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Binding Chitosan Cross-Linked with Dimethylol dihydroxyethylene Urea onto Nylon 66 Fibres for Burn Scar Management

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Chitosan is an excellent biopolymer having antimicrobial activities against various bacteria and fungi. The research aims to improve the healing characteristics and to increase the effectiveness and functions of compression fabrics used in burn scar treatments by providing infection protection with chitosan barriers. Chitosan is cross-linked with dimethylol dihydroxyethylene urea then binds onto nylon 66 fabric to progress pressure garments with permanent antimicrobial activity. The obtained nylon 66 fabric in powernet structure is analyzed *via* total reflection infrared spectroscopy and scanning electron microscopy. Finally, antimicrobial and washing tests are performed. The results show that chitosan cross-linked with dimethylol dihydroxyethylene urea onto nylon 66 fibre is feasible and the fibres bind with chitosan are shown to be antimicrobial. The maximum binding level is 0.56 wt % and the antimicrobial effectiveness of the fabric remains at around 90 % after 5 washes and 50 % after 30 washes, which will provide a long period of protection.

Keywords: Chitosan, Dimethylol dihydroxyethylene urea, Nylon 66, Crosslinking, Antimicrobial, Burn scars.

INTRODUCTION

Chitin and chitosan (CS) polymers are natural aminopolysaccharides having unique structures, multidimensional properties, highly sophisticated functions and wide ranging applications in biomedical and other industrial areas [1-3]. Chitosan is the deacetylated derivative of chitin, which is the second most abundant polysaccharide found on earth next to cellulose. Chitin is the main component in the shells of crustaceans, such as shrimp, crab and lobster. It is also found in exoskeletons of mollusks and insects and in the cell walls of some fungi [4,5]. The physical properties of chitosan arise from its crystalline polymorph and biological activities.

Chitin and chitosan are known for their excellent biological properties. Among the most important are biocompatibility with human cells, ordered regeneration of wounded tissues, immune enhancing activity, induction of immediate hemostasis, radical scavenging activity and antimicrobial activity [6]. The antimicrobial activities of chitosan against various bacteria and fungi are well known. Several different mechanisms for microbial inhibition by chitosan have been proposed, but the exact mechanism is still not known. The most accepted one is the interaction of the positively charged chitosan with the negatively charged residues at the cell surface of many fungi and bacteria, which cause extensive cell surface alterations and reduce cell permeability [7,8].

The positive attributes of excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity and versatile biological activities, such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development [9-14]. Varan *et al.* [15-18] used silver, polyhexamethylenebiguanide, triclosan and quaternary ammonium compounds to impart durable antimicrobial properties to nylon/spandex compression fabrics for the rehabilitation of hypertrophic burn scars. These garments are in direct contact with the skin, but should not cause skin irritation and provide a hygienic environment to prevent infections during pressure garment therapy (PGT).

The present study aims at binding chitosan onto nylon 66 fibres by crosslinking with dimethylol dihydroxyethylene urea and characterizing the resultant antimicrobial activity. Yang *et al.* [19] studied the mechanism of bonding a hydroxy-functional organophosphorous oligomer (HFPO) to nylon 66 fabric using the formaldehyde derivatives of urea and melamine, including dimethylol dihydroxyethylene urea (DMDHEU) and trimethylolmelamine (TMM), as the bonding agents. The percent phosphorus retention of the treated nylon increased as the DMDHEU or TMM concentration was increased.

In antibacterial studies, chitosan was blended with nylon 6 by combining solvent evaporation and a phase-inversion technique, followed by chelation with silver ions [20]. Han *et al.* [21] have performed the surface modification of

polybenzoimidazole (PBI) membrane with chitosan chains using 4-isocyanato-4'-(3,3'-dimethyl-2,4-dioxoazetidino)-diphenylmethane (IDD) as a coupling agent to build up chemical linkages between the polybenzoimidazole membrane surface and chitosan chains. Polybenzoimidazole-chitosan increases its surface hydrophilicity and enhances pervaporation/dehydration on isopropanol/aqueous solutions and shows high pervaporation separation indexes, which are about 4-fold greater than the value measured with the neat polybenzoimidazole membrane. Glampedaki *et al.* [22] have functionalized the surface of polyamide 6,6 fabrics using chitosan-based hydrogels and investigated the moisture absorption capacity of the new fabrics. The hydrogel embedded thermosensitive microparticles of poly (N-isopropylacrylamideco-acrylic acid) incorporation into the fabric surface layer was achieved by crosslinking the primary amine groups of chitosan with the end amine groups of the polyamide, using the natural crosslinker genipin.

