Preparation of N, N-dialkyl quaternary ammonium chitosan and its long-lasting antibacterial finishing process for rabbit fur fabric

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Abstract. N,N-dialkylchitosan (N,N-CTS) was modified by n-butyraldehyde using Schiff base method, it is then synthesized with N,N-CTS by etherification with 3-chloro-2-hydroxypropyltriethylammonium chloride (CHPTAC-ethyl). FTIR and spectrophotometry were used to characterize their chemical structure and physical and chemical properties. The minimum antibacterial activity of the modified chitosan was determined by the minimum bacteriostatic method, and the minimum effective bacteriostatic concentration (MIC) of Escherichia coli was 0.2 g/L, which was better than that of natural chitosan. The antibacterial effect and washing resistance of rabbit wool fabric were investigated by using modified chitosan. After washing for 10 times, the results showed that the inhibition rate of N,N-CCTS on escherichia coli was still more than 99.9%.

1. Introduction
Rabbit fur fabric has good comfort, warmth, moisture absorption and moisturizing effects, and has a natural health effect. However, due to the small amount of impurities in rabbit wool fabrics, there is a large number of microorganisms without special finishing such as washing in the fabric making process, making rabbit wool fabrics more susceptible to microorganisms, and there are few studies on antibacterial finishing of rabbit wool fabrics[1-4].

Chitosan (CTS) is the only biological polymer with amino group in nature. The amino group in the solution dissolved by chitosan is generally easy to be positively charged. By combining with the negative electrons on the cell membrane surface of bacteria, the permeability of the cell membrane of bacteria can be changed so as to inhibit the growth of bacteria. Chitosan is insoluble in water but soluble in weak acids due to intermolecular hydrogen bonding. The amino chemical properties of chitosan are active, and the reaction of chitosan and aldehyde group under acidic conditions produces Schiff base, and then the stable n-alkylated chitosan is reduced by sodium borohydride. 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 group with strong steric hindrance and hydration ability at the hydroxyl group position can also greatly weaken the intermolecular hydrogen bond of chitosan and increase the water solubility of chitosan derivatives[5-8].
Based on this, chitosan was alkylated with n-butyraldehyde, and further modified with etherification agent chptac-ethyl. Finally, N,N-CCTS was obtained. This paper studied the antibacterial activity of N,N-CCTS and applied it to the antibacterial finishing of rabbit fur fabric. The antibacterial activity of rabbit fur fabric before and after finishing was tested, and the feasibility and prospect of N,N-CCTS in the antibacterial finishing of rabbit fur fabric were discussed.

2. Materials and methods

2.1. Materials
Chitosan was purchased from sinopharcine chemical reagent co., ltd. (Shanghai, China), and hydrochloric acid and anhydrous ethanol were provided by Tianjin Fengchuan chemical reagent science and technology co., Ltd.(China). Sodium borohydride and chptac-ethyl were purchased from Shanghai Macklin biochemical co., Ltd. (China), Tianjin Kemiou chemical reagent co., Ltd. (China) provides glacial acetic acid, n-butyraldehyde, sodium lauryl sulfate, sodium hydroxide, sodium hypophosphate, citric acid. The other reagents were of analytical grade.

2.2. Synthesis of chitosan derivatives
The synthesis route of N-CCTS is shown in Figure 1.

Firstly, dissolve chitosan in 5% acetic acid solution, stir at room temperature until dissolved, add n-butyraldehyde of equal molar mass and 2% sodium lauryl sulfate, and stir at room temperature for 8h. Add sodium hydroxide solution to adjust the pH=7. Slowly add 10% NaBH₄ solution (1.5 times of aldehyde), stir the reaction for 2h, and then adjust the pH=4.5 with glacial acetic acid. Add the aldehyde a second time and repeat the above reaction step. When the secondary reduction is completed, adjust the pH value to neutral with NaOH solution. Add 70% ethanol to the precipitated product and let it stand for centrifugation. The product is freeze-dried to obtain pure N,N-dialkyl chitosan(N,N-CCTS).

