

**Synthesis, Structure-Bioactivity Relationship, and Application
of Antimicrobial Materials**

by

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Abstract

Antimicrobial N-halamine compounds were designed, synthesized and applied onto various materials to investigate and develop several properties of the N-halamine-based technologies. There are five projects covered in the study.

In the first two projects several novel derivatives of a commercial N-halamine coating were synthesized to improve the several properties of the coating. The antibacterial activity, stability, and ultraviolet (UV) light resistance of the different derivatives were compared to attempt to ascertain the influence of electronic, steric, and hydrophobic/hydrophilic effects for the N-halamine biocidal materials.

In the third project, a previously declared problem, the decomposition of para-aramid polymer when treated with bleach was investigated. Four mimics, representing the acid and the amine parts, of the para-aramid and meta-aramid polymers were synthesized. The degradation mechanism of the para-aramid polymer was stated.

In the fourth project, efforts were employed to provide antimicrobial property on commercial products. In the first part, a commercial nanofilter was treated with two N-halamine coatings to provide contact biocidal property. The treatment conditions were optimized considering the commercial manufacturing. The antibacterial activity and filtration efficiency were evaluated

after the treatments. In the second part, a commercial fiber containing N-H functionality was treated with acid and bleach to provide antimicrobial property. The antibacterial activity, UV light stability, and storage stability of the treated fibers were evaluated.

In the last project; cellulose, starch and a commercial polymer containing N-H functionality were dissolved in a common solvent and then extruded into fibers. The physical properties and water absorbency characteristics of the composite fibers were evaluated. The antibacterial activity and UV light resistance of the chlorinated composite fibers were examined.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Infectious diseases have been the number one killer in human history, except the very early stages.¹ Giralamo Fracastoro first theorized in 1546 that diseases were transmitted by tiny agents, cannot be seen by the naked eye, which he called “seminaria” means the seeds of disease. A century and a half later the theory, with the improvements in microscopy, Antonie van Leeuwenhoek described the number of animalcules (microorganisms) in the scurf of a tooth might exceed the number of men in a kingdom. Two hundred years later the visual observation of microorganisms, Louis Pasteur put the existence of germs and the germ theory of disease together.¹ The new science of microbiology started just on time because the nineteenth century was the era of urbanization. The majority of people came to live in densely crowded cities because of the rapid industrialization. Crowded cities proved to be ideal environments for the spread of infectious microorganisms and produced many epidemics and pandemics (smallpox, cholera, tuberculosis, several yellow fever, Spanish flu, etc.). There was optimism around the world about infectious diseases after World War II due to development in antibiotics, antimicrobials, and better sanitation. The golden period of antibiotics was between 1945

to 1970 when highly effective agents were discovered and developed. However, since the 1980s, the introduction of new agents has declined. Parallel to this there has been an alarming increase in pandemics and bacterial resistance to existing agents.¹⁹

Nowadays, globalization (increasing international travel, global commerce, etc.) has unleashed microorganisms and spread them everywhere like a computer virus on the internet.¹ In recent years, several lethal outbreaks have raised public fear. Besides the wide-ranging geographic spread, the speed of spreading was dramatic as shown by SARS in 2003, Bird flu in 2004-2008, and Swine flu in 2009. Infectious diseases are the leading cause of death worldwide and the third leading cause in the United States.² Moreover, most of the factors that contribute to the spread of infectious disease will continue in the future, such as increasing international travel, lack of adequate health care, urbanization, population growth, etc.² Beside the normal course of events, some of the infectious diseases may be deliberately spread by acts of bioterrorism involving pathogens. In this regard, the protection bar should be raised even higher by this unreasonable prospect.

