

Influence of Acetylation on the Antimicrobial Properties of Chitosan Non-Woven Fabrics

Hye Kyoung Shin, Mira Park,[†] Hak-Yong Kim,^{†,*} Fan-Long Jin,[‡] Heung Soap Choi,[§]
Keziah H. Kim,[#] David S. Kim,[¶] and Soo-Jin Park^{*}

Department of Chemistry, Inha University, Nam-gu, Incheon 402-751, Korea. *E-mail: sjpark@inha.ac.kr

[†]Department of Textile Engineering, Chonbuk National University, Chonju 561-756, Korea. *E-mail: khy@chonbuk.ac.kr

[‡]Department of Polymer Materials, Jilin Institute of Chemical Technology, Jilin City 132022, People's Republic of China

[§]Department of Mechanical and Design Engineering, Hongik University, Sejong 339-701, Korea

[#]Flintridge Preparatory School, 4543 Crown Avenue La Canada Flintridge, CA 91011, USA

[¶]Daegu International School, 1555 Bongmu-dong, Dong-gu, Daegu 701-170, Korea

Received April 11, 2013, Accepted May 22, 2013

Chitosan non-woven fabrics were acetylated to improve their antimicrobial properties. The active chlorine content, antimicrobial properties, storage stability, and surface properties of acetylated chitosan non-woven fabrics were investigated. The active chlorine content of the fabrics increased upon reduction of the degree of the acetylation or increase in sodium hypochlorite concentration. Acetylated chitosan non-woven fabrics showed powerful antimicrobial activity by efficiently killing *Escherichia coli* and forming a growth inhibition zone for *Staphylococcus aureus*. Furthermore, scanning electron microscopy observations demonstrated that the acetylated chitosan non-woven fabrics were not damaged in sodium hypochlorite solution.

Key Words : Chitosan, Non-woven fabrics, Active chlorine content, Antimicrobial activity, Surface properties

Introduction

Non-woven fabrics are widely used in environments like medical and health care facilities. However, due to their high surface area, non-woven fabrics are easily contaminated by bacteria such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus faecalis*, and *Clostridium per-fringens*, which can cause smelliness, reduced product quality, and bacteria cross-contamination. When using non-woven fabric-based dishcloths contaminated by bacteria to wipe or clean surfaces, hands or surfaces will enhance the contamination by bacterial transfer. Nowadays, non-woven fabric-based air filters, masks, and gowns used in hospitals are an important defense against bacteria, but these items are still not 100% effective because they lack proper antibacterial activity. Thus, non-woven fabrics should have antibacterial activity to prevent contamination by germs.¹⁻⁶

Chitosan, a natural antimicrobial copolymer of β -1,4-linked-D-glucosamine and N-acetyl-D-glucosamine, is produced by partial deacetylation of chitin. In addition to its antimicrobial activity, chitosan is hypoallergenic, biocompatible, biodegradable, and has excellent blood-clotting functions that make it an attractive candidate for a wide range of biomedical applications. Nevertheless, the antimicrobial activity of chitosan is only moderate, which may not be powerful enough for many applications. Thus, a number of chitosan derivatives have been synthesized to further improve the potency of its inhibitory effect. Physical and chemical properties, biological characteristics, and the degree of biodegradation are significantly affected by the degree of acetylation (DA). Thus, the adjustment of DA is

an important factor for applications of chitosan.⁷⁻¹³

In a previous report, we presented the preparation and characterization of chlorinated cross-linked chitosan/cotton knits for biomedical applications.¹⁴ In this study, acetylated chitosan non-woven fabrics were prepared, and their antimicrobial activity, surface properties, and storage stability were investigated.

Experimental

Materials. Chitosan non-woven fabrics (93% deacetylated) used in this study were purchased from Tesan Met Tech. (Korea). Acetic anhydride, sodium hypochlorite solution, hydrochloric acid, standard sodium thiosulfate, potassium iodide, and methanol were supplied by Aldrich Chemical.

Acetylation of Chitosan Non-Woven Fabrics. Chitosan non-woven fabrics (5 g) and acetic anhydride were reacted in 100 mL methanol at room temperature for 24 h. After the reaction, the precipitate was washed with deionized water and air-dried for 24 h.

