EFFECTS OF CHITOSAN AND ITS OLIGOSACCHARIDES ON RABBIT'S SERUM LYSOZYME ACTIVITY IN THE INTRAVENOUS AND ORAL ADMINISTRATIONS, AND IN THE *in vitro* BLOOD CULTURE

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Abstract

Effects of chitosan (MW 250,000) and its oligosaccharides (MW 304-1,306) on serum lysozyme activity were examined by intravenous (i.v.) and oral administrations in rabbits and by an *in vitro* blood culture. In the daily i.v. administration of a mixture of chitosan oligosaccharides at a dose of 8.2-9.4 mg kg^{-1} per day for 5 days, the serum lysozyme activity was enhanced about 3 times that of the control. In this i.v. injection, rabbits showed some abnormal physiological symptoms, but the symptoms disappeared within 1-2 days after the stop of the injection. The single i.v. administration of polymeric chitosan (MW 250,000) at a dose 4.5 mg kg^{-1} resulted in the death of rabbits within a few days. The serum lysozyme activity was not enhanced by the daily oral administration of a 2% chitosan-supplemented diet (60-100 g/day/rabbit) for 48 days and by an *in vitro* blood culture in the presence of chitosan oligosaccharides (0.001-1.000 mg ml^{-1}) for 72 hr. These results indicate that orally administrated chitosan is not absorbed in the form of depolymerized or oligosaccharides into the blood.

Keywords: blood culture, chitosan, chitosan-oligosaccharides, oral and i.v. administrations, serum lysozyme activity

Chitin, (1→4)-2-acetamido-2-deoxy-β-D-glucan, is one of naturally abundant biopolymers, and chitosan is the N-deacetylated derivative of chitin. These biopolymers have various biofunctions including an elicitor for pathogenesis-related (PR) proteins in plants [1,2], an activator for animal immune systems [3-5], antibacterial [6] and hypocholesterolemic functions [7], a wound tissue-healing function for animals [8,9], human beings [10] and plants [11], and a metal chelating action in animal organs [12]. The i.v. and parenteral administrations of the oligosaccharides of chitin or chitosan at a dose of 1-50 mg kg^{-1} stimulate macrophages, and the growth of Sarcoma 180 MN46 [13], Meth-A [14] solid tumors and Lewis lung carcinoma [15] is inhibited in mice, and the infection of *Listeria monocytogenes* is prevented [16]. Orally administrated polymeric chitosan plays a hypocholesterolemic action [7, 17,18], but the i.v.

administration of chitosan oligosaccharides does not [19,20]. The i.v. administration of chitosan oligosaccharides enhances serum lysozyme activity in rabbits, but that of chitin oligosaccharides does not [19,20]. Practical uses of chitin and chitosan have just been started in various fields including biomedical, cosmetic, textile, biotechnological, food and feed additives, and agricultural fields [21]. One of the questions in the functional health food field is whether the serum lysozyme activity is enhanced or not by the oral administration of polymeric chitosan.

We now report that the serum lysozyme activity is enhanced by the i.v. injection of chitosan oligosaccharides, but not by the oral administration of polymeric chitosan in rabbits, and that chitosan oligosaccharides do not enhance the serum lysozyme activity in the *in vitro* rabbit's blood culture.

Materials

Crab shell chitosan (d.s. 0.25 for NAc, MW 8×10^5) was a product of Kyowa Technos Co. (Chiba, Japan) and used as a feed additive for rabbits. Polymeric chitosan (d.s. 0.02 for NAc, MW 250,000), chitosan oligosaccharides (MW 304-1,306, lot no. 60-015) and chitin oligosaccharides (MW 382-1,146, lot no. 870126) were products from Katakura Chikkarin Co. Ltd., Tokyo, a mixture of (1→4)-linked α-D-galactosaminan oligosaccharides from Higeta Shoyu Co. Ltd. Chiba, and a lyophilized powdered sample (No. M-3770) of *Micrococcus lysodeikticus* cells from Sigma Co., St.Louis. Sodium CM-chitin salt {d.s. for CM 0.31, $[\alpha]_D^{24}$ -6° (*c* 1.0, water)} was prepared from sodium chitin salt and chloroacetic acid [22].

Methods

Rabbits and breeding method

Male rabbits (Shimizu Experimental Animal Agriculture Corp., Shizuoka, Japan) were individually raised in a cage, fed a diet once a day, and had freely access to drinking water. A commercial granular diet ('Labo-R-stock', Nihon Nosan Kogyo, Inc., Tokyo) was used as a basal diet for a control experiment. A 2% chitosan-supplemented diet was prepared by adding 20 g of crab shell chitosan to 1 kg of the basal diet. The rabbits fed 60 g (1.2 g chitosan) of the diet per day from 1st to 5th day, 70 g (1.4 g chitosan) from 6th to 8th day, 80 g (1.6 g chitosan) from 9th to 11th day, 90 g (1.8 g chitosan) from 12th to 13th day, and 100 g (2 g chitosan) from 13th to 48th day. The body weight of rabbits was

analyzed once a week.

Intravenous (i.v.) injection

A mixture of chitosan oligosaccharides was dissolved at 10 mg/ml in physiological saline, and i.v. injected daily into rabbit's auricular vein through a 0.2 μm fitter (Steradisc 25, Kurabo Inc., Osaka) at a dose of 8.2-9.4 mg kg^{-1} per day. Its total fed amount was 150 mg per rabbit for 5 days. A sterilized polymeric chitosan solution in 2% aq. acetic acid was i.v. injected at a dose of 4.5 mg kg^{-1}.

A crude enzyme solution

Rabbit's blood-letting was performed from their auricular vein and abdominal arterial vein after fasting for 12 h. The blood was put into a plastic centrifuging tube and kept in an ice bath for about 30 min, and centrifuged at 1500xg at 0 °C for 10 min. The clear supernatant serum was dialyzed against 0.05M McIlvain buffer solution (pH 5.0) at 4-5 °C for 24 h, in which the buffer solution was changed one time with a fresh one. The dialyzed solution was used as a crude enzyme solution for the analysis of lysozyme activity.

Protein and hexosamine analyses

Protein was analyzed by the Lowry method [23] using bovine serum albumin as standard, and hexosamine was analyzed by a Blix modified Elson-Morgan method [24] after hydrolysis with 6N HCl at 100 °C for 20h.

Blood culture

Portion (about 5 ml) of rabbit's blood was mixed with 0.5 ml of a blood anticoagulant solution [sodium citrate (2.630 g), citric acid (0.327), D-glucose (2.320g) and $NaHPO_4$ H_2O (0.251 g) per 100 ml]. Each 0.2 ml-portion of the above solution was put into a plastic centrifuging tube. Fetal calf serum (1.5 ml), a RPMI 1640 medium (13.0 ml) and a solution (0.5 ml) of several fixed amounts of chitosan oligosaccharides was added. The tube was incubated at 37 °C under 5% CO_2 gas with gentle stirring once a day. After 72 h-incubation, the tube was centrifuged at 1500xg at 4 °C for 20 min, and the supernatant serum was used for the analysis of lysozyme activity.

Lysozyme activity

Portion (0.2 ml) of the serum sample obtained above was mixed with 2.8 ml of a suspension mixture (0.25 mg/ml) of a lyophilized powdered sample of *M. lysodeikticus cells* in 0.1 M phosphate buffer solution (pH 7.4). The mixture was incubated at 37 °C with mechanical stirring for 60 min, and the decrease of the turbidity was monitored at 600 nm [19]. One unit (U) for the lysozyme activity is defined as a decrease in the absorption of 0.001 per min.

Results and Discussion

Rabbit's serum lysozyme activity by the daily i. v. injection of a mixture of chitosan-oligosaccharides

As shown in Table 1, rabbit's serum lysozyme activities increased at about 2.7 times that of control after the daily i.v. injection of chitosan oligosaccharides (8.2-9.4 mg kg^{-1} per day) for 5 days, and the serum hexosamine value reached to the normal value (5.4-7.7 umol/ml) after 7 days. However, the serum hexosamine value kept the normal value even after the five i.v. injections of chitin oligosaccharides [19]. These data indicate that chitosanolytic activity is weaker then chitinolytic activity in rabbit's blood. The serum lysozyme activity was almost not enhanced by the daily oral administration of chitosan (0.58-0.87 g kg^{-1} per day) for 48 days.

Table 1
Rabbit's serum lysozyme activities in the daily i.v. injection of a mixture of chitosan oligosaccharides for 5 days and in the daily oral administration of the 2% chitosan-supplemented diet for 48 days

Chitosan	Administration method	Dose (kg^{-1} per day)	Period (days)	Lysozyme activities[a] (U/ml serum)
Oligosaccharides	i.v.	8.2-9.4 mg	5	12.0±4.0
	i.v.	4.5 mg	5	9.2±2.2[b]
Polymers	oral	0.59-0.87 g	48	4.3±1.2
Control				4.5±0.9

[a]Mean±SD, n=3-7. [b]Ref. 19.

Rabbits showed abnormal physiological symptoms (screaming, slow physical movement and poor appetite) after the five i.v. injections of a mixture of chitosan oligosaccharides at a dose of 8.2-9.4 mg kg^{-1} per day. These abnormal physiological symptoms disappeared within 1-2 days after the stop of the injection. However, no abnormal physiological symptoms were observed with rabbits, who had the five i.v. injections of chitin oligosaccharides at the same dose.

On the other hand, only one injection of a sterilized polymeric chitosan solution in aq. acetic acid at a dose of 4.5 mg kg^{-1} in rabbits resulted in their death within a few days. The polymeric chitosan probably enhances both the blood coagulation factors and the chelating with Fe ions present in

the blood. In fact, the bright reddish blood color was turned into dark color after the i.v. injection of the polymeric chitosan.

Table 2
Lysozyme activities in the *in vitro* rabbit's blood culture in the presence of oligosaccharides of chitin and chitosan[a]

Experiment[b]	Oligosaccharide mixture (mg ml^{-1})				
	0 (control)	1	0.1	0.01	0.001
A	4.5±0.9	3.7±0.1	4.1±0.4	3.9±0.2	3.9±0.1
B	4.5±0.2	3.7±0.1	4.3±0.3	4.7±0.3	n.d.

[a]U ml^{-1} serum, mean ±SD and n=2-3.
[b]Chitosan oligosaccharides (A) and chitin oligosaccharides (B) was added into the culture media (see Text).

Table 3
The body weight of rabbits fed the basal diet and the 2% chitosan-supplemented diet

Feeding period (days)	Body weight (kg)[a]	
	2% chitosan-supplemented diet	Basal diet
0	2.01±0.22	2.10±0.16
7	2.07±0.26	2.18±0.20
14	2.20±0.31	2.26±0.20
21	2.30±0.25	2.41±0.35
28	2.38±0.36	2.48±0.24
35	2.45±0.25	2.60±0.32
42	2.47±0.37	2.62±0.36
49	2.67±0.47	2.78±0.38

[a]Means±SD and n=3.

Lysozyme activity in the *in vitro* rabbit's blood culture in the presence of the oligosaccharides of chitosan or chitin

Serum lysozyme activity was not enhanced by treatment with each oligosaccharide mixture (0.001-1.000 mg ml^{-1}) of chitosan and chitin in the *in vitro* blood cultures (Table 2). These data indicate that blood erythrocytes, leukocytes and

thrombocytes do not secrete chitinolytic enzymes into the blood.

Table 4

Hexosamine values in the serum of rabbits fed daily each the basal and 2% chitosan-supplemented diets for 48 days

Diet	Hexosamine values in serum[a] (μmol/ml of serum)
Basal	6.8±1.0
2% chitosan-supplemented	7.0±1.1

[a]mean±SD, n=3.

Table 5

Rabbit's serum lysozyme activity and physiological symptoms in the oral and i.v. administrations and in the *in vitro* rabbit's blood culture[a]

Compounds	Dose (kg^{-1} per day)	Lysozyme activity enhancement	Physiological symptoms
Oral administration			
polymeric chitosan	0.58-0.87 g	inactive	normal
LMW-chitosan	0.58-0.87 g	n.d.	normal
chitosan oligosaccharides	0.58-0.87 g	n.d.	normal
i.v. administration			
polymeric chitosan	4.5 mg	n.d.	abnormal[b]
chitin-oligosaccharides	4.5 mg	inactive	normal
galactosaminan oligosaccharides	4.5 mg	inactive	normal
LMW-chitosan	4.5 mg	active	abnormal
chitosan-oligosaccharides	4.5 mg	active	normal[c]
chitosan-oligosaccharides	8.2-9.4 mg	active	abnormal[c]
in vitro blood culture (0.001-1.000 mg ml^{-1})			
chitosan-oligosaccharides		inactive	
chitin-oligosaccharides		inactive	

[a]n.d., not determined. [b]Died after a few days.
[c]Recovered within a few days.

