PREPARATION AND APPLICATION OF REGENERABLE N-HALAMINE

BIOCIDAL MATERIALS

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PREPARATION AND APPLICATION OF REGENERABLE N-HALAMINE

BIOCIDAL MATERIAL

Lei Kou

A Dissertation

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PREPARATION AND APPLICATION OF REGENERABLE N-HALAMINE

BIOCIDAL MATERIALS

Lei Kou

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VITA

Lei Kou, daughter of Mingcai Kou and Yunping Wu, was born in Wudu, Gansu Province, the People's Republic of China, on Jun 3, 1979. She attended Lanzhou University in China, where she graduated with a Bachelor of Science degree in Chemistry in July 2001 and a Master of Science degree in Physical Chemistry in July 2004. She also entered the Ph.D program in the Department of Chemistry and Biochemistry at Auburn University in September 2004 where she worked under the supervision of Dr. S. D. Worley. She married Tiansheng Mei, son of Yuechang Mei and Meiqin Chen, in 2006.

DISSERTATION ABSTRACT

PREPARATION AND APPLICATION OF REGENERABLE N-HALAMINE

BIOCIDAL MATERIALS

Lei Kou

Doctor of Philosophy, August 10, 2009
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Copolymers of an N-halamine siloxane and a quaternary ammonium salt siloxane were prepared using 5,5-dimethylhydantoin and trimethylamine as functional groups. The solubility of this siloxane copolymer in water was dramatically better than that of the hydantoinyl siloxane homopolymer reported previously. The stability of the oxidative chlorine loading on cotton swatches was not affected by the presence of the very hydrophilic quaternary functional group. Utilizing the new copolymer in an antimicrobial coating application offers the advantage that no organic solvent is required when preparing a coating bath for textile materials because copolymer is adequately soluble in water whereas most siloxanes are only soluble in organic solvent.

The monomers of the compounds 6-phenyl-3-(3'-triethoxysilylpropyl)-1,3,5-

triazinane-2,4-dione and 6,6-dimethyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,
4-dione were synthesized and coated on the surfaces of silica gel particles and cellulose.

Exposure to diluted sodium hypochlorite solutions rendered these polymers antimicrobial.

The treated polymers inactivated 7 log concentrations of both *Staphylococcus aureus* and

Escherichia coli O157:H7 with brief contact times. The coated cotton fabrics showed

great stability and rechargeability following exposure to UV and ambient light.

Several functionalized N-halamine monomers were synthesized for this study, namely

5-aminomethyl-5-methyl-hydantoin, 5-methyl-5-hydroxylmethylhydantoin, and

5-chlormethyl-5-methylhydantoin. The first two of theses N-halamine precursors can be

coated onto cotton surfaces with the addition of the cross-linking agent

butanetetracarboxylic acid (BTCA), and 5-chlormethyl-5-methylhydantoin can also be

coated onto cotton surfaces with the aid of sodium hydroxide as a catalyst. All of these

treated cotton swatches were rendered biocidal by exposure to halogen solutions either

before or after curing the coating or material.

The preparation of several new hydantoin diols and tetraols is also reported here.

These were copolymerized with commercial polyol and diisocyanate to form different

polyurethane films that could be painted onto a surface. Activation by chlorination

produced biocidal polyurethane films.

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The author would like to express her deepest appreciation to her advisor, Dr. Worley, for his guidance, support, encouragement, and insight throughout this investigation. This dissertation would never been completed without his support and invaluable academic guidance.   The author would like to thank committee members, Dr. V. Cammarata, Dr. S. Striegler, and Dr. R.M. Broughton for their valuable suggestions and discussions. I would like to thank my group members: Dr. Liang, Dr. Ren, Dr. Akdag, Dr. Barnes, Dr. Lee, Changyun Zhu, and Kocer Hasan for discussion and help. Special thanks to Dr. Huang of the Department of Nutrition and Food Science for conducting antimicrobial testing and serving as her outside reader. I would like to thank the Department of Chemistry and Biochemistry, VansonHalosource, Inc., Seattle, WA and the US Airforce for funding support.

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Chapter 1

Introduction

Archaeological evidence indicates that human beings have used biocides as preservatives for food (salt and natural spices) and water since the earliest civilizations.[1] Salt, honey, and sugar solutions have long been known to have preservative action for food. As early as 450 BC, the Persians used copper or silver vessels to store water to keep the water from spoiling. Ancient Egyptians developed the art of mummification to treat the dead, using natural alkalis, native sodium carbonate, oils, and balsams. Pitch from oil seepage was also used by Egyptian and Chinese physicians in the treatment of wounds, and wine, vinegar, rose oil, and honey were used for wound dressing in medieval times. At around the same time mercuric chloride was prepared and used by Arab physicians as an antiseptic. Later, wood tar and coal tar were introduced as disinfectants. The use of chlorine solutions, iodine, and phenol has also been reported for wound dressing and wound disinfectants.[1]

The need for effective disinfectants was discovered during the development of antiseptic surgery. A number of chemicals, including wood tar, mercuric chloride, copper sulphate, hydrogen peroxide, and chlorine-releasing agents, were used as biocides to control the spread of disease in hospitals.[1-4]

Biocides are mainly used to preserve food and medicine and to maintain sterility of pharmaceutical products. They are very important chemicals which provide different levels of disinfection and sterilization of a wide range of surfaces and materials, especially, in hospital or industrial environments. Furthermore, growing concern about contamination due to microorganisms, personal hygiene and the spread of disease have led to the application of biocides in home environments.[2]

Generally, biocides kill or inhibit growth or inactivate the microorganisms that cause and spread disease, degrade perishable goods, contaminate water, interfere with equipment and harm living creatures. Biocides can be divided into two categories: antiseptics and disinfectants. Chemical antiseptics are used on or in living tissue, while disinfectants are used on inorganic surfaces such as medical devices or surfaces in hospitals, homes or schools.[5-6]

A good biocide should have the following characteristics: an acceptable price, high efficiency coupled with a wide spectrum of activity, a long shelf life, fast killing speed at relatively low concentrations, no undesirable smell, and be neither corrosive nor toxic to the environment.

There are over 5,000 EPA-registered formulations in use today, and some of the active ingredients most commonly found in the topically-applied products currently used in the medical, food service, and consumer markets will be discussed in the following section.

Topical Biocides

Chlorhexidine

Chlorhexidine-1,6-di(4'-chlorophenyldiguanido)hexane, a bisbiguanide (CHX) is most commonly found in products as a salt of gluconic acid (CHG) (Figure 1). CHX has a high level of antimicrobial activity and low toxicity to mammalian cells,[5-6] with a strong affinity for binding to the skin that leaves a residue of antimicrobial activity after application. It has been used for treating acute and chronic wounds, as a surgical or healthcare personnel handwash, and also as a preservative and a disinfectant of medical instruments and environmental surfaces.[7] However, microorganisms readily become resistant to CHX.[5-6]
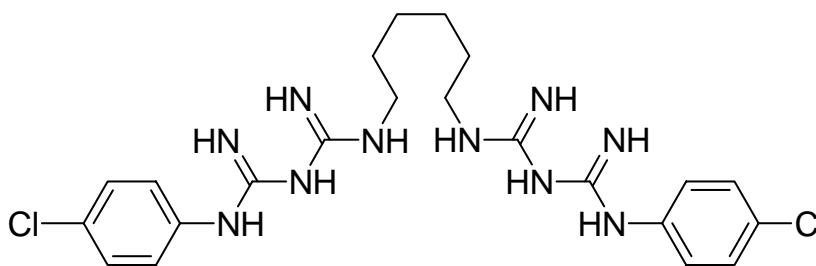
Figure 1. Structure of CHX

Halophenols

Parachlorometaxylenol (PCMX) and Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) are both examples of chlorinated phenols (Figure 2). Halophenols have relatively low antimicrobial efficacy. Both PCMX and Triclosan provide a fairly immediate and persistent effect, and leave no residual antimicrobial activity. PCMX products are mainly formulated for healthcare personnel handwashes. Triclosan has a wide range of applications that includes underarm deodorants, sock deodorizers, handwash, handsoaps,

3

toothpaste, shower gels, and body cleansers. *E. coli* and *S. aureus* show some resistance to Triclosan,[8] and there is evidence to suggest that it accumulates in the environment.[7]

PCMX   5-chloro-2-(2,4-dichlorophenoxy)phenol
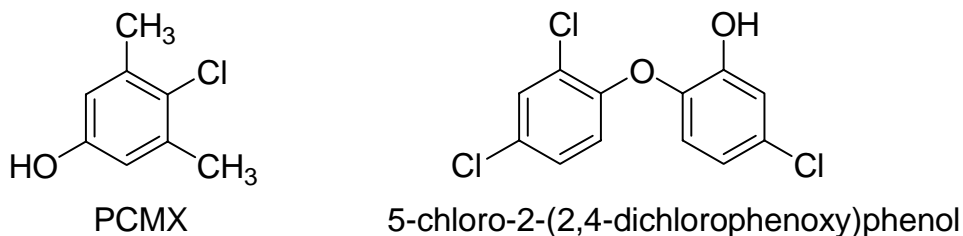
Figure 2. Structures of PCMX and Triclosan

Quaternary Ammonium Compounds (Quats or QACs)

The biocidal activity of quaternary ammonium compounds (quats) was first reported in 1916.[1] Quaternary ammonium compounds (quats) have been utilized as active ingredients in many types of applications: household cleansers, institutional disinfectants, skin and hair care formulation and preservatives in eye drops and mouthwash.

Quats (Figure 3) are odor-free but show a limited scope of efficacy because they are not effective against Gram-negative bacteria and spores. Also, some bacteria such as *Staphylococci* and *Lactobacilli* become resistant to compounds in this class.[8, 9-10] Quaternary ammonium salts require at least one lengthy alkyl group (C12-C16) to achieve biocidal function.[11-14]
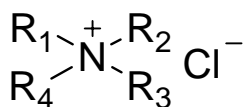
R_1-R_4=alkyl or aromatic substituents

Figure 3. Structure of quaternary ammonium compounds

Alcohols

Alcohols are widely used in medical, food service, and consumer products. However, they need a significant concentration (usually 15-70 %) to be effective as a biocide. Alcohols, such as ethanol, isopropanol, and n-propanol, are popular for their "near-instant" antimicrobial activity. Alcohols generally inactivate spores and viruses with fatty outer coats such as mycobacterium. Alcohols are popular in hand cleansers and hand sanitizers due to their speedy action, but although ethanol, isopropanol, and n-propanol show excellent immediate biocidal activity, they evaporate rapidly and so lack persistent antimicrobial activity. They also lead to problems with dry skin with frequent use.[7]

Heavy Metals

Zinc, mercury, silver, tin, copper, arsenic and their salts are toxic to most life forms.[15] Although their salts are cheap and effective, they are persistent and toxic. For example, mercury chloride is an effective way to kill bacterial spores, but its use was banned in the 1950s as a result of the growing environmental problems. Some studies have shown that bacteria become resistant to heavy metal salts after long term exposure.[16]

Aldehydes

Formaldehyde has been used for hundreds of years as an embalming agent. It is effective in killing water-borne bacteria, and its vapor has been applied to the sterilization of inanimate surfaces such as chicken houses and equipment.[17] However, formaldehyde is a potent carcinogen, so there are restrictions on its use.[18] The most commonly used "cold sterilant" is glutaraldehyde (Figure 4), which is used to sterilize surgical instruments as an alternative to heat sterilization in autoclaves.[17]
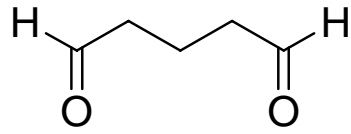
Figure 4. Structure of Glutaraldehyde

Peroxides and other forms of oxygen

As a powerful biocidal agent, hydrogen peroxide functions as a wound cleaner, surface cleaner, surface disinfectant, and for sterilization applications (in liquid or gas forms).[19] It is environmentally friendly and degrades rapidly into water and oxygen, leaving no harmful residues.[19-20] It also has a broad spectrum activity of killing bacteria, fungi, and spores.[21-22]

Peracetic acid and ozone are also powerful biocidal agents.[20] Peracetic acid is mainly used in liquid formulation disinfectants, while ozone is used in gaseous forms. Ozone is considered to be the most reactive and short-lived oxidizing agent for biocidal applications, although it also has adverse health effects for people who come into contact with high concentrations.[23]

Halogens

There is a long history of the use of halogens as antiseptics.[7] Iodine has poor solubility in water and good solubility in alcohols, ketones, and carbonic acids, but its solubility in water can be increased by adding alkali to convert the iodine into triiodides and polyiodides. Iodophor is the most popular form of iodine for most applications. This is a complex of triiodine and a carrier such as polyacrylic acid, polyether glycols, and polyamides. Iodine is commonly used in skin antiseptics, surgical scrub solutions, and disinfectants for medical equipment. It is also used to treat wound infections.[7]

Cl_2, Br_2, I_2, $HOCl$, and ClO_2 are cheap and effective broad spectrum biocides. Chlorine, hypochlorite, and ClO_2 have been used in swimming pools for a long time.[20] Hypochlorite is degraded by UV light and reacts with many organic compounds to produce toxic $CHCl_3$. ClO_2 is not stable so it must be generated on-site, which can be done using a variety of chemical and electrochemical methods.[20] It has been widely used as a skin disinfectant, and for surface disinfection, medical/dental sterilization, and building decontamination.[20]

Bacteria

Bacteria are classified as prokaryotic cells as they do not contain a nucleus bounded by a membrane, but instead have a nuclear filament.[24-25]

Bacteria size and forms

Bacteria display a wide variety of shapes and sizes. The lengths of most of the medically important bacterial species range from 0.4 to 2 micrometers and may take the form of spheres, rods or spirals.[7, 26] *Staphylococcus* is spherical in appearance and often aggregates into grape-like clusters,[27] while *Escherichia coli* is rod-shaped.[28]

Gram-staining

Bacteria are also classified as being either Gram-positive or Gram-negative based on the chemical and physical properties of their cell walls, as determined using the Gram staining method.[25] Gram staining ranks among the most useful and important approaches for identifying bacteria. Gram-positive bacteria stain purple, as they are able to retain the crystal violet stain due to the high amount of peptidoglycan in the cell wall. In contrast, Gram-negative bacteria cannot retain the crystal violet stain, instead taking up the

counterstain (safranin) and appearing red or pink. In general, the difference between Gram-positive and Gram-negative bacteria is that Gram-negative bacteria have dense outer membranes and Gram-positive bacteria do not.[25]

Mechanism of action of biocides

The structures of both Gram positive and Gram negative bacteria are shown in Figure 5.[29] All bacteria species have cell walls, cytoplasmic membranes, and cytoplasm, and Gram-negative bacteria have an extra outer membrane made of lipopolysaccharide. Although the structures and compositions of the outer envelopes differ widely, the cytoplasmic membranes are largely the same, with only subtle differences. The rich matrix of balanced interactions between phospholipid and enzymic/structural protein in the cytoplasmic membrane controls its semipermeability properties, intracellular homeostasis, and the transfer of solutes and metabolites in and out of the cytoplasm. The cytoplasm consists of the cell's nucleic acid, ribosomes and various enzymes. Among the cell wall, cytoplasmic membrane, and cytoplasm, the cytoplasmic membrane is thought to be the major attack site for biocides.[30-32]
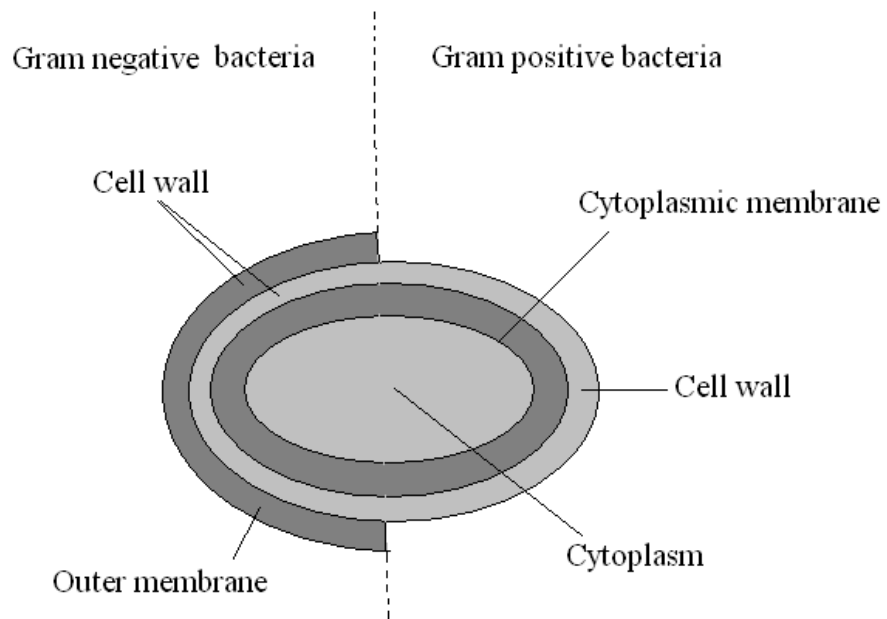
Figure 5. Structures of Gram positive and Gram negative bacteria

The interaction between biocides and microorganisms includes the following steps: the initial binding and accumulation of biocides on the cell surface, the alteration of the outer layer, the penetration of the biocides, and the interaction of biocide and target sites.[2]

Damage caused by the biocide will manifest in various ways,[29] including leakage of intramolecular components such as potassium (K^+), inorganic phosphates, pentoses, nucleic acids and proteins through the disruption of cytoplasmic membranes; lysis; disruption of the proton motive force and inhibition of active transport; inhibition of metabolic mechanisms such as respiration or catabolic/anabolic reactions; disruption of replication; and coagulation of intracellular materials.

CHX shows a bacteriostatic effect at relatively low bacterial concentrations. At higher concentrations, it exerts bactericidal effects very rapidly. Bacterial cells carry

negative charges, so cationic CHG is absorbed onto the phosphate-containing cell walls with an ionic interaction, penetrating the cell wall and being attracted to the cytoplasmic membrane, resulting in the leakage of intracellular compounds. Quaternary ammonium compounds (quats) have a similar biocidal mechanism, while aliphatic alcohols dissolve the phospholipids in the cytoplasmic membrane to disrupt the membrane's integrity and again cause leakage.[29] Phenols, halophenols, and biguanides also cause cell lysis by physical interaction. Weak acids like sorbic and benzoic acid also change the pH balance of cell membranes to acidify the cell interior and disrupt metabolism.[29, 33]

Hypochlorites, organochlorine derivatives, hydrogen peroxide, peracetic acid, organomercurials and heavy metal salts oxidize the thiol groups in proteins, which are very important for the activity of many enzymes. This oxidation of thiol groups leads to cell inhibition or cell inactivation.[34] Hypochlorite and chlorine-releasing agents may also halogenate the amino groups in the proteins to inhibit the microorganism's metabolism.[29]

Glutaraldehyde, *ortho*-phthalaldehyde, and formaldehyde interact with the amino, imino, amide, carboxyl and thiol groups in microorganisms, and have a strong tendency to react and cross-link with primary amines. All of these damage cell walls and inhibit metabolism and replication.[29, 35]

N-halamines

N-halamines have been found to be particularly efficacious because of their nontoxicity to humans and the environment, long-term stability and regenerability, and efficient biocidal activity against a broad spectrum of microorganisms. It is also not possible for organisms to develop a resistance to them because halogen is the active

oxidant.

N-halamine compounds containing nitrogen-halogen bonds are known to be especially effective biocides, and inorganic N-halamines based on inorganic substitute groups are often used. Three typical inorganic N-halamines (N-chlorosulfamic acid, sodium N-chloroimidodisulfonate, and trichloroimidometaphosphate), along with the general chloroamine formula, are shown in Figure 6. These inorganic N-halamines release hypochlorous acid slowly by hydrolysis, and have been substituted for chlorine gas in drinking water systems in some areas due to their long-lasting germicidal properties.[36-37]
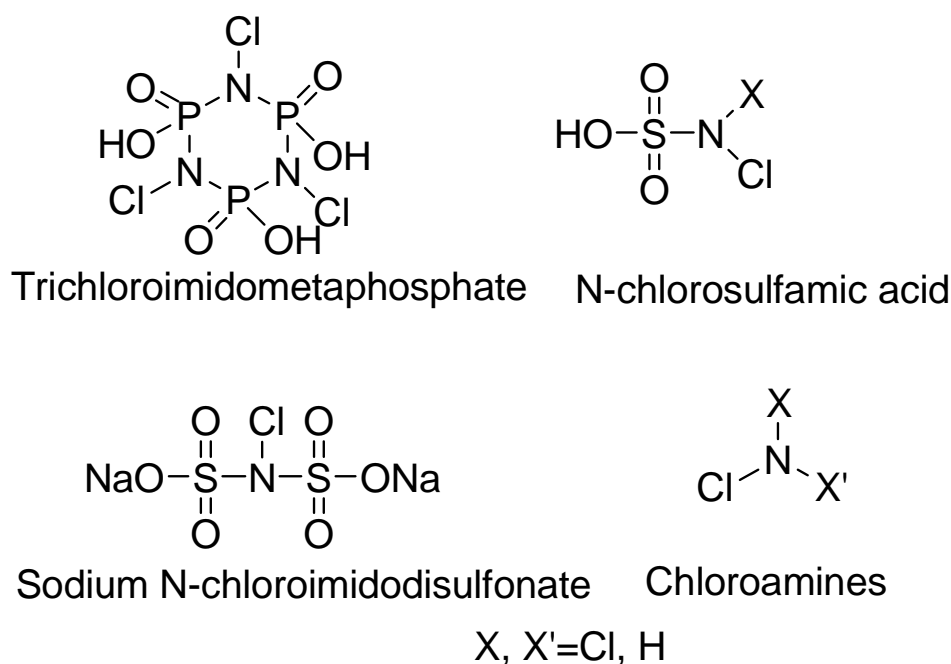
Figure 6. Structures of inorganic N-halamines

Organic N-halamines contain at least one organic substituent group. There are three types of N-Cl moieties, amine, amide, and imide, and the stabilities of their N-Cl bonds

and biocidal efficacy vary. There are two kinds of biocidal mechanisms available: the dissociation of polar N-X in water, generating hypochlorous acid or hypochlorite anion; and the transfer of the oxidative chlorine to the bacteria, which kills the bacteria. The latter mechanism is most effective, as organic N-halamine is generally very stable in solution.[38-40]

The stability of N-Cl bonds

Hydrolysis of N-Cl bonds produces hypochlorite, and the dissociation constants of different N-Cl bonds are shown in Table 1 below.[39]
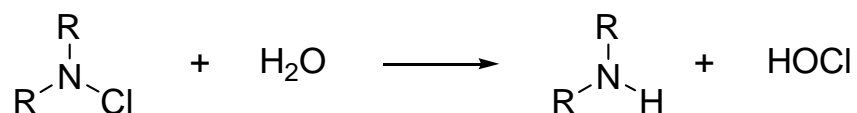
Table 1. The dissociation constants of different N-Cl bonds

N-halamine	Dissociation constant
amine	$<10^{-12}$
amide	$<10^{-9}$
imide	$<10^{-4}$

The nature of the N-Cl moiety determines the order of the inactivation of the bacteria. Imide N-halamine is less stable than amide N-halamine, which is less stable than amine N-halamine, so the killing speed of bacteria of N-halamine is imide> amide>amine.[40-41] The same trend concerning the stability of N-halamine was confirmed by high-level calculations, although it was surprising to find the strongest amine N-halamine bond has the longest bond length.[42] Overall, imide N-halamine bonds have the greatest ionic character and the shortest bond lengths.[42] It has been suggested that the

stability order of the imide and amide moieties could be reversed based on the steric effect of the substituent on the 5 position of a hydantoin ring.[42]

Most stable and applicable N-halamines are cyclic structures that lack an α-hydrogen, because α-hydrogen dehydrohalogenates to produce a stable C-N double bond.[43-44] This process is accelerated by heat and UV-light.[43-44] Cyclic N-halamines are very efficient broad-spectrum biocides with a long shelf life.[43-44] They are stable in aqueous solutions because there is a low concentration of "free chlorine" leached into water. They will not react with organic compounds to produce toxic $CHCl_3$.[44]

$$R-\underset{\underset{\displaystyle }{|}}{\overset{\overset{\displaystyle X}{|}}{N}}-CH_2R' \xrightarrow[\text{-HX}]{\text{heat or UV}} R-N=CHR'$$
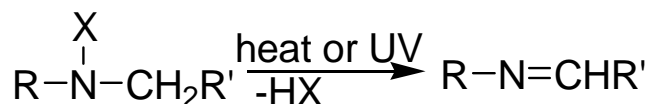
The Worley group has focused on the study and development of N-halamines since the 1980s, and have synthesized numerous N-halamine compounds.[45-48] These include several hydantoin derivatives (**1**), 3-halo-4,4-dimethyl-2-oxazolidione [Agent 1] (**2**), 1,3-dihalotetramethyl-2-imidazolidinone [Agent A] (**3**), and 1,3-dihalo-2,2,5,5-tetramethylimidazolindin-4-one [TMIO derivatives] (**4**),[45-48] all of which have shown excellent biocidal activity and very good stability, both in solution and dry storage.

