

Antibacterial Coating of Cellulose by Iso-bifunctional Reactive N-halamine with the Dyeing Process of Reactive Dye

Tong Mu¹, Nengyu Pan¹, Yingfeng Wang¹, Xuehong Ren^{1*}, and Tung-Shi Huang²

¹Key Laboratory of Eco-textiles of Ministry of Education, College of Textiles and Clothing, Jiangnan University, Jiangsu 214122, China

²Department of Poultry Science, Auburn University, Auburn 36849, AL, USA

(Received July 13, 2018; Revised August 12, 2018; Accepted September 3, 2018)

Abstract: In this study, we synthesized a novel N-halamine precursor, sulfuric acid mono-[2-(4-[4-chloro-6-(2-[4,4-dimethyl-2,5-dioxo-imidazolidin-1-yl]-ethylamino)-[1,3,5]triazin-2-ylamino]-benzenesulfonyl)-ethyl] ester sodium (TB), which contains two reactive groups of monochloro triazine reactive groups and bis-sulphatoethylsolphone reactive groups. The structure of TB is similar to iso-bifunctional group reactive dyes and could be coated on cotton fabrics by covalent bonds through a reactive dyeing process. The cotton coated with TB was characterized by FTIR and SEM. After chlorination, the treated cotton fabrics showed excellent antibacterial efficacy and inactivated all inoculated *S. aureus* (ATCC 6538) and *E. coli* O157: H7 (ATCC 43895) within 1 min of contact. Over 85 % of tensile strength retained both in warp and weft directions after treatment and chlorination. Almost 80 % of active chlorine can be regained by treating with household bleach after extensive washing and long time storage.

Keywords: Iso-bifunctional group, N-halamine, Antibacterial, Cellulose, Dyeing process

Introduction

Cellulose, such as cotton, is one of the most abundant natural materials on the earth and has been used to produce many products which impact human life in different fields directly [1]. Good biocompatibility and hydrophilia of cotton provides a conducive environment that can potentially support the growth of bacteria [2]. In order to decrease or even eliminate this risk, various materials finished by different antimicrobial agents have been researched [1,3]. N-halamine compounds have attracted much attention due to its unique properties such as high efficacies, regenerabilities, broad-spectra, and stabilities for inactivating bacteria among various antibacterial agents [4-8].

Due to its excellent disinfection efficacies in water treatment and sterilizing hard surface, N-halamine has been studied in recent years [9]. The antibacterial activity of N-halamines mainly depends on the active chlorine arising from the covalent bonds between nitrogen and halogen (N-X) [10]. The N-X bonds become N-H and release active chlorine to inactivate bacteria, and the N-H can be reserved to N-X in a dilute hypochlorite aqueous solution [11]. In order to expand further application, the siloxane group had been introduced into N-halamine structure and used to treat materials such as sand, mesoporous molecular sieves, graphene oxide and textiles [12-16]. Some insoluble N-halamine polymers were synthesized and applied on fabrics for antibacterial activity [10,17-19]. Several potential N-halamine precursors, 2,2,6,6-tetramethyl-4-piperidinol (TMP) derivatives, 5,5-dimethylhydantoin (DMH) derivatives, and 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (TTDD),

have also been synthesized and utilized in different domains [4,5,16,20,21]. Most of these N-halamine agents containing functional groups can be attached onto the surface of fabrics with the help of a cross-linking agent through a drying and curing process [10]. But the high temperatures in the finishing process have greatly affected the physical and mechanical properties, especially the strength of fabrics. A series of reactive N-halamine agents containing s-triazine ring have been synthesized and could be attached onto the cotton at low temperatures with small loss of breaking strength after dyeing [10,19]. However, large amounts of salts have been utilized for desirable antimicrobial efficacies, which has an adverse impact on the environment.

