Preparation of Chitosan Biguanidine Hydrochloride and Application in Antimicrobial Finish of Wool Fabric

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ABSTRACT

Chitosan biguanidine hydrochloride (CGH) has been synthesized by the guanidinylation reaction of chitosan with dicyandiamide. Its synthetic mechanism was discussed. The structures of CGH were characterized by FT-IR and ^{13}CNMR. In this study, we used citric acid (CA) as a crosslinking agent, mixed with CGH to perform a pad-dry-cure treatment on wool fabric to study its antimicrobial effects with the help of scanning electron microscopy (SEM). The result showed that there was no obvious sign that CGH adhered to the wool fabric if the wool fabrics were not oxidized by hydrogen peroxide. The surface crosslinks of the oxidized wool fibers were relatively coarse, which beneficial for the antimicrobial and antiseptic effects of the wool fabrics.

Key words: chitosan biguanidine hydrochloride; synthesis; wool; antimicrobial

INTRODUCTION

Marine polysaccharide drugs have attracted much attention. Chitosan, one of the most important marine polysaccharides, has many peculiar biological activities such as immunity, norcholesterol, and antibacterial effect, and thus has prospective application in the fields of medicine, textile and biotechnology. Some antibacterial activities have been described with chitosan and modified chitosan derivatives [1-5]. However, chitosan shows its biological activity only in acidic medium because of its poor solubility above pH 6.5. Thus, water soluble chitosan derivatives which are soluble in both acid and basic physiologic circumstances might be good candidates for a polycationic biocide [6-9].

Chitosan possesses primary amino groups in its structure and the number of these amino groups is related to the rate of antimicrobial activity. The introduction of asparagine to chitosan oligosaccharide significantly improved the bactericidal activity and minimum inhibitory concentration; this probably indicates that the higher the number of amino groups, the higher the antimicrobial activity [10]. Guanidinium salts have attracted increasing interest in recent years, the guanidine derivatives with antimicrobial and antifungal activity have been investigated as medical and crop protection agents and antiseptics for industry products, food and other goods for daily use [11-15]. Few studies have investigated the feasibility of guanidinylated chitosan [16-17].

Possibility of guanidinylated chitosan with multi-amino groups seemed attractive. Richard F. Stockel showed a general method for preparing

aminosaccharide biguanides over a decade age. Aminosaccharide biguanides are prepared by reacting an aminosaccharide with a mono- or multi-functional cyanoguanidine. However, this method needs the lengthier reaction time of greater than 6 hours if the reaction is conducted at 100℃.

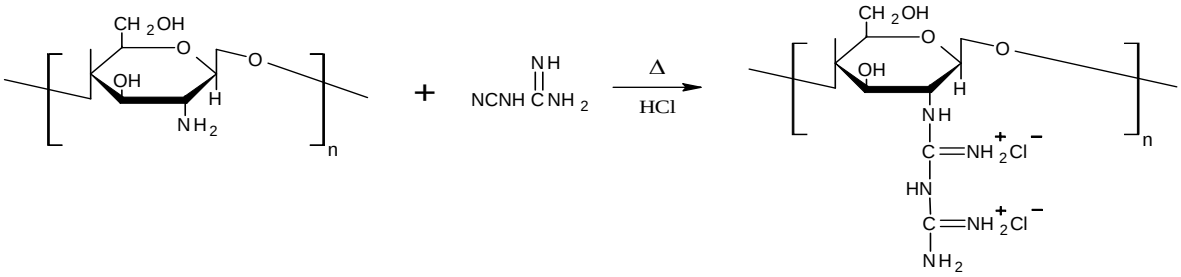


FIGURE 1. The synthesis of chitosan biguanidine hydrochloride (CGH)

We report here a novel guanidinylated chitosan derivative: chitosan biguanidine hydrochloride CGH (*Figure 1*), which was prepared by chitiosan with dicyandiamide in 0.15mol/L hydrochloric acid solution, expecting to improve its water solubility and antibacterial activity further and reduce the abovementioned reaction time. Derivate was characterized by FT-IR and ^{13}CNMR. In this paper, citric acid (CA) was used as a crosslinking agent to study the antimicrobial treatment of wool fabrics through the mixture of CA with CGH. Wash fast and antimicrobial tests were also conducted to evaluate the wool fabric antimicrobial treatment.

