PREPARATION OF CHITO-OLIGOSACCHARIDE AS AN ANTIMICROBIAL AGENT AND ITS EFFECT ON COTTON FABRICS

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Introduction

The major classes of antimicrobial agents for textiles include organo-metallics, phenols, quaternary ammonium salts, and organo-silicones. These finishes should be durable, have selective activity towards undesirable organisms, be compatible with other finishes and dyes, and be nontoxic to man [1]. Chitosan, as a deacetylated derivative of chitin, is a natural, non-toxic and biodegradable polymer. Chitosan is also known as an antimicrobial polysaccharide due to antimicrobial action of the amino group at the C-2 position of the glucosamine residue.

Recently, one of the various researches of applying chitosan to textile was its use in the preparation of a blend fiber having an antimicrobial property [2]. There are some reports about the utility of chitosan polymer in textile finishing to impart antimicrobial activity. For example, chitosan salt produced by organic acid was mechanically held to the surface of textiles by a tremendous amount of resin which forms crosslinks. However, this resin presents possible environmental and toxicological problems. This may also impart an undesirable stiffness to the treated fabric [3].

Currently, little work has been published on the utilization of chito-oligosaccharide (COS) to produce antimicrobial activity in textile finishing. In this study, COSs were prepared by depolymerizing chitosan and were applied to cotton fabric by pad-dry-cure process, and the antimicrobial activity of treated fabric was evaluated.

Experimental

1. MATERIAL

A scoured and bleached, 100% cotton fabric was used in this investigation. A chitosan of an initial 83.9% degree of deacetylation was obtained from Kumho Chemicals Co., Ltd., Korea. The reagent grade sodium nitrite used for the depolymerization of chitosan was obtained from Showa Chemical Inc. All the other chemicals were of reagent grade and used without further purification. Glucosamine hydrochloride and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) were purchased from Aldrich Chem. Co. to determine the number average degree of polymerization of COS. Nutrient broth and trypton glucose extract agar obtained from DIFCO Laboratories were used for antimicrobial activity test on the treated cotton fabric.

2. PROCEDURES

PREPARATION OF FULLY DEACETYLATED CHITOSAN

The fully deacetylated chitosan was prepared by the method of Mima *et al.* [4]. The degree of deacetylation (DD) of the samples was measured by alkali titration method [5]. The IR spectra of chitosan films were obtained by using a FT-IR Spectrometer made by MIDAC Co., USA.

PREPARATION AND ANALYSIS OF COS

A solution of chitosan was prepared by adding 3 g of the fully deacetylated chitosan into 100 ml of a 2% (w/w) aqueous acetic acid solution. An aqueous solution containing 0.429 or 0.143 g of sodium nitrite, which corresponds to 1/3 or 1/9 mole equivalent to a glucosamine unit of chitosan, was slowly stirred into the chitosan solution over a half hour period. The reaction mixture was stirred an additional 2.5 hours at room temperature, and then neturalized with dilute NaOH solution. Excess water was evaporated with a Shimazu rotary evaporator at 40°C while applying a vacumm to make the soultion concentrate to 10% of total volume. To extract the COS, the concentrated solution was poured into excess methanol. The precipitates were collected by filtration and washed with methanol. Then the precipitates were washed several times with acetone, dried in vacumm at 40°C, and kept in a refrigerator.

The number average degree of polymerization (DPn) of COS was determined by colorimetric titration. Aldehyde group of 2,5-anhydro-D-mannose produced in one end of COS reacted with MBTH and ferric chloride to yield an intense blue color [6].

TREATMENT OF COS ON COTTON FABRIC

COS was applied to the cotton fabrics by pad-dry-cure process. The pad bath consisted of COS, acetic acid which is mole equivalent to glucosamine of COS, $MgCl_2 \cdot 6\ H_2O$ as a catalyst, 0.1% Triton X-100 as a penetrating agent, and 0.1% softening agent. The pressure on mangle was adjusted to produce 80% wet pick-up. The padded sample was dried at 60°C for 3 minutes and then cured. The treated cotton samples were rinsed with tap water thoroughly for 30 minutes at 50°C and dried at room temperature.

The amount of COS combined to the cotton fabric was determined by using the above colorimetric method for determining the DPn of COS.

ANTIMICROBIAL ACTIVITY

Dow Corning Corporate Test Method (CTM)-0923, Antimicrobial Activity Dynamic Test of Surfaces, was used for determining antimicrobial activity of the treated cotton fabrics.

Results and Discussion

DEACETYLATION OF CHITOSAN

Table I shows the effect of deacetylation treatment on the DD and the viscosity average molecular weight (Mv) of chitosan. The final of the three treatments leads to an almost fully deacetylated chitosan without serious degradation of the molecular chain.

