# Manufacture and Properties of Chitosan/*N,O*-Carboxymethylated Chitosan/Viscose Rayon Antibacterial Fibers

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**ABSTRACT:** Chitosan/*N,O*-carboxymethylated chitosan/viscose rayon antibacterial fibers (CNVFs) were prepared by blending chitosan emulsion, *N,O*-carboxymethylated chitosan (*N,O*-CMC), and viscose rayon together for spinning. The fibers were characterized by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA). TEM micrographs showed that chitosan microparticles dispersed uniformly along the oriented direction with the mean size ranging from 0.1 to 0.5 $\mu$m. DSC spectra of these fibers showed that no significant change in thermal property was caused by adding chitosan and *N,O*-CMC into the viscose rayon. TGA spectra showed that the good moisture retentivity was not affected by the addition of chitosan and *N,O*-CMC. Both DSC and TGA suggested that the decomposing tendency of the viscose rayon above 250°C seemed to be weakened by the chitosan. The fibers' mechanical properties and antibacterial activities against *Escherchia coli, Staphylococcus aureus,* and *Candida albicans* were measured. Although the addition of chitosan slightly reduced the mechanical properties, the antibacterial fibers' properties were obtained and were found to meet commercial requirements. CNVF exhibited excellent antibacterial activity against *E. coli, S. aureus,* and *C. albicans.* The antibacterial activity increased along with the chitosan concentration and was not greatly affected by 15 washings in water. Scanning electron microscopy (SEM) was used to observe the morphology of bacteria cells incubated together with the antibacterial or reference fibers. SEM micrographs demonstrated that greater amounts of bacteria could be adsorbed by the antibacterial fiber than by the reference fiber; these bacteria were overwhelmingly destroyed and killed.  J Appl Polym Sci 84: 2049–2059, 2002; DOI 10.1002/app.10501

**Key words:** chitosan; *N,O*-carboxymethylated chitosan; viscose rayon; antibacterial fiber

## INTRODUCTION

Chitosan, a copolymer of glucosamine and *N*-acetyglucosamine units linked by 1-4 glucosidic bonds, is obtained by N-deacetylation of chitin, which is the second most naturally occurring biopolymer (after cellulose).[1] Because of its special biological, chemical, and physical properties, chitosan has found applications in many industrial and agricultural enterprises.[2–6] Chitosan is also a biocompatible polymer reported to exhibit a great variety of useful biological properties such as anticholesteremic[7] and ion-sequestering actions.[8] Its antifibroblastic activity may aid in wound healing.[9] Investigation into the use of chitosan for the controlled delivery of drugs[10,11] has

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**Table I Antimicrobial Activities of Chitosan**

| Microorganism | MIC[a] (ppm) |
|---|---|
| Bacteria | |
| *Agrobacterium tumefaciens* | 100 |
| *Bacillus cereus* | 1000 |
| *Corinebacterium michiganence* | 10 |
| *Erwinia* ssp. | 500 |
| *Erwinia carotovora* ssp. | 200 |
| *Escherchia coli* | 20 |
| *Klebsiella pneumoniae* | 700 |
| *Micrococcus luteus* | 20 |
| *Pseudomonas fluorescens* | 500 |
| *Staphylococcus aureus* | 20 |
| *Xanthomonas campestris* | 500 |
| Fungi | |
| *Botrytis cinerea* | 10 |
| *Drechstera sorokiana* | 100 |
| *Fusarium oxysporum* | 10 |
| *Micronectriella nivalis* | 10 |
| *Piricularia oryzae* | 5000 |
| *Rhizoctonia solani* | 1000 |
| *Trichophyton equinum* | 2500 |

[a] MIC, minimum growth inhibitory concentration.

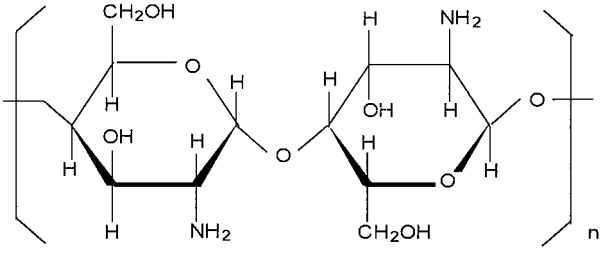
been intensive, and chitosan membranes have been explored as edible packing materials for foods.[12]

Recently, the antibacterial and antifungal activities of chitosan have been followed with great interest. Chitosan inhibits the growth of a wide variety of bacteria and fungi[13–20] (see Table I), showing broad spectra of antibacterial activity, high killing rate, and low toxicity toward mammalian cells.[21,22]

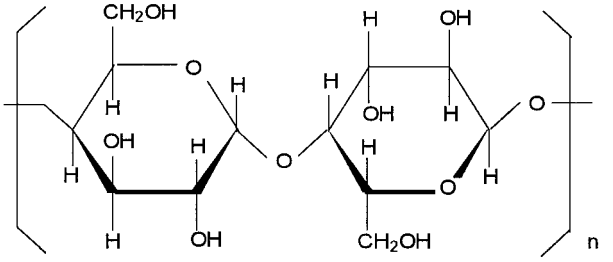
There are two proposed modes of action for microbial growth inhibition. In one mechanism, the polycationic nature of chitosan interferes with the negatively charged residues of macromolecules at the cell membrane surface.[23] Chitosan interacts with the membrane to alter cell permeability,[24] even to cause the leakage of the intracellular components.[25] The other mechanism involves the binding of chitosan with DNA to inhibit RNA synthesis.[26]

Because of the antibacterial activity and little skin reaction over a wide range of biomedical investigation,[17] chitosan can be used in antibacterial next-to-skin fabrics. Cotton fiber and viscose rayon are the undergarment materials of choice because of their demonstrated safety to the body and the comfort that results from their high moisture retentivity. Cellulose, the main ingredient of cotton and viscose rayon, has a molecular structure similar to that of chitosan. The difference between the two polysaccharides is that chitosan has the amino group instead of the hydroxyl group at the $C_2$ position of every pyranose ring (Fig. 1). This similarity is expected to give high compatibility between these two polymers. Moreover, the formation of intermolecular hydrogen bonding between them also contributes to the compatibility. Blending cellulose and chitosan is expected to be a useful method to introduce antibacterial activity into cellulosic materials for its functionalization.