In this study, a novel N-halamine precursor sulfuric acid mono-[2-(4-[4-chloro-6-(2-[4,4-dimethyl-2,5-dioxo-imidazolidin-1-yl]-ethylamino)-[1,3,5]triazin-2-ylamino]-benzenesulfonyl)-ethyl] ester sodium (TB) (Figure 1) was synthesized and characterized. The structure of TB, with two different reactive groups, enables the attachment onto cotton fabrics through covalent bonds under lower temperatures and with less salt. The antibacterial efficacies of the treated cotton swatches against *S. aureus* and *E. coli* O157:H7 were evaluated according to a modified American Association of Textile Chemist and Colorists (AATCC) test method. The stabilities and rechargeabilities of the N-Cl bonds of the reactive N-halamine coatings have been investigated. The breaking strength of the TB-coated cotton fabrics has also been measured.

Experimental

Materials

Hundred percent bleached cotton fabrics were purchased

*Corresponding author: xhren@jiangnan.edu.cn

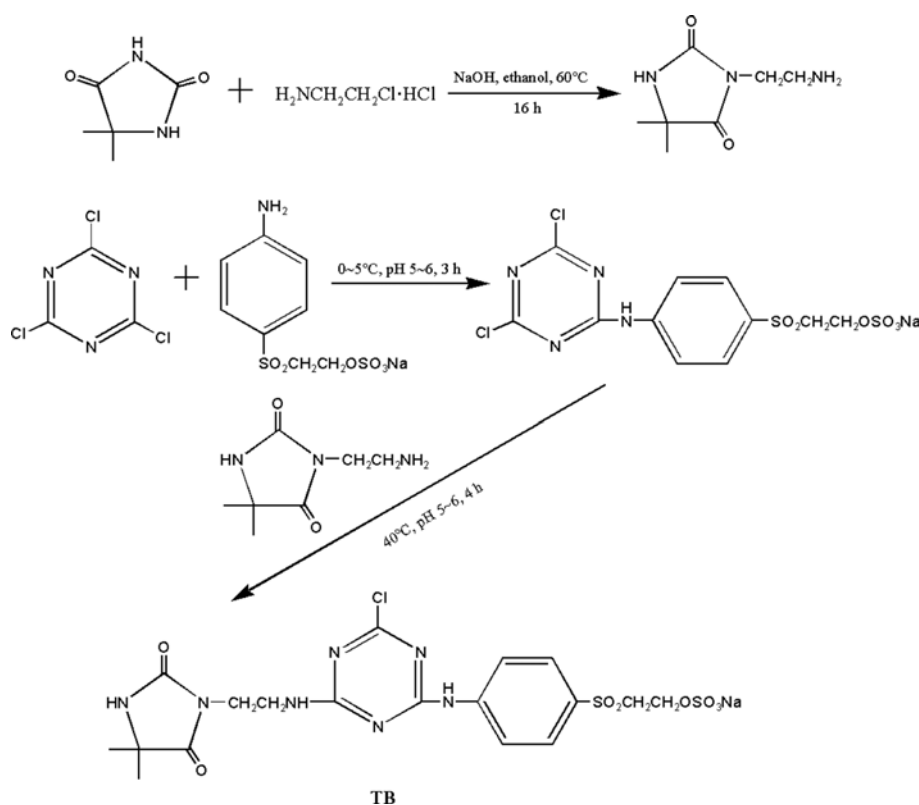


Figure 1. Synthesis route of TB.

from Zhejiang Guandong Textile Dyeing Garment Co., Ltd., Zhejiang, China. Cyanuric chloride and 2-chloroethylamine hydrochloride were purchased from J&K Chemical Co., Ltd., Shanghai, China. p-(β -Sulfato ethyl sulfonyl)aniline was purchased from Xiaogan Shenyuan Chempharm Co., Ltd., Hubei, China. 5,5-Dimethylhydantoin was provided by Hebei Yaguang Fine Chemical Co., Ltd., Hebei, China. Other chemicals were from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China and used without further purification.

