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Crosslinking chitosan into H_3PO_4/HNO_3–$NANO_2$ oxidized cellulose fabrics as antibacterial-finished material

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ABSTRACT

The primary hydroxyl groups on C6 position in glucose units of cellulose with H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation produced monocarboxy cellulose and binding sites, subsequent amide reaction with chitosan solution to obtain chitosan crosslinked cotton fabrics. Scanning electron microscope and FT-IR spectroscopy were used to detect the fiber morphology and chemical bonding between chitosan and oxidized cellulose, respectively. The influences of H_3PO_4/HNO_3–$NaNO_2$ oxidation and chitosan treatment on physical properties of cotton fabrics were examined by determining carboxyl content, weight loss and mechanical properties of fabrics, as well as chitosan content in the composite fabrics. Antibacterial performance of chitosan–cellulose fabrics against *Escherichia coli* and *Staphylococcus aureus* was evaluated. As a result, chitosan was bonded into cotton fiber via the amido bond of C—N formed between amino groups of chitosan and carboxyl groups on oxidized cellulose, and these resultant chitosan–cotton fabrics showed high antimicrobial activity and excellent antibacterial washing durability.

1. Introduction

Cellulose is the most abundant natural and renewable carbon-based polymer on the earth. By conservative estimates, well over 10^{12} tons of cellulose and related polysaccharides are produced in our world each year due to natural processes (Hon, 1994; Schurz, 1999). For millennia, it has been used as a functional, low cost and biodegradable raw material in all kinds of practical applications. Cellulosic polymers have specific and advantageous characteristics of the following: they are generally strong, hydrophilic, stable to chemicals, safe to living bodies, reproducible and recyclable, which are consistent with environmental concerns for exploiting sustainable green products of cellulose materials. In recent years, a number of modification techniques have been carried out to reinforce these original properties or to add new functionalities for cellulose, and presently chemical modifications have been positioned at the interesting field of the synthesis of novel cellulose-based materials (Chang & Robyt, 1996; Tahiri & Vignon, 2000).

The three hydroxyl groups of the cellulose polymers responsible for reaction activity can undergo chemical reactions common to all primary and secondary groups, such as esterification, nitration, etherification and oxidation. The chemical modification of cellulose with oxidizing agents is a quite frequent procedure in cellulose chemistry, and aldehyde, ketone and carboxyl groups may be generated in the cellulose molecules during the oxidation, depending on the nature of the oxidant and the conditions of oxidation. Some oxidations proceed with rather high selective reaction, one of the selective oxidations cleaves the C2—C3 bond of the glucopyranoside ring and introduces two aldehyde groups at C2 and C3 positions (Calvini, Conio, Princi, Vicini, & Pedemonte, 2006; Kim, Kuga, Wada, Okano, & Kondo, 2000; Potthast, Schiehser, Rosenau, & Kostic, 2009), the other causes selective oxidation of primary hydroxyl groups at C6 in anhydroglucose units to carboxyl groups via the intermediate aldehyde stage (de Nooy, Besemer, & van Bekkum, 1995; Kumar & Yang, 2002; Painter, Cesaro, Delben, & Paoletti, 1985). One of the significant advantages of selective oxidations over other oxidations is that they have high reaction rate and selectivity, minimize degradation and retain the mechanical and morphological properties of the starting polysaccharides (Nevell, 1963). On the other hand, the selective oxidation may introduce functionalities and improve properties of native celluloses by effective introduction of aldehyde and carboxyl groups into fibrous celluloses, or they can be used as reactive "chemical hooks" for further surface chemical modification.

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On such way obtained cellulose by selective oxidation can be widely used as an immobilizing matrix for various enzymes (Varavinit, Chaokasem, & Shobsngob, 2001), proteins (Xu, Huang, & Wang, 2013), and amine drugs (Zimatkina, Yurkshtovich, Zimatkin, & Kaputsky, 1996) by reaction with their amino functions, or as such for specific uses (Kim & Kuga, 2002).

Chitin, a major component of the cuticles of crab and shrimp, is the most abundant natural amino polysaccharides with a large unexplored resources. Chitosan is partially or completely N-deacetylated chitin, and mainly consists of β-(1,4) linked 2-amino-2-deoxy-β-D-glucopyranose. Unlike cellulose, chitosan contains active amino and acetoamino groups. These amino polysaccharides have received much attention as possible substitutes for synthetic polymers due to their biocompatibility, biodegradation, non-toxicity and antimicrobial properties. Recently, a wide variety of applications in antimicrobial activity of chitosan have displayed that chitosan is effective in inhibiting growth of bacteria and fungi, chitosan has been studied in controlling common postharvest fungal phytopathogens (Pacheco et al., 2008; Teli & Sheikh, 2012). The antibacterial properties of chitosan depend on its molecular weight, degree of deacetylation and the type of bacterium (Chung et al., 2004; No, Park, Lee, & Meyers, 2002). In this sense, the chemical fixation of the chitosan by crosslinking agents into the cellulose matrices may improve the endurance and efficiency of the resultant antibacterial textile (Eltahlawy, El-bendary, Elhendawy, & Hudson, 2005; Zhang, Chen, Ji, Huang, & Chen, 2003). However, the reported procedures to attain the covalent linkage of the chitosan into cellulose often involved the use of toxic cross-linkers or additives (Schiffman & Schauer, 2007; Zhang et al., 2003), which would cause some risks associated with the human health and environment. It has been established that periodate oxidized cellulose reacts through its aldehyde groups with the amino groups of chitosan under specific conditions, giving the corresponding Schiff base (Hou, Liu, Liu, Duan, & Bai, 2008; Liu, Nishi, Tokura, & Sakairi, 2001). Much of the interest has been focused on obtaining the antibacterial cellulose–chitosan fibers through grafting of chitosan onto the dialdehyde oxycellulose fibers or bonding chitosan into cellulose fibers with a chemical crosslinking agent, but there is no data about using monocarboxy cellulose fibers to produce antibacterial composite fibers.