In our previous study,[27,28] a blend film of chitosan, *N,O*-carboxymethylated chitosan (*N,O*-CMC), and viscose rayon was investigated. Results suggested partial compatibility of chitosan with viscose, which could be further improved by the addition of suitable *N,O*-CMC. In this study, a novel chitosan/*N,O*-CMC/viscose rayon antibacterial fiber (CNVF) was prepared and characterized by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA). The mechanical properties and antibacterial activities against *Escherchia coli, Staphylococcus aureus,* and *Candida albicans* were also investigated. The mor-




(a) Chitosan




(b) Cellulose

**Figure 1** Structures of chitosan and cellulose.

phology of bacteria cells growing in the presence of CNVF and viscose rayon was observed by scanning electron microscope (SEM).

## EXPERIMENTAL

### Materials

Original chitosan (MW $1.08 \times 10^6$; degree of deacetylation 0.85), provided by Qingdao Medicine Institute, China, was depolymerized by γ-irradiation degradation to a lower molecular weight of $2.0 \times 10^5$. To synthesize *N,O*-CMC, chitosan (20 g) was suspended in 200 mL isopropanol, 50.4 mL sodium hydroxide solution (10 mol/L), and 24 g monochloroacetic acid. The system reacted at 65°C for 1 h and the pH was adjusted to 7.0 with glacial acetic acid. After filtration, the solid product was washed with methanol. The resultant *N,O*-CMC was oven-dried at 60°C.[29] The degree of carboxymethylation determined by pH titration was 0.86. *E. coli* (8099), *S. aureus* (ATCC 6538), and *C. albicans* (ATCC 10231) were provided by the School of Biology Science, NanKai University, Tianjin, China, and stored at 4°C.

### Spinning Method

Different amounts of chitosan emulsion and *N,O*-CMC were added into viscose to form a series of precursor mixtures with different chitosan concentrations. After fully stirring, a vacuum treatment was performed for 2 h to eliminate air bubbles. Then the mixed viscose was spun through a viscose-type spinneret (30 holes with 0.08 mm diameter). The spinning was performed on viscose fiber production facilities (Nanjin Jinling Chemical Fiber Co., Jiangsu Province, China) with a stretching ratio of 1.25.

### Measurement

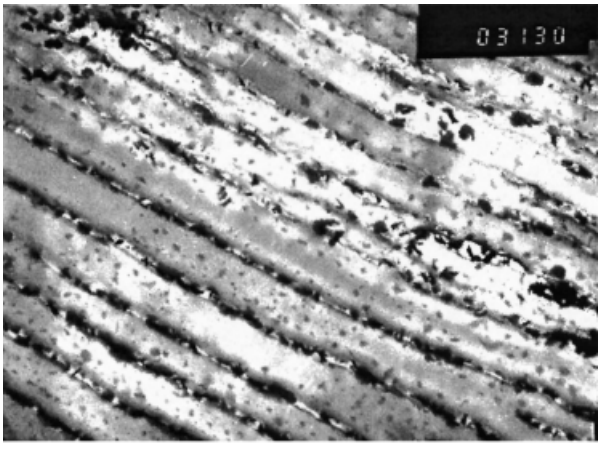
A differential scanning calorimeter (Model DSC7; Perkin Elmer Cetus Instruments, Norwalk, CT) was used for measurement. Each sample (5–10 mg) was run under nitrogen atmosphere at a scanning rate of 5°C/min. The temperature for the first scan ranged from 20 to 170°C. Then the samples were quenched and scanned the second time, with the temperature ranging from 20 to 300°C.

TEM observation was performed to investigate the morphology of the cross-sectional area in CNVF with a JEM-100CX instrument. Samples were prepared by being dissected in liquid nitrogen and stained with $OsO_4$.

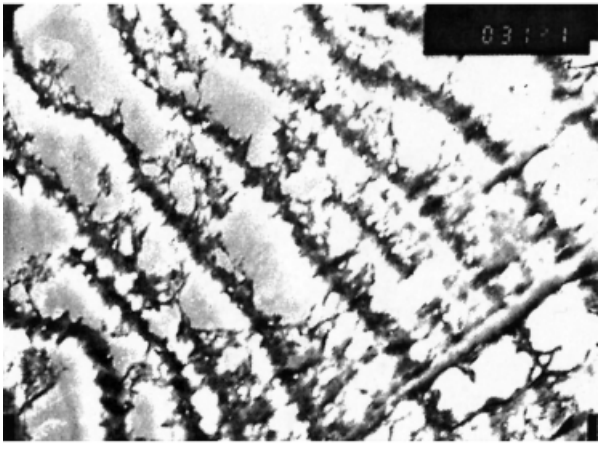
TGA was performed with a Netzsch TG 209 apparatus. Each sample (about 7 mg) was run under nitrogen atmosphere at a scanning rate of 5°C/min from 20 to 350°C.

