Synthesis of 2-Hydroxypropyl Trimethylammonium Chloride Chitosan and its Application in Bamboo Fiber Paper

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To obtain a functional composite paper with antibacterial activity, 2-hydroxypropyl trimethylammonium chloride chitosan (HACC) was prepared and sprayed onto the surface of paper made of bamboo fiber. HACC was synthesized from chitosan with 2,3-epoxypropyl trimethylammonium chloride (GTAMC), and the optimal preparation conditions were selected by single-factor tests. The highest degree of substitution (DS) of HACC reached 0.868, when the ratio of chitosan to GTAMC was 1:4, and the water solubility was noticeably improved. The structural characterization demonstrated the successful modification on the original chitosan with a decrease in heat stability and the peak correlated with hydroxypropyl trimethylammonium chloride groups in FTIR. The addition of HACC in the bamboo fiber paper greatly increased the antibacterial activity, and water absorption was higher as well. These results may serve as a basis for the modification and the preparation of chitosan antibacterial agents.

Keywords: Chitosan; Water solubility; Bamboo fiber paper; Antibacterial activity

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INTRODUCTION

Bamboo fiber, with features of renewability and biodegradability, is a natural non-wood plant fiber. As one of the fastest renewable plants with a maturity cycle of 3 to 4 years, there is no anxiety over ground pollution even if it is buried in the earth. Considering the serious environmental problems, the popularity of its utilization in daily necessities is increasing. Due to its outstanding breathability (Wang *et al*. 2003) and hygroscopicity (Li *et al*. 1994; Ray *et al*. 2005), bamboo fiber can be a kind of ideal material of some tissue paper products such as sanitary pads and napkins.

However, the high porosity of the surface of these paper products results in the adhesion of large amounts of bacteria. Hence, adding an antibacterial ingredient on paper to prepare a new kind of composite has a huge potential for development.

Chitosan, poly-β-(1→4)-N-acetyl-D-glucosamine is readily obtained from chitin and is the most abundant polymer after cellulose, hemicellulose, and lignin (Rinaudo 2007; Song *et al*. 2011; Zheng *et al*. 2015); it exists largely in the exoskeleton of arthropods or in the cell walls of fungi and yeast (Aranaz *et al*. 2009; Rane and Hoover 2009; Aranaz *et al*. 2010). Due to its excellent properties, such as low pollution, antibacterial activity, biocompatibility, film forming property, and nontoxicity (Li *et al*. 2005; Eldin *et al*. 2008; Elsabee *et al*. 2012), chitosan has drawn considerable interest in recent years. However, poor solubility in neutral water and alkaline solutions greatly limits the scope of its applications (Croisier and Jérôme 2013).

When the pH value is close to neutral, a large fraction of the amino groups in chitosan will be in their uncharged form. Thus, the number of positive charges on the entire chain are correspondingly reduced, and the water solubility decreases accordingly. To overcome this pH limitation, many researchers are exploring water-soluble chitosan *via* chemical modification (Shahidi *et al*. 1999; Varma *et al*. 2004; Guo *et al*. 2007; Anitha *et al*. 2009). Due to its outstanding water solubility and antibacterial property (Suzuki *et al*. 2000; Lim and Hudson 2004), introduction of quaternary ammonium salts onto the chitosan backbone will be one of the best methods.

N-trimethyl chitosan can be synthesized directly by the reaction between the $-NH_2$ of chitosan and iodomethane under alkaline conditions (Zhang *et al*. 2006). However, iodomethane is a highly volatile, carcinogenic and expensive reagent, which limits its range of application. Ester exchange reaction is a way to form quaternary ammonium salt of chitosan as well, starting from chitosan, trimethylamine, and ethyl chloroacetate (Fang *et al*. 2005).