The H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation of cellulose can produce monocarboxy cellulose in which the primary hydroxyl groups of C6 site in the glucose residues are converted into the carboxyl groups (Kumar & Yang, 2002; Painter et al., 1985; Wang, Xiang, & Zou, 2009). 6-Monocarboxy cellulose is nontoxic, bioabsorbable, biodegradable and affinity to skin, which has been recommended as a matrix for the immobilization of drugs and proteins. The carboxyl groups of monocarboxy cellulose can be further modified by an amine reaction with amino groups of chitosan, so that chitosan can be bonded onto the oxycellulose fibers. In this paper, we report the oxidation of cotton fabrics with H_3PO_4/HNO_3–$NaNO_2$ under aqueous mild conditions in order to achieve an antimicrobial fabric by subsequent immobilization of chitosan, in this grafting process of carboxyl groups in monocarboxy cellulose fabrics with amino groups of chitosan no synthetic cross-linkers prepared using high-molecular weight polymers needs to be added. Furthermore, it may not involve environmental unfriendly chemical reactions. Our data demonstrates that chitosan grafted onto monocarboxy oxycellulose fabrics without any requirement of crosslinking agents have antimicrobial capability attributed to the characteristic of the cationic amino groups of chitosan. These produced cotton fabrics would be utilized for various biomedical products, antimicrobial decorating and package materials.

2. Experimental

2.1. Materials

Samples of chitosan with a deacetylation degree (DD) of 88% and average molecular weight (M_w) of 253906 Da (denoted as CS_1) and chitosan with a deacetylation degree (DD) of 92% and average molecular weight (M_w) of 4750 Da (denoted as CS_2) were supplied by the Aldrich (USA) and used. Raw cotton woven fabrics (118 g/m^2) and cotton fibers were obtained from Intertek Group (Shanghai, China) and used with preliminary treatment to remove any additives. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) obtained from American Type Culture Collection (ATCC) were used for antibacterial activity test. Nutrient agar and triptone were supplied by the Shengyuan Biological and Technological Co. Ltd., Beijing, China. H_3PO_4 (85%, w/v), HNO_3 (69.7%, w/v), $NaNO_2$ and acetone were purchased from Sinopharm Chemical Reagent Co. Ltd. (China) and used as supplied. All chemicals used for the following investigations were of analytical grade.

2.2. Oxidation of cellulose fabrics with H_3PO_4/HNO_3–$NaNO_2$

A sample of cotton fabric with a liquor ratio of 1:30 (w/v) was immersed in a mixture solution of phosphoric acid and nitric acid (1:2, v/v), subsequently 1.4% (w/v) of sodium nitrite was added all at once. The mixture was occasionally oscillated in the brown container covered with a Petri dish for preventing the release of reddish brown fumes to the air (the whole assembly was placed in a draught chamber equipped with a two-ply filter adsorbing the acids and organic solvents), at 40 °C, for 15, 30, 45, 60, 120, 180, 300 and 360 min under the absence of light. After completion of the oxidation, the cotton fabrics were washed with deionized water several times and soaked in 0.5% (w/w) of propanetriol solution for 20 min at ambient temperature to remove the oxidant. Finally, these oxidized fabrics were washed with acetone and then used for the chitosan reaction without drying.

2.3. Crosslinking chitosan into the oxidized cellulose fabrics

A chitosan solution was prepared by dispersing chitosan in 2% (v/v) aqueous acetic acid solution for 1 h at 60 °C. The above mentioned oxidized cotton fabrics were immersed in chitosan solution (concentration range from 0.5 to 4.0%, w/w) for 30–300 min at five different temperatures (40, 50, 60, 70 and 80 °C), with constant stirring. After chitosan treatment, the fabrics were rinsed thoroughly with deionized water at ambient temperature. The resulting cellulose fabrics were dried at 60 °C under vacuum for 3 h to produce the composite cellulose–chitosan fabric.

2.4. Measurement

2.4.1. Fourier transform infrared spectroscopy

The Fourier transform infrared spectra of samples were recorded on a Bruker VERTEX-80 FT-IR spectrophotometer (Germany) using KBr pellets, and samples were analyzed in transmittance, equipped with the Omnic data processing software. The measurements were performed at 20 °C and a relative humidity of 65%.

2.4.2. Determination of carboxyl group in the monocarboxy cellulose

The carboxyl groups of the oxidized cellulose react with the salts of weaker acids such as calcium acetate, forming a salt of the oxidized cellulose and releasing an equivalent amount of the weaker acid. On this basis as well as by the method described in the United States Pharmacopeia (USP, 1995), for determination of carboxyl

content in oxidized cellulose fibers, the following method was developed. The cellulose sample should be obtained in the acidic form by replacement of its cations by hydrogen ions by the treatment of cellulose samples (0.5 g) with 0.01 M HCl for 1 h, followed by washing with deionized water. In the next step to the oxidized cellulose 50 ml of a 2% (w/w) calcium acetate solution were added. After frequent shaking for 30 min, the mixture was titrated with 0.1 M standardized solution of sodium hydroxide, using phenolphthalein as an indicator. The volume of NaOH solution consumed was corrected for the blank. The carboxyl content in the sample was calculated as follows:

$$\text{Carboxyl content}(\%, \text{w/w}) = \frac{0.1M \times V_{\text{NaOH}} \times MW_{\text{COOH}}}{m \times (1 - w/100)} \times 100 \quad (1)$$

where $0.1M$ is the concentration of NaOH solution, V_{NaOH} is the volume (ml) of NaOH solution used in titration after correcting for the blank, m is the weight of oxidized cotton fiber sample (mg), and w is the moisture content (%).

2.4.3. Determination of weight loss

The formation of soluble fragments, as a result of the cellulose destruction caused by oxidation reaction, was determined by measuring the weight loss of oxidized fabric samples by applying the direct gravimetric method (Koblyakov, 1989).

2.4.4. Scanning electron microscopy (SEM) analysis

Scanning electron microscopy photographs were taken on a Japan Hitachi X-650 electronic instrument operating at 5 kV after sputtering the samples with gold.

2.4.5. Chitosan content in the synthetic cellulose–chitosan fabrics

The chitosan content incorporation to the composite cotton fabrics was calculated from the nitrogen percentage on the basis of the calibration curve for the weight of chitosan and titration value. The nitrogen content in the chitosan and cellulose–chitosan fabrics was determined by Kjeldahl nitrogen analysis in a DS-20 apparatus (Tecator Instrument Co. Ltd., Sweden), according to the standard method (ISO 937, 1978). All determinations were carried out by triplicates.

2.4.6. Tensile properties measurement

The tensile strength and elongation at break of cotton fabrics were performed with a YG(B)026D-250 electronic fabric tension tester (Ningbo Textile Instrument Factory, China), by following this usual procedure: the test length of the sample fabric was 300 mm, speed was 200 mm/min, and the tensile strength and elongation of the fabrics were measured as the mean of 10 individual fabric. The samples were conditioned in a room (20 °C, a relative humidity of 65%) for 48 h before measurement.