### Antibacterial Assessment

Antibacterial activities of the series of CNVFs against *E. coli* and *S. aureus* were qualitatively evaluated by using the optical density method described as follows. A representative bacteria colony was picked off, placed in a nutrient broth




(a)




(b)

**Figure 2** TEM micrographs of CNVF: (a) ×8000; (b) ×15,000.

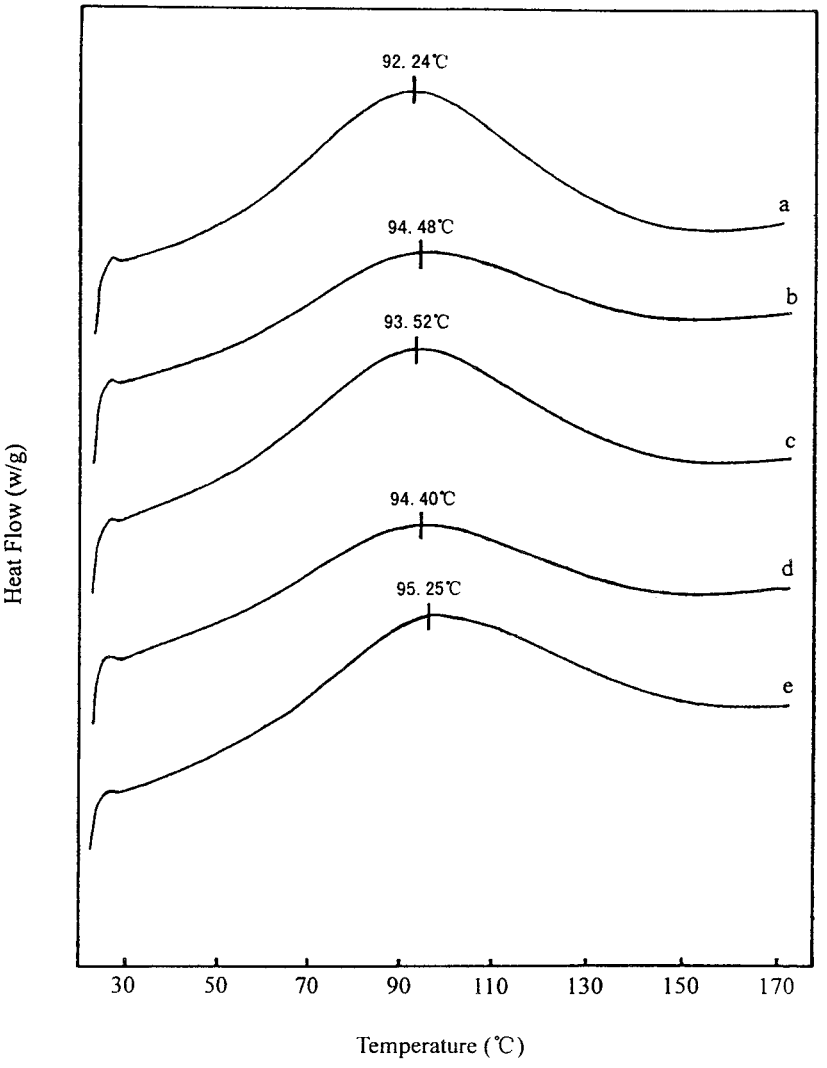



**Figure 3** DSC spectra of viscose rayon (a) and CNVFs 1–4 (b–d) obtained in the first scan.

(peptone, 10 g; beef extract, 3 g; NaCl, 3 g in 1000 mL distilled water, pH 7.0) and incubated at 37°C for 24 h. Then the obtained fresh culture in which bacteria cells grew prolifically was ready for the antibacterial test. A 0.2-mL aliquot of the fresh culture was inoculated to the medium (9.8 mL) containing viscose rayon (reference) or antibacterial fiber (0.1 g) and incubated in a shaking bed (150 rpm) at 37°C for 24 h. During incubation, the turbidity of the medium was measured at 610 nm (by 756MC UV–Vis spectrophotometer; Shanghai, China) five separate times. The antibacterial activity of CNVF 2 washed for 0, 5, 10, and 15 times was also similarly determined. [The washing of fibers was performed according to the method provided by the Chinese Textile Industry Standard FJ-54P-85. CNVF 2 (300 g) was immersed in 9 kg water containing 18 g detergent and washed in a washing machine for 25 min, then rinsed for 2 min and spin-dried, then rinsed and spin-dried once more, and dried at 80°C for 30 min. This whole process was defined as a single washing for the fiber.] After 24-h incubation, the morphology of bacteria cells growing in the presence of CNVF 2 and viscose rayon was observed by scanning electron microscope (Hitachi-X510, Japan).

The shake-flask method was used to quantitatively evaluate the antibacterial activity of CNVF 2 against *E. coli, S. aureus,* and *C. albicans* in terms of bacterial reduction rate. A 0.5-mL aliquot of noted fresh culture was added to 70 mL of

