concentration and were slightly lower in two-bath than in one-bath (Fig. 2(b)). It may be related to cellulose heat treatment, which causes oxidation and scission of cellulosic chains. The presence of aldehyde groups in oxidized cellulose makes the cellulose unstable and causes yellowing of the fabrics. The crosslinking reaction may also cause an additional yellowing for the treated fabrics (El-tahlawy, El-bendary, Elhendawy, & Hudson, 2005). In addition, CA is partially converted to α,β-unsaturated acids (*cis*-aconitic acid and *trans*-aconitic acid) on the fabric by dehydration during the curing, which causes fabric yellowing (Andrews & Trask-Morrell, 1991). The larger the amount of α,β-unsaturated acids is, the lower WI will be.

3.3. The effect of concentration of O-QCTSS

Fig. 2 shows the effect of O-QCTSS concentration on the amount of fixation of O-QCTSS on the treated fabric using CA as cross-linker with one and two bath process. It can be observed that the concentration of O-QCTSS has a great impact on cotton fabric in adsorption equilibrium. Increasing the amount of O-QCTSS incorporated in the finishing bath is accompanied by substantial increase in the fixation amount of O-QCTSS on the treated cotton fabrics when the O-QCTSS concentration was <3% in two-bath. Further increase in its concentration only slightly changed the fixation amount of O-QCTSS on the treated fabric. This is due to the fact that both the hydrogen bond on cellulose and the cross-linking agents on the fabrics were limited. And also because there was no creative bond on the O-QCTSS, the adsorbed O-QCTSS without cross-linking would be washed away during the following wash process. In addition, WI of the treated cotton fabrics was marginally decreased when the O-QCTSS concentration was over 3% due to partially oxidation and scission of chitosan derivative chains. Thus optimal concentration of O-QCTSS should be around 3%.

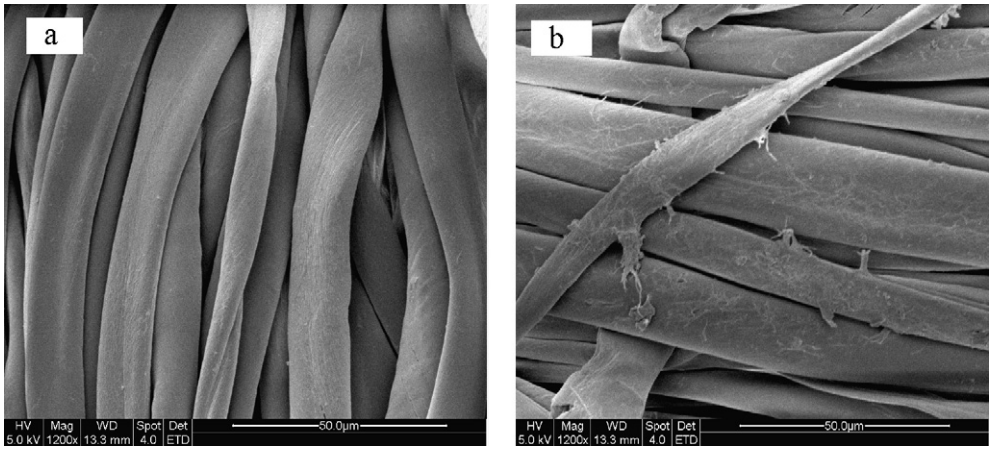


Fig. 3. SEM images of the surface of cotton fiber before (a) and after (b) finish with O-QCTSS.

3.4. The effect of cure temperature and time

The fixation amount of O-QCTSS on fabrics finished depends greatly on the curing temperature and time. Whiteness and the fixation amount of O-QCTSS of the treated cotton fabrics cured at different temperature (120–160 °C) for 1–5 min with two-bath process are listed in Table 1. The results showed that the curing temperature and time were proportional to the amount of O-QCTSS on the finished fabrics at 120–140 °C for 1–3 min, but when at 160 °C, it was lower than that at 140 °C. The former is due to the fact that increasing curing temperature and/or prolonging curing time improve the extent of crosslinking of finishing agent to cellulose and increase the linked chitosan, and if the temperature is too low and/or curing time is too short, the crosslinking was not sufficient and the substantial O-QCTSS linked through weak chemical bond was washed away in the washing process. Latter phenomenon may be owing to the degradation (oxidation and chain scission) of chitosan derivatives caused by high temperature. Besides, the samples treated at 160 °C showed uneven distribution of dye on the fabrics and different depth of shade between front and back of the fabrics due to migration. Excessively high curing temperature made the chitosan derivatives have no time to penetrate into fibers and migrate onto the fabric surface.