2.5. Assays for antibacterial activity of cellulose fabrics

The antibacterial property for cotton fabric samples was measured according to the method described in literature (Höfer, 2006). Gram-negative bacteria *E. coli* ATCC 25922 and Gram-positive bacteria *S. aureus* ATCC 25923 were used for antibacterial activity determination of chitosan crosslinked cotton fabrics. Antibacterial property of synthetic chitosan–cellulose fabrics was assayed as follows: each of the bacterium (10^5–10^6 log N/ml) was inoculated into 9 ml sterile potassium hydrogen phosphate buffer solution (pH 7.2) at 37 °C for 24 h. Then modified cotton fabric samples were added in solution and incubated at 37 °C for 48 h. The raw cotton fabric was also added into solution using the same procedure to produce the control sample. Viable cells (log N/ml) were enumerated on TSA agar by pour plating 1 ml of serial dilutions of physiological solution followed by incubation at 37 °C for 48 h. The average values of the duplicates were converted to colony forming units per milliliter. The percentage inhibition ratio of bacteria can be calculated by the following equation:

$$\text{Inhibition ratio}(\%) = \frac{A - B}{A} \times 100 \quad (2)$$

where A and B are bacteria amount per milliliter for the control (raw cotton fabrics) and chitosan crosslinked cotton fabrics test samples, respectively. In this way, by directly comparing a reference material with the treated sample, it is always possible to record the direct effect of the antimicrobial treatment, because external factors (e.g. supply of nutrients) can largely be excluded and, because of the characteristics of the sample and reference materials, it can be assumed that any potential growth pattern will be the same.

The effects of washing on stability of antibacterial activity of the chitosan modified cellulose fabrics were studied. Cotton fabric samples were put into the solution with 5 mg/ml of soaping agent at a liquor ratio of 1:50 and washed for 10 min at 40 °C, subsequently the fabric samples were rinsed with cold deionized water, which was repeated certain times, and the samples were dried at 60 °C, then the antibacterial property of the washed cotton fabrics was tested according to the above antibacterial assay process.

3. Results and discussion

3.1. Obtaining of monocarboxy cellulose fabrics

The first stage of preparing chitosan–cellulose fabrics involves the formation of monocarboxy cellulose by the H_3PO_4/HNO_3–$NaNO_2$ oxidation of cotton fabrics. The oxidation of polysaccharide compounds by HNO_3 alone is sluggish and frequently requires a catalyst (e.g. H_2SO_4) and an initiator (e.g. NO_2 or HNO_2). With the H_2SO_4/HNO_3–$NaNO_2$ oxidation, this method reportedly decreased the oxidation rate and oxidation level of cellulose at low concentrations of H_2SO_4 (Wanleg, 1956). An increase of the H_2SO_4 concentration in H_2SO_4/HNO_3–$NaNO_2$ mixture would cause further degradation of cellulose and a further decrease in the yield of oxycellulose. However, as a weak acid, H_3PO_4 slowly hydrolyzed cellulose and hence, produced oxycellulose in high yields. In our studies, the oxidation of cellulose by the use of HNO_3 in combination with H_3PO_4 and $NaNO_2$ was investigated. The influences of H_3PO_4/HNO_3–$NaNO_2$ oxidation on cotton fabrics were initially assessed by determining the carboxyl group content, weight loss and breaking strength of cellulose fabric samples.

In the H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation, the C6 primary hydroxyl groups of cellulose are converted to carboxyl groups. The carboxyl group content of oxidized cotton fabrics by H_3PO_4/HNO_3–$NaNO_2$ reaction system is shown in Fig. 1. The curve exhibited increase in carboxyl group content ranging from 1.35 to 12.52% with the increase of oxidation time. During the first stage, the carboxyl content in the oxidized fabric increased linearly with relatively high rate when reaction time was increased from 15 to 60 min ($R^2 = 0.9972$). A further increase in the oxidation time from 60 to 360 min, the cotton fabrics oxidized with H_3PO_4/HNO_3–$NaNO_2$ had higher carboxyl group content compared to the fabrics oxidized in the initial time, but the rate of carboxyl content gradually diminished at the oxidation time over 300 min. This can be explained by the fact that nitrogen oxides (e.g. HNO_2, NO_2, NO and N_2O_3) are the oxidants in the oxidation of cellulose by H_3PO_4/HNO_3–$NaNO_2$ mixture, since nitrogen oxides (NO_2 and NO) are odd-electron species, they can initiate the oxidation by abstracting a α-hydrogen atom at C6 site from cellulose to generate Cell–C(•)H—OH, further attack by NO_2• and subsequent release of NO •and HNO_2 forms Cell–$CH(OH)_2$ and Cell–CHO intermediates, respectively. Undergoing a hydrogen abstraction reaction

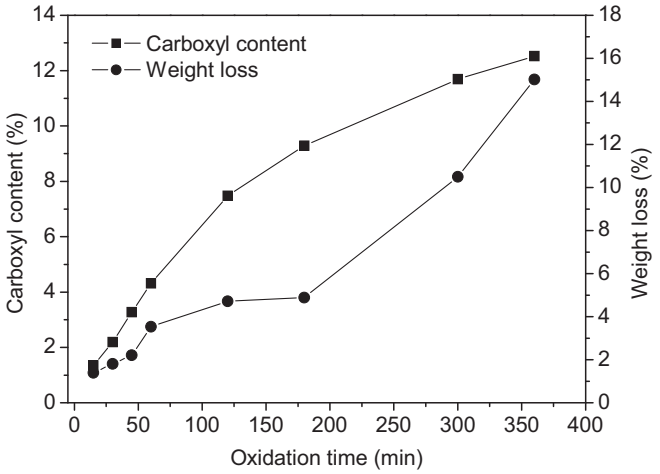


Fig. 1. Relationship between the carboxyl group content, weight loss of the oxidized cotton fabric and the oxidation time of H_3PO_4/HNO_3–$NaNO_2$.