Instruments

An AVANCE III 400 MHz Digital NMR Spectrometer (Bruker AXS GmbH, Karlsruhe, Germany) was used for 128 scans to characterize the synthesized product (TB). FTIR spectra of cotton and the treated cotton were recorded by a Nicolet NEXUS 470 spectrometer (Nicolet Instrument Corporation, Madison, WI, USA) in the optical range of 500-4000 cm^{-1} by averaging 32 scans at a resolution of 4 cm^{-1} . Surface morphologies of cotton and the treated cotton fabrics were investigated by a SU1510 field-emission SEM (Hitachi, Tokyo, Japan).

Synthesis of TB

Synthesis of 3-(2-aminoethyl)-5,5-dimethylhydantoin was followed to a previous research with modifications [19]. A

mixture of 5,5-dimethylhydantoin, sodium hydroxide, and 2-chloroethylaminehydrochloride (molar ratio=2:1:2) was added in ethanol and stirred at 60 °C for 16 h. After reaction, the transparent viscous oil product was obtained after filtration and evaporation. The yield was 96%. $^1\text{H-NMR}(\text{D}_2\text{O-d}_2)$: δ 1.21-1.26 (6H), δ 2.67 (2H), δ 3.44 (2H).

Cyanuric chloride was dissolved in acetone with stirring for 30 min in an ice bath. Then, equimolar quantity sodium salt of p-(β -Sulfato ethyl sulfonyl)aniline (prepared from para-ester and semi-molar quantities sodium carbonate) was added into above solution and the pH was maintained at 2.5-3.5 using 1.25 M sodium carbonate solution. The reaction was carried out at 0-5 °C for 3 h. The water solution prepared by equimolar quantity of 3-(2-aminoethyl)-5,5-dimethylhydantoin was added to the above reaction system at pH 7-8 and stirred at 40 °C for 4 h. The sample was filtered and a crude off-white product was obtained by removing the solvent under reduced pressure distillation from the filtrate. Afterward, the product was washed with acetone and ethanol. The yield was 62%. $^1\text{H-NMR}(\text{D}_2\text{O-d}_2)$: δ 1.46 (6H), δ 3.70 (2H), δ 3.85 (2H), δ 4.39 (2H), δ 4.55 (2H), 7.90-7.99 (4H).

Cotton Fabrics Finishing and Chlorination Procedure

The finishing solution was prepared as follows: 0.1 g TB and 1 g Na_2SO_4 were dissolved in 20 ml of deionized water. Cotton swatches were soaked in the treatment solution at

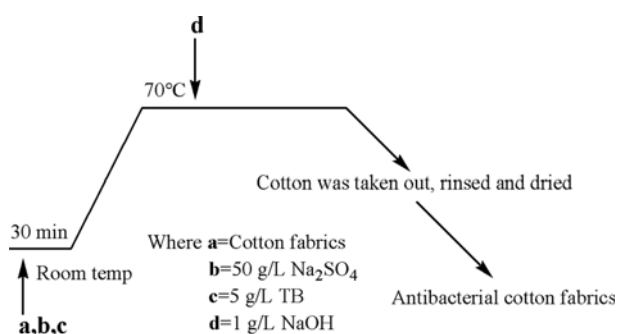


Figure 2. The finishing procedure of TB on cotton fabrics.

room temperature for 30 min. Then 0.02 g NaOH was added to the solution after slowly increasing the temperature to 70 °C. After 2-4 h, the samples were taken out and washed with water to remove the unfixed TBs, and dried at room temperature. The process was shown in Figure 2.

The modified cotton samples were immersed in a 10 % commercial aqueous sodium hypochlorite solution, pH 7 at room temperature for 1 h. Then, the chlorinated samples were removed, and washed with distilled water and dried at 45 °C for 1 h. The chlorine loads of samples were determined by the iodometric/thiosulfate titration method. The $[Cl^+]$ % on the cottons watches was calculated according the following equation:

$$[Cl^+] \% = \frac{35.45NV}{2W} \times 100\%$$

where $[Cl^+]$ % is the wt% 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 (g).

