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Antimicrobial Cellulose: Preparation and Application of 5-methyl-5-aminomethylhydantoin

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Abstract

A new N-halamine precursor, 5-methyl-5-aminomethylhydantoin (AH), was synthesized. This N-halamine precursor can be coated onto cotton surfaces with addition of the cross-linking agent butanetetracarboxylic acid (BTCA) and rendered biocidal by exposure to halogen solutions either before or after curing the coating or material. Standard washing tests show that covalently bound AH/BTCA on the cotton swatches can survive repeated washing cycles. After 50 washing cycles, chlorinated cotton swatches had lost 98.7 % of oxidative Cl^+, but after rechlorination, almost 43.5 % of Cl^+ was regained. Biocidal tests indicated that the cotton swatches coated with chlorinated AH/BTCA were effective against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*.

Key words: N-halamine, biocides, biocidal coating, textiles

1. Introduction

Increased concerns about the protection of healthcare workers are developing because cross-transmission is a major medical complication in that environment. Medical protective gears, such as in uniforms and gowns, can not provide enough protection for doctors and nurses, and the gear may be responsible for the spread of infectious disease because some microorganism such as MRSA can exist and survive on it for long periods of time [1,4,6-8,10]. Thus antimicrobial textiles and polymers are in great demand for protection against infectious disease pathogens.

Several coating methods have been developed in these laboratories and elsewhere; the most promising way is to covalently incorporate biocidal compounds onto various surfaces. Sun and his coworkers have reported grafted 3-allyl-5,5-dimethylhydantoin (ADMH) and 1-acryloyl-2,2,5,5-tetramethylimidazolidin-4-one (ACTMIO) by a continuous process [9]. Kang and his coworkers treated a PET texture with an oxygen plasma glow discharge to produce peroxides on its surface as well as grafted polymerization of acrylic acid. Chitosan and quaternized chitosan (QC) were covalently linked to PET-A (Acrylic acid grafted onto oxygen-plasma-treated PET) by ester bonds [2]. Worley and coworkers have patented the methods of the synthesis and use of silane and siloxane compounds for incorporating N-halamine moieties onto coatings and materials, rendering them biocidal. Chemical bonds are formed by condensation reactions of OH groups on the siloxane with OH groups on cellulose [11]. Several hydantoin derivatives such as 1,3-dimethylol-5,5-dimethylhydantoin and 3-methyl-2,2,5,5-tetramethyl-4-imidazolidinone reacting with OH groups on cellulose

have been synthesized and coated onto cotton [5].

This paper describes a new procedure used to incorporate a hydantoin moiety onto cotton fibers. 5-aminomethyl-5-methyl-hydantoin (AH) has been synthesized and linked to cotton by addition of crosslinker butanetetracarboxylic acid (BTCA). BTCA has been widely used in the presence of the catalyst sodium hypophosphite for superior durability in repeated laundry cycles and wrinkle resistance for cotton without obviously effecting mechanical strength or whiteness [3]. 5-aminomethyl-5-methylhydantoin was linked to cotton at the site of the amino group, rather than at the usual imide N moiety on the hydantoin ring. Imide N-Cl and amide N-Cl can thus exist simultaneously after chlorination. Imide N-Cl bonds are less stable than amide ones, so they release active chlorine to microbes relatively more rapidly and kill the pathogens in the shortest time. Amide N-Cl bonds being relatively more stable than imide N-Cl ones offer increased efficacy over a long time period, even under washing conditions.

2. Experimental

2.1. Materials

All chemicals were purchased from the Aldrich Chemical Company, Milwaukee, WI and used as they were received without further purification unless otherwise noted. The fabric used was Style 400 Bleached 100% Cotton print Cloth (Testfabics, Inc., West Pittston, PA). Chlorination was accomplished with household bleach (Clorox, Inc., Oakland, CA). The bacteria used were *S. aureus* ATCC 6538 and *E. coli* O157:H7 ATCC 43895 (American Type Culture Collection, Rockville, MD). The Trypticase soy agar employed was from Difco Laboratories, Detroit, MI.

