

Antibacterial Activity of Cationically Modified Cotton Fabric with Carboxymethyl Chitosan

Amira M. El-Shafei,¹ Moustafa M.G. Fouda,¹ Dierk Knittel,² Eckhard Schollmeyer²

¹Textile Research Division, National Research Centre, Dokki, Cairo, Egypt

²German Textile Research Centre, D-47798 Krefeld, Germany

Received 25 June 2007; accepted 21 January 2008

DOI 10.1002/app.28352

Published online 16 July 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A water-soluble carboxymethyl chitosan was prepared with a view to develop a multifunctional finish on cotton. Carboxymethyl chitosan (CMCTS) was synthesized by chemical reaction of chitosan with monochloroacetic acid under alkaline condition. The water soluble CMCTS was applied to cationized cotton with different concentrations. The treated fabrics were characterized through monitoring the textile physical properties and for the antibacterial activity against *Escherichia*

coli DSMZ 498 and *Micrococcus luteus* ATCC 9341. The results obtained show that the physical properties of the treated fabrics are improved by increasing the CMCTS concentration, as well as the antibacterial activity. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 110: 1289–1296, 2008

Key words: cationized cotton; carboxymethyl chitosan; textile finishing; antibacterial activity; polyelectrolyte

INTRODUCTION

Chitosan is the deacetylated derivative of chitin, which is the second most abundant polysaccharide found on earth next to cellulose. Chitin, a major component of the shell of crab and shrimp, is one of the most abundant natural polysaccharides with a large unexplored commercial potential. Chitosan is found in some fungi, but its quantity is so limited that it is mainly produced commercially by alkaline deacetylation of chitin.¹ Chitosan is a partially deacetylated polymer of acetyl glucosamine obtained after alkaline deacetylation of chitin. It comprises copolymers of glucosamine and *N*-acetyl glucosamine² and has a combination of many unique properties such as nontoxicity, biocompatibility, and biodegradability.³ Chitosan has got wide application in textile dyeing and finishing as a substitute for the various chemicals used in textile processing.⁴ It has been used as a pretreatment agent in dyeing of cotton, in textile printing, wool dyeing, and shrink proofing, and in durable press finish. However, the application of chitosan in textiles is limited due to its poor solubility above pH 6.5.⁵ Another major problem is

its poor durability on cotton textile due to lack of strong bonding forces between the two polysaccharides.⁶ It was hypothesized that if a water-soluble chitosan derivative could be developed it can lead to development of a multifunctional finish. Cotton can be made antimicrobial, its dyeability can be modified or enhanced and treatment with chitosan can also impart wrinkle recovery property to it, and because of this water-soluble carboxymethyl chitosan (CMCTS) was prepared.

The carboxymethyl derivatives *N*,*O*-substituted derivatives are water soluble and contain an amino group either as the primary ($-\text{NH}_2$) or as secondary amine ($-\text{NH}-\text{CH}_2\text{COOH}$).⁷ The degree of water solubility of this derivative *N*- and *O*-substituted appears to vary with the molecular weight of the material as well as the degree of substitution. The multifunctional chitosan derivative will be favorable to find new kind of polysaccharide with multifunction such as antibacterial character.⁸

The textile industries continue to look for eco-friendly processes that substitute for toxic textile chemicals. In this point of view, chitosan and carboxymethyl chitosan are selected to be useful as excellent candidates for an ecofriendly textile chemical.⁴ The major problem of chitosan as antibacterial agent is the loss of cationic nature and antibacterial activity under alkaline condition and due to this, carboxymethyl chitosan can afford a suitable water-soluble substitute derivative that can work properly under alkaline condition.

This work is undertaken with a view of imparting cotton fabric with antibacterial activity. The fabric

Correspondence to: A. M. El-Shafei (mayamira2001@yahoo.com).

Contract grant sponsor: Federal Ministry for Education and Research (BMBF); contract grant numbers: 033459, 0312689C.

Contract grant sponsor: Deutsche Forschungsgemeinschaft (DFG); contract grant number: 445 AGY-112/37/04.

was first cationized by a commercially available cationic agent, namely Quab[®] 151 (epoxypropyl-trimethyl ammonium chloride).⁹ Cationized cotton was then treated with carboxymethyl chitosan as a polyelectrolyte of opposite charge to form ionic crosslinks in cellulose structure, thereby can enhance cotton characteristics. Besides the antibacterial activity of the fabric, other properties such as crease recovery angles (CRA), tensile strength, as well as acid and reactive dyeability will be examined.