DEPOLYMERIZATION OF FULLY DEACETYLATED CHITOSAN

The overall stoichiometry of the reaction between chitosan and HONO is shown in Scheme I [7]. Figure 1 shows the IR spectra of the COS I and II. Spectra have the carbonyl band of aldehyde group at about

1700 cm^{-1}. From a calibration curve of the concentrations of glucosamine measured against absorbance at 650nm, the number average degree of polymerization (DPn) of the two COS I and II was determined and the result given in Table II.

Table I . Effect of repeated deacetylation treatment on the DD and the Mv of chitosan

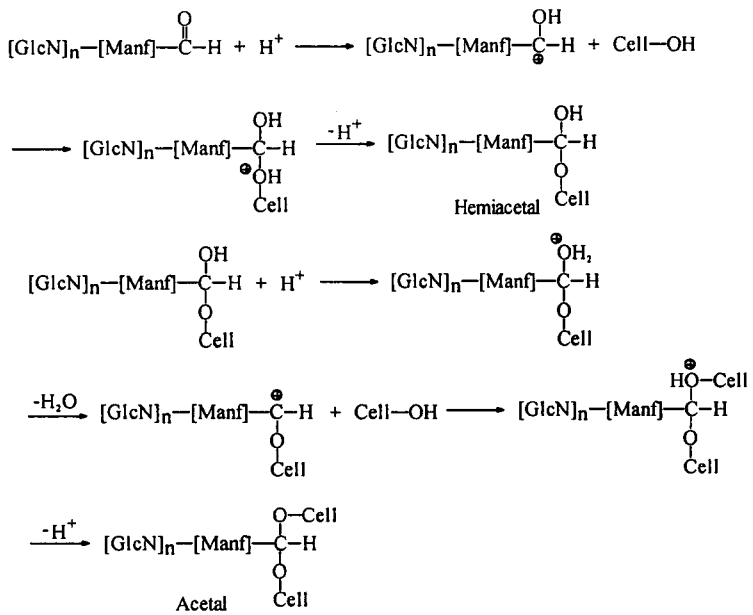
Sample	DD[b] (%)	Mv[c] ($\times 10^5$)
OC[a]	83.87	4.76
2DC	94.24	2.27
3DC	99.35	2.22

aOC : original chitosan, 2DC : 2times deacetylated chitosan,
3DC : 3times deacetylatd chitosan
bDegree of deacetylation
cThe viscosity average molecular weight.

Table II .Number average degree of polymerization (DPn) of chito-oligosaccharides

Expected DPn	$NaNO_2$[a] (mol.equiv.)	Absorbance[b] (Y)	Mole of aldehyde[c] (X, micromol/ml)	1/X	DPn
2	1/3	1.1327	0.2484	4.026	3
8	1/9	0.4157	0.0912	10.965	10

aAmount of $NaNO_2$ was calculated by [1/(expected DPn+1)] mole
bSample concentration was 1g/*l*
cXs were calculated from the calibration curve of glucosamine.



Scheme I. Reaction of chito-oligosaccharide with cellulose.

TREATMENT OF COS ON COTTON FABRIC

The COS I was chosen to determine the optimal curing conditions of temperature and time. Figure 2 shows the effect of curing temperature on the fixed amount of COS I in 1 g of cotton fabric at 3 minutes

Figure 3 shows the effect of curing time on the fixed amount of COS I in 1 g of cotton fabric at the optimal curing temperature of 120 °C. The fixed amount of COS I rose steeply from minutes 1 to 4 of curing time. Above 4 minutes, the curves flattened out and showed essentially no increment of the fixed amount of COS I thereafter. Figure 4 shows a plot between different concentrations of COS and the fixed amount of COS I and II in 1 g of cotton fabric, at the optimal curing conditions.

ANTIMICROBIAL ACTIVITY

Figure 5 shows the effect of concentrations of COS upon the reduction of bacteria. This result indicates that COS I and II inhibit bacterial growth. The reduction of bacteria increased gradually as the concentrations of COS I and II increased from 0% to 1.4% and 1.6%, respectively. Above the 1.4% and 1.6% levels of concentration of COS I and II, their curves flattened out and showed essentially a 100% reduction of bacteria values thereafter

Figure 6 shows the effect of repeated washing on the reduction of bacteria. The COS I and II exhibited a 100% retention of antimicrobial activity up to the fifth wash cycle. After the fifth wash cycle, the antimicrobial activity of COS II was greater than that of COS I. The COS II retained 93% of its antimicrobial activity at 20 wash cycles while the COS I retained 84%.