Chlorination of Acetylated Chitosan Non-Woven Fabrics. The pH of sodium hypochlorite solution was adjusted to 5 using hydrochloric acid, and acetylated chitosan non-woven fabrics were added to the solution and maintained at room temperature for 30 min. After the chlorination treatment, the fabrics were washed several times with deionized water and then dried at room temperature for 24 h.

Degree of Acetylation. FT-IR spectra were recorded with an AVATAR 370 spectrometer (Thermo Nicolet) using KBr pellets. The peaks of N-acetyl-D-glucosamine (1320 cm⁻¹) and -CH₂ of the sixth carbon (1420 cm⁻¹) are shown in

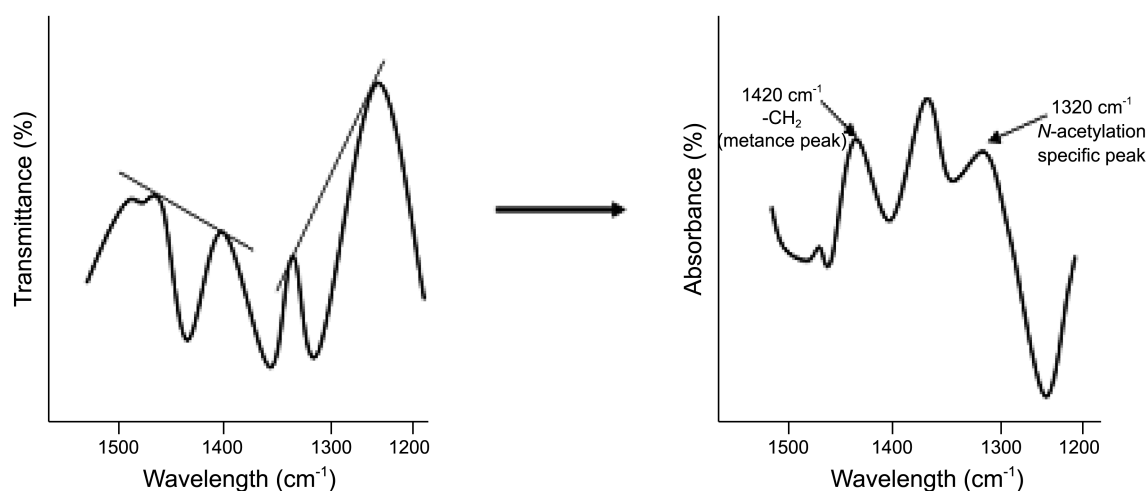


Figure 1. FT-IR spectra for DA analysis of chitosan.

Figure 1. DA values were calculated from the area of these 2 peaks according to following equation:¹⁵

$$DA = \left(\frac{A_{1320}}{A_{1420}} - 0.3822 \right) / 0.03133 \quad (1)$$

where A_{1320} and A_{1420} are the area of peaks of *N*-acetyl-D-glucosamine and $-\text{CH}_2$ of the sixth carbon, respectively.

Active Chlorine Measurement. The active chlorine content of chlorinated chitosan non-woven fabrics was measured using the iodine titration method. About 0.05 g of fabric was dispersed in 1% acetic acid solution, and 1 g of potassium iodide was added to the solution. The solution was stirred at room temperature for 1 h and then titrated with 0.01 mol/L of standard sodium thiosulfate.¹⁶ The active chlorine content (*Cl*, %) was calculated according to Eq. (2).

$$Cl(\%) = \frac{35.3}{2} \times \frac{(V_{Cl} - V_0) \times 10^{-3} \times 0.01}{W_d} \times 100 \quad (2)$$

where V_{Cl} and V_0 are the volumes of sodium thiosulfate solution consumed in the titration of the chlorinated and nonchlorinated chitosan non-woven fabrics, respectively. W_d is the weight of the chlorinated chitosan non-woven fabrics.

Antimicrobial Properties. The antimicrobial properties of chitosan non-woven fabrics were examined according to the American Association of Textile Chemists and Colorists (AATCC) test method 100 against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus*. Liquid cell cultures were prepared, divided into 2 flasks, and sterilized. The 2 bacterial species were added, and the solutions were cultivated for 24 h. The cultivated Gram's solutions were diluted to 10^5 CFU/mL using sterilized physiological salt solution. Solid cell cultures were prepared and used to inoculate the Gram's solutions. The cultures were cultivated at 37 °C for 12 h, and the growth inhibition zone of the chlorinated samples was investigated.

