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Rechargeable biocidal cellulose: Synthesis and application of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione

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ABSTRACT

An N-halamine precursor containing two hydroxyl groups (diol) has been synthesized. The N-halamine diol precursor was coated onto cotton fabrics with the assistance of the cross-linking agent BTCA. The surface of the coated cotton could be rendered biocidal by exposure to dilute hypochlorite solutions. Syntheses routes, characterization data, and antimicrobial test results will be presented. The durability and rechargeability of chlorine on the coated cotton was determined according to an AATCC standard washing test. The chlorinated cotton swatches were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) and showed excellent efficacy against these two bacterial species within a brief time of contact. Over 70% of the chlorine lost after repeated washing or UVA irradiation could be regained upon rechlorination.

1. Introduction

Textile materials are carriers of microorganisms such as fungi, bacteria, and viruses. With the favorable conditions existing for textiles, such as humidity, warmth, and nutrients, the microorganisms increase their numbers very rapidly. These microorganisms, if pathogenic or infectious, may cause health problems to users of the textile materials, especially healthcare institutional personnel. The transmission of infectious diseases that occur in hospitals and other public health related venues could be prevented, or at least minimized, by the workers wearing antimicrobial clothing.

Generally, antimicrobial textiles can be obtained by chemically or physically incorporating antimicrobial agents onto fibers or fabrics. The durability of antimicrobial textiles can be grouped into two categories, temporary or durable. Temporary antimicrobial fabrics can be achieved easily, but also their biocidal functionality is subjected to loss in laundering. N-halamine materials have been developed and shown to be effective biocides in these laboratories during the past two decades (Tsao, Williams, & Worley, 1990; Worley & Sun, 1996; Worley & Williams, 1988). The outstanding advantages of these N-halamine compounds are their durable and rechargeable antimicrobial properties when these compounds can be covalently bound to the substrates (Grunzinger et al., 2007; Makal, Wood, Ohman, & Wynne, 2006).

Natural fibers, such as cotton, are superior media to synthetic fibers for the growth of bacteria due to their hydrophilic properties. However, cotton does possess many functional groups, such as hydroxyl groups, which make it easily modified to render it antimicrobial. There are two means to prepare antimicrobial cellulose: (1) N-halamine precursors are bound chemically to cotton fibers (Barnes et al., 2006; Liang et al., 2006, 2007a,b; Liu & Sun, 2008; Ren et al., 2008a; Sun & Sun, 2001, 2002; Sun & Xu, 1999a,b,c); (2) N-halamine precursors are blended with cotton fibers before fiber extrusion (Lee, Broughton, Worley, & Huang, 2008b).

One common method to render the cotton fibers durable antibacterial activity is to bind the precursor moieties to the fibers by covalent bonds. Durable and regenerable antibacterial finishing of cotton by using monomethylo1-5,5-dimethylhydantoin (MDMH) and 1,3-dimethylo1-5,5-dimethylhydantoin (DMDMH) have been reported by Sun and his coworkers (Sun & Xu, 1999a,b,c). Durable and regenerable antibacterial agents like MDMH and DMDMH can be chemically bound to cellulose by using a wet finishing technique. The DMDMH treated fabrics also improved durable press functions. Grafting hydantoin-containing monomers onto cotton cellulose has been reported also (Liu & Sun, 2008; Sun & Sun, 2001, 2002). A heterocyclic monomer, 3-allyl-5,5-dimethylhydantoin (ADMH) was synthesized and employed in grafting cotton fabrics (Sun & Sun, 2001). A continuous "dip-pad" finishing process was developed to graft ADMH onto

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cotton (Liu & Sun, 2008; Sun & Sun, 2002), with the advantages of lower initiator usages, higher graft efficiencies, and shorter reaction times than the previously used exhaustion process. Recently, in these laboratories a series of N-halamine siloxane coatings were prepared and reported to produce biocidal cellulose (Barnes et al., 2006; Liang et al., 2006, 2007a,b). These coatings of cotton proved to be quite stable to machine washing as the lost chlorine could be regained partially. Most recently, the admicellar polymerization technique was employed to coat N-halamine precursor monomer onto cotton to produce upon chlorination antimicrobial cellulose (Ren et al., 2008a).