In all cases, the presence of chitosan increased the polyamide fabric wetting times significantly [22]. Shi [23] oxidized chitosan and nylon 6 fibre using potassium persulfate and the antibacterial rating of the fabric remained at around 90 % after being washed 50 times. Tseng *et al.* [24] showed nylon textiles bonded with chitosan polymer had better antibacterial performances than those bonded with chitosan oligomers activated by an open air plasma.

In this study, DMDHEU was used as the binding agent. The prepared fabric was characterized *via* attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and scanning electron microscopy (SEM). The antimicrobial properties of the samples were also determined by the AATCC Test Method 100.

EXPERIMENTAL

Several pieces of gray chinlon cloth (average weight = 3 g; BSN Medical Inc.), chitosan (weight average molecular weight = 1.5×10^5) (Vanson Inc.) and other reagents used were analytically pure.

Methods: 150 g of gray chinlon cloth was weighed and scoured (0.5 g/L sequestering agent, 2 g/L nonionic, octylphenol ethoxylate surfactant penetrating agent, 2 g/L sodium carbonate (Table-1) in a 1:20 bath ratio) using the Dupont Procedure in Fig. 1. The scouring lasted for 60 min at 80 °C. The cloth was then rinsed in water at 38 °C for 90 min. and dried in a convection oven at 50 °C for 30 min, 1 wt. % and 0.5 wt. % chitosans were added to four solutions of 2 % lactic acid separately and were constantly stirred at 20.5 °C until the chitosan was fully dissolved. 0.1 % w/v and, 0.08 % w/v DMDHEU, 0.1 wt % ethylenediamine tetraacetic acid (EDTA) and 0.1 wt. % polyethyleneglycol *p*-(1,1,3,3-tetramethylbutyl)phenylether (Triton X-100) were then reacted with 1.0 and 0.5 % w/v chitosan as each mixtures were stirred for 24 h at 20.5 °C (Table-2). The combined solutions were used for binding chitosan onto chinlon. For each experiment, 3 g of cloth was weighed and a 1:16 bath ratio was used. The cloth samples were placed into each of the four solutions separately. The treatment lasted for 20 min at 20.5 °C. The cloth samples were then dried in a convection oven for 30 min at 50 °C and subsequently cured in an oven for 2 min at 130 °C. Finally, the cloth samples were weighed using an electronic balance.

TABLE-1
SCOURING AGENTS

Agents	Stock solution % (g/L)
Questial (sequestering agent)	0.5
Soda ash	2.0
Triton X-100	2.0

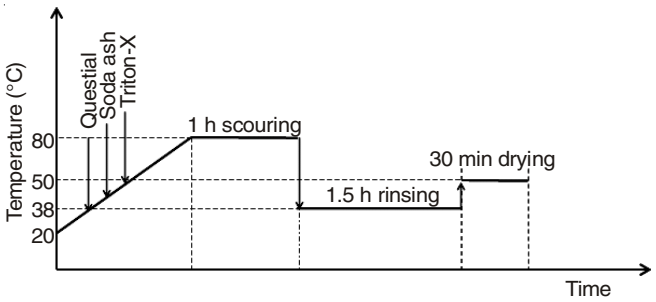

Fig. 1. Scouring of the samples using the dupont procedure

The scouring agents are listed in Table-1. To chitosan/DMDHEU reactant solution, EDTA and Triton X-100 (1 g/L) were added separately (sample B and D) and for sample C, 0.08 g/L DMDHEU was added to change the degree of binding and to observe the effect on the antimicrobial activity and wash durability (Table-2). The pH of the solutions was controlled to below pH 6.5, since chitosan shows its antimicrobial activity only in acidic conditions because of its poor solubility above pH 6.5.