Storage Stability and Rechargeability. The storage stability of chitosan non-woven fabrics as a function of storage time was examined by measuring the active chlorine

content at 25 °C and 65% relative humidity. After 30 days, the chitosan non-woven fabrics were re-chlorinated using a 0.8% sodium hypochlorite solution, pH 5 for 30 min. The active chlorine content of the re-chlorinated chitosan non-woven fabrics was measured using the iodine titration.

Results and Discussion

Acetylation of Chitosan Non-Woven Fabrics. FT-IR spectra were measured on varying chitosan/acetic anhydride (C/A) ratio samples. Figure 2 shows the FT-IR spectra of

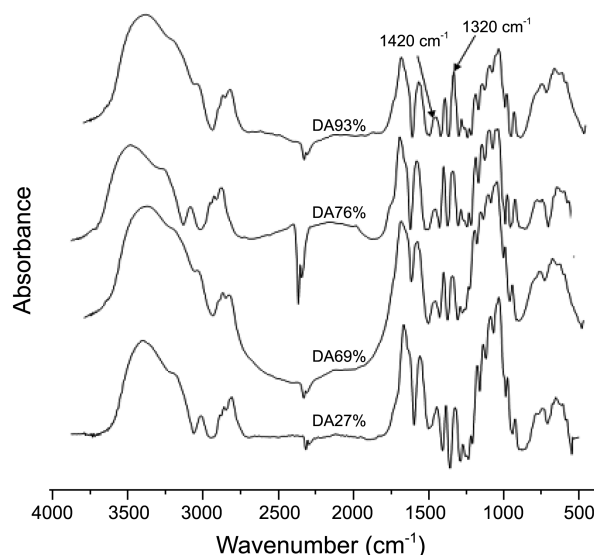


Figure 2. FT-IR spectra of acetylated chitosan non-woven fabrics as a function of DA.

Table 1. DA values of acetylated chitosan non-woven fabrics as a function of acetic anhydride concentration

Concentration of acetic anhydride	0.2	0.5	1.0	2.0
DA (%)	26.73	63.24	75.58	92.33

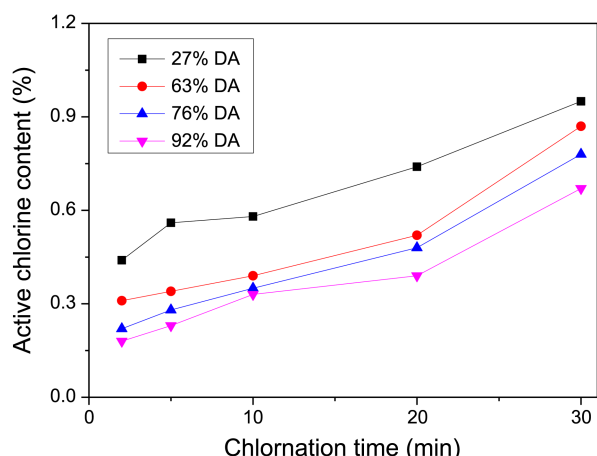


Figure 3. Effect of chlorination time on active chlorine content of acetylated chitosan non-woven fabrics having various DA.

acetylated chitosan non-woven fabrics. DA was calculated by taking the ratio of the area of the CH_2 peak and *N*-acetyl-D-glucosamine peaks at 1420 cm^{-1} and 1320 cm^{-1} , respectively. The results of the DA values for acetylated chitosan non-woven fabrics as a function of acetic anhydride concentration are shown in Table 1. The DA of the fabrics increased with increasing concentration of acetic anhydride. The DA of the fabrics was 27% for C/A ratio of 0.2%, 63% for C/A ratio of 0.5%, 76% for C/A ratio of 1.0%, and 92% for C/A ratio of 2.0%.^{15,17}

Active Chlorine Content. Figure 3 shows the effect of chlorination time on the active chlorine content of acetylated chitosan non-woven fabrics having various DAs at 0.8% sodium hypochlorite and pH 5. The active chlorine content of the fabrics decreased with increasing DA, while it increased with increasing chlorination time. When the chlorination time reached 30 min, the active chlorine content was 0.93% for 27% DA, 0.86% for 63% DA, 0.77% for 76% DA, and 0.67% for 92% DA.¹⁶

Table 2 shows the active chlorine content of acetylated chitosan non-woven fabrics measured by energy dispersive spectroscopy (EDS). Similar to the results from the iodine titration experiment, the active chlorine content of the fabrics decreased with increasing DA.

