Preparation of n-alkylated quaternary ammonium chitosan and its application in antibacterial finishing of rabbit hair fabric

Yingxue Jiang, Yi Zhang, Hao Zhang, Ruoying Zhu*, Yuhan Bai, Ye Yu
School of Textile Science and Engineering, Tiangong University, Tianjin 300387, China

Abstract. Alkylated chitosan was obtained by alkylation of chitosan(CTS) with n-butyraldehyde, the product was then reacted with trichlorodihydroxypropyl triethyl ammonium chloride(CHPTAC-ethyl) to form n-alkylated quaternary ammonium chitosan. Its chemical structure was characterized by FTIR, and its physicochemical properties, such as viscosity-average molecular weight and solubility, were determined by viscosity and spectrophotometry. The antimicrobial activity of the modified chitosan was determined by the minimal antimicrobial method, and the minimum effective antimicrobial concentration (MIC) of the product against escherichia coli was 0.15g/L, which was better than the MIC value of the natural chitosan. Using citric acid as cross-linking agent and sodium hypophosphate as catalyst, the antibacterial finishing of rabbit hair fabric with modified chitosan showed that the antibacterial rate of n-alkylated quaternary ammonium chitosan to Escherichia coli was over 99.9%, and the antibacterial rate was better than that of natural chitosan, and it was a kind of good natural macromolecule antibacterial finishing agent for animal hair fabric.

1. Introduction
Rabbit hair fabric has good comfortablity, warmth retention, moisture absorption and moisture retention. However, rabbit hairs and fabric contain less impurities compared with wool, no specific washing is carried out during the manufacture of rabbit hair textile, so rabbit hair fabric is more vulnerable to microbial damage. Moreover, there are few studies on antibacterial finishing of rabbit hair fabric[1-4]. Chitosan is a natural non-toxic, biodegradable, biocompatible and renewable high quality natural resource, which has its own unique molecular structure, excellent physiological activity and other functions. Therefore, using chitosan as a high-quality natural resource, suitable targeted chemical modification of using natural chitosan as a high-quality natural resource is performed to prepare a series of different types of new high-efficiency, non-toxic, biocompatible green chitosan surfactants and antibacterial products characterized of high-efficiency and biocompatibility, as well as exploration of the relationship between its structure and performance of the modified chitosan have aroused , which has both academic interest due to great social and economic benefits[5-8].

Chitosan is water insoluble, while has considerable solubility in weak acids due to intermolecular hydrogen bonding. The amino chemical properties of chitosan are active. E.V. Lasareva et al.[9] reacted chitosan with aldehyde group to form schiff base under acidic conditions, and then reduced by sodium borohydride to form stable n-alkylated chitosan. After the introduction of alkyl group into chitosan molecules, the intermolecular hydrogen bond of chitosan was significantly weakened and the solubility of chitosan was improved. The exposed hydroxyl groups on chitosan also have active
chemical properties. The introduction of quaternary ammonium salt groups with high steric hindrance and strong hydration ability at the position of hydroxyl groups can also greatly weaken the intermolecular hydrogen bonds of chitosan and increase the water solubility of chitosan derivatives\cite{10-13}. Based on this, chitosan was alkylated with n-butyraldehyde, and the product was further modified with etherification agent 3-chloro-2-hydroxypropyl triethyl ammonium chloride (CHPTAC-ethyl), and n-alkylated quaternary ammonium chitosan was finally obtained. In this paper, the antibacterial activity of modified chitosan was studied and applied to the antibacterial finishing of rabbit hair fabric. The antibacterial activity of rabbit hair fabric before and after finishing was tested. The feasibility and prospect of the modified chitosan in the antibacterial finishing of rabbit hair fabric were discussed.

2. Synthesis and Characterization

2.1. Materials
Chitosan was supplied by sinopharm chemical reagent Co., Ltd. (Shanghai, China); Hydrochloric acid, anhydrous ethanol, sodium borohydride, CHPTAC-ethyl, acetic acid, n-butyraldehyde, sodium dodecyl sulfate, sodium hydroxide, citric acid, etc. were provided by Shanghai Macklin biochemical Co., Ltd. (Tianjin, China). Rabbit wool fabric was supplied by Weileik Clothing Co., Ltd. (Shandong, China).