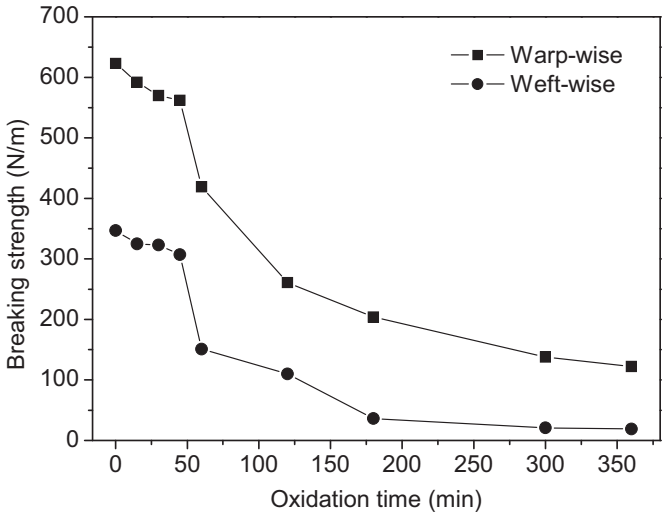


Fig. 2. The effect of oxidation time on the warp-wise and weft-wise breaking strength of the oxidized cotton fabric.

followed by an attack of NO_2 •, subsequent elimination of HNO_2 for Cell–$CH(OH)_2$ and subsequent hydrolysis of the addition product for Cell–CHO, lead to the monocarboxy cellulose formation of Cell–COOH according to a concerted mechanism (Ogata, 1978). In addition, the first oxidation was a fast phase involving easy access to the cellulose chain-molecules in amorphous regions, then a slower oxidation reaction was thought to proceed in the inner core of crystal regions of cellulose, due to high crystallinity index and more complex fine and micro structure of cotton with cellulose I crystal structure (native cellulose), which is in agreement with literature data (Kreze, Jeler, & Strnad, 2005; Tahiri & Vignon, 2000).

As a result of H_3PO_4/HNO_3–$NaNO_2$ oxidation, cellulose molecules would undergo scission with the production of soluble fragments containing highly oxidized cellulose molecules and/or some of low-molecular weight products obtained due to proceeding beta-elimination at the C6 aldehyde intermediate and/or depolymerization by some active species such as hydroxyl radicals formed in situ as side reactions. The formation of soluble fragments was demonstrated by measuring of the weight loss of oxidized cotton fabrics, which could characterize the severity of oxidation. Fig. 1 shows that the loss in weight of oxidized fabrics is more pronounced with the prolongation of oxidation time. The weight loss for cotton fabrics oxidized with 180 min in H_3PO_4/HNO_3–$NaNO_2$ mediated system is 4.89%, while for the fabrics treated under severe conditions (i.e. during 360 min), the value of weight loss is 3.07 times higher (15.02%). That is a predicted result, due to the fact that in the case of the H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation of native celluloses, the oxidation initially takes place in accessible or disordered regions and then proceeds inside crystal phases in cellulose after extended reaction time. The end units of cellulose chains are more susceptible to hydrolysis than the glycosidic linkages, resulting in remarkable decrease of the molecular weight. Additionally, increasing reaction time at lower pH the oxidant becomes overly aggressive, so that the cotton fabrics suffered from a so deep structural damage and depolymerization (de Nooy et al., 1995).

The oxidation process of H_3PO_4/HNO_3–$NaNO_2$ had a significant influence on the mechanical properties of cotton fabrics. As it could be seen from Fig. 2, the warp-wise and weft-wise breaking strength of oxidized cotton fabrics were weakened with increasing oxidation time. The breaking strength of the oxidized fabrics did not change markedly when the oxidation time was in the range of 0–45 min, whereas it significantly decreased at the oxidation time over 45 min, probably attributing to breaking the crystalline structure of the original cotton cellulose (Kumar & Yang, 2002; Saito & Isogai, 2004), as well as remarkable increase in weight loss (see Fig. 1). The H_3PO_4/HNO_3–$NaNO_2$ oxidation system attacks the crystal regions of cellulose already with extending degree of oxidation, causing the disruption of hydrogen bonding between cellulose chains, which affects its physical properties. Therefore, applying severe conditions in the case of H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation of cotton fabrics, results in loosing fibrous structure and for textile applications only oxidation under mild conditions should be used, and H_3PO_4/HNO_3–$NaNO_2$ oxidation time of 45 min was chosen for further investigations.

The above mentioned weight loss of the oxidized cotton fabrics is mainly responsible for changes in the oxidized fiber surface, as it is shown in Fig. 3a and b, respectively. The surface of the oxidized fiber becomes very coarse, and many narrow and long lines can be easily seen, indicating that the cotton fibers were suffered from corrosion of H_3PO_4/HNO_3–$NaNO_2$ in the oxidation process, and prolonged oxidation time increased the degrees of corrosion for cotton fibers, which are in accordance with the weight loss data. From the microscopic analysis it is clear that we obtained cotton fibers with developed surface, that enhanced the ability of chitosan combination and could be important for crosslinking chitosan.

3.2. Preparation and characterization of chitosan crosslinked cellulose fabrics

In the second stage, chitosan modified cotton fabric was prepared by subsequent reaction of the oxidized cotton fabrics with a solution of chitosan in aqueous acetic acid. The composite process is displayed in Scheme 1. According to the reported procedure (Kumar & Yang, 2002; Ogata, 1978), the H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation of D-glucopyranose units in cotton cellulose molecules (a) selectively converted the C6 primary hydroxyl group into the carboxyl group, giving the monocarboxy cellulose (b), then the free amino group of chitosan reacted with a carboxyl group to achieve the corresponding C—N of amido bond with high degrees of substitution (c). The synthetic reaction process was characterized by infrared spectroscopy as shown in Fig. 4. Compared the infrared spectrum of original cotton cellulose, the characteristic absorption band of the H_3PO_4/HNO_3–$NaNO_2$ oxidized cellulose clearly appeared at 1735.8 cm^{-1} due to the stretching vibration of the C=O double bond of the carboxyl and carbonyl groups in the oxidized cellulose fabrics, and became inapparent after reaction with chitosan (see Fig. 4c and d). Meanwhile, the absorption peaks at near 1373.2 cm^{-1} and 1282.5 cm^{-1} as well as 892.9 cm^{-1} respectively