Healthcare-associated infections (nosocomial infections) are an increasingly important problem for medical facilities in spite of expanding infection control efforts.^{3,4}

Nosocomial infections are a result of treatment in a healthcare service, but secondary to the patient's original condition. In this regard, numerous bacteria involved in nosocomial infections are multidrug-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and *Clostridium difficile* have relatively long survival times on surfaces and become resistant to antimicrobial agents over time.^{5,6} Overuse and misuse of antimicrobial agents are main contributors to the antimicrobial resistance.² For example,

the rate of *C. difficile* infection is estimated to be 13% in patients who stay in the hospital for two weeks, and increases to 50% in those who stay four weeks.⁷

Contaminated materials serve as important sources of cross-infections due to liberating of microorganisms into the air or transferring to the surroundings through direct or indirect contact.^{3,4} The transmission of the microorganisms through contamination of surfaces in the environment among patients and healthcare workers can be minimized by deactivating them on the contaminated surfaces within sufficiently brief time intervals. A hospital study found that a universal bleach-based cleaning protocol could reduce the rate of nosocomial *C. difficile* infections by two-thirds.⁸

Antimicrobial materials have the potential to reduce the risk of the next SARS agents, and particularly antimicrobial textiles can limit the survival of microbes incriminated in the nosocomial infection phenomenon. Antimicrobial agents which can limit the spreading rate of the infectious diseases have a cost; however, prevention from infectious diseases is easier, cheaper, and healthier than curing them afterwards. Numerous antimicrobial agents have been designed to minimize the spread of infections in medicinal facilities, in other public venues, and in the home environments.

Antimicrobial agent usage in healthcare facilities is critical, and is increasing in household applications. There is a need for new and better antimicrobial agents for various applications and for emerging microorganisms.

1.2 Bacterial Cells and Inactivating Mechanism of Antibacterial Agents

Bacteria are divided mainly into two classes: Gram-positive and Gram-negative. They both have similar internal (including cell membrane), but very different external

structures as detailed in Figure 1.1. Gram-positive bacteria (i.e. *S. aureus*) have a thick cell wall containing peptidoglycan and teichoic acids. Gram-negative bacteria (i.e. *E. coli*) have a relatively thinner cell wall consist of peptidoglycan surrounded by a second lipid membrane (outer membrane) containing lipopolysaccharides. Gram-negative bacteria are generally more resistant to antibacterial agents than are Gram-positive ones because of the extra layer, the outer membrane. The outer membrane can restrict the uptake of the agents and limit the concentration of active biocide which can reach the target site(s).⁹ The outer membrane also can perform as a molecular filter for hydrophilic compounds.

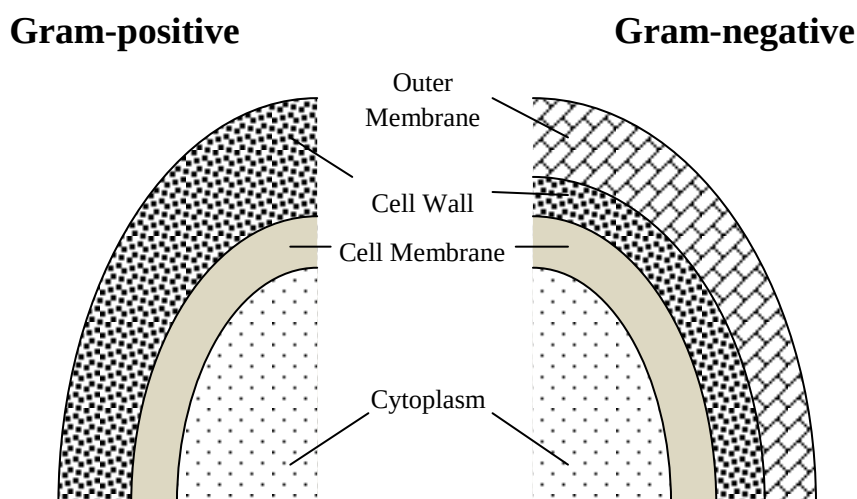


Figure 1.1. Cell structures of Gram-positive and Gram-negative bacteria.