Despite the advantage that the product is easy to separate and purify, the complex reaction processes and long reaction time cannot be avoided. Schiff base, as the intermediate, has been regarded as a vital role in another way to form chitosan quaternary ammonium salt. Firstly, N-alkyl chitosan were prepared by introducing an alkyl group into the amine groups of chitosan *via* Schiff's base intermediates. Then N-Alkyl chitosan derivatives were reacted with iodomethane to produce water-soluble cationic polyelectrolytes (Kim *et al*. 1997). But the same problem is the hazardous and high-cost reagent-iodomethane. In addition to the above methods, 2-hydroxypropyl trimethylammonium chloride chitosan (HACC), was prepared by reacting chitosan with glycidyltrimethylammonium chloride (GTAMC) in a neutral condition (Lim *et al*. 2004). As a quaternization reagent, GTAMC is easy to react with amino groups of chitosan. The reaction process is relatively straightforward with the high yield (up to 100%). And at the same time, GTAMC has a range of diverse sources because of industrialized production. Therefore, we chose GTAMC as a modifier to prepare HACC.

Due to the excellent antibacterial activity, chitosan with its derivatives has been mixed with cellulose to prepare antibacterial materials. Spraying 2-hydroxypropyl trimethylammonium chloride chitosan (HACC) onto the softwood pulp to prepare antibacterial paper is one of the methods (Zhang and Guo 2008). Moreover, chitosan/bamboo charcoal composite films have been prepared using a spin coater machine (Nitayaphat *et al*. 2009). Recently, N, O-carboxymethyl chitosan (N, O-CMCS) has been combined with bamboo fiber to prepare blended films (Zheng *et al*. 2014). However, there are few reports on preparing antibacterial bamboo fiber paper using HACC as an antibacterial agent.

In this paper, HACC was synthesized using chitosan and 2,3-epoxypropyl trimethylammonium chloride (GTAMC). Antibacterial paper was prepared using HACC as an antibacterial agent. The structures of chitosan, HACC, and composite paper were characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). Moreover, the antibacterial activity of composite paper against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was examined.

EXPERIMENTAL

Materials

Chitosan, whose molecular weight is 7.5×10^5 and the degree of deacetylation 0.95, was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sichuan Vanov New Material Co., Ltd. (Chengdu, China) supplied the bamboo fiber tissue paper. All other reagents were analytical grade and used without further purification.

Methods

Preparation of HACC

Chitosan (6 g) was added into 60 mL of distilled water, and a certain amount of GTAMC was added every 2 h, for a total of 3 times. The mixture was allowed to sit at the desired temperature for a period of time in a water bath. The resultant clear yellow liquid was poured into 200 mL of cold acetone while stirring, and the mixture was stored at 4 °C overnight. The next day, the acetone was poured out, and the gelatinous substance was dissolved in 100 mL of methanol. The white product was then filtered *via* suction and was precipitated with acetone-ethanol (v/v, 4:1). It was then dried at 70 °C to obtain the final desired HACC product.

Degree of substitution (DS) measurement

The degree of substitution was measured by the silver nitrate titration (Wen *et al*. 2012). The HACC (0.2 g) was accurately weighed and dissolved in 50 mL of distilled water, and K_2CrO_4 solution was added as an indicator. The 0.05 mol/L $AgNO_3$ standard solution was employed to titrate the solution until a brick red precipitate first appeared. Another 50 mL of distilled water was used as the blank test. Each sample was titrated 3 times, and the average was calculated. The DS of HACC was calculated using Eq. 1,

$$DS = \frac{(V_2 - V_1) \times c \times 10^{-3})}{\frac{m}{314}} \tag{1}$$

where V_1 is the volume of $AgNO_3$ (mL) consumed by distilled water, V_2 is the volume of $AgNO_3$ (mL) consumed by samples, c is the molar concentration of the standard $AgNO_3$ solution (mol/L), m is the weight of HACC (g), and 314 is the molecular weight of HACC (g).

Water solubility

The solubility of HACC with different DS was evaluated by the turbidity. HACC (40 mg) was dissolved in 20 mL of 1% (v/v) CH_3COOH solution. Next, 4% (v/v) NaOH solution was slowly added to adjust the pH, which was recorded simultaneously. The original solution at pH 3 was used as a reference. The transmittance of the solution was recorded on a Hitachi U-1000 spectrophotometer (Tokyo, Japan) using a quartz cell with an optical path length of 1 cm at 600 nm.