Body weight, and serum lysozyme activity and serum hexosamine values in rabbits fed polymeric chitosan

Any abnormal physiological symptoms were not observed with

rabbits fed the 2% chitosan-supplemented diet (0.58-0.87 g of chitosan kg^{-1} per day) for 48 days. Each rabbit fed about 88.6 g of chitosan during 48 days. No significant difference was observed with the body weight (Table 3), the serum lysozyme activity (Table 1) and the serum hexosamine (Table 4) value between the two groups of rabbits fed the basal diet and the 2% chitosan-supplemented diet.

Conclusion

Table 5 summarizes the serum lysozyme activities and physiological symptoms in the oral and i.v. administrations, and the serum lysozyme activities in the *in vitro* blood culture in the presence of each of chitosan and its oligosaccharides.

The blood serum lysozyme activity was enhanced by the i.v. administration of chitosan oligosaccharides, but the enhancement was not found in both the oral administration of polymeric chitosan and in the *in vitro* blood culture. The results indicate that blood erythrocytes, leukocytes and thrombocytes do not secrete lysozyme. The blood lysozyme is probably originated from the blood vascular muscle cells and organ tissue cells. Low-molecular-weight (LMW) chitosan (MW 3,000) and chitosan oligosaccharides (MW 304-1,305) [25] stimulate mitogenic response to platelet-derived growth factor in the culture of vascular smooth muscle cells [26]. Extracellular lysozyme activity was enhanced by treatment with chitosan oligosaccharides in the *in vitro* culture of rat vascular smooth muscle, chicken embryo fibroblast, monkey kidney vero and dog skin fibroblast cells [27]. Orally administrated chitosan is digested in intestines [18], the resulting oligosaccharides do not enter into the blood through intestinal organs, and the orally administrated chitosan is utilized probably by intestinal bacteria as a nutrient, resulting in a change of the intestinal bacterial flora. Lysozyme is secreted from animal cells [27], and its activity is found in bloods [28] and tears [27]. Chitinase activity is also found in human blood serum [29,30]. The chitinolytic activities in the blood are probably enrolling on a biological-self defense function for animals to protect the host cells and tissues against pathogen infection by digesting the cell walls. The enhancement of lysozyme activity in tissues and body fluids will be expected as the result of the enhancement of blood lysozyme. In our previous experiment [19], almost no abnormal physiological symptoms were observed with rabbits i.v. injected with less than 4.5 mg kg^{-1} of chitosan oligosaccharides. However, the present

data indicate that the i.v. administration of excess amount of chitosan oligosaccharides is dangerous.

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EFFECT OF CHITOSAN DERIVATIVES ON THE INFECTION OF PLANTS BY PATHOGENIC BACTERIA

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Abstract

Chitosan derivatives were examined to determine their inhibition of Pseudomonas syringae pv tomato (Pst) growth and induction of tomato resistance to bacterial infection. Chitosan and low-molecular deaminated chitosan showed dual antibacterial activity inhibiting the growth of bacteria and bacterial infection.
Microcrystalline chitosan and chitosan oligomers did not inhibit the growth of Pst but induced resistance of tomato to Pst infection whereas anionic chitosan derivatives did not displayed antibacterial activity. This is first report on the induction of plant resistance to bacterial infection by chitosan derivatives.

Keywords: chitosan derivatives, phytopathogenic bacteria, inhibition of bacterial infection, induction of plant resistance

Introduction

Chitosan, a deacetylated derivative of chitin, has been found only in the cell walls of a limited group of fungi, including phytopathogenic fungi (1). In spite of this limited occurrence in nature, much attention has been paid to chitosan and its oligomers because of their high biological activity. Chitosan is obtained by a chemical deacetylation of chitin crustaceans. Chitosan derived from cell walls of phytopathogenic fungi or from shrimp shell chitin actively induces the broad spectrum of defense responses in plants (2). Chitosan inhibited the growth of phytopathogenic fungi and bacteria (3,4) and induced resistance of plants to fungal, viral and viroid infections (5,6,7). This report describes a preliminary study on the induction of natural plant resistance to bacterial infection by chitosans and chitosan oligomers.

Material and Methods

The bacterial strain of Pseudomonas syringae pv tomato (Pst) used in this study was isolated and idenified in our laboratory. Chitosan derivatives i.e. standard chitosan (MW=150.000, DA=15%), low molecular deaminated chitosan, microcrystalline chitosan, sulphate chitosan and carboxymethyl-chitosan were obtained from Institute of Chemical Fibres, Łódź, Poland. Chitosan was dissolved in acetic acid and the pH of the solution was adjusted to 5.5-5.7 with 1.0 N KOH solution. The hydrogel of microcrystalline chitosan was suspended in water using a homogenizer. Deaminated chitosan, sulphate chitosan and carboxymethylchitosan were dissolved in water. Chitosan

oligomers were prepared from 15% acetylated chitosan by degradation by fungus Aspergillus fumigatus. To obtain the A. fumigatus degraded chitosan, 50 ml of M9 medium supplemented with 0.25% of chitosan (final concentration) was inoculated with fungus in Erlenmeyer flaks. The culture was incubated at 37°C for 4 days on rotary shaker and after that mycelium was removed by filtration. The enzymatic degradation of chitosan was stopped by boiling the filtrate for 5 min. and precipitate was removed by centifugation. The filtrate of Aspergillus degraded chitosan was used as a crude mixture of chitosan. Chitin oligomers were analysed by HPLC. Antibacterial activity of chitosan derivatives were tested in two aspects: the inhibition of the growth of bacteria and the inhibition of viral infection. The effect of chitosan on the growth of bacteria was determinated by minimum inhibitory concentration (MIC) test. Drops of chitosans, at different concentrations were applied to the surface of agarose plates containing culture of Pst strain in Nutrient Dextrose medium. The MIC was defined as the lowest concentration of chitosans that inhibited bacterial growth after overnight incubation on the agarose plate at 37°C. For the determination of the induction of resistance of plants to bacterial infection tomato plants and Pst were used. The effect of chitosan on Pst infection was calculated as the percentage of reduction of the number of local lesions produced by pathogen on the chitosan treated leaves in comparision to control.

Results and Discussion

The antibacterial activity of chitosan derivatives are summarized in Table 1.

Table 1

Inhibition of the growth and infectivity of Pseudomonas syringae pv tomato by chitosans

CHITOSANS	MIC	INHIBITION OF INFECTION (%)
Standard chitosan	0.1	70
Sulphate chitosan	(1.0)	0
Carboxymethylchitosan	(1.0)	0
Deaminated chitosan	0.1	60
Chitosan oligomers	(1.0)	60
Microcrystalline chitosan	(0.5)	60

The tomato leaves were sprayed with 0.1% chitosan 1 day before inoculation.

Activity of the standard chitosan (solution in acetic acid) and low-molecular deaminated chitosan were manifested dually: by inhibition of the bacterial growth and the inhibition of bacterial infection. Chitosan has formed a hard washable film on the treated leaves and in such a case the inhibition of bacterial infection might be due to the presence of residual amounts of chitosan, which can act directly on bacteria. It is interesting that microcrystalline chitosan whose particles prevent its penetration into cells of the treated leaf, also inhibited the bacterial infection. It may suggest that its inhibitory activity is mediated by a soluble components, probably chitosan oligomers or chitosan which could induce a messenger able to activate defense mechanisms. Chitosan degraded by

Aspergillus fumigatus to chitosan oligomers lost ability to inhibit the growth of bacteria but protected plants from the bacterial infection. These results strongly suggested that the effect of chitosan on the bacterial infection was rather due to the induction of natural resistance of plants to bacterial infection that direct inactivation of bacteria. The inhibition of the bacterial infection developed in the chitosan treated leaves almost immediately after treatment and the same inhibiting effect was observed either after 5 hrs or 2 days chitosan application (Table 2).

Table 2

Inhibition of Pseudomonas syringae pv tomato infection by chitosan applied at different times and concentrations.

Interval between chitosan treatment and bacteria inoculation	**INHIBITION OF INFECTION (%)**			
	CONCENTRATION OF CHITOSAN (%)			
	0.2	**0.1**	**0.05**	**0.01**
5 hours	70	70	60	50
24 hours	70	70	60	50
48 hours	60	60	50	50

The tomato leaves were sprayed with the 0.1% solution of chitosan

The efficiency of chitosan in the inhibition of bacterial infection varied from 70 to 50% and depended on its concentration and time of application. Chitosan at concetration of 0.01 mg/ml inhibited bacterial infection in 50%. Anionic chitosan derivatives i.e. sulphate chitosan and carboxymethyl-chitosan did not displayed activity against Pst.

Conclusion

We present the first report that chitosan has the potential to induce resistance of plants to bacterial infection. The antibacterial activity of chitosan makes chitosan a potential agent for the control of bacterial diseases.

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Functional Finishing of Cotton Fabrics by Treatment with Chitosan

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Abstract

Cotton fabric was treated with chitosan solution by pad-dry(-cure) method to impart antimicrobial properties. Four chitosans of different degree of deacetylation(DAC: 65~95 %) with similar molecular weight(ca. 50,000) and one chitosan oligomer(MW 1,800, DAC 86 %) were used. Antimicrobial activity against *Staphylococcus aureus* was evaluated by the Shake Flask Method. Treated fabrics were laundered up to 20 times according to AATCC Test Method 60-1986 and antimicrobial activity of laundered fabrics was evaluated. The antimicrobial activity was increased with the concentration and DAC of chitosan used. And the cured samples showed better durability to laundering than not-cured samples. Crosslinker and binder decreased the antimicrobial activity of fabrics treated with chitosan oligomer and were not effective to improve the durability to washing.

Keywords: chitosan, chitosan oligomer, degree of deacetylation, antimicrobial activity, durability to washing, crosslinker, binder.

Introduction

Recently, consumers concern about sanitary property of textile products and their safety toward human and environment. Biodegradable natural polymers such as chitin and chitosan draw much attention on this respect. Chitosan is prepared from the deacetylation of chitin, in which hydroxyl groups on the No. 2 carbon atom are replaced by amino groups. Chitosan fiber has been produced and used as a suture or a dressing for wound treatment (1,2). The application of chitosan as an antimicrobial finishing agent has been studied in textile area(3,4). However, use of chitosan as a finishing agent is limited in the aftertreatment of textile fabrics due to its weak

binding. Accordingly, problems in binding of chitosan to textile material should be solved for expanding chitosan uses.

In this study, cotton fabric was treated with chitosan, and the effect of treatment condition and DAC on the antimicrobial activity was investigated. And the effect of curing and additives such as binder or crosslinker on the durability to laundering was also examined.

Experimental

Materials

A desized, scoured and bleached 100 % cotton fabric was used. Four chitosan samples of similar molecular weight(ca. 50,000) with different degree of deacetylation(65, 78, 84, and 95 %, respectively) and one chitosan oligomer(MW 1,800, DAC 86%) were used. Polyurethane emulsion(TX-B-202F, Takamatsu Oils & Fats Co., Ltd., Japan) and DMDHEU(dimethylol-dihydroxyethylene urea, BASF Co., Ltd.) with catalyst($MgCl_2$) were used as a nonionic binder and bifunctional crosslinker, respectively. Other reagents used were of laboratory grade.

Treatment of fabrics

Cotton fabrics were padded with chitosan(MW 50,000) solution dissolved in 2 % acetic acid. The padded samples were dried at 100 ℃ for 5 min and some of dried samples were further cured at 150 ℃ for 3 min. Treated samples were rinsed and conditioned at the standard conditions.

In other procedure of treatment, chitosan oligomer was dissolved in distilled water, and binder or DMDHEU with catalyst was added in the padding bath. Cotton fabric was treated at the same condition as described earlier.

Evaluation of treated fabrics

Add-on was calculated from the difference in sample weight before and after chitosan treatment.

Antimicrobial activity of treated fabrics was evaluated by the Shake Flask Method in terms of the reduction rates in the number of colonies of a gram-positive test bacterium, *Staphylococus aureus*(ATCC No. 6538).

Treated fabrics were laundered 5, 10, 15, and 20 times according to AATCC Test Method 60-1986. After laundering, antimicrobial activity was evaluated as described earlier.

Results and Discussion

Add-on of treated fabrics with chitosan

Table 1 gives the effect of treatment conditions and DAC on the add-on of fabrics treated with chitosan of MW 50,000. As shown in Table 1, add-on increase with the chitosan concentration, but it is not dependent on curing and DAC.

Table 2 shows add-on of fabrics treated with chitosan oligomer. At same concentration, add-on of fabrics treated with chitosan of MW 50,000 is higher than that of treated fabrics chitosan oligomer. Addition of binder or DMDHEU increases add-on of treated fabrics slightly.