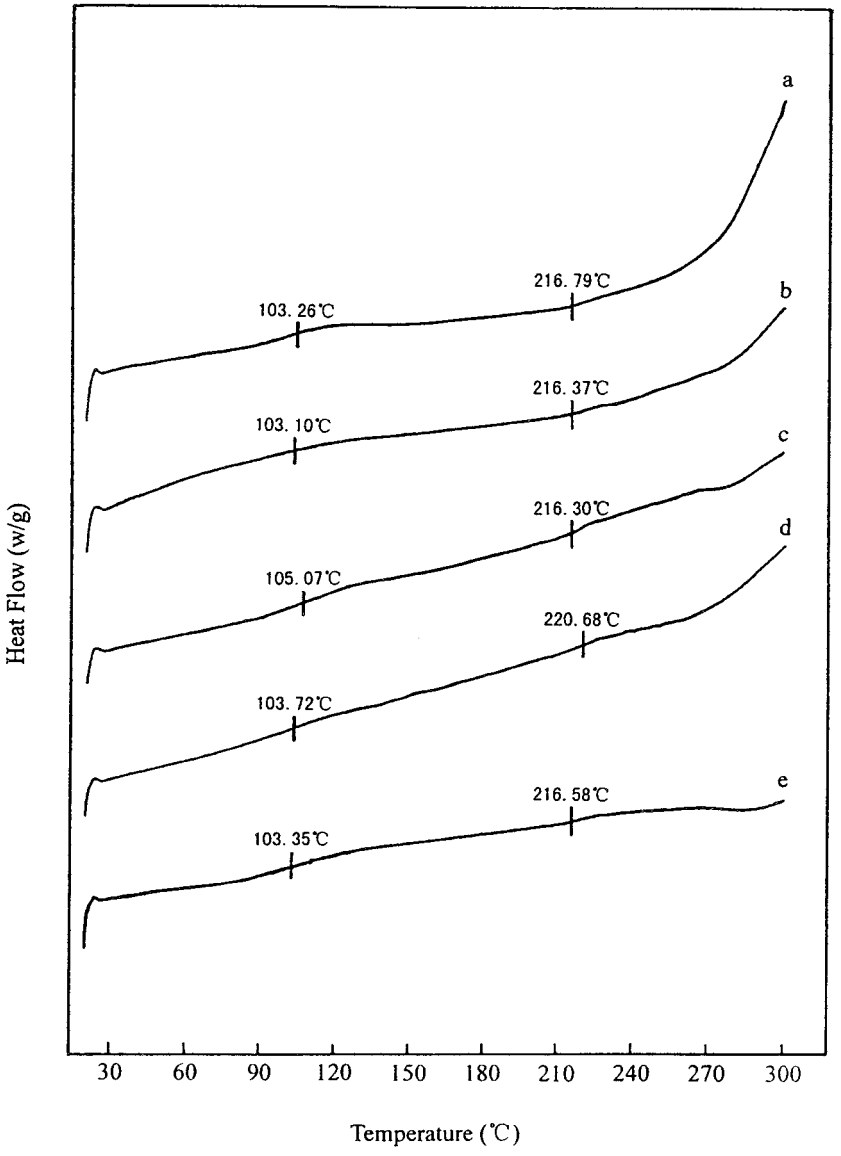



**Figure 4** DSC spectra of viscose rayon (a) and CNVFs 1–4 (b–d) obtained in the second scan.

phosphate-buffered saline (PBS, 0.03 mol/L, pH 7.2–7.4) containing 0.75 g antibacterial or control fiber. After the cultivation was shaken (300 rpm) at 37°C for 1 h, 0.5 mL of the obtained mixture was picked off, diluted with PBS, and spread on the nutrient agar (agar, 15 g; peptone, 10 g; beef extract, 3 g; NaCl, 3 g in 1000 mL distilled water, pH 7.0) plate to give the single colonies. After being incubated at 37°C for 24 h, the number of survivors was counted. The number of bacteria in 0.5 mL of fresh culture was also determined by means of this plate-counting method.

## RESULTS AND DISCUSSION

### TEM Analysis

In our previous study,[27,28] the morphology of regenerated films consisting of chitosan, *N,O*-CMC, and viscose rayon was investigated. A higher degree of microspheric phase separation was observed in the TEM micrograph of the binary film composed of chitosan and viscose rayon than that of the ternary film composed of chitosan, viscose rayon, and a small amount of *N,O*-CMC (*N,O*-CMC/chitosan = 1/10, w/w). Also, the diameters

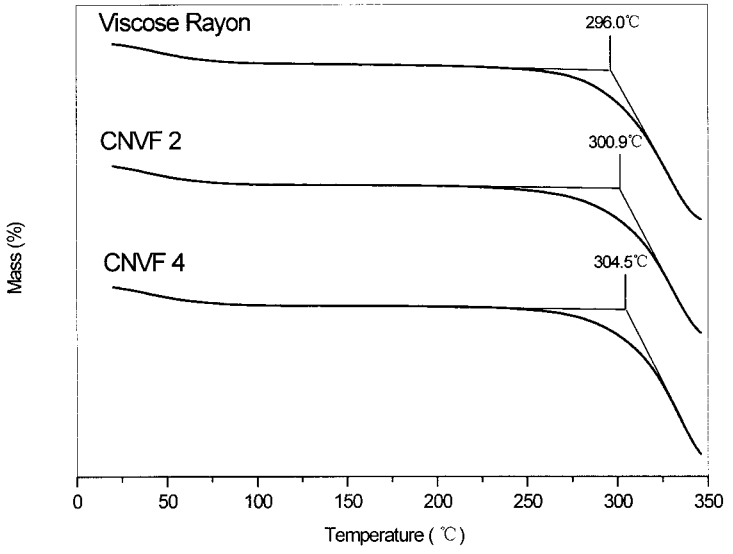



**Figure 5** TGA spectra of viscose rayon and CNVFs.

of chitosan microparticles in the binary film are much larger than those in the ternary film. In the light of the results of DSC, DMA, and WAXS, a conclusion was thus reached that the compatibility between chitosan and viscose rayon could be improved by a small amount of *N,O*-CMC.