Antimicrobial Efficacy Testing

According to AATCC Text Method 100-2004, control fabric and treated fabric swatches with/without chlorination were challenged with *S. aureus* and *E. coli* O157:H7. In the test, 25 ml of bacterial suspensions were added in the middle of two pieces of swatches. Then, the residual oxidative chlorine was neutralized by adding 5.0 ml of sterile 0.02 N sodium thiosulfate solution after 1, 5, 10, and 30 min treatments, respectively. The surviving bacteria were rinsed off from the swatches by stirring, and a 10-fold serial dilution method of bacterial suspensions were made and each dilution was plated on trypticase soy agar plates. After incubation at 37 °C for 24 h, the bacterial colonies on the plates were counted for antimicrobial efficacy analysis.

Stability Testing

The treated cotton swatches after chlorination were immersed in 0.15 % AATCC detergent water solution with 50 stainless steel balls, rotated with 42 rpm at 49 °C in a Launder Ometer according to AATCC Test Method 61-

1996. After the equivalents of 5, 10, 25, and 50 machine washes, the cotton samples were removed, washed with distilled water and dried at room temperature. The quantities of remaining chorines on the cotton samples were titrated using the iodometric/thiosulfate titration method. For the storage stability test, the chlorinated TB-coated cotton fabrics were stored in a dark condition at room temperature for 30 d, then, the chorine on the fabrics was measured.

Breaking Strength Testing

Tensile strength of the treated fabrics was tested according to GB/T3923-1997. Untreated cotton fabrics and the treated cotton fabrics with/without chlorination were cut into 250×50 mm². Each sample was tested three times and the average value was calculated.

Results and Discussion

Syntheses of TB

5,5-Dimethylhydantoin (DMH) contains two nitrogen hydrogen bonds (N-H), and the nitrogen hydrogen bonds can transform to N-Cl after chlorination, allowing good antibacterial property. 2-Chloroethylamine hydrochloride has reactive chlorine and amino group, which can react with the sodium salt of 5,5-dimethylhydantoin obtained from the reaction between 5,5-dimethylhydantoin and sodium hydroxide in ethanol. 2,4,6-Trichloro-1,3,5-triazine was the reactive group in this study. The compound has three chlorines and could be substituted at different temperatures. The first chlorine of s-triazine rings was substituted with 3-(2-aminoethyl)-5,5-dimethylhydantoin at 0-5°C in weak acidic conditions [22,23]. To improve the reactiveness of the final product, p-(β-sulfato ethyl sulfonyl)aniline was added to substitute the second chlorine of cyanuric chloride at 40 °C.

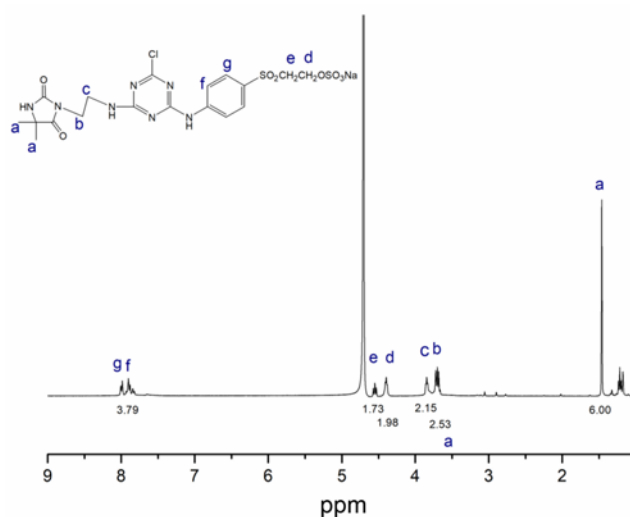


Figure 3. Proton nuclear magnetic resonance spectrum of TB.

