Molecular Weight Effect on Antimicrobial Activity of Chitosan Treated Cotton Fabrics

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Received 30 May 2000; accepted 22 August 2000

ABSTRACT: The effect of the molecular weight of chitosan on antimicrobial activity was investigated using three chitosans of different molecular weights [1800 (water soluble), 100,000, and 210,000] and similar degrees of deacetylation (86–89%). Cotton fabrics were treated with chitosan by the pad–dry–cure method. The molecular weight dependence of the antimicrobial activity of chitosan was more pronounced at a low treatment concentration. Chitosans with molecular weight of 100,000 and 210,000 effectively inhibited *Staphylococcus aureus* at a 0.5% treatment concentration. Chitosan with a molecular weight of 1800 was effective against *S. aureus* at a 1.0% treatment concentration. *Escherichia coli* was effectively inhibited by chitosan with a molecular weight of 210,000 at a 0.3% treatment concentration and by chitosans with a molecular weight of 1800 and 100,000 at a 1.0% treatment concentration. *Proteus vulgaris* was effectively inhibited by chitosans with molecular weight of 100,000 and 210,000 at a 0.3% treatment concentration and by chitosan with a molecular weight of 1800 at a 0.5% treatment concentration. None of the chitosans significantly inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* below a 1.0% treatment concentration. Chitosans with high molecular weights were more effective in inhibiting bacterial growth than chitosans with low molecular weights. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2495–2501, 2001

Key words: chitosan; cotton fabric; degree of deacetylation; bacterial reduction rate; molecular weight dependence

INTRODUCTION

Antimicrobial treatment is rapidly becoming a standard finish for some categories of textile products such as medical, institutional, and hygienic uses.[1] Recently, it became popular in sportswear, women's wear, aesthetic clothing, to impart anti-odor or biostatic properties. Antimicrobial finishes provide protection against odor, staining, deterioration, allergies, and infectious diseases. In addition, these finishes should be safe and durable and not adversely affect other properties of the textile product.[2]

The efficacy of chitosan for use as an antimicrobial finishing agent was confirmed in several studies.[3–6] However, the use of chitosan as an antimicrobial agent in textile areas is limited because the hand of fabrics is adversely affected by treating with chitosan, especially of high molecular weight (MW). Low laundering durability of the chitosan treated fabrics is another problem to be solved. It is important to clarify the effect of the MW on antimicrobial activity for facilitating

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Table I Molecular Weights (MW, M_n) and Degree of Deacetylation (DDA) of Chitosans

Chitosan	DDA (%)	MW	M_n	Dispersity
A	86	1,828	1,795	1.01
B	89	101,300	51,200	1.98
C	89	207,900	193,800	1.07

M_n, number-average MW.

the use of chitosan for an after treatment agent for textiles. The MW of chitosan should be selected according to the end use of the treated fabrics.

A previous study[7] showed that the antimicrobial activity of chitosan oligomer depends on the strains of bacteria. Some bacteria were effectively inhibited by chitosan oligomer, but some other bacteria were not. In this study we investigated the MW dependence of the antimicrobial activity of chitosan against five strains of bacteria.

EXPERIMENTAL

Chitosan (MW 1800) was prepared by the acid degradation method, which was described in a previous study.[7] The MW 100,000 and 210,000 chitosans were obtained commercially. The MW of the chitosan was determined by a gel-permeation chromatograph (LCSS-905, Jasco, Co.) with a Shodex OHpak SB-801+SB-802+SB-803 column using 0.1*M* NaCl in 0.2% acetic acid as a mobile phase at a flow rate of 1 mL/min.[7] The colloid titration technique[8] was used to estimate the degree of deacetylation (DDA).