Table 1. Add-on(%) of fabrics treated with chitosan of MW 50,000[a]

DAC of chitosan (%)	Concentration of chitosan (%)			
	0.1	0.3	0.5	1.0
65	1.12	1.33	1.83	2.16
78	1.11	1.31	1.85	2.15
84	1.10	1.33	1.84	2.14
95	1.11	1.34	1.83	2.14

[a] cured samples

Effect of chitosan concentration on the antimicrobial activity

The effect of chitosan concentration on the antimicrobial activity is shown in Fig. 1. Antimicrobial activity of MW 50,000 chitosan is increased with concentration and approached to a maximum value at 0.5 %, and then it is not changed significantly. Chitosan of DAC 65 % shows reduction rate below 40 % even if chitosan concentration increases, which means it is not effective against *S. aureus*. Chitosan of DAC 78 % displays the maximum reduction rate of 93 % at 0.5 % chitosan concentration and it is not increased thereafter. Chitosan of DAC above 84 % shows similar reduction rates and reaches to the reduction rate of 100 % above 0.5 % chitosan concentration. Regardless of DAC, reduction rate is not increased anymore above 0.5 % of treatment concentration for chitosan of MW 50,000. Chitosan oligomer displays ca. 80 % of reduction rate at 0.5 % concentration

Table 2. Add-on(%) of fabrics treated with chitosan oligomer

Additive conc. (%)		Concentration of chitosan (%)	
		1.0	1.5
None		1.21	1.76
Binder	0.1	1.38	1.63
	0.3	1.34	1.80
	0.5	1.33	1.73
DMDHEU[a]	0.1	1.36	1.88
	0.3	1.30	1.92
	0.5	1.38	2.01

[a] with 6% $MgCl_2$ on the weight of DMDHEU

Effect of degree of deacetylation on the antimicrobial activity

As shown in Fig. 2, reduction rate is increased with DAC at the same chitosan concentration. The increase of DAC plays a positive role onto antimicrobial activity because amine groups imparting antimicrobial activity increase as DAC increases. However, antimicrobial activity is not significantly increased above DAC 84 %. Effect of DAC on the antimicrobial activity is more evident at lower chitosan concentration.

Chitosan of DAC 65 % does not show significant antimicrobial activity. And chitosan of DAC 78 % shows significant antimicrobial activity at the concentration above 0.5 %, but does not reach to 100 % reduction rate up to 1.0 % of chitosan concentration.

Effect of curing on the antimicrobial activity

Reduction rate of cured samples is slightly lower than that of not-cured samples at 0.1 % chitosan concentration, but there is no difference in reduction rate between cured and not-cured samples at above 0.5 % chitosan concentration. Therefore, curing does not affect significantly on the antimicrobial activity of treated fabrics. This fact is advantageous when chitosan is applied together with other finishes which curing is required, such as durable press finish.

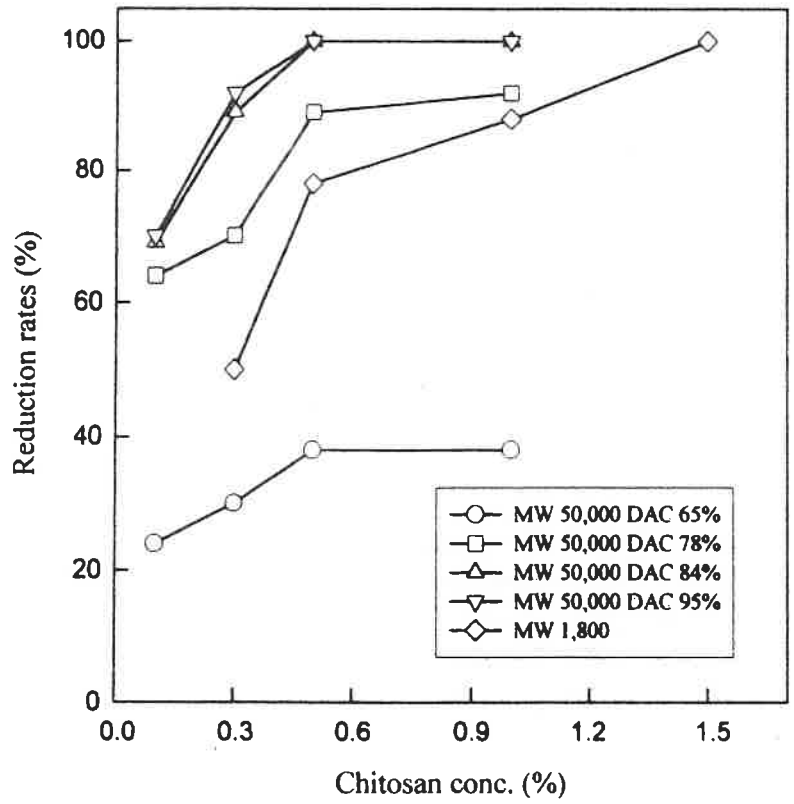

Fig. 1. Effect of chitosan concentration on the antimicrobial activity.

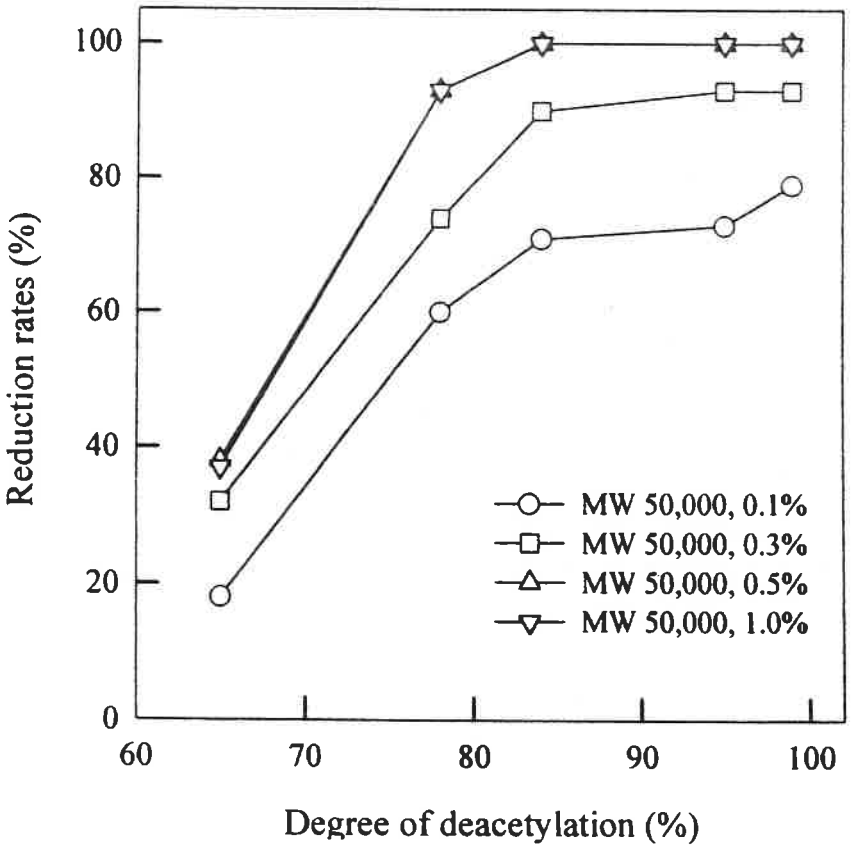

Fig. 2. Effect of degree of deacetylation on the antimicrobial activity.

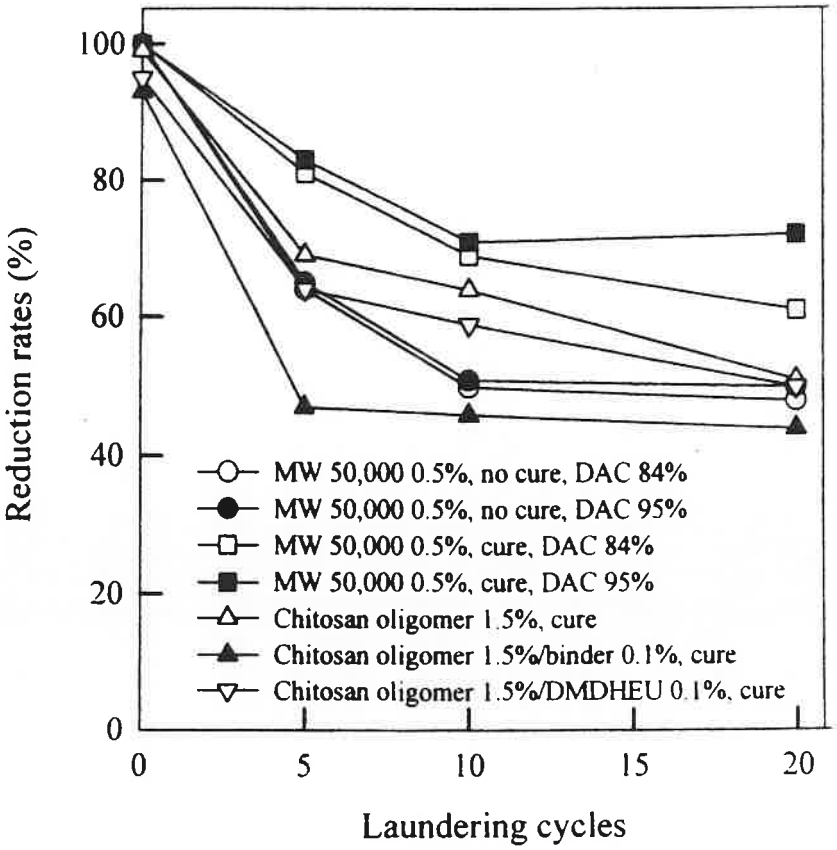

Fig. 3. Effect of binder or DMDHEU on the antimicrobial activity.

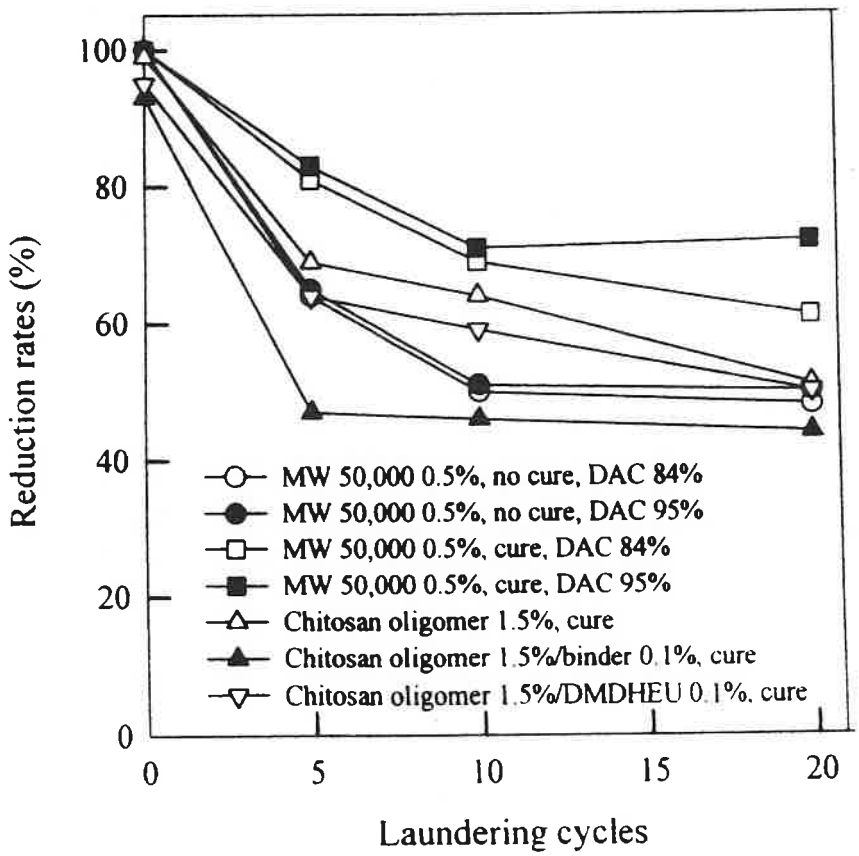

Fig. 4. Effect of treatment condition on the durability to washing.

Effect of additives on the antimicrobial activity

Antimicrobial activity is decreased with the concentration of binder or crosslinker(DMDHEU) as shown in Fig. 3. This means that DMDHEU binds with hydroxyl groups in cellulose and amine groups in chitosan resulting the decrease of antimicrobial activity. Polyurethane binder seems to mask amine groups. DMDHEU is less detrimental to antimicrobial activity compared with polyurethane binder.

Durability to laundering of treated fabrics

The effect of curing on the durability to laundering of antimicrobial activity is shown in Fig. 4. At any treatment condition and DAC, reduction rate decreases with the increase in laundering cycle. Reduction rate decreases sharply up to 5 laundering cycles and reaches to an equilibrium state at 20 laundering cycles. Cured samples show superior durability to not-cured samples. The effect of curing is more remarkably at higher chitosan concentration. In the case of chitosan oligomer, binding to cellulose fiber seems to be weaker than chitosan of MW 50,000. Compared with the fabrics treated with chitosan oligomer only, binder or crosslinker is not effective to improve the durability to laundering of chitosan treated fabrics.