The most common inactivating mechanism of biocides is damaging the integrity of cell membrane (cytoplasmic membrane).^{10,11} First, biocides bind and accumulate on the cell surface, then penetrate the outer membrane and cell wall, and finally interact with the target site(s). The final attack occurs mainly in two ways:

(1) The rupture in the cell membrane causes membrane malfunction and leakage of cytoplasmic constituents which eventually result in death of the bacteria. Quaternary ammonium salts, phenols, and biguanides have this kind of biocidal mechanism which lyse the cell by physical (ionic) interactions.¹²⁻¹⁴

(2) Chemical interaction between biocides and the cytoplasmic constituents (i.e. proteins, RNA, and DNA) may occur to inhibit the growth or inactivate the bacteria.^{14,15}

For example, chlorine affects respiration and DNA activity.⁵⁹ For the chloramines, once chloramine penetrates the cell wall, it readily reacts with four amino acids (cysteine, cystine, methionine, and tryptophan) by oxidation of thiol groups. The inactivation mechanism for chloramines is therefore involving inhibition of proteins or protein-mediated processes such as respiration.^{17,18}

1.3 Common Biocides

A biocide is a chemical substance capable of killing living organisms, whereas an antimicrobial is a substance that kills or inhibits the growth of microorganisms. A biocide can be a pesticide including fungicides, insecticides, and algicides, or an antimicrobial including antibacterials, antivirals, antifungals.¹⁹ In this regard, antimicrobial is commonly used for substances that kill microorganisms. Antimicrobial agent as used in the study most properly matches with disinfectant, a term that is applied to non-living materials that destroy microorganisms, and generally is distinguished from antibiotics that destroy microorganisms within the body, and from antiseptics which destroy microorganisms on living tissues. However, disinfectants are closely related to antibiotics

and antiseptics because their main purpose is to inhibit infections. A perfect disinfectant would offer complete sterilization without harming other forms of life, and would also possess the qualities of a long lifespan, being inexpensive, and non-corrosive.

A microorganism may be transmitted by more than one route. The main routes of transmissions are contact (direct or indirect), droplet (coughing, talking, etc.), airborne (evaporated droplets), common vehicle (medical devices), and vector borne (mosquitoes, flies, etc.). Antimicrobial agents can limit the transmission rate by limiting some of the routes mentioned above.

The most common biocides include biguanides, quaternary ammonium salts, peroxides, alcohols, heavy metals and halogens. These antimicrobial agents have different physical properties and inactivate microorganisms via different mechanisms, therefore, have different limitations.

Biguanides are cationic biocides and demonstrate good antimicrobial activities by cell membrane disruption. Several polybiguanides are used as disinfectants in contact lens cleaning solutions. One of the most common biocidal biguanide is Chlorhexidine (CHX) which has a strong affinity to bind various surfaces and leaves a residue of antimicrobial activity.^{12,20,21} It has been used in treatment of acute and chronic wounds, antimicrobial soaps, and antiseptic hand gels. However, microorganisms can become resistant to CHX over time.²⁰

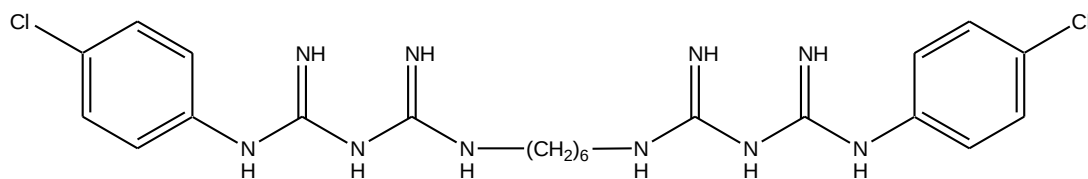


Figure 1.2. Structure of CHX.

