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The antimicrobial activity of cotton fabrics treated with different crosslinking agents and chitosan

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Abstract

Cotton fabrics were treated with two different crosslinking agents [butanetetracarboxylic acid (BTCA) and Arcofix NEC (low formaldehyde content)] in the presence of chitosan to provide the cotton fabrics a durable press finishing and antimicrobial properties by chemical linking of chitosan to the cellulose structure. Both type and concentration of finishing agent in the presence of chitosan as well as the treatment conditions significantly affected the performance properties and antimicrobial activity of treated cotton fabrics. The treated cotton fabrics showed broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria and fungi tested. Treatment of cotton fabrics with BTCA in the presence of chitosan strengthened the antimicrobial activity more than the fabrics treated with Arcofix NEC. The maximum antimicrobial activity was obtained when the cotton fabrics were treated with 0.5–0.75% chitosan of molecular weight 1.5–5 kDa, and cured at 160 °C for 2–3 min. Application of different metal ions to cotton fabrics treated with finishing agent and chitosan showed a negligible effect on the antimicrobial activity. Partial replacement of Arcofix NEC with BTCA enhanced antimicrobial activity of the treated fabrics in comparison with that of Arcofix NEC alone. Transmission electron microscopy showed that the exposure of bacteria and yeast to chitosan treated fabrics resulted in deformation and shrinkage of cell membranes. The site of chitosan action is probably the microbial membrane and subsequent death of the cell.

Keywords: Antimicrobial finish; Chitosan; Cotton; Butanetetracarboxylic acid; Arcofix NEC

1. Introduction

Polycarboxylic acids can crosslink cotton fabric in the presence of alkali metal salts of phosphorous containing acids (Kang, Deivasigamani, & Sarmadi, 2004; Sricharussin, Ryo-Aree, Intasen, & Poungraksakirt, 2004), producing cotton fabric with high levels of resiliency, high strength retention and good durability of their smooth drying properties after laundering. BTCA is the most effective but expensive finishing agent. Sodium hypophosphite is the most effective catalyst known, but has disadvantages such as high cost and causing some shade changes in most sulfur and certain reactive dyes. The difference between finishing agents based on polycarboxylic acids and those based on reaction products of amides and formaldehyde is that the polycarboxylic acids form ester linkage with the cotton cellulose and the amide derivatives form ethers.

Chitosan, a polysaccharide comprising copolymers of glucosamine and N-acetyl-glucosamine, is obtained by alkaline deacetylation of the chitin derived from the exoskeletons of crustaceans and arthropods (Li, Dunn, Grandmaison, & Goosen, 1997). It has attracted considerable interests due to their biological activities such as antimicrobial (Lim & Hudson, 2003), antitumor and immuno-enhancing effects (Choi et al., 2001; Hirano & Nagao, 1989; Jeon, Park, & Kim, 2001; Jumaa, Furkert, & Muller, 2002; Liu, Du, Yang, & Zhu, 2004; Liu, Guan, Yang, Li, & Yao, 2001; No, Pak, Lee, & Meyers, 2002; Shon, 2001; Suzuki, 1996; Tsukada et al., 1990; Zheng & Zhu, 2003)

Chitosan is inexpensive, non-toxic, biodegradable and possesses reactive amino groups. It has been useful in many

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areas of applications, such as wastewater treatment, food and textile industry and recently in drug industry and as a hydrating agent in cosmetics. (Chung, Lee, & Kim, 1998; Davidson & Xue, 1994; Kim, Choi, & Yoon, 1998; Rippon, 1984; Tsuragai, Yoshikawa, Tajima, & Ishii, 1991). In the last decade, the textile applications of chitosan have attracted many researchers. Cotton textiles have poor resistance to microorganisms, so the antimicrobial finishing of cotton fabrics is an economical way to prevent harm to the human body (Chung et al., 1998; Kim et al., 1998; Seventekin & Ucarci, 1993).

Several mechanisms were proposed for the antimicrobial activity by chitosan: (1) Polycationic structure of chitosan which can be expected to interact with the predominantly anionic components (lipopoly-saccharides and proteins of microorganism surface) resulting in changes in permeability which causes death of the cell by inducing leakage of intracellular components (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Lim & Hudson, 2004; Nikaido, 1996Vaara & Vaara, 1983; Wang, Du, & Liu, 2004). (2) The chitosan on the surface of the cell can form a polymer membrane which prevents nutrients from entering the cell (Helander et al., 2001; Liu et al., 2004; Zheng, Zhu, & Sun, 2000). (3) The chitosan of lower molecular weight enters the cell, binding to DNA and inhibits RNA and protein synthesis (Liu et al., 2001). (4) Since chitosan could adsorb the electronegative substance in the cell and flocculate them, it disturbs the physiological activities of the microorganism leading to death of the cells (Zheng & Zhu, 2003).