EXPERIMENTAL

Materials

Chitosan (molecular weight 800 KDa; the degree of deacetylation 96.0%) and dicyandiamide (CP) were supplied by Guoyao chemical reagent Co. Ltd (Shanghai, China). Beef extract, agar and peptone were supplied by Yijia chemical reagent Co. Ltd (Shanghai, China).All other reagents are analytical grade provided by Beihong Yijia chemical reagent Co. Ltd (Tianjin, China).

Preparation of chitosan biguanidine hydrochloride (CGH)

Chitosan (2% w/v) were dissolved in 0.15mol/L hydrochloric acid, and the desired amount of dicyandiamide (corresponding to a molar ratio of 1:2 compared with chitosan residue) was added at 100℃ for 2h and then it was cooled to room temperature. The mixture was washed thoroughly with ethanol, and then dried under vacuum to constant weight to give product chitosan biguanidine hydrochloride (CGH).

Fabrics treatment

The wool fabrics were padded two dips and nips (100% wet pick up) in a solution containing 1%CGH or 1%CGH, 1%CA and 1% hypophosphite. After treatment, the wool fabrics were dried at 80℃ for 5 min and cured at 120℃ for 2 min, then thoroughly rinsed in 50℃ hot water and air dried in a standard atmosphere for testing (20±1℃ and 65±2RH).

Prior to antimicrobial treatment, all wool fabrics were

pretreated for 1h at 70℃ in baths containing 2g/L of sodium silicate and 20g/L of hydrogen peroxide (30%) with a 25:1 liquor-to-goods ratio, pH: 9, then thoroughly rinsed in distilled water and air dried. We carried out the crosslinking treatment of the wool fabric with a solution containing 1%CGH, 1%CA and 1% hypophosphite for curing the wool fabric with the previous conditions.

Testing and analysis

Infrared (IR) spectra were recorded in the range of 4000-500cm^{-1} on a Nicolet NEXUS-670 Infra red spectrophotometer (Nicolet Ltd, USA). ^{13}CNMR spectra were recorded on a Bruker Avance 400 NMR spectrophotometer（Bruker Ltd, Switzerland）. SEM analysis was done on JEOL JSM-5600LV machine (JEOL Ltd, Japan). The antimicrobial test was carried out according to the antimicrobial standard of the Japan association for the evaluation of textile and was measured according to quantification methods JIS L1902-2002(Adsorption method). An evaluation method in which test bacterial suspension is inoculated directly onto samples. A bacteriostatic value (S) greater than 2.0 means that the test sample is bacteriostatic, and an antiseptic value (L) greater than 0 means that the test sample possesses antiseptic effects. Bacterial growth activity value (F) greater than 1.5 means that the test result is effective.

$$S=M_b-M_c \quad L=M_a-M_c \quad F=M_b-M_a$$

M_a is the average common logarithm for the number of bacteria, obtained from three standard samples immediately after inoculation. M_b is the average common logarithm for the number of bacteria, obtained from three standard samples after 18 h incubation. M_c is the average common logarithm for the number of bacteria, obtained from three antibacterial-treated test samples after 18 h incubation.

Absorption rate **test: Adsorption rate of** chitosan biguanidine hydrochloride on wool **was estimated by measuring the changes in the dry weights of the sample:** absorption rate **(%) = (dry weight after treatment- dry weight before treatment)/ dry weight before treatment×100.** The durability of the treated wool fabric against repeated launderings was evaluated by washing wool fabrics according to AATCC test method 124.