Quaternary ammonium salts (QASs) target the inner membrane of the microorganisms by ionic interaction with phospholipids resulting in cell-wall leakage and intercellular coagulation.¹⁴ The inactivation mechanism is an electrostatic interaction of the N^+ site with the negatively charged cell surface allowing the lipophilic chain to penetrate through the cell wall and disrupt it which is a relatively slow inactivation mechanism. QASs exhibit maximum activity when they have at least one alkyl substituent at the nitronium site, which is usually eight to nineteen carbon atoms.²² QASs are not highly effective against Gram-negative bacteria and are ineffective against spores. Additionally, several bacteria can become resistant to QAS.^{20,23} QASs are used in cosmetics, the food industry, and household disinfection.

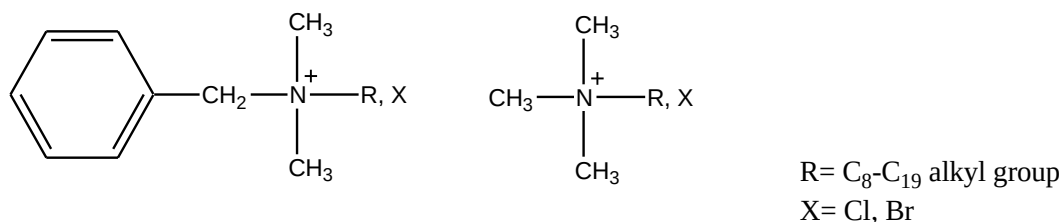


Figure 1.3. General structure of quaternary ammonium salts.

Peroxides are powerful antimicrobial agents against bacteria, fungi, and spores.^{24,25} They disrupt enzymes and proteins by oxidizing thiol groups. They are used as wound cleaners, surface cleaners for sterilization applications, and in sewage and swimming pools for

odor control. Hydrogen peroxide vapor is used as a room disinfectant. The disadvantages of peroxides are that they readily evaporate, have no residual effect, and are fairly toxic.

Alcohols also cannot provide long-term biocidal activity because of their low boiling points. In general, short chain alcohols, such as ethanol and isopropanol, are mostly used for instant antimicrobial applications. They need a significant concentration 50-70 wt% to be effective.¹² Alcohols are organic solvents and can dissolve the coating of microbes to damage their membrane integrity which results in leakage.²⁵ In addition to that alcohols can also form hydrogen bonds with proteins/enzymes, denature them in bacteria, and inactivate their catalytic functions.¹² Alcohols are, however, not effective against resistant fungal and bacterial spores, or endospore forming bacteria such as *C. difficile*.²⁶ Alcohols are widely used in disinfecting sprays in hospitals and in hand sanitizers due to their fast action.

Heavy metals such as copper, silver, zinc, and their salts are toxic to most living beings. Heavy metals ions (cation) penetrate the cell into the cytoplasm to inhibit the growth of bacteria by cleavage of the disulfide bonds within proteins. They also form complexes with proteins or precipitate out the proteins. They are cheap and effective. However, they are very toxic to human beings (i.e., mercuric chloride). Silver and copper are relatively less toxic, however their inactivation rate is very slow. Silver atoms have been introduced into polymers in zeolite carriers (nanoaluminosilicate particles). The silver ions exchange with other positive ions (often sodium) from the moisture in the environment as shown in Figure 1.4. Recently commercialized silver care washing machines have been designed using this technology.