Binding yield: The binding yield (BY) refers to the weight increase due to crosslinked chitosan by weight in grams of the nylon fabric after padding w/w % (g) (M_{bp}), compared with the initial weight of the nylon fabric before padding w/w % (g) (M_p) (eqn. 1).

$$\text{Binding yield (\%)} = [\ (M_{bp} - M_p)/(M_p)\ ] \times 100 \quad (1)$$

Binding rate determination: The crosslinking residual rate (R_{or}) refers to the percentage of chitosan that exhausts onto nylon fibre where chitosan on fibre by weight in grams w/w % (g) (M_b) compared with the chitosan in solution w/w % (g) (M_{bo}) (eqn. 2).

$$R_{or}(\%) = (M_b/M_{bo}) \times 100 \quad (2)$$

The binding rate (R_b) refers to the weight increase due to chitosan where (M_a) w/w % (g) percentage of the dry weight,

TABLE-2
SOLUTION PROPERTIES

Sample ID	Solution temperature (°C)	pH
(A) (1.0 % w/v) Chitosan, (0.10 % w/v) DMDHEU	20.5	3.1
(B) (1.0 % w/v) Chitosan, (0.10 % w/v) DMDHEU, (0.1 % w/v) EDTA	20.5	5.5
(C) (0.5 % w/v) Chitosan, (0.08 % w/v) DMDHEU	20.5	1.0
(D) (1.0 % w/v) Chitosan, (0.10 % w/v) DMDHEU, (0.1 % w/v) Triton X-100	20.5	3.0

without the chinlon crosslinked mass, after the treatment with chitosan (M_a–$M_{ao}R_{or}$) and that of the original chinlon before the treatment w/w % (g) (M_{ao}) from each solutions in Table-2 (eqn. 3).

$$R_b(\%) = [(M_a - M_{ao}R_{or})]/[(M_{ao}] \times 100 \quad (3)$$

Analyses and characterization: Infrared spectroscopy was performed on the chinlon fabric samples using the Nicolet Nexus 470 Spectrophotometer FTIR infrared analyzer with AVATAR omni sampler in the attenuated total reflectance (ATR) mode. The microstructures of the samples were observed using a SEM, JEOL JSM 5900-LV scanning electron microscope at an accelerating voltage of 15 kV. The antimicrobial properties of the samples were tested by the NC State TECS Tissue Lab in collaboration with Biotech Testing Services (report no. 20146120/1-6). The experimental method used to determine the antimicrobial effects was AATCC Test Method 100: 2004 "Assessment of Antibacterial Finishes on Textiles" Standards, using *Staphylococcus aureus* ATCC 6538 (2.00×10^5 CFU/mL) test inoculum.

RESULTS AND DISCUSSION

The binding of chitosan with DMDHEU onto nylon 66 is probably by the formation of a crosslinked network on the surface of the nylon filaments. The ratio of chinlon to chitosan was controlled at 10:1. A series of crosslinked samples were obtained by binding the modified chitosan onto nylon 66 fibre by changing properties of the solutions and concentrations. Sample E was prepared by keeping the solution overnight in a closed petri dish to see the effects of ageing on binding. Several representative samples were characterized. The binding rates of the chitosan bound with nylon 66 samples are presented in Table-3.

TABLE-3
BINDING RATES OF THE CHITOSAN BOUND ONTO NYLON 66 SAMPLES

Samples	O-BR	A-BR	B-BR	C-BR	D-BR	E-BR
Binding rate (%)	0	0.56	0.53	0.56	0.48	0.51

Fig. 2 presents the binding yield percentage as a function of the reaction time when chitosan was crosslinked with DMDHEU onto nylon 66 in different percentages and with the addition of nonionic surfactants and chelating agents.

Fig. 3 presents the rates of binding for each sample. Binding rate curves for chitosan crosslinked with DMDHEU in different percentages onto nylon with the addition of EDTA (0.1 % w/v) and Triton-X (0.1 % w/v) are included in each of these figures for comparison.

0-BR was the pure nylon 66 sample that was used in the experiment. The binding rates from samples B-BR to A-BR changed from 0.53 to 0.56 wt %. The binding rate changed with percentages of DMDHEU, EDTA, Triton X-100, reaction pH and oxidation. A slight decrease was observed when EDTA and Triton X-100 were added separately. The addition of a binder (EDTA) increased binding rate, but reduced durability. Also the effects of changes in pH were observed and are presented in Fig. 3. In acidic media (pH < 6.5), most of the amino groups