EXPERIMENTAL

Materials and methods

Scoured and rinsed cotton (Style S/400, 106 g/m², plain weave) were obtained from TESTEX, Germany, and Chitosan coded as Bilb 99 was obtained from Bremerhaven GmbH, Germany. 2,3-Epoxy propyltrimethyl ammonium chloride (Quab[®] 151) was obtained from Degussa (Düsseldorf, Germany). Monochloroacetic acid and 2,3,5-triphenyltetrazolium chloride (TTC) were supplied by Merck (Darmstadt, Germany). Two types of dyestuff were used, acid dye namely, Alizarin Red S monohydrate and reactive dye namely, Cibacron, Tuerkis, 2G-E, kindly supplied by Ciba-Geigy. *Escherichia coli* (*E. coli*) DSMZ 498 and *Micrococcus luteus* ATCC 9341, details of their cultivation and inoculation are described in Ref. 10, statistical studies was done, number of experiments was ($n = 5$) and $SD = 0.7$.

Standard I-nutrient broth medium (SI) and Standard I-nutrient agar (SI-agar) were purchased from Merck, Germany. All other chemicals and solvents are of laboratory grade.

Characterization procedures

Determination of chitosan molecular weight

The average molecular weight (M_w) of chitosan used was determined by gel permeation chromatography (GPC); a laser light scattering device PN-3000 (15° and 90°) together with a refractive index detector PN-1000 were from Postnova Analytics, Eresing, Germany. GPC measurements of chitosan solutions were performed using commercially available columns Nucleogel GFC 1000-8 (Macherey-Nagel GmbH, KG, Düren, Germany) as well as Gral 3000 by PSS (Polymer Standards Service GmbH, Mainz, Germany). For calibration standards of pullulan of M_w 100, 200, and 500 kDa were used. Aqueous buffer solution (acetic acid/sodium acetate 0.5/0.3M/L) as a mobile phase with flow rate of 1 mL/min was also used.

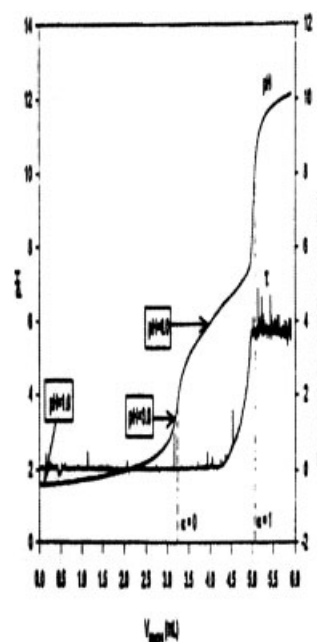


Figure 1 Back titration of chitosanhydrochloride.

Degree of deacetylation

To determine the degree of deacetylation (DDA) of chitosan bilb 99, potentiometric titration method proposed by Koetz and Kosmella¹¹ was employed after slight modification. Fifty milligram of chitosan was dissolved in 5 mL of freshly prepared 0.1M HCl. To the excess component (chitosanhydrochloride), a defined solution of an oppositely charged titrant component (NaOH 0.1M) was slowly added under stirring at a flow rate of 1.2 mL/min to maintain equilibrium condition. The changes in turbidity, conductance, and the pH were automatically registered as functions of the titrant volume described in Figure 1. The potentiometric back titration of chitosanhydrochloride solution is given together with the turbidimetric titration curve. The first equivalence point of the pH-curve directly corresponds to the degree of dissociation ($\alpha = 0$). The second equivalence point ($\alpha = 1$) is in good agreement with the flocculation point; that is the maximum of the turbidity curve. From the NaOH consumption between ($\alpha = 0$) and ($\alpha = 1$), the DDA is directly determined to be 86%.

Synthesis of carboxymethyl chitosan

The experimental technique adopted for carboxymethylation of chitosan was as follows: certain volume of sodium hydroxide solution (30% w/v) was added to 16 g chitosan suspended in isopropyl alcohol. The mixture was left under stirring for 30 min at room temperature. To this mixture, 34 g of monochloroacetic acid was added and the content of the flask was

subjected to continuous stirring for 3 h. At the end, the excess alkali was neutralized using glacial acetic acid and the chitosan was precipitated by adding acetone. Finally, the modified chitosan was filtered and washed with isopropyl alcohol/water (70 : 30) several times and dried at 60°C.¹² The final product was soluble in water.