Other well known N-halamines include 1,3,5-trihalo-6,6-dimethyl-1,3,5-triazinane-2,4-dione (**5**),[44] chlorinated isocyanurates (trichloro and dichloro) (**6**),[49-50] 3-chloro-2,2,4,4-tetramethyloxazolidine (**7**),[43, 51-52] 1,4-dihalo-2,2,5,5-tetramethyl-3,6-piperazinedines (**8**),[43, 51-52] N-halosuccinimides (**9**),[53] halogenated piperidones (**10**),[54] halogenated melamine (**11**),[44] haloglycourils (**12**),[54] and halogenated piperidines (**13**)[53, 55] (bold numbers refer to labels in Figure 7).
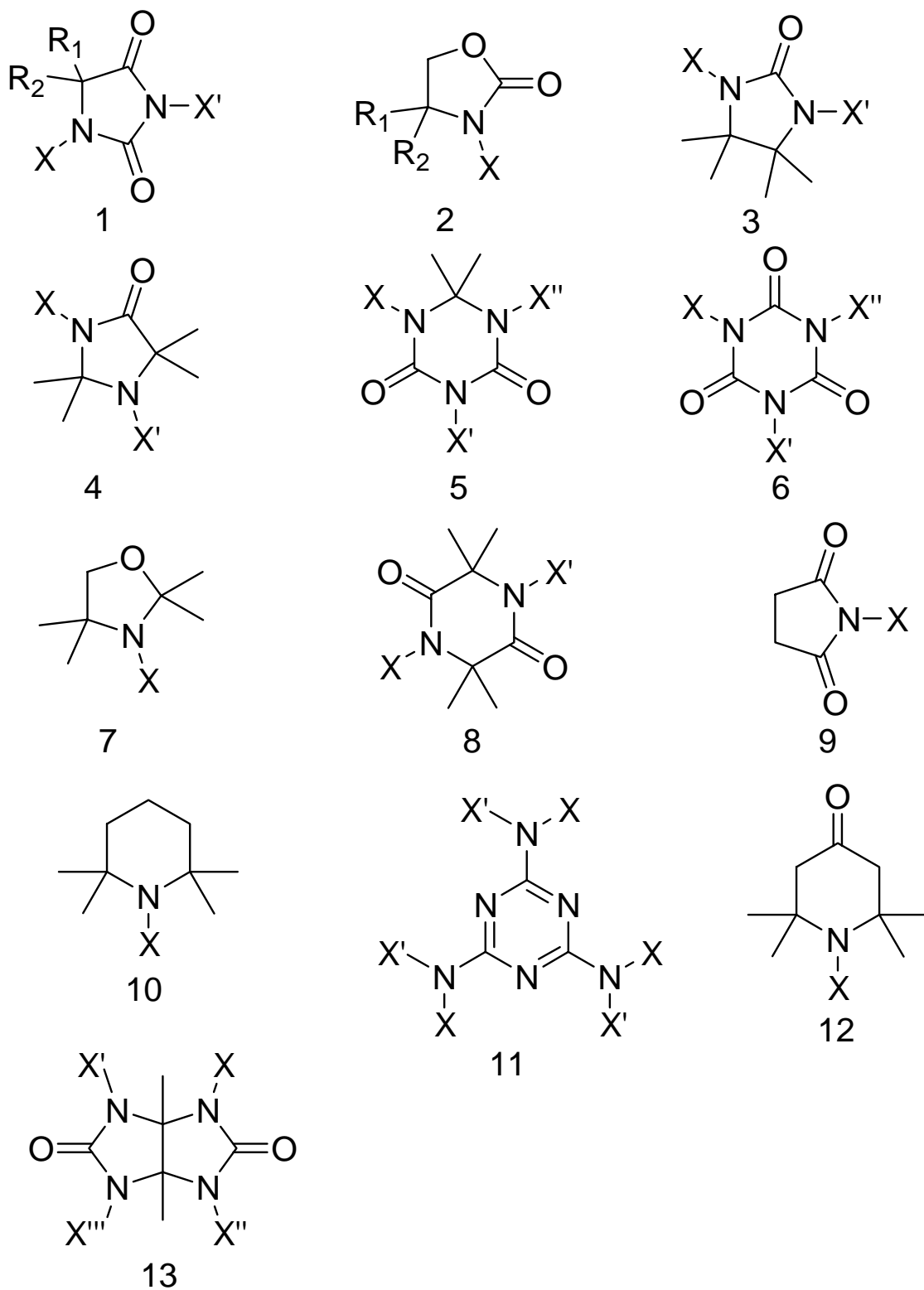
Figure 7. Structures of Organic N-halamines

The formation of a hydantoin ring (Bucherer-Bergs reaction)[56] is commonly is used in our group. The mechanism is shown below (Scheme 1)[56]:
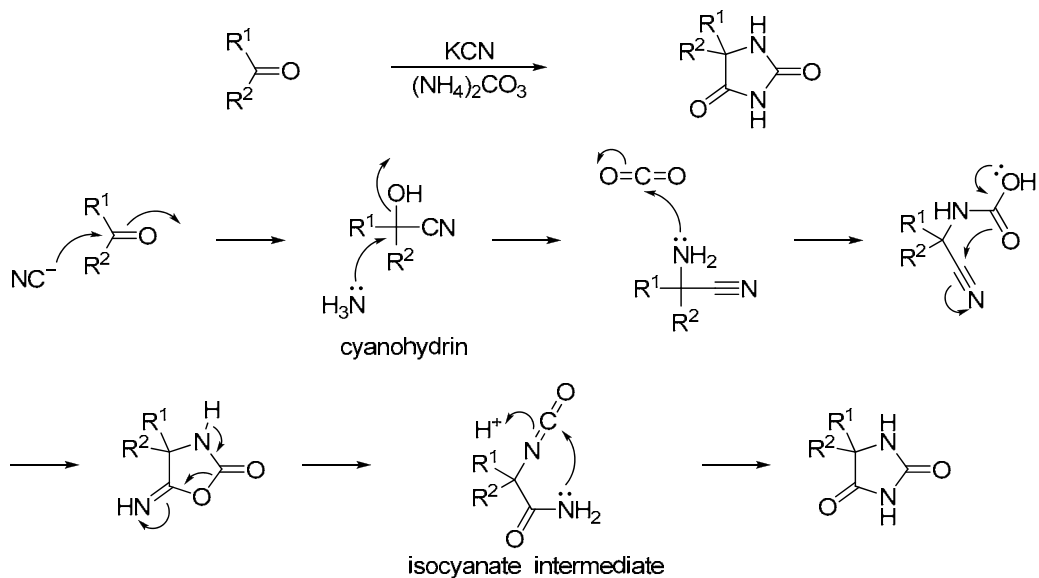
Scheme 1. The mechanism of the Bucherer-Bergs reaction

There are three main approaches used to the manufacture of polymeric biocides. The first is to polymerize the N-halamine monomers, either alone or with other monomers, to produce various antimicrobial polymers.[57-58] The second is to graft or coat N-halamine precursor monomers onto the polymers, or to modify the polymer units by chemical reaction to form N-halamine derivatives (Figure 8).[59-64] The third is to add N-halamine monomers or N-halamine polymers to the host polymers before polymer processing or fiber extrusion, although this can lead to problems with leaching.[65]
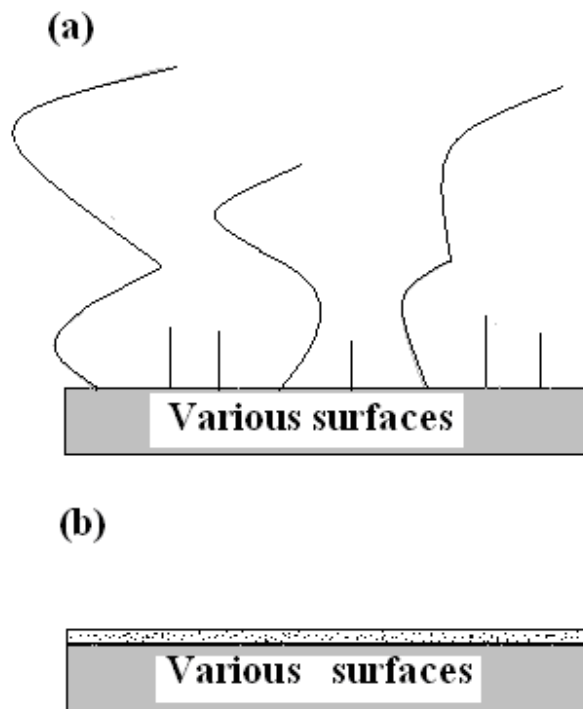
(a) Polymers are grown from or attached to surfaces.

(b) Thin coating of antimicrobial polymers applied to a variety of different materials

Figure 8. Grafting or coating N-halamine precursor monomers onto various surfaces

Biocidal polymer materials

Lambert and coworkers have prepared and studied quaternary ammonium anion-exchange resin-triiodide from the strong base Dowex 1-X8 (Figure 9), which can release on demand enough iodine to be extremely effective in killing both Gram-positive and Gram-negative bacteria, as well as RNA and DNA viruses.[66-67]
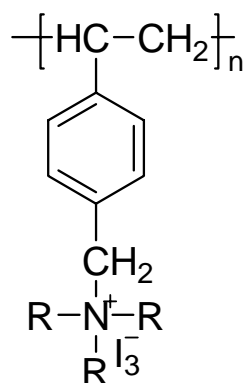
Figure 9. Structure of the Quaternary ammonium anion-exchange resin-triiodide

Sun and Worley developed insoluble polymers poly(1,3-dichloro-5-methyl-5-(4'-vinylphenyl)-hydantoin) (poly I) and poly(1,3,5-trichloro-6-(4'-vinylphenyl)-1,3,5-triazine-2,4-dione) (poly-CTD) (Figure 10).[59-63] Chlorination by bubbling chlorine gas in sodium hydroxide solution renders all of these biocidal. None are soluble in water and release less than 0.1mg/L free chlorine into flowing water. These polymers have been shown to inactivate numerous species of bacteria, fungi, and even rotavirus within a few seconds. Once the oxidative chlorine has been exhausted, it can be replenished by exposure to free chlorine. They have already found many applications in water disinfection, although they suffer from limitations due to problems with uncontrolled particle size and irregular size distribution.[59-63]

Chen and his coworkers used highly cross-linked polystyrene beads with a uniform size to overcome the above limitations.[64] The resulting poly(styrenehydantoin) beads can be chlorinated or brominated, and the chlorine or bromine loading controlled by the pH value of the chlorination/bromination solution. For example, when the pH is below 8, the chlorine loading is 20.0 wt % (dichlorinated), 14 wt % chlorine at pH 8.0-8.5, and 10 wt % chlorine at pH 12.5 (monochlorinated).[68]

Chen also prepared chlorinated methylated polystyrene hydantoin beads (PHY)[69-70], chlorinated methylated polystyrene hydroxymethylhydantoin beads (PMHY)[70], and chlorinated methylated polystyrene imidazolidinone beads (PI)[70] (Figure 10). PHY-Cl contained 6.2 wt % chlorine loading at pH 7.5, which inactivated *S. aureus* and *E. coli* within 2 seconds. PHY-Br contained 8.2% by weight of oxidative bromine (equivalent to 3.6 wt % oxidative chlorine) and inactivated both species of bacteria within 1 second. The N-Br bond is weaker than the N-Cl bond, so bromine is easily transferred to the bacteria cell. However, the stability of the chlorinated N-halamine moiety is better than that of the brominated N-halamine moiety. Chen also compared the biocidal efficacy of PHY-Cl and two polyquat beads **PQ1** and **PQ2** (shown in Figure 10) and reported N-chlorinated methylated polystyrene hydantoin beads to be much more efficient in killing the bacteria in aqueous solution than structurally similar polyquat beads.[69-70]
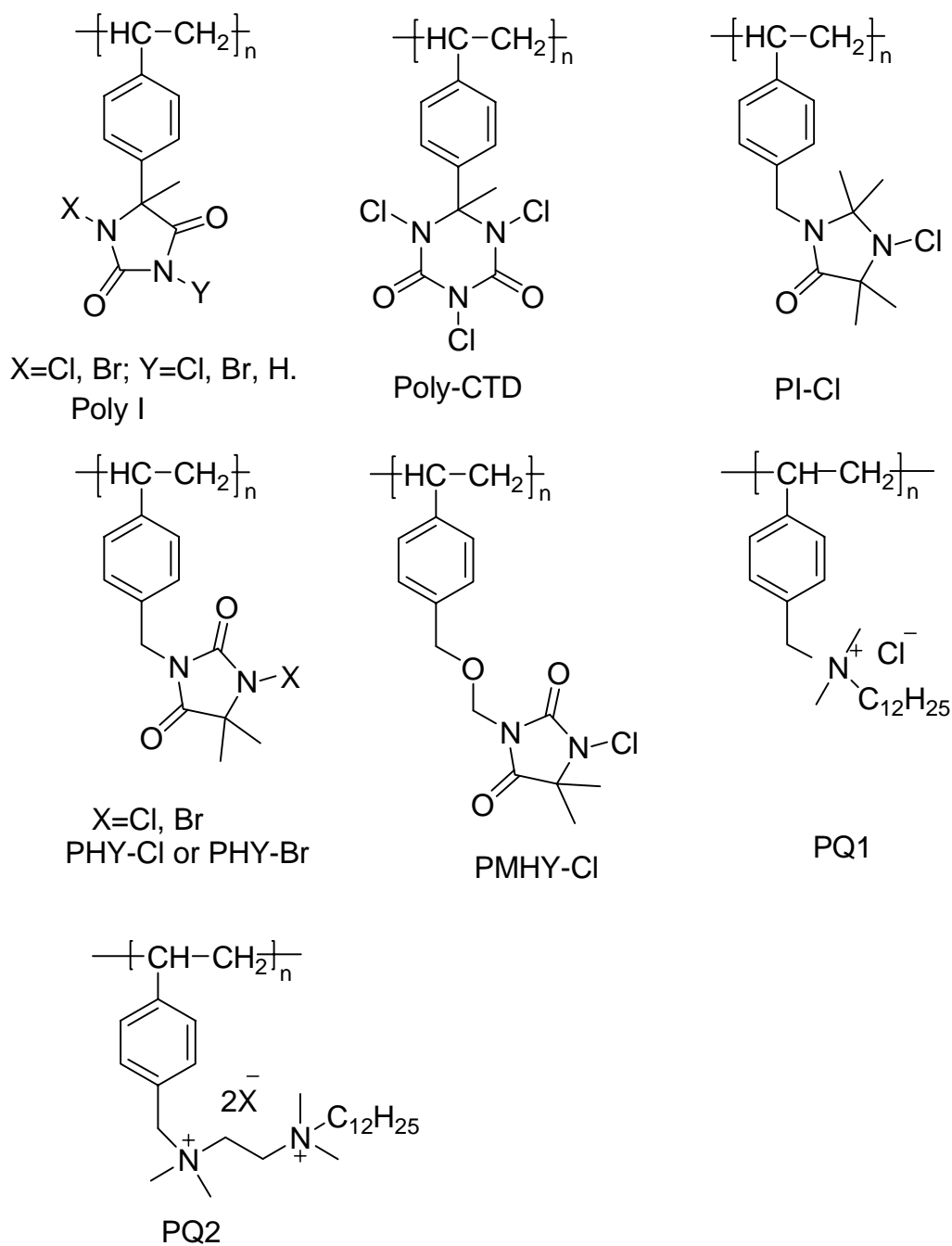
Figure 10. Structures of polystyrene N-halamine beads

Eknoian synthesized 3-halo-4-[[(alkylacryl)oxy]methyl]-4-ethyl-2-oxazolidinones (Figure 11)[57-58, 71]. All of these monomers could also be copolymerized with numerous

commercial monomers such as acrylonitrile and vinyl acetate or grafted onto polymer backbones to produce granular polymers. These granular polymers were tested and confirmed to be efficient biocides. Eknoian noted that the amount of N-halamine should be kept below 5 % if the monomer is to be incorporated into any polymer framework to avoid adversely affecting the polymer performance. For specific applications, it is preferable to graft the monomer to preexisting polymer backbones, which we already charaterized. [57-58, 71]
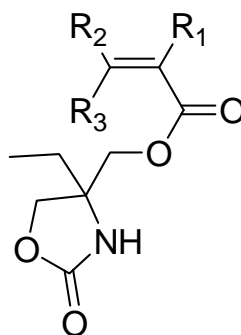
Xa $R_1=R_2=R_3=H$
Xb $R_1=R_3=H$, $R_2=CH_3$
Xc $R_1=R_2=H$, $R_3=CH_3$
Xd $R_1=H$, $R_2=R_3=CH_3$

Figure 11. Structure of 3-halo-4-[[(alkylacryl)oxy]methyl]-4-ethyl-2-oxazolidinones

Eknoian also linked N-halamine moieties to a poly (ethylene glycol) (PEG) backbone to obtain the water soluble polymers **14a** and **14b** (Figure 12). Biocidal tests showed that the PEG-hydantoin polymer achieved a 6-log reduction (99.9999% inactivation) of the bacteria within 10 minutes. [72-73]

14a   14b
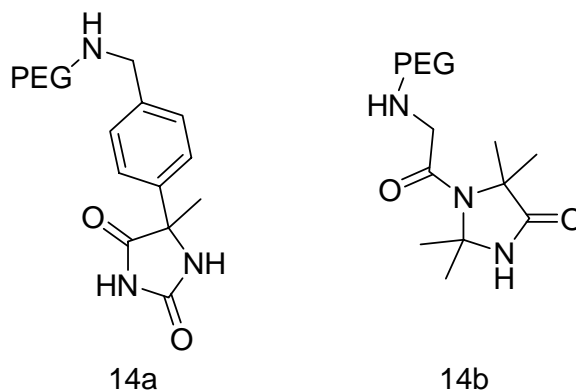
Figure 12. Structures of N-halamine- PEG polymer precursors

Li prepared several acrylic monomers containing the hydantoin moiety (Figure 13), which were then polymerized via radical polymerization initiated by 2, 2'-azobisbutyronitrile in organic solutions at 60°C to obtain solid copolymers. These were shown to be efficient biocides after chlorination.[74]

R=H or CH$_3$
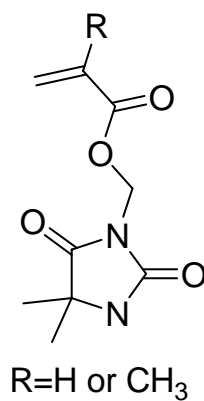
Figure 13. Structure of an acrylic monomer containing the hydantoin moie

Sauvet and coworkers synthesized some biocidal QAS-containing polysiloxanes (Figure 14) by direct quaternization of tertiary amines by chloropropyl or bromopropyl groups attached to polysiloxane chains. These siloxane polymers showed good

bactericidal activity against both gram-negative bacteria such as *Escherichia coli* and gram-positive bacteria such as *Staphylococcus aureus*.[75-77]
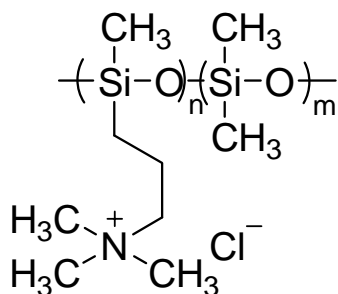
Figure 14. QAS-containing polysiloxane

Tiller and coworkers prepared poly (alkyloxazoline) telechelics with one quaternary N, N-dimethyldodecylammonium (DDA) end group (Figure 15).[77-79]
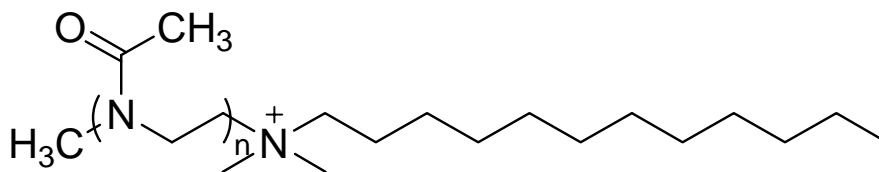
Figure 15. Structure of poly((alkyloxazoline) telechelics with one quaternary N,N-dimethyldodecylammonium end group

Wynne and coworkers prepared polyurethane polymer with 4, 4′-methylenebis(cyclohexyl isocyanate) (HMDI), 1,4-Butanediol (BD), and the terpolymer telechelic P[MOx:Hy4Ox:3FOx-75:20:5] (Figure 16). The monomer, 5, 5-dimethyl-3-(2-((3-methyloxetan-3-yl)methoxy)ethyl)-imidazolidine-2,4-dione (Figure 17) underwent cationic ring-opening copolymerization with two other monomers, 3-(2,2,2-trifluoroethoxy)-3-methyloxetane and 3-methoxymethyl-3-methyloxetane, to form terpolymer telechelic P[MOx:Hy4Ox:3FOx-75:20:5], thus incorporating hydantoin

rings into the telechelic. The pre-biocidal polyurethane polymer was dissolved in THF and then used to produce a thin coating by spreading the solution onto the surface of the substrate and then evaporating the solvent.[80-82] The same group also prepared a telechelic incorporating quaternary ammonium salts (Figure 18), which they then used to create a prebiocidal polyurethane films.[83]
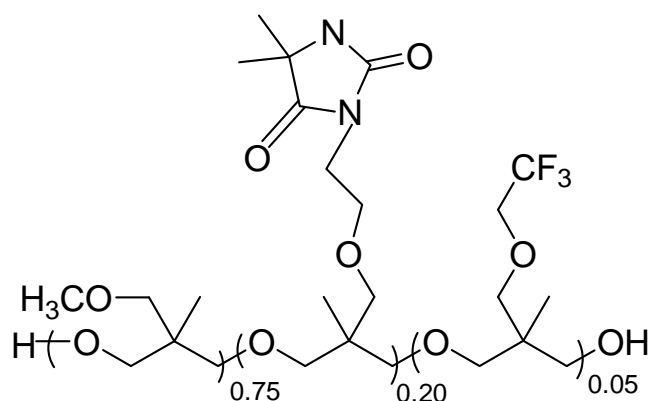
Figure 16. Structure of the terpolymer telechelic P[MOx:Hy4Ox:3FOx-75:20:5]
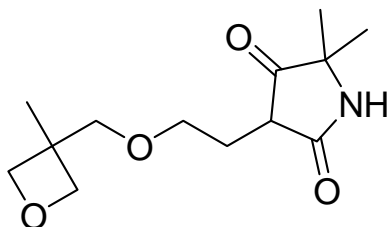
Figure 17. Structure of oxetane incorporating the hydantoin moiety

$$R=OCH_2CF_3 \text{ or } (OCH_2CH_2)_2OCH_3$$
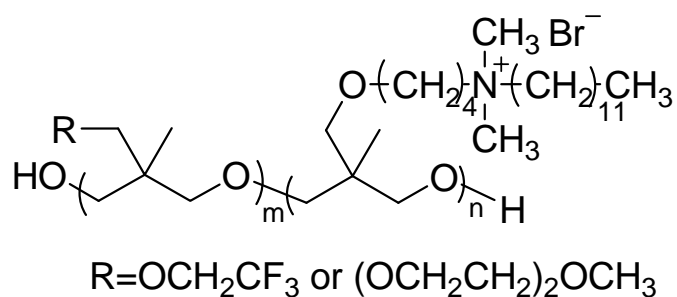
Figure 18 The structure of telechelic incorporating quaternary ammonium

functionalities

Tew and coworkers prepared polyguanidinium oxanorbornene (PGON) (Figure 19) from norbornene monomers via ring-opening metathesis polymerization. This polymer showed strongly antibacterial properties against both Gram-negative and Gram-positive bacteria.[84]
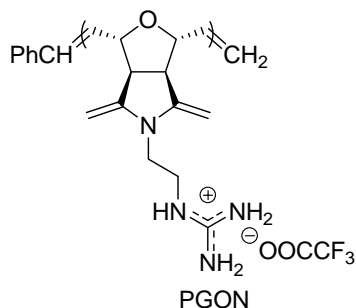
Figure 19. Structure of PGON

Kenawy and coworkers incorporated quaternary ammonium salts or phosphonium salts into polymethacrylate[85] or polyamide[86] backbones (Figures 20 and 21). Of the resulting products, the polymer synthesized with tributyl phosphonium salt was demonstrated to be the most effective against several species of bacteria.
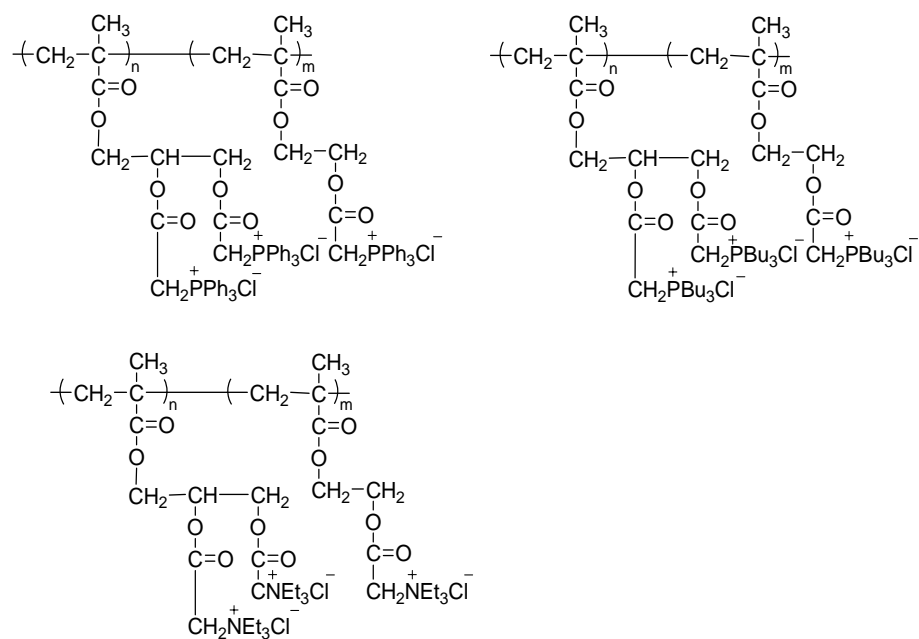
Figure 20. Polymethacrylate backbone incorporating quaternary ammonium or
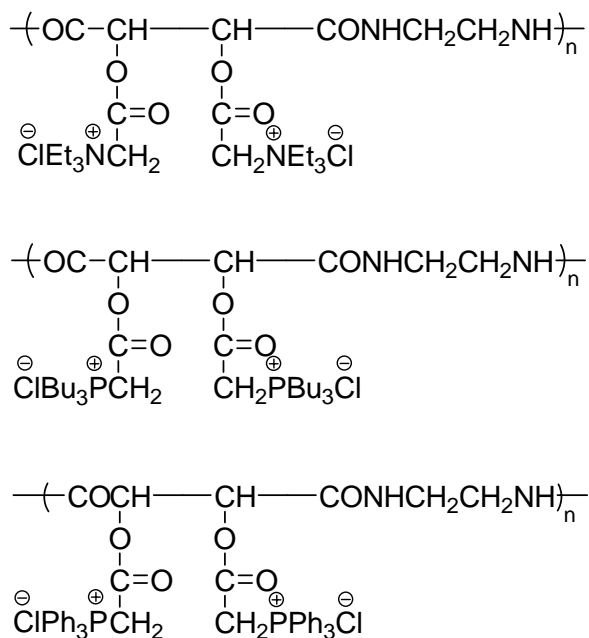
phosphonium salts

Figure 21. Polyamide backbone incorporating quaternary ammonium or phosphonium

salts

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Biocidal surface coating and films

Research into antimicrobial surface coatings and films has been an active field for many years due to increasing concerns about the hazards of bacterial contamination from potential exposure.[87-89] The biocidal coatings and films developed have been applied on a wide range of surfaces, including walls, floors, medical devices, health care products, hospital equipment, food packaging, and food storage facilities. They are used to inhibit bacterial growth in health care or food service centers, and control odors and mildew.