Preparation of HACC-bamboo fiber composite paper

The optimal group of HACC was employed to prepare an antibacterial paper with bamboo fiber paper. The 3 wt.% HACC in relation to the content of bamboo fiber paper was dissolved in the distilled water and uniformly sprayed on both sides of the original paper. The resultant paper was oven-dried at 105 °C to achieve constant weight.

FTIR spectroscopy

The FTIR spectra of HACC and chitosan were recorded on a Tensor 27 spectrometer (Bruker, Germany) with KBr pellets from 4000 cm^{-1} to 400 cm^{-1}.

Thermal analysis

The thermal behaviors of HACC and chitosan were evaluated with a TGA device (GDT-60, Shimadzu, Kyoto, Japan). The samples weighing around 8 mg were heated from 50 to 500 °C at a heating rate of 10 °C/min under an inert atmosphere of N_2, the flow rate of which was 10 mL/min.

SEM analysis

Scanning electron microscopy was used to investigate the surface morphology of chitosan, HACC, and the composite paper. Pieces were cut from paper, coated with gold, and observed with an electron microscope (S-3000N, Hitachi, Tokyo, Japan) at an acceleration voltage of 10 kV.

Detection of paper properties

The mechanical properties of paper sheets were tested by standard methods: GB/T 461.1(2002) (water absorption) and GB/T 8942(2002) (softness).

Antibacterial activity determination

The GB/T 20944.3 (2007) test method was applied for determining the antibacterial activity. *E. coli* and *S. aureus* were selected as the test bacteria.

RESULTS AND DISCUSSION

Single-factor Experiments of HACC Preparation

HACC was synthesized from chitosan *via* a ring-opening reaction with GTAMC. The reaction schematic of HACC preparation is shown in Fig.1.

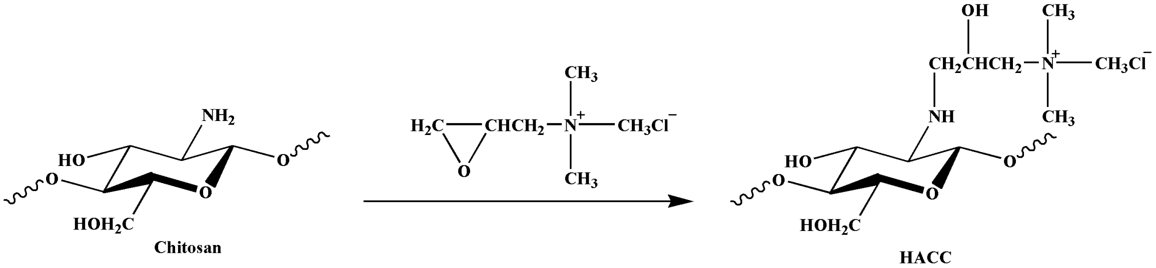

Fig. 1. Reaction schematic of HACC preparation

The mass ratio of chitosan to GTAMC, reaction temperature, and reaction time were selected to evaluate the effect of the DS on the HACC preparation. The results are summarized in Table 1. The DS of HACC ranged from 0.6885 to 0.8675 under different conditions. In this system, -NH_2 was the highest active group, followed by -OH in C-3 and C-6. In the neutral aqueous condition the hydroxyl groups of chitosan are not sufficiently nucleophilic to induce ring opening of GTAMC, whereas the amino group of chitosan is nucleophilic enough to do that (Roberts 1992). Accordingly, the reaction mostly took place at -NH_2 in C-2. The ring-opening reaction occurred on GTAMC, which substituted H at -NH_2 to introduce quaternary ammonium groups onto chitosan.