Therefore, a novel N-halamine precursor with an iso-bifunctional group (TB) was synthesized. TB has a similar structure as reactive dyes, and the dyeing process could be used for the antibacterial finishing of cotton fabrics at low temperatures. The structure of TB was confirmed by $^1\text{H-NMR}$ spectrum (Figure 3).

Characterization of TB Coated Cotton Fabrics

The structure of TB contained two reactive groups, which could be covalently bonded onto cellulose. These bindings are through the reaction of nucleophilic substitution of monochloro-s-triazinyl reactive group at 60-90 °C [10,24]. TB could also be attached onto cotton through a nucleophilic addition reaction due to the vinyl sulfone reactive group in TB. In the finishing process, the repulsion forces between the agent and cotton fabrics decreased by adding neutral salt, and the addition of base was to produce cellulosate anions and facilitate both the nucleophilic substitution and nucleophilic addition reaction [24,25]. In order to obtain antimicrobial properties, the treated samples were chlorinated by 10 % diluted commercial sodium hypochlorite solution. The chlorine loading was used to evaluate the degree of exhaustion of the antimicrobial agent. In this study, the chlorine loading could reach up to 0.32 %.

FTIR spectra of cotton and cotton coated with TB before and after chlorination are shown in Figure 4. Compared with untreated cotton fabrics, two peaks at 1561 and 1695 cm^{-1} on the TB treated cotton fabrics were corresponded to the carbonyl group on hydantoinyl and benzene rings, respectively [26-28]. Two vibrational bonds shifted to 1563 and 1707 cm^{-1} in the chlorinated samples caused by an electron-withdrawing effect of oxidation after chlorination. It demonstrated that TB was attached onto cotton fabrics through covalent bond successfully.

Figure 5 shows the SEM micrographs of the surface of

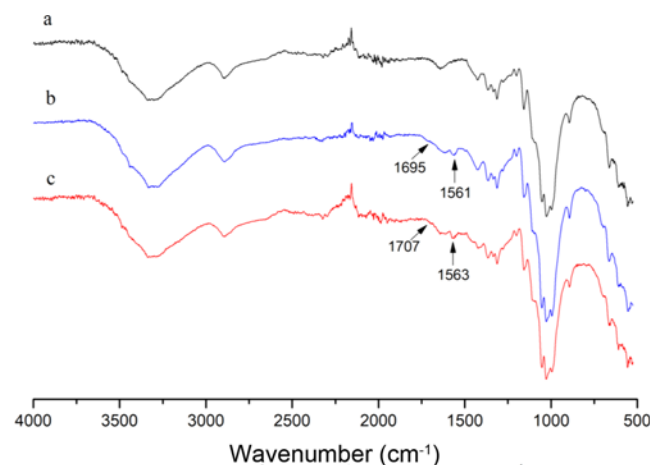


Figure 4. Fourier transform infrared spectra of (a) cotton fabrics, (b) cotton coated with TB, and (c) cotton coated with TB after chlorination.

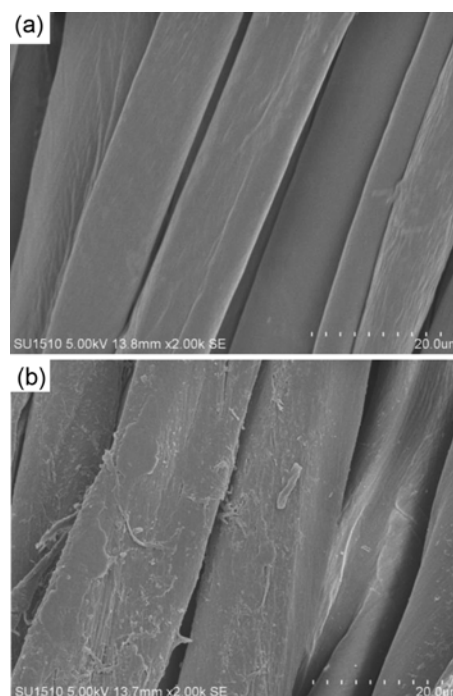


Figure 5. Scanning electron microscope micrographs of (a) cotton and (b) cotton coated with TB.

cotton fabrics and the TB treated cotton fabrics. It was found that the surface of TB-coated cotton fabrics became rougher compared with those in the control samples. This phenomenon provides evidence that these N-halamine precursors were attached onto cotton fibers.