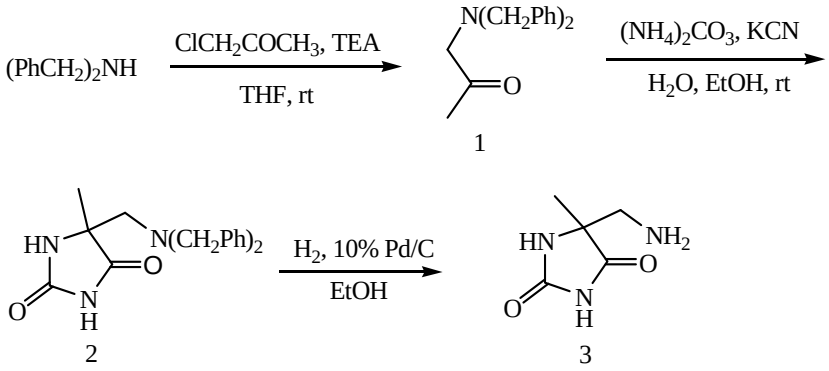
2.2. Instruments

The NMR spectra were obtained using a Bruker 400 MHz spectrometer;

the IR data were obtained with a Shimadzu IR Prestige-21 FTIR spectrometer.

2.3. Preparation of 5-methyl-5-aminomethylhydantoin

The preparation procedure for 5-methyl-5-aminometyhlhydantoin is illustrated in Scheme 1, which is based upon a modified literature method [5].



Scheme 1. Synthesis of 5-methyl-5-aminomethylhydantoin (AH)

l-(Dibenzylamino)-2-propanone (1). In a 500 ml Erlenmeyer flask l-chloro-2-propanone (20.0 g, 215 mmol), dibenzylamine (21.5 g, 110 mmol), triethylamine (13.2 g, 130 mmol), and 150 ml DMF were stirred at ambient temperature for 72 h under a N_2 atmosphere. The precipitate was filtered, and the solvent was removed by rotary evaporation to produce a brown oil. The residue was dissolved in 200 ml of chloroform. This solution was washed sequentially with saturated sodium bicarbonate (2 × 100 ml) and dried over sodium sulfate. The solvent was removed under reduced pressure to afford **1** (26.4 g, 104 mmol, 95 %): ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.01 (s, 3 H), 3.20 (s, 2 H), 3.60 (s, 4 H), 7.16-7.43 (m, 10 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 27.39, 57.49, 62.72, 126.98, 128.55, 128.43, 138.03, 207.74.

5-[(Dibenzylamino)methyl]-5-methyl-2,4-imidazolidinedione (2). In a 50 ml Erlenmeyer

flask **1** (26.4 g, 104 mmol)**,** potassium cyanide (13.58 g, 208 mmol), ammonium carbonate (39.98 g, 416mmol), and 200 mL of aqueous ethanol (1:1) were stirred at ambient temperature for 3 d. The precipitate was filtered and dried in the air, affording 30 g of crude product. The residue was recrystallized from 95% ethanol to afford pure **2** as white crystals: mp 202-204^{0}C; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.15 (s, 3 H), 2.77 (q, 2 H), 3.59 (q, 4 H), 7.22~7.33 (m, 10 H), 7.90 (s, 1H), 10.71 (s, 1 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 21.14, 57.53, 58.79, 63.73, 126.78, 128.04, 128.69, 138.20, 156.69, 178.21.

5-(Aminomethyl)-5-methyl-2,4-imidazolidinedione (AH)(3). In a 500-mL Parr hydrogenation reactor, compound **2** (3.00 g 9.27 mmol), 0.24 g of 10% Pd/C, and 100 mL absolute ethanol were reacted in the presence of 60 psi of H_2. The mixture was shaken while heating (IR lamp) for 1.5 h. Upon cooling to room temperature, the catalyst was removed by filtration, and the solvent was evaporated to give a white solid **3** (1.32 g, 9.27 mmol, 100 %): mp 187-188^{0}C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.16 (s, 3 H), 2.63 (q, 2 H), 3.34 (s, 1 H), 7.71(s, 1 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 20.62, 47.88, 64.28, 158.06, 178.06.