In this study the influences of crosslinking agents such as BTCA and Arcofix NEC in the presence of chitosan as well as the finishing conditions on the performance properties and the antimicrobial activity of the treated cotton fabrics were studied. Also the effect of dyeing on the antimicrobial activity of treated cotton fabrics was studied.

2. Materials and methods

2.1. Chitosan characterization

Chitosan was hydrolyzed with 1N HCl/isopropanol mixture at 90 °C for different reaction durations, filtered, neutralized with dilute sodium hydroxide, then washed with distilled water till neutral. The viscosity average molecular weights of hydrolyzed chitosan were shown as 1.5, 5, 30 and 50 kDa.

2.2. Fabrics treatment

The cotton fabrics were padded two dips and nips (90–95% wet pick up) in a solution containing finishing agents (6–12%), chitosan (0–1%) dissolved in an equivalent amount of HCl. The finishing agents used are BTCA and Arcofix NEC (Low formaldehyde) independently in the presence of sodium hypophosphite (SHP) and chitosan hydrochloride as catalyst, respectively. Chitosan hydrochloride (Ch-HCl) has a dual function, since it provides cationic sites to the cotton cellulose for both BTCA and Arcofix NEC and as a catalyst in the case of Arcofix NEC. After treatment, the cotton fabrics were dried at 85 °C for 5 min and cured at 160 °C for 3 min.

2.3. Dyeing process

The cotton fabrics treated with finishing agent in the presence of chitosan as mentioned above were dyed using reactive and basic dyes, namely reactive red 88 (6-Amino-*n*-toluene sulfonic acid) and basic dye 21(N,N-Dimethyl-P-nitrosoaniline hydrochloride), respectively, according to the conventional exhaustion method. A dyeing bath solution containing 2% dye (owf), 50 g/L sodium sulfate and 15 g/L sodium carbonate was used. The dyeing process was performed at 95 °C for 1 h using material-to-liquor ratio 1:20. After dyeing, the fabrics were thoroughly washed with 2 g/L of non-ionic detergent for 30 min at 90 °C and then washed with cold water. The dyed fabrics were dried and finally evaluated for their antimicrobial activity.

2.4. Metal treatments

The treated cotton fabrics were impregnated in different solutions containing Zn^{2+}, Cu^{2+} and Ag^{+} of 0.5 g/L. The treated metal fabrics were washed several times with distilled water, and then dried at room temperature.

2.5. Testing and analysis

The wrinkle recovery angle (WRA) was measured according to AATCC test method 66-1989 and tensile strength (TS) according to ASTM D1682-75 (1985). The whiteness index (WI) of the washed samples was measured using Hunter Lab D25M optical sensor (USA) and the stiffness using Indroof Stiffness Tester (Totoseiki, Japan). The chemical properties of the treated fabric were monitored via determining the nitrogen content (N %) according to Kjeldal method (Vogel, 1966) and the acidic properties of the treated fabrics were assessed using acid-base titration according to the reported method of Yang and Wang (2000).

2.6. Durability test

The durability of the treated cotton fabric against repeated launderings were evaluated by washing cotton fabrics according to AATCC test method 61(2A)-1996. All the treated fabrics were subjected to 1, 10 and 20 consecutive launderings in the presence of non-ionic detergent. Home laundering is according to AATCC test method 124 (AATCC, 2000).

2.7. Antimicrobial activity of the treated fabrics

2.7.1. Microorganisms used

Microorganisms used in this study are shown in the following table. Five bacterial and one fungal species were subjected to antimicrobial activity test of treated cotton fabrics. These microorganisms were obtained from Microbial Chemistry Department, National Research Center, Cairo, Egypt.

Test microorganism	Classification	Abbreviation
Bacillus subtilis	Gram-positive bacteria	*Bs*
Bacillus cereus	Gram-positive bacteria	*Bc*
Escherichia coli	Gram-negative bacteria	*Ec*
Pseudomonas aeruginosa	Gram-negative bacteria	*Pa*
Staphylococcus aureus	Gram-positive bacteria	*Sa*
Candida albicans	Yeast	*Ca*

2.7.2. Media used

Nutrient broth/agar medium: It contains (g/L), (5) peptone, (3) beef extract. For solid medium 15 g/L agar was added.

Malt broth/agar medium: It contains (g/L), (5) peptone, (24) malt extract. For solid medium 15 g/L agar was added.

2.7.3. Growth conditions

An inoculum of each bacterial strain was suspended in 25 mL of nutrient broth medium and shaken for 24 h at 37 °C. For yeast, malt broth was inoculated with test organism and incubated at 28 °C for 24 h.