Biocidal Efficacy

The biocidal efficacies of cotton coated with TB against *S. aureus* and *E. coli* O157:H7 are presented in Table 1. Cotton and TB-cotton showed some degree of bacterial reduction due to caused by the adhesion of bacteria to the cotton fabrics. TB-coated fabrics showed a higher bacterial reduction than that in the cotton fabric only due to the increase of hydrophilicity and roughness after coated with

Table 1. Biocidal efficacy against *S. aureus* and *E. coli* O157:H7

Sample	Contact time (min)	Bacterial reduction			
		<i>S. aureus</i> ^a		<i>E. coli</i> O157:H7 ^b	
		%	Log	%	Log
Cotton	30	55.11	0.35	77.67	0.65
TB-cotton	30	98.30	1.17	95.91	1.39
TB-cotton-Cl	1	100	6.00	100	6.26
(Cl ⁺ %=0.24)	5	100	6.00	100	6.26
	10	100	6.00	100	6.26

^aInoculum was 1.00×10^6 CFU per sample and ^binoculum was 1.80×10^6 CFU per sample.

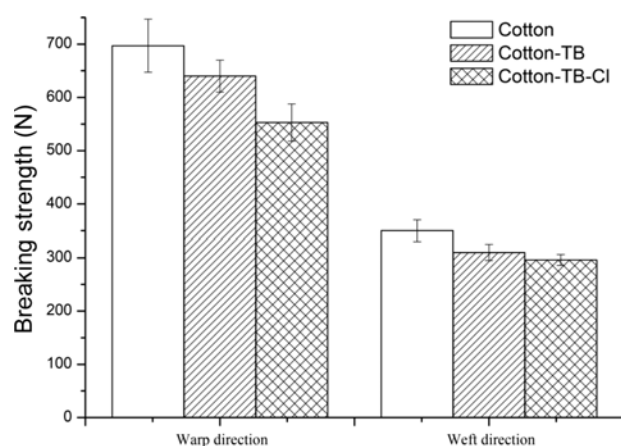


Figure 6. Breaking strength of cotton fabrics coated with TB.

TB resulting in the increase of bacterial absorption [10]. Chlorinated TB-coated cotton fabrics completely inactivated all inoculated 6 log *S. aureus* and 6.26 log *E. coli* O157:H7 within 1 min, respectively.

Tensile Strength

Figure 6 shows the tensile strength of untreated and treated cotton fabrics. The TB-coated cotton fabrics treated by the traditional pad-dry-cure finishing method retained about 90 % of tensile strength both in warp and weft direction. The reduction of breaking strength caused by the finishing process was due to the alkaline condition and the loss of the intermolecular and intramolecular hydrogen bonds on cotton fabrics resulting from the substitution of hydroxyl group [10]. After chlorination, there was a small degree of tensile strength reduction compared with unchlorinated samples due to the oxidation of chlorine.

Washing and Storage Stability

The washing and storage stability of the treated cotton fabrics are very important after applying antimicrobial materials. The N-Cl of N-halamine compounds could be hydrolyzed to N-H and hypochlorous acid in water and cause the loss of antibacterial property [20]. The stability toward hydrolysis of the N-Cl bonds in various molecular structures is in the order of amine>amide>imide halamine [20]. N-halamine contained in the structure of TB is an amide structure. The washing stabilities of the chlorinated TB-coated cotton fabrics were shown in Table 2. Each washing cycle in this tested method was equivalent to five household machine washings. Only 20 % chlorine was retained after 5 washing cycles, and most of the chlorine was lost (from 0.15 to 0.01) after 50 washing cycles. However, more than 80 % of the chlorine (0.12 %) could be regained after rechlorination, which indicated that the decrease of oxidative chlorine was ascribed to the dissociation of N-Cl and most of the covalent bonds between TB and cotton

Table 2. Washing test of the cotton coated with TB

Washing cycle ^a	C	R
0	0.15	0.15
5	0.05	0.13
10	0.02	0.13
25	0.01	0.13
50	0.01	0.12

C: chlorination before washing (Cl⁺%), R: rechlorination after each washing cycle (Cl⁺%). ^aEach cycle of washing in this method is equivalent to five machine washings.