Conclusion

Add-on of treated fabrics is increased with chitosan concentration, but is not dependent on curing and DAC. Antimicrobial activity is increased with chitosan concentration and reached to a maximum at 0.3~0.5 % depending on DAC. Antimicrobial activity is increased sharply with DAC up to 84 %. Curing improves the durability to laundering of antimicrobial activity of fabrics treated with chitosan of MW 50,000. Addition of binder or cross-linker increases add-on, but decreases the antimicrobial activity of treated fabrics with chitosan oligomer. Binder or crosslinker seems not effective to improve durability to laundering of the fabrics treated with chitosan oligomer.

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Antimicrobial Finishing of Polypropylene Nonwoven Fabric by Treatment with Chitosan

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Abstract

Three chitosans of different molecular weight having similar degree of deacetylation(DAC, 85~90 %) were used; ca. 1,800(oligomer), 40,000 and 180,000. PP nonwoven fabrics were treated with chitosan solution by pad-dry method. Antimicrobial activity was measured by the Shake Flask Method. PP nonwoven fabrics treated with chitosan oligomer showed high antimicrobial activities against *S. aureus*, *E. coli* and *P. vulgaris* at 100~500 ppm treatment concentration. However, it was not effective against *K. pneumoniae* and *P. aeruginosa* below 0.1 %. Chitosan of MW 40,000 was effective against *S. aureus* and *P. vulgaris* at 0.3 %, and *E. coli* at 0.5 %. Chitosan of MW 180,000 was effective against *S. aureus*, *E. coli* and *P. vulgaris* at 500 ppm, *K. pneumoniae* at 1.0 %, and *P. aeruginosa* at 0.5 %.

Keywords: Chitosan oligomer, chitosan, antimicrobial activity, polypropylene nonwoven fabric

Introduction

Chitosan, deacetylated chitin, is the second most plentiful natural polymer. Currently, chitin and chitosan are manufactured commercially in large scale from shellfish waste. They are being used in medical, hygienic, agricultural, food, cosmetics, waste treatment and textile areas because of its biocompatibility, non-toxcity, antimicrobial property, and so on (1-5).

Nonwoven fabric is a rapidly growing segment of textile industry. The applications of nonwoven fabrics range from disposable sanitary products through sophisticated medical fabrics, along with automotive filtration, and even fabrics used in spacecraft. Materials used for manufacturing nonwoven

fabrics are rayon, polyester, polypropylene, glass fiber, etc. Among them, polypropylene is easy to manufacture nonwoven fabrics by thermal bonding method.

The objective of this study is to investigate the applicability of chitosan to impart antimicrobial properties to PP nonwoven fabrics.

Material and Methods

Materials

100 % PP nonwoven fabric(thermal bonded, 26 g/m^2, 0.13 mm thickness) was used. Three chitosans of different molecular weight with similar degree of deacetylation(DAC, 85~90 %) were used; 1,800(oligomer), 40,000 and 180,000. Oligomeric chitosan sample was prepared in laboratory and chitosan of MW 40,000(Keum-Ho Hwasung Chem. Co., Korea) and 180,000(Protan, Norway) were obtained from commercial sources.

Treatment of nonwoven fabrics

Chitosan oligomer(MW 1,800) was dissolved in distilled water and other two chitosans in 2 % acetic acid solution. Fabric samples were padded with chitosan solution of various concentration to give 90~110 % pick-up. The padded samples were dried at 100 ℃ for 3 min and rinsed in distilled water except samples treated with chitosan oligomer.

Evaluation of treated fabrics

Add-on was calculated from the difference in sample weight before and after treatment. Antimicrobial activity of treated fabrics was evaluated by the Shake Flask Method in terms of bacteria reduction rate. Bacteria used are *Staphylococcus aureus*(ATCC 6538), *Escherichia coli*(ATCC 8473), *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (ATCC 13388). Air permeability was measured by Frazier apparatus according to ASTM D-737-75. Stiffness was measured by Cantilevel method according to ASTM D-1388-55. In order to test the absorbancy of treated nonwoven fabrics, liquid strike-through time was measured according to ERT 150.2-93 method using simulated urine.

Results and Discussion

Effect of chitosan concentration on the antimicrobial activity

Chitosan oligomer shows antimicrobial activity above 90 % reduction rate against *S. aureus* and *E. coli* at 500 ppm level and against *P. vulgaris* at 100 ppm of chitosan concentration as shown in Fig. 1. But it is not effective against *K. pneumoniae* and *P. aeruginosa* below 0.1 % of chitosan concentration. Chitosan oligomer is most effective against *P. vulgaris* causing diaper rashes.

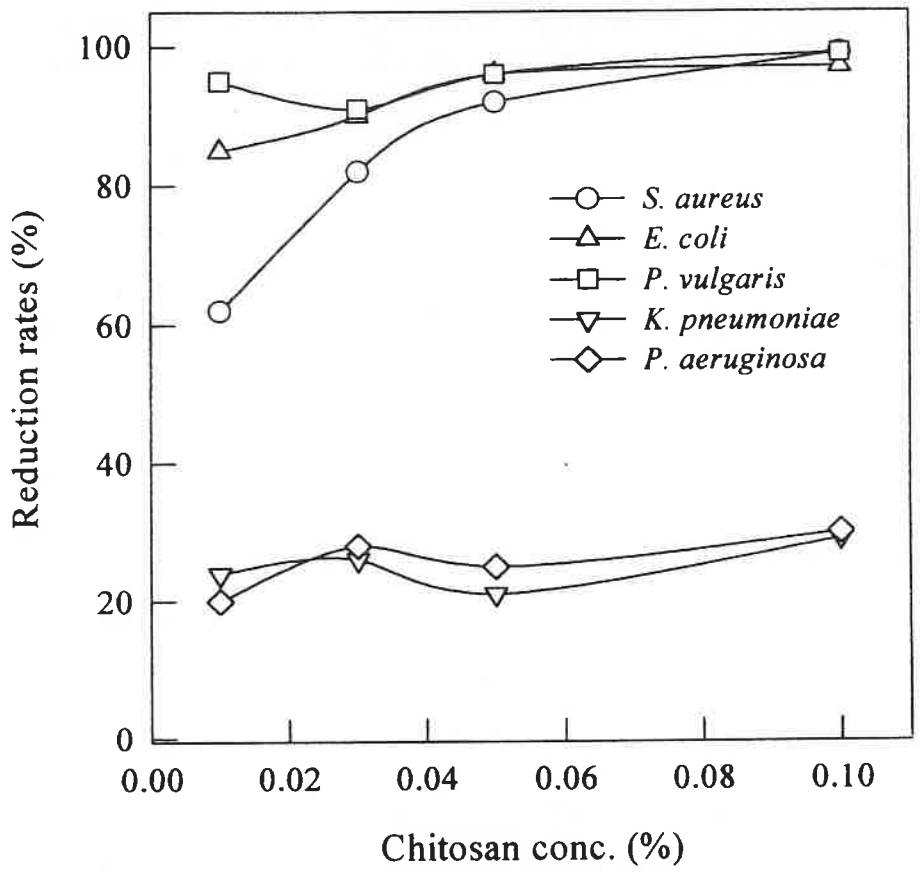

Fig. 1. Antimicrobial activity of chitosan oligomer.

Fig. 2 indicates that chitosan of MW 40,000 displays antimicrobial activity

against *S. aureus*, *E. coli* and *P. vulgaris* at 0.5 % level, which is 3~10 times higher concentration compared with chitosan oligomer. It is not effective against *K. pneumoniae* and *P. aeruginosa* below 1 %.

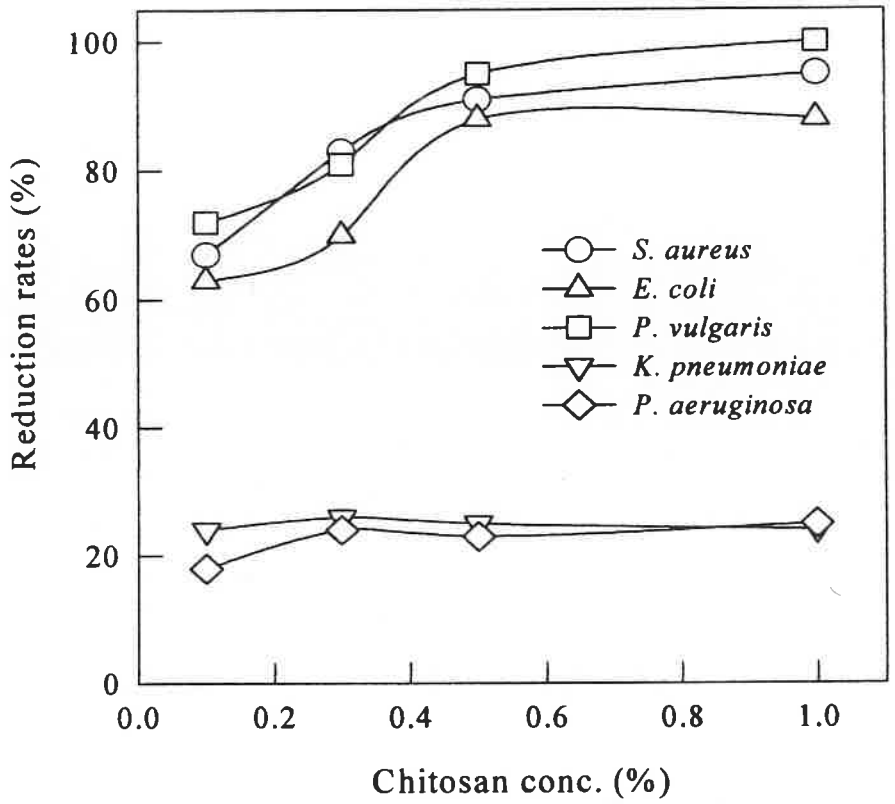

Fig. 2. Antimicrobial activity of chitosan of MW 40,000.

As shown in Fig. 3, chitosan of MW 180,000 is effective against *S. aureus*, *E. coli* and *P. vulgaris* at 500 ppm level, showing almost 100 % reduction rate. And it shows antimicrobial activity above 70 % of against *K. pneumoniae* and *P. aeruginosa* at 0.3~0.5 % level.

Effect of wetting agent on the antimicrobial activity

Wetting agent usually is added in the finishing formulation to improve the penetration of finishing solution. We tried to find the effect of wetting agent on the antimicrobial activity of chitosan. At 0.1 % level of Triton X-100(alkylaryl polyether alcohol), reduction rate of *S. aureus* is decreased up to 30~40 % as shown in Fig. 4. It is thought that electrostatic

interactions between cationic amine groups in chitosan and weak anionic ethylene oxide groups in wetting agent would be occurred. This causes to decrease the antimicrobial activity imparted by amine groups in chitosan.

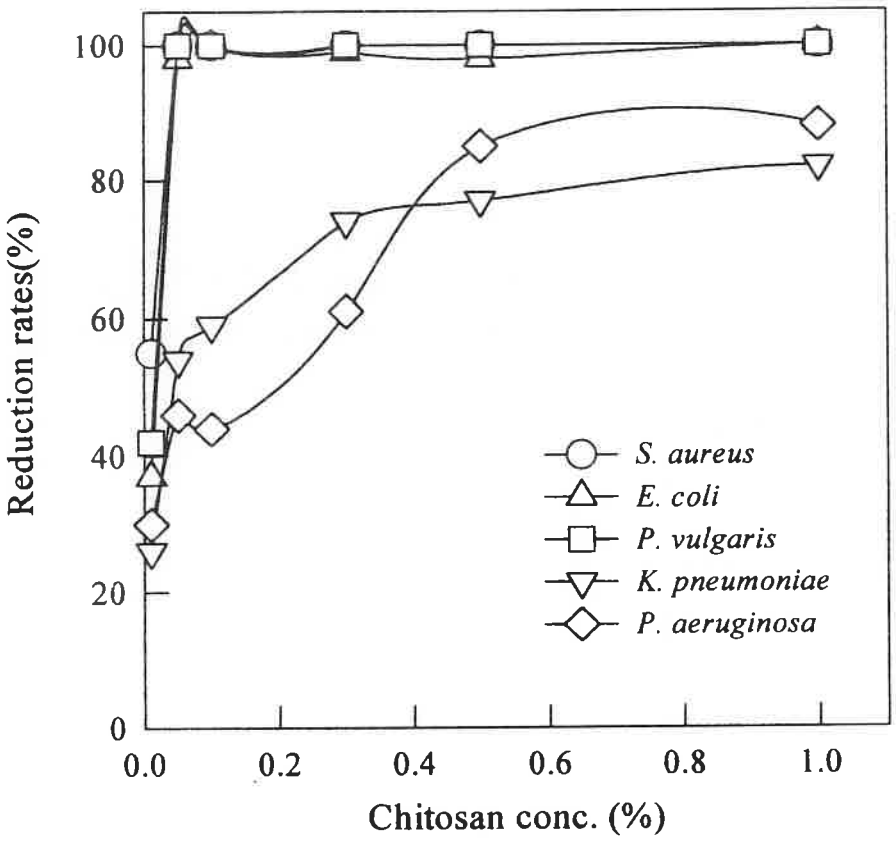

Fig 3. Antimicrobial activity of chitosan of MW 180,000.