2.7.4. Antimicrobial activity test

Disc diffusion method with some modification was used for screening the cotton fabric samples for antimicrobial activity (Ericsson & Sherris, 1971). Nutrient agar (for bacteria) or malt agar (for yeast) plates were inoculated with 0.1 mL of an appropriate dilution of the tested culture. Cotton fabric samples (1 cm diameter) were placed on the surface of the inoculated plates. The plates were incubated at the appropriate temperature for 24 h. Diameter of inhibition zone (mm) including the disc diameter was measured for each treatment.

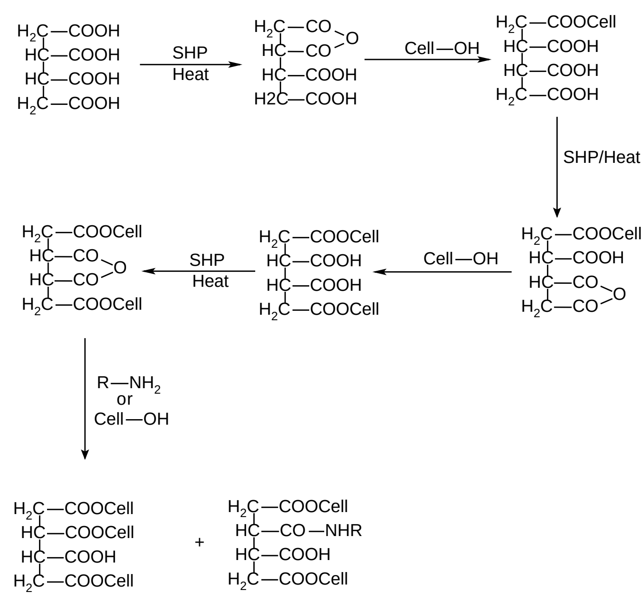
2.8. Transmission electron microscopy (TEM)

The microorganisms were grown for 24 h in liquid media for using as inocula. The media containing 0.75 g of the textile sample were inoculated with 1 mL of each culture and shaken for 24 h at 100 rpm. The cultures were centrifuged and washed once with 0.9% saline solution and resuspended in 0.9% saline then examined by EM 10 Model transmission electron microscope (Zeiss, West Germany).

3. Results and discussion

The mechanism of crosslinking of cotton fabrics with polycarboxylic acids is well known (El-Tahlawy, 1999; Gu & Yang, 1998; Hsieh, Chen, & Wei, 2003), since the reaction of polycarboxylic acid with cellulosic polymers occurs through esterification of three hydroxyls, although not all the carboxylic acids will be able to react with the cellulosic substrate. The most reactive polycarboxylic acids are those capable of forming five membered anhydride rings. Also, at least three carboxylic groups must be present in each acid molecule if it is to crosslink cellulose, since one unesterified carboxyl group remains after an anhydride ring has esterified a hydroxyl group of cellulose.

The addition of chitosan hydrochloride (Ch-HCl) in the finishing bath formulation provides the cellulose cationic properties, as in the case of BTCA, in the presence of SHP as a suitable catalyst. While using Arcofix NEC, chitosan has a dual function, since it acts as a catalyst as well as a cationizing agent. During the drying and curing reaction, part of the finishing agent is consumed in linking chitosan to the cellulosic substrates through esterification (as in BTCA) or etherification reactions (as in Arcofix NEC).



The crosslinking mechanism of BTCA in the presence of chitosan.

The effect of finishing conditions, finishing agent type and dyeability on antimicrobial activity of Ch-HCl, were studied in detail. Also the durability of Ch-HCl as an antimicrobial finish against repeated washing was also studied.

3.1. Effect of finishing agent concentration

Table 1 shows the effect of finishing agent concentration (BTCA or Arcofix NEC) on the performance properties of the treated cotton fabrics. Irregardless of the finishing agent

Table 1
Effect of finishing agents concentration on the performance properties of the treated fabrics

Finishing agent used	Finishing agent (%)	Physical properties					Chemical properties	
		WRW (w+f)°	TS (kg)	E. at break (cm)	WI	Stiffness	N (%)	Acidic properties
Untreated	–	143	86	3.8	89.1	1.43	0	9
BTCA	6	262	53.3	2.1	84	2.1	0.007	49.5
	8	282	49.3	1.8	83.3	2.29	0.009	56.8
	10	292	41	1.7	82.2	3.02	0.015	74.3
	12	300	36.5	1.7	80.3	3.73	0.02	96
Arcofix NEC	6	228	66.2	2.2	82.1	2.67	0.26	23.4
	8	246	63.4	2	81.5	2.41	0.43	28.1
	10	250	60	1.9	80.6	2.84	0.6	30.5
	12	264	49	1.8	78.8	3.2	1.06	33.4