Generally, when ammonia is present in active chlorine-containing solutions in the pH range of 4.5–8.5, monochloramines and dichloramines are also present. Acetylated chitosan non-woven fabrics show different active chlorine contents at various DAs. In fact, at lower DA values, the amount of dichloramines is higher than that of acetyl amides, thus conferring the fabrics a higher active chlorine content.

Table 2. Active chlorine content of acetylated chitosan non-woven fabrics measured by EDS

DA (%)	27	63	76	92
Cl (%)	2.51	2.21	2.19	1.76

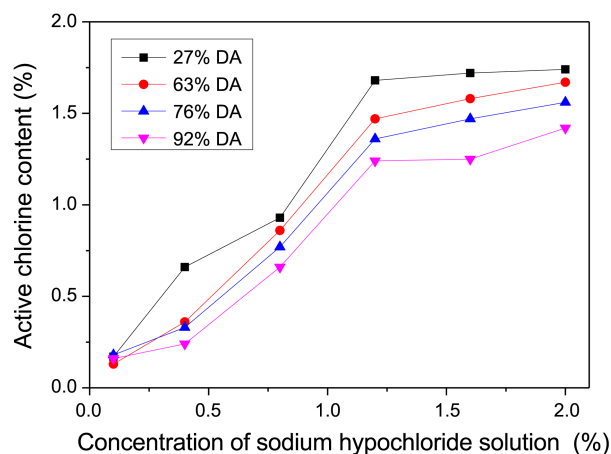


Figure 4. Effect of concentration of sodium hypochlorite solution on active chlorine content of acetylated chitosan non-woven fabrics with various DA.

In the same chlorination conditions, acetylated chitosan non-woven fabrics at 27% DA showed a lower active chlorine content (0.93%) compared that of chlorinated cross-linked chitosan fiber (6.45%) or cross-linked chitosan films (6.86%). In contrast, as explained below, these active chlorine-containing acetylated chitosan non-woven fabrics showed high antimicrobial activity against both gram-negative and gram-negative bacteria in the antimicrobial test.

Figure 4 shows the effect of the concentration of the sodium hypochlorite solution on the active chlorine content of acetylated chitosan non-woven fabrics at various DAs. The active chlorine content of the fabrics significantly increased with increasing concentrations of sodium hypo-

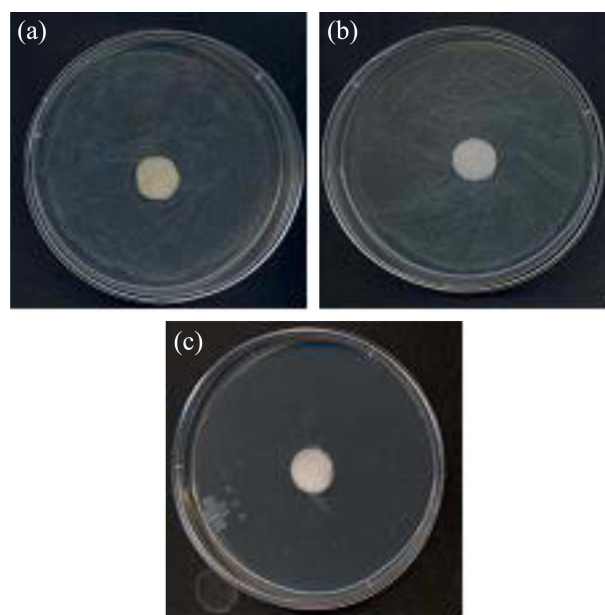


Figure 5. Antimicrobial properties of unchlorinated and chlorinated samples against *E. coli*. (a) unchlorinated chitosan non-woven fabric; (b), unchlorinated acetylated chitosan non-woven fabrics having DA 92%; (c) chlorinated acetylated chitosan non-woven fabrics having DA 92%.