Cationization of cotton fabric followed by treatment with CMCTS

Cationization of cotton fabrics were carried out using pad-dry-cure method.¹³ The experimental conditions were adopted as follows: cotton fabrics were padded in solution containing Quab[®] 151 (50–140 g/L) and NaOH (1 : 1M ratio), acetone was added as proper solvent for the reaction medium, and then the fabrics were squeezed between two nips and dips to a wet pick up 100%. The cotton fabrics were dried at 80°C for 3 min, and then cured at 120°C for 8 min. At this end, the cationized fabrics were padded again in solution containing CMCTS (20–50 g/L). The fabrics were then batched in plastic bags at 60°C overnight. At the end, the finished fabrics were washed several times with water, and finally dried at ambient laboratory conditions.

Dyeing of treated cotton fabrics

Dyeing of the cationized cotton fabrics before and after treatment with CMCTS was carried out without the addition of salt. The dyeing bath concentration either for acid or reactive dye was 500 mg/L and the material to liquor ratio was 1 : 50. The aqueous solution of the dye containing the samples was subjected to shaking at 80°C for 1 h. At the end of dyeing, samples were washed several times with boiling water containing 0.1% Marlipal[®] (nonionic wetting agent), and finally washed with cold water and dried at ambient conditions.

Testing and analysis

CRA of the treated and untreated samples were measured according to DIN 53 890 standard test method.¹⁴ Tensile strength and elongation at break were determined according to DIN standard test method.¹⁵ Color measurements as *K/S* were carried out using Datacolor Type International, Dietlekol, Schweiz.¹⁶ The wettability test according to TEGAWA was applied.¹⁷ Chitosan derivative (CMCTS) was characterized by solid-state ¹³C NMR spectroscopic analysis. The treated cotton samples were also characterized by FTIR spectroscopy. Antimicrobial activity for the treated cotton was carried out using TTC test method, which is described in detail in Ref. 10.

RESULTS AND DISCUSSION

Textile goods, especially those made from natural fabrics can provide an excellent environment for microorganisms to grow and thereby reducing its exhaustion property, because of their large surface area and ability to retain moisture. Therefore to impart the natural fibers, a high antimicrobial activity and its chemical modification has been found to be an highly effective method.¹⁸ The durable wrinkle-free cotton¹⁹ and durable fire-resistant cotton²⁰ are examples of some of the chemical modifications of cotton fabric. The major problem encountered with the cotton cellulose is its lack of attractive sites. To overcome this problem, we have prepared cationic cotton to make an ionic crosslinking with CMCTS as anionic compound and form antibacterial cotton.

The aforementioned cationized- and cationized-CMCTS-treated cotton were used for cotton finishing. The effects of application of such finishes on fabric performance as assessed by nitrogen percent, tensile strength, elongation at break, crease recovery, dyeing performance, and antibacterial activity were studied. Given below are the results obtained along with appropriate discussion.

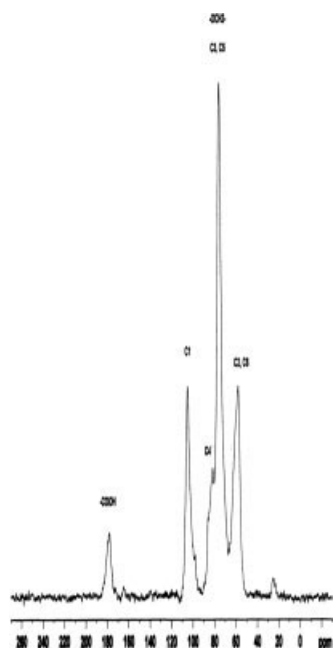
Characterization of carboxymethyl chitosan by solid state ¹³C NMR