Scoured and bleached cotton fabric (plain weave, 32 × 32/cm^2, 110 g/m^2, 0.23 mm) was used. Fabric samples were padded with a chitosan solution of various concentrations to give 100% pickup. The padded samples were dried at 100°C for 3 min, cured at 150°C for 3 min, and washed in distilled water followed by drying. The antimicrobial activity of the treated fabrics was evaluated by the shake flask method[8] in terms of the bacterial reduction rate. The bacteria used were *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8473), *Proteus vulgaris* (ATCC 6059), *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* (ATCC 13388).

The performance properties of the treated samples were evaluated using published standard procedures including the tensile strength by cut strip method (ASTM D 1682-64), the stiffness by cantilever method (ASTM D 1388-55), and moisture regain (ASTM D 629-59).

RESULTS AND DISCUSSION

Analysis of Chitosan

Gel-permeation chromatographic data are shown in Table I. The DDA of our chitosans determined by the colloid titration technique was similar (86–89%). Thus, the effect of the DDA on antimicrobial activity was excluded. Figure 1 shows the IR spectra of the chitosans used. The IR spectrum of chitin shows C═O and N—H bands at 1650 and 1550–1560 cm^{-1}, respectively, because of acetamide groups. The acetamide groups in chitin are changed into amide groups in chitosan by deacetylation. Therefore, the intensities of the amide I and II bands at 1650 and 1550–1560 cm^{-1} gradually decrease and the intensity of the NH_2 formation band at 1590 cm^{-1} increases in the IR spectra of MW 100,000 and 240,000 chitosans. On the other hand, the IR spectrum of the MW 1800 chitosan shows bands at 1530 and 1620 cm^{-1}, which are slightly shifted compared to the other two chitosans due to salt formation between the amide groups in the chitosan and the remaining acetic acid used for lowering the MW.

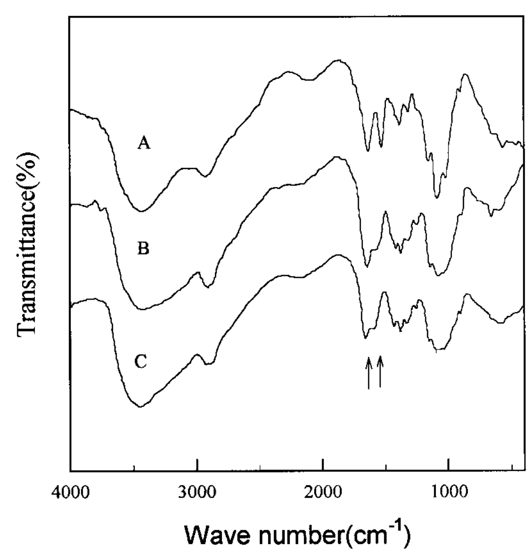

Figure 1 The IR spectra of chitosans at 1800 (spectrum A), 100,000 (spectrum B), and 240,000 (spectrum C).

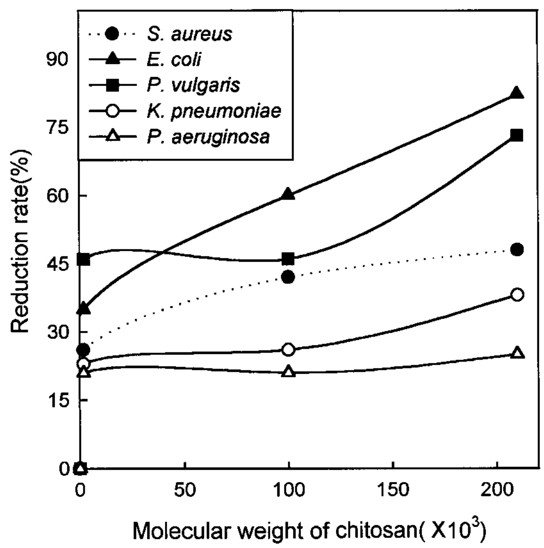

Figure 2 The molecular weight effect of chitosan on the antimicrobial activity of the treated fabrics (0.1% solution).