2.4. Coating procedure

5-methyl-5-aminomethylhydantoin (AH) was coated onto the surfaces of cotton swatches (Style 400 Bleached 100 % Cotton print Cloth, Testfabrics, Inc., West Pittston, PA) by soaking the swatches for 15 min in baths containing about 0.349 mol/L AH (mass content 5 %) and 0.349 mol/L BTCA dissolved in distilled water. After the soaking procedure, the coated swatches were generally cured at 80^{0}C for 5 min and then 125, 135, and 145^{0}C for 5~35 min. Then the swatches were washed in 0.5 % detergent solution for 15 min, followed by several water rinses to remove any weakly bonded coating or physically absorbed species.

2.5. Chlorination procedure

The coated cotton swatches were chlorinated by soaking them in a 1 % aqueous solution of NaOCl household bleach at ambient temperature for 1 h. The chlorinated swatches were washed with distilled water and dried at 45^0C for 1 h to remove any free chlorine. The loading of bound chlorine on the swatches was determined as described below.

2.6. Analytical titration procedure

The chlorine contents of the cotton swatches were analyzed by a traditional iodometric titration method. A small cotton sample (about 0.15 g) was immersed in 50 ml distilled water, and then 10 drops of 4 N acetic acid were added into the solution, which was stirred. Then 0.25 g KI was added to the solution, and then 10 drops of starch solution as an indicator. The solution became dark blue, and the mixture was titrated with 0.0375 N standard sodium thiosulfate until the blue color disappeared. The amount of active chlorine percent was calculated using the following equation:

$$Cl\ \% = [N \times V \times 35.5/(2 \times W)] \times 100\%$$

where N and V are the concentration of standard sodium thiosulfate (eqv/L) and volume of the consumed $Na_2S_2O_3$ (L), and W is the weight of the cotton swatch sample (g).

2.7. Biocidal efficacy testing

Dried swatches were then challenged with either *S. aureus* ATCC 6538 or *E. coli* O157:H7 ATCC 43895 using a ‘‘sandwich test.’ In this test 25 µL of bacterial suspension were placed in the center of a swatch, and a second identical swatch was laid upon it, held in place by a sterile weight to insure good contact of the swatches with the inoculum. The bacterial suspensions employed for the tests contained from 10^6 to 10^7 colony forming units (CFU), the

actual number determined by counting after spread-plating on Trypticase soy agar (Difco Laboratories, Detroit, MI) plates. After contact times of 1.0, 5.0, and 10.0 min, the various swatches were placed in sterile conical centrifuge tubes, each containing 5 mL of sterile 0.01 M sodium thiosulfate to quench any oxidative free chlorine which might have been present, and vortexed for 2 min to remove bacteria. Then the swatches were removed, and serial dilutions of the quenched solutions were plated on Trypticase soy agar. The plates were incubated at 37.1^{0}C for 24 h and then counted for viable CFU's of bacteria.

2.8. Laundry durability and regenerability

Laundry durability and regenerability of the biocidal functions were conducted according to the AATCC Test Method 61 (Test 2A Procedure). The cotton samples were laundered at 5, 10, 25, and 50 washing cycles in a washing machine and then rinsed with tap water and dried in air. Unchlorinated swatches and half of the prechlorinated swatches from each washing cycle were recharged with 1% bleach solution. The chlorine percent was measured by iodometric /thiosulfate titration.

3. Results and discussion

3.1. Coating procedure

The FTIR spectrum of 5-aminomethyl-5-methylhydantoin is shown in Figure 1. The bands at 1670 cm^{-1} and 1612 cm^{-1} correspond to the carbonyl groups of the hydantoin function which can form an intramolecular hydrogen bond with the exocyclic amino group. These bands disappear when the amino group is linked to cellulose through BTCA.