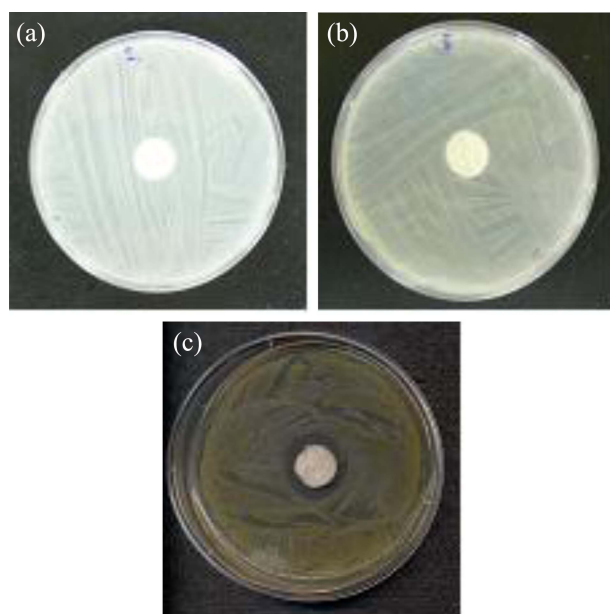


Figure 6. Antimicrobial properties of unchlorinated and chlorinated samples against *S. aureus*. (a) unchlorinated chitosan non-woven fabric; (b) unchlorinated acetylated chitosan non-woven fabrics having DA 92%; (c) chlorinated acetylated chitosan non-woven fabrics having DA 92%.

chlorite solution up to 1.2%, and after that value, it only slightly increased.¹⁸

Antimicrobial Properties. Figure 5 represents the antimicrobial properties of non-chlorinated and chlorinated chitosan non-woven fabrics at various DAs against *E. coli*. The active chlorine content of acetylated chitosan non-woven fabrics was lower than that of chlorinated cross-linked chitosan fiber or cross-linked chitosan film, as explained above. However, when the active chlorine content was higher than 0.64%, the acetylated chitosan non-woven fabrics completely eliminated *E. coli* and demonstrated powerful antimicrobial activity for various DA values.¹⁹

Figure 6 represents the antimicrobial properties of non-chlorinated and chlorinated chitosan non-woven fabrics against *S. aureus* at various DAs. The acetylated chitosan non-woven fabrics did not totally kill *S. aureus* but instead formed a growth inhibition zone. Table 3 shows the growth inhibition zone of the fabrics for *E. coli* and *S. aureus*. Due to the decreased transfer amount of chlorinated chitosan non-woven fabrics containing *N*-halamine, the growth inhibition zone decreased at increasing DA.²⁰

Surface Properties. Figure 7 shows SEM images of acetylated chitosan non-woven fabrics at various DAs. The

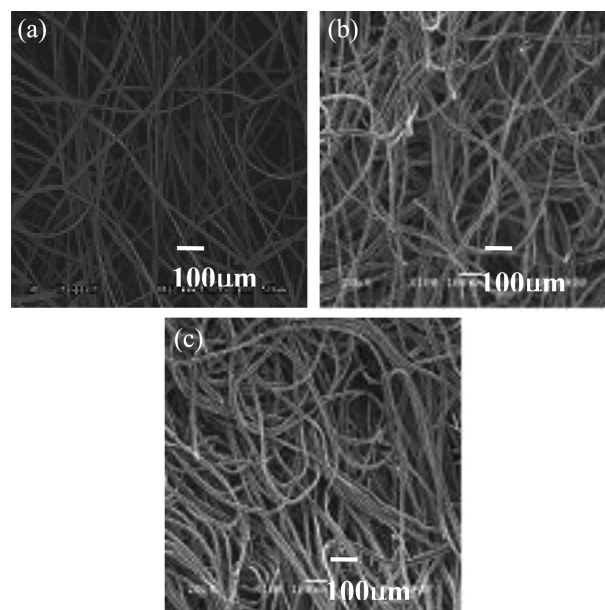


Figure 7. SEM images of acetylated chitosan non-woven fabrics. (a) unchlorinated chitosan non-woven fabric; (b) unchlorinated chitosan non-woven fabrics with DA 92%; (c) chlorinated chitosan non-woven fabrics with DA 92%.