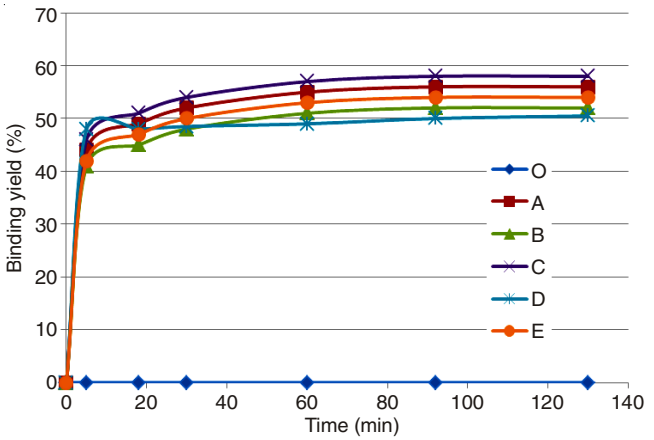

Fig. 2. Binding yield percentage; (O) pure nylon 66, (A) 1 % Ch, 0.1 % DMDHEU, (B) 1 % Ch, 0.1 % DMDHEU, 0.1 % EDTA, (C) 0.5 % Ch, 0.08 % DMDHE, (D) 1 % Ch 0.1 % DMDHEU, 0.10 % polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether, (E) 1 % Ch, 0.1 % DMDHEU (Kept overnight in closed petri dish)

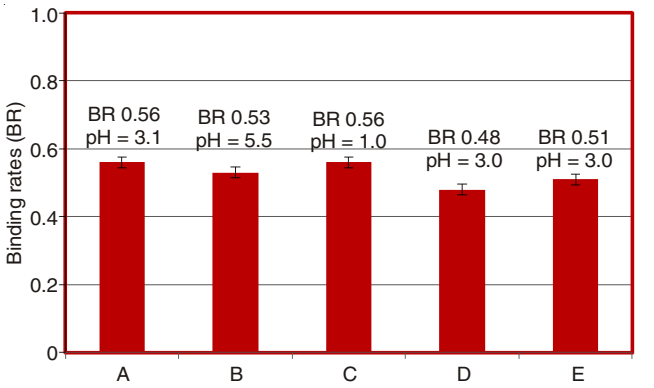

Fig. 3. Rates of binding (A) 1 % Ch, 0.1 % DMDHEU, (B) 1 % Ch, 0.1 % DMDHEU, 0.1 % EDTA, (C) 0.5 % Ch, 0.08 % DMDHEU, (D) 1 % Ch 0.1 % DMDHEU, 0.1 % polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether, (E) 1 % Ch, 0.1 % DMDHEU (Kept overnight in closed petri dish)

of chitosan become protonated. This allows the formation of electrostatic interactions involving the NH_3^+ groups of chitosan. Chitosan, which was crosslinked with DMDHEU and then bound onto nylon 66 and their antimicrobial properties were analyzed.

Infrared spectroscopy: The infrared spectra of samples O, A, B and D are presented in Fig. 4. The results are consistent with those in the literature [19]. The absorption peak at 3300 cm^{-1} can be attributed to the stretching vibration of amide groups of nylon 66. Two absorption peaks at 2935 and 2860 cm^{-1} can be attributed to the asymmetrical and symmetrical stretching vibrations of amide groups of nylon 66, respectively. The absorption peak at 1635 cm^{-1} can be attributed to the stretching vibration of the amide carbonyl group also present in DMDHEU; furthermore, the absorption peak at 1536 cm^{-1} can be attributed to the bending vibration of secondary amide groups. The intensity and height of the absorption peak at 1536 cm^{-1} was found to increase after binding of the samples. This finding showed that the peak intensity of the amino group decreased, which suggests that the amino group concentration was reduced. Some of the amino groups were involved in the reaction. A tertiary amide was obtained from the reaction of a