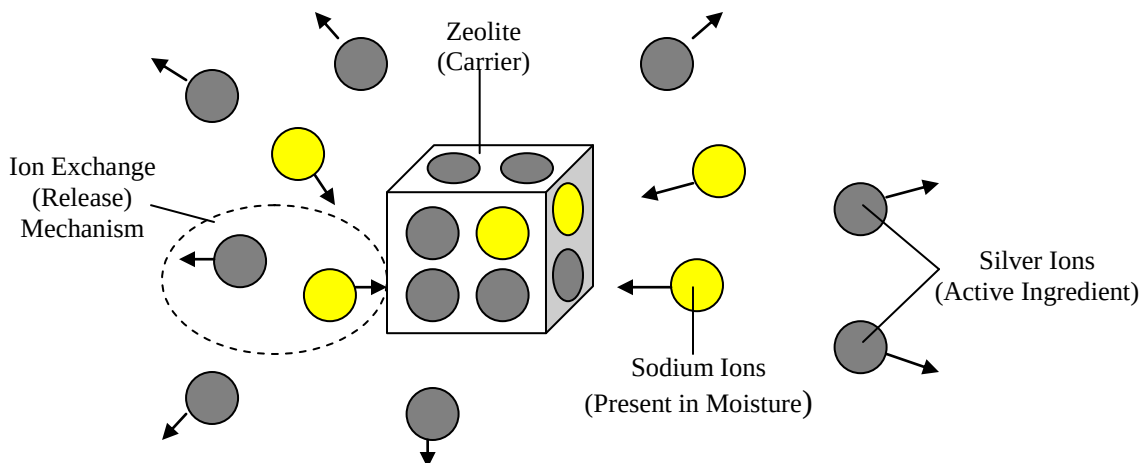


Figure 1.4. Release of silver ions from multi-faced carrier.²⁷

Halogens such as Cl_2 , Br_2 , I_2 , NaOCl , and ClO_2 react with the amino groups in proteins and oxidize the proteins in the protoplasm.¹² The oxidation causes irreversible destruction of enzymatic activities and thus inactivates the bacteria.²⁸ Halogens are inexpensive and very effective against broad-spectrum microorganisms; bacteria, spores, and viruses.^{18,25}

Chlorine has been widely used for water disinfection and in swimming pools for decades due to strong biocidal activity. The bleaching properties of chlorine gas in water solution led to its first practical application in textile bleaching, followed by commercial production in 1785.²⁵ In 1825, Labarraque first used chlorinated soda solution (sodium hypochlorite) as a disinfectant. Chlorine exists as hypochlorous acid (HOCl) below pH 7.6 and as hypochlorite ion (OCl^-) above pH 7.6, and both HOCl and OCl^- are called free chlorine. HOCl is stronger in bactericidal action than OCl^- .²⁵ One of the most common household uses of free chlorine is bleach containing around 5-6 wt% sodium hypochlorite. The use of chlorine in aqueous media is being questioned because of the toxic disinfection by-products, such as chlorinated hydrocarbons and oxidized organic

compounds.^{29,30} However, by far the most effective home disinfectant is the commonly used chlorine bleach which is effective against most common pathogens and is inexpensive. Chlorine bleaches do not cause bacterial resistance because they act as a general chemical oxidant on multiple cell components in a very direct physical way.²⁶ Numerous antimicrobial agents based on different technology have been designed for various applications according to target properties such as rate of inactivation, durability, cost, etc. For example, there are three levels of disinfection in hospitals; high-level, intermediate-level, and low-level disinfection. A high-level disinfectant is effective against bacteria, viruses, and bacterial endospores. Hydrogen peroxide, formaldehyde/alcohol, and ethylene oxide can be considered as high-level disinfectants and are often used to treat medical and surgical materials. Intermediate-level disinfectant is effective against broad-spectrum of microorganisms except bacterial endospores. Iodine+alcohol, chlorine compounds, and phenolic compounds can be given as examples for intermediate-level disinfectants. Low-level disinfectants are ineffective against bacterial endospores, *Tubercle bacillus* (mycobacteria), and small nonlipid viruses. Quaternary ammonium compounds are low-level disinfectants with relatively poor activity against Gram-negative bacteria.²⁵

1.4 N-Halamines

Halogens are very effective biocides, however they are not very stable in water and on surfaces. N-halamines (combined halogen compound) are much more stable than are free halogens. N-halamines are well known as halogen “stabilizers” to retard the loss of

halogen from aqueous medium, e.g. swimming pools. They react with halogens to abstract and retain an oxidative halogen in a relatively stable, noncorrosive form. The bond between nitrogen and halogen atoms is temporary and this “stored” halogen is released in response to contact with microorganism.³¹ The stability also means that the N-halamine does not degrade under the normal conditions of use and storage, until the oxidative chlorine is needed. N-halamines have been demonstrated to be one of the most efficacious biocidal agents due to their long-term stability, non-toxicity to humans, biocidal function against a broad range of microorganisms, and regenerable properties upon exposure to washing cycles.³²⁻³⁴ In addition, more stable N-halamines release very low concentration of free chlorine into aqueous medium decreasing the amount of halogenated hydrocarbon by-products produced in bleach disinfection.