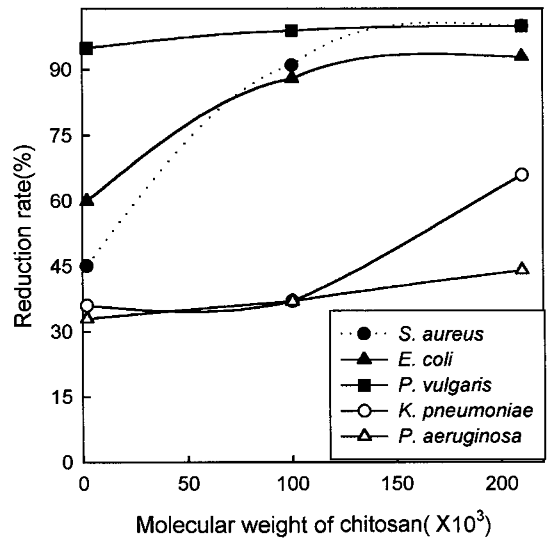

Figure 4 The molecular weight effect of chitosan on the antimicrobial activity of the treated fabrics (0.5% solution).

MW Effect on Antimicrobial Activity

Figures 2–5 show the effect of the MW on the antimicrobial activity of the fabrics treated with chitosan against five strains of bacteria. The untreated fabric showed a 13–40% reduction rate, depending on the strains of bacteria. This was due to adsorption of bacteria on the cotton fabric itself.[3] We considered the antimicrobial activity to be efficacious when the bacterial reduction rate was above 70%. The MW dependence of the antimicrobial activity varied with the strains of bacteria. In general, the bacterial reduction rate increased with the increase of the MW, although it depended on the strains of bacteria. The amino groups in chitosan interfere with bacterial metabolism by stacking at the cell surface and binding with DNA to inhibit mRNA synthesis.[9,10] Chitosan of higher MW shows more of a tendency to deposit on the surface of fabrics, resulting in

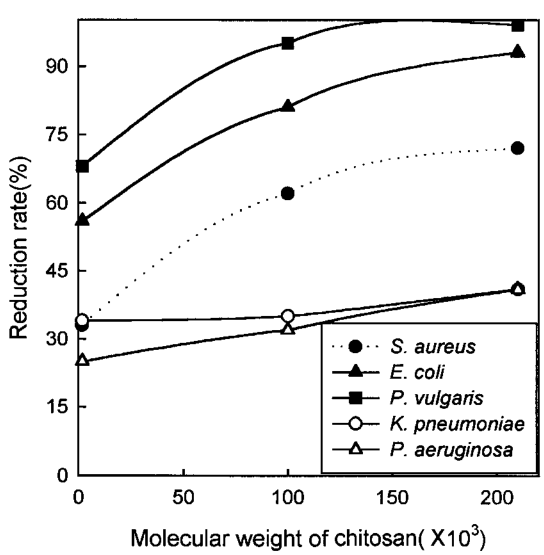

Figure 3 The molecular weight effect of chitosan on the antimicrobial activity of the treated fabrics (0.3% solution).

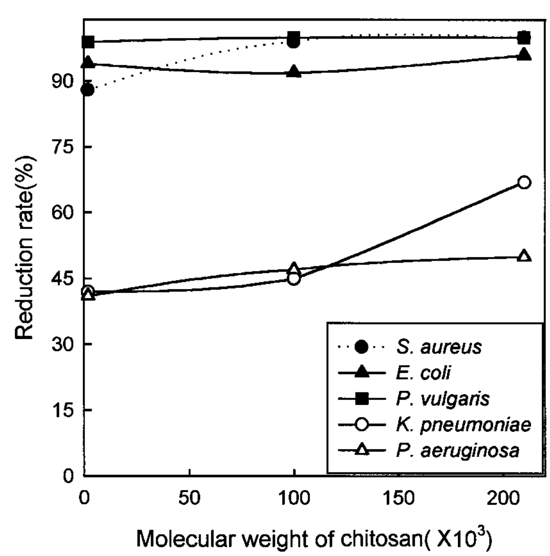

Figure 5 The molecular weight effect of chitosan on the antimicrobial activity of the treated fabrics (1.0% solution).