Table 1. Single-factor Experiments

	Chitosan: GTAMC (Molar Mass Ratio)	Temperature (°C)	Time (h)	Degree of Substitution
1	1:3	85	8	0.8626
2	1:4	85	8	0.8675
3	1:5	85	8	0.8354
4	1:6	85	8	0.8283
5	1:7	85	8	0.7973
6	1:3	75	8	0.7318
7	1:3	80	8	0.8335
8	1:3	90	8	0.7986
9	1:3	95	8	0.7652
10	1:3	85	6	0.7791
11	1:3	85	7	0.8335
12	1:3	85	9	0.7522
13	1:3	85	10	0.6885

Figure 2 shows the effect of the mass ratio of chitosan to GTAMC on the DS of HACC at 85 °C for 8 h. As the amount of GTAMC increased, the DS first rose and then decreased because the amount of NH_2 that was substituted increased along with the increased amount of GTAMC. However, the trend fell due to the increased steric hindrance. Moreover, excessive GTAMC may cause self-polymerization that hindered the reaction. (Wen *et al.* 2012). In conclusion, the reasonable molar mass ratio of chitosan to GTAMC was 1:4.

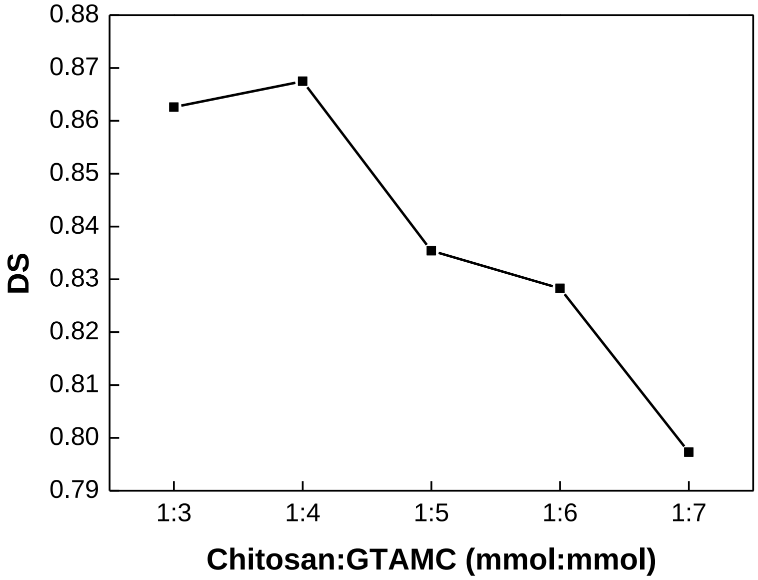

Fig. 2. Effect of mass ratio to HACC on DS

Figure 3 shows the effect of the temperature on the DS of HACC when chitosan-GTAMC (m/m, 1:4) was used for 8 h. The DS of HACC first increased, peaked when the temperature was 85 °C, and then gradually decreased. With an increase in temperature, the amorphous area of chitosan absorbed water and gradually swelled, and the crystalline beam became elastic. In addition, the higher temperature enhanced the flow ability of the reaction system, which also enlarged the effective contact area, so the DS of HACC increased. However, the DS decreased after 85 °C. A partial explanation for this may be that excessive temperatures promoted the ineffective decomposition of chitosan and GTAMC, which was disadvantageous to the reaction.