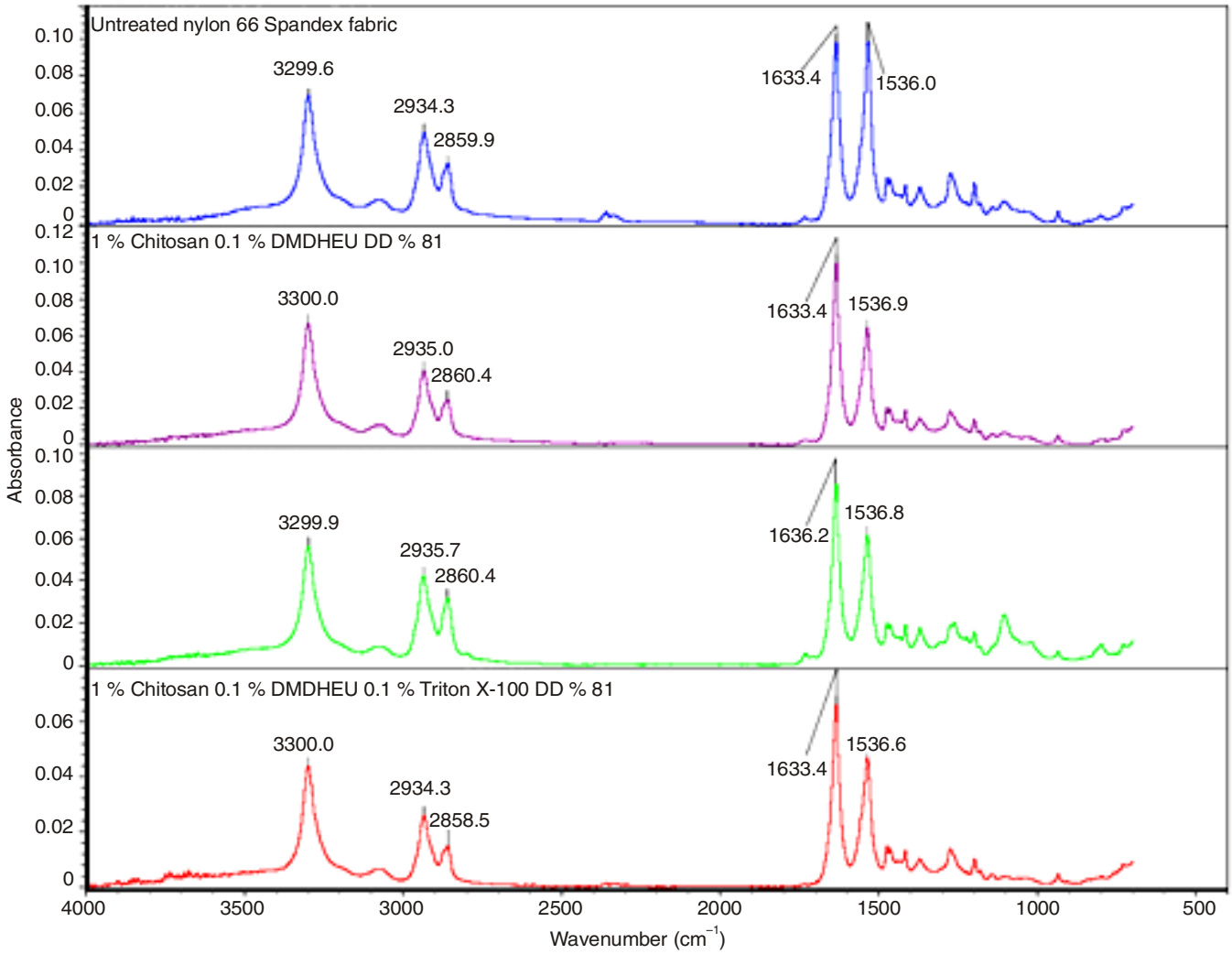

Fig. 4. Infrared spectra of the chitosan bound with nylon 66 samples; (A) untreated nylon 66, (B) 1 % Ch, 0.1 % DMDHEU, (C) 1 % Ch, 0.1 % DMDHEU, 0.1 % EDTA, (D) 1 % Ch 0.1 % DMDHEU, 0.1 % Triton X-100

portion of the secondary amide groups. A tertiary amide has no amide N-H bonds so its IR spectrum does not have amide N-H stretching or bending peaks and therefore no absorptions in the functional group region. As a result, the absorption peak intensity at 1536 cm^{-1} was weakened after bonding chitosan on the nylon 66 fabric samples.

SEM analysis of the microstructure: The photographs of untreated nylon 66 and nylon 66 bound to chitosan were acquired from a JEOL JSM 5900-LV scanning electron microscope using an accelerating voltage of 15 kV and are presented in Fig. 5. The microstructure of the untreated nylon 66 fibre is shown in Fig. 5A. The fibre surface is smooth and featureless. The treated microstructure is shown in Fig. 5B. Chitosan is clearly seen on the surface of the nylon 66 fibre. This sample has a mean percentage of binding yield (BY %) of 0.54 (Fig. 3).