Chitosan, 0.5%; Chitosan M.Wt, 5 kDa; [SHP], 6%; Curing temp., 160 °C for 3 min; Drying temp., 85 °C for 5 min.

used, the wrinkle recovery angle (WRA) increased by increasing the finishing agent concentration. The values of WRA of the samples treated with BTCA are much higher than Arcofix NEC treated fabrics. The enhancement in the WRA is acceptable, since increasing the finishing agent concentration will increase the availability of crosslinking molecules and consequently increase its accessibility to crosslink the cellulosic hydroxyls. Also the higher values of BTCA are expected, since it has four carboxyl groups and can crosslink three cellulosic hydroxyls.

On the other hand, increasing the finishing agent concentration leads to a decrement in the values of tensile strength (TS) and whiteness index (WI). The decrement in the tensile strength is due to the hydrolytic action of the finishing agent at higher temperature (Sricharussin et al., 2004; Xu & Li, 2000). The losses in the TS of BTCA treated fabrics are much higher than that of Arcofix NEC treated fabrics. This may be due to the higher hydrolytic action of BTCA than Arcofix NEC. The decrease in the WI of the treated cotton fabric may be related to cellulose heat treatment, which causes oxidation and scission of cellulosic chains. The presence of aldehyde groups in oxidized cellulose make it unstable and causes yellowing of the fabrics. Also the crosslinking reaction may cause an additional yellowing for the treated fabrics.

Table 1 shows the influence of finishing agent concentration on the chemical properties (N %, acidic properties) of the treated fabrics. Both N % and acidic properties increase by increasing the finishing agent concentration. These chemical properties represent the extent of crosslinking and amount of chitosan linked to cellulose through formation of ester bond, H-bonding and/or Van der Wal forces.

The antimicrobial activity of cotton fabrics treated with different concentrations of Arcofix NEC or BTCA in the presence of Ch-HCl is shown in Table 2. It is clear that BTCA treated fabrics has higher antimicrobial activity against gram-positive and gram-negative bacteria and yeast tested than that of the Arcofix NEC treated fabrics. This may be related to the higher power of BTCA as a crosslinking agent than Arcofix NEC. Also increasing the finishing agent concentration increases the amount of chitosan linked to cellulose and consequently increases the antimicrobial properties of the treated fabrics. The disappearance of antimicrobial activity of cotton fabrics treated with BTCA or Arcofix NEC in the presence of chitosan was observed after alkaline launderings (data not shown). This is due to loss of the necessary acidic properties of Ch-HCl and the residual carboxylic groups. Therefore, it is very important to reacidify the treated fabrics after launderings.

3.2. Effect of chitosan molecular weight

Table 3 shows the effect of chitosan molecular weight on the physical and chemical properties of the treated cotton fabrics when applied at 10% level. It is clear that increasing the molecular weight of chitosan from 1.5 to 50 kDa is accompanied by a decrease in the WRA and marginal

Table 2
Antimicrobial activity of the cotton fabrics treated with different concentrations of BTCA or Arcofix NEC in the presence of Ch-HCl

Microorganism tested	Antimicrobial activity (inhibition zone in mm)							
	BTCA (%)				Arcofix NEC (%)			
	6	8	10	12	6	8	10	12
Ec	12	14	14	15	11	12	12	12
Pa	14	16	17	17	11	12	13	13
Sa	15	17	20	22	12	13	14	15
Bs	15	16	17	18	11	12	13	12
Bc	13	15	15	17	11	13	13	13
Ca	13	16	16	18	10	10	11	11

Table 3
Effect of chitosan molecular weight on the performance properties of the treated fabrics

Finishing agent used	Chitosan molecular weight (kDa)	Physical properties					Chemical properties	
		WRA (w+f)°	TS (kg)	E. at break (cm)	WI	Stiffness	N (%)	Acidic properties
Untreated		143	86	3.8	89.1	1.43	0	9
BTCA	1.5	306	38	1.7	76.9	2.47	0.016	78.1
	5	292	41	1.7	82.2	3.02	0.015	74.3
	30	284	43	2	84.2	3.23	0.013	65.4
	50	268	46	2.1	86.3	3.64	0.01	60.1
Arcofix NEC	1.5	259	58	2.3	73.5	2.77	0.61	35.4
	5	250	60	2.5	80.6	2.84	0.6	30.5
	30	244	65	2.6	83	3.1	0.58	26.9
	50	206	70	2.6	85.2	3.34	0.55	25