RESULTS AND DISCUSSION

Synthesis reaction mechanism of chitosan biguanidine hydrochloride (CGH)

Novel chitosan biguanidine hydrochloride having unique structures and properties are prepared by reaction of the chitosan with a dicyandiamide. *Figure 2* showed that reaction mechanism between chitosan and dicyandiamide was the nucleophilic addition reaction, the free amino group in the chitosan adds to the triple bond of the cyano group present in the dicyandiamide reactant. This reaction can be readily carried out in aqueous media in the presence of a protonating agent, HCl. It is found the side reaction of dicyandiamide hydrolyzing to guanylurea during the synthesis of chitosan biguanidine hydrochloride. The trend of the hydrolysis is significant with the increasing acidity. The hydrolysis reaction was showed in *Figure 3*.

Characterization of chitosan biguanidine hydrochloride (CGH)

The synthesis of the chitosan biguanidine hydrochloride was achieved by a nucleophilic addition reaction of the chitosan with dicyandiamide. FT-IR, ^{13}CNMR analyses indicated the success of the guanidylation reaction.

Structural changes of chitosan and its derivative were confirmed by FT-IR spectra (*Figure 4*).

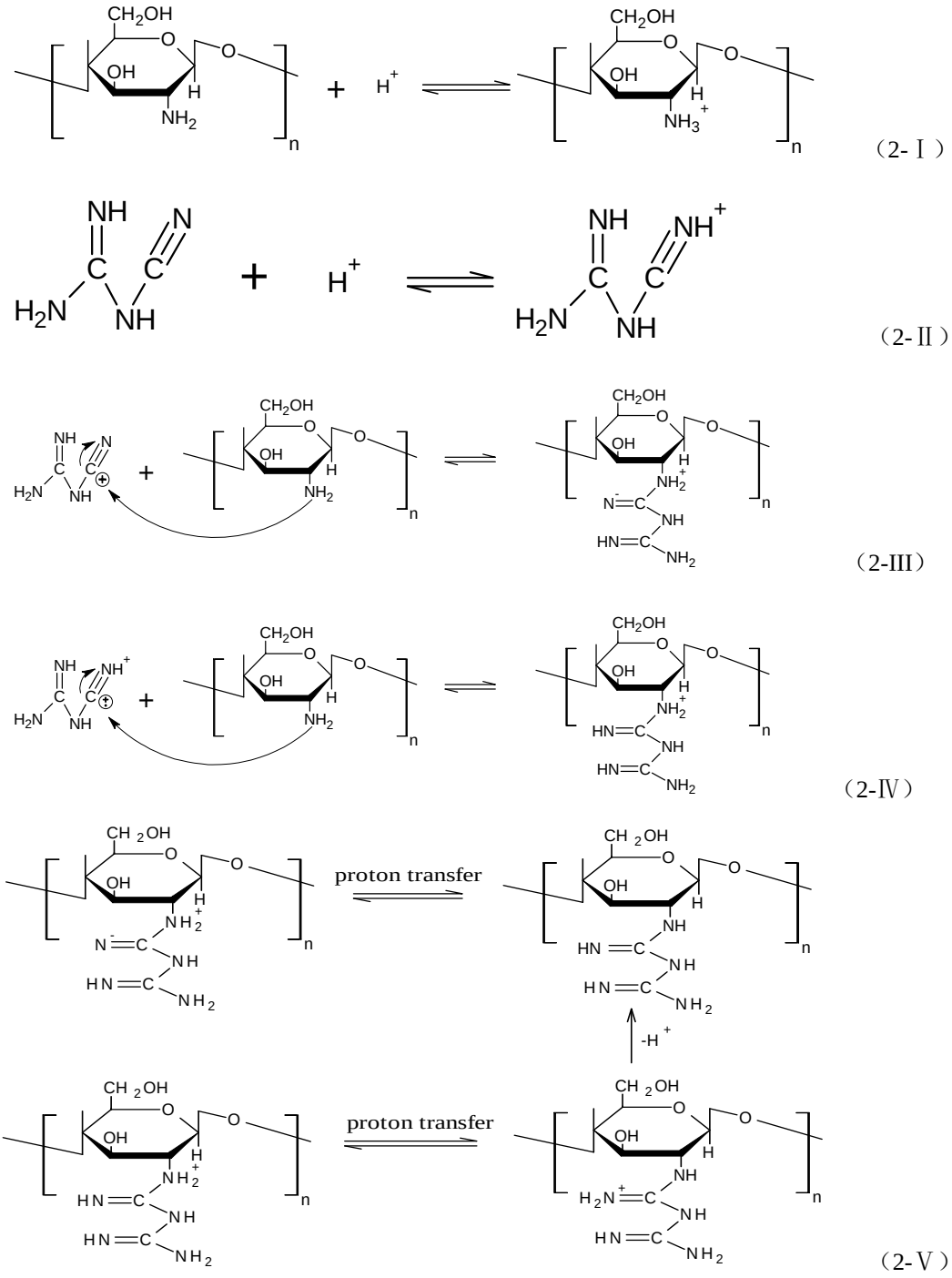


FIGURE 2. Synthesis reaction mechanism of chitosan biguanidine hydrochloride (CGH)

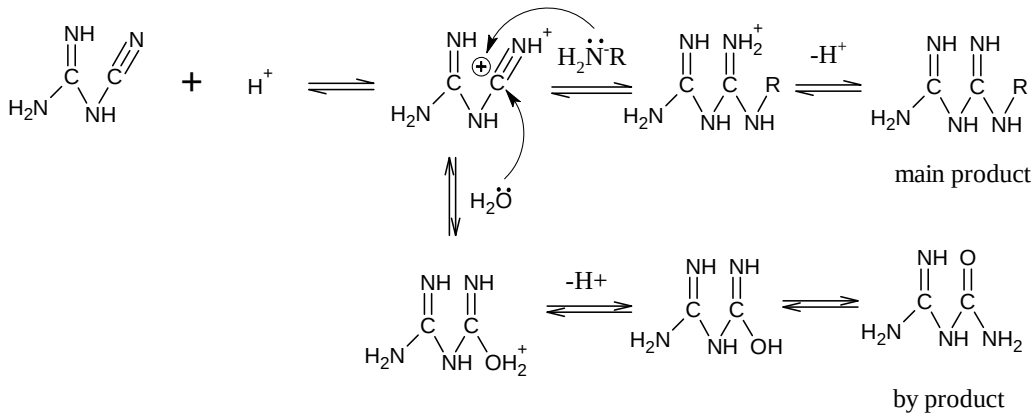


FIGURE 3. The nucleophilic addition reaction of chitosan, water and dicyandiamide

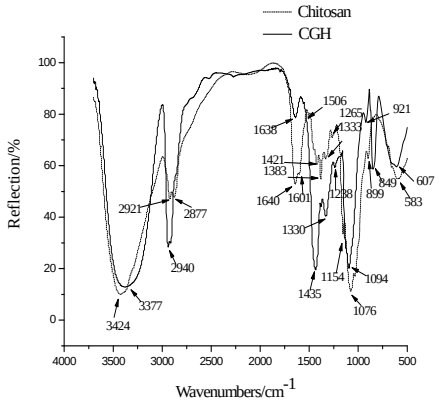


FIGURE 4. FT-IR spectra of chitosan and chitosan biguanidine hydrochloride (CGH)

The IR spectra of CGH showed a new peak at 1638cm^{-1} assigned to the stretching vibration of C=NH in guanidine group [-HNC（=NH）NH_2], the peak at 1601cm^{-1} that had been assigned to the binding vibration of –NH_2 group of chitosan had disappeared, the new stronger peak at 1435 cm^{-1} was assigned to the stretching vibration of C-N-C and the peak at 1330 cm^{-1} was assigned to the stretching vibration of C-N, it can be deduced that guanidinylation had been successful.