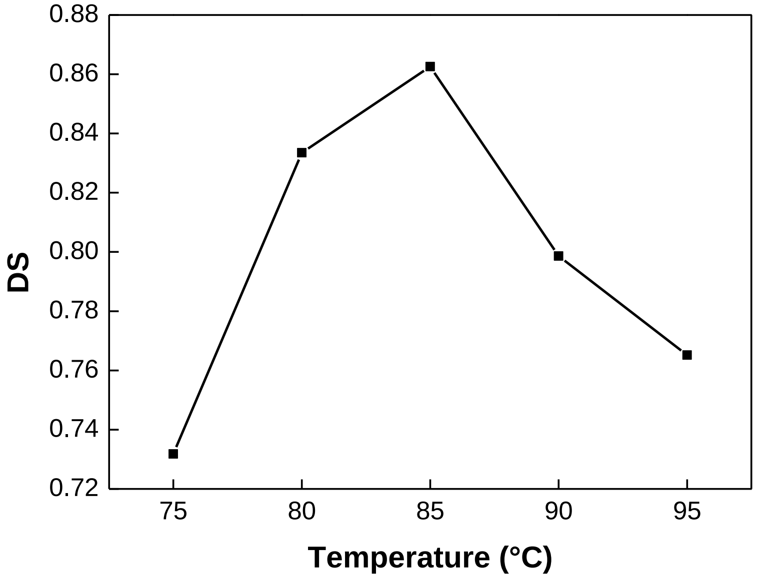

Fig. 3. Effect of temperature to HACC on DS

Figure 4 shows the effect of the time on the DS of HACC when the chitosan-GTAMC (m/m, 1:4) was used at 85 °C. Similarly, the peak of DS appeared at 8 h, reaching 0.863. A longer time can lead to chitosan solution becoming sticky, which obstructed the GTAMC from infiltrating into the chitosan and resulted in less reaction efficiency.

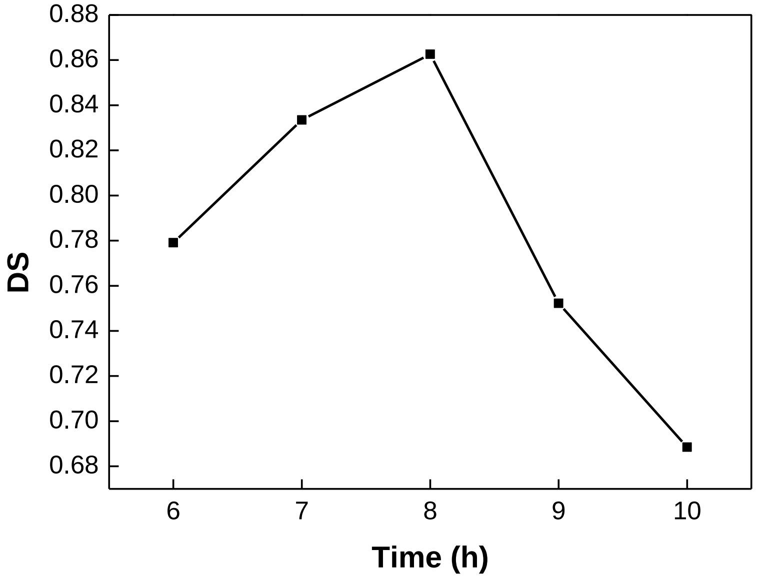

Fig. 4. Effect of time to HACC on DS

The optimal reaction conditions were as follows: molar mass ratio of chitosan to GTAMC, 1:4; reaction temperature, 85 °C; and reaction time, 8 h. The best group of HACC achieved under these conditions was chosen to prepare the antibacterial paper.

Water Solubility

The water solubility results are presented in Fig. 5. Chitosan and all of its products were soluble in water under acidic conditions. When the pH was over 7, the solubility of chitosan declined. In contrast, all HACC products were stable over a wide range of pH values; thus, the solubility and transmittance of HACC, of which the DS results were at the range of 0.6885 to 0.8626, were all numerically high.