Chitosan, 0.5%; Finishing agent, 10%; [SHP], 6%; Curing temp., 160 °C for 3 min; Drying temp., 85 °C for 5 min.

increase in the TS, WI, and stiffness. Increasing the chitosan molecular weight in the finishing bath leads to an increase in the apparent viscosity of the finishing bath; consequently decreasing the penetrability of the reactive ingredient to the sub surface of the cotton fabrics. This also increases the probability of chitosan side reactions. This means that the finishing agents may react with chitosan (mobile phase) rather than cotton hydroxyls (immobile phase). Using chitosan with higher molecular weight imparts significant stiffness properties to the treated fabrics as compared with untreated ones. This is probably due to the surface coating action of chitosan of higher molecular weight.

The chemical properties of the treated cotton fabrics show that N % and the acidic properties are marginally decreased with increasing the chitosan molecular weight. This is related to the decrement in the crosslinking efficiency as well as the amount of chitosan linked to the cotton cellulose.

Table 4 shows the antimicrobial effect of cotton fabrics treated with BTCA in the presence of chitosan of different molecular weights. Treatment of cotton fabrics with Arcofix NEC in the presence of chitosan of different molecular weights did not enhance the antimicrobial activity. The results indicated that the maximum antimicrobial effect against *Ec*, *Pa*, *Sa* and *Ca* was for the cotton fabrics treated with 1.5 or 5 kDa chitosan. For *Bs*, the cotton fabrics treated with chitosan of different molecular weights showed the same inhibition zone. However, cotton fabrics treated with chitosan of higher molecular weights had a slightly enhanced antimicrobial effect against *Bc*. The data shows a meager reduction in the antimicrobial activity after the twentieth laundering cycle. These results verify that chitosan is firmly bound to the cotton fabrics.

It has been reported that the optimum molecular weight of chitosan for antimicrobial activity was 1.5 kDa (Jeon & Kim, 2000). In contrast to this report, Ueno, Yamaguchi, Sakairi, Nishi, and Tokura (1997) reported that chitosan with molecular weight less than 2.2 kDa had little effect on microbial growth and the minimal inhibitory concentration of chitosan. Other reports stated that the low molecular weight of chitosan ranging between 5 and 10 kDa showed strong bactericidal and fungicidal effects (Kumar, Varadaraj, Lalitha, & Tharanathan, 2004; Shon, 2001).

3.3. Effect of chitosan concentration

The effect of chitosan concentration on the performance properties of the treated fabrics is shown in Table 5.

Table 4
Antimicrobial activity of the cotton fabrics treated with chitosan of different molecular weights after successive washing (AATCC laundery)

Chitosan molecular weight (kDa)	No. of washing cycles	Antimicrobial activity expressed as inhibition zone (mm) against					
		Ec	*Pa*	*Sa*	*Bs*	*Bc*	*Ca*
1.5	1	19	18	18	15	18	20
	10	18	18	18	15	18	19
	20	18	17	18	14	17	17
5	1	18	17	18	15	18	20
	10	17	17	17	15	18	17
	20	17	15	17	14	18	17
30	1	15	15	15	15	20	15
	10	15	14	14	15	20	15
	20	14	14	14	15	19	14
50	1	15	15	12	15	20	15
	10	15	15	12	15	20	15
	20	15	15	12	14	18	13

Table 5
Effect of chitosan concentration on the performance properties of the treated fabrics

Finishing agent used	Chitosan conc. (%)	Physical properties					Chemical properties	
		WRA (w+f)°	TS (kg)	E. at break (cm)	WI	Stiffness	N (%)	Acidic properties
Untreated		143	86	3.8	89.1	1.43	0	9
BTCA	0	295	42	2.5	85.7	2.06	0	69.4
	0.25	300	40	2.3	82.9	2.26	0.006	72.1
	0.5	306	38	1.7	76.9	2.47	0.016	78.1
	0.75	300	37	1.7	72.7	2.66	0.019	71.2
	1	292	35	1.6	71	2.93	0.023	66.8
Arcofix NEC	0	242	68	3	85.7	2.5	0.15	29.9
	0.25	250	63	2.8	80	2.63	0.43	32.4
	0.5	259	58	2.3	73.5	2.77	0.61	35.4
	0.75	230	44	2.1	70.2	3.11	0.63	32.8
	1	201	40	2	69.1	3.45	0.65	30.1

Chitoan M.Wt, 1.5 kDa; Finishing agent, 10%; [SHP], 6%; Curing temp., 160 °C for 3 min.; Drying temp., 85 °C for 5 min.