In this investigation the mixture of chitosan emulsion, *N,O*-CMC, and viscose rayon was used for spinning. When the chitosan emulsion and *N,O*-CMC were added, the viscosity of viscose was slightly reduced. The mixed viscose was easy to spin on the production facilities and hybrid fibers were obtained. TEM micrographs of CNVF are shown in Figure 2. In these micrographs the dark areas ($OsO_4$ stained) are the chitosan phase and the bright ones are the viscose rayon phase. There was no obvious phase separation found, thus confirming the good compatibility between cellulose and chitosan with the addition of *N,O*-CMC. Chitosan microparticles dispersed uniformly along the oriented direction in the fiber, with the mean size ranging from 0.1 to 0.5 $\mu$m.

## DSC Analysis

The thermal transitions of viscose rayon and CNVFs were determined by DSC analysis. Fiber ingredients are listed in Table III (see below). For the first scan, the temperature was raised from 20 to 170°C (Fig. 3), ensuring that the samples did not decompose. Because of a certain amount of free water contained in the samples, all of the spectra gave a significant transition at about 100°C. From the identity of this transition peak we can conclude that the good moisture retentivity of viscose rayon was not affected by the addition of chitosan and *N,O*-CMC.

After the first scan, the free water was eliminated and the thermal transitions of each fiber could be seen clearly in the spectra of the second scan (Fig. 4). The five spectra in Figure 4 are almost identical, indicating that no significant change in thermal property was caused by adding chitosan and *N,O*-CMC into the viscose rayon. For viscose rayon, the reference sample, two transitions are observed at 103.26 and 216.79°C [Fig. 4(a)]. Figure 4(b)–(e), which represent the CNVFs with different chitosan concentrations, also show similar transitions at about both temperatures. This similarity illustrates the good compatibility between chitosan and viscose rayon in the presence of *N,O*-CMC. When the temperature reached 250°C, the viscose rayon decomposed significantly as the temperature increased. It is worth noting, however, that this tendency was greatly reduced in the antibacterial fibers. This difference could also be seen in TGA spectra.

## TGA Analysis

The moisture retentivity and decomposition temperatures of viscose rayon and antibacterial fibers were determined by TGA (c.f. Fig. 5). The mass changes at some temperatures are listed in Table II.

**Table II Mass Change at Some Temperatures Determined in TGA**

| Temperature (°C) | 40 | 60 | 80 | 100 | 120 | 150 |
|---|---|---|---|---|---|---|
| Mass change (%) | | | | | | |
| Viscose rayon | −4.182 | −6.889 | −8.310 | −9.041 | −9.226 | −9.361 |
| CNVF 2 | −4.257 | −7.001 | −8.638 | −9.084 | −9.275 | −9.384 |
| CNVF 4 | −4.196 | −6.931 | −8.353 | −8.988 | −9.203 | −9.315 |

The data of all three samples gave the same result that the mass deduced along the temperature before 100°C and kept nearly constant between 100 and 150°C.

The mass loss before 100°C is attributed to the free water contained in the fibers. As we mentioned, viscose rayon has a good moisture retentivity attributed to the large amount of –OH on the cellulose molecular chain. Given that both chitosan and *N,O*-CMC have good hydrophilicity, their addition will not weaken the good moisture retentivity of the fibers. This could be confirmed by TGA spectra. From Table II we can see that the mass change of CNVF before 100°C showed no significant difference with viscose rayon.

The decomposition temperature of the sample was defined as the point of the intersection of two tangents, as shown in Figure 5, which was automatically calculated by the instrument. The decomposition temperatures of viscose rayon, CNVF 2, and CNVF 4 are 296.0, 300.9, and 304.5°C, respectively. The decomposition temperature increased along the chitosan concentration in the fiber. This result agreed with the DSC analysis, which suggested that the decomposition tendency was weakened by the addition of chitosan and *N,O*-CMC. Investigation of the mechanism of this phenomenon is now under way.

## Mechanical Properties of the Antibacterial Fibers

Some mechanical properties of the fibers are shown in Table III. Data demonstrate that tenacity, break extension, and wet tenacity of the CNVFs are a little lower than those of viscose rayon. This is attributed to the addition of chitosan and *N,O*-CMC, which slightly hamper the crystallization of cellulose molecules during the spinning process. Despite the slight decrease in mechanical properties of antibacterial fibers, they are still better than those established by the Chinese National Standard (GB/T 13758-92), as shown in Table III, indicating that antibacterial fibers can meet commercial requirements.

## Antibacterial Properties

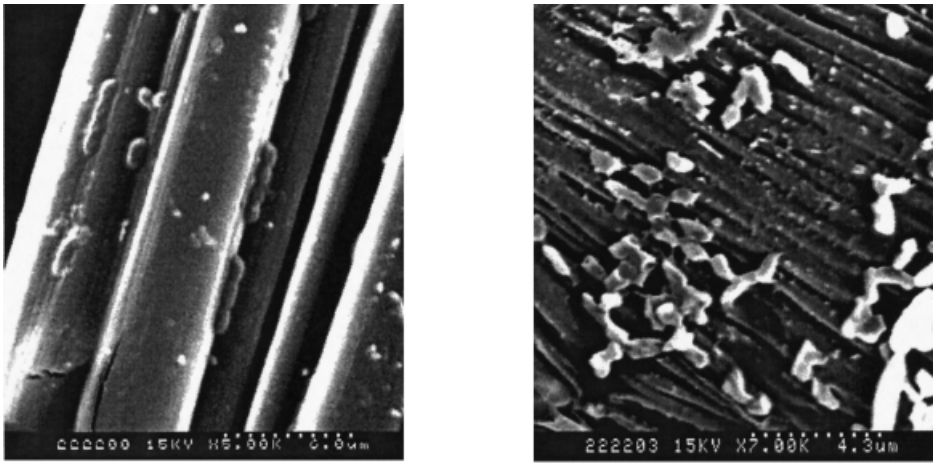
### *SEM Observation*

Figure 6 and Figure 7 show the *E. coli* or *S. aureus* cells, respectively, adsorbed on the viscose rayon or CNVF after a 24-h shaken incubation. In the SEM micrographs displayed in Figure 6(a) and Figure 7(a), only a few of the bacteria cells were adsorbed on the surface of the viscose rayon, in which the chitosan concentration is 0. These bacteria cells grew normally without any deformation. Figure 6(b) and Figure 7(b) show that a considerable amount of bacteria was adsorbed on the antibacterial fibers, aggregated, and displayed significant destruction and deformation of the cells. CNVF exhibits distinct antibacterial activity.

