Modification of Silica Gel, Cellulose, and Polyurethane with a Sterically Hindered *N*-Halamine Moiety to Produce Antimicrobial Activity

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ABSTRACT: The sterically hindered amine monomer 4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine has been synthesized and covalently bonded to the surfaces of silica gel particles and cellulose (cotton) and copolymerized in a polyurethane coating formulation. Upon exposure to dilute sodium hypochlorite (household bleach), a very stable *N*-Cl bond is formed *in situ* at the hindered amine nitrogen site. This source of oxidative chlorine provides an antimicrobial function to the silica gel, cotton, and polyurethane. Stability, regenerability, and biocidal efficacy data are presented. The new *N*-halamine materials were remarkably effective against *Staphylococcus aureus* and *Escherichia coli* O157 : H7 in brief periods of contact. The materials should find application in water treatment and medical applications. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 2306–2313, 2007

Key words: biofibers; biological applications of polymers; biomaterials; biopolymers; functionalization of polymers

INTRODUCTION

Inspired by the pioneering studies of Higuchi and coworkers[1] and Emerson et al.[2] in the 1970s, a new area of disinfectant technology based upon the synthesis and testing of heterocyclic *N*-halamine compounds was developed primarily in these laboratories during the period 1980 to 1990 (for example, see references quoted in Ref. 3). It was found that oxidative chlorine or bromine could be bonded to nitrogen moieties of three types (amine, amide, or imide) in the heterocyclic molecules. The strength of the nitrogen-halogen bonds in the structures generally follows the order amine *N*-X > amide *N*-X > imide *N*-X, which is the same order as their resistance to hydrolysis in aqueous solution, and inversely related to the ease of transfer to bacterial cells, and thus to the contact time necessary to cause inactivation of the cells. Therefore, it was possible to design *N*-halamine structures which fit the requirements of a particular disinfection application, for example, rapid cell inactivation, but lower stability in solution, versus longer contact times for cell inactivation, but also greater stability over time. Then, from the late 1980s, *N*-halamine technology has been extended to functionalization of polymers, in these laboratories and elsewhere, to produce a wide variety of useful biocidal coating materials such as for polymer-supported antimicrobial water filters,[4–7] textiles,[8–12] polyurethane films,[13,14] elastomers,[15] and siloxane tethers.[16,17] Such *N*-halamine polymers have a number of advantages over other types of biocidal polymers, for example, rapid biocidal action upon contact, regenerability by exposure to free halogen, and no known resistance within pathogens since the active agent is oxidative halogen.

In this work the studies have been extended to novel polymers incorporating the molecule 4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine (see structure **I** in reaction Scheme 1). This structural unit contains a sterically hindered amine nitrogen which binds oxidative chlorine very firmly, yet as will be shown herein, still functions as an effective biocide. The unchlorinated precursor has been employed as a light stabilizer for various polymeric compositions, but never as biocidal precursor.[18] Another hindered *N*-halamine copolymer has recently been shown to provide excellent resistance to biofilm formation.[19]

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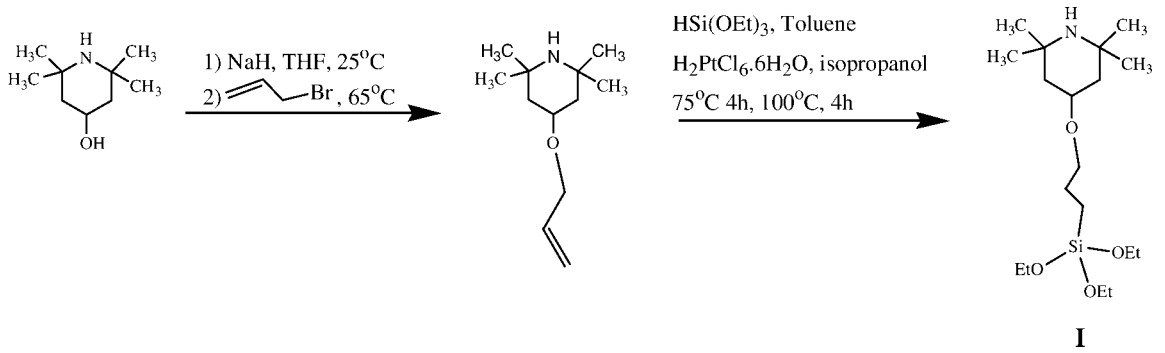


Scheme 1 Synthesis of 4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine (**I**).