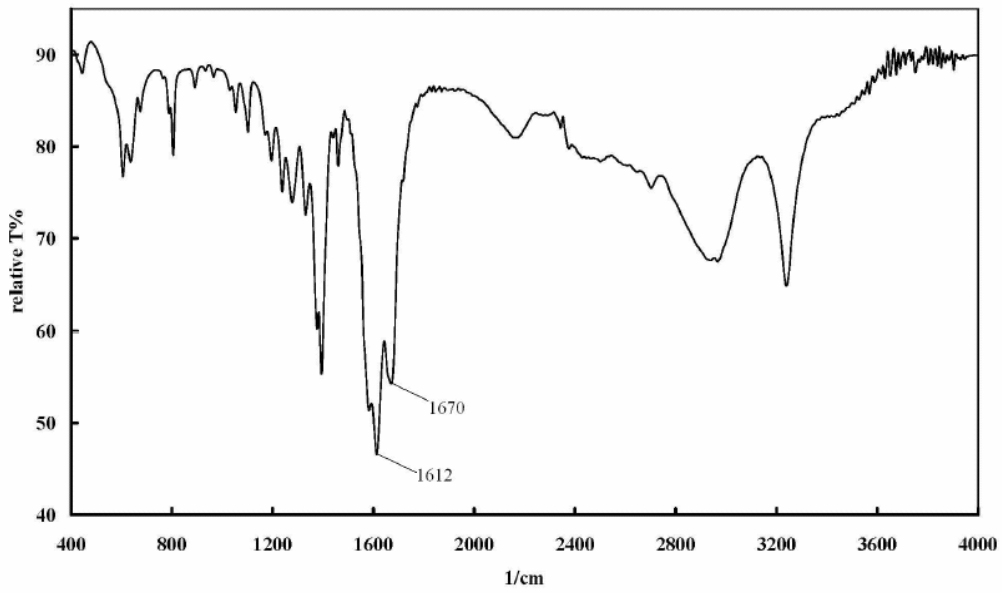


Figure 1. FTIR spectrum of 5-methyl-5-aminomethylhydantoin (AH)

Figure 2 shows the FTIR Spectra of cotton swatches before and after treatment with AH and BTCA, and after chlorination. The band of 1720 cm^{-1} in figure 2b provides evidence that the hydantoin functional group is present in the coating. After chlorination, this peak shifted to 1724 cm^{-1} (Figure 2c). A new band arises at 1578 cm^{-1} due to the formation of C=N bonds (see Scheme 2). This C=N bond formation results from dehydrohalogenation of the exocyclic N-chlorinated amide group.