Regardless of the finishing agent used, increasing the amount of chitosan incorporated in the finishing bath is accompanied by a decrease in the tensile strength and WI and an increase in the N % and the stiffness of the treated cotton fabrics. The loss of tensile strength, and WI, may be due to the coating behavior of chitosan at higher concentration. The values of WRA and the acidic properties increase by increasing the chitosan concentration from 0 to 0.5%, then decrease by further increase in the chitosan concentration.

The antimicrobial activity of the cotton fabrics treated with BTCA and different concentrations is shown in Table 6. Treatment of fabrics with Arcofix NEC in the presence of different concentrations of chitosan showed non-consistence antimicrobial activity. The results in Table 6 indicate that the antimicrobial activity was strengthened as the concentration of chitosan increased from 0.0 to 0.5%. Treatment with 0.75% chitosan showed the same antimicrobial activity as 0.5%. Increasing chitosan concentration to 1% leads to a decrement in the antimicrobial activity. It is known that BTCA can exhibit acceptable antimicrobial activity in the absence of chitosan (Kim et al., 2003). So addition of chitosan to the finishing bath increases the probability of its binding to cellulose structure through the ester bond, consequently, enhancing the antimicrobial properties of the treated fabrics (see N % in Table 5). While the loss of antimicrobial behavior of Ch-HCl treated fabrics at higher chitosan concentration owing to the reaction of finishing agents with chitosan rather than crosslinking of cellulose (see acidic properties in Table 5) was observed. In agreement with these results, it was reported that 0.5% chitosan could inhibit the growth of *Ec* completely (Jeon & Kim, 2000). In another study, Zheng and Zhu (2003) indicated the antimicrobial activity of 0.5% chitosan solution against *Sa* was much greater than *Ec*.

3.4. Effect of curing temperature and time

Improvement in textile properties of fabrics finished with different finishing agents depends greatly upon the curing temperature and time. Table 7 shows the performance

Table 6
Antimicrobial activity of the cotton fabrics treated with different chitosan concentrations after successive washing (AATCC laundry)

Chistosan concentration	No of washing cycles	Antimicrobial activity expressed as inhibition zone (mm) against					
		Ec	*Pa*	*Sa*	*Bs*	*Bc*	*Ca*
0	1	14	14	17	15	13	19
	10	14	14	17	14	12	19
	20	14	13	16	14	12	19
0.25	1	16	16	20	16	15	25
	10	15	16	19	15	14	24
	20	15	15	15	15	13	24
0.5	1	19	20	20	19	19	30
	10	19	20	20	19	19	25
	20	18	19	15	18	18	20
0.75	1	20	19	19	18	20	20
	10	20	20	16	17	19	28
	20	20	17	15	17	18	27
1	1	15	19	18	16	12	23
	10	15	18	16	15	12	22
	20	15	18	15	15	11	22

Table 7
Effect of curing temperature and time on the performance properties of the treated fabrics

Finishing agent used	Curing temp.(°C)	Curing Time (min)	Physical properties					Chemical properties	
			WRA (w+f)°	TS (kg)	E. at break (cm)	WI	Stiffness	N (%)	Acidic properties
Untreated			143	86	3.8	89.1	1.43	0	9
BTCA	150	1	189	58	2.3	81	1.78	0.004	45
		2	242	49	2.2	79.3	2.0	0.005	48.6
		3	275	42	2	77.1	2.19	0.007	55.3
	160	1	210	56	2.2	79	1.99	0.008	50.3
		2	278	52	2	77.4	2.23	0.01	70.2
		3	306	38	1.7	76.9	2.47	0.013	78.1
	170	1	260	44	2.1	76	2.1	0.012	71.9
		2	288	40	2	75	2.38	0.015	75.1
		3	320	37	2	73.8	2.47	0.022	80
Arcofix NEC	150	1	156	66.9	2.6	79	2.07	0.29	26.5
		2	178	64.6	2.51	77.2	2.31	0.32	28.6
		3	199	62.1	2.39	75.6	2.62	0.41	30.5
	160	1	178	65.4	2.48	75.9	2.21	0.39	29
		2	230	63.5	2.4	74.3	2.49	0.42	31.2
		3	259	58	2.3	73.5	2.77	0.52	35.4
	170	1	189	61.5	2.35	73.5	2.39	0.49	33.5
		2	240	59.8	2.16	71.6	2.51	0.55	36.2
		3	268	56.4	2.0	70	2.76	0.62	39.8

Chitoan, 0.5%,; Chitosan M.Wt, 1.5 kDa; Finishing agent, 10%; [SHP], 6%; Drying temp., 85 °C for 5 min.

properties of the treated cotton fabrics cured at different temperature (150–170 °C) for 1–3 min.