Determination of antimicrobial activity: The antimicrobial properties of the chitosan bound with nylon 66 samples were determined *via* the AATCC Test Method 100 without washing and after 5, 10 and 30 washes and are presented in Table-4. The AATCC Test Method 61 (2A): 2010 "Colorfastness to laundering: Accelerated" was followed to evaluate the washing durability. The total population of *Staphylococcus aureus* ATCC 6538 on each sample was determined. After the

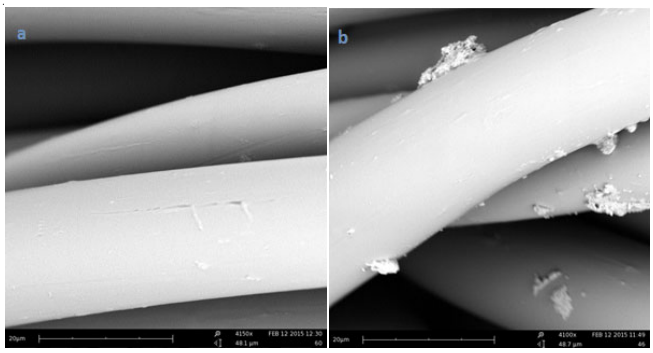

Fig. 5. SEM observed microstructures of the samples (A) pure nylon 66 sample, (B) after bonding (1.0 % w/v) chitosan crosslinked with DMDHEU (0.1 % w/v) onto nylon 66 fiber (solution A; from Table-2)

antimicrobial tests were performed, the live vibrio concentration of the standard blank sample at zero contact time, as well as that of a standard blank sample oscillated for 24h and that of the antimicrobial fabric sample oscillated for 24 h, were compared. The inhibition rate was calculated. For sample A, the chitosan binding rate was 0.56 % and the percentage reduction of bacteria (R) was 92 %. The percentage reduction of bacteria (R) for sample B with EDTA was 49 % even after 5 washes. The antimicrobial properties of the chitosan bound

TABLE-4
ANTIMICROBIAL TEST RESULTS OF THE CHITOSAN BOUND WITH NYLON 66 SAMPLES

Treatment sample	Test organism: ATCC 6538 *Staphylococcus aureus* (CFU/sample)		Percentage reduction of bacteria (R)
	Inoculated sample at 0 contact time (cfu/mL)	Inoculated sample at 24 h oscillation (cfu/mL)	
Before Washing			
A	1.95×10^5	1.51×10^4	92
B	1.92×10^5	9.80×10^4	49
D	1.93×10^5	9.60×10^4	50
5 Washes			
A	1.94×10^5	1.40×10^3	99
B	1.94×10^5	9.80×10^4	49
D	1.93×10^5	9.20×10^4	52
10 Washes			
A	1.92×10^5	1.20×10^5	38
B	1.89×10^5	60	100
D	1.92×10^5	9.80×10^4	49
20 Washes			
A	1.92×10^5	1.20×10^5	38
B	1.92×10^5	1.08×10^4	92
D	1.92×10^5	1.20×10^5	38
30 Washes			
A	1.93×10^5	9.20×10^4	52
B	1.93×10^5	1.03×10^4	95
D	1.92×10^5	1.20×10^5	38

with nylon 66 fibre after 5, 10, 20 and 30 washes were also determined. The antimicrobial activity of the modified fabrics showed more durability even after 30 washes. The addition of Triton X-100 (sample D) caused a 50 % decrease in antimicrobial activity. The antimicrobial properties of the unwashed chitosan bound with nylon 66 fibres and those washed 5, 10, 20 and 30 times were compared with each other. It has found that chitosan was strongly fastened to nylon 66 was expected because chitosan was bound onto the nylon 66 fibre *via* chemical bonding. Therefore, the modified nylon 66 fibre has excellent antimicrobial activity even after 5 washings and showed good durability up to 30 washings.

Conclusion

Chitosan was crosslinked with dimethylol dihydroxyethylene urea and subsequently bound onto nylon 66 fabric. This crosslinked structure was characterized *via* total reflection infrared spectroscopy and scanning electron microscopy and its antimicrobial properties were tested. The results showed that bonding chitosan crosslinked with dimethylol dihydroxyethylene urea onto nylon 66 was feasible, with a binding rate of 0.56 wt %. The binding rate indicates the antimicrobial activity of the fabric remained at around 90 % after 5 washes and at 50 % after 30 washes.

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