As mentioned earlier, Eknoian made several copolymers by polymerizing 4-[[(alkylacryl)oxy]methyl]-4-ethyl-2-oxazolidinones with other monomers and then coating the resulting polymers onto various surfaces by dissolving the polymers in organic solvents, applying these solutions to the surfaces and then heating to remove the solvents.[58, 71] Seeking to avoid the use of organic solvents, Eknoian went on to utilize emulsion polymerizations of oxazolidione monomers, either with commercial monomers or grafted onto a variety of polymer backbones in water, and adding surfactants to disperse the polymer particles in water. The resulting solution could then be used directly for coating. The clear coatings produced effectively inactivated both Gram-positive and Gram-negative bacterial.[57-58,71]

Sun and coworkers prepared two novel cyclic-amine monomers, 3-allyl-5,5-dimethylhydantoin (ADMH) and 7,8-benzo-3-allyl-1,3-diazasprio[4.5]decane-2,4-dione (BADDD) (Figure 22), which were copolymerized with acrylonitrile (AN), vinyl acetate (VAC), and methyl methacrylate (MMA). Although there were difficulties in preparing ADMH and BADDD homopolymers, the halogenated polymers and films (only VAC copolymer would be made into film) showed good

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biocidal activity, good stability and regenerability.[90]
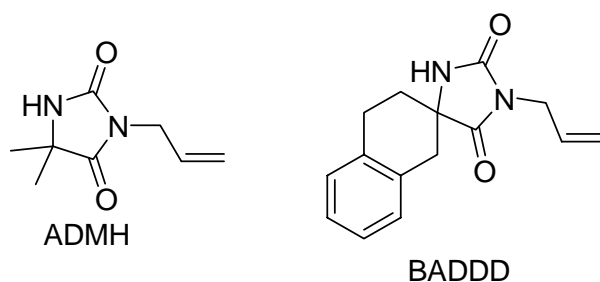
Figure 22. Structure of ADMH and BADDD

Sun and coworkers also prepared 3-(4'-vinylbenzyl)-5,5-dimethylhydantoin (VBDMH) (Figure 23) which can easily polymerize either with itself or with vinyl acetate, acrylonitrile, and methyl methacrylate.[91] He synthesized 1-acryloyl-2,2,5,5-tetramethylimidazolidin-4-one (ACTMIO) (Figure 23), and its copolymers with acrylic and vinyl monomers were readily prepared under mild conditions. These copolymers were then cast into polymer films, all of which proved to be stable and efficient biocidal materials.[91]

The same group grafted monomer ADMH and ACTMIO onto various textile materials such as nylon, polyester (PET), polypropylene, polyester/cotton blends (PET/cotton) and pure cotton print cloth with the presence of crosslinker triallyl-1,3,5-triazine-2,4,6 (1H,3H,5H)-trione (TATAT)[92]. The group drew particular attention to the influence of the hydrophilic and hydrophobic properties of the fabric on the resulting antibacterial activity. They reported that cellulose, nylon, and PET/cotton provided faster inactivation due to their more hydrophilic properties and suggested that more hydrophobic fabrics prevented good contact between the aqueous suspension of bacteria and the surface of the fiber polymers.[92]
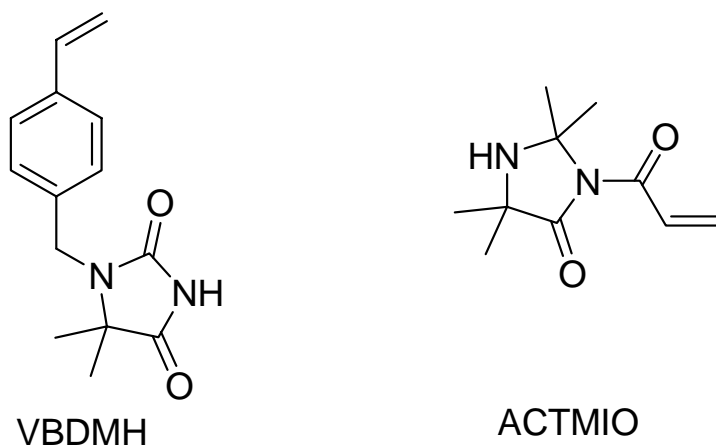
VBDMH

ACTMIO

Figure 23. Structure of VBDMH and ACTMIO

Qian and Sun grafted 3-methylol-2,2,5,5-tetramethylimidazolidin-4-one (MTMIO)[39] and 1,3-Dimethylol-5,5-dimethylhydantoin (DMDMH)[93] (Figure 24) onto cellulose-containing fabric, both separately and in some mixed ratios. MTMIO inactivated bacteria at a relatively slower speed that DMDMH because amine halamine N-Cl bonds are more stable than amide N-Cl bonds. However, MTMIO showed better stability than DMDMH during the washing tests. A combination of DMDMH and MTMIO brings amine, amide, and imide halamine structures together on cellulose, which can improve both the power and the stability of the biocidal functions of treated cellulose-containing fabrics.
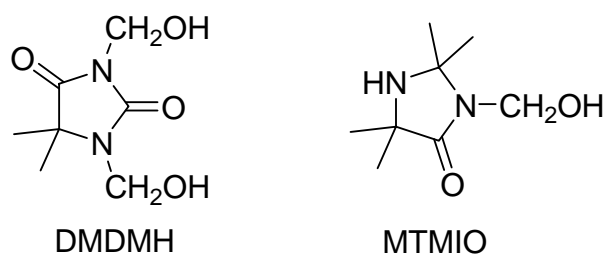
DMDMH

MTMIO

Figure 24. Structures of DMDMH and MTMIO

Sun and coworkers modified polyamide (nylon 66) by reacting carboxylic end groups with biocidal quaternary ammonium salts such as cetylpyridinium chloride (CPC) and benzyldimethylhexadecyl ammonium chloride (BDHAC) (Figure 25). Because of the lack of reactive groups or sites on the polyamide chain, chemical treatment was needed for the modification. Under basic conditions, the carboxylic ends on the polyamide turned into anionic carboxylate. The ionic reaction between carboxylic end groups and cationic quaternary ammonium salt facilitates the development of durable antimicrobial functions.[94]

Cetylpyridinium chloride(CPC)

Benzyldimethylhexadecyl ammonium chloride (BDHAC)
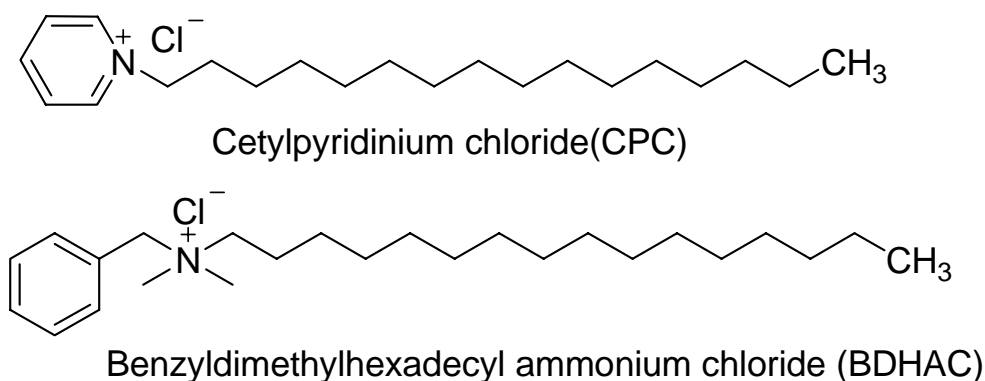
Figure 25. Structures of quaternary ammonium salts

Alkoxy silane is a very useful surface coupling agent with OH groups such as cellulose, and several precursor N-halamine moieties have been successfully attached to siloxanes.[95-98, 99-103, 105-107] For example, Both 5,5-dimethyl-3-(3'-triethoxysilylpropyl) hydantoin (BA-1)[99-103, 105-106] and 3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8 -triazaspiro[4.5]decane-2,4-dione (TTDD) (Figure 26)[104] are now being employed in industrial settings. N-chlorohydantoinyl siloxanes are prolific N-halamine compounds that can be applied to a variety of surfaces, including cellulose,[98-103] PET, [107] silica gel,[99,]

BA-1

TTDD Siloxane

BA-1 derivatives

$R_1=CH_3, R_2=C_3H_7$
$R_1=CH_3, R_2=C_5H_{11}$
$R_1=CH_3, R_2=C_6H_{13}$
$R_1=CH_3, R_2=C_7H_{15}$
$R_1=CH_3, R_2=C_6H_5$
$R_1=CH_3, R_2=p-CH_3C_6H_4$
$R_1=CH_3, R_2=p-t-ButylC_6H_4$
$R_1=C_6H_5, R_2=C_6H_5$

TMIO-Siloxane

Ham-Sil
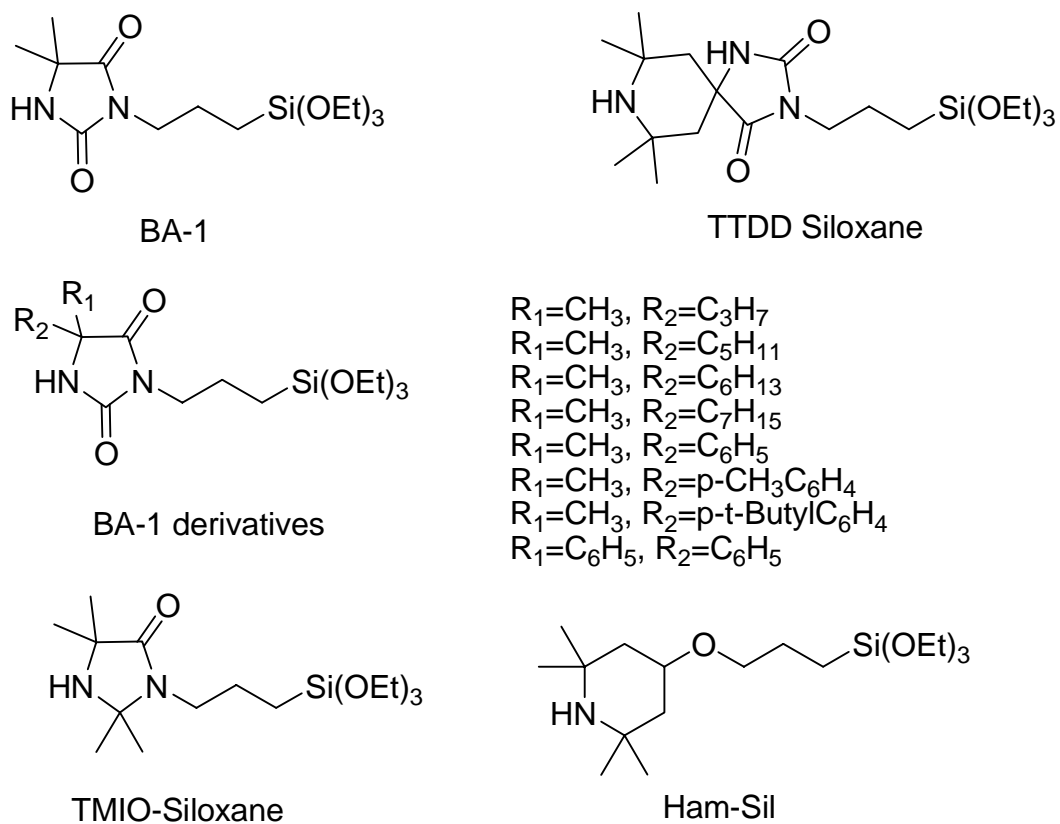
Figure 26. Structures of N-halamine siloxanes

BA-1 derivatives

OH-containing surface

R = hydantoin ring
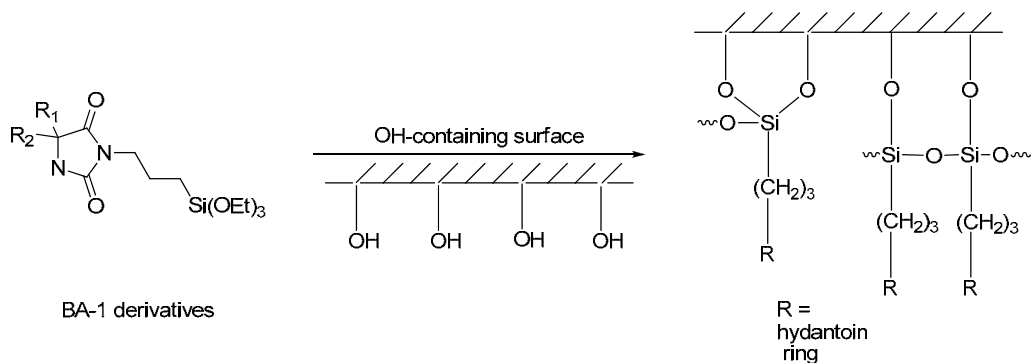
Scheme 2. Coating hydantoinyl siloxane onto OH-containing surfaces

Epoxides

Another useful series of N-halamines for biocidal surface coating is composed of hydantoinylepoxide derivatives, which can be synthesized by reacting the sodium salts of the appropriate hydantoins with equimolar concentrations of commercial epichlorohydrin at room temperature for 6–10 h in aqueous solution.[108-109] This aqueous solution can be used directly as a coating solution. Hydantoinyl epoxide can be bonded to a cotton substrate via tether groups (Scheme 3).

$R_1=CH_3$, $R_2=CH_3$; $R_1=CH_3$, $R_2=C_3H_7$; $R_1=CH_3$, $R_2=C_6H_{13}$;
$R_1=CH_3$, $R_2=C_6H_5$; R_1, R_2=Pentamethylene
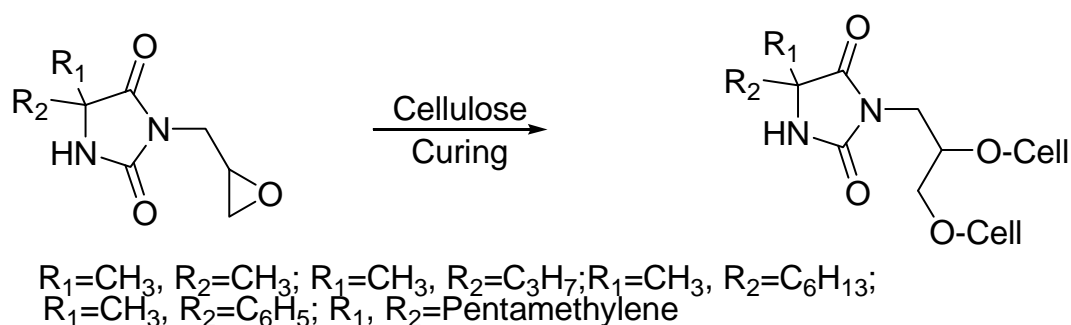
Scheme 3. Coating of hydantoinyl epoxide onto the cellulose

Polyurethane films

Li[110] prepared a new N-halamine-containing diol, 5,5-dimethyl-3-(N, N-di-β-hydroxyl-ethyl-aminomethyl)hydantoin (Figure 27) and polymerized the new hydantoin diol with commercial water-borne acrylic polyol and diisocyanate to form a polyurethane on various surfaces, all of which then showed good biocidal activity.[110] However, Li later found this compound is not stable over a long-term storage.[97]
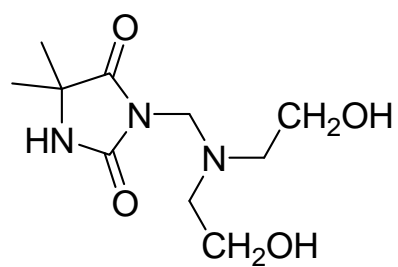
Figure 27. Structure of a novel N-halamine-containing diol precursor

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Chapter 2

Synthesis of a Water Soluble Siloxane Copolymer and Its Application for Antimicrobial Coatings

Introduction

N-chlorohydantoinyl siloxanes can be employed either as monomers or as oligomers. However, in neither form can they be dissolved appreciably in water, so an ethanol/water mixture must be utilized as a coating bath. This poses obvious drawbacks for its use in an industrial setting, as an aqueous bath is always preferred over one containing an organic solvent. Previous work by our group has extended the technology to develop a siloxane copolymer incorporating both an N-halamine and a quaternary ammonium functionality.[1] The presence of the dimethyldodecyl ammonium quat functional group renders the siloxane copolymer soluble in water, but when the hydantoin:quat ratio is 3:1, the solubility is as low as 3%. Biocidal efficacies show that quat functional units do little to improve the disinfection capability, hence the primary function of the quat units is to improve the solubility of the copolymer in water.[1]

The current work focuses on the preparation of a less expensive hydantoinyl quat siloxane copolymer that is more soluble in water and can be deposited onto the surface of cellulose and rendered biocidal upon chlorination. Solubility, stability, rechargeability,

and biocidal efficacies were therefore evaluated for the new copolymer coating material (Scheme 4).
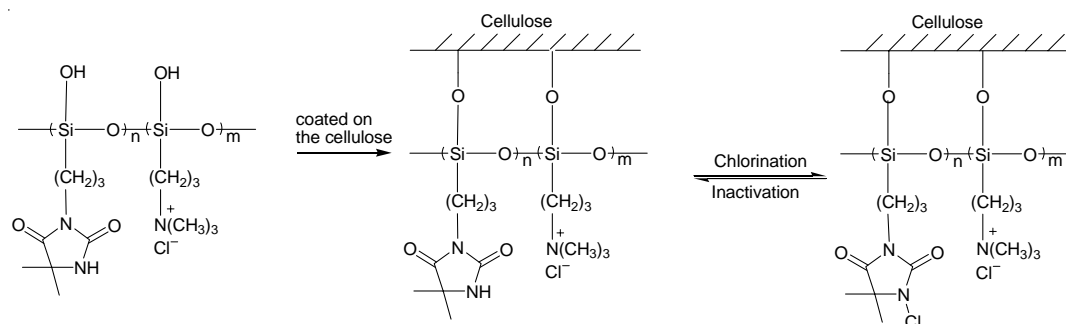
Scheme 4. The new copolymer coating material

Experimental

Preparation of Poly[3-(5,5-dimethylhydantoinylpropyl)siloxane-co-trimethyl ammoniumpropylsiloxane chloride] (PHQS). The starting material poly(3-chloropropylsiloxane) (PCPS) was synthesized by mixing 0.1 mol 3-chloropropyltriethoxysilane (Aldrich Chemical Company, Milwaukee, WI) and 80 mL 0.1 N HCl and stirring the mixture at room temperature for 2 h and then removing H_2O by evaporation in vaccum.

PHQS copolymers were prepared by both two-step processes and a one-step process. In a two-step process (Scheme 5), the approximate amount of hydantoin salt and PCPS reacted to produce a desired value of coefficient n in the first step, and then an excess amount of trimethylamine was added to react with the first step product to give a final product with the desired m value. For example, to produce a PHQS with the ratio of hydantion: quat (n: m)= 9:1, PCPS (13.86 g, 0.10 mol) was mixed with the potassium salt

of 5,5-dimethylhydantoin (14.96 g, 0.09 mol) in 100 mL DMF. After stirring the

mixture at 100 $^{\circ}$C for 5 h, and removing KCl (6.52 g) precipitate by filtration,

trimethylamine (8 mL, 40 wt% solution in water) was added, and the reaction mixture

was stirred at room temperature for 24 h and then at 60 $^{\circ}$C for 24 h. After removing

most of the DMF by evaporation in vacuum, the reaction residue was washed with CHCl$_3$

or CH$_2$Cl$_2$ to remove trace DMF to produce a white solid product (the overall yield by

weight was 87%).
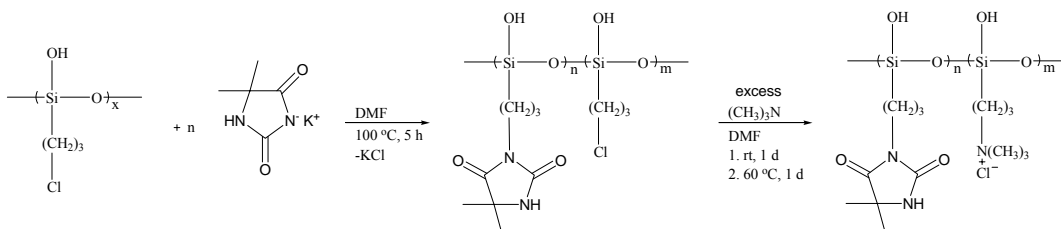
Scheme 5. Preparation of PHQS by a two-step process; n and m are the number of

repeating units of hydantoin and quat, respectively.

In the other two-step reaction, PCPS was reacted with trimethylamine first and then with

the hydantoin salt (Scheme 6).   The overall yield by weight was 80%.
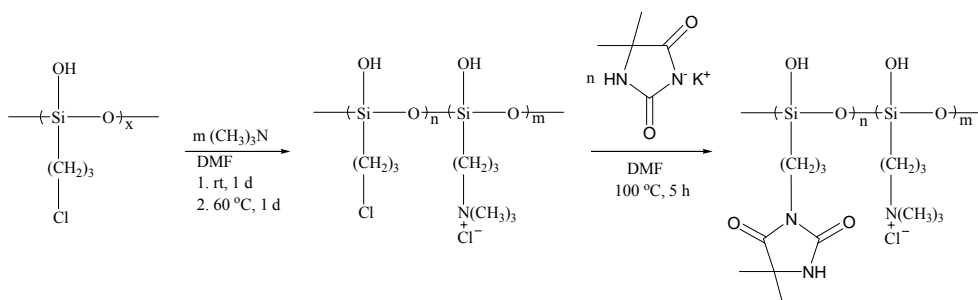
Scheme 6. Preparation of PHQS by a second two-step process.