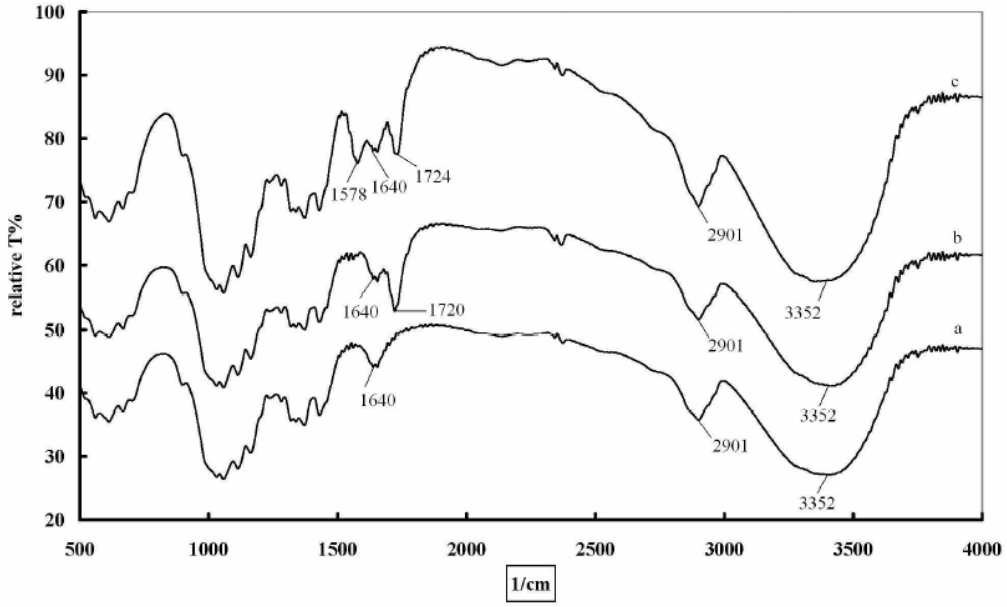
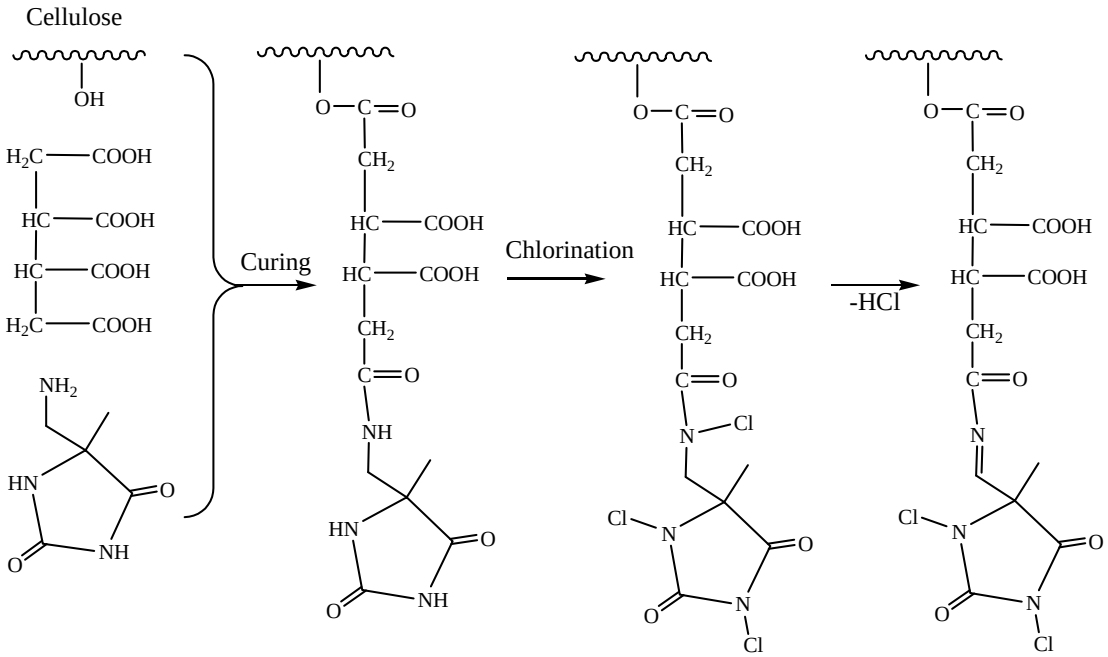


Figure 2. FTIR spectra of cotton control, treated cotton, and treated cotton after chlorination

a- cotton control

b- treated without chlorination

c- treated with chlorination



Scheme 2. Attachment of AH/BTCA to cellulose.

3.2. The effect of BTCA and a possible mechanism for attachment

Figure 3 and Table 1 show that AH should not be directly used for coating cotton because the amino group of AH clearly reacts poorly with OH groups of cellulose. When BTCA is used to tether the AH to cotton, the results are much improved as indicated by the increased final chlorine loading. The mechanism for the process is shown in Scheme 2. The amino group of AH reacts with a COOH group of BTCA to form an amide linkage and a second COOH reacts with an OH group on cellulose to produce the coating.

Table 1. The effect of curing time with and without BTCA [a]

Curing Time (min)	Chlorine weight percent on cotton samples	
	Cl^+% [b]	Cl^+% [c]
5	0.03	0.10
15	0.04	0.53
25	0.04	0.74
30	0.04	0.94
35	0.04	0.89

[a]The cotton samples were cured at 145^0C for 5~35 min. A 1% Clorox solution was used for chlorination without pH adjustment.

[b]Coating solution: 5 % AH in distilled water.

[c]Coating solution: 5 % AH and mol_{AH}:mol_{BTCA}=1:1 in distilled water.