amino groups more easily accessible to bacteria.[11] It is known that cotton fiber treated with chitosan can be dyed with acid dyes because amino groups in the chitosan provide dyeing sites for the acid dye. Dye adsorption also increases with the increase of the MW of chitosan because of the increase of amino groups accessible to acid dye molecules.[11]

The MW dependence of antimicrobial activity at a 0.1% treatment concentration is shown in Figure 2. The reduction rates of *E. coli* and *Prot. vulgaris* increased remarkably with the increase of the MW, exhibiting antimicrobial activity at MW 210,000. The reduction rates of *S. aureus* and *K. pneumoniae* increased gradually as the MW increased, but antimicrobial activity against them was not exhibited. On the other hand, *Pseu. aeruginosa* did not show a MW dependence. Tokura et al.[12] investigated the mechanism of antimicrobial activity using MW 2200 and 9300 chitosan oligomers and found that chitosan oligomers showed different growth inhibition mechanisms, depending on the MW and strains of bacteria.

Compared with the results at a 0.1% treatment concentration, the MW dependence of the antimicrobial activity became more pronounced at a 0.3% treatment concentration (Fig. 3). The reduction rates of *S. aureus, E. coli,* and *Prot. vulgaris* increased distinctively with the increase of the MW. The MW 210,000 chitosan showed high antimicrobial activity against *E. coli* and *Prot. vulgaris,* exhibiting above a 90% reduction rate and showing antimicrobial activity against *S. aureus.* The reduction rates of *K. pneumoniae* and *Pseu. aeruginosa* increased slightly with the increase of the MW.

Figure 4 shows the MW dependence of the antimicrobial activity at a 0.5% treatment concentration. Reduction rates for *E. coli, S. aureus,* and *K. pneumoniae* increased with the increase of the MW. *Proteus vulgaris* was effectively inhibited by all three chitosans, showing above a 90% reduction rate. The MW dependence became less pronounced on *Prot. vulgaris* at above a 0.5% treatment concentration. On the other hand, *Pseu. aeruginosa* was not inhibited by any MW of chitosan.

At a 1.0% treatment concentration (Fig. 5) the MW dependence of the antimicrobial activity was not present except for *K. pneumoniae.* Regardless of the MW, *E. coli, Prot. vulgaris,* and *S. aureus* were inhibited effectively showing above a 90% reduction rate. None of the chitosans showed any

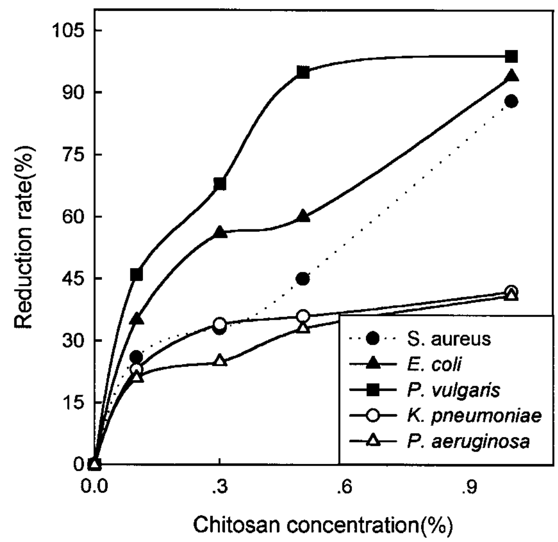

Figure 6 The effect of the chitosan concentration on the bacterial reduction rate of the treated fabrics (MW 1800).

significant antimicrobial activity against *K. pneumoniae* and *P. aeruginosa.*

The above results showed that the effectiveness of the antimicrobial activity depended on the strains of bacteria. Also, the antimicrobial activity of chitosan was found to depend on the MW of chitosan. In general, a chitosan with a higher MW was more effective than chitosan with a low MW. The MW dependence of the antimicrobial activity of chitosan was more remarkable at a low treatment concentration.