It is well known that the cross-linking agent, 1,3-dimethylo1-5,5-dimethylhydantoin (DMDMH), has been used in permanent-press finishing of cotton fabric. The slow release of free formaldehyde from DMDMH during wearing and using, however, is an inherent disadvantage in using this chemical. Cross-linking agents, such as polycarboxylic acids free of formaldehyde, have been developed as an alternative for the DMDMH in durable press finishing of cellulose in the last decade (Udokichdecha, Kittinaovarat, Thanasoonthornroek, & Potiyaraj, 2003; Yang, 1991; Yang & Wang, 1996; Yang, Wang, & Kang, 1997). This technique has been used to produce antimicrobial cellulose by bonding the aminohydantoin-containing precursors onto cotton with the aid of polycarboxylic acids (Kou et al., 2006; Lee, Broughton, Akdag, Worley, & Huang, 2008a). In this study, a new N-halamine precursor, 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione, was synthesized and attached to cellulose via the cross-linking agent 1,2,3,4-butanetetracarboxylic acid (BTCA). The chlorinated coated cotton samples will be shown to demonstrate excellent antimicrobial properties in inactivating *Staphylococcus aureus* and *Escherichia coli* O157:H7. The stabilities of the coated samples during washing and upon UV irradiation were also investigated.

2. Experimental

2.1. Materials

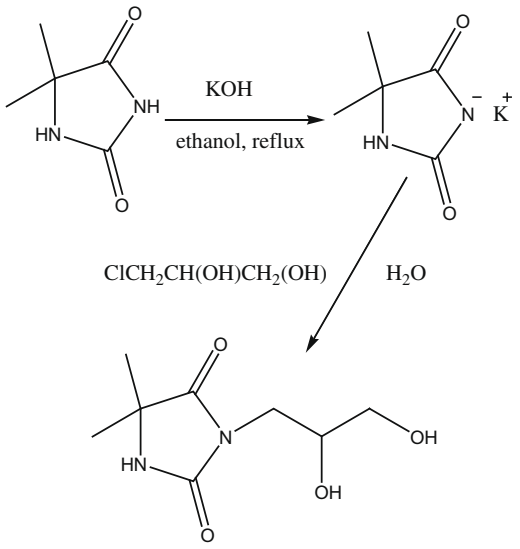
Fabric of 100% bleached cotton was purchased from Testfabrics, Inc. (West Pittston, PA). All chemicals used in this research were purchased from Fisher Scientific (Fair Lawn, NJ) or Aldrich Chemicals (Milwaukee, WI) and employed without further purification.

2.2. Instruments

The NMR spectra of the synthesized compound were recorded by a Bruker AV-400 (400 MHz) spectrometer. FTIR spectra of cotton, coated cotton, and chlorinated coated cotton were obtained with a Nicolet 6700 FT-IR spectrometer.

2.3. Synthesis of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione (Scheme 1)

A mixture of 0.05 mol of 5,5-dimethylhydantoin and 0.05 mol of potassium hydroxide in 100 mL ethanol was refluxed for 10 min. The above solution was mixed with an equimolar quantity of 3-chloropropanediol in 25 mL water and then stirred for 16 h at ambient temperature. After the reaction, ethanol and water were removed, and 50 mL of acetone were added to the flask. Potassium chloride produced in the reaction was removed by filtration. The solvent acetone was evaporated producing a transparent viscous oil, which was shown to be the desired product. The experimental yield was 90%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (6H), 3.29 (4H), 3.70 (1H); ^{13}C NMR (DMSO-d_6), δ 25.08, 25.09, 41.94, 58.06, 64.64, 68.73, 156.18, 178.07 (Scheme 1).