On the other hand, whiteness of treated fabrics decreases with increasing curing temperature and time. The loss of whiteness is due to the higher oxidation of cellulose and dehydration of CA (Eltahlawy et al., 2005). The concentration of dehydrated products gradually increases as the curing temperature increases and the curing time prolonging (Lu & Yang, 1999).

From the above results, the optimal finishing conditions obtained for O-QCTSS finish cotton fabric were as follows: two-bath process using material-to-liquor ratio 1:20, CA crosslinking agent 14% o.w.f, O-QCTSS 3% o.w.f, catalyst NaH_2PO_2 6%, o.w.f., penetrant JFC 1%, o.w.f, dry 80 °C × 3 min and cure 140 °C × 3 min. Under optimal finishing conditions, the fixation amount of the chitosan derivatives on treated fabrics is shown in Table 2. According to Table 2, the fixation amount of the chitosan derivative on treated fabrics was comparatively high for O-HTCC-N-DEBn. Possible reason is that there are two water-soluble quaternary ammonium groups in O-HTCC-N-DEBn, the crosslinking reaction of CA and chitosan derivative is smoother in homogeneous system.

Table 1
The effect of curing temperature and time on the amount of fixation on the treated fabrics[a].

Curing temp (°C)	Curing time (min)	The amount of fixed O-QCTSS (mmol/kg fiber)	Whiteness
120	3	10.57 ± 0.09	90.53 ± 0.11
140	1	8.84 ± 0.10	90.61 ± 0.09
140	3	12.23 ± 0.09	90.49 ± 0.10
140	5	12.34 ± 0.10	89.62 ± 0.15
160	3	12.14 ± 0.09	89.79 ± 0.16

[a] O-QCTSS (3% o.w.f.); crosslinking agent CA (14%, o.w.f.); catalyst NaH_2PO_2 (6%, o.w.f); penetrant JFC (1%, o.w.f); finishing method, two-bath process.

3.5. The surface morphology of treated fabrics

The surfaces of cotton fabrics were morphologically observed by SEM. Fig. 3 shows the surface appearances of O-QCTSS treated samples and the untreated ones. The photographs reveal that before the treatment (a), cotton fiber surface was smooth and flat, while the finished (b) fiber surface has an appearance of small grains as well as the fiber mesh layer. From the SEM pictures, it was clear that the treatment of cotton with O-QCTSS results in a unique morphological form, having a more textured surface than the unmodified one. This result is caused by the deposition of chitosan derivative on the surface of cotton fabric by crosslinking agent.

3.6. Antibacterial properties of fabrics treated with chitosan derivates

We investigated effects of the structure and concentration of chitosan derivatives and the concentration of the crosslinker on the antibacterial activities of finished fabrics to obtain effective antibacterial textiles. The tested results are shown in Table 3. It can be seen from the Table 3 that all treated fabrics possess good antimicrobial activity under the optimum finishing conditions. O-QCTS-DEBn exhibit particularly high activity. This result can be

Table 2
Amount of fixation of the chitosan derivatives on treated fabrics under optimal finishing process.

Chitosan derivatives	Amount of fixation of chitosan derivatives (mmol/kg fiber)
O-QCTSS	14.23 ± 0.09
O-HTCC-N-Bn	12.46 ± 0.10
O-HTCC-N-DEBn	15.20 ± 0.09

Table 3
The antibacterial activity of fabrics treated with chitosan derivatives[a].