EXPERIMENTAL

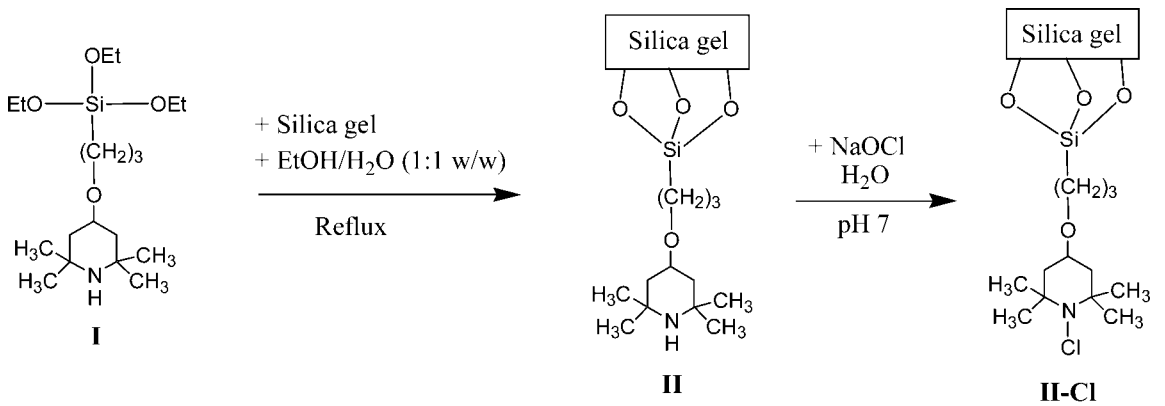
Materials and instrumentation

Starting chemicals and solvents were purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Fabric swatches (Style 400 Bleached 100% Cotton Print Cloth) were obtained from Testfabrics (West Pittston, PA). Bacterial cultures of *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* O157 : H7 ATCC 43,895 were obtained from the American Type Culture Collection (Rockville, MD), and Trypticase soy agar from Difco Laboratories (Detroit, MI). NMR, IR, and mass spectral data were obtained with a Bruker 250 MHz spectrometer, a Shimadzu Prestige 21 FTIR, and a Micro Mass Trio 2000, respectively.

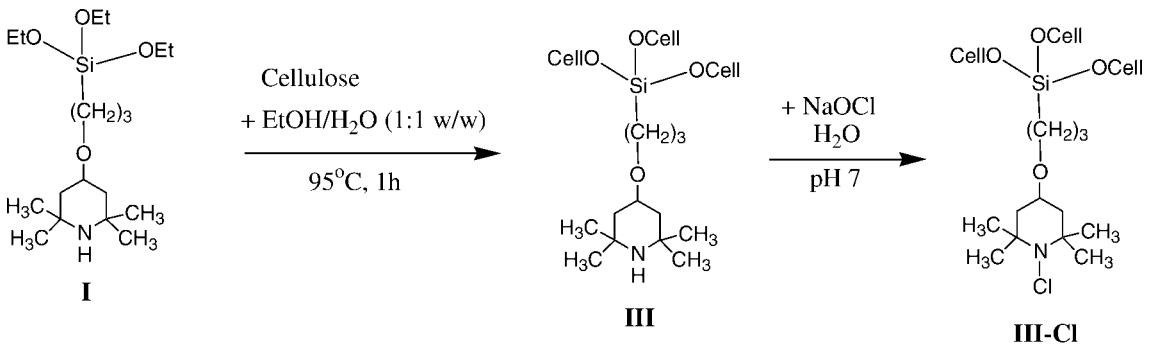
Preparation of 4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine (I)