Properties of treated fabrics

As shown in Table 1, add-on of samples treated with chitosan oligomer is not changed significantly up to 0.1 % treatment concentration. Add-on for the samples treated with chitosans of MW 40,000 and MW 180,000 is increased with the treatment concentration. Efficiency of add-on is higher for the chitosan of larger molecular weight.

Stiffness of samples is increased with the molecular weight of chitosan and treatment concentration. Chitosan of high molecular weight gives extensive surface deposition and makes fabrics stiffer.

Absorbency, indicated by strike-through time, is generally increased with

the treatment concentration increases. However, compared with the untreated, chitosan treatment makes fabrics less absorbent. Chitosan of low molecular weight makes treated fabrics more absorbent than those of high molecular weight. Therefore, chitosan oligomer is considered desirable for diaper end uses.

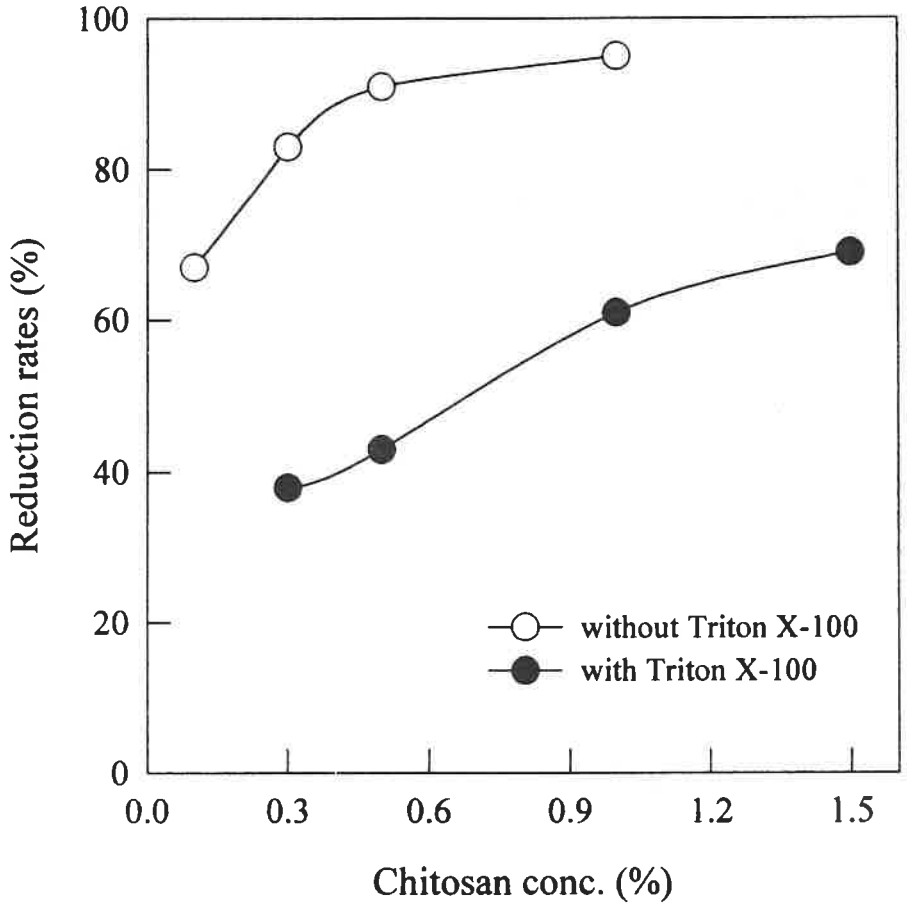

Fig. 4. Effect of wetting agent on the antimicrobial activity (MW 40,000, *S. aureus*).

Air permeability decreases as chitosan concentration increases. And the higher molecular weight of chitosan, air permeability decreases more at same chitosan concentration.

Table 1. Properties of nonwoven fabrics treated with chitosan

Chitosan sample (MW)	Treatment conc. (%)	Add-on (%)	Stiffness (cm)	Air permeability (cc/㎠ · sec)	Strike-through time(sec)
Untreated	-	-	2.55	269	2.62
1800	0.05	-	2.75	271	3.60
	0.1	-	2.85	264	3.23
	0.5	-	2.87	260	3.02
	1.0	-	2.98	243	2.93
40,000	0.1	0.32	3.13	259	5.86
	0.3	0.31	3.33	254	5.73
	0.5	0.38	3.45	243	5.17
	1.0	1.14	3.63	240	4.34
180,000	0.05	0.33	2.70	263	7.15
	0.1	0.36	3.04	250	6,54
	0.5	0.77	3.82	228	6.05
	1.0	1.43	3.95	221	6.07

Conclusion

Chitosan oligomer displays antimicrobial activity above 90 % of reduction rate against *S. aureus* and *E. coli* at 500 ppm of treatment concentration and against *P. vulgaris* at 100 ppm. But it is not effective against *K. pneumoniae* and *P. aeruginosa* below 0.1 % of treatment concentration. Chitosan of MW 40,000 shows reduction rate above 90 % against *S. aureus*, *E. coli* and *P. vulgaris* at 0.5 % level. It is not effective against *K. pneumoniae* and *P. aeruginosa* below 1 % of treatment concentration. Chitosan of MW 180,000 is effective against *S. aureus*, *E. coli* and *P. vulgaris* at 500 ppm level showing almost 100% reduction rate. And it shows reduction rate above 70 % against *K. pneumoniae* and *P. aeruginosa*.

Chitosan treated fabrics shows higher add-on, stiffer handle, higher absorbency and lower air permeability with the increase of molecular weight and treatment concentration.

Acknowledgements

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DEGRADABLE CHAFF FROM CHITOSAN FIBRES

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ABSTRACT

An engineering study is being conducted in order to develop environmentally friendly fibres to be metallized and used in the form of radar-countermeasure chaff. Presently, chaff is made of glass fibres coated with aluminum. In order to be degradable, the substrate glass must be replaced with an environmentally friendly material. The material of choice for this project is chitosan. To generate fibres from chitosan, a solution spinning process is required.[1] A wet spinning system has been constructed to accomplish this task. Typically, 20 filament yarns are spun using a 5% by weight chitosan in 2% by volume acetic acid solution extruded into a 1M potassium hydroxide bath. Average denier of the yarns is 70-80 denier or about 23 micrometers in diameter. The average moduli of the yarns is 40-50 g/denier. Chaff fibres, however, require moduli that are greater than 100 g/denier. This paper discusses an improved wet spinning system and preliminary results concerning crosslinked chitosan fibres for improved moduli.

Keywords: chitosan, chaff, fibres, coagulation, drying, crosslinking, glutaraldehyde.

INTRODUCTION

Chaff is a radar countermeasure system. Chaff is typically packaged as bundles of very small glass filaments coated with aluminum. The overall size of the filaments is approximately 20 micrometers in diameter. These filaments are cut to specific radio wavelengths. When chaff is dispersed behind an aircraft, the metallized filaments create a decoy for enemy radar. This is illustrated in Figure 1.

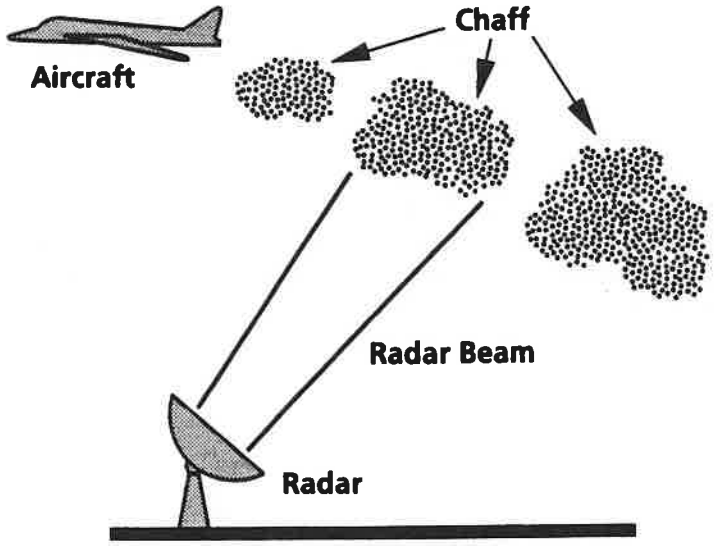


Figure 1. An illustration of an aircraft dispensing chaff.

The problem with chaff is that it may be harmful to people and the environment. The solution is to make a degradable chaff filament. Such a degradable filament can be achieved using chitosan to replace the glass substrate in chaff.

In this paper, we describe an improved wet spinning system for fabricating chitosan fibres and some preliminary crosslinking results.

MATERIALS AND METHOD

Chitosan, lot # 740026, was obtained from Pronova Biopolymer of Redmond, WA as a 60 mesh powder. This was a crab based chitosan with a claimed viscosity of 1000 mPas. Its degree of deacetylation was verified using a method of UV spectrophotometry as developed by Muzzarelli.[2] The main absorbance occurred in the range of 200-204 nm and the DD value was found to be 84.9 + 0.2%. The average molecular weight, Mv, was determined based on intrinsic viscosity as measured in a Ubblelohde viscometer with a solvent of 0.1M acetic acid/0.2M NaCl and was calculated to be 7.73 x 105 g/mol. The viscosity average molecular weight was calculated based on the Mark-Houwink results of Rinaudo.[3] The following chemicals were all obtained and used as reagent grade from Aldrich: glacial acetic acid, sodium hydroxide pellets, ethanol, methanol, propanol, acetone, potassium hydroxide pellets, ammonium hydroxide, lithium hydroxide powder, tri-sodium orthophosphate powder, and sodium bicarbonate powder.

Building of the Wet Spinning System

In order to fabricate the chitosan fibres, a wet spinning system has been set up following the method of Qin.[4] The wet spinning system is illustrated in Figure 2.

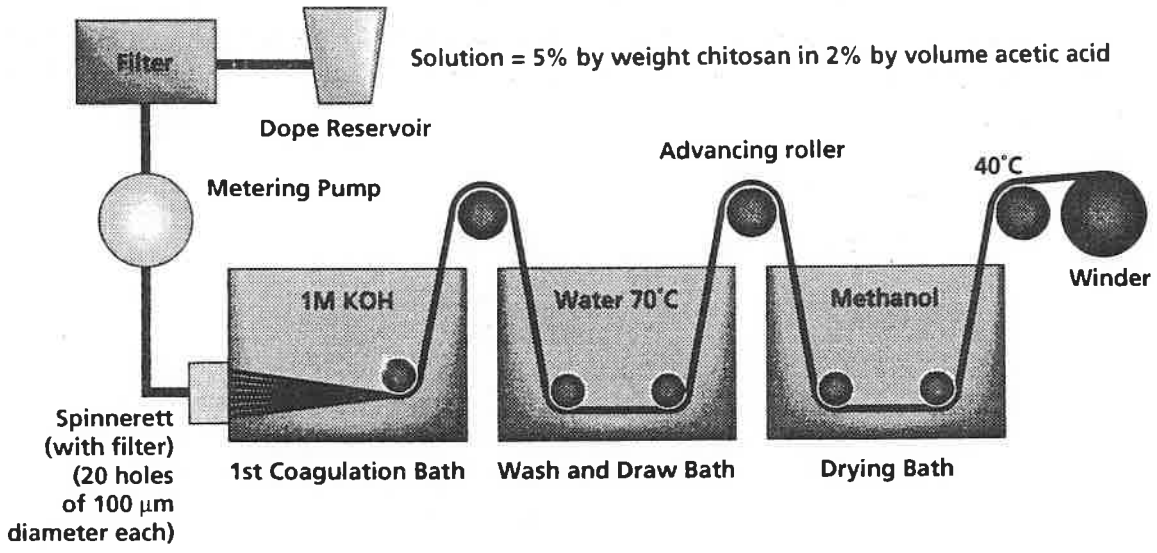
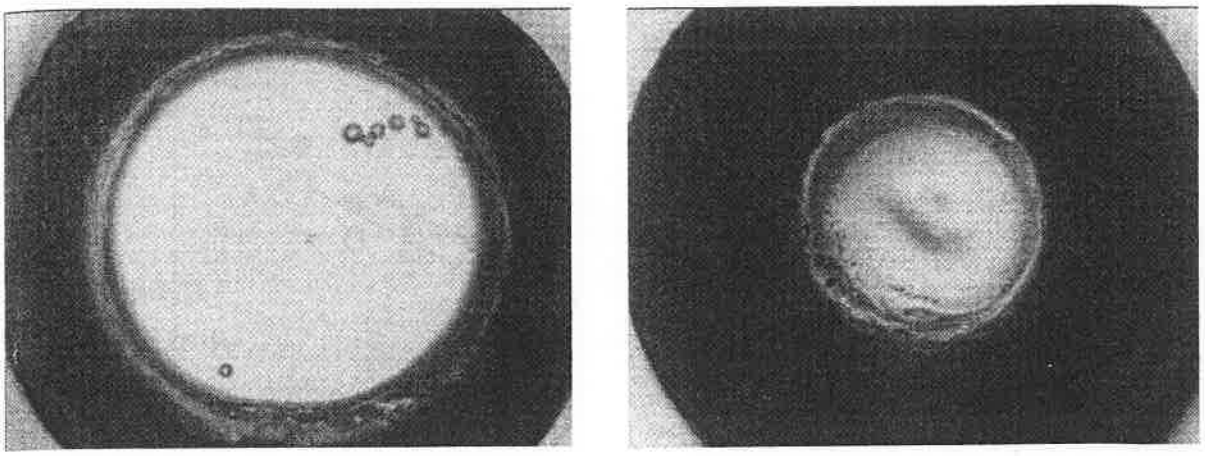


Figure 2. An illustration of the wet spinning system.