Chitosan derivatives	Amount of chitosan derivatives (o.w.f)	Amount of CA (o.w.f)	Reduction (%)	
			S. aureus	*E. coli*
O-QCTSS	0	14	48.70 ± 6.32	36.00 ± 7.11
	0.5	14	84.12 ± 4.25	77.50 ± 5.18
	1	14	93.98 ± 3.67	89.25 ± 5.45
	2	14	99.73	97.68 ± 3.21
	3	0	87.86 ± 5.12	73.20 ± 5.63
	3	4	88.27 ± 4.38	74.50 ± 6.02
	3	8	90.60 ± 5.01	81.48 ± 5.86
	3	10	92.30 ± 4.25	90.50 ± 4.51
	3	14	100	99.30
	3	20	100	99.45
	5	14	100	99.68
O-QCTS-Bn	3	14	99.28	96.19 ± 2.08
O-QCTS-DEBn	3	14	100	99.71

[a] Finishing process: catalyst NaH_2PO_2 (6%, o.w.f); penetrant JFC (1%, o.w.f); cure 140 °C × 3 min.

interpreted that the high cationic charge density of O-QCTS-DEBn with two cationic charge of ammonium salt can favor interaction with negative residues at bacterial cell surface. Quaternary ammonium salt can act on the bacterial cell membrane, make cell membrane lose its barrier function, and then kill the bacteria. As a typical Gram-positive bacteria, *S. aureus* cell wall contains negative substances, such as proteins, teichoic acid and lipopolysaccharides (LP). The cationic $-N^+(CH_3)_3Cl^-$ can charge cell surface in flocculation, undermine the role of cell plasma membrane permeability barrier, destroy cell integrity, and make cell lose nutrients to achieve antibacterial activity (Lim & Hudson, 2003; Li et al., 2010). Table 3 also shows the antimicrobial activity against the bacterium increased with the increase of the concentration of chitosan derivative (O-QCTSS). When the concentration of O-QCTSS was up to 2%, the antimicrobial efficiency against *S. aureus* and *E. coli* were 99.7% and 97.7%, respectively. Increasing the concentration to 3% made the antimicrobial rate against *S. aureus* reach 100% and the efficiency against *E. coli* close to 100%. The excellent antimicrobial activity of O-QCTSS was due to the structure containing two different antimicrobial groups of quaternary ammonium group and the Schiff base group. The exact antimicrobial mechanism of Schiff base group is still unknown. Its ability to bind to critical components such as metal ions, sulfhydryl and amino groups of proteins associated with transport and membrane function might explain their antibacterial activity (da Silva et al., 2010; Ramos-Nino, Clifford, & Adams, 1996). For Gram-negative bacteria *E. coli*, a thick layer of extracellular type LP can prevent foreign macromolecules. O-QCTSS antibacterial effect on *E. coli* may be attributed to the synergy actions of the quaternary ammonium group and the Schiff base group. The fabric treated with O-QCTS-Bn, by contrast, showed a slight decrease in antimicrobial activity because of the electron-donating effect of N-benzyl reduced protonation of amino.

On the other hand, the antibacterial activities of O-QCTSS treated fabric in presence of CA cross-linking agent was higher than in absence of CA as compared with fabric given the same treatment and increased with the increase of the concentration of CA, but the increase amplitude is gradually decreased. This

Table 4
The washing resistance of the chitosan derivatives treated fabric[a].

Chitosan derivatives (3%, o.w.f)	Amount of CA (o.w.f)	Wash times	Reduction (%)	
			S. aureus	*E. coli*
O-QCTSS	14	0	100	99.30
	0	5	23.03 ± 10.11	–
	14	5	96.71 ± 2.30	93.40 ± 4.63
	14	10	92.95 ± 5.10	88.83 ± 4.98
	14	15	84.37 ± 6.23	80.26 ± 6.01
	4	20	31.65 ± 8.95	28.70 ± 9.12
	8	20	43.76 ± 8.02	39.1 ± 8.96
	10	20	54.41 ± 7.36	49.6 ± 6.74
	14	20	78.34 ± 6.12	75.78 ± 5.23
	20	20	79.48 ± 5.14	76.05 ± 4.95
O-QCTS-Bn	14	0	99.28	96.19 ± 3.02
	0	5	22.10 ± 10.65	–
	14	5	81.13 ± 6.86	78.74 ± 5.45
	14	10	70.69 ± 5.21	66.32 ± 6.01
	14	15	57.93 ± 4.87	54.90 ± 5.05
	14	20	<20	<20
O-QCTS-DEBn	14	0	100	99.71
	0	5	20.20 ± 10.14	–
	14	5	80.48 ± 5.21	80.35 ± 5.34
	14	10	68.91 ± 5.76	65.20 ± 5.52
	14	15	53.80 ± 5.18	48.76 ± 6.03
	14	20	<20	<20

[a] Finishing process: chitosan derivatives (3%, o.w.f); catalyst NaH_2PO_2 (6%, o.w.f); penetrant JFC (1%, o.w.f); cure 140 °C × 3 min.

indicates that CA helps to establish chemical bonding between chitosan derivatives and cotton fabric and the amount of O-QCTSS bonded to fabric reached maximum when the concentration of CA was up to 14%. The samples with CA treated only, also have some antibacterial activity on *S. aureus* and *E. coli* since CA is an antibiotic (Cherrington, Hinton, Mead, & Chopra, 1991).