Scheme 1 shows the reactions employed to prepare the sterically hindered amine siloxane (**I**). The procedures used for the two reactions were modifications of literature methods.[18,20] Briefly, in a typical preparation, 7.94 g (0.05 mol) of 2,2,6,6-tetramethyl-4-piperidinol (99%) was mixed with 100 mL of freshly distilled anhydrous THF. The mixture was stirred in a sealed flask under nitrogen at ambient temperature until it became clear. It was then poured into a flask containing 1.20 g (0.05 mol) of sodium hydride. The reactants were stirred for 30 min at ambient temperature under a nitrogen atmosphere. Then 6.11 g (0.05 mol) of allyl bromide (99%) were added in bulk. The reactants were stirred for 30 min at ambient temperature and then for 4 h at 65°C under a nitrogen atmosphere. The sodium bromide byproduct was removed by filtration and the THF solvent by distillation. The procedure produced 8.63 g of the desired product 4-(allyloxy)-2,2,6,6-tetramethylpiperidine as an oil corresponding to a yield of 87%. Spectroscopic data for this intermediate product were: ^{1}H-NMR (d_6 DMSO) δ 0.96 (m, 2H), 1.02 (s, 6H), 1.10 (s, 6H), 1.84 (m, 2H), 3.73 (m, 1H), 3.99 (d, 2H), 5.12 (d, 1H). 5.26 (d, 1H), 5.87 (m, 1H); ^{13}C-NMR (d_6 DMSO) δ 29.73, 35.27, 44.69, 51.19, 68.25, 72.09, 115.88, 136.50; IR(KBr) 1130, 1423, 1458, 1645, 1821, 2927, 2956, 3319 cm^{-1}; *m/z* 197.

The final product **I** was then prepared by adding 9.86 g (0.05 mol) of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine, prepared as described above, in 200 mL of anhydrous toluene to a flask under nitrogen atmosphere, followed by 8.65 g (0.05 mol) of triethoxysilane (95%). This solution was allowed to stir at ambient temperature under nitrogen until it became clear. Then 20 μL of a 2% by weight solution of chloroplatinic acid (Spier catalyst) dissolved in isopropyl alcohol were added to the mixture, and it was held with stirring at 75°C for 4 h, and then at 100°C for an addi-



Scheme 2 Preparation of biocidal silica gel.



Scheme 3 Preparation of biocidal cellulose.

tional 4 h. Disappearance of an IR band at 1645 cm^{-1} was used as evidence that the 4-(allyloxy)-2,2,6,6-tetramethylpiperidine had completely reacted. The resulting oil was distilled under vacuum with the product **I** collected at 95°C at 0.1 mm. The yield of the reaction was 75%. Spectroscopic data for the product were: ^{1}H-NMR ($CDCl_3$) δ 0.61, 0.94, 1.08, 1.14, 1.20, 1.62, 1.89, 3.40, 3.60, 3.75; ^{13}C-NMR ($CDCl_3$) δ 6.58, 18.31, 23.53, 29.04, 35.01, 44.99, 51.39, 58.33, 70.10, 72.33; IR(KBr) 952, 1081, 1186, 1237, 2924, 2972, 3317 cm^{-1}; *m/z* 361.

Coating procedures

For silica gel, 0.25 to 1.25 g of **I** were mixed with generally 2.5 g of silica gel (30-60 mesh) in 10.0 g of ethanol/water (1 : 1 w/w) in a flask, and the mixture was heated at reflux for times varying from 0.5 to 7.0 h. The functionalized silica gel (**II** in Scheme 2) was isolated by vacuum filtration, rinsed thrice with 100 g portions of ethanol/water (1 : 1 w/w), and dried at ambient temperature in air.

For cellulose, cotton swatches were soaked in a bath containing 5% by weight **I** in ethanol/water (1 : 1 w/w) for 15 min. Then they were cured at 95°C for 1 h, soaked in 0.5% detergent solution for 15 min, and rinsed several times with water to remove any weakly attached coating. Following drying in air, typical add-on weight percentages of **III** (Scheme 3) were about 4.6%.

For polyurethane, a recipe of 0.05 to 0.25 g **I**, 2.00 g of a commercial water-borne acrylic polyol formulation (Ser. 297, Part A, Enviro-tread, TNEMEC, Kansas City, MO), 0.50 g of a commercial isocyanate (Ser. 297, Part B, Enviro-glaze, TNEMEC), and 1.00 g of water to aid mixing of the components was prepared. After vigorous stirring, the mixture was spread onto the surfaces of transparency slides. Drying in air at ambient temperature for 16 h produced a smooth, tightly bound polyurethane copolymer coating. The cured coatings were rinsed several times with distilled water to remove any unbound **I**.