In a one-step process (Scheme 7), PCPS, the potassium salt of 5,5-dimethylhydantoin,

and trimethylamine were simply mixed in a molar ratio of 0.1:0.09:0.01. The reaction was carried out at room temperature for 24 h and then at 60 °C for 24 additional hours, followed by heating at 100 °C for 5 h. The overall yield by weight was 78.5%.
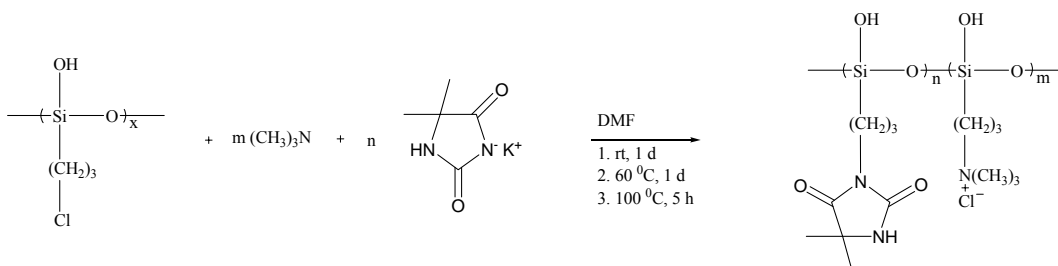
Scheme 7. Preparation of PHQS by a one-step process.

The above three methods gave very similar products of PHQS with the ratio of hydantion: quat (n: m) = 9:1 as determined by ^{1}H NMR and IR. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.09-3.34 (29H), 1.54-1.70 (20H), 1.25-1.26 (54H), 0.53 (20H) ppm; IR 689, 749, 771, 1091, 1217, 1279, 1350, 1420, 1448, 1693, 1763, 2885, 2935, 2976, 3000-3700 cm^{-1}.

Preparation of Homopolymer PQS. PCPS (4.16 g, 0.030 mol), trimethylamine (6 mL, 40 wt% aqueous solution), and 30 mL DMF were combined, and the reaction mixture was stirred at room temperature for 24 h and then at 60 °C for 24 h. After removing most of DMF by evaporation in vacuum, the reaction residue was washed with CHCl$_3$ or CH$_2$Cl$_2$ to remove trace of DMF to yield a white solid product (the yield was 90 %) (Scheme 8). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.39-3.51 (2H), 3.17 (9H), 2.10 (2H), 0.57 (2H) ppm; IR 908, 950, 968, 1480, 2892, 2951, 3000-3700 cm^{-1}.

The chemical scheme shows the reaction:

Left structure: a silicon-oxygen backbone with OH group on Si, and a $(CH_2)_3$ chain terminating in Cl, repeat unit x: $+Si-O+_x$

$+\ m\ (CH_3)_3N$

Conditions over arrow: DMF; 1. rt, 1 d; 2. 60 °C, 1 d

Right structure: silicon-oxygen backbone with OH on Si, $(CH_2)_3$ chain terminating in $N(CH_3)_3^+ Cl^-$, repeat unit m: $+Si-O+_m$
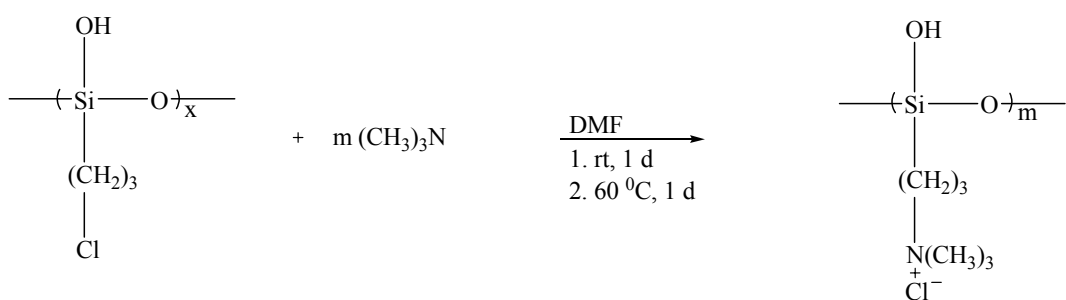
Scheme 8. Preparation of PQS.

Coating Procedure. Swatches (390 cm^2) of 100% desized, scoured, and bleached cotton fabric (purchased from Testfabrics, Inc., West Pittston, PA) were soaked in baths containing 0.5 - 10% copolymer aqueous solution for 15 min. The cotton swatches were dried at 95 °C for 1 h and cured at 145 °C for 20 min. Then the treated swatches were washed with 0.5% detergent solution for 15 min, rinsed with water several times to remove any weakly bonded compounds, and dried at 45 °C for 1 h. The amount of polymer in the coating was estimated by analytic titration following chlorination. This would represent a minimum value since some amide nitrogen might not be chlorinated.

Chlorination Procedure. The coated cotton swatches were chlorinated with 10% commercial household bleach solution (NaOCl) at pH 7 for 1 h, rinsed with tap water, and then with distilled water thoroughly to remove free chlorine. The chlorinated cotton swatches were dried at 45 °C for 1 h.

Analytical Procedure. A modified iodometric/thiosulfate titration procedure was employed to determine the oxidative chlorine loading of PHQS in which 90 mL of ethanol and 10 mL of 0.1 N acetic acid were used instead of distilled water only. This was done to expedite the removal of oxidative chlorine in the procedure. The bound chlorine

loading was calculated by the following equation:

$$Cl^+\% = \frac{0.0375 \times V \times 35.45}{2 \times W} \times 100 \qquad (1)$$

where 0.0375 eqv/L is the normality of the $Na_2S_2O_3$, V is the volume (L) of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in grams of the cotton swatch samples.

A modified ion association titration procedure was used to determine the weight percentage of quat functional group on the copolymer.[1-2] 0.020-0.050 g PHQS or PQS was dissolved in 50 mL 0.05 N acetic acid solution. Three drops 0.5% bromophenol blue/ethanol indicator were added, and the solution was titrated with 0.01 N sodium tetraphenylborate until a light yellow color appeared at the end point. The weight of quat functional group of copolymer was determined from the equation below:

$$quat\% = \frac{0.01 \times V \times M}{W} \times 100 \qquad (2)$$

where 0.01 N is the normality of sodium tetraphenylborate solution, V is the volume (L) consumed of sodium tetraphenylborate solution, and M is the molecular weight of the repeating unit of quat functional group, and W is the weight of the PHQS or PQS sample. The method of ion associate titration is based on the solvent extraction of R_4N^+ with an ion associate reagent.[2] Bromophenol was used here as an indicatior.

$$\text{HBP} \rightleftharpoons \overset{+}{\text{H}} \ + \ \overset{-}{\text{BP}}$$
yellow　　　　　　　blue

$$\overset{+}{\text{R}_4\text{N}} + \ \overset{-}{\text{BP}} \longrightarrow (\text{R}_4\overset{+}{\text{N}})\,(\overset{-}{\text{BP}})$$
　　　　blue　　　　　　blue
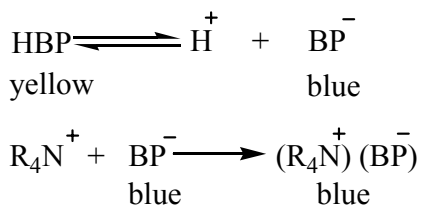
The color change for R_4N^+ at the equivalent point by adding STPB⁻(titrant)

$$\overset{-}{\text{STPB}} \ + \ \overset{+}{\text{H}} \ + \ (\text{R}_4\overset{+}{\text{N}})\,(\overset{-}{\text{BP}}) \longrightarrow (\text{STP}\overset{-}{\text{B}})\,(\text{R}_4\overset{+}{\text{N}}) \ + \text{HBP}$$
　　　　　　　　　blue　　　　　　　　　colorless　　　yellow
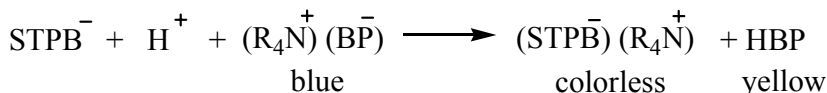
Biocidal Efficacy Testing.　Cotton swatches were challenged with Gram-positive *Staphylococcus aureus* ATCC 6538 and Gram-negative *Escherichia coli* O157:H7 ATCC 43895 for antimicrobial efficacy analysis using a "sandwich test" (see below). Untreated (control I), treated but unchlorinated (control II), and chlorinated samples were used in this study. A 25 μL bacterial suspension was placed in the center of a pair of 6.45 cm square cotton swatches held in place by a sterile weight to insure good contact of the swatches. After contact times of 1, 5.0, 15.0, and 30.0 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution in 50 mL sterile conical centrifuge tubes to remove all oxidative chlorine and then vortexed for 2 min vigorously. Serial dilutions of the quenched solutions were made using pH 7, 100 μM phosphate buffer, and these were plated on Trypticase soy agar plates. The plates were incubated at 37 °C for 24 h, and viable bacterial colonies were counted for biocidal analysis.

Washing Testing.　Laundering tests were performed to evaluate the stability and rechargeability of chlorine on the fabric samples by applying American Association of Textile Chemists and Colorist (AATCC) Test Method 61. 19.35 cm square cotton swatches were washed for the equivalents of 5, 10, 25, and 50 washing cycles. The

chlorine loadings on the samples before and after washing were determined by titration; in some cases samples were recharged for analytical oxidative chlorine determination.

Results and Discussion

    Characteriazation of PHQS and PQS.   The quat siloxane homopolymer displayed a broad signal at 0.56 ppm which can be assigned to the protons on the CH_2 group bonded to Si.   The protons on the CH_3 and CH_2 groups of the quarternary ammonium salt gave broad bands at 3.16 and 3.42 ppm, respectively, at relatively lower field.   The third CH_2 groups exhibited a ^1H NMR signal at 1.85 ppm.   N-halamine/quat siloxane copolymers (n:m = 5:1 and 9:1) provided similar signals to the quat siloxane polymer due to the overlap of CH_2 group proton signals for the two repeating units and an extra signal for the two methyl group protons bonded to the hydantoin rings at ca. 1.25 ppm (Figure 28).

a. Quat siloxane polymer

b. Hydantoinyl/quat siloxane copolymer (5:1)

c. Hydantoinyl/quat siloxane copolymer (9:1)
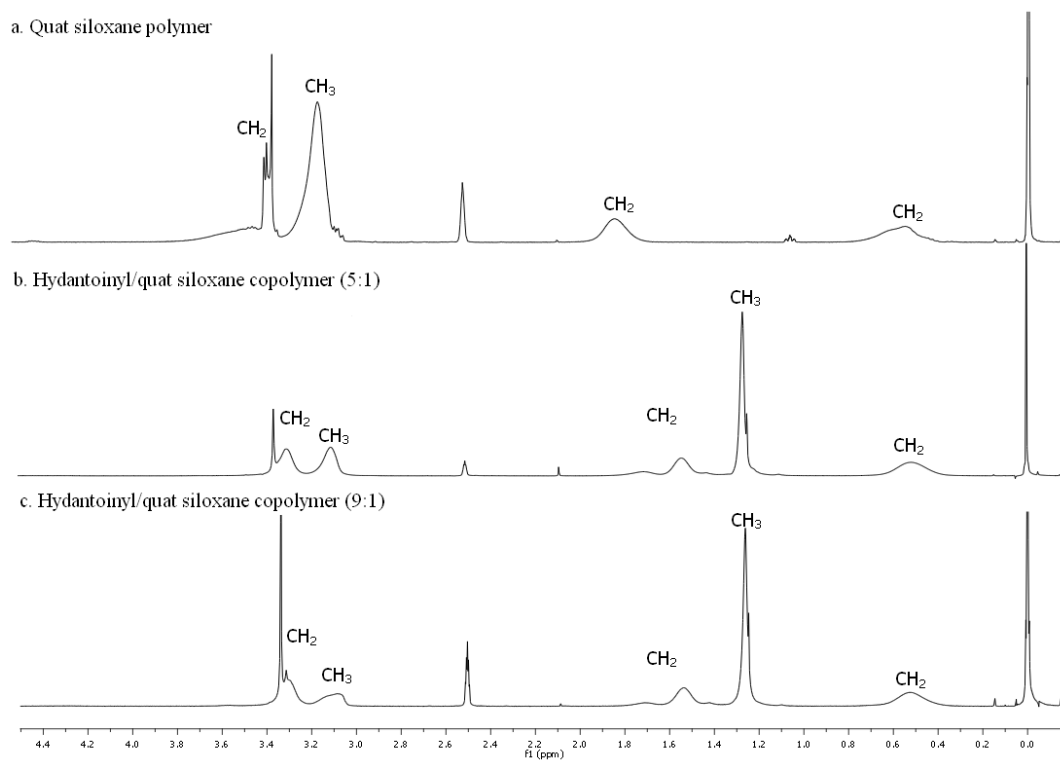
Figure 28. *¹H NMR spectra of quat siloxane homopolymer and hydantoinyl/quat siloxane copolymers.*

In Figure 29, for the quat siloxane homopolymer, the CH IR stretching modes of the CH_2 groups bonded to the ammonium functionality was observed at 2935 and 2892 cm^{-1}. The CH IR stretching mode of the CH_3 groups of the quat functionality was observed at 2976 cm^{-1}. The 1480 cm^{-1} band can be assigned to vibrational modes corresponding to the deformation of CH_2 and CH_3 groups of the quaternary amine. There was a band observed at 907-911 cm^{-1} due to the asymmetric C-N stretching mode of the quat functionality.[3] For hydantoinyl/quat siloxane copolymers 1:1 and 9:1, two extra bands at 1763 and 1692-1696 cm^{-1} indicate the presence of imide and amide groups of the hydantoin ring. The N-Cl stretching vibrational mode band which should occur in the 600-800 cm^{-1}

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region could not be identified due to the many overlapping bands in that region.
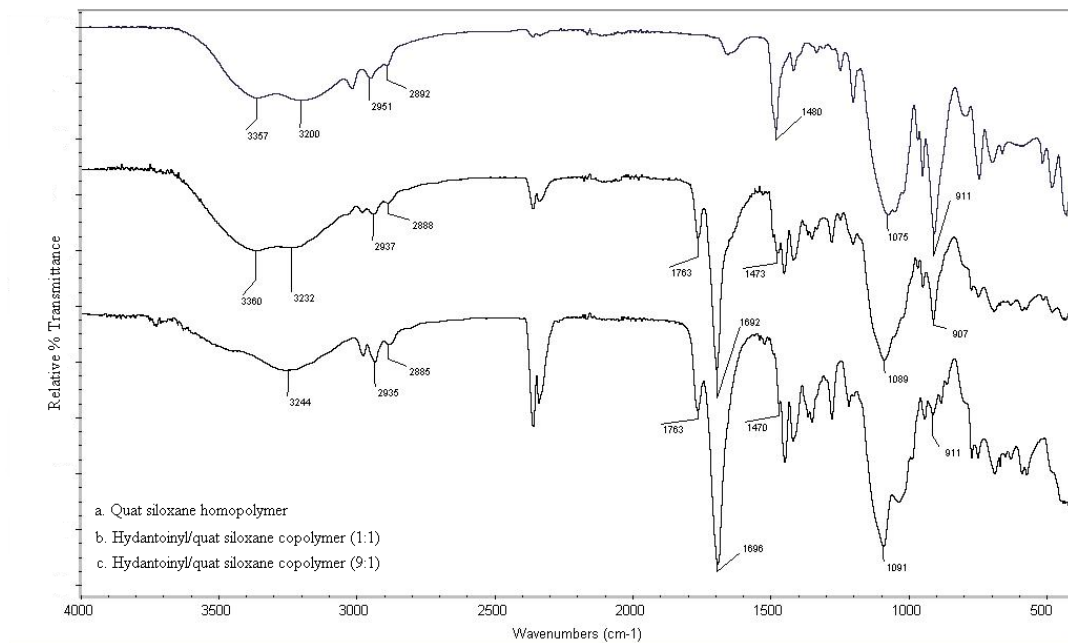
Figure 29.   IR spectra of quat siloxane homopolymer and hydantoinyl/quat siloxane

copolymers.

The percentage of quat functional group was also estimated by a modified ion association titration method.   The presence of a quat functionality caused bromophenol blue/ethanol solution to become blue in color.   The data obtained from this method is compared with the predicted weight percent of quat functional group from mixing ratios in Table 2.

Table 2.   Determination of Quat Moiety Portion by Ion Assoiciation Titration

Hydantoin:Quat	Quat%	
n:m	Determined by titration	Predicted Theoretical
1:1	42.3	46.2
3:1	28.4	22.3
5:1	10.1	8.71
9:1	16.4	14.7
0:100	89.0	89.4

It should be noted that triethylamine was also used to attempt to prepare hydantoinyl/quat siloxane copolymer, but triethylamine did not react with the polymer chain appreciably probably due to steric hindrance of the three ethyl groups.

Solubility Test Results.   Polyhydantoinyl siloxane (PHS) can only be dissolved in water and ethanol mixtures.   Water is a more economical and environmentally friendly solvent than any organic solvent.   Much effort has been made to enhance the solubility of polyhydantoinyl siloxane in water so that use of an organic solvent can be avoided. In the previous work done by Liang and his coworkers, dimethyldodecylamine was used to prepare hydantoinyl/quat copolymer to improve solubility in water and possibly provide additional biocidal activity at the same time.[1] The solubility of PHQS (3:1) in that work was around 3% .[1] Greater solubility of PHQS in water found in this work is shown in Table 3.   With the higher n value, the solubility decreased.   For ratios of 1:1 and 3:1 PHQS, the solubility was 80% and 70%, respectively; even for a 9:1 mole ratio of

hydantoin: quat, the solubility was still 15%, which is sufficient for an industrial application.

Table 3.   Solubility of PHQS (wt%) with different n:m ratios in water

n:m ratio	Solubility
1:1	80%
3:1	70%
5:1	45%
9:1	15%

The Effect of dfifferent ratios of n/m (9:1, 5:1, 3:1 and 1:1) on the Chlorine Loading.   In this experiment, 12.90 cm^2 cotton swatches were soaked in baths containing 0.1 g PHQS.   From Table 4 it can be seen that the chlorine loading of the cotton swatches coated with 1:1 and 3:1 mole ratios of hydantion: quat are independent of the concentration of the coating solutions.

Table 4. The effect of different n and m mole ratios on the chlorine loading of PHQS[a]

Coating Solution concentration	9:1 Cl$^+$ wt%	5:1 Cl$^+$ wt%	3:1 Cl$^+$ wt%	1:1 Cl$^+$ wt%
0.5%	0.16			
1%	0.26			
3%	0.58	0.20	0.21	
5%	0.69	0.41	0.23	0.13
8%		0.43	0.23	0.13
10%		0.47	0.24	0.14

[a] Data represents three trials; the error in the measurements was ±0.02 weight percent.

As mentioned earlier, 80 % solubility of PHQS in water will increase hydrolyses from the surface to yield lower chlorine loadings.   Actually, the cotton swatches having 0.20 Cl$^+$ % loading can provide efficient biocidal efficacy.   For 5:1 and 3:1 mole ratios within PHQS, the concentration of the coating solution can be 3%, and for a 9:1 mole ratio, a 1 % coating solution is sufficient.

Biocidal Testing.   The biocidal efficacy tests were conducted in duplicate, and the data are shown in Table 5. The cotton was coated in baths containing 1% PHQS (9:1).   It is assumed that untreated cotton and treated but unchlorinated cotton caused 1-2 log reductions (90-99%) for *S. aureus* due to adhesion of bacteria to the cotton swatches instead of inactivation. The cotton swatches with 0.23 % and 0.26 % Cl$^+$ % inactivated

both *S. aureus* and *E. coli* within 1-5 min with complete 7 log reductions (99.9999%). Biocidal efficacies of the cotton swatches coated with PHQS with mole ratio of hydantoin: quat=9: 1, were similar to those reported for the hydantoinyl siloxane oligomer.[4] Only the N-halamine structures performed a biocidal function since the quat units only contained methyl groups to enhance solubility.[5]

Table 5.   The efficacies of coated cotton swatches against S. aureus and E. coli O157:H7

Samples	Contact Time (mins)	Log reduction of bacteria			
		S. aureus		*E. coli* O157:H7	
		Run 1	Run 2	Run 1	Run 2
Cotton	30	1.05	1.92	0.43	0.58
Cotton-copolymer	30	0.66	2.41	0.35	0.92
Cotton-copolymer-Cl Run1 Cl^+ % = 0.23 % Run2 Cl^+ % = 0.26 %	1	6.92	3.72	6.97	7.09
	5	6.92	7.02	6.97	7.09
	10	6.92	7.02	6.97	7.09
	30	6.92	7.02	6.97	7.09

Washing Testing.   The data pertaining to the stability and rechargeability of chlorine on the fabric during washing in a Launder-Ometer are shown in Table 6.

Table 6. *Stability and rechargeability of cotton swatches coated with PHQS and subjected to a machine washing process*

Machine washes[a]	[c]H:Q = 9:1 (1% bath)			H:Q = 5:1 (5% bath)		
	X[b]	Y[b]	Z[b]	X	Y	Z
0	0.30			0.47		
5	0.05	0.12	0.12	0.18	0.31	0.09
10	0.02	0.11	0.07	0.13	0.22	0.09
25	0.00	0.07	0.04	0.04	0.17	0.07
50	0.00	0.06	0.04	0.03	0.13	0.05

[a]One machine cycle is equivalent to five machine washes.

[b]X-Chlorination before washing (Cl^+wt%); Y-Rechlorination after washing (Cl^+wt%); Z-Chlorination only after washing (Cl^+wt%).

[c]H: Q is the mole ratio of Hydantoin: Quat.

[d]The error in the measured Cl^+ weight percentage values was ±0.02.

Each cycle of washing in this test was equivalent to five machine washings. It can be seen that the prechlorinated samples (Group X) dramatically lost chlorine during the first washing cycle (equivalent to 5 machine washes). Compared with the rechlorinated samples (Group Y) and the chlorinated samples after washing (Group Z), prechlorination did more to prevent hydrolysis of the coating for the fabric coated with PHQS 5:1 than for the samples coated with PHQS 9:1. It has been noted that generally prechlorination increases the hydrophobicity of surfaces with substantially higher initial chlorine loading and helps prevent the coating from hydrolysis.[6-7] After rechlorination, loss of chlorine

compared to the initial chlorine loading indicated that the coating to some extent does hydrolyze. The result is consistent with washing test data of pure hydantoinyl siloxane,[6] so that the presence of more hydrophilic groups of quaternary ammonium salt did not significantly enhance hydrolysis of the coating.

Conclusions

A series of hydantoinyl/quat siloxane copolymers with different ratios of hydantoinyl and quat groups were prepared. Solubility of these copolymers in water varies from 15% - 80%, and organic solvents would thus be avoided for coating fabrics greatly enhancing their industrial application. The cotton swatches coated with PHQS with 0.23% and 0.26% chlorine loading could achieve around 7 log inactivation within 1-5 min for both Gram-positive *S. aureus* and Gram-negative *E. coli* O157:H7. The results of both biocidal testing and washing testing were consistent with those for the hydantoinyl siloxane oligomer studied previously.[5-6] The quat functional group (trimethylammonium salt) improves solubility of the copoplymers in water for industrial application and does not affect any biocidal activity, stability, and rechargeability of the fabric coated with these copolymers.

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Chapter 3

Antimicrobial Cellulose: Preparation and Application of

5-aminomethyl-5-methylhydantoin

Introduction

A new technology was extended to incorporate a hydantoin moiety onto cotton fibers. 5-aminomethyl-5-methyl-hydantoin (Figure 30) was synthesized and linked to cotton using the 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 enhancing wrinkle resistance for cotton without severely affecting its mechanical strength or whiteness.[1] Here, 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. This allows imide N-Cl and amide N-Cl to exist simultaneously after chlorination. Imide N-Cl bonds are less stable than the equivalent amide bonds, so they release active chlorine to microbes relatively more rapidly and kill the pathogens in a shorter time. However, amide N-Cl bonds are relatively more stable than imide N-Cl bonds and so offer increased efficacy over a long time period, even under washing conditions.
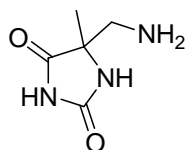
5-aminomethyl-5-methyl-hydantoin (AH)

Figure 30. Structure of 5-aminomethyl-5-methyl-hydantoin

Experimental

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.

Instruments. The NMR spectra were obtained using a Bruker 400 MHz spectrometer; the IR data were obtained with a Shimadzu IR Prestige-21 FTIR using KBr pellets.