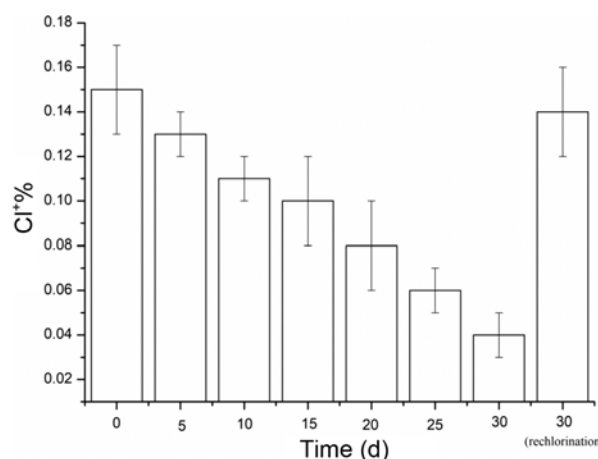


Figure 7. Storage stability of cotton coated with TB.

fabrics were retained after 50 washing cycles.

Figure 7 shows the storage stability of active chlorine on the chlorinated TB-coated cotton fabrics. After storage at ambient temperature for 30 d, 73 % of the chlorine on the TB-coated cotton fabrics was lost (from 0.15 to 0.04), but the antimicrobial activity was still [19]. And almost all of the active chlorine (0.14 %) could be regenerated after rechlorination. This result indicated that the loss of chlorine on the cotton fabrics was mainly due to the reduction of oxidative chlorine in N-Cl into N-H and the covalent bonds between TB and cotton fabrics was stable.

Conclusion

A novel N-halamine precursor with iso-bifunctional group was synthesized and characterized by ¹H-NMR. The synthesized TB was used to finish the cotton through the dip dyeing process of reactive dyes at low temperatures. After exposure to dilute sodium hypochlorite solution, the TB-coated cotton fabrics showed excellent antibacterial property against *S. aureus* and *E. coli* O157:H7 resulting in 6 logs of the bacterial inactivation within 1 min contact time. The result of washing stability test showed that after 50 washing cycles, more than 80 % of the chlorine on the treated cotton

fabrics could be restored by dilute sodium hypochlorite solution. The chlorinated TB-coated cotton fabrics still had good antimicrobial activity after 30 days of storage and most of the chlorine load could be recovered after rechlorination. The treated cotton fabrics showed a small loss of tensile strength both in the warp and weft direction (about 14 %).

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities (No. JUSRP51722B), the Project of Jiangsu Science and Technological Innovation Team, national first-class discipline program of Light Industry Technology and Engineering (LITE2018-2), 111 Projects (B17021), and the Fundamental Research Funds for the Central Universities (JUSRP11806).