Chlorination procedures

The functionalized silica gel **II** was chlorinated by soaking it for 30 min in a 10% aqueous solution of sodium hypochlorite bleach buffered to pH 7 by dropwise addition of 6*N* HCl. The product (**II-Cl** in scheme 3) was rinsed with three portions of deionized water (50 mL per 2.5 g **II-Cl**) and then dried at 45°C for 2 h to remove occluded free chlorine. The oxidative chlorine content (% by weight Cl^+) was determined by a modified iodometric/thiosulfate titration procedure which has been described previously.[21] Equation (1) below was employed, where

$$\%Cl^+ = \frac{NV35.34}{2W}100 \quad (1)$$

N and *V* are the normality (eqiv./L) and volume (L), respectively, of the $Na_2S_2O_3$ consumed in the titration, and *W* is the weight (g) of the sample of **II-Cl**.

The derivatized cellulose was generally chlorinated by a procedure analogous to that described above for silica gel. However, in some cases the cotton swatches **II** were chlorinated using about 200 ppm of Cl^+ in a commercial washing machine. A standard testing procedure was used for these experiments (AATCC Test Method 61, Test 2A). In this way the cotton swatches could be evaluated for chlorine content after 5, 10, 25, and 50 washing cycles, which provided an assessment of the retention of the coating on the cellulose. The samples were dried in a household drier at 65°C before analytical chlorine determination. The pH of the washing water was also measured before and after each set of laundering cycles. The percent by weight Cl^+ on the cotton swatches was determined by iodometric/thiosulfate titration, and eq. (1) again was employed for **III-Cl** samples.

For the polyurethane copolymer films, the chlorination procedure was performed at varying aqueous bleach concentrations (1%, 5%, 10%) and times of exposure (0.5–4.5 h). After exposure to the bleach solution, the films were rinsed thoroughly with distilled water and then dried in air at ambient temperature for 24 h, and then at 45°C for 5 h. In this case, eq. (2) was

employed to determine the oxidative chlorine loading, where

$$Cl^{+}(\text{atoms/cm}^2) = \frac{6.02 \times 10^{23} NV}{2A} \quad (2)$$

N and V are the normality (eqv/L) and volume (L), respectively, of the $Na_2S_2O_3$ consumed in the titration, and A is the area in cm^2 of the slide coated with chlorinated polyurethane copolymer.

Stability and regeneration of chlorine

Silica gel **II-Cl** was packed into a glass column (25.0 cm length, 1.0 cm inside diameter) to a height of 18.0 cm with an empty-bed volume of about 4.0 mL. Distilled water was pumped continuously through the column at a rate of 8.0 mL/min for 168 h. The chlorine content of the **II-Cl** was determined before and after the experiment, and a recharge was performed using the same procedure as employed initially for chlorination to assess the stability of the coating on the silica gel particles.

Stability and regeneration for the cellulose samples **III-Cl** were evaluated using AATCC Test Method 61, Test 2A. Some cotton swatches were chlorinated before washing, some after, and some both, in addition to the related studies described above for chlorination during the washing procedure.

Stability and regeneration experiments for the polyurethane samples will be performed in the future.

Biocidal efficacy testing

For the silica gel samples, columns of uncoated silica gel (control), coated silica gel **II** (control), and chlorinated coated silica gel **II-Cl** were prepared as described above. A peristaltic pump (MasterFlex L/S, Cole Palmer, Vernon, IL) was used to circulate inocula of *Staphylococcus aureus* and *Escherichia coli* O157 : H7 containing about 10^6 CFU in 50 mL of sterile pH 7 buffer through the columns at a flow rate commensurate with about 1 s per pass for the cells. Contact times could thus be varied by varying the number of circulations through the columns. At a given contact time, aliquots (25 μL) were collected in sterile tubes and immediately quenched with 0.02*N* sodium thiosulfate to ensure that any oxidative chlorine leaching out of the column (less than 0.2 ppm Cl^+) was not present to continue bacterial inactivation. Serial dilutions of the quenched effluent solutions were plated onto Trypticase soy agar. Following incubation at 37°C for 24 h, the plates were counted to determine the numbers of any viable CFUs.