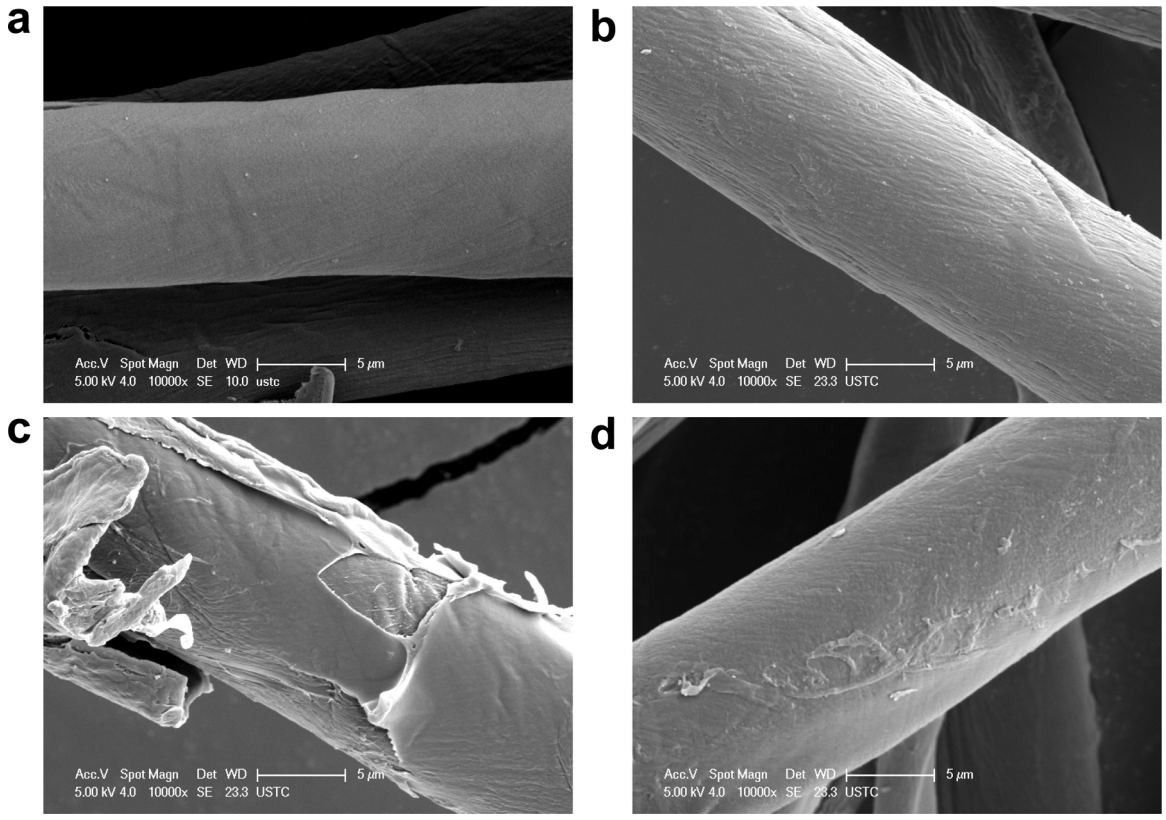
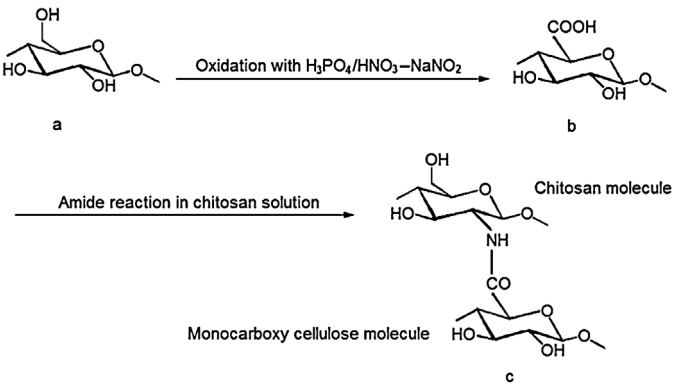


Fig. 3. SEM images of the surface of: (a) original cotton fiber; (b) oxidized cotton fiber; (c) chitosan CS_1 and (d) chitosan CS_2 crosslinked oxidized cotton fiber, respectively.



Scheme 1. Preparation of the chitosan crosslinked monocarboxy cellulose fabric.

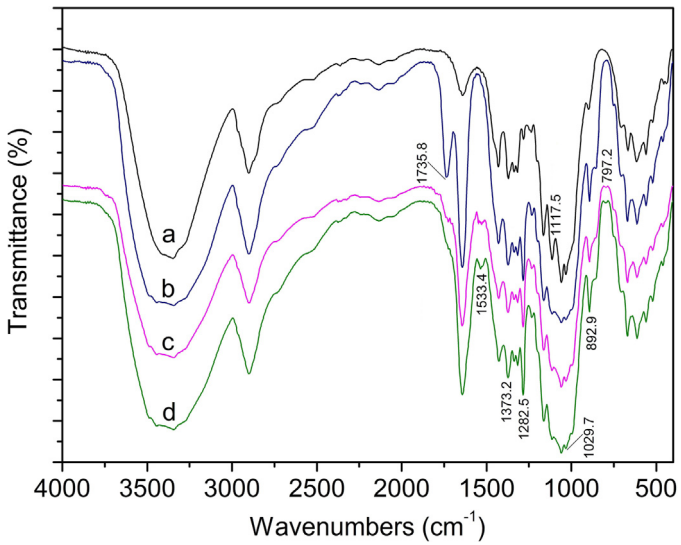


Fig. 4. Infrared spectra of: (a) original cotton fabric; (b) oxidized cotton fabric; (c) chitosan CS_1 and (d) chitosan CS_2 crosslinked oxidized cotton fabric, respectively.

indicates the —CH bending and —OH out-plane bending, reinforced as shown in the IR spectra of the oxidized fabric and chitosan modified fabrics, whereas the stretching vibration peaks of asymmetric ring at 1117.5 cm^{-1} and C—O of primary alcohol at 1029.7 cm^{-1} were weakened. After chitosan graft, the characteristic absorption peak at 1533.4 cm^{-1} corresponding to the C—N stretching of amide II in the IR spectra c and d of the synthetic cotton fibers reveals that the amido bond was formed between amino groups in chitosan and carboxyl groups on the oxidized cellulose. Furthermore, a faintish amide characteristic absorption peak at 797.2 cm^{-1} presented in Fig. 4c and d of chitosan crosslinked cotton fibers. This finding leads to the conclusion that chitosan has been crosslinked to the oxidized monocarboxy cotton fabric by the reaction of the amino group of chitosan with the carboxyl group of the oxidized cellulose.