Scheme 1. Synthesis of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione.

2.4. Coating of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione onto cotton

Cotton swatches were soaked in aqueous solutions containing 3–5% diol and equimolar quantities of BTCA for 15 min. These swatches were dried at 100 °C for 5 min, cured at 170–180 °C for 2–3 min, soaked in 0.5% detergent solution for 15 min, washed with water, and dried in air.

2.5. Chlorination and analytical titration

The cured N-halamine diol-coated cotton fabric swatches were immersed in a 10% commercial aqueous sodium hypochlorite (NaOCl) solution at pH 11 at room temperature for 1 h. The chlorinated cotton samples were washed thoroughly with distilled water and dried at 45 °C for 1 h to remove any remaining free chlorine from the surface of the fabric. The loaded chlorine concentration on the samples was determined by the iodometric/thiosulfate titration method. The Cl^+ % on the cotton swatches was calculated from the following equation:

$$Cl^+\ \% = \frac{N \times V \times 35.45}{W \times 2} \times 100$$

where Cl^+ (%) is the weight percent of oxidative chlorine on the samples, N and V are the normality (equiv/L) and volume (L) of the titrant sodium thiosulfate, respectively, and W is the weight of the cotton samples in grams.

2.6. Biocidal efficacy testing

Control and chlorinated cotton fabrics were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) using a modified AATCC Test Method 100-1999. Twenty-five microliters of the bacterial suspensions buffered at pH 7 were added to the center of two pieces of 1 in.^{2} cotton swatches which were held in place by sterile weights. After the contact times of 1, 5, and 10 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution to remove all oxidative chlorine and vortexed. The sodium thiosulfate employed in these studies in a control experiment did not cause a reduction of either bacterium. Serial dilutions of the quenched samples were made using pH 7, 100 μM phosphate buffer, and they were plated

on Trypticase soy agar. The plates were incubated at 37 °C for 24 h, and viable bacterial colonies were recorded for biocidal efficacy analysis. Unchlorinated coated swatches treated in the same manner served as control samples.

2.7. Stability testing of chlorine on coated cotton

AATCC Test Method 61-1996 was used to evaluate the stabilities of chlorine after repeated standard washing. The cotton swatches were washed for the equivalents of 5, 10, 25, and 50 machine washes in a Launder-Ometer. The Cl^+ % loadings of samples after the washings were determined by the titration procedure addressed above.

2.8. UVA light stability testing

UVA light stabilities of chlorinated cotton fabrics coated with the hydantoin diol were measured using an Accelerated Weathering Tester (The Q-panel Company, USA). The chlorinated coated cotton fabrics were placed in the UV light (Type A, 315–400 nm) chamber for contact times in the range from 1 to 24 h. After a specific time of exposure to UVA light, the cotton samples were removed from the UV chamber and titrated, or rechlorinated and titrated.

3. Results and discussion

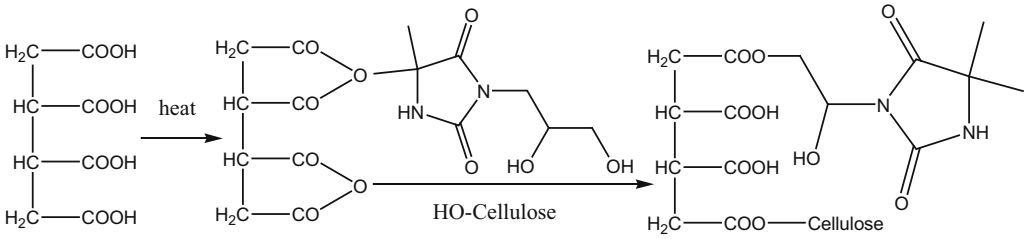
3.1. Characterization of cotton coated with N-halamine diol

Cotton cellulose has numerous hydroxyl groups which can react with polycarboxylic acids such as BTCA by forming ester bonds. The synthesized N-halamine precursor, 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione, contains two hydroxyl groups which can also react with BTCA through esterification. BTCA, acting as a cross-linking agent, links the N-halamine diol to the cotton cellulose through the curing process (Scheme 2). The coated cotton fabrics contained numerous amide functional groups which could be rendered antimicrobial by exposure to diluted household bleach (Scheme 3).