Because the chitosan in CNVF cannot be dissolved by the medium, this result can be explained by the first antibacterial mechanism

**Table III Some Physical Properties of Chitosan/Viscose Rayon**

| Filament | Chitosan (%) | *N,O*-CM (%) | Viscose Rayon (%) | Tenacity (CN/dtex) | Break Extension (%) | Wet Tenacity (CN/dtex) |
|---|---|---|---|---|---|---|
| GB/T13758-92 | 0 | 0 | 100 | ≥1.52 | 17.0–22.0 | ≥0.69 |
| Viscose rayon | 0 | 0 | 100 | 1.65 | 19.0 | 0.90 |
| CNVF 1 | 0.4 | 0.1 | 99.5 | 1.64 | 21.6 | 0.88 |
| CNVF 2 | 0.8 | 0.2 | 99 | 1.59 | 22.9 | 0.84 |
| CNVF 3 | 1.2 | 0.3 | 98.5 | 1.52 | 18.0 | 0.80 |
| CNVF 4 | 1.6 | 0.4 | 98 | 1.53 | 19.5 | 0.80 |



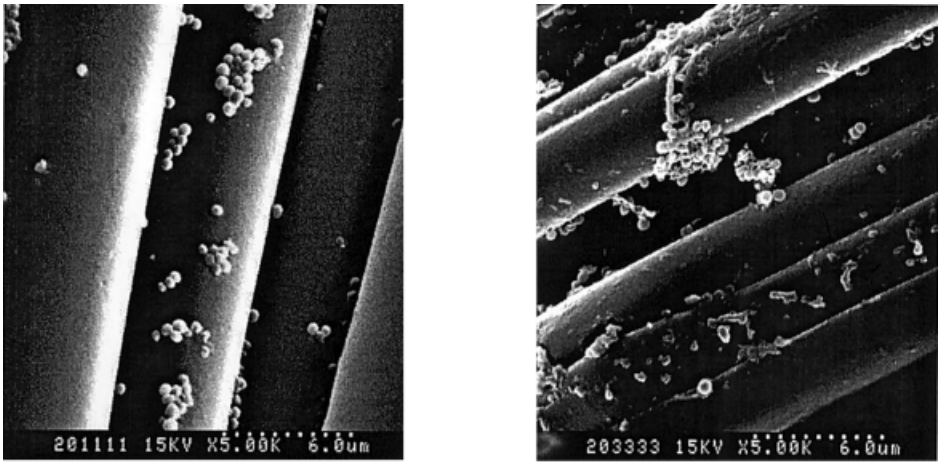
(a) 5000× (b) 7000×

**Figure 6** SEM micrographs of *E. coli* on viscose rayon (a) and CNVF (b).

mentioned above. The amino groups on the chitosan molecular chain can be easily protonated to form $-NH_3^+$, which gave antibacterial fibers their polycationic nature. $-NH_3^+$ interferes with the negatively charged residues of macromolecules at the cell membrane surface, destroys the cell membrane, and causes considerable leakage of the intracellular components. This result is in agreement with that of the study by Leuba and Stossel[25] on chitosan's antibacterial activity, in which the leakage of proteinaceous materials from bacteria caused by chitosan was observed by means of UV absorption.

### *Qualitative Antibacterial Assessment of CNVF Against* E. coli *and* S. aureus

Figure 8 and Figure 9 give curves of optical density (OD) versus culture time for the viscose rayon and CNVFs against *E. coli* and *S. aureus,* respectively. Because the bacteria cell is opaque, the medium became turbid as the bacteria propagated. Therefore, the optical density can be used as a criterion by which to measure the antibacterial activity of the fibers. The smaller the OD of the medium, the higher the antibacterial activity of the fiber.