The amount of chitosan introduced into the oxidized cotton fabrics was determined by the Kjeldahl nitrogen analysis. Fig. 5 shows the relationship between the amount of chitosan incorporated to the oxidized fabrics and the preparing conditions for the novel modified cotton fabrics. The general trend was that the amount of chitosan fixed to the oxidized fabrics increased with the concentration of chitosan aqueous solution, the crosslinking time and the reaction temperature till the reaction between the amino groups of chitosan and the carboxyl groups of oxidized cotton fabrics was completed. From Fig. 5a, it could be seen that 2% (w/w) chitosan aqueous solution supplied enough chitosan to couple with the oxidized cotton fabric. Additionally, Fig. 5b and c indicated that the

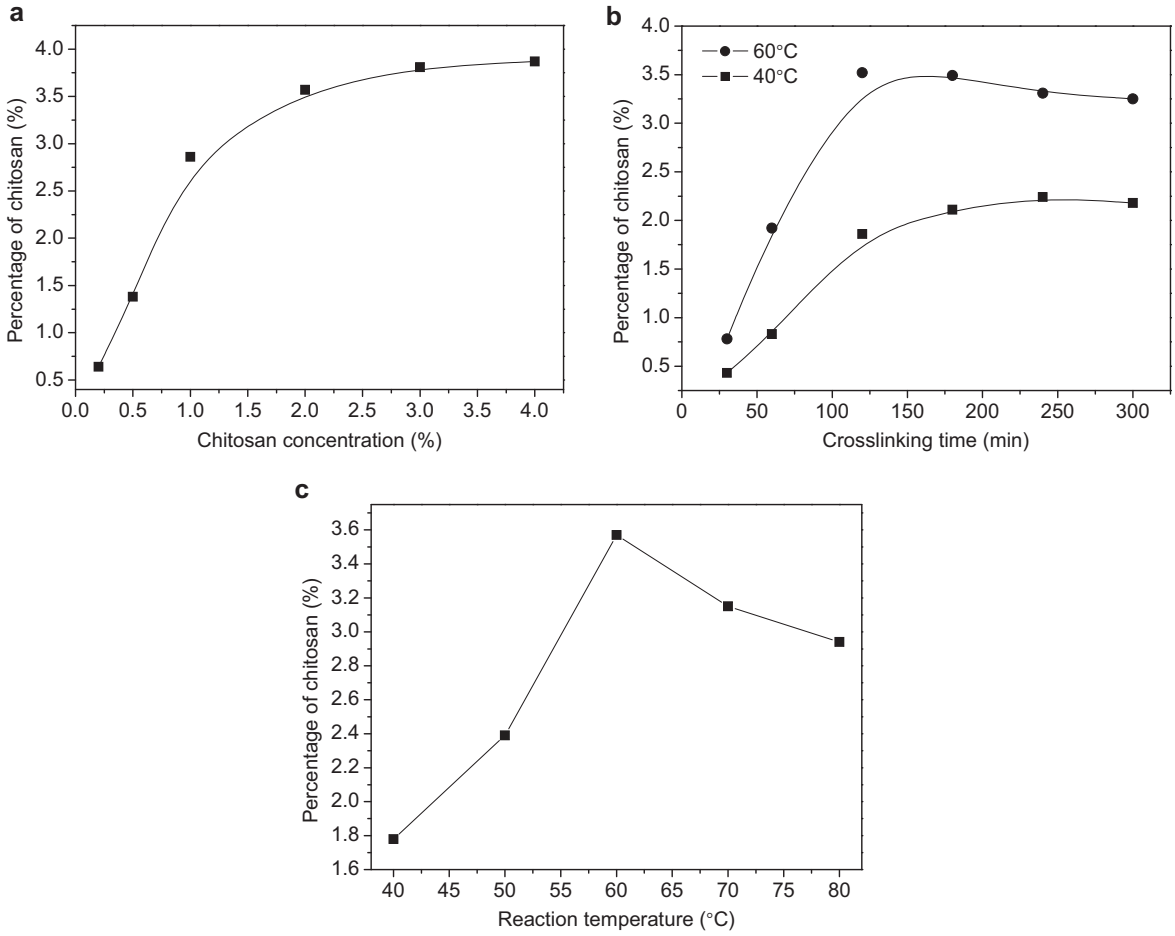


Fig. 5. Relationship between the amount of chitosan CS_2 introduced into oxidized cotton fabrics and the concentration of chitosan aqueous solution, crosslinking time and reaction temperature used in preparing composite chitosan–cellulose fabrics.

heat is an important factor, high temperature may quicken the reaction of the amino group of chitosan with the carboxyl group of the oxidized cellulose and reduce the reaction time. However, the acidic solution of chitosan produced over corrosion for the cotton fabrics under the reaction temperature exceeding 60 °C. According to the experimental results, the optimal preparing conditions for the chitosan concentration of 2%, the reaction time of 120 min and the reaction temperature of 60 °C were determined to obtain the synthetic chitosan–cotton fabrics.

The carboxyl group content of the monocarboxy cellulose reflects not only the oxidation degree of the cellulose fabrics but also the ability of linked chitosan. Theoretically, increasing the oxidation time of H_3PO_4/HNO_3–$NaNO_2$ may increase the carboxyl groups of the oxidized cellulose and subsequent amount of the chitosan introduced into cotton fabrics. Fig. 6 shows the effect of the time in preceded oxidation by H_3PO_4/HNO_3–$NaNO_2$ on the amount of chitosan incorporated to the oxidized cotton fabrics. As an exciting result, it is discovered that short oxidation time yields adequate carboxyl groups for the oxidized cotton fabric to ensure sufficient amount of chitosan linked to the oxidized cotton fabric for achieving high antibacterial activity when investigated in antimicrobial examining. The maximum amount of fixed chitosan was 3.57% of the weight of fabrics. Thus the cotton fabric is oxidized in mixture solution of phosphoric acid and nitric acid (1:2, v/v) containing 1.4% (w/v) of sodium nitrite at 40 °C for 45 min to receive such carboxyl content. As shown in Fig. 6, the maximal amount of bonded chitosan was 1.54 times higher for the fabrics treated in the solution of chitosan with lower molecular

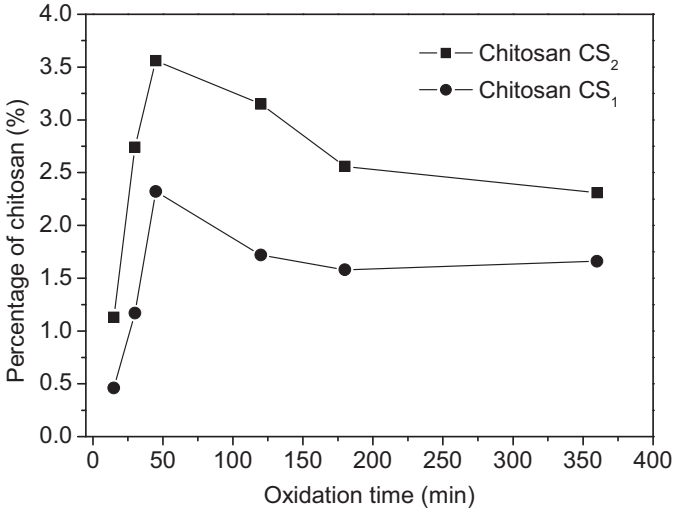


Fig. 6. Relationship between the amount of chitosan crosslinked into cotton fabrics and chitosan molecular weight and time of the H_3PO_4/HNO_3–$NaNO_2$ oxidation.