Effect of Treatment Concentration on Antimicrobial Activity

We thought that the effect of the treatment concentration on the antimicrobial activity would be different, depending on the MW of the chitosan and strains of bacteria. It is known that the minimum inhibitory concentration of chitosan is different, depending on the strains of bacteria.[4] Figure 6 shows the effect of the treatment concentration on the antimicrobial activity of the fabrics treated with MW 1800 chitosan. The bacterial reduction rate of the treated fabrics increased with the increase of chitosan concentration, even though the effectiveness of growth inhibition depended on the strains of bacteria. All bacteria showed a 21–46% reduction rate at a 0.1% treatment concentration. The reduction rate of *Prot. vulgaris* increased to 95% at a 0.5% treatment concentration, but those of the other bacteria

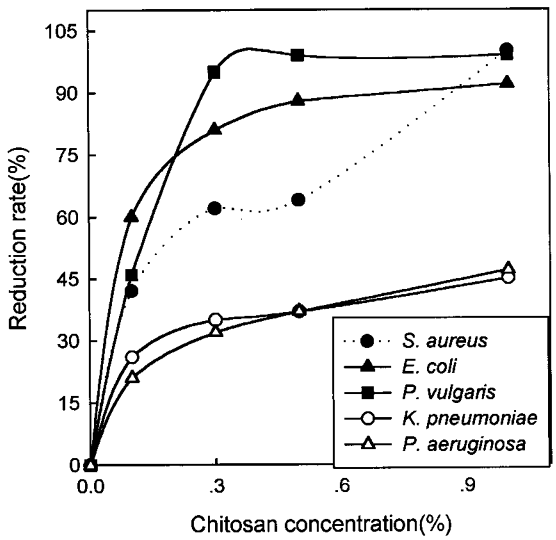

Figure 7 The effect of the chitosan concentration on the bacterial reduction rate of the treated fabrics (MW 100,000).

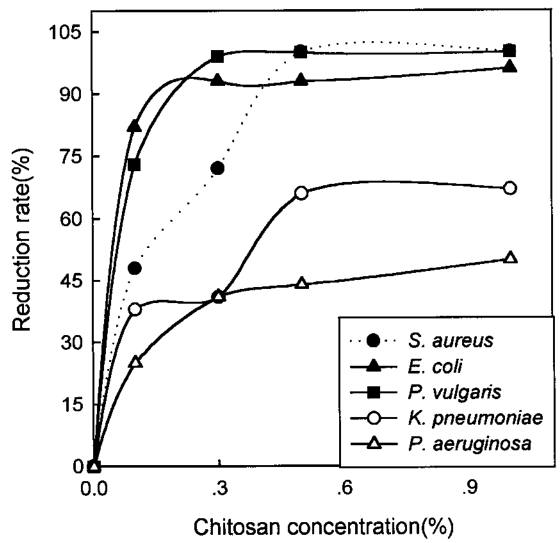

Figure 8 The effect of the chitosan concentration on the bacterial reduction rate of the treated fabrics (MW 210,000).

were below 60%. As the treatment concentration increased to 1.0%, the reduction rates of *Prot. vulgaris, E. coli,* and *S. aureus* increased to 88–99%. In a previous study[7] using nonabsorbent polypropylene nonwoven fabric, MW 1800 chitosan showed high antimicrobial activity against *Prot. vulgaris, S. aureus,* and *E. coli* at 0.01 and 0.05% treatment levels and showed above a 90% reduction rate. Compared with the results of this study, the MW 1800 chitosan seemed to be more than 10 times as effective when treated on polypropylene fabric than on cotton fabric. We speculate that the chitosan oligomer molecules penetrate better into the cotton fiber, resulting in less amino groups being available on the surface of the cotton fabric than the polypropylene fabric at the same treatment concentration. This makes the chitosan oligomer less effective on cotton fiber in terms of antimicrobial activity.