Secondly, put a certain amount of alkylated modified chitosan, distilled water, NaOH into the flask, and continue to stir, alkalinize at 45 °C for 30min, and slowly add equal molar mass of CHPTAC-ethyl. After the reaction is completed, the pH is adjusted to neutral with a hydrochloric acid solution. After cooling to room temperature, an equal volume of absolute ethanol is added and the mixture is continuously stirred, and the precipitate is left to stand. After the product is completely precipitated, it is washed with a 50% ethanol solution, centrifuged, and the product is freeze-dried to obtain a relatively pure solid N,N-CCTS.

![Figure 1. Preparation route of N,N-CCTS.](image-url)
2.3. Structure and performance characterization

The infrared analysis test of the samples was performed on a Nicolet IS50 infrared spectrometer with a resolution of 4 cm⁻¹, a scanning range of 4000-400 cm⁻¹, and KBr tabling. The sample concentration was 7.5%[9-10].

Determination of the solubility of the sample. Using different concentrations of citric acid solution as a solvent to prepare a 0.5% sample solution, using an ultraviolet spectrophotometer to measure the transmittance of the sample solution at a wavelength of 620 nm[11-12].

2.4. Minimum Inhibitory Concentration

A 0.5% sample solution was prepared by using 0.5% acetic acid solution as a solvent, and the appropriate concentrations of the medium and the appropriate sample solution were mixed to obtain concentrations of 0.05mol/L, 0.1mol/L, 0.15mol/L, 0.2mol/L, and 0.3mol/L, 0.4mol/L, 0.5mol/L mixed medium, after coagulation, 1-2mL of E. coli bacteria suspension was dropped on the plate. Invert the plate in an incubator for 24 hours and observe the growth of the bacteria. The concentration of the test solution that completely inhibits the growth of the colony is the MIC value of the sample to the test bacteria[13-14]. Then prepare the above plate with a mixed concentration of 0.2g / L for sample comparison tests.

2.5. Rabbit fur fabric finishing

Dissolve 2g of finishing agent, 3g of citric acid, and 3g of sodium hypophosphite in 200mL of distilled water, add 3g of rabbit fur fabric, and perform initial soaking at 50 °C for 20min. After soaking, the fabric was taken out for head rolling with the residual rate of 100%, and the fabric was soaked for a second time at 90°C for 40min. Then the fabric was taken out and rolled for a second time, so that the final liquid rate of the fabric was 200%. After that, it was prebaked at 50°C for 5min, and then heated up to 90°C until absolutely dry.

2.6. Determination of antibacterial activity of rabbit fur fabric

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

The 0.75±0.05g fabric and the antibacterial treated fabric were respectively loaded into triangular bottles containing the test bacteria solution and exposed to shock at 150rpm and 37°C for 12h. Take it out, dilute it to an appropriate concentration, spread the plate, and incubate in a biochemical incubator at 37 °C for 18 hours. Observe the colony growth of the blank group, control group and test group, respectively. The antibacterial effect was determined based on the results of repeated tests. Bacteriostatic rate is calculated as follows:

$$R = \frac{B - A}{B} \times 100\%$$

Where R is the bacterial inhibition rate, and B and A are the number of viable bacterial cells before and after shaking the bottle[15-16].

3. Results and Discussion

3.1. Structure and performance analysis

FTIR of raw materials and products measured by KBr tabling method are shown in Figure 2. Compared with a, the bending peak of the primary amine N-H of b near 1600 cm⁻¹ disappeared, indicating that substitution occurred at -NH₂, and the in-plane bending vibration peaks of -CH₃ and -CH₂ appearing near 1400cm⁻¹ and the stretching vibration peaks of C-N of secondary amine at 1160cm⁻¹, indicating that the alkyl group was successfully introduced at the amino position of chitosan to obtain N,N-CTS. Compared with a and b, the C-O stretching vibration peak of c in primary alcohols near 1060cm⁻¹ basically disappeared, and the absorption peak of dialkyl ether near 1150cm⁻¹ was
enhanced, indicating that the quaternary ammonium group was introduced into chitosan molecules by forming ether bonds with the hydroxyl group on C-6, and N,N-CCTS was finally obtained.