The ^{13}CNMR spectra of chitosan and chitosan biguanidine hydrochloride in HCl/D_2O were showed in *Figure 5*.

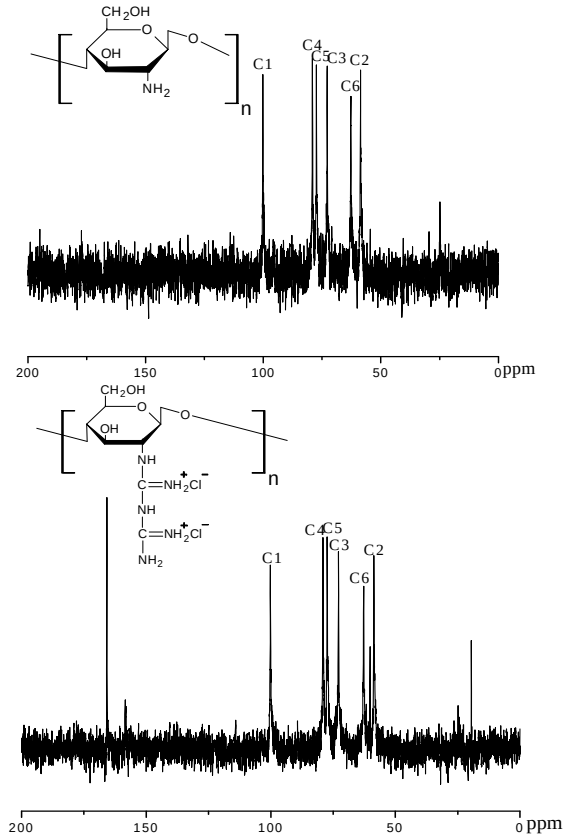


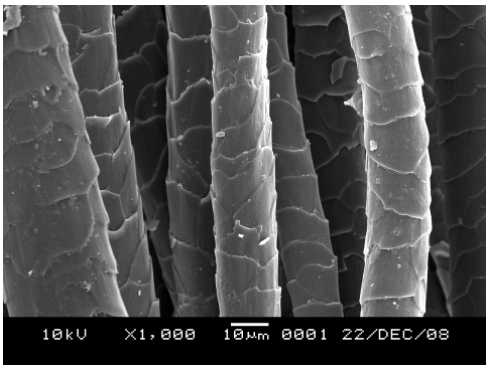
FIGURE 5. ^{13}CNMR spectra of chitosan and chitosan biguanidine hydrochloride (CGH)

Comparing the ^{13}CNMR spectrum of chitosan with that of chitosan biguanidine hydrochloride, the distinct signals at 158.4 and 165.8 ppm were assigned to the carbons of biguanidine

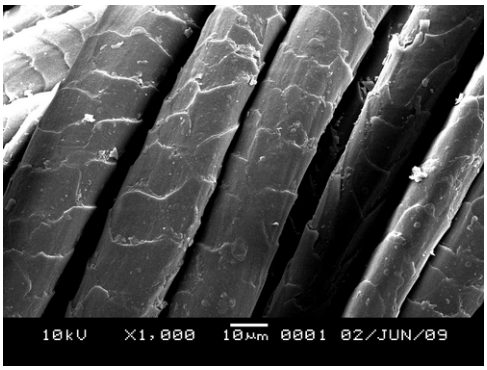
groups[17], and the ^{13}CNMR chemical shifts for chitosan at 58.6(C2), 62.7(C6), 72.8(C3), 77.3(C5) and 79.0(C4) and 100.1(C1) (ppm) were detected. In contrast to chitosan, the signals of chitosan biguanidine hydrochloride showing at 58.6(C2), 62.7(C6), 72.8(C3), 77.3(C5) and 79.0(C4) and 100.1(C1) (ppm) were attributed to the polysaccharide structures. The ^{13}CNMR spectra confirmed that the amino groups of chitosan being partly guanidinylated.