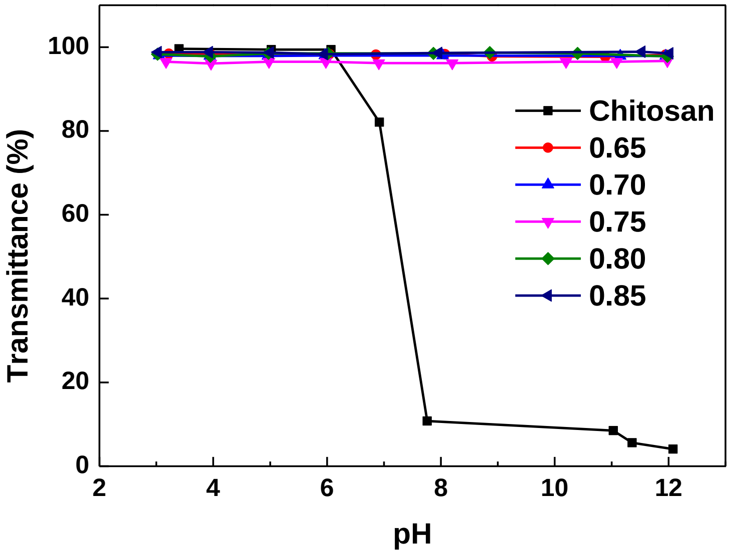

Fig. 5. pH effect of water solubility of chitosan and HACC of different DS: 0.65, 0.70, 0.75, 0.80, and 0.85

Chitosan was insoluble in neutral water and alkaline solutions, but HACC with outstanding solubility in the above-mentioned solutions overcame this pH restriction. This was mainly because the -NH_2 of C-2 was substituted by a quaternary ammonium group of GTAMC that was of high steric hindrance, which weakened hydrogen-bonding actions within or between the molecules. The original crystal structure was broken, leading to much higher water solubility.

TG Analysis of HACC and Chitosan

TGA was used to ascertain the thermal stability of HACC and chitosan. The thermal behavior of HACC (with the highest DS) and chitosan are compared in Fig. 6. The TG curve of the pyrolysis process was divided into three weight loss stages. When the temperature was increased from 50 °C to 240 °C, the mass loss was ascribed to the decomposition of the free water and crystal water. Second, a maximum weight loss of over 45% from 240 °C to 320 °C was observed as a result of decomposition, oxidation, and combustion. The samples continued to decompose at a slower rate, which corresponded to the further ablation of molecular chains and oxidation of residual carbon. The results demonstrated that the thermal stability of HACC was lower than that of chitosan. This result was attributed to the introduction of quaternary ammonium groups, which destroyed the original crystalline structure of chitosan. Consequently, intramolecular and intermolecular hydrogen bonds were weakened.

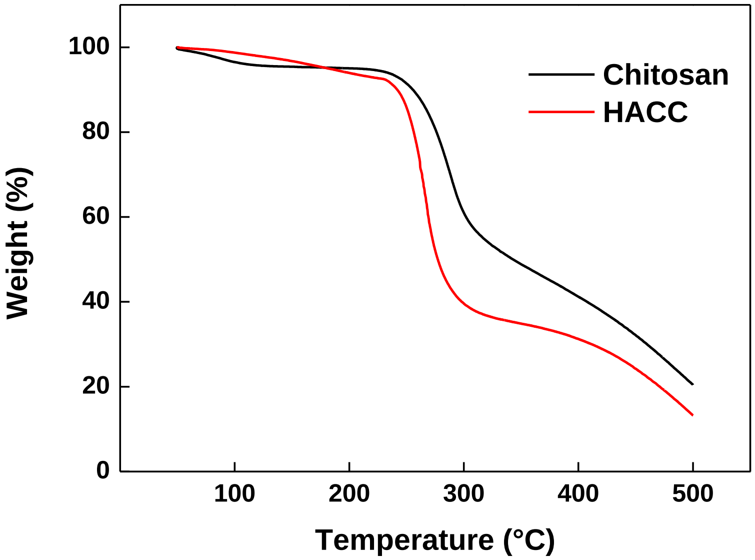

Fig. 6. TG of chitosan and HACC

FTIR of HACC and Chitosan

The structure of HACC (with the highest DS) and chitosan was investigated by FTIR spectroscopy to identify whether chitosan was successfully modified. The spectra are shown in Fig. 7.