Preparation of 5-methyl-5-aminomethylhydantoin. The preparation procedure for 5-methyl-5-aminometyhlhydantoin is illustrated in Scheme 9, which is based upon a modified literature method.[2]
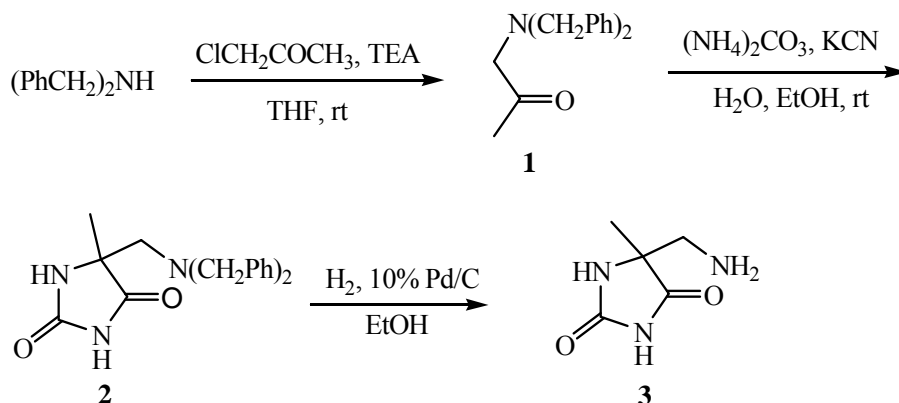
Scheme 9. Synthesis of 5-methyl-5-aminomethylhydantoin

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, DMSO-d_6) 2.01 (s, 3 H), 3.20 (s, 2 H), 3.60 (s, 4 H), 7.16-7.43 (m, 10 H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) 27.4, 57.5, 62.7, 127.0, 128.6, 128.4, 138.0, 207.7 ppm.

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°C; ^{1}H NMR (400 MHz, DMSO-d_6) 1.15 (s, 3

H), 2.65 (d, J = 13.0Hz, 1 H), 2.87 (d, J = 13.0Hz, 1 H), 3.59 (q, J = 13.0Hz, 4 H), 7.22-7.33 (m, 10 H), 7.90 (s, 1H), 10.71 (s, 1 H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) 21.1, 57.5, 58.8, 63.7, 126.8, 128.0, 128.7, 138.2, 156.7, 178.2 ppm.

5-(Aminomethyl)-5-methyl-2,4-imidazolidinedione (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, 250 watts) 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°C. ^{1}H NMR (400 MHz, DMSO-d_6) 1.16 (s, 3 H), 2.57 (d, J = 13.2 Hz, 1 H), 2.69 (d, J = 13.2 Hz, 1 H), 3.34 (s, 1 H), 7.71 (s, 1 H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) 20.6, 47.9, 64.3, 158.1, 178.01 ppm.

Coating procedure. 5-aminomethyl-5-methylhydantoin (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 °C for 5 min and then 125, 135, and 145 °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.

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 °C for 1 h to remove any free chlorine. The loading of bound chlorine on the swatches was determined

as described below.

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×V×35.45/(2 ×W)] ×100%

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

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'' (see blow). 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 ± 2.0 sec, 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

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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°C for 24 h and then counted for viable CFU's of bacteria.

Laundry durability and regenerability. Laundry durability and regenerability of the biocidal functions were conducted according to the AATCC (American Associate of Textile Chemists and Colorists) Test Method 61 (Test 2A Procedure). The cotton samples were laundered at 5, 10, 25, and 50 equivalent 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.

Results and discussion

Characterization of AH and the cotton swatches coated with AH. The FTIR spectrum of 5-aminomethyl-5-methylhydantoin is shown in Figure 31. 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 do not appear when the amino group is linked to cellulose through BTCA.
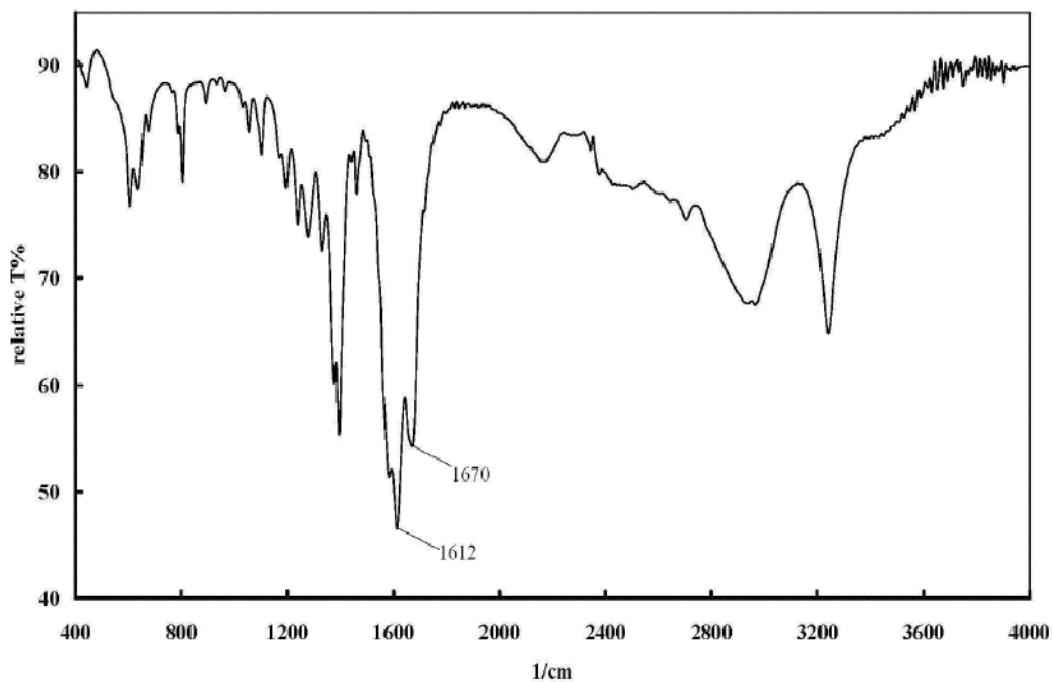
Figure 31. FTIR spectrum of 5-aminomethyl-5-methylhydantoin (AH)

Figure 32 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 32b provides evidence that the hydantoin functional group is present in the coating. The band at 1724 cm^{-1} may be due to the vibration band of the carboxylic group of BTCA and the carbonyl group of hydantoin ring overlapping.
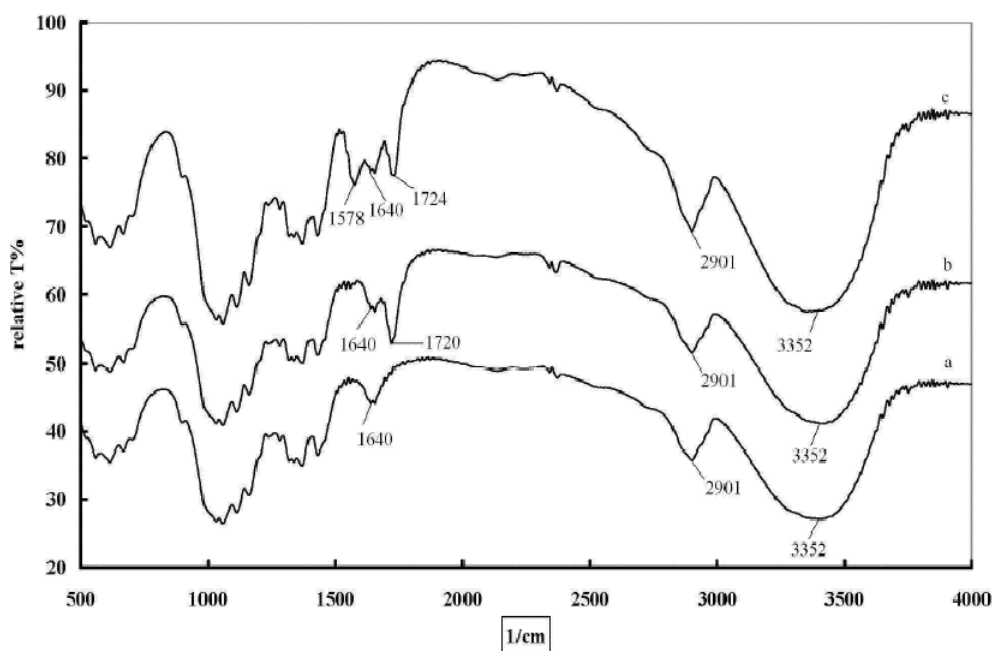
Figure 32. FTIR spectra of cotton control, treated cotton, and treated cotton after

chlorination

a- **cotton control**

b- **treated without chlorination**

c- **treated with chlorination**

The effect of BTCA and a possible mechanism for attachment

Figure 33 and Table 7 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. A possible mechanism for the process is shown in Scheme 10. 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.

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Curing Time (min)	Chlorine weight percent on cotton samples[d]	
	$Cl^+\%$ [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^oC 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.

[d]Data represents three trials; the error in the measurements was ±0.02 weight percent.
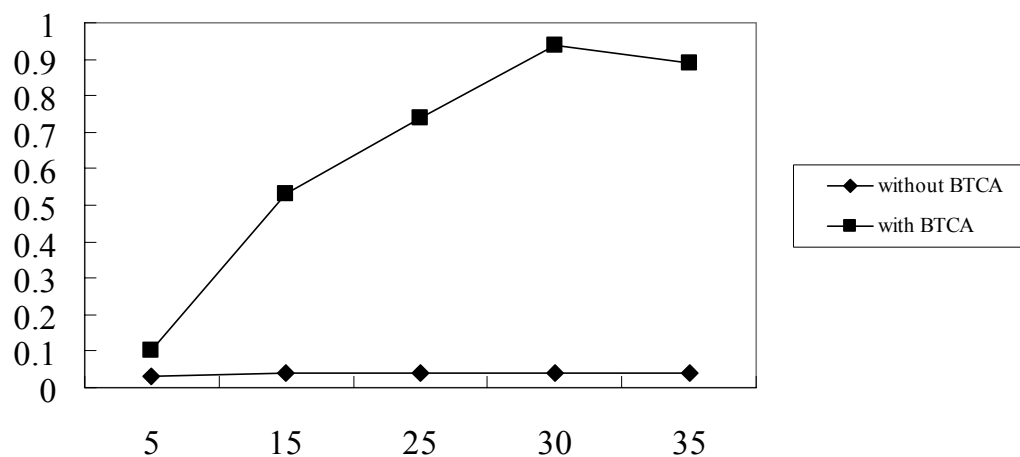
Figure 33. The effect of curing time with and without BTCA
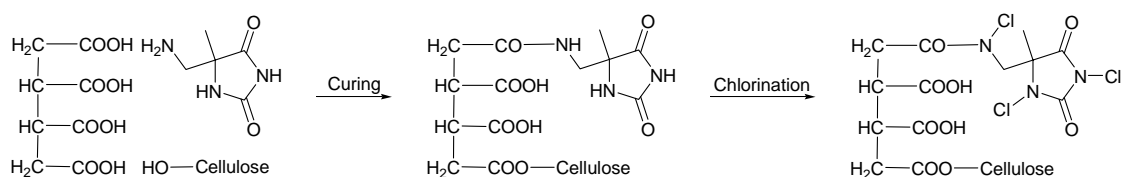
Scheme 10. Attachment of AH/BTCA to cellulose

The effect of the concentration of coating solution. Table 8 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 8. The effect of the concentration of coating solution [a]

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

[a]Cotton was coated in 5% AH (mol_{AH}:mol_{BTCA} =1:1) for 15 min, the cloth samples were cured at 145 $^{\circ}$C for 35 min, and then chlorinated with 1% Clorox without pH adjustment.

[b]Data represents three trials; the error in the measurements was ±0.02 weight percent.

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 9. Higher curing temperature causes increased chlorine percent because it facilitates reaction between antimicrobial agents and the fibers. The optimum curing conditions were $145^{o}C$ for 30 min.

Table 9. The effect of curing temperature and curing time[a]

Time		Chlorine weight Percent[b] (%)
5 min	125°C	0.02
	135°C	0.05
	145°C	0.10
15 min	125°C	0.18
	135°C	0.33
	145°C	0.53
25 min	125°C	0.34
	135°C	0.35
	145°C	0.74
30 min	125°C	0.44
	135°C	0.56
	145°C	0.94
35 min	125°C	0.50
	135°C	0.64
	145°C	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.

[b]Data represents three trials; the error in the measurements was ±0.02 weight percent.

The effect of pH of the chlorination solution. Table 10 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 our laboratory 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.[3] 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 10. The effect of bleach concentration[a]

Clorox solution	pH		Chlorine weight percent
	Initial[b]	After[c]	(%) Loaded on the cotton[d]
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°C 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.

[d]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Washing tests. The data in Table 11 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 11. Washing tests of the chlorinated cotton swatches

Washing cycles	Chlorine weight percent (%) loaded on the cotton[a]		
	A	B	C
0	0.77	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.

[a]The error in the measured Cl^+ weight percentage values was ±0.02.

Biocidal tests. Tables 12 and 13 show that after chlorination, the fabric swatches were effective against both Gram-positive *S. aureus* and Gram-negative *E. coli*. With the presence of chlorinated hydantoinyl functional groups, the swatches achieved about 7 log reduction within 1 min of contact, while fabric swatches without chlorination, and cotton itself, showed small log reductions. Once chlorinated, the fabric possessed very good biocidal efficacy.

Table 12. Biocidal test for the microorganism: E. coli **O157:H7**

Sample	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
Cotton Control	0	9.00×10^6	0
	1	7.24×10^6	0.09
	5	6.30×10^6	0.16
	10	6.10×10^6	0.17
Cotton-AH Control	0	9.00×10^6	0
	1	5.36×10^6	0.23
	5	4.89×10^6	0.30
	10	4.49×10^6	0
Cotton-AH-Cl	0	9.00×10^6	0
	1	nd[a]	6.95
	5	nd[a]	6.95
	10	nd[a]	6.95

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

Table 13. Biocidal test for the microorganism: S. aureus

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

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

Conclusion

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* O157:H7 (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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Chapter 4

Novel N-halamine Siloxanes

Introduction

Due to the stabilizing effect of six-membered rings, novel N-halamine moieties incorporating six-member rings are expected to be promising candidates. This possibility led to the synthesis of 6-phenyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione (MTPTD) (Figure 34) in the third project reported here. The precursor to this siloxane compound was 6-phenyl-1,3,5-triazinane-2,4-dione, which contains two amide nitrogens and one imide nitrogen. The imide nitrogen moiety binds to the silane moiety because of its greater acidity compared to the amide groups. The new siloxane thus contains two amide hydrogens, both of which are available for chlorination, and the existence of a benzene ring-electron donor should stabilize the N-Cl bonds upon chlorination. Although there is one alpha hydrogen at which dehydrohalogenation could occur, dehydrohalogenation could occur only once, leaving a single N-Cl to be stabilized by the benzene ring. 6,6-dimethyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione (DMTPTD) (Figure 34) was also synthesized to compare its stability with that of MTPTD, although the DMTPTD was expected to have only limited practical application

because of the high expense of the starting materials. Thus, in this study 6-phenyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione and 6,6-dimethyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione were synthesized and coated onto cotton fabric swatches and silica gel particles. The coated samples were rendered antimicrobial by chlorination, and then the chlorinated cotton swatches and silica gel were evaluated for biocidal efficacy against *E. coli* O157:H7 and *S. aureus*. The stabilities and rechargeabilities of the N-Cl bonds of the N-halamine siloxane coating were evaluated using washing tests and exposure to UV and ambient lighting.

6-phenyl-3-(3-(triethoxysilyl)propyl)-1,3,5-triazinane-2,4-dione
(MTPTD)

6,6-dimethyl-3-(3-(triethoxysilyl)propyl)-1,3,5-triazinane-2,4-dione
(DMTPTD)
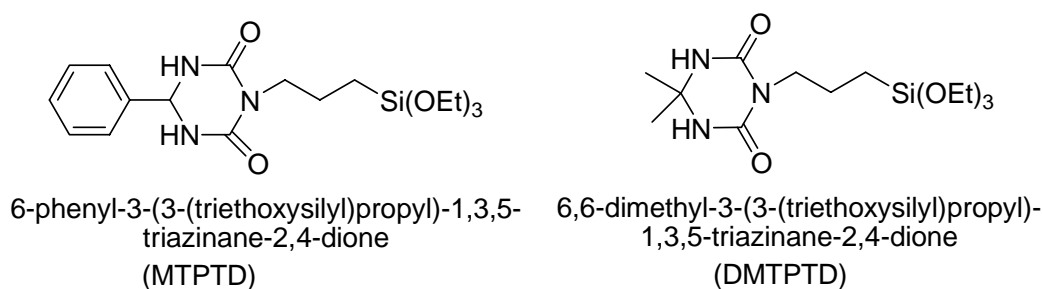
Figure 34. Structures of MTPTD and DMTPTD

Experimental

Preparation of 6-phenyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione (Scheme 11). A mixture of 10.5 g biuret (97%), 10.6 g benzaldehyde, and 95.1 g HCl (37%) was stirred at room temperature for 1 day. After completion of the reaction, ice water (200 mL) was added. After filtration, the solid was collected and washed with distilled water until the pH became 5. The yield of the product 6-phenyl-1,3,5-triazinane-2,4-dione was 80%.

A mixture of 3.82 g (20 mmol) 6-phenyl-1,3,5-triazinane-2,4-dione and 1.30 g 86% KOH (20 mmol) was refluxed in an EtOH (50 mL) solution for 10 min, a solid potassium salt being obtained after removal of solvent under reduced pressure and drying under vacuum at 60°C for 2 days. Then the dry salt and 5.06 g 3-chloropropyltriethoxysilane (20 mmol) in DMF (25 mL) were mixed and heated at 95°C overnight. After the solution cooled to ambient temperature, KCl was removed by filtration, and then DMF was removed at reduced pressure to yield a light yellow solid. The solid was dissolved in ethyl acetate (60 mL), and unreacted 6-phenyl-1,3,5-triazinane-2, 4-dione and its salt were removed by filtration. After removal of the ethyl acetate by evaporation, a small portion of ethyl ether was added which allowed recovery of a pure white solid identified as the siloxane MTPTD.

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44-7.46 (m, 5H), 5.51 (s, 1H), 3.81 (q, J = 7.0 Hz, 6 H), 3.70 (t, J = 7.6 Hz, 2H), 1.71 (m, 2H), 1.23 (t, J = 7.0 Hz, 9H), 0.63 (t, J = 8.6 Hz, 2H) ppm; ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.3, 137.8, 130.4, 129.5, 126.9, 63.7, 58.6, 43.4, 22.2, 18.5, 7.8 ppm.
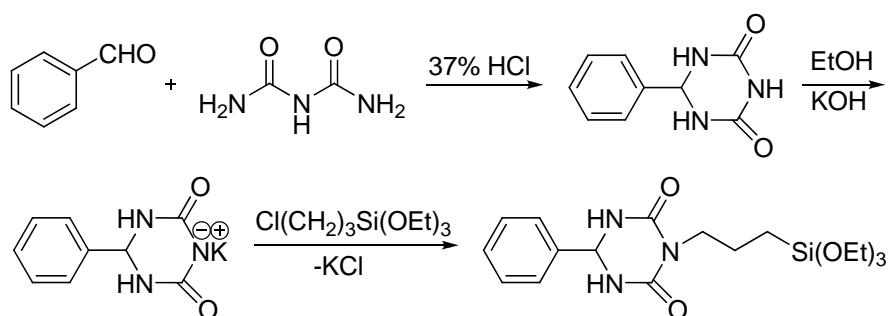
Scheme 11. Synthesis of MTPTD

Preparation of 6,6-dimethyl-1,3,5-triazinane-2,4-dione. A literature synthetic procedure was followed (Scheme 12).[1] 1.35 g (0.010 mol) dithiobiuret were added to a solution of 0.65 g acetone and 8 mL EtOH. Dry HCl was bubbled through the solution for 30 min with stirring at room temperature for an additional 30 min. Excess 1 M NaOH was added to render the solution clear. Neutralization with acetic acid at room temperature caused precipitation of the product, which was isolated by filtration and dried to obtain 1.54 g of pure product. The yield was 88 %.

Then 1.75 g (0.010mol) 6,6-dimethyl-1,3,5-triazine-2,4(1H,3H)-dithione was dissolved in 17.6 mL NaOH (2.5 M), and the solution was heated to 40-45°C. 9.1 g 30wt% H_2O_2 aqueous solution were added dropwise while the temperature was maintained at 40-45°C, and then the temperature was raised to 80°C. Neutralization with 2 N H_2SO_4 at room temperature caused the precipitation of the product, which was isolated by filtration and dried to obtain 1.21 g of product. The yield of 6,6-dimethyl-1,3,5-triazinane-2,4-dione was 85%.

Preparation of 6,6-dimethyl-3-(3'-triethoxysilypropyl)-1,3,5-triazinane-2,4-dione (Scheme 12). 1.43 g (10 mmol) 6,6-dimethyl-1,3,5-triazine-2,4(1H,3H)-dione and 0.65 g 86% KOH (10 mmol) were suspended in 40 mL of EtOH solution, and the mixture was refluxed for 10 min. The solvent was removed at ambient temperature under reduced pressure to obtain the solid potassium salt, which was isolated by filtration and dried under vacuum at 60°C for 2 d. Then the dry potassium salt and 2.53 g 3-chloropropyltriethoxysilane (95%) were suspended in 15 mL of DMF, and the solution was heated at 95°C overnight. The KCl salt produced was removed by filtration, and then DMF was removed at reduced pressure to obtain 3.44 g of a light yellow solid. The yield

of DMTPTD was 99%.

^{1}H NMR (250 MHz, DMSO-d$_6$) δ 7.92 (s, 2H), 3.74 (q, $J = 7.0$ Hz, 6H), 3.51 (t, $J = 7.1$ Hz, 2H), 1.50 (m, 2H), 1.30 (s, 6H), 1.14 (t, $J = 7.0$ Hz, 9H), 0.49 (t, $J = 8.4$ Hz, 2H) ppm; ^{13}C-NMR (62 MHz, DMSO-d$_6$) δ 152.9, 63.4, 58.2, 42.1, 29.7, 22.3, 18.7, 7.7 ppm.
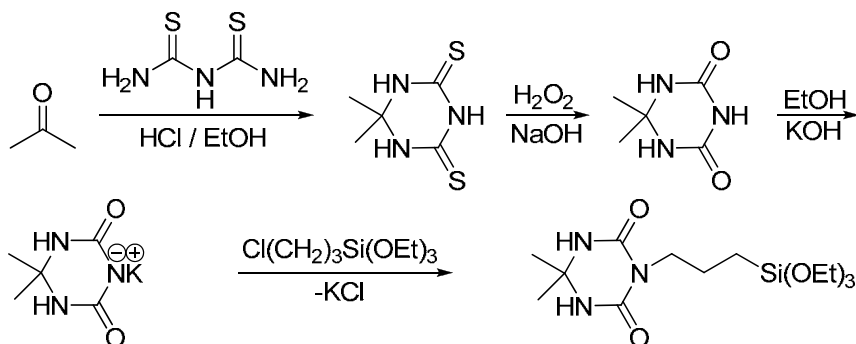
Scheme 12. Synthesis of DMTPTD

Coating procedure. For MTPTD siloxane, cotton swatches were soaked in solutions containing 2.5% by weight MTPTD in ethanol/1 N acetic acid (2:1 w/w) for 15 min. For DMTPTD siloxane, cotton swatches were soaked in solution containing 5% by weight DMTPTD in ethanol/water (1:1 w/w) for 15 min. The coated swatches were cured at 95°C for 1 h and then further at 145°C for 20 min. The swatches were washed in 0.5% detergent solution for 15 min, rinsed with water, and then dried in air.

MTPTD siloxane was also coated on the silica gel particles by refluxing 25 g silica gel in baths containing 8 g MTPTD and 100 g EtOH/H$_2$O (1:1 w/w) for 10 to 18 h. The coated silica gel was filtered and washed with 400 mL EtOH and H$_2$O (1:1 w/w).

Chlorination procedure. The coated cotton swatches were soaked in a 10% aqueous solution of NaOCl household bleach buffered to pH = 7 at ambient temperature for 1 h. The chlorinated swatches were washed with tap water and distilled water, and then dried at 45°C for 1 h to remove any free chlorine. The silica gel was chlorinated in a

10% Clorox solution (pH = 7) for 1 h, washed with tap water and distilled water, and dried at 45°C for 2 h.

Analytical titration procedure. The loading of bound chlorine (Cl$^+$) on the swatches and silica gel was determined by a standard iodometric/thiosulfate titration procedure. The percent weight of Cl$^+$ on the cotton swatches and silica gel were determined from the equation below:

$$Cl^+\% = \frac{0.0375 \times V \times 35.45}{2000 \times W} \times 100$$

where V is the volume (mL) of the Na$_2$S$_2$O$_3$ consumed in the titration, and W is the weight in grams of the cotton swatch or silica gel sample. The normality of the sodium thiosulfate was 0.0375 meq/mL.

Biocidal efficacy testing. The cotton swatches and silica gel were challenged with Gram-positive *Staphylococcus aureus* ATCC 6538 and Gram-negative *Escherichia coli* O157:H7 ATCC 43895 for antimicrobial efficacy analysis. Both chlorinated and unchlorinated control samples were used in this study.