SEM analysis

The wool fabrics were padded two dips and nips (100% wet pick up) in a solution containing CGH and CA. After treatment, the wool fabrics were dried at 80℃ for 5 min and cured at 120℃ for 2 min. Surface morphological structure of untreated wool and treated wool were shown in *Figure 6*.



(a)



(b)

FIGURE 6. SEM photographs of the untreated wool (a) and treated wool (b)

There was no obvious sign that CGH adhered to the wool fabric because of the obstruction caused by the rigid scales on the surface and cuticle of the wool fabric [18] and so there was no obvious adherence of CGH to the surface of the fiber. The wool fabric was oxidized by hydrogen peroxide (20g/L), as shown in *Figure 7*, showed that hydrogen peroxide pretreatment has a light damage on surface scalelike structure of wool, the oxidized wool fibers were relatively coarse compared to untreated wool, which is the result of oxidation and scission of the numerous disulfide bonds in the exocuticle of the wool.

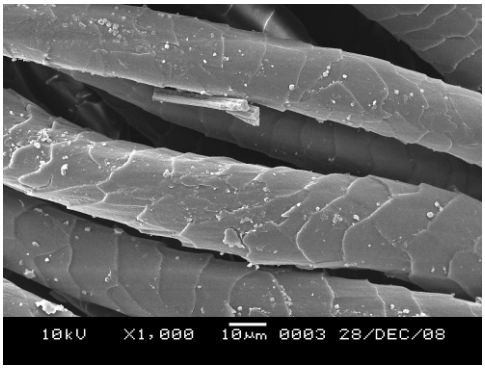


FIGURE 7. SEM photographs of the wool oxidized with hydrogen peroxide

When wool fabrics were oxidized with hydrogen peroxide and then treated with CGH and CA, the results were shown in *Figure 8*.

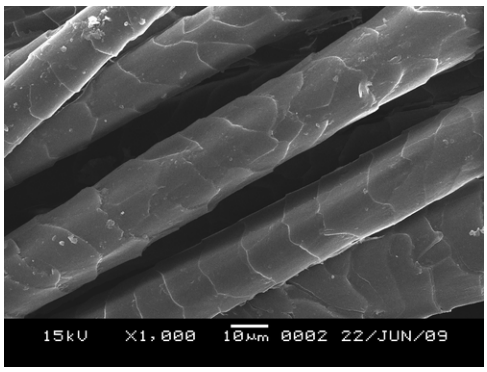


FIGURE 8. SEM photographs of the wool oxidized with hydrogen peroxide and then treated with CGH and CA

The surfaces of oxidized wool fibers were coarse. Compared to *Figure 7*, *Figure 8* showed a relatively smoother surface substantiated presence of the CGH compound on the surface of the wool. Once the surface of scales on the wool fabric was damaged by hydrogen peroxide, the CGH adhered to the wool fabric.

Antimicrobial and wash fast analysis

For wool fabric, according to the Japan Association for the Evaluation of Textile antimicrobial standard, a bacteriostatic value greater than 2.0 means that the test sample is bacteriostatic, and an antiseptic value greater than 0 means that the sample possesses antiseptic effects. In the experiment, as *Table I* shows, when the bacteriostatic value was less than 0 and the antiseptic value was less than 0, the CGH did not adhere to the fabric, which resulted in no antimicrobial or antiseptic properties. The protected the inner cortex when it reacted with the CA crosslinking agent and CGH heat treatment. We hoped to produce esterification between the wool fabric and CA/ CGH, so the other free carboxyl would form an amide bond between the CGH amino group or wool amino group and become fixed. Because of the scale hindrance, it could not completely react with the hydroxyl and amino group crosslinks within the wool fiber, which resulted in poor crosslinking, so we added CA, which caused the CGH to be unable to adhere [18].