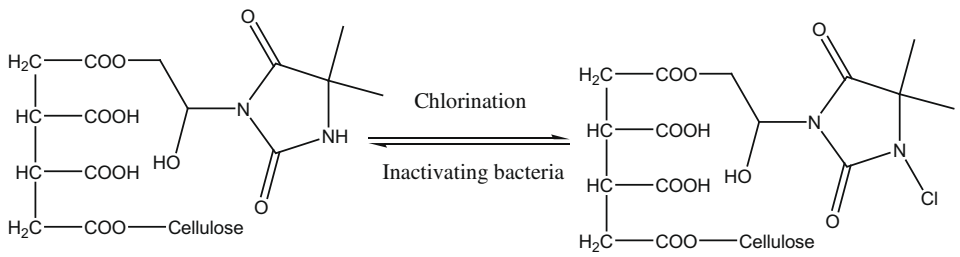
The FTIR spectra of cotton, cotton coated with BTCA, and cotton coated with BTCA and N-halamine diol before and after chlorination are shown in Fig. 1. The characteristic vibrational band of cotton coated with BTCA appears at 1712 cm^{-1} in Fig. 1 (B), which corresponds to the carbonyl vibrational modes of BTCA. This band was not observed in Fig. 1(A) for cotton. The cotton coated with BTCA and N-halamine diol shows a new band at 1704 cm^{-1} (in Fig. 1(C)). This vibrational band shifts to 1724 cm^{-1} after chlorination (in Fig. 1(D)). The shifts to higher wave number of the hydantoin carbonyl bands upon chlorination have been reported from these laboratories for other N-halamines (Chen et al., 2003; Ren et al., 2008a).

3.2. Biocidal testing

The treated cotton swatches coated with unchlorinated and subsequently chlorinated hydantoin diol were challenged with the bacteria *S. aureus* (Gram-positive) and *E. coli* O157:H7 (Gram-negative). The biocidal efficacy results are shown in Tables 1 and 2. The unchlorinated coated cotton control samples showed some degree of log reduction of the bacteria *E. coli* O157:H7 and *S. aureus*. The reductions for the unchlorinated samples were due to the adhesion of the bacteria to the surface of the cotton fibers, rather than to inactivation. Cultures of the control fiber samples showed that live bacteria were present. As mentioned earlier, the sodium thiosulfate quenching solution did not cause inactivation of either bacterium. The bacterial log reduction for *S. aureus* after 10 min contact was 1.80, which was more significant than that for *E. coli* O157:H7 (0.47). The chlorinated coated cotton samples could inactivate 100% of the bacteria *S. aureus* within 1 min with log reduction of 6.99, while the chlorinated coated cotton samples inactivated 99.94% *E. coli* O157:H7 in 1 min and 100% in 5 min, or a 6.89 log reduction with a contact time from 1 to 5 min. This is consistent with our previously published results for other N-halamine coatings that have indicated that *E .coli* O157:H7 is generally more resistant to inactivation than is *S. aureus*. Inactivation within 5 min on cotton coated with the N-halamine moieties indicates that the coating described herein could have great potential for use in healthcare environments.



Scheme 2. Attachment of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione to cellulose.



Scheme 3. Production of antimicrobial cellulose.