For efficacy testing for the coated cellulose samples, 1 inch square cotton swatches were prepared. The controls employed contained no coating and unchlorinated coating (**III**). The swatches were challenged with *Staphylococcus aureus* and *Escherichia coli* O157 : H7 containing about 10^5–10^7 CFU, dependent upon the experiment, in a ''sandwich test.'' In this test 25 μL of inoculum were placed in the center of a swatch sample, and an identical swatch was placed upon the inoculated swatch held down by a sterile weight to ensure efficient contact. Then after contact times of 5.0, 10,0, and 30.0 min, the various swatches were placed in sterile tubes containing 5.0 mL of 0.02*N* sodium thiosulfate to quench biocidal action. The tubes were vortexed for 150 s to remove bacteria from the swatches, and serial dilutions of the solution were plated onto Trypticase soy agar. The plates were incubated at 37°C for 24 h, and then counted for viable CFUs.

The polyurethane coatings on the transparency slides were challenged in a ''sandwich test'' as described above. However, in this case lower concentrations of inocula were employed (about 10^3–10^5 CFU) and longer contact times (1.0, 2.0, 3.0 h) because higher bacterial concentrations or shorter contact times did not display complete inactivation due to lower surface areas of the coatings involved.

RESULTS AND DISCUSSION

Silica gel

As expected, the amount of **I** which can be coated onto silica gel particles to produce **II**, as measured by analytical determination of oxidative chlorine on **II-Cl,** depends upon the weight of **I** employed and the time of reaction. Table I shows the effect of coating reaction time for production of **II** for a weight ratio (silica gel to **I**) of 3.33 at pH 7.0. As can be seen, the coating efficiency increases dramatically with time of reaction up to about 5 h, with only a small increase at 7 h. In previous work in these laboratories in which silica gel was coated with a silylpropylhydantoin derivative under the same experimental conditions, a somewhat higher chlorine loading of 1.15% Cl^+ was obtained.[22] It was found that greater chlorine loadings

TABLE I
Coating Efficiency of I on Silica Gel to form II and then II-Cl Upon Chlorination as a Function of Reaction Time

Reaction time (*h*)[a]	Titrated Cl^+ (wt %)
0.5	0.43
1.0	0.60
2.0	0.75
3.0	0.84
5.0	0.89
7.0	0.92

[a] Reaction of 2.5 g silica gel with 0.75 g **I** in 10 g ethanol/water (1 : 1 w/w) at pH 7.

TABLE II
Biocidal Efficacy of Coated Chlorinated Silica Gel II-Cl against *S. aureus* and *E. coli* O157 : H7

Coating material/ chlorine loading	Contact time (s)	Log reduction *S. aureus*[a]	Log reduction *E. coli* O157 : H7[b]
Exp. 1			
Silica gel control, wt % Cl^+	10	0.13	ND[c]
	30	0.09	0.09
	60	0.14	0.39[d]
II, 0 wt % Cl^+	10	0.75	0.50
	30	0.81	0.57
	60	1.01	0.89[d]
II-Cl, 0.85 wt % Cl^+	10	3.19	1.54
	30	3.49	2.08
	60	6.32	6.22[d]
Exp. 2			
Silica gel control, 0 wt % Cl^+	10	0.13	0.02
	30	0.09	0.07
	60	0.14	0.05
II, 0 wt % Cl^+	10	1.44	0.07
	30	2.06	0.13
	60	2.18	0.41
II-Cl, 1.01 wt % Cl^+	10	3.46	1.63
	30	6.28	6.12
	60	6.28	6.12

[a] Inoculum concentration was 2.07×10^6 CFU in Exp. 1; 1.93×10^6 CFU in Exp. 2.
[b] Inoculum concentration was 1.67×10^6CFU in Exp. 1; 1.33×10^6 CFU in Exp. 2.
[c] No determination.
[d] These data are for 120 s contact since all bacteria were not inactivated for *E. coli* O157 : H7 in Exp. 1; inactivation was complete at 60 s in Exp. 2.