weight (CS_2) than chitosan with higher molecular weight (CS_1). The chitosan content in the modified cotton fabric increased with the oxidation time of H_3PO_4/HNO_3–$NaNO_2$ in the initial stage, and then it decreased and became nearly constant when the time of oxidation was over 120 min. This is probably attributed to the differences in the oxidation reaction site and amide bond formation in the

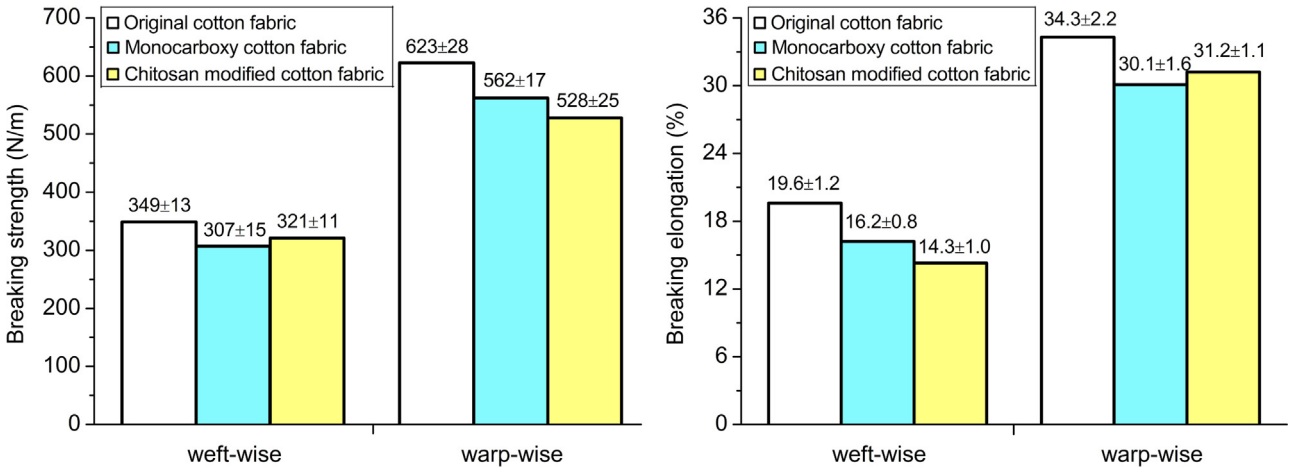


Fig. 7. Change in breaking strength (left) and breaking elongation (right) of the original, monocarboxy and chitosan CS_1-crosslinked cotton fabrics.

oxycellulose (Kim & Kuga, 2002; Liu et al., 2001). During the oxidation of H_3PO_4/HNO_3–$NaNO_2$, the small nitrogen oxide molecule may enter the cotton fiber interior and the glucose units on the surface and inside of the cotton fiber can be oxidized. On the other hand, the higher molecular chitosan CS_1 principally couples with the carboxyl groups of the oxidized fiber surface due to a huge molecule, whereas chitosan CS_2 with lower molecular weight combines with the carboxyl groups formed not only on the surface but also in small pores of the oxidized cellulose fiber. These could be seen from SEM photomicrographs of the surfaces of the chitosan CS_1 and chitosan CS_2 grafted cotton fibers, Fig. 3. The surface of the oxidized fiber is rough, affected by oxidation. The surface of chitosan CS_1 coated cotton fiber is smooth and visibly covered with a layer of chitosan as shown in Fig. 3c, while treatment with chitosan CS_2, the chitosan film of the fiber surface is relatively unapparent, also many small grains were laid on the surface of chitosan CS_2 coated cotton fiber in Fig. 3d.

The mechanical properties comparison of starting cotton fabric, oxidized and chitosan CS_1-crosslinked cotton fabrics are clarified in Fig. 7. Tensile properties of the oxidized and chitosan CS_1-modified fabrics were lower than that of starting cotton fabric. On the whole, there was no significant difference in breaking strength and breaking elongation between the monocarboxy fabric and chitosan CS_1-coated fabric. This is probably owing to the two facts: first, the chitosan liquor in aqueous acetic acid can corrade cellulose fibers, and this may decrease the breaking strength and elongation in some way; second, because chitosan was reacted with carboxyl group in amorphous zone of the oxidized cellulose, furthermore, a single chitosan molecule can bind with many carboxyl groups between monocarboxy cellulose chains to form network in the composite chitosan–cellulose fabric, so it may enhance breaking strength and elongation to some extent.