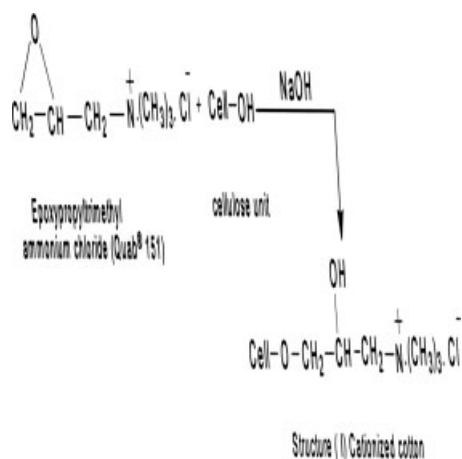
Carboxymethylation of chitosan (CTS) is achieved with monochloroacetic acid and sodium hydroxide. According to Ref. 21, this reaction takes place preferentially either at C-6 hydroxyl groups or at the NH₂-group resulting in N/O-CMCTS. The solid-state ¹³C NMR spectrum for a typical *N*-carboxymethyl chitosan shows signals attributed to the *N*-carboxymethyl substituent, at 47.7 and 168.7 ppm, for N—CH₂ and COOH, respectively.²¹

But in case of our results, the solid-state ¹³C NMR described in Figure 2 shows signals at 73 and 175 ppm, which attributed to —O—CH₂— and COOH, respectively. This downfield shift of the carbon indicates the formation of *O*-carboxymethyl chitosan. The formation of this product agrees with the higher reactivity of hydroxyl group of C6 in this heterogeneous reaction. The *N*-carboxymethyl substituent is not present because of the absence of peaks at 47 and 168 ppm for N—CH₂ and COOH, respectively.

FTIR-analysis of the CMCTS and treated cotton fabrics

The FTIR spectrum of chitosan [Fig. 3(a)] shows peaks assigned to the saccharide structure at 1149.7, 1072.6, and 897.9 cm⁻¹, and a strong amino characteristic peak concentration at around 3400 cm⁻¹.





Scheme 1 Reaction mechanism of Quab[®] 151 with cotton.

respectively, of $-\text{CH}_2$ groups. A weak peak observed around 896 cm^{-1} is attributed to the stretching of $\text{C}-\text{N}^{(+)}$ bond formed in cationized cotton (b). This peak is neither observed in the spectra of bleached cotton nor CMCTS.

- c. Weak peak at 1730 cm^{-1} is attributed to the stretching of carboxylic carbonyl groups. Also, the weak peak at 896 cm^{-1} represents the $\text{C}-\text{N}^{(+)}$ of cationized cotton.

Effect of cationized and cationized/CMCTS treated cotton on textile performance

Wet and dry crease recovery angle

Reaction of Quab[®] 151 with cotton fabrics produces cationized cotton as outlined in the following scheme reaction (Scheme 1).

Cotton samples treated with the aforementioned cationized finishes in the absence and presence of CMCTS were monitored for dry and wet CRA in the warp and weft directions. It is seen in Figure 5(a,b) that CMCTS finishes based on cationized cotton impart significant enhancement in wet and dry crease recovery of the cotton fabrics as compared with the cationized cotton. Cationized cotton bearing CMCTS may allow crosslinking if these groups are localized at two adjacent cellulose chains as suggested by structure II (Scheme 2). The magnitude of crease resistance, whether wet or dry, would rely on the degree of crosslinks, the ratio of intra-inter-chain crosslinks, and the state of the fabric during measurement of the CRA.

The reaction of CMCTS with cationized cotton samples enhances both dry and wet CRA, as can be seen in Figure 5(a,b). It is obvious that at the same concentration of Quab[®] 151 (100 g/L) the dry and wet CRA are 198° and 230° but by the application of

a CMCTS at a concentration of 40 g/L there is enhancement in dry and wet CRA to 210° and 265° , respectively, and this is due to the ionic crosslinking of the cotton fabric.

Strength properties

Results of strength properties, including tensile strength (TS) and elongation at break ($E\%$) of cotton fabrics finished are shown in Tables I and II. Obviously, CMCTS (based on cationized cotton) improves the tensile strength of the cotton fabric most probably due to the extra strength of CMCTS film. It is clear that pre-cationization of the ether-crosslinked cotton samples leads to slight improvement in the tensile strength as well as elongation at break. Such improvement may reflect the cationization effect on the post-etherifies cationized cotton cellulose according to Ref. 23, and/or the ionic crosslinking nature as it may bring the cellulose chain closer to each other, hence, an increasing in the TS is observed. For example, the tensile strength increases from 24.5 cN for untreated fabric to 32.4 cN for fabrics treated with cationized CMCTS. Such differences in the values of tensile strengths could be associated with differences