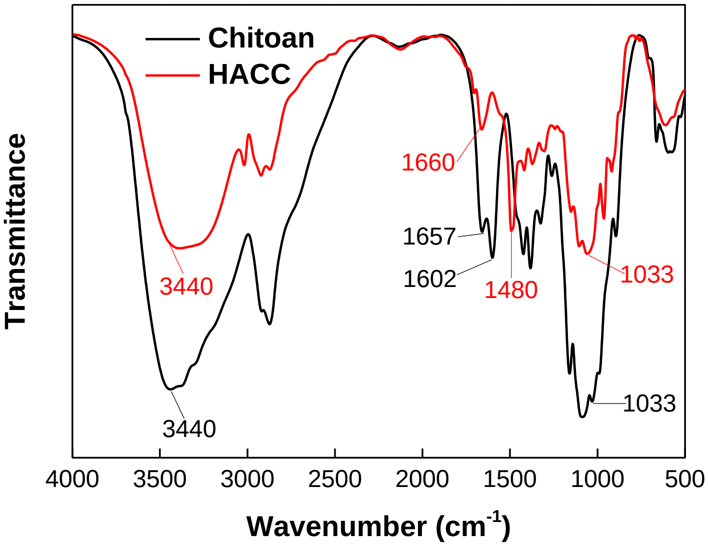

Fig. 7. FTIR spectra of chitosan and HACC

In the spectrum of chitosan, the shape of the absorption band from 1600 cm^{-1} to 1660 cm^{-1} was obviously different with that of HACC. Peak at 1657 cm^{-1} was referenced as amide I bands. However, the peak at 1602 cm^{-1} that was related to the in-plane flexural vibration of amide II disappeared in HACC. Additionally, the new band around 1480 cm^{-1}, which corresponded to the C-H stretching vibration of CH_3 at the quaternary ammonium group, existed in the spectrum of the HACC. Furthermore, compared to the original chitosan, there barely was a difference concerning three characteristic peaks of C-O-C at 1058 cm^{-1}, 1080 cm^{-1}, and 1033 cm^{-1}. These data revealed that side-chains of hydroxypropyl trimethylammonium chloride groups were introduced onto -NH_2. Furthermore, the O-H stretching peak at 3440 cm^{-1} became wider in comparison to chitosan, which showed the higher specific absorption of hydroxyl. This result indicated that the substituted reaction mostly took place at the NH_2 of chitosan.

SEM Analysis of HACC and Composite Paper

The SEM images of chitosan, HACC, and the composite paper are presented in Fig. 8. The surface of the original chitosan was rough, with many small holes. In contrast, the surface of the HACC was smooth and sturdy after modification, and the lamellar arrangement and disappearance of pores was clearer. It should be noted that the modifier, with much more steric hindrance, filled in the space and compacted the structure.

The HACC was randomly dispersed in bamboo fiber paper. As shown in the picture of original bamboo fiber paper, the surface was rough, with high porosity and loose cross-bonding. In the composite paper, some flake material was adsorbed on the surface of the bamboo fiber, indicating that HACC was connected to it.