3.3. *Antibacterial property of the synthetic chitosan–cotton fabrics*

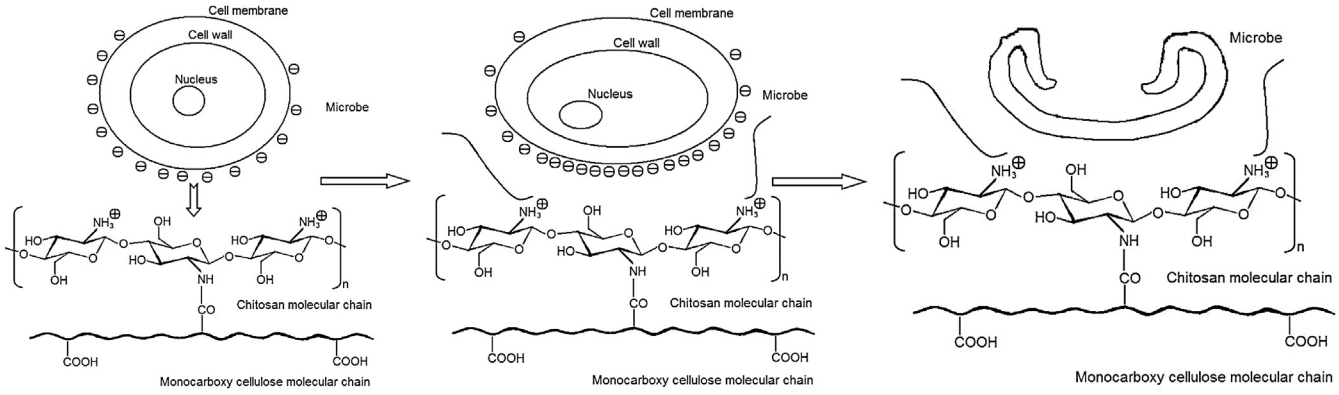
In order to understand the antibacterial efficacy on chitosan, the antimicrobial properties of the chitosan crosslinked oxidized cotton fabric and original cotton fabric were investigated through antibacterial measurement. The results are shown in Table 1. As can be seen, the chitosan treated original cotton fabrics possess some extent antibacterial capability, but in this case chitosan molecules is just physically adsorbed on the fiber surface without chemical binding, and the washing durability for antibacterial activity is very low, the bacterial inhibition ratios of samples C0-1 and C0-2 after 50 times of washes decreased dramatically. Compared with the antibacterial ratio of the original cotton fabric treated by chitosan, chitosan crosslinked cotton fabrics showed outstanding bactericidal capabilities for tested bacteria. The general tendency of inhibition growth of the composite chitosan–cotton fabrics for Gram-positive bacteria *S. aureus* was stronger than for Gram-negative bacteria *E. coli*, according with literature data (Hou et al., 2008; No et al., 2002). The chitosan crosslinked cotton fabric sample denoted as C45-2 showed the most effective sterilization against Gram-negative bacteria *E. coli*, whereas the samples denoted as C30-1, C45-1 and C45-2 displayed good antibacterial activity with the inhibition ratio above 95.54% for Gram-positive bacteria *S. aureus*. Also, the antibacterial assays against washing indicated that chitosan crosslinked cotton fabrics, namely samples denoted as C30-1, C45-1 and C45-2, still preserved rather high antibacterial ratio with increasing washing times. In such way, the chitosan molecules were combined on the fabrics surface through chemical covalent bonds and the chitosan crosslinked cotton fabrics showed high stability of achieved antimicrobial activity regard to washing. It should be mentioned that the overoxidized fabrics treated by chitosan (samples C360-1 and C360-2) show low antibacterial activity and washing stability of antibacterial property, probably due to the long oxidation time, the cellulose structure is broken and undesirable products of oxidation occur, which have negative influence on antibacterial properties.

Additionally, these antimicrobial results indicate that the H_3PO_4/HNO_3–$NaNO_2$ oxidized cotton fabrics modified with both chitosan CS_1 and CS_2 possess prominent properties against bacteria. However, the molecular weight, degree of deacetylation and the dosage of chitosan have significant effects on the antibacterial capabilities of chitosan (Chung et al., 2004). Seen from Table 1, the oxidized fabrics crosslinked by chitosan with higher molecular weight (CS_1) was more effective against bacteria than chitosan with lower molecular weight, particularly for antibacterial samples C0-1, C30-1 and C45-1. Probably, the chitosan with lower molecular weight easily penetrated into the fiber inside, while the higher molecular chitosan (CS_1) mainly fixed on the fiber surface and only slightly entered cotton fibers, which cause the amino groups of the chitosan CS_1 to be more accessible to react with bacteria (Zhang et al., 2003).

The chitosan inhibition mechanism for bacteria can be suggested in Scheme 2. The active amino group on chitosan may bind with a hydrogen ion to give the cation of NH_3^+ in the acidic solutions, the polycationic amino groups of chitosan attract the microbial cell walls consisting of electronegative phospholipid bilayer through the direct interactions of charges (Fang, Chan, Mao, & Leong, 2001). Thereby, the activity of bacteria is restrained and

Table 1
Bacterial inhibition (%) of the chitosan crosslinked original and H_3PO_4/HNO_3–$NaNO_2$ oxidized cotton fabrics.

Sample code	Oxidation time (min)	Chitosan type (chitosan content, %)	Washing times					
			0		30		50	
			E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
C0-1	0	CS_1 (0.38)	59.82	66.75	16.14	39.33	7.61	13.70
C0-2		CS_2 (0.47)	51.41	58.02	9.25	25.26	3.43	11.16
C30-1	30	CS_1 (1.17)	90.60	95.54	89.42	93.62	87.05	93.20
C30-2		CS_2 (2.74)	85.35	89.91	84.12	86.24	83.37	85.19
C45-1	45	CS_1 (2.32)	95.66	99.49	94.28	98.32	94.11	98.05
C45-2		CS_2 (3.57)	96.29	98.52	95.36	96.51	94.27	96.18
C360-1	360	CS_1 (1.66)	57.94	63.86	43.56	50.20	35.46	46.13
C360-2		CS_2 (2.31)	58.47	66.09	32.15	47.35	28.08	34.02



Scheme 2. Schematic diagram of the interaction between chitosan and bacteria.

the degradation of proteins and other intracellular constituents then take place, as well as alterations on the permeability of the microbial cell, which induces the loss of essential nutrients and eventual death for bacteria as seen in Scheme 2.

4. Conclusion

This study ensures the possibility of obtaining synthetic chitosan–cellulose fabrics using monocarboxy cotton fabrics. Herein, the cotton fabrics were oxidized by H_3PO_4/HNO_3–$NaNO_2$ mediated system to produce active carboxyl groups and then chitosan was immobilized onto the monocarboxy cellulose fabrics without use chemical crosslinking agents through the amide reaction between free amino groups in chitosan and carboxyl groups of oxidized cotton fabrics. With prolonged oxidation time of H_3PO_4/HNO_3–$NaNO_2$ system, the carboxyl group content and weight loss of oxidized cotton fabrics increased markedly, the amount of grafted chitosan increased with the oxidation time during the initial stage, but it decreased and then became nearly constant at longer oxidation time. In comparison with original cotton fabrics, the H_3PO_4/HNO_3–$NaNO_2$ mediated oxidation decreased the mechanical properties of fabrics, while the chitosan graft of the oxidized cotton fabrics did not significantly change the tensile properties of the oxidized fabrics. From the analysis of infrared spectra, it can be deduced that an amido bond was created between amino groups of chitosan and carboxyl groups in oxidized cellulose.

Chitosan crosslinked cotton fabrics generally showed stronger bactericidal capabilities for Gram-positive bacteria *S. aureus* than for Gram-negative bacteria *E. coli*. The cotton fabrics grafted with chitosan of different molecular weight displayed high antibacterial properties whereas the chitosan with higher molecular weight was more effective against bacteria than the chitosan with a lower molecular weight. Furthermore, chitosan crosslinked cotton fabrics showed stable washing durability of bactericidal activity against *E. coli* and *S. aureus*. These imply that the synthetic chitosan–cellulose fabrics may find potential applications in medical and textile industry.

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