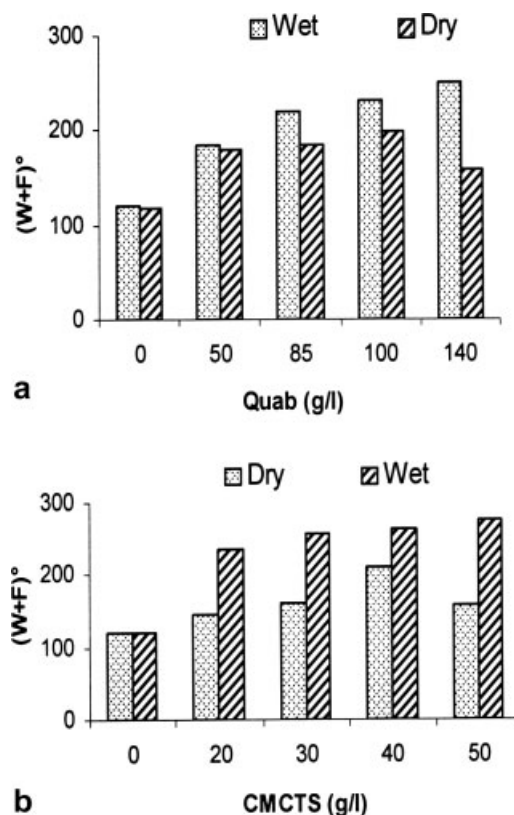
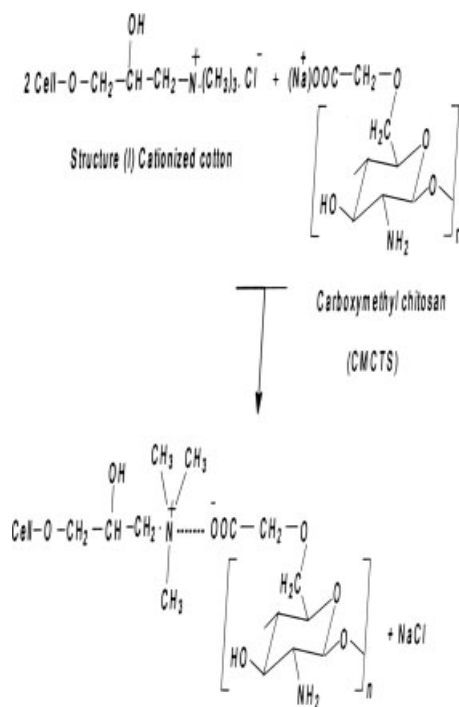


Figure 5 Effect of Quab[®] 151 (a) and CMCTS (b) concentrations (based on cationized cotton) on the crease recovery angle (CRA) wet and dry. For (b) [Quab[®] 151], 100 g/L; fixation temperature/time, $140^\circ\text{C}/3\text{ min}$.



Scheme 2 Ionic crosslinking of cotton.

in the strength of the films of the chitosan finishes which, in turn, is governed by the molecular weight of the chitosan finish, nature and distribution of its substituents, and mode of association of the finish with the cotton fabrics. Tables I and II show also that the elongation at break is not significantly affected when the fabric was treated with the cationized-CMCTS based finishes. The elongation at break displays a value ranging between 12 and 18% when the finishing was carried out using the cationized-based finishes in the presence of CMCTS.

Crosslinked cotton dyeability expressed as (K/S)

Figure 6(a,b) shows *K/S* values (measure of color strength) of cationized cotton fabric before and after treated with CMCTS. It is seen that the increasing concentration of Quab[®] 151 from 50 to 140 g/L enhances *K/S* values of dyed samples with acid and

TABLE I
Effect of Quab[®] Concentration (Based on Cationized Cotton) on the Strength Properties

Quab [®] 15 (g/L)	TS _(Kgf)	E %
0	24.5	12.0
50	24.5	13.3
85	24.0	13.1
100	23.5	12.3
140	23.5	12.4

TABLE II
Effect of CMCTS concentration (Based on Cationized Cotton) on the Strength Properties

CMCTS (g/L)	TS _(Kgf)	E%
0	24.5	13.3
20	24.7	14.9
30	25.1	15.2
40	28.0	18.7
50	32.4	18.4

[Quab[®] 151], 100 g/L; fixation temperature/time, 140 °C / 3 min.

reactive dyes, and this finding is considered as an evidence for the cationization of cotton. It is also seen that for a given Quab[®] 151 (100 g/L) concentration and with increasing CMCTS concentration there is decrease in *K/S* values of the dyed samples whether with reactive or acid dye; both have the same trend. From this, it is obvious that the *K/S* values depend largely on the magnitude of both cationization and concentration of CMCTS. Also, CMCTS may block the site of dye-uptake.