The fastness to soaping of fabrics treated in the presence and absence of CA was tested. The results are shown in Table 4. The chitosan derivatives treated fabrics in absence of CA as a cross-linking agent lost its antimicrobial activity after five launderings. The poor durability may be due to the weak adhesive strength between the chitosan derivatives and fabric surface by Van Der Waal's forces and/or hydrogen bonding. However, the durability of O-QCTSS, O-QCTS-Bn and O-QCTS-DEBn treated fabrics in presence of CA still kept over 93%, 78% and 79% of bacterial reduction, respectively. This phenomenon ensures that the chitosan derivatives were fixed on the cotton fabric covalently. In addition, the washfastness of the chitosan derivatives treated fabrics decreased with increasing washing times. The antibacterial activities of O-QCTS-Bn and O-QCTS-DEBn treated fabrics decreased significantly after twenty launderings, whereas O-QCTSS treated fabrics kept over 75% after fifteen launderings. This may be related with the water-solubility, so, the better the water-solubility of chitosan derivatives is, the more easily it is washed away from the fabric, and the worse the durable antimicrobial activity becomes. Because the order of solubility of the chitosan derivatives was O-QCTS-DEBn > O-QCTS-Bn > O-QCTSS, the durable antimicrobial activity of O-QCTS-Bn and O-QCTS-DEBn was lower than that of O-QCTSS.

4. Conclusion

Three water-soluble chitosan derivatives were synthesized and characterized using FT-IR. Applications of these chitosan derivatives to antimicrobial finishing of cotton fabric were investigated for the first time using CA as a crosslinking agent. The desirable conditions for the application of the chitosan derivatives to cotton fabrics are that concentration of chitosan derivatives is 3% (o.w.f), CA (14%, o.w.f) is used as a cross linking agent, the catalyst is NaH_2PO_2 (6%. o.w.f), and the drying and curing conditions are 80 °C × 3 min and 140 °C × 3 min using a two-bath process and material-to-liquor ratio 1:20. The cotton fabrics treated with chitosan derivatives exhibited high antimicrobial activity and good washing resistance against *E. coli* and *S. aureus* The antimicrobial efficiency of O-QCTSS treated one was still over 75% after 20 consecutive home launderings. The results showed that sufficient washing durability and high antimicrobial effects could be obtained for fabrics treated with O-QCTSS.

Acknowledgement

The authors thank the Natural Science Foundation of Jiangnan University (project number 20091437) for financial support.

References

Andrews, B. A. K., & Trask-Morrell, B. J. (1991). Esterification crosslink finishing of cotton fabric with tricarboxylic acids. *American Dyestuff Reporter*, *80*, 26–31.

Cherrington, C. A., Hinton, M., Mead, G. C., & Chopra, I. (1991). Organic acids: chemistry, antibacterial activity and practical applications. *Advances in Microbial Physiology*, *32*, 87–108.

da Silva, C. M., da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, Cleide V. B., et al. Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced Research*, in press, doi:10.1016/j.jare.2010.05.004.

Daly, W. H., & Guerrini, M. M. (1998). Antimicrobial properties of quaternary ammonium cellulose and chitosan derivatives. *Polymeric Materials Science and Engineering*, *79*, 220–221.

Diana, A., Miquel, G., & Keiko, S. (2009). Cross-linking chitosan into UV-irradiated cellulose fibers for the preparation of antimicrobial-finished textiles. *Carbohydrate Polymers*, *77*, 536–543.

El-tahlawy, K. F., El-bendary, M. A., Elhendawy, A. G., & Hudson, S. M. (2005). The antimicrobial activity of cotton fabrics treated with different crosslinking agents and chitosan. *Carbohydrate Polymers*, *60*, 421–430.

Guo, Z. Y., Xing, R. E., Liu, S., Zhong, Z. M., Ji, X., Wang, L., et al. (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydrate Research*, *342*, 1329–1332.