Optimizing the Coagulation Bath

The coagulation properties of some mixtures of 5% by weight chitosan in 2% by volume aqueous acetic acid were investigated with the goal of determining the optimum coagulation conditions

for the spinning of chitosan fibres. Solutions of 5% by weight chitosan in 2% by volume aqueous acetic acid were prepared, filtered, and extruded through a large diameter hole syringe (the exact diameter of the hole was 2 mm) into coagulation baths of varying composition that were all strongly basic in nature, at least pH 12 or greater. For each coagulant, time was varied between 22 seconds and 2 minutes at 23 $\pm$ 0.3 °C. Throughout all of the experiments, a distinct moving boundary between coagulated and uncoagulated polymer was observed within the cylindrical shaped polymer fibres. This distinct moving boundary is illustrated in Figure 3.



A. Photomicrograph after 45 seconds of submersion in 70% 1M NaOH / 30% ethanol.

B. Photomicrograph after 105 seconds of submersion in 70% 1M NaOH / 30% ethanol.

Figure 3. Photomicrographs of chitosan fibre cross-sections showing moving boundary between coagulated and uncoagulated polymer.

Optimizing the Drying System

For this study, chitosan yarns were spun through the spinning system. The coagulation bath used was 1M NaOH and a wash & draw step was not incorporated. Thus, only two baths were used. In the second bath, chitosan yarns were dried using various drying agents followed by a heated roller at 40 °C, and then were wound up.

Crosslinking the Chitosan Fibres

Chitosan fibres were wound onto skeins and submerged into water-based solutions of glutaraldehyde at a concentration of 5 g/litre. Both the time of submersion and the temperature of the crosslinking solutions were varied. To measure the degree of crosslinking, the samples were placed into 5% aqueous acetic acid solutions for 24 hours. After 24 hours, the degree of swelling was analysed by measuring the fibre diameters under a microscope following the method of Wei et al.[5]

RESULTS

The results of the coagulation study are shown graphically in Figure 4.

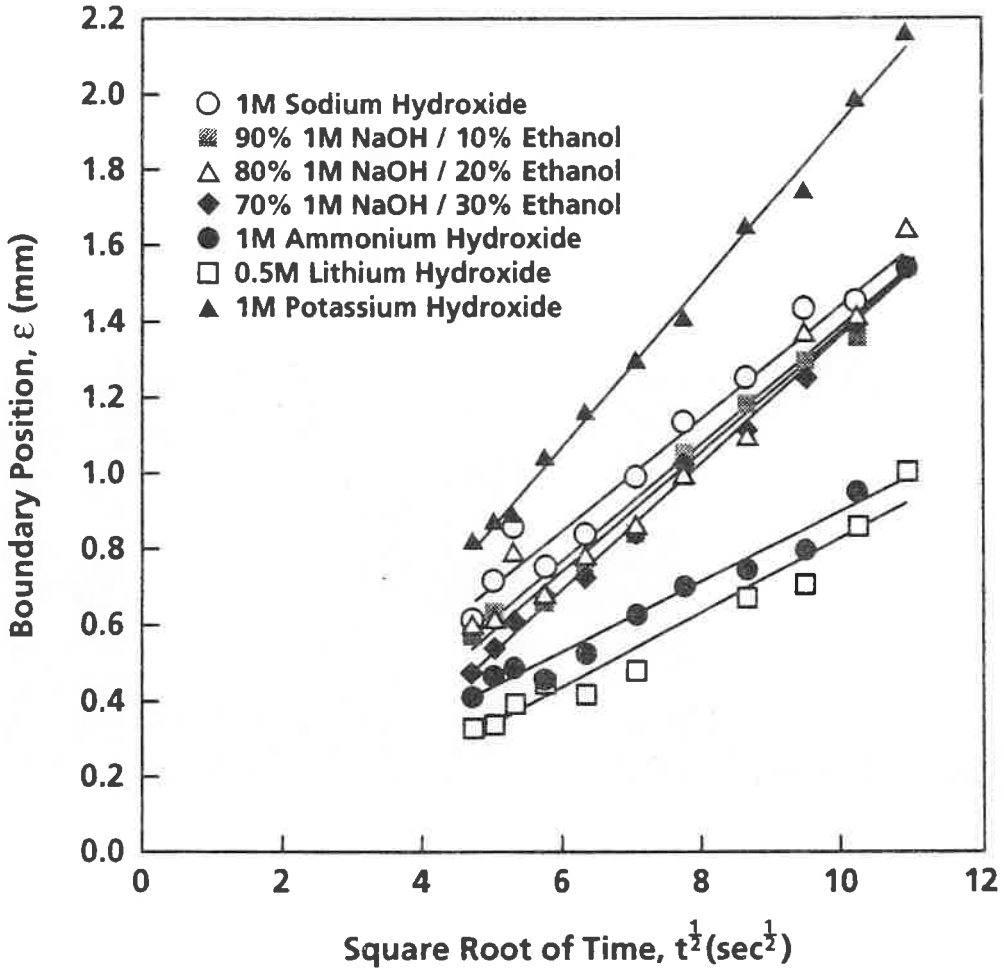


Figure 4. Boundary position as it relates to time, based on Fick's 2nd Law, for chitosan fibres in different coagulating agents.

The results of the drying study are listed in Table 1. Based on these results, it was decided to incorporate a 1 m long methanol bath into the spinning system.

Table 1: Mechanical Properties and Diameters of Fibers Produced Using Various Drying Agents in the Wet Spin Process.

Note these results are for yarns with a filament count of 18 ± 2 filaments.

Drying Agent	Filament Diameter (μm)	Initial Modulus (g/denier)	Breaking Strength (g force)	Tenacity (g/denier)	Elongation at break (%)
Acetone	41.0	7.2	39.8	0.20	16.1
Isopropanol	33.5	14.6	37.4	0.28	9.0
Ethanol	28.8	26.2	108.6	1.00	14.5
Methanol	24.9	28.2	93.5	1.05	18.5
Methanol with Draw on Yarn	21.2	40.0	91.1	1.30	5.7

The results of the crosslinking study are presented in Table 2 below. For each sample, the diameter of the dry fibre before crosslinking was compared to the diameter after swelling for 24 hours. Based on this comparison, a percentage degree of swelling was derived.

Table 2: % Swelling for Crosslinked Chitosan Fibres Submerged in 5% Aqueous Acetic Acid

Time (min.)	% swell 25°C	% swell 30°C	% swell 40°C	% swell 50°C	% swell 70°C
0	dissolved	dissolved	dissolved	dissolved	dissolved
0.5	dissolved	dissolved	dissolved	dissolved	dissolved
1	94.74	93.15	86.84	86.84	72.22
2	92.31	87.18	77.27	77.27	64.29
3	88.10	84.38	75.00	75.00	58.33
5	84.38	77.27	64.29	64.29	58.33
10	80.77	75.00	58.33	58.33	64.29
20	77.27	72.22	58.33	58.33	64.29
30	72.22	68.75	58.33	58.33	58.33
45	66.67	58.33	58.33	58.33	58.33
60	58.33	58.33	58.33	58.33	58.33

DISCUSSION

The results of the coagulation study, as seen in Figure 4, demonstrate a straight line relationship between boundary position, $\varepsilon(t)$, and the square root of time spent in the coagulation bath. This relationship is in accordance with Fick's 2nd Law. Ziabicki[6] has modeled the theory of diffusion with a moving boundary in wet spinning by assuming a linear, one-dimensional system containing component 'b' into which the other component 'a' migrates from the outside and instantaneously reacts with 'b'. Such relationships regarding boundary motion in a wet spinning system have also been studied by Hermans[7], Paul[8], and most recently by Liu[9].

In Paul's[8] 1968 results involving a copolymer of acrylonitrile and vinyl acetate, it was evident that the linear relationship between boundary position and time was valid until 30% penetration of the boundary, after which Paul's results begin to deviate from linearity. Liu[9] reported similar results for the extent of linear agreement of Fick's 2nd Law. The results presented in Figure 4 show that Fick's 2nd Law for a semi-infinite medium is valid until at least 72% penetration of the chitosan sample by the coagulant. Based on the results of the coagulation study, it was decided to use 1M KOH as the coagulant for wet spinning chitosan fibres.

The tabulated results of the drying study as seen at Table 1 demonstrated that the best fibre mechanical results were achieved by drying the yarns using either pure ethanol or pure methanol. Further measurements were taken by observing the surface morphology of the fibres under a scanning electron microscope (SEM) and by measuring moisture content using thermal gravimetric analysis (TGA). The SEM and TGA results both confirmed that methanol gave a drier and smoother sample than did drying in ethanol.

The results of the crosslinking study demonstrated swelling, however, there was a large uncertainty associated with this measurement because it is difficult to focus the microscope on the swollen fibre. Furthermore, with more crosslinking time and at higher temperatures, the fibres become more and more brown in colour suggesting that some side reactions may be occurring. Mechanical testing of the fibres was also performed but showed little indication of an increase in stiffness or initial modulus.

CONCLUSION

Fabrication of a wet spinning system for chitosan fibres has been completed. The coagulation bath was optimized and a 1M KOH agent is being used as the coagulating agent. The drying system was also optimized and a pure methanol bath followed by a heated roller is being used to dry the chitosan yarn.

With regard to the coagulation study, it was determined that of all the coagulants tested, 1M KOH gave the fastest rate to precipitation at room temperature. It is concluded also that the results of the study follow Fick's 2nd Law for a linear, semi-infinite medium to 72% penetration of the boundary.

The results of the drying study further demonstrated that methanol as a drying agent, as compared to acetone, ethanol, and isopropanol, yielded fibres with the highest mechanical properties and the smoothest surface morphology.

The results of the crosslinking experiments demonstrated an increase in fibre swelling with crosslinking time and crosslinking temperature. The uncertainties in the swelling measurements are however very large. More experimentation is required to optimize the swelling measurements and to look more closely at mechanical properties.

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The dyeing properties of chitin/ cellulose mixed fibers

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Abstract

Recently, chitin and its derivatives have been used in various fields, for example, as fibers. In this work, the fibers prepared by a wet sppining from the mixed solution of chitin viscose and cellulose viscose were examined for their dyeing properties with an acid dye (C.I.Acid Orange 7) and several reactive dyes. The chitin/ cellulose composite fibers containing the chitin content of 3%, 10%, and 20% were designated CR(3), CR(10), and CR(20), respectively. The dyeing isotherms of the dye on the fibers were measured at various pH and compared with those of silk and wool. The extent of dyeing depends markedly on pH in dyebath and increases with decreasing pH. The dye is exhausted by the fibers at the same pH of dyebath in the following order: CR(20)> wool >CR(10)> silk>_CR(3). This order corresponds to the content of amino groups in the fibers. The behavior and thermodynamic parameters_of dyeing for CR(3) were found to be very similar to those for silk. This suggests that the electrostatic force between the positively charged amino groups in the fibers and the dye anion plays a significant role in the dyeing.

_The dyeing properties of CR( ) were also examined with a monochlorotriazinyl, a dichlorotriazinyl, a sulfatoethylsulfonyl dyes, and the reactive dyes having two reactive groups .

Keywords: Chitin/cellulose, composite fibers, acid dye, reactive dye, dyeing property, dyeing isotherm, dyeing rate

Materials and Methods

CR( ), viscose rayon, and wool in yarn states were used after washing in an aqueous solution of non-ionic surfactant for 1h at 80°C. Silk scoured in an aqueous solution of sodium carbonate was used. C.I.Acid Orange 7(OR) was synthesized from diazotized sulfanilic acid and 2-naphthol and purified by Robinson-Mills method. The dyeing with OR was performed as follows: 0.01g of a fiber sample was hung on stenless steel and immersed in 100ml of an acetate buffer solution of pH4 containing various amounts of OR and was dyed at a constant temperature until equilibrium was reached. The free dye concentration was determined by

colorimetry of a residual dye solution in dyebath. The dye uptake was determined by colorimetry of the extract with formamide from a dyed sample.