(a) 5000× (b) 5000×

**Figure 7** SEM micrographs of *S. aureus* on viscose rayon (a) and CNVF (b).

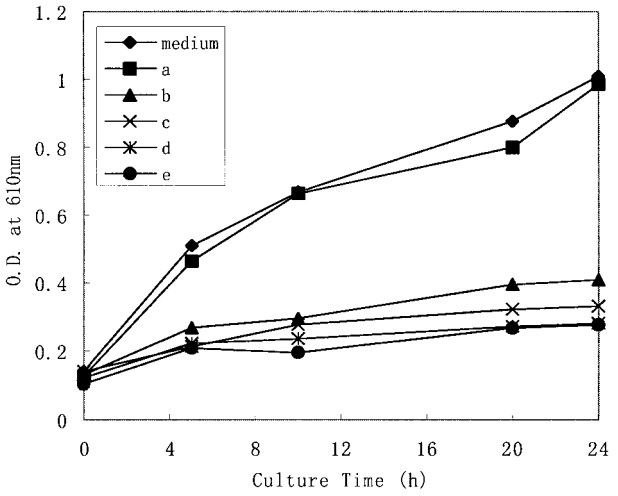



**Figure 8** Antibacterial activity of viscose rayon (a) and CNVFs 1–4 (b–e) against E. coli.

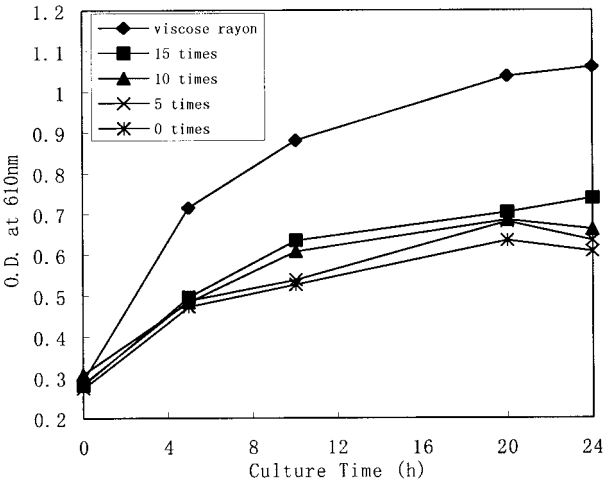



**Figure 10** Effect of washing on the antibacterial action against *E. coli* of CNVF 2.

According to Figures 8 and 9, compared to the OD of pure medium and viscose rayon, the OD values of CNVFs are much lower. Moreover, with the increase of concentration of chitosan in the fiber, OD values decrease accordingly. In other words, the antibacterial activity of CNVF may increase if the chitosan concentration is raised.

One of the most important properties in determining the usefulness of the antibacterial fiber or textile is the washing endurance. It is satisfying that the OD values of CNVF increased only slightly and were still much lower than that of the viscose rayon, even though fibers had been washed 15 times (Figs. 10 and 11). The washing-enduring antibacterial activity is attributed to the poor solubility of chitosan in water and good compatibility between chitosan and cellulose in the presence of *N,O*-CMC. The lasting antibacterial activity gives CNVF the potential of being successfully applied to industrial uses.

### *Quantitative Antibacterial Assessment of CNVF Against* E. coli, S. aureus, *and* C. albicans

Table IV gives the average number of colonies in the experimental medium containing antibacterial or reference fibers before and after 1-h shaken incubation. The bacteria reduction rate (BRR) of each fiber was calculated as follows:

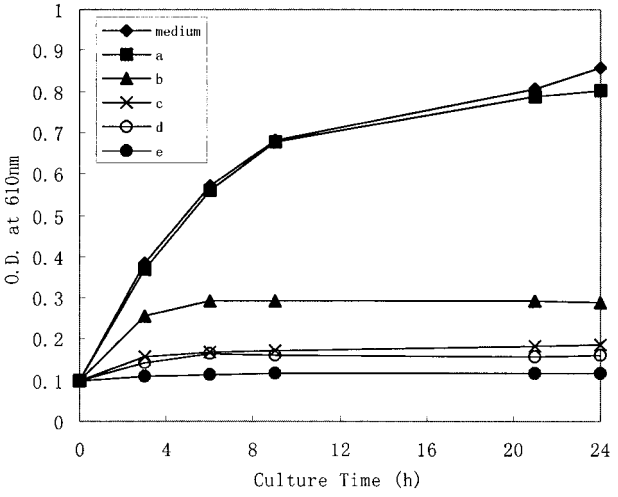



**Figure 9** Antibacterial activity of viscose rayon (a) and CNVFs 1–4 (b–e) against S. aureus.

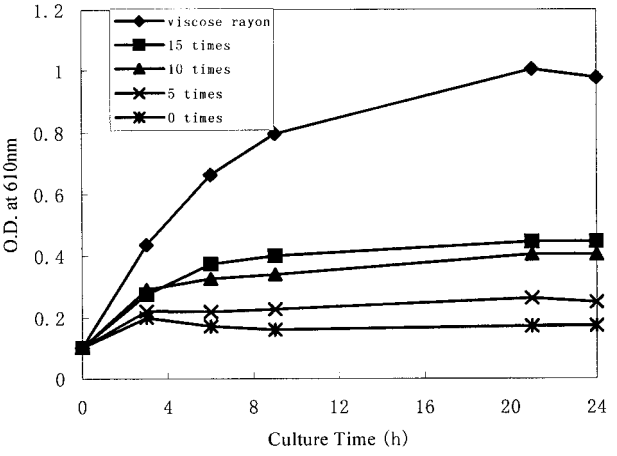



**Figure 11** Effect of washing on the antibacterial action against *S. aureus* of CNVF 2.

**Table IV Result of the Quantitative Antibacterial Assessment**

| Fiber Bacteria | $N_1$ | $N_2$ | Bacteria Reduction Rate (BRR) (%) | BRR Difference with Control (%) |
|---|---|---|---|---|
| Viscose rayon | | | | |
| *E. coli* | $1.4 \times 10^4$ | $1.361 \times 10^4$ | 2.82 | — |
| *S. aureus* | $1.4 \times 10^4$ | $1.364 \times 10^4$ | 2.56 | — |
| *C. albicans* | $1.5 \times 10^4$ | $1.454 \times 10^4$ | 3.08 | — |
| CNVF2 | | | | |
| *E. coli* | $1.7 \times 10^4$ | $0.801 \times 10^4$ | 52.90 | 50.08 |
| *S. aureus* | $1.7 \times 10^4$ | $0.889 \times 10^4$ | 47.69 | 45.13 |
| *C. albicans* | $1.6 \times 10^4$ | $0.966 \times 10^4$ | 39.64 | 36.56 |

$$\mathrm{BRR} = \frac{N_1 - N_2}{N_1} \times 100\%$$

where $N_1$ is the average number of colonies before shaken incubation and $N_2$ is the average number of colonies after shaken incubation.