Figure 2. FTIR spectra of raw materials and products.

The test was carried out by analyzing the light transmittance of the chitosan solution. The better the solubility, the greater the light transmittance; on the contrary, the light transmittance was smaller. Figure 3 shows that the transmittance of the samples increases with increasing citric acid concentration. After the introduction of an alkyl group into the chitosan molecule, the intermolecular hydrogen bonding of the chitosan was significantly weakened, and the solubility of the chitosan was improved. Therefore, the solubility of N,N-CTS in weak acids was significantly improved compared with CTS. Then, cationic quaternary ammonium groups were introduced into the alkylated chitosan molecular chain, which destroyed the hydrogen bond between groups of different molecular chains, reduced crystallinity, significantly improved solubility. This shows that the solubility of N,N-CCTS in weak acid is better than that of N,N-CTS and CTS, and it is speculated that the improvement of solubility may be the factor for the improvement of antibacterial performance of modified chitosan.

Figure 3. Transmittance of samples at different citric acid concentrations.
3.2. Minimum Inhibitory Concentration

Figure 4. Antibacterial rate and colony growth of the sample against E. coli.
(A - blank group, B - chitosan, C - N,N-CTS and D - N,N-CCTS are the colony growth situation when the sample concentration is 0.2g/L)

Figure 4 clearly shows the results of the sample's promotion, inhibition and bacterial death on E. coli. The positively charged free amino group on chitosan can be combined with the negative charge on the cell wall to form a polymer protective film on the surface of the bacteria to prevent nutrients from being transported into the bacteria. After the initial alkylation modification, the antibacterial properties of the modified chitosan were basically unchanged, and the minimum antibacterial concentration had no significant change compared with chitosan, with MIC values of 0.3g/L. After further modification of N,N-CCTS contains stronger quaternary ammonium groups than amino groups, enhanced bacteriostasis, and the MIC value increased to 0.2g/L. And the solubility is enhanced, and the formed protective film is more dense, preventing the transport of nutrients to the bacteria, and at the same time, causing the cell wall to form holes and causing the leakage of intracellular components, leading to bacterial death. Therefore, the introduction of quaternary ammonium groups further enhanced the antibacterial performance of N,N-CCTS.

3.3. Antibacterial properties of rabbit fur fabric

Figure 5. Antibacterial rate of the sample against E. coli.
(a - unfinished fabric, b - CTS finished fabric, c - N,N-CTS finished fabric, d - N,N-CCTS finished fabric were all washed for 5 times for fabric, and the test solution was diluted 10 times)
The comparison of the antibacterial effect of rabbit wool fabrics treated with different finishing agents on E. coli is shown in Figure 5. The results are approximately consistent with the antibacterial rate of the previous section, and the antibacterial rate of modified N,N-CCTS reaches over 99.9%. The amino group and hydroxyl group on the chitosan molecule react with the carboxyl group on the citric acid to form the amide bond and ester bond, which are cross-linked to the rabbit hair fiber. After washing for 5 times, the chitosan has a better bacteriostatic effect. The solubility of N,N-CTS increased, and its bacteriostatic properties did not decrease significantly compared with chitosan. The quaternary ammonium group in N,N-CCTS further improves its solubility and, as a stronger bacteriostatic group, is more prone to cross-linking reactions. Therefore, its washing resistance and bacteriostatic performance are the best.