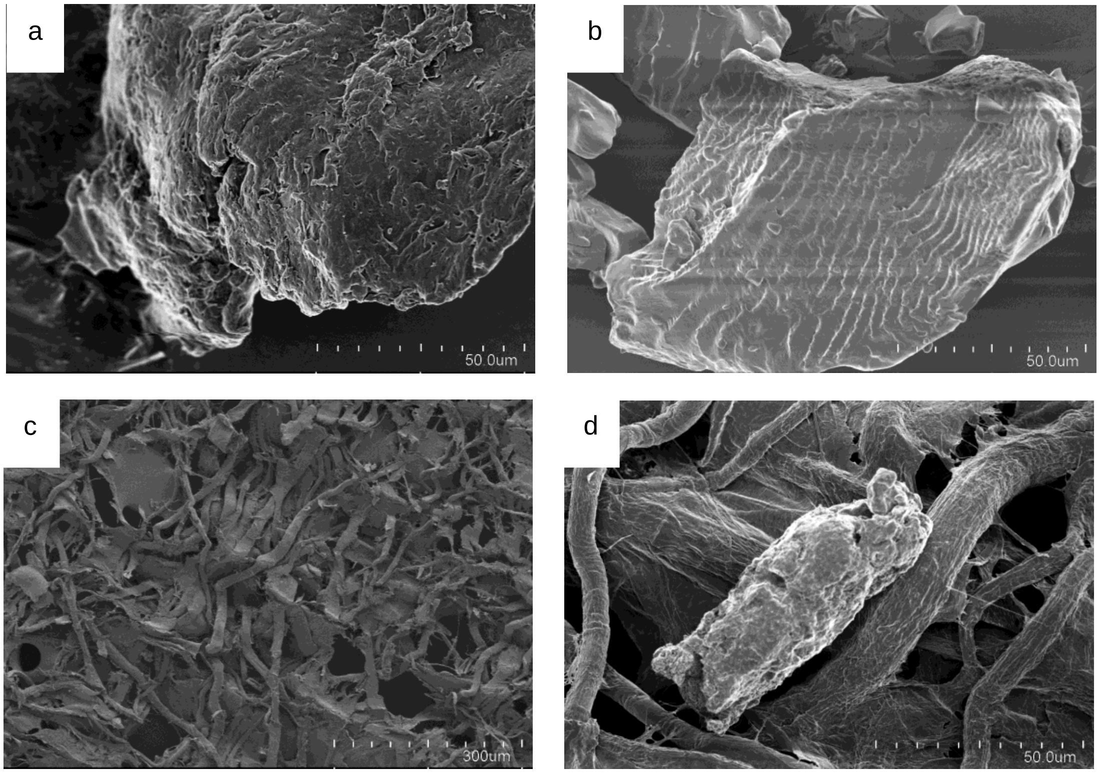

Fig. 8. SEM images of chitosan (a), HACC (b), original bamboo paper (c), and paper with HACC (d)

Water Absorption and Softness of Composite Paper

As a kind of tissue paper, water absorption and softness are two vital parameters. As can been seen from Table 2, after having been sprayed on HACC, composite paper showed little change compared with untreated bamboo paper. And it is also worth mentioning that the water absorption of composite paper was higher. HACC's good performance in hygroscopicity may lead to this result.

Antibacterial Activity Study of Composite Paper

The antibacterial activity of composite paper was tested against *E. coli* and *S. aureus*, as shown in Table 3. The antibacterial activity was greatly reinforced with the addition of HACC. This result reflected that the electrostatic attraction occurred between the quaternary ammonium salt cationic attained after modification and anions on the surface of the cell walls, which led to an unbalanced internal environment. As a result, the growth of microorganisms was suppressed.

Table 2. Water Absorption and Softness of Composite Paper

Sample	The Height of the Liquid Column in the Capillary [mm•(100s^{-1})]	Softness (mN)
Untreated Bamboo Paper	50	84
Paper with HACC	55	81

Table 3. Antibacterial Activity of Composite Paper

Sample	Antibacterial Rate Against *E. coli* (%)	Antibacterial Rate Against *S. aureus* (%)
Original Bamboo Paper	18	54
Paper with HACC	97	99

CONCLUSIONS

1. The treatment of chitosan with GTAMC led to a successful modification at -NH_2. HACC with different DS was obtained, with the highest DS at 0.8675.
2. The GTAMC content, reaction temperature, and time had major effects on the substitution degree of HACC. Consequently, the optimal conditions were the mass ratio of chitosan to GTAMC of 1:4, reaction temperature of 85 °C, and reaction time of 8 h.
3. HACC presented outstanding solubility in neutral water and alkaline solutions.
4. After modification, thermal stability of HACC was lower than that of chitosan.
5. The composite paper with HACC on the surface has better water absorption compared to the original bamboo fiber paper.
6. A functional composite paper with antibacterial activity was successfully prepared by adding HACC onto the original bamboo paper. The growth of *E. coli* and *S. aureus* were inhibited, showing satisfactory antibacterial property.
7. The composite paper has better water absorption compared to the original bamboo fiber paper, and the softness did not change a lot.

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