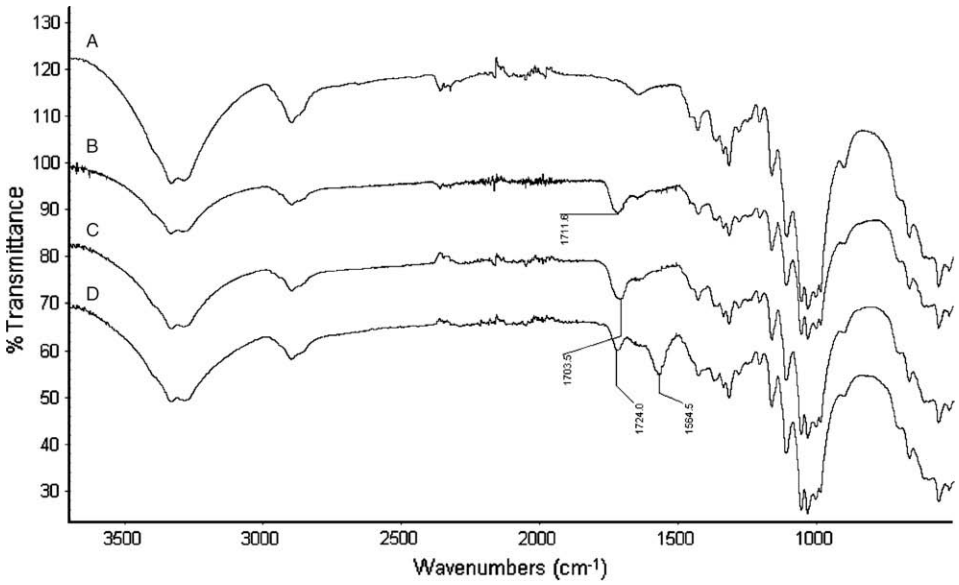


Fig. 1. FTIR spectra of (A) cotton, (B) cotton BTCA, (C) cotton hydantoin diol BTCA, and (D) chlorinated cotton hydantoin diol BTCA.

Table 1
Biocidal efficacy against *S. aureus*[a]

Sample	Contact time (min)	Bacterial log reduction
Hydantoin diol control	1	1.37
	5	1.44
	10	1.80
Hydantoin diol-Cl 0.2 Cl^+ % by wt.	1	6.99
	5	6.99
	10	6.99

[a] The inoculum concentration was 9.67×10^6 CFU.

Table 2
Biocidal efficacy against *E. coli* O157:H7[a]

Sample	Contact time (min)	Bacterial log reduction
Hydantoin diol control	1	0.39
	5	0.44
	10	0.47
Hydantoin diol-Cl 0.2 Cl^+ % by wt.	1	4.46
	5	6.89
	10	6.89

[a] The inoculum concentration was 7.67×10^6 CFU.

3.3. Stability and rechargeability

The stabilities of the oxidative chlorine and the chlorine rechargeability of the N-halamine compound on cotton fabrics were evaluated by an AATCC standard washing test addressed in Section 2. Each washing cycle in this method is equivalent to five household machine washings followed with water rinsing at 65 °C for 15 min. The retained chlorine on the cotton fabric decreased rapidly with the increase of washing cycles from 0.28% to 0.02% after 10 washing cycles, primarily due to the facile hydrolysis of the N–Cl bond with the elongation of washing times. The rechargeability of the coated cotton fabric samples, however, is excellent after exposure to numerous washing cycles (Fig. 2). After 50 washing cycles and rechlorination, over 75% of the chlorine was restored, which indicated that the coated cotton fabric would still be very effective in inactivating bacteria. In a use process the fabric could be recharged during each machine washing cycle by adding a dilute solution of household bleach.

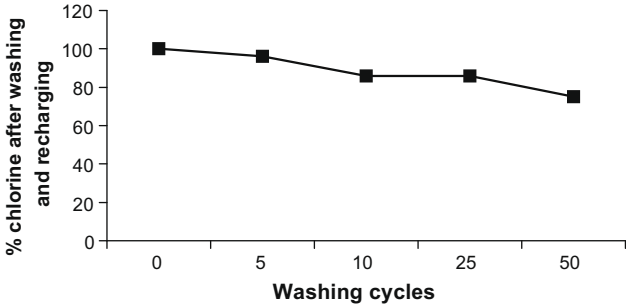


Fig. 2. Rechargeability of coated cotton after exposure to washing tests.