For the cellulose samples, 25 μL of bacterial suspension containing 10^6 to 10^7 colony forming units (CFU) were placed in the center of a pair of one inch square cotton swatches held in place by a sterile weight to insure good contact of the swatches. After contact times of 1.0, 5.0, and 15.0 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solutions in 50 mL sterile conical centrifuge tubes to remove all oxidative chlorine and vortexed for 2 min vigorously to remove the bacteria from the samples. Serial dilutions of the quenched solutions were made using pH 7, 100 μM phosphate buffer, and were plated on Trypticase soy agar plates. The plates were

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incubated at 37ºC for 24 h, and bacterial colonies were recorded for biocidal analysis.

For the silica gel samples, treated or untreated silica gel were packed in long glass columns with an inside diameter of 1.0 cm and a length of 25.0 cm. The length of the silica gel was 18.0 cm. The void volume was measured for the purpose of calculating the contact times. A peristaltic pump (MasterFlex L/S, Cole Palmer Inc., Vernon Hills, IL) was used to control the flow rate of 50 mL of aqueous bacterial suspension (pH 7, 100 mM phosphate buffer) containing about 10^7 CFU (colony forming units) of bacteria) through the columns. By repeated recirculation of the inoculum through the columns, contact times could be varied. At specific time intervals (5, 10, 30 sec), 0.05 mL of effluent was collected at each time in sterile tubes. The aliquots were immediately quenched with 0.5 mL of 0.1 N sodium thiosulfate to prevent any subsequent inactivation of the bacteria by any free chlorine that might leach out from the biocidal silica gel. Serial dilutions of the quenched effluents were plated onto Trypticase soy agar plates, and the plates were incubated at 37ºC for 24 h. The viable colonies were counted for the biocidal efficacy analysis.

UV and laboratory light stability testing. The stability of the cotton swatches coated with the chlorinated siloxanes was measured using UV-light produced by an Accelerated Weathering Tester (The Q-panel Company, USA) and ambient laboratory light. After either exposure to UVA irradiation from 1h to 28 d, or exposure to lab lighting from 1 to 4 weeks, the fabric samples were titrated immediately and also after rechlorination.

Results and discussion

Biocidal efficacies of cotton swatches coated with MTPTD and DMTPTD and biocidal efficacy of silica gel coated with MTPTD. The biocidal efficacy of the cotton swatches coated with MTPTD and DMTPTD and the silica gel coated with MTPTD for *S. aureus and E. coli* O157:H7 are shown in Tables 14 and 15. From the data in Table 14, it is demonstrated that silica gel coated with chlorinated MTPTD caused complete inactivation of *S. aureus* within 10 sec and *E. coli* O157:H7 within 5 sec. Small losses of organisms were detected for the control samples. Compared with silica gel coated with the chlorinated monomer of 5,5-dimethyl-3-(3'-triethoxysilylpropyl)hydantoin which inactivated both organisms within 30 sec,[2] the inactivation rate of the chlorinated silica gel coated with MTPTD was higher.

Table 14. Biocidal column test of silica gel coated with MTPTD and chlorinated MTPTD

Samples	Contact Time (sec)	Log reduction of bacteria	
		S. aureus	*E.coli* O157: H7
	5	0.60	0.00
Silica gel-MTPTD	10	0.81	0.13
	30	1.06	0.37
Silica gel –MTPTD –Cl	5	4.21	7.03
Cl$^+$%=2.81	10	7.03	7.03
	30	7.03	7.03

Table 15 shows the biocidal efficacies for chlorinated cotton swatches coated with

MTPTD and DMTPTD against both *S. aureus* and *E. coli* O157:H7. For the coated but unchlorinated samples, there are small log reductions due to the adhesion of bacteria to the cotton swatches. The cotton swatches coated with DMTPTD inactivated *S. aureus* within 15 min and *E. coli* O157:H7 within 1 min. For the cotton swatches coated with MTPTD, 1 min was required to provide about 7 log reductions of the two organisms. These coated cotton swatches were efficient biocidal materials.

Table 15. *Biocidal tests of cotton swatches coated with MTPTD and DMTPTD and their chlorinated derivatives*

Samples	Contact Time (mins)	Log reduction of bacteria	
		S. aureus	*E. coli*
	1	0.40	0.19
Cotton-MTPTD	5	0.52	0.22
	15	0.64	0.28
Cotton-MTPTD-Cl	1	7.03	7.07
$Cl^+\%=0.40$	5	7.03	7.07
	15	7.03	7.07
	1	0.02	0.01
Cotton-DMTPTD	5	0.05	0.04
	15	0.11	0.23
Cotton-DMTPTD-Cl	1	3.75	6.89
$Cl^+\%=0.29$	5	4.41	6.89
	15	7.08	6.89

Washing tests of the cotton swatches coated with MTPTD and DMTPTD. From Table 16, the chlorine loading of the cotton swatches coated with chlorinated MTPTD decrease from 0.41 to 0.01% after 50 washing cycles; for the samples coated with chlorinated DMTPTD, the chlorine loading decreased from 0.34% to 0.03% because of dissociation of the N-Cl bond and some loss of the coating itself. The chlorine loading was dramatically lost during the first five washing cycles. After rechlorination, 0.12%-0.13% chlorine was regained.

Table 16. Washing Test of cotton swatches of MTPTD and DMTPTD[a]

	Washing cycles	X (%)	Y (%)	Z (%)
	0	0.41	0.41	
	5	0.03	0.22	0.14
MTPTD	10	0.02	0.18	0.13
	25	0.02	0.18	0.13
	50	0.01	0.12	0.11
	0	0.34	0.34	
	5	0.08	0.34	0.11
DMTPTD	10	0.04	0.14	0.10
	25	0.04	0.14	0.10
	50	0.03	0.13	0.10

X: Chlorination before washing; Y: Chlorination before and after washing;

Z: Chlorination after washing.

[a] The error in the measured Cl^+ weight percentage values was ±0.02.

Stability of the cotton swatches coated with DMTPTD and MTPTD under UV-irradiation. The stabilities of the chlorinated DMTPTD and MTPTD coatings to UV irradiation are shown in Table 17. The Cl^+ weight percent decreased from 0.29%-0.43% to 0.01% after 24 h in the UV chamber which indicates that almost all of the N-Cl bonds decomposed within 24 h. However, after rechlorination of the samples following exposure to UV light for 24 h, 83% active chlorine was regained. After the samples were exposed to UV light for 28 d and rechlorinated, for chlorinated DMTPTD, 69% Cl^+ was regained, and for MTPTD, 42% Cl^+ was regained. In comparison to rechlorination of 5,5-dimethyl-3-(3'-triethoxysilylpropyl)hydantoin (1 in Figure 35), 3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (2 in Figure 35), 4-[3-triethoxysilylpropoxyl]-2,2,6,6-tetramethylpiperidine (3 in Figure 35),[3-4] and 3-triethoxysilylpropyl-2,2,5,5-tetramethylimidazolidin-4-one (4 in Figure 35),[4] after 30 d exposure to UV light, 75% of the initial loading of chlorine was recovered for 2, 65% for 4, 30% for 1, and 28% for 3.[4] These data show that the compounds themselves, or the N-Cl covalent bonds are varying in stability under UV irradiation. The UV stability of DMTPTD is similar to chlorinated 2 and 3. Although the structure of MTPTD contains one alpha hydrogen for which dehalogenation could take place, the unusual stability of MTPTD is presumably reasonable due to the existence of an aromatic ring.
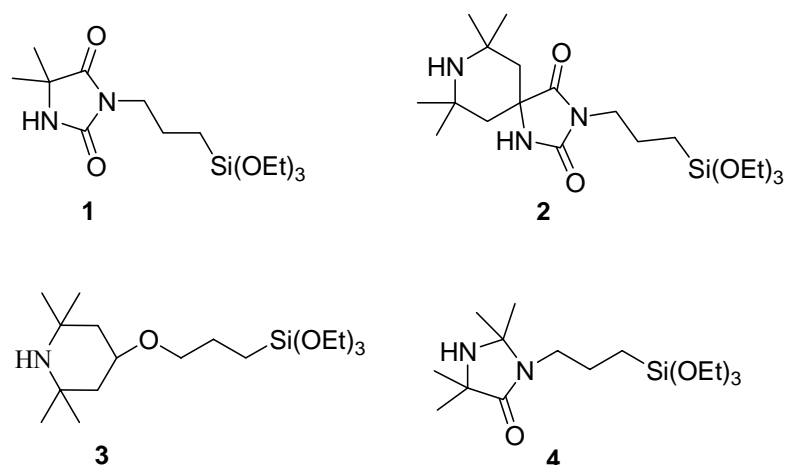
Figure 35. Structures of compounds 1-4

Table 17. UV light stability of chlorinated siloxanes coated onto cotton fabrics

Time	DMTPTD		MTPTD	
	% Cl^{+a}	rechlorination	% Cl^{+a}	rechlorination
0h	0.29		0.43	
1h	0.11		0.13	
2h	0.07		0.07	
3h	0.05		0.07	
5h	0.02	0.27	0.04	0.43
12h	0.02	0.25	0.04	0.41
24h	0.01	0.24	0.01	0.36
7d		0.24		0.36
14d		0.20		0.27
21d		0.20		0.20
28d		0.20		0.18

[a] The error in the measured Cl^+ weight percentage values was ± 0.02.

Table 18 shows the stability data for DMTPTD and MTPTD under ambient light exposure. Almost all of the N-Cl bonds decomposed after 28 d. After rechlorination of the samples, 97% Cl^+ was recovered for DMTPTD and 93% Cl^+ for MTPTD. In comparison to **1**, **2**, **3**, and **4**, 20%-78% of active chlorine was recovered in those cases.[4] The six-membered ring compounds demonstrated good stability under lab lighting.

Table 18. Laboratory light stability of chlorinated siloxanes coated onto cotton fabrics

Time	DMTPTD		MTPTD	
[d]	% Cl^{+a}	rechlorination	% Cl^{+a}	rechlorination
0	0.29		0.43	
7	0.17	0.28	0.31	0.43
14	0.13	0.28	0.12	0.43
21	0.08	0.27	0.11	0.40
28	0.06	0.28	0	0.40

[a]The error in the measured Cl^+ weight percentage values was ±0.02.

Conclusion

Two new compounds, MTPTD and DMTPTD, have been synthesized and coated onto the cotton swatches and silica gel particles. Chlorination rendered them effectively biocidal against *S. aureus* and *E. coli* O157:H7 bacterial challenge. These two compounds contain two nitrogen sites available for chlorination. After 50 washing cycles, most of chlorine was lost, but 0.12-0.13% chlorine loading could be regained after rechlorination. Stability testing in a UV-chamber showed that 69% Cl^+ was regained for

DMTPTD and 42% Cl^+ for MTPTD after rechlorination of the samples which had been exposed to UV light for 28 d. Under lab lighting, nearly all of the N-Cl bonds decomposed after 28 d, but after rechlorination of the samples, 97% Cl^+ was recovered for DMTPTD and 93% Cl^+ for MTPTD. The stabilities of both of the new compounds are good compared to other siloxane coatings studied previously. Although one alpha hydrogen exists in the structure of MTPTD which could lead to dehalogenation, the stability is improved by the presence of the aromatic ring. It will be less expensive to produce MTPTD than DMTPTD for the purposes of industrial application.

References for chapter 4

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Chapter 5

The Synthesis and Practical Applications of more Biocidal N-halamine Biocides

Introduction

The fourth project's objective was to synthesize the functionalized N-halamine compounds 5-methyl-5-hydroxylmethylhydantoin (HO-Hy) and 5-chlormethyl-5-methylhydantoin (Cl-Hy) (Figure 36). These two monomers were coated onto cotton swatches by different methods, and after chlorination their biocidal activities and stabilities were investigated.

HO-Hy   Cl-Hy
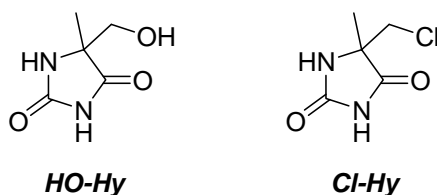
Figure 36. Structures of HO-Hy and Cl-Hy

Experimental

Preparation of 5-hydroxymethyl-5-methylhydantoin (HO-Hy) (Scheme 13). 8.22 g (0.10 mol) of 90% acetol, 13.44 g (0.20 mol) of 97% potassium cyanide, and 38.24 g (0.40 mol) of ammonium carbonate were added to 100 mL of distilled water. The solution was stirred at room temperature for 2 days and then the water was removed under reduced pressure. 200 mL of acetone were added to the remaining mixture of solids. After

stirring for 15 minutes, the insoluble inorganic compounds were filtered out. After removing the acetone under reduced pressure, 12.73 g of light yellow product were obtained. The yield was 88%.
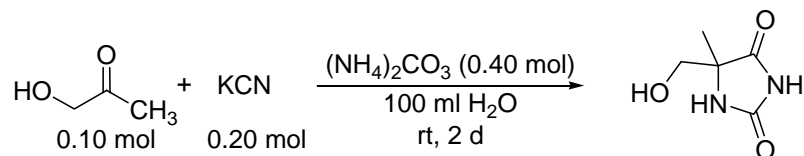
Scheme 13. Synthesis of 5-hydroxymethyl-5-methylhydantoin

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.39 (s, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.06 (d, J = 8.0 Hz, 1H), 1.40 (s, 3H) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 174.6, 157.6, 73.1, 60.5, 23.8 ppm. (Figures 37 and 38)
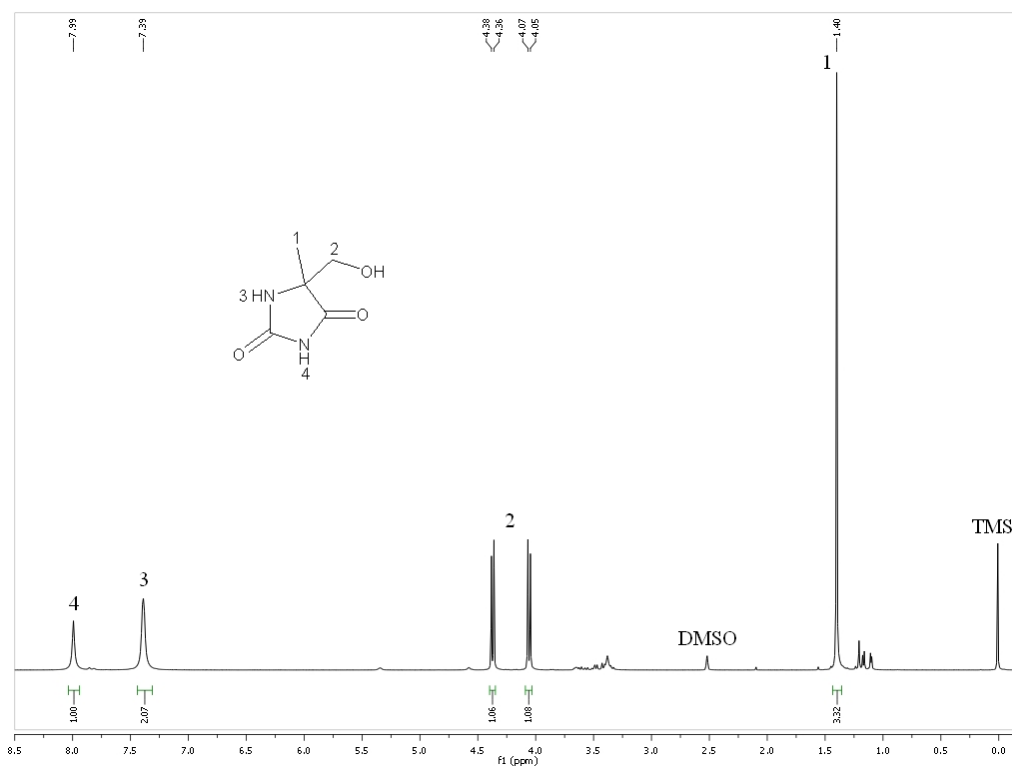
Figure 37. ^{1}H NMR of 5-hydroxylmethyl-5-methyl-hydantoin
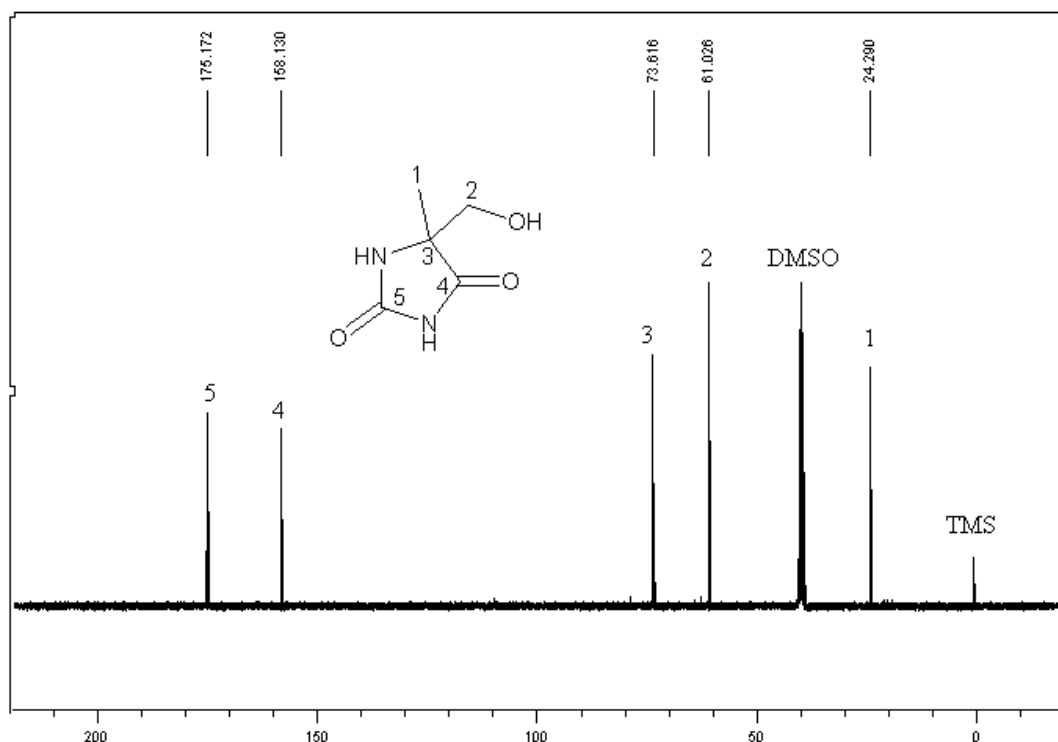
Figure 38. ^{13}C NMR of 5-hydroxylmethyl-5-methyl-hydantoin

Preparation of 5-chloromethyl-5-methylhydantoin (Cl-Hy) (Scheme 14). 9.64 g

(0.10 mol) of 96% chloroacetone and 25.20 g (0.3 mol) of sodium bicarbonate were

added to 100 mL of distilled water at $10^{o}C$ and then 6.72 g (0.10 mol) of 97% potassium

cyanide were added drop by drop over a period of 30 minutes. 19.22 g (0.20 mol) of

ammonium carbonate were added to the resulting mixture, which was then stirred at $25^{o}C$

for 5 hours. The water was removed under reduced pressure. 200 mL of acetone were

added to the solid residue. After stirring for 15 minutes the insoluble inorganic

compounds were filtered out. After removing the acetone, 13.95 g of yellow oil were

obtained. After purification by column chromatography, 5.86 g of white solid compound

were obtained.
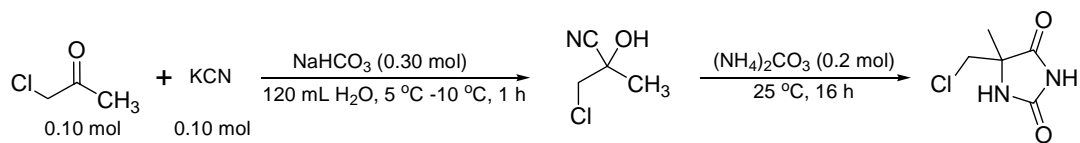
Scheme 14. Synthesis of 5-chloromethyl-5-methylhyndatoin

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.82 (s, 1H), 8.08 (s, 1H), 3.81 (d, J = 12.0 Hz, 1H), 3.63 (d, J = 12.0 Hz, 1H), 1.34 (s, 3H) ppm; ^{13}C-NMR (100 MHz, DMSO-d$_6$) δ 176.3, 157.9, 63.3, 48.5, 21.7 ppm. (Figure 39 and 40)
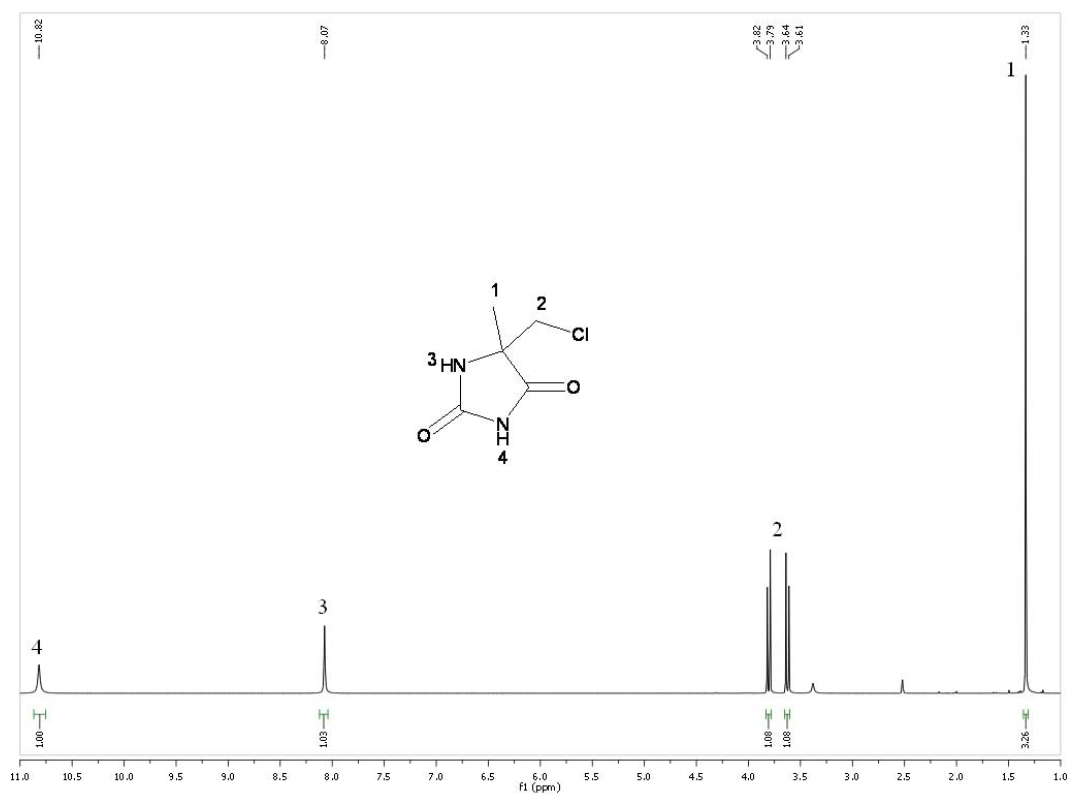
Figure 39. ^{1}H NMR of 5-chloromethyl-5-methyl-hydantoin

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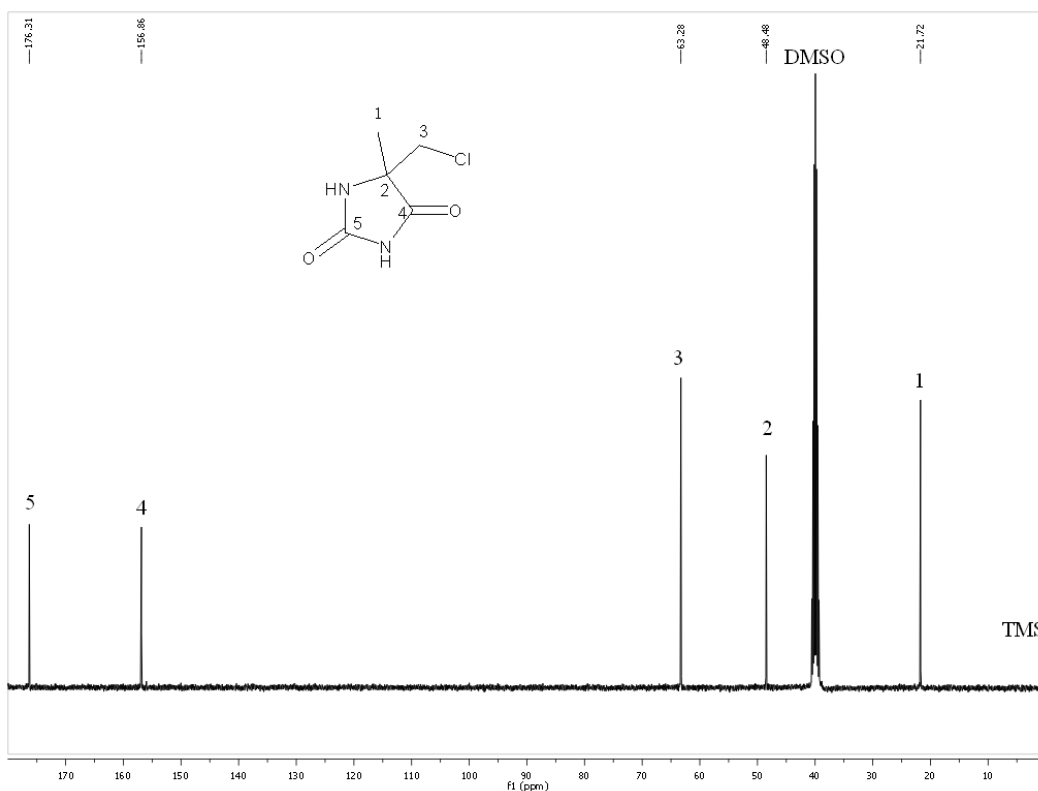
Figure 40. ^{13}C NMR of 5-chloromethyl-5-methyl-hydantoin

Coating and chlorination procedure. 1. Cotton fabric swatches were soaked for 15 minutes in baths containing either: a. 5% of HO-Hy in water; b. 5% of HO-Hy in 1 N HOAc; or c. 5% of HO-Hy in 1 N HCl. The cotton swatches were then either dried at 95 °C for 1 h and then cured at 145 °C 0.5-1 h, or immediately cured at 145°C for 0.5-2 h without drying at lower temperature, after which they were washed with 0.5% detergent solution for 15 min, rinsed with water several times to remove any weakly bonded compounds, and dried at 45 °C for 1 h. The coated cotton swatches were chlorinated with 10% commercial household bleach solution (NaOCl) at pH 7 for 1 h, rinsed with tap water, and then with distilled water thoroughly to remove free chlorine. Finally, the chlorinated cotton swatches were dried at 45 °C for 1 h.