(hence increased coating efficiencies) could be obtained for the hydantoin derivative under acidic or basic conditions;[22] only experiments at pH 7 were performed for **II** in this study. It is interesting that different *N*-halaminesiloxane derivatives (piperidine versus hydantoin) have somewhat different coating efficiencies for silica gel under the same experimental conditions. As for the stability of the coating, when water was continually pumped through a column containing **II-Cl** for 168 h, the chlorine content declined from 0.89% Cl^+ to 0.85%; regeneration produced 0.86%. Thus, over 168 h subjected to flowing water, a small amount of siloxane was hydrolyzed from the surfaces of the silica gel particles. Similar data were obtained for the hydantoin derivative.[22]

In the biocidal efficacy testing experiments (Table II), complete 6.3 log inactivation of *S. aureus* was obtained in the contact interval 30–60 s for the chlorinated coated silica gel particles in the column-flow experiment. In the case of *E. coli* O157 : H7 the results were less consistent with total inactivation being observed in the interval 10–30 s in one experiment, but 60–120 s in the other. It should be noted that a 6 log inactivation corresponds to a 99.9999% reduction in viable cells. Also, the control **II** columns absorbed 1 to 2 logs of the *S. aureus* (they could be recovered live in the column)

TABLE III
Durability of III on Cotton Exposed to Machine Washing Cycles

Number of washing cycles	Weight % Cl^+ for chlorination only before the first washing cycle	Weight % Cl^+ for chlorination only after the indicated number of washing cycles	Weight % Cl^+ for prechlorination and rechlorination after the indicated number of washing cycles
0	0.49[a]		
5	0.45	0.43	0.46
10	0.42	0.40	0.43
25	0.34	0.31	0.37
50	0.22	0.18	0.25

[a] All chlorinations were performed at pH 7 (see Experimental).

TABLE IV
Durability of III and a Hydantoin Siloxane Derivative on Cotton Exposed to Machine Washing Cycles Containing Household Bleach

Number of washing cycles	Weight % Cl^+ for chlorination of **II**[ab]	Weight % Cl^+ for chlorination of a hydantoin siloxane coating[bc]
5	0.44	0.07
10	0.46	0.09
25	0.43	0.07
50	0.30	0.06

[a] **II** was exposed to about 200 ppm Cl^+ concentration from household bleach; the pH varied from 10.30 to 9.42 during the experiment.
[b] Measured after the indicated number of washing cycles.
[c] The hydantoin siloxane derivative of Ref. 17 was exposed to about 200 ppm Cl^+ concentration from household bleach; the pH varied from 10.63 to 9.35 during the experiment.

which was a greater loss than observed for *E. coli* O157 : H7, probably due to the geometrical shapes of the cells (rods for *S. aureus*. spheres for *E. coli* O157 : H7). Nevertheless, the chlorinated particles clearly caused significant inactivation of the two pathogens, and no live bacteria could be recovered from the columns after the experiments. It should be noted that the **II-Cl** performed slightly less efficiently than did the chlorinated hydantoin siloxane mentioned above which inactivated both bacteria in the 10–30 s contact interval.[22] The latter silica gel samples contained somewhat higher chlorine loadings, and that *N*-halamine contains an amide *N*-Cl bond rather than an amine *N*-Cl bond as in **II-Cl**.

Cellulose

The data in Tables III and IV address the resistance of the coating on cotton to hydrolysis from the surface, and hence the retention of biocidal activity. Table III shows that when the coating is prechlorinated to form **III-Cl** at pH 7, that 0.49% by weight Cl^+ can be loaded onto the cloth. After 50 machine washing cycles 0.22% remained. A rechlorination after the 50 washing cycles raised the loading to 0.25% indicating that only about 50% of the coating was hydrolyzed from the surface of the fibers during 50 washing cycles. When the coating was chlorinated only after completing the washing process, 0.18% remained. Thus prechlorination provided a coating slightly more resistant to hydrolysis as might be expected since the chlorinated surface **III-Cl** is more hydrophobic than is the unchlorinated surface **III**. In contrast, the hydantoin siloxane derivative mentioned earlier initially loaded 0.42% by weight Cl^+ on cotton, but retained only 0.13% after rechlorination after 50 washing cycles.[17] Thus the piperidine

TABLE V
Biocidal Efficacy of Coated Chlorinated Cotton III-Cl against *S. aureus* and *E. coli* O157 : H7