Table 3
UV light stability of chlorinated coating on cotton fabrics

Time (h)	(Cl^+ % wt)
0	0.23
1	0.15
2	0.13
3	0.11
4	0.09
8	0.09
24	0.03
24 (rechlorination)	0.16

3.4. UVA light stability

The UVA light stability of the chlorinated cotton coated with hydantoin diols was determined according to the method mentioned in Section 2. Table 3 shows the UVA light stability results for the cotton fabrics coated with hydantoin diol following chlorination. The chlorine on the coated cotton decreased rapidly within the first hour of irradiation, and slightly decreased with the extension of time. About 40% of the chlorine remained on the coated cotton fabrics after 8 h under UVA light irradiation, but most of the chlorine was lost after 24 h of irradiation. After exposure to UV light for 24 h and exposure to an aqueous solution of sodium hypochlorite, about 70% of the chlorine on the coated cotton could be recovered, which indicates that the N-halamine hydantoin diols coated onto cotton fabrics were more stable under the UVA irradi-

ation than were the N-halamine siloxanes coated onto cotton fabrics reported previously (Ren et al, 2008b).

4. Conclusion

An N-halamine precursor, 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione, was synthesized and coated onto cotton fabrics by using BTCA as a cross-linking agent. The amide nitrogen of the hydantoin rings attached onto the cotton samples could be converted to halamines upon exposure to dilute household bleach. The cotton swatches with halamines were shown to be biocidal against *Staphylococcus aureus* and *Escherichia coli* O157:H7. The bacteria, *S. aureus* and *E. coli* O157:H7, could be inactivated within 1 min and 5 min of contact with log reductions of 6.99 and 6.89, respectively. The N-halamine structure was very stable after repeated laundry washing, and about 75% of the chlorine could be restored with dilute chlorine bleach. The UVA light stability of the chlorine on the coated samples was excellent; the chlorine was lost more slowly than that of cotton fabrics coated with N-halamine siloxanes. In addition, about 70% of the chlorine could be regained after rechlorination.

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References

Barnes, K., Liang, J., Wu, R., Worley, S. D., Lee, J., Broughton, R. M., et al. (2006). Synthesis and antimicrobial applications of 5,5′-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]. *Biomaterials, 27*, 4825–4830.

Chen, Y., Worley, S. D., Kim, J., Wei, C. I., Chen, T. Y., Suess, J., et al. (2003). Biocidal polystyrenehydantoin beads. 2. Control of chlorine loading. *Industrial and Engineering Chemistry Research, 42*, 5715–5720.

Grunzinger, S. J., Kurt, P., Brunson, K. M., Wood, L., Ohman, D. E., & Wynne, K. J. (2007). Biocidal activity of hydantoin-containing polyurethane polymeric surface modifiers. *Polymer, 48*, 4653–4662.

Kou, L., Liang, J., Worley, S. D., Lee, J., Broughton, R. M., & Huang, T. S. (2006). Antimicrobial cellulose: Preparation and application of 5-methyl-5-aminomethylhydantoin. *Research Journal of Textile and Apparel, 10*, 44–53.

Lee, J., Broughton, R. M., Akdag, K., Worley, S. D., & Huang, T. S. (2008a). Antimicrobial fibers created via polycarboxylic acid durable. *Textile Research Journal, 77*, 604–611.

Lee, J., Broughton, R. M., Worley, S. D., & Huang, T. S. (2008b). Antimicrobial polymeric materials; Cellulose and m-aramid composite fibers. *AATCC Review, 8*, 43–48.