Conclusions

This study was undertaken because of our interest in the application of chitosan as a new antimicrobial agent that has the potential for eliminating the need of binding chemical as a crosslinker or binder. Fully deacetylated chitosan was successfully attained according to Mima's method. The DPn of COSs could be well controlled by depolymerizing the fully deacetylated chitosan with sodium nitrite.

The antimicrobial activity of cotton fabric treated with COS I (DPn = 3) and II (DPn = 10) increased gradually as the concentrations increased from 0% to 1.4% and 1.6%, respectively. Above the 1.4% and 1.6% levels of concentration they showed a 100% antimicrobial activity. The COS I and II exhibited a 100% retention of antimicrobial activity up to the fifth wash cycle. The COS I and II retained 84% and 93% of their antimicrobial activity, respectively at 20 wash cycles. COSs as a new antimicrobial agent exhibited good antimicrobial activity and durability of washing without the need of binding chemical as a crosslinker.

ACKNOWLEDGEMENT

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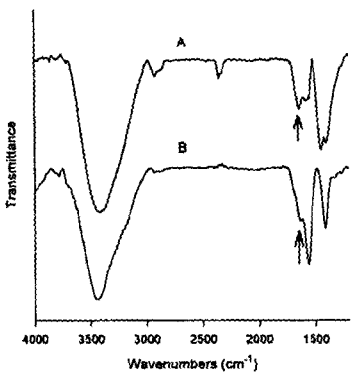

Figure 1. FT-IR spectra of chito-oligosaccharides(COSs). A:COSI,B:COSII

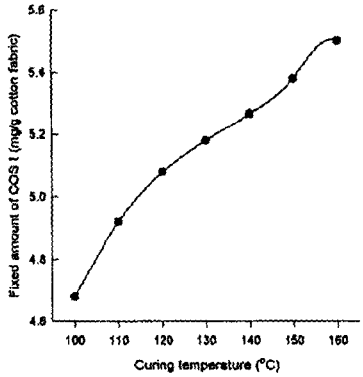

Figure 2. Effect of curing temperature on the fixed amount of COS I in 1g of cotton fabric treated with 1% COS I in the presence of $MgCl_2 \cdot 6H_2O$ at different temperatures for 3 minutes.

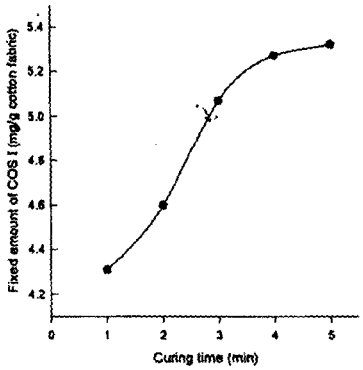

Figure 3. Effect of curing time on the fixed amount of COS I in 1g of cotton fabric treated with 1% COS I in the presence of $MgCl_2 \cdot 6H_2O$ at 120 °C for different minutes.

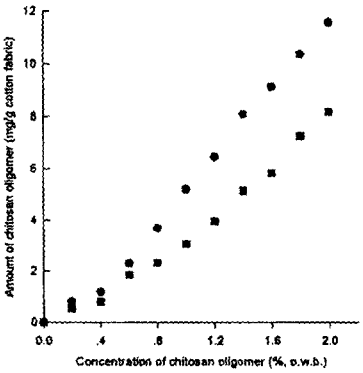

Figure 4. Plot between different concentrations of COS and the fixed amount of COS I and II in 1g of cotton fabric treated in the presence of $MgCl_2 \cdot 6H_2O$ at 120°C for 3 minutes.●: COS I, ■: COS II

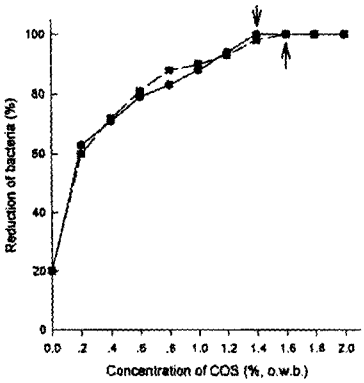

Figure 5. Effect of different concentrations of COS on the reduction of bacteria. ● : COS I, ■ : COS II

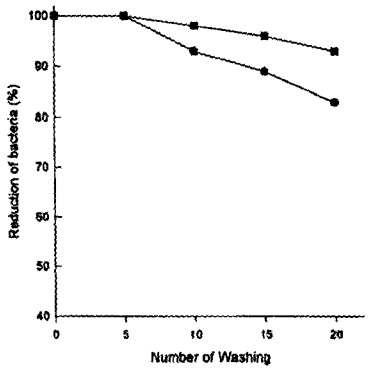

Figure 6. Effect of repeated washing on the reduction of bacteria. ● : COS I, ■ COS II.