The reactive dyes used were Cibacron Blue P-BR(C.I.Reactive Blue 5, monochlorotriazinyl dye), Remazol Brilliant Blue R(C.I.Reactive Blue 19, sulfatoethylsufonyl dye, purified by acetone-DMSO method), Procion Red M-2B(C.I.Reactive Red 1, dichlorotriazinyl dye), and C.I.Reactive Red194(monochlorotriazinyl/sulfatoethylsulfonyl dye). The dyeings with reactive dyes were performed as follows: 0.01g of fiber samples were soaked in the dye solution of various pH containing dye of 0.005g for 1h at 80°C (25°C in case of Red 1). The exhaustion was determined by substrating a residual dye amount from a initial dye amount. The fixation was determined by an additional substration of an extracted dye amount with formamide from a dyed sample.

When the exhaustion is small, this quantitative method contains large errors. Thus, the reflection of a dyed sample was measured by colorimetry at a specific wave length. The K/S was estimated from Kubelka-Munk equation(1).[1)]

$$K/S = (1-R^1)^2/2R^1 - (1-R^\circ)^2/2R^\circ \qquad (1)$$

K: Extinction coefficient, S: Scattering coefficient

R^1: Reflection of dyed sample, R°: Reflection of undyed sample

Results and discussion

Dyeing properties for an acid dye(at equilibrium)

The dye uptake by CR(20) was strongly influenced by pH of the dye bath. The adsorption of OR by CR(20) increased with the decrease of pH. The dyeing isotherms of OR on CR(3) , CR(10), and CR(20) were measured in an acetate buffer solution of pH 4 at 30°C, 40°C and 50°C. The adsorption of OR by CR( ) was the sigmoid type. The lower the temperature, the more the dye uptake, indicating that this adsorption is exothermic. The dye uptake varied in the following order: CR(20)> CR(10)> CR(3), which corresponds to the content of chitin in the composite fiber. For comparison, the dyeing isotherms of wool, silk, and viscose rayon were measured under the same conditions. The dye uptake at equilibrium decreased in the order: CR(20)> wool> CR(10)> silk> CR(3)» viscose rayon. The difference in the dye uptake are ascribed to the difference of the content of the amino groups in the amorphous region in the fiber. Therefore, the adsorption of OR by these fibers is responsible for the binding of dye anion to the protonated amino groups. Let us suppose that this adsorption is approximated by the Langmuir type. For the adsorption of Langmuir type, the Klotz equation(2) holds.[2)]

$$1/r = 1/(r \times n) \times 1/c + 1/n \qquad (2)$$

where, r: dye uptake(mol/10^5g fiber), c: the concentration of free dye(mol/L) at equilibrium, K: binding constant, n: all sites efficient to the binding

The reciprocal plot of r and c was a straight line. The first binding constant k_1(= nxK) was calculated from the slope in its line, and thermodynamic parameters for binding were determined. The thermodynamic parameters for binding of OR by CR( ), silk, and wool were listed in Table 1.

Table 1 Thermodynamic parameters for binding of C.I.Acid Orange 7 by chitin/cellulose composite fibers, silk, and wool

Fiber	Temperature (° C)	k_1 x 10^{-5}	ΔF° (cal/mol)	ΔH° (cal/mol)	ΔS° (eu)
CR(3)	30	2.564			
	40	1.904	-6330	-8260	-6.17
	50	1.103			
CR(10)	40	6.939			
CR(20)	40	7.042			
Silk	40	1.844	-5880	-8220	-7.41
Wool	40	8.621			

The behavior and thermodynamic parameters of dyeing for CR(3) were found to be very similar to those for silk. This suggests that the electrostatic force between the positively charged amino groups in the fibers and the dye anion plays a significant role in the dyeing.

The dyeing rate of OR for CR( )

Fig.1 shows the dyeing rates of OR for CR( ), wool, and silk measured for up to 900 sec in an acetate buffer solution of pH 4 at 40°C. The dyeing rates decreased in the following order: CR(20)» CR(10)» silk> CR(3) =wool. The dye easily penetrates into CR( ) fiber, whereas its penetration into the wool fiber becomes difficult owing to its scale structure.

Dyeing properties for reactive dyes

The exhaustion and the fixation of C.I.Reactive Blue 5 by CR(10) and CR(20) were measured in the buffer solution of various pH at 80°C and 1 h. For CR(10) and CR(20), the exhaustion was high at low pH and decreased with the increase of pH. The fixation was

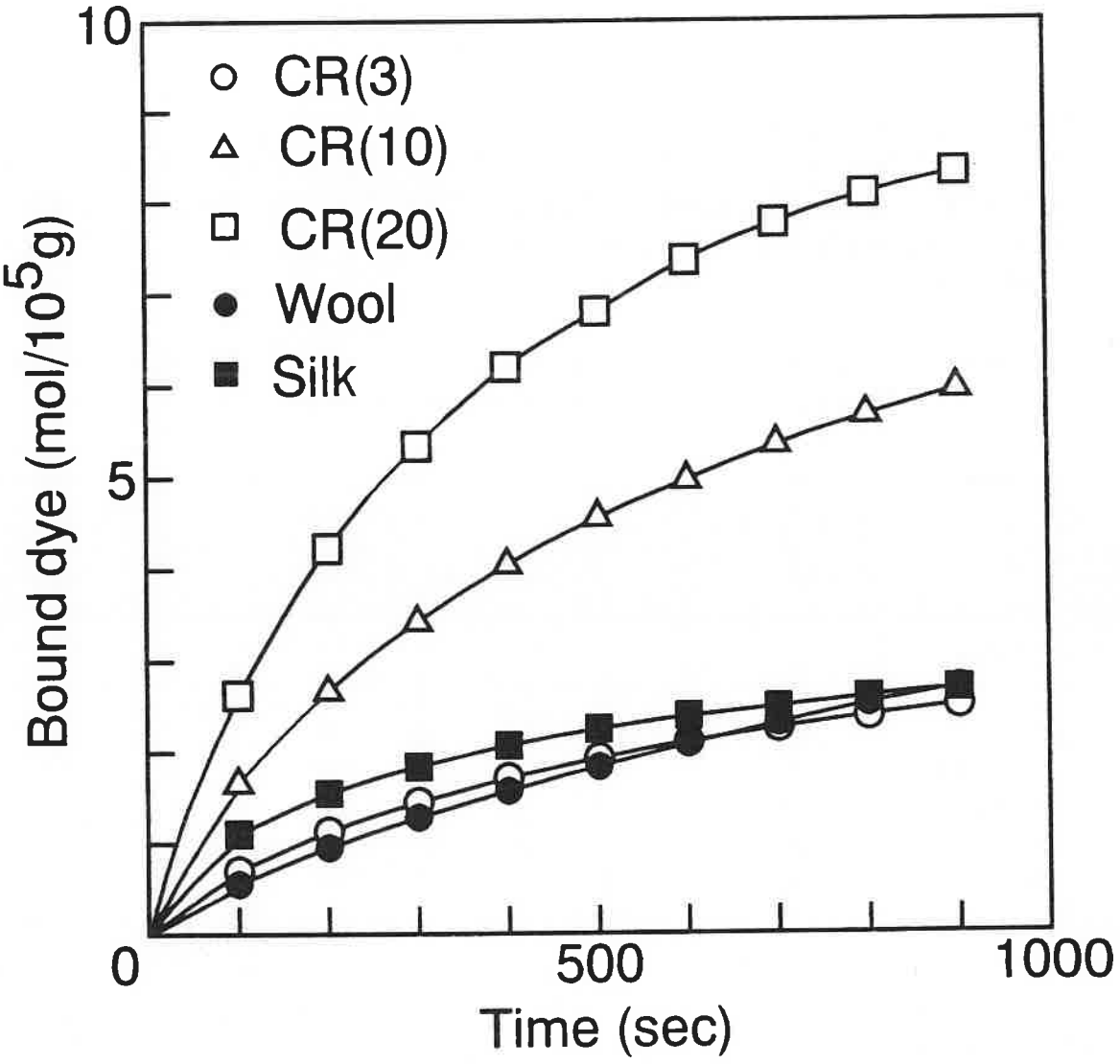

Fig.1 The dyeing rates of C.I.Acid Orange 7 for CR( ), wool, and silk in an acetate buffer solution of pH 4 at 40°C.

maximum in the vicinity of pH 6. Blue 5 was exhausted and fixed slightly by CR(20) in the range over pH10. Blue 5 did not fix for viscose rayon below pH 10 and did slightly over pH 10. It is apparent also from the measurement of K/S of the viscose rayons which were dyed with Blue 5 and followed to be treated with formamide.

The exhaustion and the fixation of C.I.Reactive Blue 19 by CR(3), CR(10), CR(20) and viscose rayon were measured. The exhaustion and the fixation were very high in weak acidic and weak alkaline regions. The exhaustion and the fixation decreased in the following order at the same pH: CR(20)> CR(10)> CR(3). Viscose rayon did not exhaust and fix Blue 19 below pH 8. The relation between K/S and pH reflects well this fact. When the partially deacetylated chitin was dyed with Blue 19, the same tendency as the case of CR( ) was observed.

few sorts of reactive dyes in weak acidic and weak alkaline regions in which viscose rayon was not exhausted and fixed.

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CHITOSAN TREATMENT ON WOOL PRETREATED WITH COLD PLASMA

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Abstract

The oxidative plasma treatments of wool at low temperature and pressure (glow discharge) modify restrictively the cuticle surface of the fibers improving its surface wettability, dyeability, fiber cohesion and shrink-resistance. However, the shrink-resistance properties obtained by plasma treatment do not impart a machine-washable finish, which is one of the end-users demands .

In this paper the biopolymer chitosan application on O_2 or air plasma treated wool is studied. The results obtained based on contact angle data, show that the surface of plasma treated wool fibers becomes hydrophilic and, therefore, the chitosan adsorption increases. Consequently the shrink-resistance of plasma treated wool is appropriatelly enhanced. A new ecological shrink-proofing wool process is proposed.

Keywords: chitosan, wool, oxidative plasma, shrink-resistance.

Introduction

The oxidative plasma treatments of wool at low temperature and pressure (glow discharge) modify restrictively the cuticle surface of the fibers improving its surface wettability. dyeability, fiber cohesion and shrink-resistance [1-4]. The use of plasma treatment in wool finishing is highly advantageous since this type of treatment is an environmentally acceptable alternative to the conventional chlorination finishing processes. However, the shrink-resistance properties obtained by plasma treatment do not impart a machine-washable finish, which is one of the end-users demands .

In order to increase the shrink-proofing or anti-felting properties a further treatment such as deposition of polymers on wool fibers is needed [5]. In recent years biopolymers have attracted a great deal of scientific and industrial interest as possible substitutes for synthetic polymers. The polysaccharide-based biopolymer chitosan, owing to its biocompatibility, biodegradability, water-binding capacity, and non toxic properties [6,7] is a possible substitute for synthetic polymer in wool finishing. Chitosan has been used as a shrink-resist agent [8,9,10] and as an agent for improving the dyeability of wool [11,12]. This paper includes the assessment of the shrink-resistance effect obtained on wool fibers pretreated with oxigen or air cold plasma and then treated with chitosan, as a possible new ecological wool finishing process. Also, the adhesion and the spreading of chitosan on the surface of wool fibres based on contact angle data and scanning electron microscopy observation are qualitatively evaluated.

Materials and methods

Two different botany knitted wool fabric were used : (A) cover factor 1.28 tex/mm supplied by the IWS (UK), and (B) cover factor 1.06 tex/mm supplied by a textil industry. Wool samples were thoroughly cleaned before use [12].

Low temperature plasma of glow discharge (GD) treatments were carried out in a plasma reactor (Model Technics Plasma 200-G) using air and O_2 as plasma gas. A sample of wool was placed in the vacuum chamber, which was evacuated to a pressure of about 0.1 mbar before air and O_2 were introduced.

Chitosan : low molecular weight , Mr 70,000, supplied by Fluka and without further purification.

Chitosan treatments: chitosan was dissolved in distilled water containing acetic acid (3g/l). Treatments were carried out by exhaustion. Liquor ratio 20:1,25°C,60 min. Treated samples were let to dry at room temperature.

Staining: a staining technique to obtain a qualitative assessment of the presence of chitosan in wool fibers was used. Wool samples were treated with the dye C.I.Reactive Red 180 at 1.2% owf , liquor ratio 60:1, 50°C, 5 min.

Color measurement of stained samples was carried out with a Macbeth Color Eye 3000 diffusion reflectance spectrophotometer under illuminant D_{65} using 10° observer.

Contact angle hysteresis were carried out with a KSV SIGMA 70 electrobalance. A single fiber was attached to an end of the arm of an electrobalance and suspended above a beaker containing water [13] . The perimeters of the fibers were determined from the wetting force with decane which wets perfectly the fiber [14] . Contact angles were calculated according to the equation : $F/L = \gamma_L.\cos\theta$;where: F=wetting force average, L=perimeter of the fiber, γ_L= surface tension of water, θ = contact angle .