Table II Performance Properties of Cotton Fabrics Treated with Chitosan

Chitosan MW	Treated Concn (%)	Add-On (%)	Moisture Regain (%)	Stiffness (cm)	TSR (%)
Control	—	0.00	6.13	2.70	100[a]
1800	0.1	0.90	5.65	2.82	99
	0.3	0.99	5.99	2.90	97
	0.5	0.99	6.22	2.95	94
	1.0	1.21	6.26	3.09	92
100,000	0.1	0.90	6.18	2.85	97
	0.3	1.05	6.42	3.35	95
	0.5	1.06	6.40	3.62	95
	1.0	1.34	6.59	5.02	92
210,000	0.1	0.90	6.51	3.10	92
	0.3	1.07	6.54	3.98	91
	0.5	1.12	6.58	5.25	90
	1.0	1.43	6.73	6.18	89

TSR, tensile strength retention.
[a] 27.91 kgf.

Figure 9 Scanning electron microscopy pictures (original magnification ×750) of cotton fabrics treated with a 0.1% chitosan solution with MWs of (A) 1800, (B) 100,000, and (C) 210,000.

The MW 100,000 chitosan appeared to be more effective than MW 1800 chitosan. Figure 7 shows that MW 100,000 chitosan showed no antimicrobial activity at a 0.1% treatment concentration, despite reduction rates that were slightly higher than those of MW 1800 chitosan at a given treatment concentration. It showed antimicrobial activity against *Prot. vulgaris* and *E. coli,* exhibiting above an 80% reduction rate from a 0.3% treatment concentration. On the other hand, the antimicrobial activity against *S. aureus* appeared at a 0.5% treatment concentration. *Klebsiella pneumoniae* and *P. aeruginosa* showed below a 45% reduction rate at a 1.0% treatment concentration; however, their reduction rates increased as the treatment concentration increased.

Figure 8 shows the effect of the treatment concentration on the antimicrobial activity of the fabrics treated with MW 210,000 chitosan. The initial slope of the graph is steeper than those of the other two chitosans. The acid dye uptake of the fabric treated with chitosan increases as the MW of chitosan increases, indicating that chitosan with a higher MW provides more easily accessible amino groups than chitosan of low MW at the same concentration.[11] This also means that chitosan with a higher MW results in higher effectiveness in terms of antimicrobial activity. As the treatment concentration increased, the reduction rate increased rapidly up to 0.3–0.5% treatment concentration, depending on the strains of bacteria, and thereafter it did not increase significantly. This chitosan inhibited *E. coli* and *Prot. vulgaris* considerably, even at a 0.1% treatment concentration. At a 0.3% treatment concentration the reduction rates of *Prot. vulgaris, E. coli,* and *S. aureus* were 99, 93, and 72%, respectively; they increased to 93–100% at a 0.5% concentration. So, it was not necessary to increase the treatment concentration above 0.5% for *Prot. vulgaris, E. coli,* and *S. aureus.* Although the reduction rates of *K. pneumoniae* and *Pseu. aeruginosa* increased gradually with the increase of treatment concentration, the MW 210,000 chitosan did not inhibit their growth significantly.

Performance Properties of Treated Cotton Fabrics

The effect of chitosan treatment on the other physical properties of the cotton fabric is shown in Table II. With the increase of treatment concentration and MW of the chitosan the add-on of treated fabrics increased. Above a 0.3% treatment concentration a higher add-on was obtained with a higher MW at the same treatment concentration. The stiffness of the treated fabrics was made stiffer as the MW and treatment concentration of the chitosan increased. According to subjective hand evaluation, the fabrics treated with MW 210,000 chitosan above a 0.5% treatment concentration were very stiff. The tensile strength retention of the treated fabrics decreased slightly as the treatment concentration and MW of the chitosan was increased.