In general, at lower cure temperatures much longer times were needed to increase the WRA of the finished fabrics. The curing temperature was inversely proportional to tensile strength, WI The increment in the WRA may be attributed to higher temperature and longer times as well as greater availability of finishing agent (BTCA, or Arcofix NEC) molecules to crosslink cellulose. While the loss of TS and WI reflects the hydrolytic effect of the finishing agent, in addition to the higher oxidation reaction of cellulose. Also increasing the curing temperature and/or prolonging the curing time improve the extent of crosslinking of finishing agent to cellulose and increase of the linked chitosan.

Effect of curing temperature and time on antimicrobial activity of cotton fabrics treated with BTCA/chitosan is shown in Table 8. Results of treatment with Arcofix NEC/chitosan were ignored due to inconsistent antimicrobial activity. In general, the most suitable curing temperatures for antimicrobial activity are 160 °C for 2–3 min or 170 °C for 1 min against all tested organisms. However, curing temperature and time

Table 8
Effect of curing temperature and time on antimicrobial activity of treated cotton fabrics

Curing temperature (°C)	Curing time (min)	No. of washing cycles	Antimicrobial activity expressed as inhibition zone (mm) against					
			Ec	*Pa*	*Sa*	*Bs*	*Bc*	*Ca*
150	1	1	19	15	15	15	15	26
		20	18	14	13	13	14	22
	2	1	19	17	17	15	15	25
		20	18	17	13	14	13	23
	3	1	20	19	17	16	15	26
		20	19	17	15	15	14	22
160	1	1	21	17	16	16	15	25
		20	19	16	16	15	13	22
	2	1	22	20	20	17	16	30
		20	20	18	15	14	15	28
	3	1	25	20	20	16	17	30
		20	24	19	17	15	15	28
170	1	1	26	20	20	16	16	28
		20	25	18	17	13	14	24
	2	1	25	18	18	17	17	26
		20	24	17	15	15	14	26
	3	1	22	17	15	18	17	20
		20	21	16	15	15	14	15

Table 9
Effect Arcofix NEC/BTCA ratios on the performance properties of the treated fabrics

BTCA/ Arcofix NEC ratios (%)	Physical properties					Chemical properties	
	WRA (w+f)°	TS (kg)	E. at break (cm)	WI	Stiffness	N (%)	Acidic properties
Untreated	143	86	3.8	89.1	1.43	0	9
100/0	278	52	2	77.4	2.23	0.01	70.2
75/25	264	55	2.1	77	2.29	0.09	60.2
50/50	248	58.5	2.2	75.6	2.38	0.2	52.3
25/75	239	60.1	2.3	75	2.41	0.31	43.5
0/100	230	63.5	2.4	74.3	2.45	0.42	31.2

Chitosan, 0.5%; Chitoan M.Wt, 1.5 kDa; Finishing agent, 10%; [SHP], 6%; Drying temp., 85 °C for 5 min Curing temp, 160 for 3 min.

Table 10
Antimicrobial activity of cotton fabrics treated with different Arcofix NEC/BTCA ratios, chitosan and metal ions against *E. coli* expressed as inhibition zone (mm) after successive washings

Metal ions used/ no of washing	Home laundry of cotton fabrics treated with Arcofix NEC/BTCA ratios (%)					AATCC laundry of cotton fabrics treated with Arcofix NEC/BTCA ratios (%)				
	0/100	25/75	50/50	75/25	100/0	0/100	25/75	50/50	75/25	100/0
None										
One wash	30	30	28	25	15	30	30	24	20	15
20 washes	28	29	24	22	14	29	26	20	18	13
Zn^{2+}										
One wash	31	30	30	25	15	30	30	28	24	15
20 washes	30	30	28	22	14	22	22	20	20	14
Cu^{2+}										
One wash	30	30	30	24	15	28	30	28	26	15
20 washes	20	24	24	22	14	22	22	18	22	14
Ag^{2+}										
One wash	30	28	30	26	15	32	30	31	28	14
20 washes	22	22	22	24	14	24	22	22	21	13

The washing was either under home laundry or AATCC laundry conditions.

appeared to have little effect on the bactericidal response of treated cotton fabrics against *Bs* and *Bc*.

3.5. Effect of arcofix NEC/BTCA ratios

It was concluded that, BTCA/Ch-HCl finished fabrics have higher WRA, WI, acidic properties and antimicrobial activity and lower TS, stiffness and nitrogen content than that of the Arcofix NEC/Ch-HCl treated fabrics.

Arcofix NEC is inexpensive and commonly used as a crosslinking agent for cotton fabrics. However, antimicrobial activity of cotton fabrics treated with Arcofix NEC/Ch-HCl is very low. Therefore mixtures of Arcofix NEC/BTCA were used to improve the performance properties and antimicrobial activity of the treated fabrics. As shown in Table 9, a partial replacement of Arcofix NEC with BTCA improve the values of WRA, TS, WI, stiffness and the acidic properties.