Liang, J., Barnes, K., Akdag, A., Worley, S. D., Lee, J., Broughton, R. M., et al. (2007a). Improved antimicrobial siloxane. *Industrial and Engineering Chemistry Research, 46*, 1861–1866.

Liang, J., Chen, Y., Barnes, K., Wu, R., Worley, S. D., & Huang, T. S. (2006). N-halamine/quat siloxane copolymers for use in biocidal coatings. *Biomaterials, 27*, 2495–2501.

Liang, J., Wu, R., Wang, J. W., Barnes, K., Worley, S. D., Cho, U., et al. (2007b). N-halamine biocidal coatings. *Journal of Industrial Microbiology and Biotechnology, 34*, 157–163.

Liu, S., & Sun, G. (2008). Radical graft functional modification of cellulose with allyl monomers: Chemistry and structure characterization. *Carbohydrate Polymers, 71*, 614–625.

Makal, U., Wood, L., Ohman, D. E., & Wynne, K. J. (2006). Polyurethane biocidal polymeric surface modifiers. *Biomaterials, 27*, 1316–1326.

Ren, X., Kou, L., Kocer, H. B., Zhu, C., Worley, S. D., Broughton, R. M., et al. (2008a). Antimicrobial coating of an N-halamine biocidal monomer on cotton fibers via admicellar polymerization. *Colloid and Surface A: Physicochemical Engineering Aspects, 317*, 711–716.

Ren, X., Kou, L., Liang, J., Worley, S. D., Tzou, Y. M., & Huang, T. S. (2008b). Antimicrobial efficacy and light stability of N-halamine siloxanes bound to cotton. *Cellulose, 15*, 593.

Sun, G., & Xu, X. (1999a). Durable and regenerable antibacterial finishing of fabrics; Fabric properties. *Textile Chemist and Colorist, 31*, 21–24.

Sun, G., & Xu, X. (1999b). Durable and regenerable antibacterial finishing of fabrics; Biocidal properties. *Textile Chemist and Colorist, 30*, 26–30.

Sun, G., & Xu, X. (1999c). Durable and regenerable antibacterial finishing of fabrics; Chemical structures. *Textile Chemist and Colorist, 31*, 31–35.

Sun, Y., & Sun, G. (2001). Novel regenerable N-halamine polymeric biocides. II. Grafting of hydantion-containing monomers onto cotton cellulose. *Journal of Applied Polymer Science, 81*, 617–624.

Sun, Y., & Sun, G. (2002). Durable and regenerable antimicrobial textile materials prepared by a continuous grafting process. *Journal of Applied Polymer Science, 84*, 1592–1599.

Tsao, T. C., Williams, D. C., & Worley, S. D. (1990). A new disinfectant compound. *Industrial and Engineering Chemistry Research, 29*, 2161–2163.

Udokichdecha, W., Kittinaovarat, S., Thanasoonthornroek, U., & Potiyaraj, P. (2003). Acrylic and maleic acids in nonformaldehyde durable press finishing of cotton fabric. *Textile Research Journal, 75*, 401–406.

Worley, S. D., & Sun, G. (1996). Biocidal polymers. *Trends in Polymer Science, 11*, 364–370.

Worley, S. D., & Williams, D. E. (1988). Halamine water disinfectants. *Critical Reviews in Environmental Control, 18*, 133–175.

Yang, C. Q. (1991). Characterizing ester crosslinkages in cotton cellulose with FTIR photoacoustic spectroscopy. *Textile Research Journal, 61*, 298–305.

Yang, C. Q., & Wang, X. (1996). Formation of cyclic anhydride intermediate and esterification of cotton cellulose by multifunctional carboxylic acids: An infrared spectroscopy study. *Textile Research Journal, 66*, 595–603.

Yang, C. Q., Wang, X., & Kang, I. S. (1997). Ester crosslinking of cotton fabric by polymeric carboxylic acids and citric acid. *Textile Research Journal, 67*, 334–472.