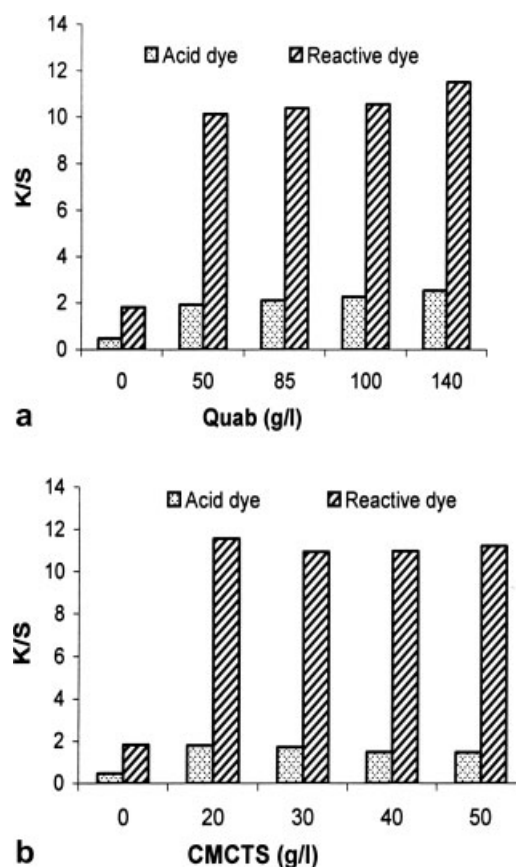


Figure 6 Effect of Quab[®] 151 (a) and CMCTS (b) concentrations (based on cationized cotton) on the dyeability of cotton. For (b) [Quab[®] 151], 100 g/L; fixation temperature/time, 140 °C/3 min.

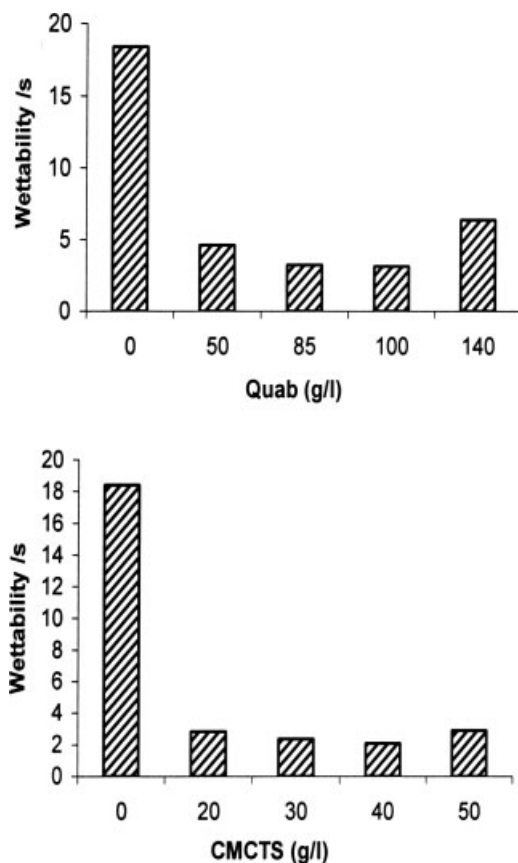


Figure 7 Effect of Quab[®] 151 (a) and CMCTS (b) concentration (based on cationized cotton) on wettability.

Wettability

Wettability measurement for different types of textile materials is important to evaluate the hydrophilicity of textile materials. This property affects the quality and hence the marketing of such textile materials. Different methods of finishing techniques are taken into consideration to improve the wettability of cotton fabrics, consequently the hydrophilic properties.

In this study, cotton fabrics treated with the aforementioned cationized finishes in the absence and presence of CMCTS were also monitored for the wettability test. The results obtained are shown in Figure 7. It is seen that CMCTS finishes based on cationized cotton impart significant enhancement in wettability of the cotton fabrics as compared with the cationized cotton. Nevertheless, in the presence of CMCTS there is a synergistic enhancement in wettability than in its absence [Fig. 7(a,b)].