TABLE I Antimicrobial activity of the wool treated with CGH and CA

Bacterial growth activity value	Antimicrobial value	Antiseptic value
2.5	＜0	＜0

The wool fabric first underwent a hydrogen peroxide preprocesses, which caused the scale to oxidize and resulted in light damage of the scale and increased the crosslinking reaction. These were then treated with CGH. Wool fabric under various conditions of heat treatment was wash-tested multiple times according to AATCC test method 124. Adsorption rate of chitosan biguanidine hydrochloride on wool is shown in *Figure 9*.

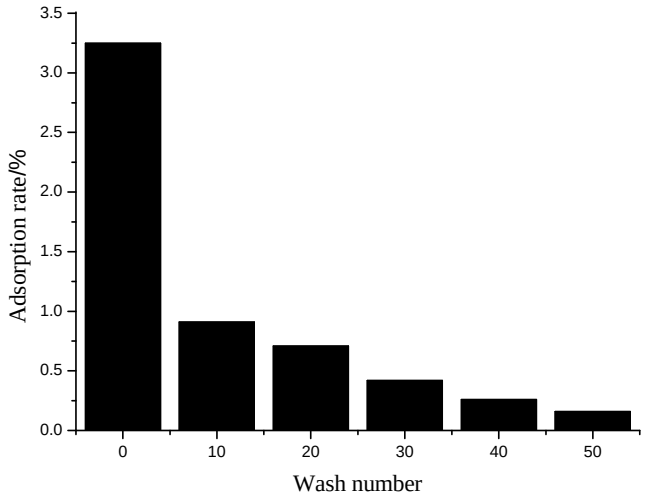


FIGURE 9. Adsorption rate of chitosan biguanidine hydrochloride on wool

Figure 9 showed that chitosan biguanidine hydrochloride adhered to the oxidized wool fabric. Adsorption rate of chitosan biguanidine hydrochloride on wool decreased with increasing number of washings. The more CGH detached from the wool fabric after 10 washings.

TABLE II. Antimicrobial activity of the wool oxidized with hydrogen peroxide and then treated with CGH and CA

Wash number	0	10	20	30	40	50
Bacterial growth activity value				2.5		
Antimicrobial value	3.6	3.1	2.8	1.9	1.4	1.0
Antiseptic value	1.2	0.6	0.3	<0	<0	<0

Note: Antimicrobial value and antiseptic value of wool oxidized with hydrogen peroxide was less than 0. When wool fabrics were oxidized with hydrogen peroxide and then treated with CGH, antimicrobial value of treated wool after 10 washings was 2.3; antiseptic value was less than 0.

Table II showed that because the amino groups of the CGH in the treatment agent easily formed an amine salt cationic, which could catch the anionic bacteria and cause its cell wall to stop growing, it showed good antimicrobial properties.

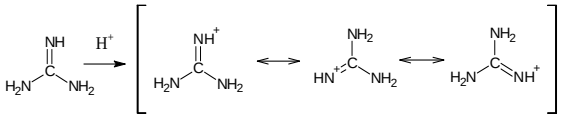


FIGURE 10. Antimicrobial mechanism of CGH

The addition of crosslinking CA will be beneficial for the antimicrobial and antiseptic effects of the wool fabrics. The antimicrobial nature decreased with increasing number of washings. When CA was added, after 20 washings, a bacteriostatic value was 2.8 an antiseptic value was 0.3, and it maintained decent antimicrobial properties, revealing a good crosslinking effect.

CONCLUSION

Chitosan biguanidine hydrochloride (CGH) with good water solubility was prepared by the guanidinylation reaction of chitosan with dicyandiamide. The mechanism of the nucleophilic addition reaction of chitosan with dicyandiamide was brought forward. Because of the scale, when the wool fabric was treated with CGH and CA solution, there was no obvious adherence of CGH to the surface of the wool fiber. After the wool was oxidized by preprocessing with hydrogen peroxide and treated with the CGH and CA, the CGH adhered to the wool fabric. The wool fabric treated with CGH and CA before oxidation did not possess antimicrobial or antiseptic properties. When oxidized and treated CGH and CA, the wool fabric obtained antimicrobial properties. The antimicrobial effect decreased with the frequency of washing.

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