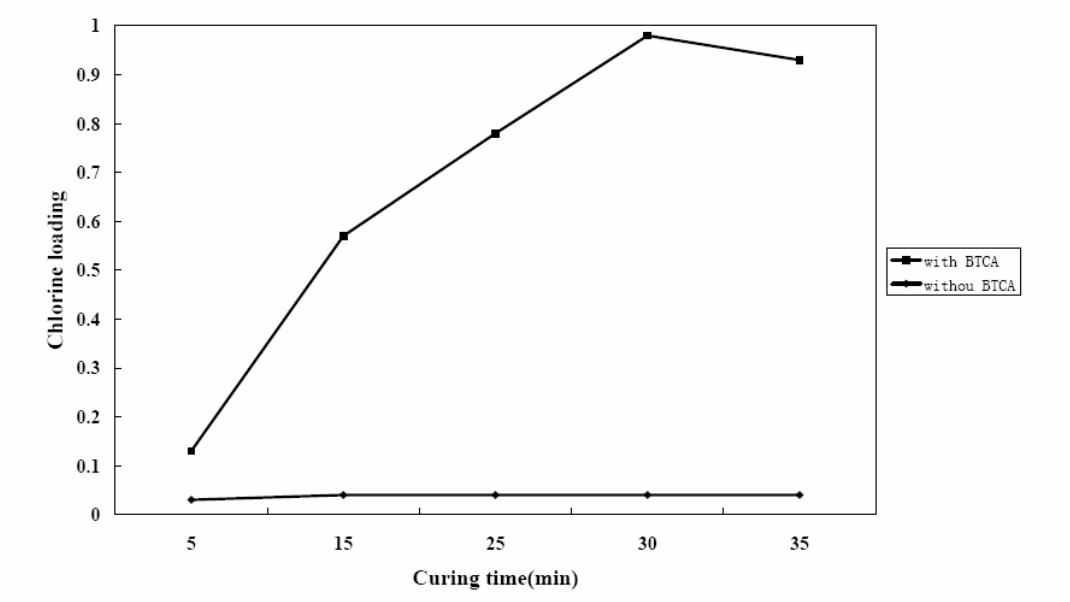


Figure 3. The effect of curing time with and without BTCA

3.3. The effect of the concentration of coating solution

Table 2 shows the maximum chlorination percent at a coating solution concentration of 5 % AH and 8.18% BTCA (mol_{AH}:mol_{BTCA}=1:1). Increasing concentrations of AH and BTCA provide increased reaction between the OH group of cellulose and the acid group of the BTCA, leading to enhanced crosslinking to cellulose. However at higher concentrations of AH and BTCA, increased reaction can occur between these two molecules leaving less free COOH groups of BTCA to react with the OH groups of cellulose, so the chlorination percent decreases at concentrations above 5 %.

Table 2. The effect of the concentration of coating solution [a]

Coating solution	1 %	2.5 %	5 %	7.5 %	10 %
Chlorine weight percent on cotton samples	0.37%	0.75 %	0.94 %	0.80 %	0.80 %

[a]The cloth samples were cured at 145^0C for 35 min and then chlorinated with 1% Clorox

without pH adjustment.

3.4. The effect of curing temperature and curing time

Curing temperature and curing time are important for the diffusion of the antimicrobial agents into the fabric and the formation of the chemical bond between the cellulose and antimicrobial agents. A set of experiments were designed to examine the effect of curing time and curing temperature. The results are shown in Table 3. Higher curing temperature causes increased chlorine percent because it facilitates the diffusion rate of antimicrobial agents into the fibers. The optimum conditions were for curing at 145^0C for 30 min.

Table 3. The effect of curing temperature and curing time[a]

Time		Chlorine weight Percent (%)
5 min	125^0C	0.02
	135^0C	0.05
	145^0C	0.10
15 min	125^0C	0.18
	135^0C	0.33
	145^0C	0.53
25 min	125^0C	0.34
	135^0C	0.35
	145^0C	0.74
30 min	125^0C	0.44
	135^0C	0.56
	145^0C	0.94
35 min	125^0C	0.50
	135^0C	0.64
	145^0C	0.89

[a]The cloth samples were coated at a solution concentration of 5 % AH (mol_{AH}:mol_{BTCA}=1:1) for 15 min and then chlorinated with 1 % Clorox without pH adjustment.