Hamman, J. H., & Katze, A. F. (2001). Effect of the type of base and number of reaction steps on the degree of quaternization and molecular weight of n-trimethyl chitosan chloride. *Drug Development and Industrial Pharmacy*, *27*, 373–380.

Jin, X. X., Wang, J. T., & Bai, J. (2009). Synthesis and antimicrobial activity of the Schiff base from chitosan and citral. *Carbohydrate Research*, *344*, 825–829.

Kurita, K., Mori, S., Nishiyama, Y., & Harata, M. (2002). N-alkylation of chitin and some characteristics of the novel derivatives. *Polymer Bulletin*, *48*, 159–166.

Li, X. F., Feng, X. Q., Yang, S., Fu, G. Q., Wang, T. P., & Su, Z. X. (2010). Chitosan kills *Escherichia coli* through damage to be of cell membrane mechanism. *Carbohydrate Polymers*, *79*, 493–499.

Lim, S. H., & Hudson, S. M. (2003). Review of chitosan and its derivatives antimicrobial agents and their uses as textile chemicals. *Journal of Macromolecular Science: Part C: Polymer Reviews C43*, 223–269.

Lim, S. H., & Hudson, S. M. (2004a). Application of a fiber-reactive chitosan derivative to cotton fabric as an antimicrobial textile finish. *Carbohydrate Polymers*, *56*, 227–234.

Lim, S. H., & Hudson, S. M. (2004b). Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. *Carbohydrate Research*, *339*, 313–319.

Lu, Y., & Yang, C. Q. (1999). Fabric yellowing caused by citric acid as crosslinking agent for cotton. *Textile Research Journal*, *69*, 685–690.

Ökem, T. (2003). Surface treatment of cotton fabric with chitosan. *Coloration Technology*, *119*, 241–246.

Ramos-Nino, M. E., Clifford, M. N., & Adams, M. R. (1996). Quantitative structure activity relationship for the effect of benzoic acids, cinnamic acids and benzaldehydes on Listeria monocytogenes. *Journal of Applied Bacteriology*, *80*, 303–310.

Roberts, G. A. F. (1997). In R. A. A. Muzzarelli, & M. G. Peter (Eds.), *Chitin handbook* (pp. 127–132). Grottammare, Italy: European Chitin Society.

Rúnarsson, Ö. V., Holappa, J., Nevalainen, T., Hjálmarsdóttir, M., Järvinen, T., Loftsson, T., et al. (2007). Synthesis of methylated chitosan containing aromatic moieties: Chemoselectivity and effect on molecular weight. *European Polymer Journal*, *43*, 2660–2671.

Sajomsang, W., Tantayanon, S., Tangpasuthadol, V., & Daly, W. H. (2008). Antibacterial activity of methylated chitosan and chitooligomer derivatives: Synthesis and structure activity relationships. *Carbohydrate Polymers*, *72*, 740–750.

Sajomsang, W., Tantayanon, S., Tangpasuthadol, V., & Daly, W. H. (2009). Quaternization of N-aryl chitosan derivatives: Synthesis, characterization, and antibacterial activity. *Carbohydrate Research*, *344*, 2502–2511.

Shin, Y., & Min, K. (1996). Antimicrobial finishing of cotton fabrics with chitosan (I) – Effect of degree of deacetylation on the antimicrobial property. *Journal of Korean Fiber Society*, *33*, 487–491.

Thanou, M., Verhoef, J. C., & Junginger, H. E. (2001). Oral drug absorption enhancement by chitosan and its derivatives. *Advance Drug Delivery Reviews*, *62*, 239–243.

Venugopala, K. N., & Jayashree, B. S. (2008). Microwave-induced synthesis of Schiff bases of aminothiazolyl bromocoumarins as antibacterials. *Indian Journal of Pharmaceutical Sciences*, *70*, 88–91.

Wan, A., Sun, Y., & Li, H. L. (2009). Characterization of novel quaternary chitosan derivative nanoparticles loaded with protein. *Journal of Applied Polymer Science*, *114*, 2639–2647.

Zhao, X. R., & Xia, W. S. (2006). Antibacterial activity of binary substituted quaternary ammonium salts of chitosan. *Journal of Food Science and Biotechnology*, *25*, 55–60.