Shrink resistance testing was carried out in a Wascator Model FOM 71 (IWS test method No. 31). When the area shrinkage is lower than 8% after two 5A cycles, wool is considered as "machine washable".

SEM observations were done with a Hitachi S-570 electron microscope. Samples were mounted and gold sputtered under vacuum previously to SEM observation.

Results and discussion

1. Shrinkage properties

1.1 Influence of O_2 or air plasma pretreatment

The area shrinkage results for "A" wool samples pretreated for 1 min in oxigen or air plasma generated at a pressure of 1mbar with different power and then aftertreated with chitosan (0.75% w/v) are given in figure 1. The area shrinkage of untreated and chitosan (0.75% w/v) treated samples are also included.

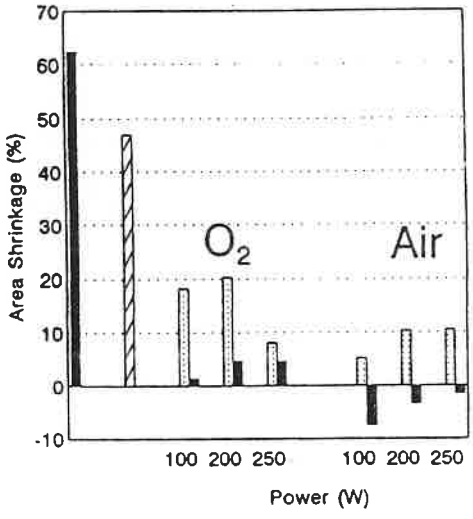


Figure 1.- Area shrinkage after two cycles of 5A Wascator shrinkage test of "A" wool: Untreated [■] , treated with chitosan [▨], treated with O_2 or Air plasma for 1 min [▣], and treated with plasma-chitosan [■].

As expected, there is a significant reduction in shrinkage area after 1 min of O_2 or air plasma pretreatment [15] . However, the shrinkage area obtained are higher than 8% after two 5A cycles Wascator shrinkage test and, consequently, it does not fulfil the end users demands.

Chitosan application on untreated wool causes only a certain reduction in area shrinkage, but chitosan application on O_2 plasma pretreated wool, provides area shrinkage values lower than 8%, which corresponds to a machine-washable finishing. Chitosan aplication on air plasma pretreated wool gives rise to an increase on the area shrinkage.

1.2 Influence of chitosan concentration

Figure 2 shows the area shrinkage of "B" wool samples treated with air plasma and air plasma-chitosan at different concentrations (0.25%, 0.50% and 0.75% w/v). Air plasma treatment were 2 or 5 minutes at 200W. For comparison purposes the results of untreated and chitosan treated "B" wool are also shown.

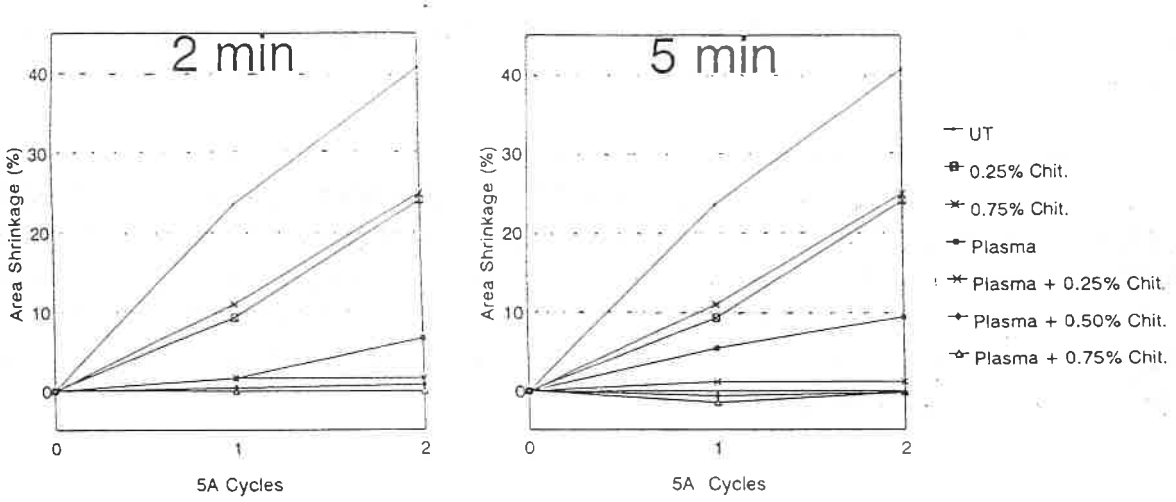


Figure 2.- Area shrinkage as a function of the number of the 5A cycles of Wascator shrinkage test of "B"wool.

The area shrinkage slightly decreases when chitosan concentration increases. A chitosan concentration of 0.25% (w/v) is enough to obtain unshrinkable wool. As expected, the level of shrink-resist effect reached is scarcely not influenced by the plasma pretreatment time.

2. Qualitative evaluation of chitosan adsorption on wool.

It was carried out by staining wool samples with C.I. Reactive Red 180, and then the color intensity was measured (Figure 3).

The color intensity of the different plasma-chitosan treated samples is similar between them but higher than those of untreated, plasma pretreated and chitosan treated wool samples. It is evident that higher chitosan adsorption occurs on plasma pretreated wool. Therefore it can be concluded that the chitosan adsorption on wool fibres is promoted by the oxidative plasma treatment.

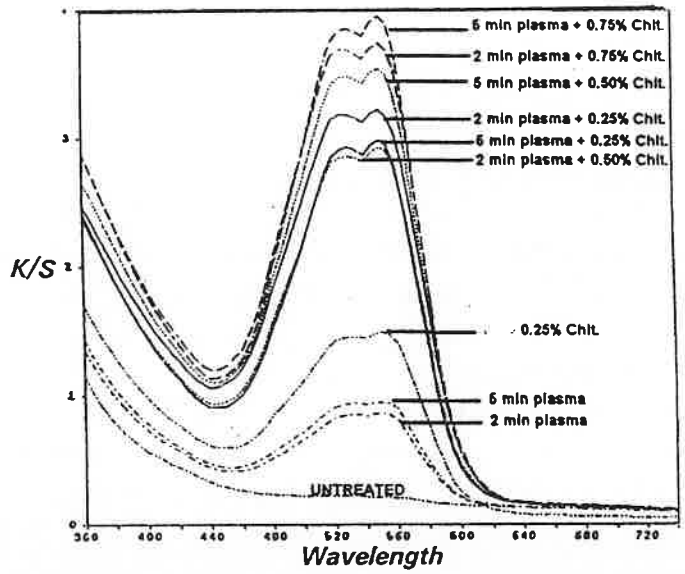


Figure 3.- The color intensity, expressed by K/S values, of untreated and treated wool samples.

3. Wettability

It is known that wool fibers subjected to an oxidative plasma process acquire on its surface new hydrophilic groups and, moreover, a partially removal of covalently bounded fatty acids belonging to the outermost surface of the fiber (fatty layer) takes place [16-18]. Considering these superficial modifications, the advancing θ_a and receding θ_r water contact angle of plasma treated wool fibres were determined and compared to untreated wool (UT) (figure 4 and table 1).

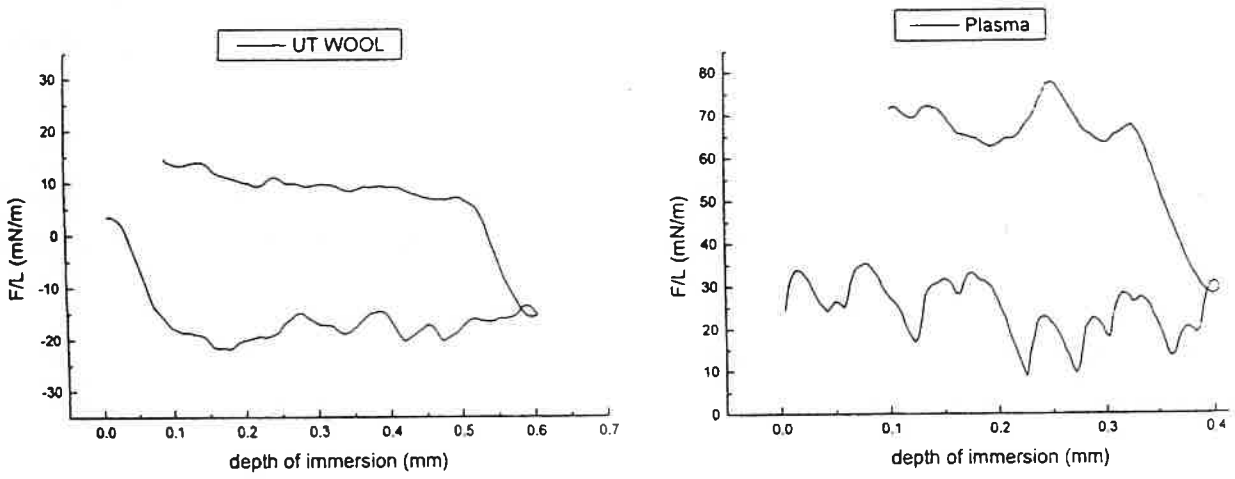


Figure 4.- The contact angle hysteresis for untreated fiber/water/air interface and for air plasma fiber/water/air interface. Air plasma treatment: 1 mbar, 200 W, 2 min.

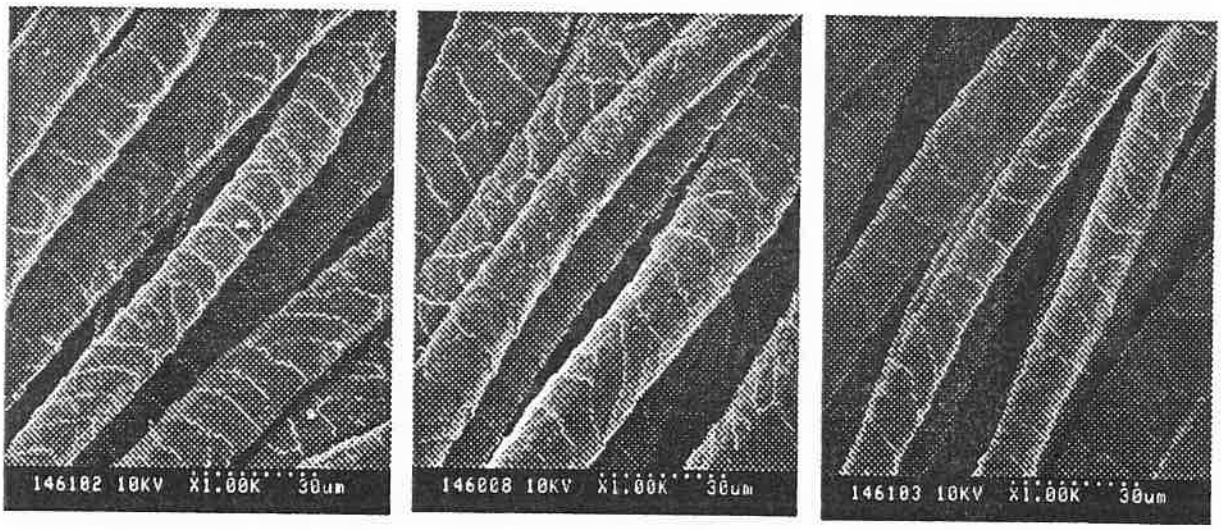
Table 1.- Values of advancing (θ_a) and receding (θ_r) water contact angle

	θ_a	θ_r
UT	104°	81°
Plasma treated	70°	19°

These results indicate that the surface of air plasma treated wool fibres becomes in hydrophilic character and consequently the spreading and adhesion of the hydrophilic chitosan could be enhanced. Similar results have been recently reported for oxigen plasma wool fibres aftertreated with different commercially shrink resist polymers [5].

4. Electron micrographs.

In order to assess the uniformity of the chitosan sorbed on wool fibre surface, SEM observations were carried out (Fig. 5).



(a) (b) (c)

Figure 5.- SEM photographs of : (a) 2min air plasma , (b) 2min air plasma + 0.25% Chit and (c) UT+0.25% Chit.

The air plasma treatment causes a minimal damage to the scale structure. Unexpectedly, chitosan is not visible neither on chitosan nor on plasma-chitosan treated wools and it seems that inter-fiber chitosan bonds do not take place. It could be assumed that the chitosan exhaustion is minimal and that it forms a very thin film on wool fibres being impossible to detect it by SEM. However the presence of the chitosan on wool surface, was evident after the staining process. This minimal quantity of chitosan sorbed on plasma wool provide an additional shrink-resist effect.

Conclusions

* The level of shrink resistance obtained with O_2 or AIR plasma treatments can be enhanced by the post-application of biopolymer chitosan to wool fabric.

* The plasma treatment promotes the spreading and adhesion of the hydrophilic chitosan polymer on wool fibers.

* It is propossed a new ecological shrink-proofing process by application of biopolymer chitosan to wool submitted previously to an oxidative cold plasma treatment.

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