2. Cotton fabric swatches were soaked in baths containing 0.50 g HO-Hy, 0.82 g butane-1,2,3,4-tetracarboxylic acid (BTCA), and 8.68 g of H_2O, dried at 80^oC for 1 h, and then either cured at 160^oC for 10-50 mins or immediately dried at 160^oC for 0.5-2 h. The coated cotton swatches were chlorinated with 1% commercial household bleach solution (NaOCl) at pH 7 for 1 h, rinsed with tap water, and then with distilled water thoroughly to remove free chlorine. Finally, the chlorinated cotton swatches were dried at 45^oC for 1 h.

3. Cotton swatches were soaked for 15 mins in baths containing either: a: 5% of Cl-Hy in water; b: 5% of Cl-Hy in 0.1 N NaOH; or c: 2 wt% Cl-Hy in 1 N NaOH. The coated cotton swatches were chlorinated in 10% Clorox solution at pH 7 for 1 h. The other steps were the same as 1 and 2.

Results and Discussion

Preparation of biocidal cotton swatches coated with HO-Hy. The data in Table 19 show that acid can catalyze the reaction between HO-Hy and hydroxyl groups on the surface of cellulose. Even though the cotton swatches coated in 5% of HO-Hy in 1 N HCl had the highest Cl^+% (0.24%), these cotton swatches become brittle after coating. 1 N HOAc was considered to be better for preparing moderate coating solutions and 0.15 Cl^+% was achieved when the cotton swatches were cured at 145^oC for longer than 1.5 h. Higher temperatures lowered the chlorine loading, as shown by the data in Table 20 for samples treated at 160 °C. Table 21 shows that 0.30 Cl^+% loading was gained in the presence of the cross linker butanetetracarboxylic acid (BTCA). Thus BTCA was always used for coating HO-Hy onto the cellulose in the following studies.

Table 19. The effect of different coating solutions of HO-Hy and different curing conditions on the chlorine loading (I)

Coating Solution	Curing condition	$Cl^+\%^a$
5% of HO-Hy in water	95 ^{o}C, 1 h; 145 ^{o}C, 0.5 h	0.05
5% of HO-Hy in 1 N HOAc	95 ^{o}C, 1 h	0.05
5% of HO-Hy in 1 N HOAc	95 ^{o}C, 1h; 145 ^{o}C, 0.5 h	0.11
5% of HO-Hy in 1 N HOAc	95 ^{o}C, 1h; 145 ^{o}C, 1.0 h	0.11
5% of HO-Hy in 1 N HOAc	95 ^{o}C, 1h; 145 ^{o}C, 1.5 h	0.15
5% of HO-Hy in 1 N HOAc	145 ^{o}C, 0.5 h	0.09
5% of HO-Hy in 1 N HOAc	145 ^{o}C, 1.0 h	0.13
5% of HO-Hy in 1 N HOAc	145 ^{o}C, 1.5 h	0.16
5% of HO-Hy in 1 N HOAc	145 ^{o}C, 2.0 h	0.16
5% of HO-Hy in 1 N HCl	95 ^{o}C, 1h; 145 ^{o}C, 0.5 h	0.24

[a]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Table 20. The effect of the same coating solution of HO-Hy and different curing conditions on the chlorine loading (II)

Coating Solution	Curing condition	$Cl^+\%^a$
5% of HO-Hy in 1 N HOAc	160 ^{o}C,10 min	0.04
5% of HO-Hy in 1 N HOAc	160 ^{o}C, 20 min	0.05
5% of HO-Hy in 1 N HOAc	160 ^{o}C, 30 min	0.07
5% of HO-Hy in 1 N HOAc	160 ^{o}C, 40 min	0.08
5% of HO-Hy in 1 N HOAc	160 ^{o}C, 50 min	0.10
5% of HO-Hy in 1 N HOAc	160 ^{o}C, 60 min	0.09

[a]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Table 21. The effect of different curing conditions on the chlorine loading for a coating solution containing BTCA

Curing condition	Cl$^+$%[a]
160 ^{o}C, 10 min	0.08
160 ^{o}C, 20 min	0.14
160 ^{o}C, 30 min	0.20
160 ^{o}C, 40 min	0.24
160 ^{o}C, 50 min	0.23
85 ^{o}C, 5 min; 160 ^{o}C, 10 min	0.12
85 ^{o}C, 5 min; 160 ^{o}C, 20 min	0.19
85 ^{o}C, 5 min; 160 ^{o}C, 30 min	0.25
85 ^{o}C, 5 min; 160 ^{o}C, 40 min	0.30
85 ^{o}C, 5 min; 160 ^{o}C, 50 min	0.30

[a]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Preparation of biocidal cotton swatches coated with Cl-Hy. As the data in Table 22 show, 1N NaOH catalyzed the reaction between the OH groups of cellulose and Cl-Hy for neutral conditions, and 0.1 N NaOH did not lead to improve the chlorine loading. 0.37 Cl$^+$% loading was achieved when the coated cotton swatches were dried at 95 ^{o}C for 1 h and then cured at 145 ^{o}C for 10 mins.

Table 22. The effect of different coating solutions of Cl-HY and different curing conditions on the chlorine loading

Coating Solution	Curing condition	Cl$^+$%[a]
5% Cl-Hy in water	95 ^{o}C, 1 h	0.03
5% Cl-Hy in water	95 ^{o}C, 1 h; 145 ^{o}C, 20 min	0.04
5% Cl-Hy in 0.1 N NaOH	95 ^{o}C, 1 h	0.04
5% Cl-Hy in 0.1 N NaOH	95 ^{o}C, 1h; 145 ^{o}C, 20 min	0.07
2% Cl-Hy in 1 N NaOH	95 ^{o}C, 1 h	0.33
2% Cl-Hy in 1 N NaOH	95 ^{o}C, 1 h; 145 ^{o}C, 10 min	0.37
2% Cl-Hy in 1 N NaOH	95 ^{o}C, 10 mins; 145 ^{o}C, 10 min	0.25

The best results were obtained for the condition 95 ^{o}C, 1 h; 145 ^{o}C, 10 min.

[a]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Characterization of the cotton swatches coated with HO-HY and Cl-HY. In Figures 41 and 42, the bands in the IR spectra at 1736 cm^{-1} and 1720 cm^{-1} indicate the presence of hydantoin moieties on the cotton swatches.[1-2] For the cotton coated with chlorinated HO-Hy, the new band at 1566 cm^{-1} is due to carboxylic salts (pH above 8). The band for the carbonyl group on the hydantoin ring shifted from 1719 cm^{-1} to 1736 cm^{-1} after chlorination because of the electron withdrawing effect of oxidative chlorine.
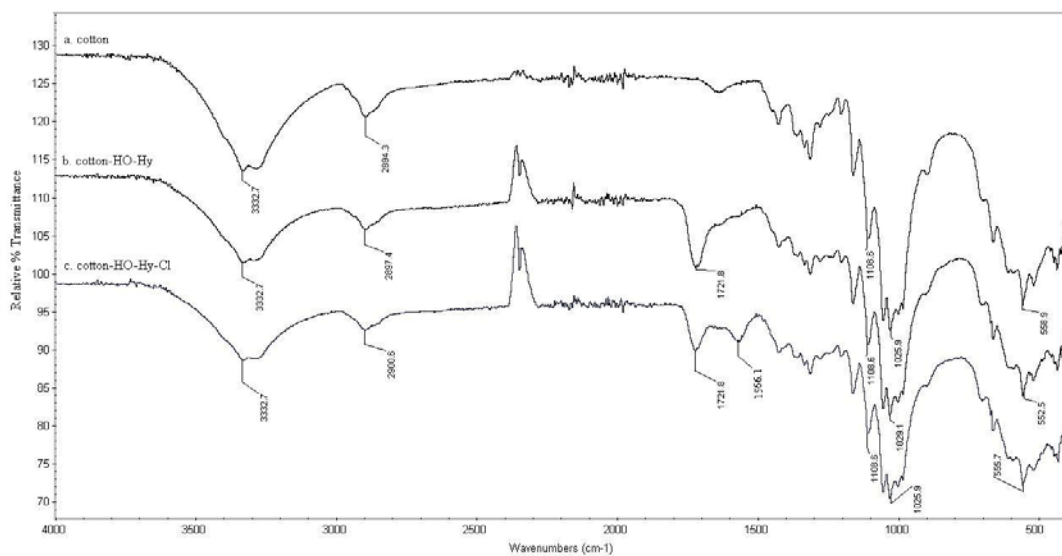
Figure 41. IR spectra of the pristine cotton and the cotton coated with HO-Hy before and after chlorination
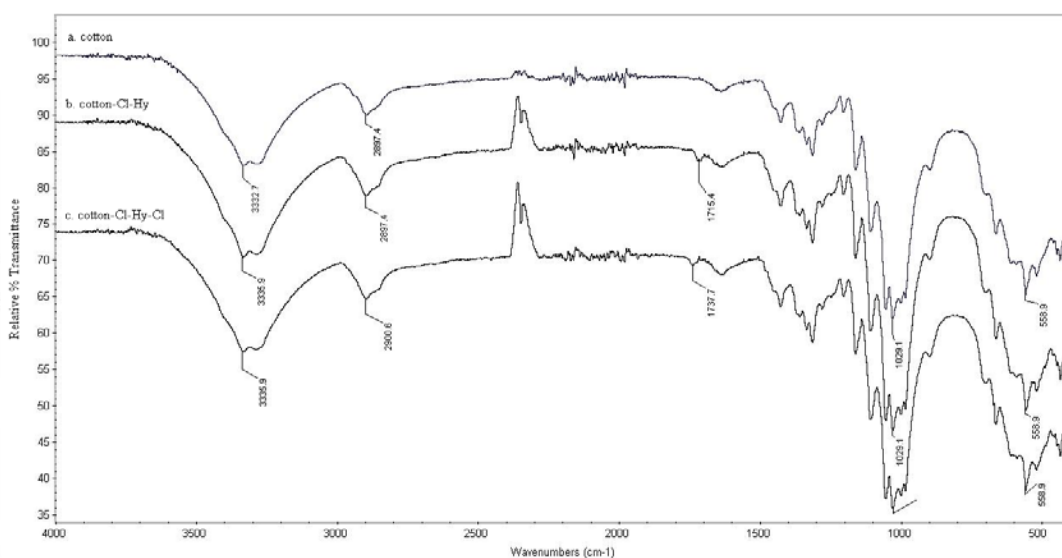
Figure 42. IR spectrum of the pristine cotton and the cotton coated with Cl-Hy before

and after chlorination

Washing test results for the cotton swatches coated with HO-HY and Cl-HY.

The greatest loss of Cl^+ was observed during the first washing cycle (equivalent to five machine washes) for both types of coated cotton swatches. The loss shown in X (in Table

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23) includes the loss of both chlorine itself and the coating. The difference between X and Y indicates the loss of the coating. The small difference between Y and Z means that in this case, prechlorination did not significantly protect the coating from hydrolysis from the surfaces of the cotton swatches.

Table 23 shows that the chlorine loading dropped dramatically during the first 5 washing cycles for both sets of samples, and nearly all of the chlorine was lost after 50 washing cycles. However, rechlorination replenished half of the oxidative chlorine for all of the samples.

Table 23. Washing testing of 5-hydroxymethyl-5-methyl-hydantoin and 5-chloromethyl-5-methyl-hydantoin

		X ($Cl^+wt\%$)[a]	Y ($Cl^+wt\%$)[a]	Z ($Cl^+wt\%$)[a]
	0	0.40	0.40	0.40
	5	0.03	0.25	0.24
HO-Hy	10	0.01	0.20	0.23
	25	0.01	0.20	0.19
	50	0.01	0.20	0.19
	0	0.27	0.27	
	5	0.03	0.18	0.18
Cl-Hy	10	0.02	0.18	0.17
	25	0.02	0.17	0.15
	50	0.01	0.17	0.14

X-Chlorination before washing; Y-Rechlorination after washing; Z-Chlorination after washing only.

[a]Data represents three trials; the error in the measurements was ±0.02 weight percent.

Biocidal testing of the cotton swatches coated with HO-Hy and Cl-Hy. Table 24 shows the results for cotton swatches coated with HO-Hy with 0.37% chlorine loading, which inactivated *S. aureus* within 1 min and *E.coli* with 5 min. The cotton swatches coated with Cl-Hy inactivated *S. aureus* and *E.coli* with 5 min. Both are thus very efficient biocides.

Table 24. Biocidal testing of cotton swatches of HO-Hy

Samples	Contact Time (min)	S. aureus		E.coli	
		Total bacteria (cfu/sample)	Log Reduction	Total bacteria (cfu/sample)	Log Reduction
HO-HY	15	6.70×10^5	1.22	5.49×10^6	0.12
	0	1.10×10^7	0	8.67×10^6	0
HO-HY-Cl (Cl^+=0.37%)	1	0	7.04	6.7×10^1	0.40
	5	0	7.04	0	6.94
	15	0	7.04	0	6.94
Cl-Hy	10	2.55×10^6	0.56	9.38×10^6	0.06
	0	9.33×10^6	0	1.07×10^7	0
Cl-Hy-Cl Cl^+=0.28%	1	2.01×10^2	4.67	0	7.03
	5	0	6.97	0	7.03
	10	0	6.97	0	7.03

Conclusions

The two compounds, 5-hydroxymethyl-5-methyl hydantoin and 5-chloromethyl-5-methylhydantoin, were coated onto cellulose substrates as evidenced by infrared spectra. The amide and imide nitrogen sites remained available for chlorination. The combination of amide and imide N-Cl showed rapid inactivation against both *S. aureus* and *E.coli*.

Cotton swatches coated with each of these two novel N-halamines also showed very good durability during the washing testing (see Table 23).

References for chapter 5

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2. Kocer, H. B.; Akdag, A.; Ren, X.; Broughton, R. M.; Worley, S. D.; Huang, T. S. Effect of alkyl derivatization on several properties of N-halamine antimicrobial siloxane coatings. *Ind. Eng. Chem. Res*. **2008***, 47*, 7558-7563.

Chapter 6

Polyurethane Films Incorporating Hydantoin Diols and a Tetraol

Introduction

Li and his coworkers prepared polyurethane paint films with a new N-halamine-containing diol, 5,5-dimethyl-3-(N,N-di-β-hydroxyl-ethyl-aminomethyl) hydantoin, commercial water-borne acrylic polyol formulation, and commercial diisocyanate formulation.[1] These films showed good biocidal efficacy. But this compound is found to not be stable over long-term storage.[2] So in this chapter, several new stable hydantoinyl diols and a tetraol (Figure 43) were synthesized and polymerized with commercial water-borne polyol and diisocyanate to form polyurethane films suitable for use as biocidal paints or biocidal coatings on a variety of surfaces. Because of the potential application as a polyurethane paint under sun light, the stability of all of these polyurethane films under UV light and laboratory light were tested.
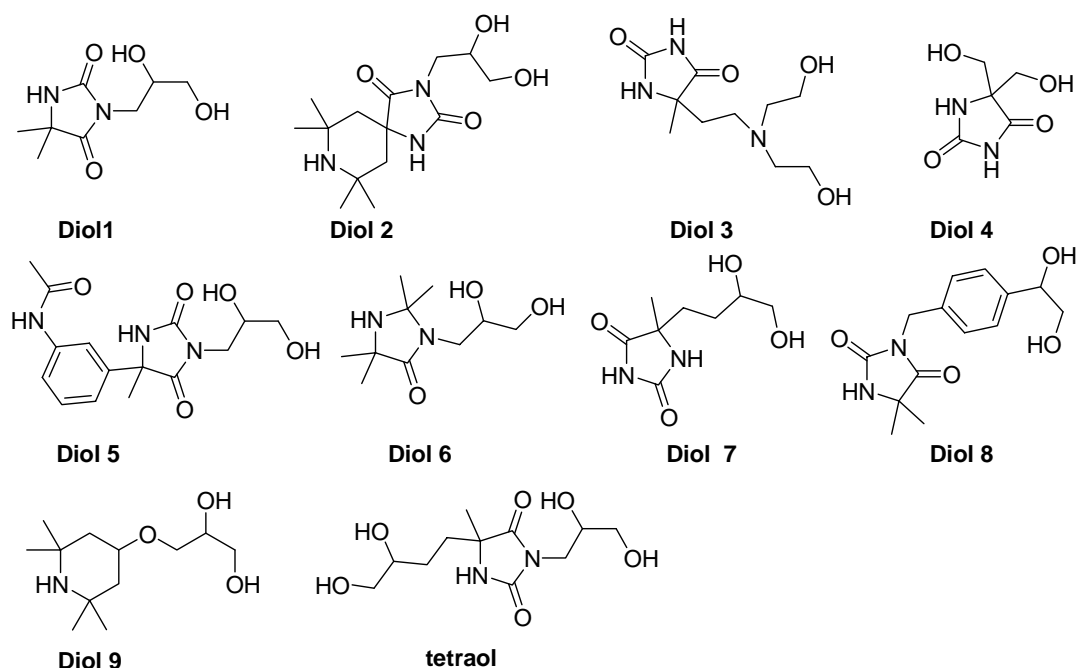
Figure 43. Structures of hydantoin diols and a tetraol

Experimental

Synthesis of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione (Diol 1). Here, a three step method (Scheme 15) was adopted: 5,5-dimethylhydantoin and equimolar quantities of KOH were added to ethanol, and the solution refluxed for 10 min. The 5,5-dimethylhydantoin potassium salts produced were isolated by removing the solvent under vacuum. Potassium salts were mixed with equimolar quantities of allyl bromide in DMF solution, and the resulting mixtures were stirred at 95°C for 16 h. After filtration, the DMF solvent was removed under vacuum. A yellowish liquid product was produced, with a yield of 99%.

11 mmol KMnO$_4$ were dissolved in 40 mL of distilled water, and then added dropwise into 40 mL of acetone in a 250 mL round flask containing 10 mmol

3-allyl-5,5-dimethylimidazolidine-2,4-dione and 20 mmol $MgSO_4$. The whole mixture was continuously stirred for 5-10 min, followed by filtration. Acetone and water were removed under reduced pressure. The $MgSO_4$ was removed by adding acetone and then filtering. After removing acetone by evaporation, a yellowish sticky product was produced with a yield of 85%.
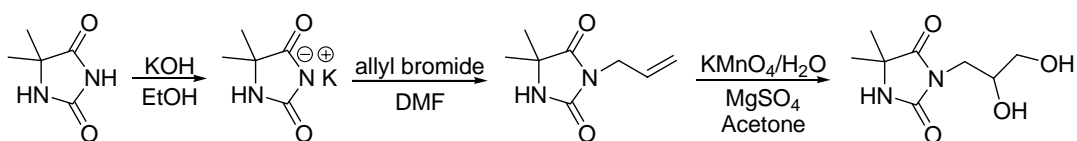
Scheme 15: The synthesis of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione (Diol 1)

A modified method (Scheme 16) was also tested: A mixture of 0.05 mol 5,5-dimethylhydantoin and 0.05 mol potassium hydroxide in 100 mL ethanol was refluxed for 10 min. This solution was mixed with equimolar quantities of 3-chloropropanediol in 25 mL of water and stirred for 16 h at ambient temperature. After the reaction was complete, ethanol and water were removed, and 50 mL of acetone were added to the flask. The potassium chloride produced in the reaction was removed by filtration. The acetone solvent was evaporated, and a transparent viscous oil was obtained. The experimental yield was 90%. Due to impurities in the product, the splitting pattern of signals were not observable.

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (6H), 3.29 (4H), 3.70 (1H) ppm; ^{13}C NMR (DMSO-d_6) δ 25.1, 41.9, 58.1, 64.6, 68.7, 156.2, 178.1 ppm.
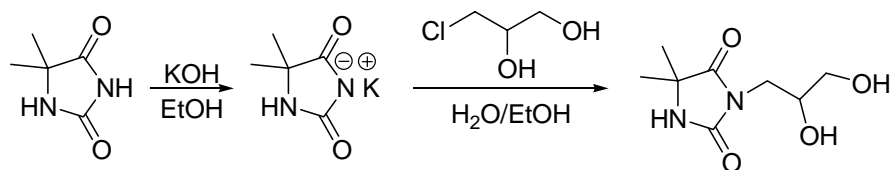
Scheme 16. A modified method to synthesize 3-(2,3-dihydroxypropyl)-5,5-dimethyl imidazolidine-2,4-dione (Diol 1)

Synthesis of 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (TTDD) (Scheme 17). 2,2,6,6-tetramethyl-4-piperidone, potassium cyanide, and ammonium carbonate were mixed in a molar ratio of 1:2:4, respectively, in ethanol/water (1:1 v/v) and stirred at room temperature for 3 days. After filtration, a white solid, 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione, was obtained. The yield was 93%.

Synthesis of 3-(2,3-dihydroxypropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro[4,5] decane-2,4-dione (Diol 2) (Scheme 17). A mixture of 0.05 mol of TTDD and 0.05 mol of potassium hydroxide in 100 mL ethanol was refluxed for 10 min. The solution was then mixed with equimolar quantities of 3-chloropropanediol in 25 mL water and stirred for 16 h at ambient temperature. Ethanol and water were removed, and 50 mL of acetone were added to the flask. The potassium chloride produced in the reaction was removed by filtration. The solvent, acetone, was evaporated under reduced pressure, producing a transparent viscous oil that was shown to be the desired product. The experimental yield was 67%.

Diol 2: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 (6H), 1.20 (6H), 1.45 (2H), 1.55 (2H), 3.30 (4H), 3.72 (1H), 4.70 (3H), 8.32 (1H) ppm; ^{13}C NMR (DMSO-d6), δ 30.2, 35.4,

42.2, 43.2, 48.7, 61.3, 64.6, 68.7, 156.8, 178.0 ppm.
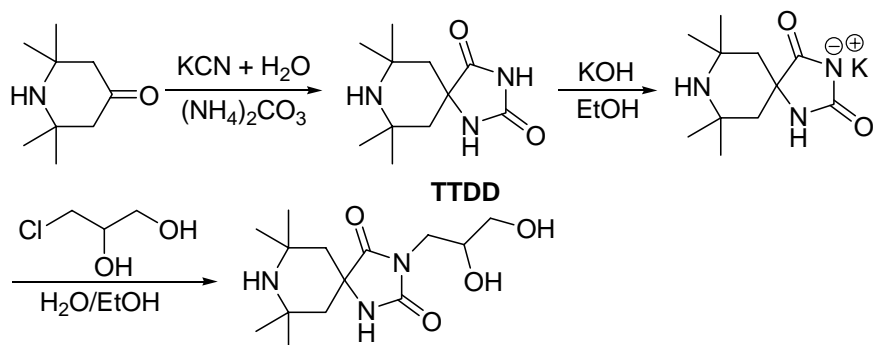
Scheme 17. A modified method to synthesize 3-(2,3-dihydroxypropyl)-7,7,9,9-tetramethyl -1,3,8 -triazaspiro[4.5]decane-2,4- dione (Diol 2)

Synthesis of 5,5-dihydroxymethylimidazolidine-2,4-dione (Diol 4) (Scheme 18).
2.25 g dihydroxyacetone, 3.26 g KCN, and 9.61 g ammonium carbonate were added to 10 mL H_2O, and the mixture was stirred at ambient temperature for 2 days. The mixture was extracted with 1-butanol. The solvent was evaporated under reduced pressure, and a slightly yellow compound was obtained. The yield was 98%.