References

1. D. Chmielewska and B. Sartowska, *Radiat. Phys. Chem.*, **81**, 1244 (2012).
2. X. H. Ren, H. B. Kocer, S. D. Worley, R. M. Broughton, and T. S. Huang, *Carbohydr. Polym.*, **75**, 683 (2009).
3. X. H. Ren, A. Akdag, H. B. Kocer, S. D. Worley, R. M. Broughton, and T. S. Huang, *Carbohydr. Polym.*, **78**, 220 (2009).
4. J. Liang, K. Barnes, A. Akdag, S. D. Worley, J. Lee, R. M. Broughton, and T. S. Huang, *Ind. Eng. Chem. Res.*, **46**, 1861 (2007).
5. I. Cerkez, H. B. Kocer, S. D. Worley, R. M. Broughton, and T. S. Huang, *Langmuir*, **27**, 4091 (2011).
6. Z. Q. Jie, X. F. Yan, L. H. Zhao, S. D. Worley, and J. Liang, *React. Funct. Polymers*, **73**, 1580 (2013).
7. H. B. Kocer, S. D. Worley, R. M. Broughton, and T. S. Huang, *React. Func. Poly.*, **71**, 561 (2011).
8. I. Cerkez, S. D. Worley, R. M. Broughton, and T. S. Huang, *React. Func. Poly.*, **73**, 1412 (2013).
9. T. C. Tsao, D. E. Williams, C. G. Worley, and S. D. Worley, *Biotechnol. Progr.*, **7**, 60 (1991).
10. Z. M. Jiang, K. K. Ma, J. M. Du, R. Li, X. H. Ren, and T. S. Huang, *Appl. Surf. Sci.*, **288**, 518 (2014).
11. Y. Gao and R. Cranston, *Text. Res. J.*, **78**, 60 (2008).
12. Z. M. Jiang, B. Demir, R. M. Broughton, X. H. Ren, T. S. Huang, and S. D. Worley, *J. Appl. Polym. Sci.*, **133** (2016).
13. Y. F. Wang, L. Li, Y. Liu, X. H. Ren, and J. Liang, *Mat. Sci. Eng. C-Mater.*, **69**, 1075 (2016).
14. N. Y. Pan, Y. Liu, X. Y. Fan, Z. M. Jiang, X. H. Ren, and J. Liang, *J. Mater. Sci.*, **52**, 1996 (2017).
15. S. D. Worley, Y. Chen, J. W. Wang, and R. Wu, *Surf. Coat. Int. B-C.*, **88**, 93 (2005).
16. X. H. Ren, L. Kou, J. Liang, S. D. Worley, Y. M. Tzou, and T. S. Huang, *Cellulose*, **15**, 593 (2008).
17. G. Sun, T. Y. Chen, and S. D. Worley, *Polymer*, **37**, 3753 (1996).
18. G. Sun, L. C. Allen, E. P. Luckie, W. B. Wheatley, and S. D. Worley, *Ind. Eng. Chem. Res.*, **34**, 4106 (1995).
19. Z. M. Jiang, Y. Liu, R. Li, X. H. Ren, and T. S. Huang, *Polym. Adv. Technol.*, **27**, 460 (2016).
20. A. Akdag, S. Okur, M. L. McKee, and S. D. Worley, *J. Chem. Theory Comput.*, **2**, 879 (2006).
21. Z. B. Cao and Y. Y. Sun, *ACS Appl. Mater. Inter.*, **1**, 494 (2009).
22. M. Braun, W. Frank, and C. Ganter, *J. Organomet. Chem.*, **696**, 3580 (2011).
23. F. Hamon, G. Prie, F. Lecornue, and S. Papot, *Tetrahedron Lett.*, **50**, 6800 (2009).
24. J. Lee, R. M. Broughton, A. Akdag, S. D. Worley, and T. S. Huang, *Fiber. Polym.*, **8**, 148 (2007).
25. T. K. Kim, S. H. Yoon, and Y. A. Son, *Dyes Pigm.*, **60**, 121 (2004).
26. G. Sun, X. J. Xu, J. R. Bickett, and J. F. Williams, *Ind. Eng. Chem. Res.*, **40**, 1016 (2001).
27. B. Zhang, Y. C. Jiao, Z. Z. Kang, K. K. Ma, X. H. Ren, and J. Liang, *Cellulose*, **20**, 3067 (2013).
28. C. M. Feng, Y. Zhang, S. W. Liu, Z. G. Chi, and J. R. Xu, *J. Appl. Polym. Sci.*, **123**, 3208 (2012).