Coating material/ chlorine loading	Contact time (min)	Log reduction *S. aureus*[a]	Log reduction *E. coli* O157 : H7[b]
Exp. 1			
Cotton control, 0 wt % Cl^+	5	ND[c]	ND[c]
	10	0.54	0.09
	30	0.71	0.19[d]
III, 0 wt % Cl^+	5	ND[c]	ND[c]
	10	1.60	0.11
	30	2.08	0.28[d]
III-Cl[d], 0.33 wt % Cl^+	5	6.99	6.94
	10	6.99	6.94
	30	6.99	6.94
Exp. 2			
Cotton control, 0 wt % Cl^+	5	0.03	0.04
	10	0.04	0.24
	30	0.20	0.24
III, 0 wt % Cl^+	5	0.48	0.71
	10	0.49	0.93
	30	1.22	1.41
III-Cl,[d] 0.34 wt % Cl^+	5	6.88	6.75
	10	6.88	6.75
	30	6.88	6.75

[a] Inoculum concentration was 9.67×10^6 CFU in Exp. 1; 7.67×10^6 CFU in Exp. 2.
[b] Inoculum concentration was 8.67×10^6 CFU in Exp. 1; 5.67×10^6 CFU in Exp. 2.
[c] No determination.
[d] Chlorination of **III** for this experiment was performed in a 10% solution of aqueous household bleach buffered to pH 7.

TABLE VI
Chlorine Loading on Polyurethane Copolymer Films as a Function of the Amount of I Added to the Formulation

Amount of I added to the formulation (g)[a]	Loading of Cl^+ (atoms/cm^2)
0.05	9.00×10^{16}
0.10	1.08×10^{17}
0.15	1.29×10^{17}
0.20	1.38×10^{17}
0.25	1.50×10^{17}

[a] The Formulation also contained 2.00 g of commercial water-borne acrylic polyol, 0.50 g of commercial isocyanate, and 1.00 g of water.

siloxane derivative is more resistant to hydrolysis from the surface of cotton fibers than is the hydantoin siloxane analog.

When the chlorination process was performed in the washing machine during each washing cycle, a dramatic difference between the two siloxane derivatives was observed. Table IV shows that both derivatives hydrolyzed away from the surface of the cotton fibers to a lesser extent with this procedure which mimics the probable use pattern for the coated materials, but the piperidine siloxane derivative was able to load substantially more weight % Cl^+ (0.30–0.46) than was the hydantoin siloxane derivative (0.06–0.09). The reason for this observation is that the pH in the washing water to which about 200 ppm of Cl^+ had been added at each washing cycle was about 10 instead of 7. The amine nitrogen in **II** chlorinates to about the same extent at pH 10 and 7; however, the amide nitrogen in the hydantoin siloxane derivative clearly chlorinates to a much greater extent at pH 7 than at pH 10.

Biocidal efficacy data were obtained for cotton swatches coated with **III-Cl** for pH 7 chlorination in a bath and for pH 10 chlorination during a washing cycle in a commercial washing machine. The results were the same for both methods, that is, complete inactivation of both bacterial species in a contact time of less than 5 min. The data for the bath chlorination at pH 7 are shown in Table V. As for the coated silica gel, the unchlorinated cotton **III** control effected a more significant log reduction of *S. aureus* than for *E. coli* O157 : H7, but in both cases the bacteria were absorbed, but not killed by the unchlorinated control. Whereas **III-Cl** effected a more than a 6 log reduction of both bacterial species within a contact time of 5 min, the hydantoin siloxane derivative required a contact time interval of 30–60 min for *S. aureus* and 60 to 120 min for *E. coli* O157 : H7.[17] The chlorine loading for the hydantoin siloxane studies was somewhat higher (0.49% Cl^+) than for the current **III-Cl** experiments, so it would appear that the hindered amine siloxane is superior to the amide siloxane for producing antimicrobial cotton if it is competitive economically.

Polyurethane

Table VI shows that the chlorine loading on the hindered amine siloxane polyurethane copolymer films is dependent on the amount of the siloxane in the formulation as expected. It is also dependent upon the time of chlorination (Table VII), but after 4 h contact with household bleach, the surface sites are filled, since the same loading of Cl^+ is obtained for 1, 5, and 10% aqueous bleach solutions at that time. Changes of weight or strength after bleaching were not measured, but there were no obvious changes in appearance.