2.2. Synthesis of Chitosan Derivatives
Chitosan was dissolved in a solution of 5% acetic acid and stirred at room temperature until dissolved thoroughly. Followed by addition of equal molar amount of n-butyraldehyde and a small amount of sodium lauryl sulfate, under stirring at room temperature for 6h. After adding sodium hydroxide solution to adjust pH to weak acidity, slowly drop an appropriate amount of 10% NaBH₄ solution, stir the reaction for 2h, add ethanol for static precipitation, centrifuge, repeat washing for 3 times, the product was freeze-dried to obtain a relatively pure n-alkylated modified chitosan (N-CTS). The product N-CTS was reacted with n-butyraldehyde in the same way, and after 6 hours, an appropriate amount of 10%NaBH₄ solution was added to reduce the reaction for 2h to obtain n, n-alkylation modified chitosan (N,N-CTS).

Alkalinate was carried out by adding alkylated modified chitosan, distilled water, NaOH in a flask, at 45°C under stirring, for 30min. Then an appropriate amount of CHPTAC-ethyl was added to the flask slowly, and adjust pH=8 with hydrochloric acid solution after the reaction. As cooling to room temperature, add equal volume of anhydrous ethanol and stir constantly. After a complete precipitation, a relatively pure solid N-CCTS or N,N-CCTS was obtained by following processing, including washing with 50% ethanol solution, centrifugation, and three times rinse as well as freeze-drying of the precipitant.

2.3. Characterization of chemical structure and physicochemical properties
IR analysis of different samples was carried out by KBr powder tablet method using Nicolet IS50 Infrared Spectrometer. The 10mg sample and 120mg dry KBr were ground evenly, and the sample was made into tablet by tablet pressing mechanism and tested in the infrared spectrometer\cite{14,15}.

The 0.5% sample solution was prepared with citric acid solutions of different concentrations as solvents, and the visible light transmission of the sample solution was determined using Evolution 201 UV-Visible Spectrophotometer at the wavelength of 620nm\cite{16,17}.

HAc solution of 0.1mol/L and NaCl solution of 0.2mol/L were prepared as solvents, and the molecular weight of the sample was determined by viscosity method\cite{18}.

2.4. Determination of Minimum Inhibitory Concentration
0.5% acetic acid solution was used as the solvent to prepare 0.5% sample solution. Appropriate medium was mixed with appropriate sample solution to prepare the mixed medium with
concentrations of 0.05mol/L, 0.1mol/L, 0.15mol/L, 0.2mol/L, 0.3mol/L, 0.4mol/L and 0.5mol/L, respectively. After solidification, 1-2ml escherichia coli suspension droplets were taken into the plate. Place the inoculated plate into the incubator and culture upside down for 24 hours to observe the growth of bacteria. The concentration of the test solution that completely inhibited the growth of bacterial colony was the MIC value of the sample to the tested bacteria\(^{[19,20]}\).

The 0.2g sample was dissolved in 40ml sterile acetic acid solution with a volume fraction of 0.5%, and the sterile acetic acid solution with a volume fraction of 0.5% was used as the control group. 19.4mL medium and 0.6mL sample solution (concentration 0.15g/L) were added to the plate at the same time, and fully mixed. After solidification, 1ml escherichia coli suspension (the bacterial quantity is about 10\(^6\)cfu/ml) was taken and dropped onto the plate. The inoculated plate was placed in an incubator and cultured upside down for 24 hours to observe the growth of bacteria.

### 2.5. Finishing and antibacterial activity determination of rabbit wool fabric

2g finishing agent, 3g citric acid and 3G sodium hypophosphate were dissolved in 200mL distilled water. 3g rabbit hair fabric was added, and soaked for 20min at 50°C for the first time. After soaking, the fabric was taken out and the excess liquid was pressed, with the pressing rate controlled at 70%. Raise the temperature to 90 and soak the fabric for 40 minutes, then remove and press out the excess liquid. After that, pre-bake at 50 for 5min, then bake at 90 until dry.