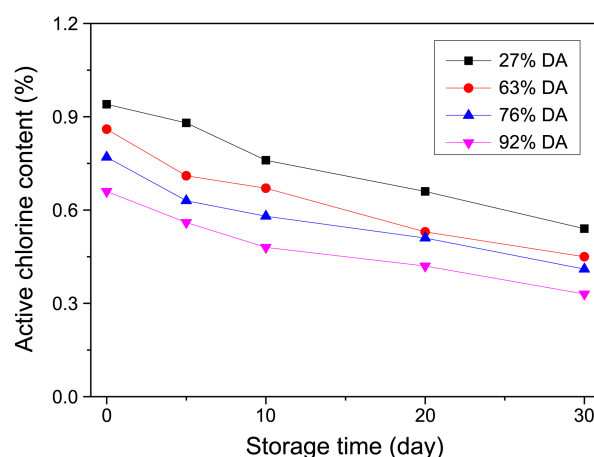


Figure 8. Storage stability of active chlorine for chlorinated chitosan non-woven fabrics having various DA.

surface of acetylated chitosan non-woven fabrics did not significantly vary after chlorination. Since non-cross-linked chitosan is swollen or dissolved in the sodium hypochlorite solution, it is difficult to transfer the *N*-halamine. Thus, the chlorination of chitosan derivatives must be carried out after the cross-linking treatment. However, the acetylated chitosan non-woven fabrics are not damaged by swelling or

Table 3. Growth inhibition zone (mm) of acetylated chitosan non-woven fabrics for *E. coli* and *S. aureus*

DA (%)	<i>E. coli</i>				<i>S. aureus</i>			
	27	62	76	92	27	62	76	92
un-chlorination	0	0	0	0	0	0	0	0
chlorination	Totally killed	Totally killed	Totally killed	Totally killed	66	45	37	17

dissolution even at the lowest DA (27%).²¹

Storage Stability. Figure 8 shows the storage stability of active chlorine for chlorinated chitosan non-woven fabrics having various DAs. The Initial active chlorine contents were 0.94%, 0.86%, 0.77%, and 0.67% for 27% DA, 63% DA, 76% DA, and 92% DA, respectively. After 30 days, the active chlorine contents of the fabrics were 43% lower (0.53%) for 27% DA, 47% lower (0.45%) for 63% DA, 48% lower (0.40%) for 76% DA, and 51% lower (0.33%) for 92% DA.

Due to chitosan hydrophilicity, the active chlorine content of the fabrics decreased with increased storage time. The storage stability of the N-Cl covalent bond also decreased with increasing DA. When the DA of chlorinated chitosan non-woven fabrics was increased, the concentration of $-\text{CH}_3\text{CONH}$ became higher than that of $-\text{NH}_2$ and the amount of amide halamine was higher than that of amine halamine. Since the strength of the N-Cl covalent bond in amide halamines is weaker than that in amine halamines, the storage stability of the chlorinated chitosan non-woven fabrics at high DAs is low. These results indicate that chlorinated chitosan non-woven fabrics are characterized by high storage stability when the amount of amine halamine is higher than that of amide halamine.²²

Conclusions

We investigated active chlorine content, antimicrobial properties, storage stability, and surface properties of acetylated chitosan non-woven fabrics. The active chlorine content of the fabrics increased with decreasing DA or increasing concentration of sodium hypochlorite solution. The acetylated chitosan non-woven fabrics completely eliminated *E. coli* and formed a growth inhibition zone for *S. aureus*. SEM images indicated that the acetylated chitosan non-woven fabrics were not damaged by the sodium hypochlorite solution. These results indicate that acetylated chitosan non-woven fabrics have powerful antimicrobial activity and may have a wide range of applications in medical and health care

facilities.

Acknowledgments. This research was supported by a grant from the Fundamental R&D Program for Core Technology of Materials funded by the Ministry of Knowledge Economy, Korea.

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