Biocidal data for the polyurethane copolymer films are presented in Table VIII. Both of the bacterial species were inactivated in the contact time interval of 1–2 h. The significantly longer time of contact necessary for inactivation of less bacteria than for **II-Cl** and **III-Cl** samples can be attributed to much less surface area exposure to the bacterial cells for the nonporous

TABLE VII
Chlorine Loading on Polyurethane Copolymer Films as a Function of the Time of Chlorination and Concentration of Household Bleach Employed

Time of chlorination (h)[a]	Loading of Cl^+ (atoms/cm^2) for 1% bleach solution	Loading of Cl^+ (atoms/cm^2) for 5% bleach solution	Loading of Cl^+ (atoms/cm^2) for 10% bleach solution
0.5	2.48×10^{16}	2.79×10^{16}	2.61×10^{16}
1.0	4.03×10^{16}	4.65×10^{16}	4.96×10^{16}
1.5	5.58×10^{16}	6.21×10^{16}	6.52×10^{16}
2.0	7.26×10^{16}	8.38×10^{16}	7.76×10^{16}
2.5	8.69×10^{16}	1.05×10^{17}	1.02×10^{17}
3.0	1.06×10^{17}	1.28×10^{17}	1.30×10^{17}
3.5	1.15×10^{17}	1.27×10^{17}	1.31×10^{17}
4.0	1.29×10^{17}	1.31×10^{17}	1.33×10^{17}
4.5	1.31×10^{17}	1.30×10^{17}	1.33×10^{17}

[a] The formulation also contained 0.15 g of **I**, 2.00 g of commercial water-borne acrylic polyol, 0.50 g of commercial isocyanate, and 1.00 g of water.

TABLE VIII
Biocidal Efficacy of Coated Chlorinated Polyurethane-I Films against *S. aureus* and *E. coli* O157 : H7

Coating material/chlorine loading	Contact time (*h*)	Log reduction *S. aureus*[a]	Log reduction *E. coli* O157 : H7[b]
Control film, 0 atoms/cm^2 Cl^+	1	1.23	0.84
	2	1.48	0.84
	3	1.56	0.97
Chlorinated film, 1.27×10^{17} atoms/cm^2 Cl^+	1	2.37	0.97
	2	4.67	3.27
	3	4.67	3.27

[a] Inoculum concentration was 4.67×10^4 CFU.
[b] Inoculum concentration was 1.87×10^3 CFU.

polyurethane copolymer films. Again, recovery of *S. aureus* CFUs from the control films was less than for *E. coli* O157 : H7, but the bacteria were not killed by the controls. The control polyurethane films were unexpectedly adept at adsorbing live bacteria with the number increasing with time of exposure. The Cl^+ loading obtained for a hydantoin siloxane polyurethane copolymer of similar formulation studied earlier in these laboratories was 8.49×10^{16} atoms/cm^2, but these films were not tested for efficacy against the two bacterial species.[17]

CONCLUSIONS

A new sterically hindered amine siloxane (4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine) has been synthesized and coated onto the surfaces of silica gel particles and cellulose (cotton swatches), and it has also been copolymerized in a polyurethane formulation for coating onto transparency slides. Upon chlorination with sodium hypochlorite (household bleach) solutions, all of the coated surfaces became antibacterial. The chlorinated coated silica gel particles provided a greater than 6 log inactivation of *S. aureus* and *E. coli* O157 : H7 in a column filter application in the 30–60 s contact time range. For chlorinated cotton the contact time necessary for a 7 log inactivation of the two bacteria in a ''sandwich test'' was less than 5 min. For the chlorinated polyurethane films a contact time of 1–2 h was necessary to provide a 3–4 log inactivation of the two bacteria. The hydantoin siloxane derivative reported previously[17] is probably the better option for silica gel and polyurethane coatings because of its lower production cost. However, for cotton the new compound reported herein is clearly the better option because of its chlorination capacity in a commercial washing machine at pH 10 and its superior stability toward hydrolysis from the surfaces of the fibers.

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