GB/T 20944.3-2008 was used to evaluate the antibacterial property of rabbit wool fabric.

### 3. Results and Discussion

#### 3.1. Reaction principle

![Figure 1. Synthesis route of alkylated chitosan](image)

The preparation principle of N-CTS and N, N-CTS is shown in Figure 1. First, the amino group on the chitosan reacts with the aldehyde group to form a Schiff base, and then N-CTS is obtained by sodium borohydride reduction. The amino group on N-CTS was condensed with aldehyde group, and the N, N-CTS was obtained by reduction.
Figure 2. Synthesis route of alkylated quaternary ammonium chitosan

Figure 2 shows the synthesis principle of N-CCTS and N, N-CCTS. First, etherifying agent CHPTMAC-ethyl is eliminated by β-halide elimination reaction in alkaline environment to produce epoxy hydroxypropyl triethyl ammonium chloride (GTA). Epoxy group has high reactivity, reacts with hydroxyl group on glucosamine ring to form ether bond, thus introducing quaternary ammonium group into chitosan macromolecule to generate N-CCTS or N, N-CCTS.

3.2. FTIR Analysis

FTIR of raw materials and products measured by KBr pressing method is shown in Figure 3. Compared with curve a, the bending peak of the primary amine N-H of curve b and c near 1600cm\(^{-1}\) disappears, indicating that substitution has occurred in \(-\text{NH}_2\), and the in-plane bending vibration peaks of \(-\text{CH}_3\) and \(-\text{CH}_2\) appearing near 1400cm\(^{-1}\) and the stretching vibration peaks of C-N of secondary amine at 1160cm\(^{-1}\), which indicate that the alkyl group was successfully introduced at the amino position of chitosan to obtain N-CCTS and N, N-CCTS. The in-plane bending vibration of \(-\text{CH}_3\) and \(-\text{CH}_2\) appearing near 1400cm\(^{-1}\) in curve c is stronger than that in curve b, indicating that the alkyl group was
introduced again in N-CTS to obtain N, N-CTS. Compared with curve a, b and c, the C-O stretching vibration peaks of primary alcohols in curve d and e near 1060 cm\(^{-1}\) basically disappeared, and the absorption peaks of dialkyl ether near 1150 cm\(^{-1}\) were enhanced. It is shown that the quaternary ammonium group is introduced into the chitosan molecule by forming an ether bond with a hydroxyl group on the glucose ring of C-6, and finally N-CCTS and N, N-CCTS are obtained.

3.3. Solubility Analysis
The test was carried out by analyzing the visible light transmittance of the chitosan solution. The better the solubility was, the greater the light transmittance was; on the contrary, the light transmittance was smaller. Figure 4 shows that the transmittance of the samples increases with the increase of the concentration of citric acid solution. As the alkyl group is introduced into the chitosan molecule, the intermolecular hydrogen bonding of the chitosan is significantly weakened, and the solubility of the chitosan is improved. Therefore, the solubility of N-CTS and N, N-CTS in weak acids is significantly improved compared to CTS. Then, cationic quaternary ammonium groups were introduced into the alkylated chitosan molecular chain, and the ionic groups and electrons mutually repel each other. Due to the enhanced space steric hindrance effect of macromolecular spacing, the hydrogen bond between groups in different molecular chains was destroyed, the crystallinity was reduced, and the dissolution was promoted. The solubility was significantly improved as the number of hydrophilic groups increased. This shows that the solubility of N-CCTS and N, N-CCTS in weak acid is better than that of N-CTS and N, N-CTS. Since more alkyl chains are introduced into N, N-CCTS, the solvent property of N, N-CCTS is lower than that of N-CCTS. It is speculated that the improvement of solubility may be a factor for the improvement of antibacterial properties of modified chitosan.