Effect of CMCTS (based on cationized cotton) on the antibacterial activity

Cotton fabric with good antibacterial activity is obtained by the cationization of cotton using Quab[®] 151 in the presence of alkali. Different concentrations

of Quab[®] 151 are applied to the cotton fabrics using the pad-dry-cure method. The antibacterial activity of the treated cotton fabrics are evaluated against *E. coli* and *M. luteus* according to the TTC-test method.¹⁰ In Figure 8, the absorbance of formazan is directly proportional to the number of active cells. The activity of the cells of both microorganisms decreased by increasing the concentration of Quab[®] 151 in comparison with the control, hence, the antibacterial activity of the treated cotton fabrics increased. The antibacterial activity of CMCTS (based on cationized cotton fabrics) was also evaluated, and the results in Figure 8 shows that the activity of the cells decreased by increasing the concentration of CMCTS based on cationized cotton. This finding results confirm structure (II) found in Scheme 2.

There are two proposed mechanisms for the antibacterial activity of CTS. In one mechanism, the

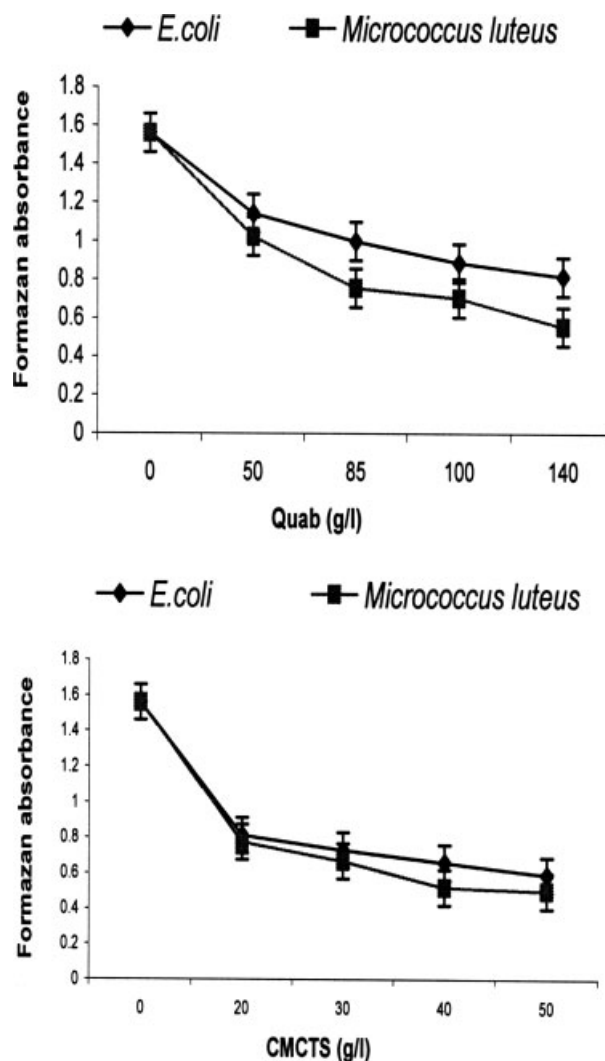


Figure 8 Effect of Quab[®] 151 (a) and CMCTS (b) concentrations (based on cationized cotton) on the antibacterial activity. For (b) [Quab[®] 151], 100 g/L.

polycationic nature of CMCTS based on the amino groups present in CTS molecules in addition to the permanent positive charge arising from the cationized cotton, both interact with the negative charged residues present at the cell wall of bacteria leading to alteration of the cell wall permeability, consequently, interfere with the bacterial metabolism and result in the death of cells.²⁴ Another mechanism assumed that the antibacterial activities of chitosan derivative can also be closely correlated to the formation of hydrophobic microareas. At pH 7, the degree of protonation of NH_2 is very low and thus the repulsion of NH_3^+ is weak, so the strong intermolecular and intramolecular hydrogen bond results in the formation of hydrophobic microareas in the polymer chains.²⁵ At the same time, the carboxyl groups in the polymer chains are strongly hydrophilic. Therefore, the polymer chains have hydrophobic and hydrophilic parts. This amphiphilic structure provides structure affinity between the cell walls of bacteria and the chitosan derivative. On the other hand, carboxylate groups having strong polarity can react with phospholipids in the cell membranes and the nonpolar parts of the derivatives can insert the hydrophobic areas. The cell membranes will be broken because of the strong electrostatic interaction and result in the death of cells. The result was compared with other results of cotton fabrics finished with quaternized chitosan. It is found that the antibacterial efficiency increased in the case of cotton finished with quaternized chitosan than with chitosan alone. These findings prove that the former mechanism should be considered to be closer to the real mechanism.