Diol 4: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (s, 2H), 4.38 (q, 2H), 3.55 (q, 2H) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 173.9, 158.5, 69.2, 65.8, 64.7 ppm.
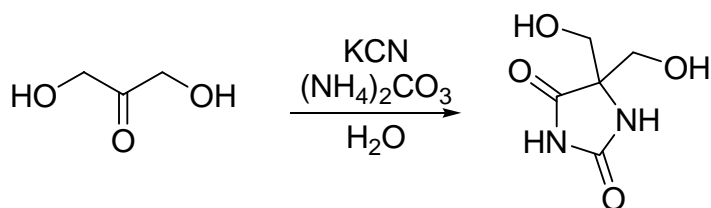
Scheme 18. Synthesis of dihydroxymethylhydantoin

Preparation of polyurethane coatings. The polyurethane paint samples were

prepared as follows: to 2 g of commercial water-borne acrylic polyol formulation (Series 297 part A, Tnemec Company, Inc) was added 0.15 g of diol or tetrol, with stirring for 2 min. Then 0.50 g of commercial isocyanate formulation (297-B converter Series 297 part B Enviro-Glaze, Tnemec Company, Inc) was mixed in, followed by the addition and mixing of 1.50 g of distilled water. The resulting formulation was immediately spread onto the surfaces of transparency film (570 cm^2) by a paint roller, and then dried in air at ambient temperature for 16 h.

The polyurethane film samples were prepared as follows: 0.6 g diisocyanate (297-B converter Series 297 part B Enviro-Glaze, Tnemec Company, Inc) , 0.3 g polyol (Adura 100 Polyol Resin, Air Products and Chemicals Inc), 0.15 g hydantoin compounds (Diol **1**, Diol **2**, BA-1 oligomer, or TTDD-siloxane) were mixed and heated at 60°C while stirring for 3 h. The resulting solution was coated onto transparency film, and a clear thin film formed after air drying overnight.

Chlorination procedure. The painted slides were chlorinated by immersion into a 10% aqueous solution of commercial bleach for 3 h. After rinsing thoroughly with distilled water, the painted slides were air dried at ambient temperature for 20 h and then at 45°C for 5 h. Similarly, the polyurethane films were chlorinated by immersion into a 10% commercial bleach solution buffered to pH 7 for 1 h. After rinsing with distilled water, the coated slides were air dried at room temperature overnight and then at 45°C for 7 h.

The bound oxidative chlorine was quantitatively determined using a modified iodometric/thiosulfate titration method. The surface concentration of the bound oxidative chlorine was calculated according to the following equation:

$$Cl^+ \left(atom/cm^2\right) = \frac{6.02 \times 10^{23} \times N \times V}{2 \times A}$$

where N is the normality (eqv/L), and V is the volume (L) of the sodium thiosulfate solution, and A is the surface area in cm^2 of the slides.

Biocidal efficacy. Both chlorinated and unchlorinated paint samples were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895). A "sandwich test" was employed to evaluate the efficacies of the samples (see blow). Here, for each sample, 25 μL of the bacterial suspension were placed in the center of a one inch square slide in a sterile Petri dish and covered with a second identical slide. After contact times of 30, 60, and 120 min, the slides were placed in sterile conical centrifuge tubes containing 5.0 mL of sterile 0.02 N sodium thiosulfate to quench any oxidative chlorine and vortexed for 2 min. The slides were then removed and the quenched dilutions diluted with pH 7, 100 μM phosphate buffer, after which they were plated on Trypticase soy agar. The plates were incubated at 37°C for 24 h, and then viable CFU of bacteria were counted.

UVA and laboratory light stability testing. Stability of the chlorinated paint films was measured using UV-light (Type A, 315-400 nm) produced by an Accelerated Weathering Tester instrument (The Q-panel Company, OH, USA) and laboratory light. After exposure, the films were titrated immediately and again after rechlorination.

Results and Discussion.

Results of biocidal testing. As shown by the data in Tables 25-28, chlorinated Diols **1-3**, diols **8-9**, and tetraol inactivated both *S. aureus* and *E. coli* O157:H7 within 60 min

and caused around a 5 log reduction. Diols **5-7** inactivated both bacteria within 120 min, but although diol **4** inactivated *E. coli* within 120 min, it could not kill *S. aureus* within the same time. The results for the films on transparency slides were different from those on cotton. This is likely to be because the bacterial suspension has a good contact with cotton, but on transparency slides the bacterial suspension solution can only make contact with the surface of the films. The hydantoin moieties of Diol **4** was fixed in the polyurethane chains more tightly than those of the other diols. This, combined with the tendency of *Staphylococcus* aggregates to form in grape-like clusters[2] that adversely affected their ability to make good contact with the surface, probably led to the inability of Diol **4** to kill *Staphylococcus* within the 120 min time limit.

Table 25. The efficacies of biocidal polyurethane paint with diols 1, 2, and 3 against S. aureus and E. coli O157:H7

[a]Sample	Contact time (min)	E. coli O157:H7		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Diol 1-Cl Cl^+atom/cm^2 $=2.38\times10^{17}$	0	5×10^4	0	6.67×10^4	0
	30	1.34×10^2	3.23	0	4.82
	60	0	4.70	0	4.82
	120	0	4.70	0	4.82
Diol 1	120	1.84×10^4	1.10	5.90×10^4	0.05
Diol 2-Cl Cl^+atom/cm^2 $=2.38\times10^{17}$	0	6.67×10^4	0	1.07×10^5	0
	30	1.47×10^3	1.66	0	5.03
	60	0	4.82	0	5.03
	120	0	4.82	0	5.03
Diol 2	120	2.88×10^4	0.36	6.83×10^4	0.19
Diol 3-Cl Cl^+atom/cm^2 $=9.05\times10^{16}$	0	2.17×10^5	0	3.37×10^5	0
	30	1.47×10^3	2.17	4.02×10^2	2.92
	60	0	5.34	0	5.53
	120	0	5.34	0	5.53
Diol 3	120	6.70×10^4	0.51	2.68×10^5	0.25

[a]See Figure 43 for the structure of compounds.

Table 26. The efficacies of biocidal polyurethane paint with diols 4, 5, and 6 against S. aureus and E. coli O157:H7

[a]Sample	Contact time (min)	E. coli O157:H7		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Diol 4-Cl Cl^+atom/cm^2 $=1.10\times10^{17}$	0	2.22×10^5	0	2.22×10^5	0
	30	6.70×10^2	2.53	1.87×10^4	1.07
	60	6.7×10^1	3.53	3.48×10^3	1.80
	120	0	5.36	9.38×10^2	2.37
Diol 4	120	1.27×10^5	0.25	1.74×10^5	0.10
Diol 5-Cl Cl^+atom/cm^2 $=1.53\times10^{17}$	0	1.67×10^5	0	1.73×10^5	0
	30	8.71×10^4	0.28	3.28×10^3	1.72
	60	1.94×10^3	1.94	1.14×10^3	2.18
	120	0	5.22	0	5.24
Diol 5	120	8.04×10^4	0.51	5.16×10^4	0.53
Diol 6-Cl Cl^+atom/cm^2 $=2.00\times10^{17}$	0	1.23×10^5	0	1.70×10^5	0
	30	1.07×10^5	0.06	1.14×10^3	2.17
	60	1.27×10^3	1.99	2.01×10^2	2.93
	120	0	5.09	0	5.23
Diol 6	120	5.72×10^4	0.33	1.15×10^5	0.17

[a]See Figure 43 for the structure of compounds.

Table 27. The efficacies of biocidal polyurethane paint with diols 7, 8, and 9 against S. aureus and E. coli O157:H7

[a]Sample	Contact time (min)	E. coli O157:H7		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Diol 7-Cl Cl^+atom/cm^2 2.50×10^{17}	0	7.67×10^4	0	1.20×10^5	0
	30	3.28×10^3	1.25	3.35×10^2	2.55
	60	2.41×10^3	1.38	0	5.08
	120	0	4.89	0	5.08
Diol 7	120	2.21×10^4	0.42	8.04×10^4	0.17
Diol 8-Cl Cl^+atom/cm^2 1.55×10^{17}	0	6.67×10^4	0	1.07×10^5	0
	30	0	4.82	0	5.03
	60	0	4.82	0	5.03
	120	0	4.82	0	5.03
Diol 8	120	3.28×10^4	0.51	5.70×10^4	0.27
Diol 9-Cl Cl^+atom/cm^2 2.50×10^{17}	0	9.33×10^4	0	1.20×10^5	0
	30	2.7×10^1	2.84	2.7×10^1	5.08
	60	0	4.97	0	5.08
	120	0	4.97	0	5.08
Diol 9	120	4.62×10^4	0.31	1.07×10^5	0.05

[a]See Figure 43 for the structure of compounds.

Table 28. The efficacies of biocidal polyurethane paint with tetraol against S. aureus and E. coli O157:H7

[a]Sample	Contact time (min)	E. coli		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Tetraol-Cl Cl^+atom/cm^2 $=2.32\times10^{17}$	0	7.67×10^4	0	7.31×10^4	0
	30	4.02×10^3	1.16	0	4.87
	60	0	4.89	0	4.87
	120	0	4.89	0	4.87
Tetraol	120	1.27×10^5	0.25	1.74×10^5	0.10

[a]See Figure 43 for the structure of compounds.

Table 29 and 30 show the biocidal data for the activity of the polyurethane films against *S. aureus* and *E. coli* O157:H7. The polyurethane film that incorporated Diol-**1** inactivated *S. aureus* and *E. coli* within 30 min. Films that incorporated TTDD diol with $1.49\times10^{18}\,Cl^+atom/cm^2$ inactivated the *E.coli* within 30 min, but for the films with TTDD oligomer, 120 min was required to kill *E.coli*. Neither could inactivate *S. aureus* within 120 min. The polyurethane films with BA-1 oligomer inactivated *S. aureu* within 60 min. There was a small log reduction for the films with no hydantoin moiety at around $1.00\times10^{17}\,Cl^+atom/cm^2$ due to the adhesion of the bacteria. Among all these films, the films with Diol **1** performed best. This may be due to the hydrophilic properties of Diol **1**; the structure of TTDD is more hydrophobic than the hydantoin ring, and the diol is more hydrophilic than the siloxane.

Table 29. Biocidal testing of polyurethane films against S. aureus and E. coli O157:H7

Sample	Contact time (min)	E. coli O157:H7		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Polyurethane film with Diol 1-Cl Cl^+atom/cm^2 $=7.51\times10^{17}$	0	1.77×10^6	0	1.43×10^6	0
	10	1.01×10^3	3.25	3.35×10^2	3.63
	30	0	6.25	0	6.16
	60	0	6.25	0	6.16
Polyurethane film with Diol 2-Cl Cl^+atom/cm^2 1.49×10^{18}	0	1.83×10^6	0	1.63×10^5	0
	30	0	6.26	3.15×10^3	2.71
	60	0	6.26	1.07×10^3	3.18
	120	0	6.26	3.35×10^2	3.69
Polyurethane film with BA-1 oligomer Cl^+atom/cm^2 5.92×10^{17}	0	4.67×10^6	0	1.43×10^6	0
	10	6.70×10^5	0.84	2.35×10^5	0.79
	30	1.27×10^3	3.57	2.81×10^4	1.71
	60	0	6.67	1.41×10^4	2.01
Polyurethane film with TTDD oligomer Cl^+atom/cm^2 2.16×10^{18}	0	1.83×10^6	0	1.43×10^6	0
	30	1.07×10^4	2.23	3.75×10^4	1.46
	60	2.68×10^2	3.83	2.35×10^3	2.66
	120	0	6.26	1.34×10^3	2.90

Table 30. Biocidal testing of polyurethane films with no hydantoin moiety against S. aureus and E. coli O157:H7

Sample	Contact time (min)	E. coli O157:H7		S. aureus	
		Total Bacteria	Log reduction	Total Bacteria	Log reduction
Polyurethane film without Diol	0	1.77×10^6	0	1.43×10^6	0
	120	6.70×10^4	0.510	2.88×10^5	0.695

Stability of polyurethane films incorporating hydantoinyl moieties exposed to UV light. As shown by the data in Tables 31, 32, and 33, the N-Cl bonds of Diol 1 (amide N-Cl) are the most stable, with 46% active chlorine remaining after exposure to UVA for 48 h. Diol 6 (amine N-Cl) exhibited a slower dissociation of N-Cl for the first 6 h, but then degraded rapidly after 6 h. For Diol 2-8 and tetraol, a small amount of oxidative chlorine remained even after exposure to UVA for 24 h, which indicated that most of N-Cl bonds had dissociated. There are both amide N-Cl and amine N-Cl bonds in Diol 2, both amide N-Cl and imide N-Cl in Diol 3, 4, and 7, but only amide N-Cl for 5, 8, 10. This result is consistent with the data obtained for the UV light stability of the cotton fabrics coated with the N-halamine siloxanes. Here, TTDD siloxane lost all of its chlorine in the shortest time, and BA-1 was the most stable among BA-1, TTDD, and TMIO-SIL.[3] The reason for this is not yet clear, and more research is needed.

After rechlorination, all of the oxidative chlorine is regained. Some of the data even show levels a little higher than the initial readings, possibly because the paint films are becoming more porous and hydantoin moieties in the inner film also take up more

chlorine.

Table 31. The stability and rechargeability of paint samples containing Diols 1 and 2 under UV light

[a]Diols	Time	Cl^+ atom/cm^2	After recharge
Diol 1	0 h	$2.63×10^{17}$	
	4 h	$2.30×10^{17}$	
	7.5 h	$2.05×10^{17}$	
	24.5 h	$1.46×10^{17}$	
	48 h	$1.21×10^{17}$	
	72.5 h	$7.94×10^{16}$	
	96 h	$7.94×10^{16}$	$2.84×10^{17}$
Diol 2	0h	$2.90×10^{17}$	
	1h	$1.90×10^{17}$	
	2h	$1.34×10^{17}$	
	3h	$1.16×10^{17}$	
	4h	$3.88×10^{16}$	
	5h	$3.88×10^{16}$	
	6h	$3.17×10^{16}$	
	24h	$9.56×10^{15}$	$3.00×10^{17}$

[a]See Figure 43 for the structure of compounds.

Table 32. The stability and rechargeability of paint samples containing Diols 3, 4, and 5 under UV light

[a]Diols	Time	Cl^+ atom/cm^2	After recharge
Diol 3	0 h	2.07×10^{17}	
	3 h	3.74×10^{16}	
	4 h	3.14×10^{16}	
	6 h	3.14×10^{16}	
	8 h	1.25×10^{16}	
	24 h	1.25×10^{16}	1.93×10^{17}
Diol 4	0 h	8.15×10^{16}	
	4 h	7.53×10^{16}	
	7.5 h	7.53×10^{16}	
	24.5 h	3.76×10^{16}	
	48 h	1.88×10^{16}	1.00×10^{17}
Diol 5	0 h	8.72×10^{16}	
	2 h	6.90×10^{16}	
	6 h	5.42×10^{16}	
	25 h	2.08×10^{16}	2.76×10^{17}

[a]See Figure 43 for the structure of compounds.

Table 33. The stability and rechargebility of paint samples containing Diols 6, 7, and 8 and tetraol under UV light

[a]Diols	Diol 6	Diol 7	Diol 8	Tetraol
0h	2.17×10^{17}	1.13×10^{17}	1.55×10^{17}	1.21×10^{17}
1h	1.75×10^{17}	4.80×10^{16}	1.09×10^{17}	9.94×10^{16}
2h	1.60×10^{17}	2.82×10^{16}	9.27×10^{16}	7.35×10^{16}
3h	1.38×10^{17}	2.25×10^{16}	8.06×10^{16}	5.18×10^{16}
4h	1.37×10^{17}	2.25×10^{16}	6.49×10^{16}	5.18×10^{16}
5h	1.25×10^{17}	1.88×10^{16}	5.93×10^{16}	3.03×10^{16}
6h	1.25×10^{17}	1.69×10^{16}	5.64×10^{16}	2.60×10^{16}
24h	5.69×10^{16}	1.69×10^{16}	2.82×10^{16}	2.07×10^{16}
After Recharge	2.13×10^{17}	2.26×10^{17}	1.57×10^{17}	2.26×10^{17}

[a]See Figure 43 for the structure of compounds.

Stability of polyurethane films incorporating hydantoinyl moieties under lab light. As Tables 34 and 35 show, Diol 1 is still the best performer, retaining 91% of its chlorine after 16 d. Diol 4 also showed good stability under lab light, with 85% chlorine remaining after 7 d, and Diol 6 was another good performer, probably due to its strong amine-chlorine bond.

Table 34. The stability and rechargebility of paint samples containing Diols 1, 2, 3, and 4 under lab light

Diols	Time (days)	Cl^+ atom/cm^2	recharge
Diol 1	0	2.21×10^{17}	
	2	2.20×10^{17}	
	8	1.82×10^{17}	2.21×10^{17}
	16	2.01×10^{17}	2.39×10^{17}
Diol 2	0	3.16×10^{17}	
	2	2.89×10^{17}	
	3	1.82×10^{17}	
	4	1.70×10^{17}	
	5	8.23×10^{16}	
	7	5.70×10^{16}	3.11×10^{17}
Diol 3	0	8.73×10^{16}	
	2	5.29×10^{16}	
	8	0	8.73×10^{16}
	16	0	9.41×10^{16}
Diol 4	0	1.07×10^{17}	
	7	9.11×10^{16}	1.35×10^{17}
	14	7.92×10^{16}	1.13×10^{17}
	21	7.53×10^{16}	1.34×10^{17}

Table 35. The stability and rechargebility of paint samples containing Diols 5, 6, 7, and 8 and tetraol under lab light

	Diol 5	Diol 6	Diol 7	Diol 8	Tetraol
0d	3.16×10^{17}	2.17×10^{17}	1.16×10^{17}	1.93×10^{17}	1.21×10^{17}
1d	2.90×10^{17}	2.00×10^{17}	7.34×10^{16}	1.89×10^{17}	1.02×10^{17}
2d	2.89×10^{17}	1.80×10^{17}	4.52×10^{16}	1.85×10^{17}	6.24×10^{16}
3d	1.82×10^{17}	1.60×10^{17}	3.95×10^{16}	1.63×10^{17}	6.00×10^{16}
4d	1.70×10^{17}	1.60×10^{17}	3.10×10^{16}	9.48×10^{16}	4.16×10^{16}
5d	8.23×10^{16}	1.30×10^{17}	2.26×10^{16}	9.13×10^{16}	2.88×10^{16}
6d	6.84×10^{16}	1.23×10^{17}	1.89×10^{16}	8.78×10^{16}	2.72×10^{16}
7d	5.70×10^{16}	1.23×10^{17}	1.63×10^{16}	1.93×10^{17}	1.21×10^{17}
21d		3.06×10^{16}			
After recharge	3.11×10^{17}	2.82×10^{17}		1.98×10^{17}	2.50×10^{17}

Diol 6 was recharged after 21 d, and diols 5, 7, 8, and tetraol were recharged after 7 d.

Conclusions

Most of chlorinated polyurethane paint with diols and a tetraol deactivate around 5 logs of *S. aureu* and *E. coli* O157:H7 within 120 min. Another clear polyurethane film incorporating Diol 1 inactivated both of *S. aureus* and *E. coli* O157:H7 within 30 min, while the clear films which incorporated Diol 2, BA-1 oligomer, and TTDD siloxane could not kill both *S. aureus* and *E. coli* within 120 min. These films also showed good stability exposed to UV irradiation and under lab light. Almost all of the chlorine was

regained after rechlorination.

References for chapter 6

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Chapter 7

Overall Conclusions and Recommendations for Future Work

In chapter two a question was proposed for copolymers of N-halamine siloxane and a quarternary ammonium salt siloxane. Could the solubility of the copolymers incorporating shorter chain quat groups (such as three methyl groups) be increased? Based on the previous research on long-chain quat copolymers, the solubility of this siloxane copolymer in water was increased considerably as compared to that of the hydantoinyl siloxane homopolymer. But it was not sufficient for industrial application. Trimethylamine has now been used to form these copolymers, and the results showed that these copolymers could be dissolved in only water to form aqueous baths with an extensive range of concentrations. The rapid inactivation of *S. aureus* and *E. coli* was due to the presence of N-halamine functional groups. Although the presence of the quat functional unit greatly improves the solubility of the copolymers in water, it does not affect the stability of chlorine loading on the cotton swatches during washing tests. Meanwhile, utilization of trimethylamine rather than dimethyldodecylamine greatly decreases the expense of application.

In chapter three, 5-Aminomethyl-5-methylhydantoin was designed to have both imide N-Cl and amide N-Cl bonds after coating onto the cotton swatches using the

crosslinker butanetetracarboxylic acid (BTCA) and subsequent chlorination. Utilization of BTCA increased the hydrophilic properties of the cotton swatches which assisted the inactivation of bacteria with high chlorine loading within 1 min. It also moderated the chlorination condition, as 1% original Clorox solution produced the same chlorine loading as a Clorox solution with pH adjustment to 7. The simulated washing tests showed that these kinds of crosslinking bonds provided a more durable coating, and less hydantoin moiety was washed off the surface of cotton swatches.

Some N-halamine siloxane coatings have been widely used in industrial applications, so much effort has been focused on developing more stable and durable N-halamine coatings. In chapter four, 6-phenyl-3-(3'-triethoxysilylpropyl)-1,3,5-triazinane-2,4-dione (MTPTD) was designed to have six-member rings rather than hydantoin rings and have more available N-H sites for chlorination. 6,6-Dimethyl-3-(3'-triethoxysilylpropyl)-1,3,5 -triazinane-2,4-dione was also synthesized to compare its stability with MTPTD. The results of stability testing under UV-chamber and lab lighting showed that the stability of these six-member rings is quite good compared to hydantoinyl siloxanes. Although an alpha hydrogen exists in the structure of MTPTD, the presence of the aromatic ring does enhance the stability of MTPTD. MTPTD was also employed to treat the surface of silica gel, and chlorination rendered these materials biocidal. The presence of two N-Cl amide bonds of MTPTD showed rapid inactivation of both *S. aureus* and *E. coli*.

Other N-halamine biocides were employed to form durable coatings as discussed in chapter five. Combination of imide N-Cl and amide N-Cl of the hydantoin ring showed good stability and biocidal activity in the previous research. 5-Hydroxymethyl-5-methylhydantoin (HO-Hy) and 5-chloromethyl-5-methylhydantoin

(Cl-Hy) were designed to form durable coatings with both imide N-H and amide N-H available in the hydantoin rings. BTCA could be used as a crosslinker for HO-Hy, or HCl could catalyze the reaction between cellulose and the hydroxyl group of HO-Hy. Cl-Hy would be coated onto the cotton swatches utilizing the catalysis of 1 N NaOH. Both of these two compounds provide stable and durable coatings on the cotton swatches. Utilization of BTCA as a crosslinker between cellulose and hydantoin moieties provided a new technique to make coatings which could find more practical uses in an industrial setting.

Several hydanotin diols and a tetraol were designed to prepare polyurethane films on various surfaces by copolymerization with commercial polyol and diisocyante in chapter six. These films could be coated on the walls, wood, cotton swatches, *etc*. Chlorination renders all of these surfaces biocidal, and the coatings showed good stability. Diol **1** seems to be suitable for two different polyurethane films discussed. It has very good solubility in water. The polyurethane films incorporated with Diol **1** shows great stability under UV and laboratory light, and it inactivated bacteria rapidly. It seems that these types of coatings could have potential application for improving the quality of living for human beings.

Future work

More work could be focused on the biocidal polyurethane films, such as for polyurethane painting. Copolymers of N-halamine siloxane and a quarternary ammonium salt siloxane could be directly mixed with some water-based latex paints because of good solubility of the copolymers in water. Also, some hydantoins with long chains could be

synthesized, and the chlorinated compounds could be directly mixed with some hydrophobic paints like acrylic paints. Physical interactions between the paints and long chains of hydantoin compounds should be strong which makes less loss of hydantoin compounds from the surfaces. Oxidative chlorine on the surface would inactivate bacteria. Good results have been obtained for coating some hydantoin compounds with hydroxyl groups onto cotton swatches using 1,2,3,4-butanetetracarboxylic acid. This technique could be also extended to treating other surfaces with active hydroxyl groups such as silica gel since the expense of the synthesis of some hydantoin diols is reasonable.