The moisture regain of the treated fabrics increased as the treatment concentration and MW of the chitosan were increased. All the treated samples, except samples treated with MW 1800 chitosan at 0.1 and 0.3% concentrations, had higher moisture regain than the untreated sample. This was due to the amino and hydroxyl groups of chitosan, which provide reactive sites for moisture. Furthermore, a coating of chitosan of high MW on the surface of the fiber (Fig. 9) made it easier for water molecules to approach reactive sites, resulting in higher moisture regain than chitosan of low MW.

SUMMARY

In order to investigate the effect of the MW on the antimicrobial activity of chitosan, cotton fabrics were treated with three chitosans with different MWs and similar DDA. The antimicrobial activity of the treated fabrics was tested in terms of the reduction rate using five strains of bacteria. In addition, the performance properties of the treated fabrics were evaluated.

The bacterial reduction rate increased as the MW increased. The MW effect on the antimicrobial activity of chitosan was more distinctive at low treatment concentrations. Regardless of the MW, the antimicrobial activity increased with the increase of treatment concentration. *Staphylococcus aureus* was effectively inhibited by MW 210,000 and 100,000 chitosans at a 0.5% treatment concentration, exhibiting above a 90% reduction rate. The MW 1800 chitosan was effective against *S. aureus* at a 1.0% treatment concentration. *Escherichia coli* was effectively inhibited by MW 210,000 chitosan at a 0.3% treatment concentration and by MW 1800 and 100,000 chitosans at a 1.0% treatment concentration, showing above a 90% reduction rate. *Proteus vulgaris* was effectively inhibited by MW 100,000 and 210,000 chitosans at a 0.3% treatment concentration and by MW 1800 chitosan at a 0.5% treatment concentration; the reduction rate was above 90%. None of the chitosans were effective against *K. pneumonia* and *Pseu. aeruginosa* below a 1.0% treatment concentration. We concluded that chitosan of high MW is more effective in inhibiting bacterial growth compared with chitosan of low MW. The antimicrobial activity of chitosan seemed to be more affected by treatment concentration than MW. However, the optimum MW for the chitosan should be decided by the end uses of the textile product.

As the treatment concentration and MW of the chitosan increased, the add-on, stiffness, and moisture regain of the treated fabrics increased and the tensile strength retention decreased slightly.

REFERENCES

1. Perkins, W. S. Text Chem Color Am Dye Rep 2000, 32, 24.
2. Kim, Y. H.; Sun, G. In Proceedings of the International Conference & Exhibition, 1998, AATCC, Philadelphia, p. 437.
3. Shin, Y.; Min, K. J Kor Fiber Soc 1996, 33, 487.
4. Seo, H.; Mitsuhashi, K.; Tanibe, H. In Advances in Chitin and Chitosan; Brine, C. J.; Sandford, P. A.; Zikakis, J. P., Eds.; Elsevier Science: New York, 1992; p. 34.
5. Uchida, Y.; Izume, M.; Ohtakara, A. In Chitin and Chitosan; Skjak-Braek, G.; Anthonsen, T.; Sandford, P., Eds.; Elsevier Science: New York, 1989; p. 373.
6. Yalpani, M.; Johnson, F.; Robinson, L. E. In Advances in Chitin and Chitosan; Brine, C. J.; Sandford, P. A.; Zikakis, J. P., Eds.; Elsevier Science: New York, 1992; p. 543.
7. Shin, Y.; Yoo, D. I.; Min, K. J Appl Polym Sci 1999, 74, 2911.
8. Hirai, A.; Odani, H.; Nakajima, A. Polym Bull 1991, 26, 87.
9. Kendra, D. F. Exp Mycol 1984, 8, 276.
10. Uchida, Y. Food Chem 1988, 2, 22.
11. Shin, Y.; Yoo, D. I. J Appl Polym Sci 1998, 67, 1515.
12. Tokura, S.; Ueno, K.; Miyazaki, S.; Nishi, N. Macromol Symp 1997, 120, 1.