CONCLUSIONS

Ionic crosslinking of cotton was carried out to solve the problems associated with chitosan such as limited solubility, antimicrobial activity, and poor laundering durability when applied as pure chitosan to cotton fabrics. A water-soluble chitosan derivative, namely carboxymethyl chitosan was successfully prepared from chitosan and monochloroacetic acid in the presence of alkali. The water-soluble product was characterized by solid-state ^{13}C NMR spectroscopy. Cationization of cotton was carried out using 2,3-epoxypropyltrimethyl ammonium chloride

(Quab[®] 151) in the presence of alkaline aqueous solution. Then, carboxymethyl chitosan was applied to the pre-cationized cotton using cold-pad-batch method. As a result of this treatment ionic crosslinked cotton was obtained. The treated cotton fabrics showed significant increases in CRA without strength loss. Also, the wettability of the treated cotton samples increased significantly. The antibacterial activity of the ionically crosslinked cotton also increased. Therefore, ionic crosslinking, as presented, of cotton improved the physical and performance properties of cotton fabrics combined with a moderate antibacterial activity.

References

1. Roberts, G. A. F. *Chitin Chemistry*; Macmillan Press: London, 1992.
2. Mishra, B.; Jayanth, P.; Sankar, C. *Indian Drugs* 2003, 40, 695.
3. Singla, A. K.; Chawla, M. *J Pharm Pharmacol* 2001, 53, 1047.
4. Lim, S. H.; Hudson, S. M. *J Macromol Sci Polym C43* 2003, 2, 223.
5. Jia, Z.; Shen, D.; Xu, W. *Carbohydr Res* 2001, 333, 1.
6. Lim, S. H.; Hudson, S. M. *Carbohydr Res* 2004, 339, 313.
7. Ernest, R.; Hayes, R. US Pat. 4,619,995 (1988).
8. Xie, W.; Xu, P.; Wang, W.; Liu, Q. *Carbohydr Polym* 2002, 50, 35.
9. Hashem, M.; Hauser, P.; Smith, B. *Text Res J* 2003, 73, 762.
10. Fouda, M.; Knittel, D.; Zimehl, R.; Hipler, C.; Schollmeyer, E. *Adv Chitin Sci* 2005, 8, 418.
11. Koetz, J.; Kosmella, S. *Adv Chitin Sci* 1997, 2, 476.
12. Hebeish, A.; Waly, A.; Higazy, A.; El-Shafei, A. M. First International Conference of Textile Research Division, NRC, Cairo, Egypt, March 2-4, 2004.
13. Hashem, M.; El-Aref, A. T.; Refai, R. *RJTA* 2004, 8, 76.
14. German DIN Book-Methods of Analysis, 53890, January 1972.
15. German DIN Book-Methods of Analysis, 255-262, 1979.
16. Deane, B. J.; Wyszecski, G. *Color in Business Science*; Wiley: New York, 1975.
17. Breichter, S.; Wurster, P.; Schmidt, G. *Melliand Text Berichte* 1987, 8, 581.
18. Holme, I. *J Text Chem* 1993, 84, 520.
19. Welch, C. M. *Text Chem Color* 1990, 22, 13.
20. Lecoceur, E.; Vorman, I.; Bourdigot, S.; Lam, T. M.; Delobel, R. *Polym Degrad Stab* 2001, 74, 487.
21. Kurita, K. In *Chitin in Nature and Technology*; Muzzarelli, R. A. A.; Jeuniaux, C.; Gooday, G. W., Eds.; Plenum Press: New York, 1986; p 287.
22. Liu, X. F.; Guan, Y. L.; Yang, D. Z.; Li, Z.; Yao, K. D. *J Appl Polym Sci* 2001, 79, 1320.
23. Rowland, S. P.; Brannan, M. A. F. *Text Res J* 1970, 40, 607.
24. Zhou, C.; Li, Y.; Meng, L. *Dyeing Finish* 1996, 22, 33.
25. Chen, T.; Zhang, X. H.; Guo, R. *Acta Phys Chim Sin* 2000, 16, 1039.