The difference of BRR between CNVF 2 and viscose rayon, listed in Table IV, shows that chitosan/*N,O*-CMC/viscose rayon hybrid fiber exhibits excellent antibacterial activity. All of the three bacteria could hardly propagate in the presence of the antibacterial fiber. Because *E. coli, S. aureus,* and *C. albicans* are common bacteria that exist on the human skin and always lead to serious illness, CNVF holds promise for favorable use in the manufacture of antibacterial undergarments.

## CONCLUSIONS

Chitosan/*N,O*-carboxymethylated chitosan/viscose rayon antibacterial fibers were prepared by blending chitosan emulsion, *N,O*-CMC, and viscose rayon together for spinning. TEM micrographs showed that chitosan microparticles dispersed uniformly along the oriented direction in the fibers, with the mean size ranging from 0.1 to 0.5 $\mu$m. The DSC spectra of these fibers showed that no significant change in thermal property was caused by adding chitosan and *N,O*-CMC into the viscose rayon. TGA spectra showed that the good moisture retentivity was not affected by the addition of chitosan and *N,O*-CMC. Both DSC and TGA suggested that the decomposing tendency of the viscose rayon above 250°C seemed to be weakened by the additives. Despite the slight decrease in mechanical properties of antibacterial fibers, they still exceed those established by the Chinese National Standard. Antibacterial fibers exhibit excellent antibacterial activity against *Escherchia coli, Staphylococcus aureus,* and *Candida albicans.* SEM micrographs demonstrated that greater amounts of bacteria were adsorbed by the antibacterial fiber than by the reference fiber; these bacteria cells were overwhelmingly destroyed and killed. The antibacterial activity increased along with the increase of chitosan concentration and was not greatly affected by the 15 washings.

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## REFERENCES

1. Bartnicki-Garcia, S. Ann Microbiol 1968, 22, 87.
2. Kurta, K.; Koyama, Y.; Taniguchi, A. J Appl Polym Sci 1986, 31, 1169.
3. Koyama, Y.; Taniguchi, A. J Appl Polym Sci 1986, 31, 1951.
4. Kurta, K.; Koyama, Y.; Chikoka, S. Polym J 1986, 16, 1083.
5. Jeuniaux, C. in Chitin in Nature and Technology; Muzzarelli, R. A. A.; Jeuniaux, C.; Gooday, C., Eds.; Plenum Press: New York, 1985; p 546.
6. Hadwinger, L. A.; Fristensky, B.; Riggleman, R. C. in Chitin, Chitosan and Related Enzymes; Zikakis, J. P., Ed.; Academic: New York, 1984; p 291.
7. Muzzarelli, R. A. A. Carbohydr Polym 1996, 29, 309.
8. Peter, M. G. J Macromol Sci Pure Appl Chem 1995, A32, 629.
9. Hirz, G. A.; Gardener, P. J.; Christensen, G. R. Ann Ophthalmol 1996, 28, 158.

10. Orienti, I.; Aiedeh, K.; Gianasi, E. J. Microencapsulation 1996, 13, 463.
11. Thacharodi, D.; Rao, K. P. Biomaterials 1996, 17, 1307.
12. Balwin, E. A.; Nisperos-Carriedo, M. O.; Baker, R. A. HortScience 1995, 30, 35.
13. Chun, H. K.; Jang, W. C.; Heung, J. C.; Kyu, S. C. Polym Bull 1997, 38, 387.
14. Fang, S. W.; Li, C. F.; Shin, D. Y. C. J Food Prot 1994, 57, 136.
15. Uchida, Y. Gekkan Fudo Kemikaru 1988, 4, 22.
16. Hiroshi, S. Sensshoku Kogyo 1993, 41, 177.
17. Seo, H.; Mitsuhashi, K.; Tanibe, H. in Proceedings of the 5th International Conference on Chitin and Chitosan, Princeton, NJ, 1991; p 34.
18. Uchida, Y.; Izume, M.; Ohtakara, A. in Proceedings of the 4th International Conference on Chitin and Chitosan, Trondheim, Norway, 1988; p 373.
19. Yalpani, M.; Jhonson, F.; Robinson, L. E. in Proceedings of the 5th International Conference on Chitin and Chitosan, Princeton, NJ, 1991; p 543.
20. Liu, X. F.; Guan, Y. L.; Yao, K. D. J Appl Polym Sci 2001, 79, 1324.
21. Franklin, T. J.; Snow, G. A. Biochemistry of Antimicrobial Action; Chapman & Hall: London, 1981.
22. Takemono, K.; Sunamoto, J.; Akasi, M. Polymers and Medical Care; Mita: Tokyo, 1989; Chapter IV.
23. Young, D. H.; Kauss, H. Plant Physiol 1983, 73, 698.
24. Young, D. H.; Kohle, H.; Kauss, H. Plant Physiol 1982, 70, 1499.
25. Leuba, S.; Stossel, P. in Chitin in Nature and Technology; Muzzarelli, R. A. A.; Jeuniaux, C.; Gooday, C., Eds.; Plenum Press: New York, 1985; p 217.
26. Hadwiger, L. A.; Kendra, D. F.; Fristensky, B. W. in Chitin in Nature and Technology; Muzzarelli, R. A. A.; Jeuniaux, C.; Gooday, C., Eds.; Plenum Press: New York, 1985; p 210.
27. Guan, Y. L.; Liu, X. F.; Zhang, Y. P. J Appl Polym Sci 1998, 67, 1965.
28. Guan, Y. L.; Liu, X. F.; Fu, Q.; Li, Z. Carbohydr Polym 1998, 36, 61.
29. Hayes, E. R. U.S. Pat. 4,619, 995, 1986.