![Figure 4. Transmission ratios of samples with different citric acid concentrations](image)

3.4. Viscosity-average Molecular Weight
Figure 5. The linear fitting curve of the relationship between sample viscosity and concentration

Table 1. Viscosity-average molecular weight of raw materials and products

| The Sample Name | CTS | N-CTS | N,N-CTS | N-CCTS | N,N-CCTS |
|-----------------|-----|-------|---------|--------|----------|
| Viscous-average molecular weight | $3.07 \times 10^5$ | $3.93 \times 10^5$ | $4.03 \times 10^5$ | $5.87 \times 10^5$ | $5.97 \times 10^5$ |

Figure 5 shows the linear fitting curve of the relationship between viscosity and concentration of raw materials and products. The molecular weight is calculated by extrapolation. At $C = 0$, the intrinsic viscosity is $[\eta]$. From formula $[\eta] = KM^\alpha$, the molecular weight of the sample can be deduced. Among them, the value of $K$ is taken as $1.81 \times 10^{-3}$, and the value of $\alpha$ is taken as 0.93 [21]. The calculated viscosity average molecular weight of the sample is shown in Table 1.

It was concluded that chitosan introduced alkyl groups and N-CTS and N, N-CTS successfully grafted cationic quaternary ammonium groups, and the molecular weight gradually increased. On the one hand, alkyl chains and macromolecular quaternary ammonium groups are introduced into chitosan. On the other hand, the increase of solubility is conducive to the full diffusion of macromolecules, mutual entanglement, the increase of characteristic viscosity leads to the increase of molecular weight.

3.5. Analysis of Minimum Inhibitory Concentration

MIC values of raw materials and products are shown in Figure 6, and the promotion, inhibition and killing effects of samples on the growth of Escherichia coli are shown in the figure. It is well known that the amino group on chitosan has certain bacteriostatic effect. After preliminary alkylation modification, the long alkyl chain penetrates the cell wall and destroys the cytoplasmic membrane through the hydrophobic interaction with the membrane hydrophobic substance such as phospholipid, so the MIC value is increased from 0.4 g/L to 0.3 g/L.

After further modification, the quaternary ammonium groups of N-CCTS and N, N-CCTS were stronger than the amino and basic groups, and the bacteriostatic properties were enhanced. In addition, the increased solubility and molecular weight can prevent the absorption of external nutrients by bacteria, and at the same time lead to the formation of holes in the cell wall and the leakage of intracellular components, thus leading to the death of bacteria. Therefore, the introduction of quaternary ammonium groups further enhanced the antibacterial properties of N-CCTS and N, N-CCTS. The solubility of N-CCTS is better than that of N, N-CCTS, which may be the reason why the bacteriostasis of N-CCTS is better than that of N, N-CCTS.
3.6. Antibacterial properties of fabric

The comparison of the antibacterial effect of rabbit hair fabric treated with different chitosan samples on Escherichia coli is shown in Figure 7. The results are roughly consistent with the antibacterial rate of the upper section. The antibacterial rate of the modified N-CTS, and N,N-CCTS can reach 99.9%. Chitosan is soluble in weak acid, and its amino group has some antibacterial properties. The solubility of N-CTS and N, N-CTS was improved, and the contact between the polymer chain and the bacterial cell wall was promoted under the action of lipophilic alkyl substitution chain, so the bacteriostatic performance was improved. The quaternary ammonium groups in N-CTS and N, N-CCTS further improve its solubility and antibacterial property, so the antibacterial effect is the best.

4. Conclusion

CTS and N, N-CTS were synthesized by the reaction of chitosan and n-butyraldehyde by schiff alkali method. And was etherized with chptac-ethyl in order to get N-CCTS and N,N-CCTS. IR spectroscopy investigation showed, that the alkyl chain and quaternary ammonium groups have been introduced onto the chitosan molecule chains successfully with the etherizing treatment. Viscosity-average molecular weight and light transmittance of different samples were measured, and the results showed that molecular weight and solubility of N-CCTS and N, N-CCTS synthesized in the research were increased compared with that of natural chitosan. The minimum bacteriostatic method
was used to determine the antibacterial activity of the samples. The MIC values of N-CCTS and N,N-CCTS were better than natural chitosan, and the MIC values of N-CCTS against escherichia coli were 0.15g/L. Therefore, it is implied that the improvement of solubility with the introduction of alkyl chain, the introduction of quaternary ammonium group as well as the increase of molecular weight of modified chitosan may be the internal factors for improvement of bacteriostatic property of modified chitosan.