The effect of treatment of cotton fabrics with Arcofix NEC/BTCA ratios, metal ions, and successive launderings on antimicrobial activity are shown in Table 10. The results show no significant differences in antimicrobial activity between the treatment of fabrics with different metal ions and untreated ones. However, partial replacement of ArcofixNEC with BTCA strengthened the antimicrobial activity compared to Arcofix NEC alone. The durability of the antimicrobial effect is dependent on the laundering type and microorganism tested. In general, non-metal treated samples under home laundering showed much better or the same antimicrobial durability as the AATCC laundering even after 20 washes. Treatment of cotton samples with chitosan before or after dyeing does not affect the antimicrobial activity or its durability (data not shown).

Fig. 1. Transmission electron photograph of *E. coli* exposed to chitosan treated cotton fabrics for 24 h showing slightly deformed cell membrane. × 20,000.

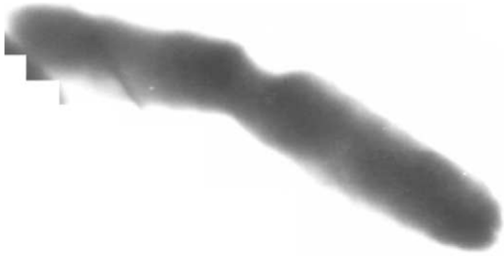


Fig. 2. Transmission electron photograph of *Pseudomonas aeruginosa* exposed to chitosan treated cotton fabrics for 24 h showing deformed cell membrane. × 10,000.

3.6. Transmission Electron Microscopy

Morphological changes of the inoculated cells of each strain after 24 h contact with chitosan treated cotton fabric were examined by transmission electron microscope (TEM). (Figs. 1–3) show slightly deformed cell membranes of gram-negative and gram-positive bacteria. However, significant morphological change and shrinking of *Candida albicans* cells were observed (Fig. 4).

Kim et al. (1997) reported that at high concentrations, cationic biocides show a bactericidal activity and bacterial cells become shrunken and deformed when exposed. The results obtained by TEM, therefore, strongly suggest that chitosan treated cotton fabrics under the experimental conditions has bactericidal and fungicidal activities.

4. Conclusion

Cotton fabrics were treated with two different cross-linking agents [butanetetracarboxylic acid (BTCA) and Arcofix NEC (low formaldehyde content)] in the presence of chitosan to provide the cotton fabrics a durable press finishing and antimicrobial properties by chemical linking of chitosan to the cellulose structure. Both type and concentration of finishing agent in the presence of chitosan as well as the treatment conditions significantly affected the performance properties and antimicrobial activity of treated cotton fabrics. The treated cotton fabrics showed broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria and fungi tested. Treatment of cotton fabrics with BTCA in the presence of chitosan strengthened the antimicrobial activity more than the fabrics treated with Arcofix NEC. The maximum antimicrobial activity was obtained when the cotton fabrics were treated with 0.5–0.75% chitosan of molecular weight 1.5–5 kDa, and cured at 160 °C for 2–3 min. Application of different metal ions to cotton fabrics treated with finishing agent and chitosan showed a negligible effect on the antimicrobial activity. Partial replacement of Arcofix NEC with BTCA enhanced antimicrobial activity of the treated fabrics in comparison with that of Arcofix NEC alone. Transmission electron microscopy showed that the exposure of bacteria and yeast to chitosan treated fabrics resulted in deformation and shrinkage of cell membranes. The site of chitosan action is probably the microbial membrane and subsequent death of the cell.

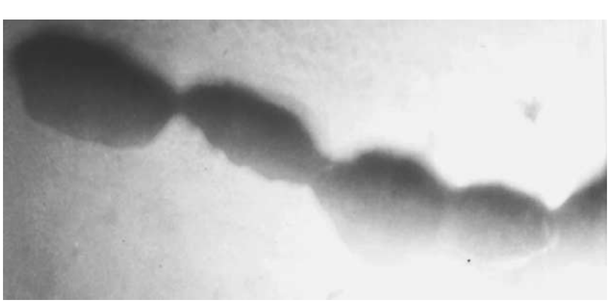


Fig. 3. Transmission electron photograph of *Bacillus cereus* exposed to chitosan treated cotton fabrics for 24 h showing deformed and shrinked cells. × 20,000.

Fig. 4. Transmission electron photograph of *Candida albicans* exposed to chitosan treated cotton fabrics for 24 h showing significant deformation and shrinking of yeast cell. × 20,000.

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