3.5. The effect of pH of the chlorination solution

Table 4 shows that chlorine loading was surprisingly independent of the concentration of the bleach solution such that a 1% solution was sufficient. Also, a pH adjustment to 7.0 had little effect on the final chlorine loadings on the cloth. Previous research in these laboratories has generally shown that chlorination of heterocyclic precursor biocidal compounds proceeds to greater extent at lower pH than at the natural pH of bleaching solutions. It is not clear why this is not the case here, but the result is welcome because it means that optimum biocidal functionality can be obtained by chlorination during a washing process without pH adjustment.

Table 4. The effect of bleach concentration[a]

Clorox solution	pH		Chlorine weight percent
	Initial[b]	After[c]	(%) Loaded on the cotton
1 %	9.28	8.97	0.86
	7.02	7.00	0.84
5 %	10.18	9.91	0.74
	7.04	6.99	0.85
10 %	10.73	10.16	0.62
	7.00	6.84	0.86

[a]Cotton was coated in 5% AH(mol_{AH}:mol_{BTCA} =1:1) for 15 min, then curing at 145^0C for 30 min.

[b]pH of the bleach solution at the indicated concentration and after adjustment to near 7.00 using 6N HCl.

[c]pH of the solution after the uptake of oxidative chlorine by the samples.

3.6. Washing tests

The data in Table 5 show that after the laundry cycles, the chlorine percent decreased because of the slow release of active chlorine and some release of the coating. However, recharging after even 50 cycles indicates that much of the coating remains bonded to the swatches such that the biocidal function can be regenerated for the life of the fabric. In fact, if a 1% bleach solution is used at each wash, the longevity of the biocidal function should be ensured.

Table 5. Washing tests of the chlorinated cotton swatches

Washing cycles	Chlorine weight percent (%) loaded on the cotton		
	A	**B**	**C**
0	0.77		
5	0.31	0.61	0.67
10	0.24	0.43	0.51
25	0.07	0.37	0.38
50	0.01	0.34	0.32

A: Chlorination before washing; B: Recharge after washing; C: Chlorination only after washing.

3.7. Biocidal tests

Tables 6 and 7 show that after chlorination, the fabric swatches were effective against both Gram-negative *E. coli* and Gram-positive *S. aureus*. With the presence of chlorinatied

Table 6. Biocidal test for the microorganism: *E. coli O157:H7*

Sample	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
Cotton Control	0 min	9.00×10^6	0
	1 min	7.24×10^6	0.09
	5 min	6.30×10^6	0.16
	10 min	6.10×10^6	0.17
Cotton-AH Control	0 min	9.00×10^6	0
	1 min	5.36×10^6	0.22
	5 min	4.89×10^6	0.26
	10 min	4.49×10^6	0.30
Cotton-AH-Cl	0 min	9.00×10^6	0
	1 min	nd[a]	6.95
	5 min	nd[a]	6.95
	10 min	nd[a]	6.95

[a]No viable colonies detected; the detection limit was 40 CFU/mL.

hydantoinyl functional groups, the swatches achieved about 7 log reductions within 1min of contact, while fabric swatches without chlorination, and cotton itself, showed little log reductions. Once chlorinated, the fabric possessed very good biocidal efficacy.

Sample	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
Cotton Control	0 min	1.00×10^{7}	0
	1 min	5.76×10^{6}	0.24
	5 min	5.63×10^{6}	0.25
	10 min	5.23×10^{6}	0.28
Cotton-AH Control	0 min	1.00×10^{7}	0
	1 min	1.21×10^{6}	0.92
	5 min	1.14×10^{6}	0.94
	10 min	9.38×10^{5}	1.03
Cotton-AH-Cl	0 min	1.00×10^{7}	0
	1 min	nd[a]	7.00
	5 min	nd[a]	7.00
	10 min	nd[a]	7.00

Table 7. Biocidal test for the microorganism: *S. aureus*

[a]No viable colonies detected; the detection limit was 40 CFU/mL.

4. Conclusions

5-methyl-5-aminomethyl-hydantoin was coated onto cotton fibers with cross-linker BTCA, and after chlorination, the cotton swatches showed excellent biocidal efficacy against Gram-positive *S. aureus* and Gram-negative *E. coli* (about 7 log reductions within 1 min).

After 50 washing cycles, much of the active chlorine was regained. Thus treated cotton swatches are regenerable and stable biocidal materials, which could provide advantages for textile industry.

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