Acknowledgments
The present study was supported by China Modern Agriculture Research system (Project Number: CARS-43-E-2).

References
[1] Gao Y and Cranston R 2008 Recent advances in antimicrobial treatments of textile Text. Res. J. 78 60-72
[2] Purwar R and Joshi M 2004 Recent developments in antimicrobial finishing of textiles—a review AATCC Review 4 22–26
[3] Williams J F and HaloSource V 2005 Antimicrobial functions for synthetic fibers: recent developments AATCC Review 5 17–21
[4] Mao J W and Murphy L 2001 Durable freshness for textiles AATCC Review 1 28–31
[5] BARIKANI M and OLIAEI E 2013 Preparation and application of chitin and its derivatives: a review Iraniian Polymer Journal 23(4) 307-326
[6] YOUNES I and RINAUDO M 2015 Chitin and chitosan preparation from marine sources structure properties and applications Mar Drugs 13(3) 1133-1174
[7] KULKARNI A D and PATIEL H M 2017 N,N,N-trimethyl chitosan: an advanced polymer with myriad of opportunities in nanomedicine Carbohydr Polym 157 875-902
[8] SAHARIAH P and MASSOM M 2017 Antimicrobial chitosan and chitosan derivatives: a review of the structure–activity relationship Biomacromolecules 18(11) 3846-3868
[9] Lasareva E V and Chernuchina A I 2018 Preparation, surface activity and colloidal properties of the ionic complex of chitosan with hexadecyl-oligo-oxyethylene hemisuccinate Carbohydrate Polymers 183 123-130
[10] KAWABATA N and NISHIGUCHI M 1998 Antibacterial activity of soluble pyridinium-type polymers Appl Environ Microbiol 54(14) 2532-2535
[11] Ravi K M N V and Muzzarelli R A A 2004 Chitosan chemistry and pharmaceutical perspectives Chem Rev 104 6071-84
[12] Kamel N A and El-Messieh S L 2017 Chitosan/banana peel powder nanocomposites for wound dressing application: Preparation and characterization Mater Sci Eng C 72 543-50
[13] De Arruda I N Q and Pereira V A 2017 Application of chitosan matrix for delivery of rutin J Iran Chem Soc 14 561-6
[14] Putri R and Dwi N 2016 Preparation and properties of arenga starch-chitosan based edible film Mater Sci and Eng 107 012047
[15] Fan M, Hu Q L and Shen K 2009 Preparation and structure of chitosan soluble in wide pH range Carbohydr Polym 78 66-71
[16] MARTUNS A F and BUENO P V 2013 Synthesis, characterization, and cytotoxicity of TMC-graft-poly(vinyl alcohol) copolymers Carbohydrate Research 381 153-60
[17] CHIVANGKUL T and PENGPRECHA S 2014 Enhanced water-solubility and mucoadhesion of n,n,n-trimethyl-n-gluconate-n-homo-cysteine thiolactone chitosan Carbohydrate Polymers 108 224-31
[18] Vauchel P and Arhaliass A 2008 Decrease in Dynamic Viscosity and Average Molecular Weight of Alginate from Laminaria Digitata during Alkaline Extraction J Phycol 44(2) 515-517
[19] Marieh G and Mehran G 2019 Preparing natural biocomposites of N-quaternary chitosan with antibacterial activity to reduce consumption of antibacterial drugs J Hazard Mater 371
224-232

[20] Angela K T and Ronaldo R F 2019 Comparative antibacterial activity of commercial chitosan and chitosan extracted from Auricularia sp Biocatalysis and Agricultural Biotechnology 17 189-195

[21] Yu Z G 1984 Determination of molecular weight and distribution of polymers Shanghai Scientific and Technical Publishers (In Chinese).