4. Conclusions
N,N-CCTS has been successfully prepared. The MIC value of N,N-CCTS to E. coli was 0.2g/L, which was better than the MIC value of natural chitosan. The antibacterial activity of rabbit wool fabric treated with N,N-CCTS was significantly improved against E. coli, the bacteriostatic rate is higher than N, N-CTS and natural chitosan, reaching more than 99.9%, which indicates that N,N-CCTS is a long-lasting and excellent antibacterial finishing agent for wool fabrics, and it will have a good application prospect in antibacterial finishing for wool fabrics.

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References
[1] Gao Y, Cranston R 2008 Recent advances in antimicrobial treatments of textile *Text. Res. J.* **78** 60-72
[2] Purwar R, Joshi M 2004 Recent developments in antimicrobial finishing of textiles—a review *AATCC Review* **4** 22-26
[3] Williams J F, HaloSource V and Cho U 2005 Antimicrobial functions for synthetic fibers: recent developments *AATCC Review* **5** 17-21
[4] Mao J W, Murphy L 2001 Durable freshness for textiles *AATCC Review* **1** 28-31
[5] De Arruda I N Q, Pereira V A and Stefani R 2017 Application of chitosan matrix for delivery of rutin *J. Iran. Chem. Soc.* **14** 561-6
[6] Kamel N A, El-Messieh S L and Saleh N M 2017 Chitosan/banana peel powder nanocomposites for wound dressing application: Preparation and characterization *Mater Sci. Eng. C* **72** 543-50
[7] Ravi K M N V, Muzzarelli R A A, Muzzarelli C, Sashiwa H and Domb A J 2004 Chitosan chemistry and pharmaceutical perspectives *Chem. Rev.* **104** 6071-84
[8] KAWABATA N, NISHIGUCHI M 1998 Antimicrobial activity of soluble pyridinium-type polymers *Appl Environ Microbiol* **54**(14) 2532-2535
[9] FanM, Hu Q L and Shen K 2009 Preparation and structure of chitosan soluble in wide pH range *Carbohydr. Polym.* **78** 66-71
[10] Putri R, Dwi N, Putri L S, Putri P S, Tetty N I and Venti S 2016 Preparation and properties of arenga starch-chitosan based edible film *Mater Sci. and Eng. A* **107** 012047
[11] CHIVANGKUL T, PENGPRECHA S, PADUNGRÖS P, Siraleartmukul K, Prasongsuk S and Muangsin N 2014 Enhanced water-solubility and mucoadhesion of n, n - trimethyl - n - gluconate - n - homo - cysteine thiolactone chitosan *Carbohydr. Polym.* **108** 224-31
[12] MARTUNS A F, BUENO P V, FOLLIMANN H D, Nocchi S R, Nakamura C V, Rubira A F and Muniz E C 2013 Synthesis, characterization, and cytotoxicity of TMC - graft - poly (vinyl alcohol) copolymers *Carbohydr. Res.* **381** 153-60
[13] Marieh G, Mehran G, Sabihe S Z and Sheila B T 2019 Preparing natural biocomposites of N-
quaternary chitosan with antibacterial activity to reduce consumption of antibacterial drugs *J. Hazard. Mater.* **371** 224-232

[14] Angela K T, Ronaldo R F, Lourdes V A, Ursela G B and John P M 2019 Comparative antibacterial activity of commercial chitosan and chitosan extracted from Auricularia sp *Biocatalysis and Agricultural Biotechnology* **17** 189-195

[15] Lidija F Z, Julija V, Tijana R, Matej B, Olivera S and Tatjana K 2013 Antimicrobial and antioxidant functionalization of viscose fabric using chitosan-curcumin formulations *Text. Res. J.* **0(00)** 1-12

[16] Zhang W, Zhou J J and Dai X L 2016 Preparation and characterization of reactive chitosan quaternary ammonium salt and its application in antibacterial finishing of cotton fabric *Text. Res. J.* **0(00)** 1-7