N-halamines are defined as compounds containing one or more nitrogen-halogen covalent bond(s), N-X moiety. There are three types of N-X moieties; amine, amide, and imide. Due to their differences in chemical environment (polarity), they have different stabilities towards dissociation of the N-X moiety, in the order amine > amide > imide halamine, while the antimicrobial activity is in reverse order, Figure 1.5.³⁵

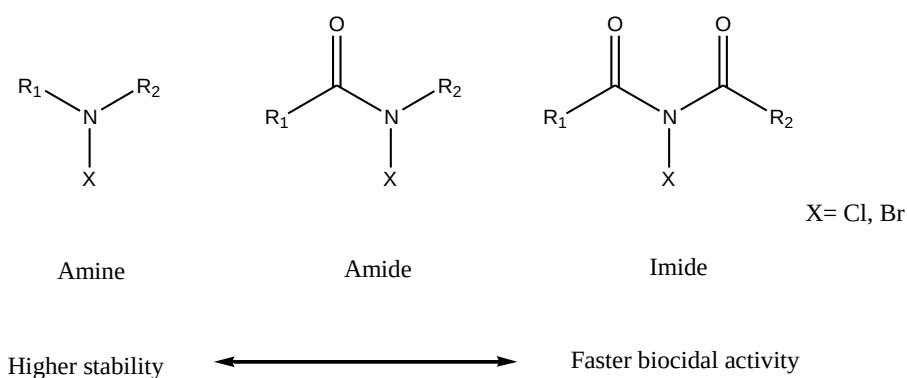


Figure 1.5. Characteristics of different N-X moieties.

The N-Cl bond dissociation constant for amine, amide, and imide moieties are $<10^{-12}$, $<10^{-9}$, and $<10^{-4}$, respectively.³⁶ In this regards, an N-halamine structure is designed considering stability or rapid activity according to application. Generally, amide N-halamines are preferred for providing both good stability and strong biocidal activity.³⁰ Electron withdrawing groups (i.e. carbonyl) next to N-X moiety weakens the N-X covalent bond. On the other hand, electron donating groups (i.e. alkyl) substituted onto the carbon atom adjacent to the N-X moiety strengthen the N-X covalent bond.³⁵

Among various N-halamine compounds, heterocyclic structures such as hydantoins, oxazolidinones, imidazolidinones, and triazines have been studied intensively to achieve higher stability compared to aliphatic structures.³⁷ Figure 1.7 shows the structures of numerous cyclic N-halamine compounds; containing amine group(s) (1-3), amide group(s) (4-6), imide group(s) (6, 9), both amide and imide groups (10, 11), and amine, amide and imide groups(s) (12). In general, imidazolidinones are more stable than oxazolidinones.³⁸ Among various N-halamine compounds, particularly cyclic N-halamines containing no α -hydrogen (the hydrogen on the carbon atom adjacent to N-X moiety) are preferred to avoid from alpha dehydrohalogenation reaction.³⁹

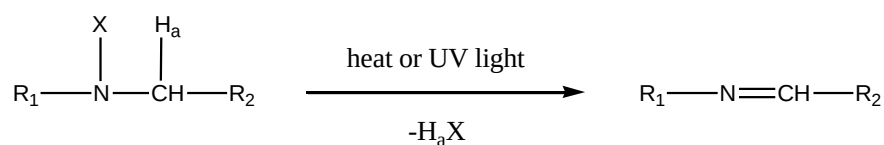


Figure 1.6. Alpha dehydrohalogenation.

^a H_a: alpha hydrogen.

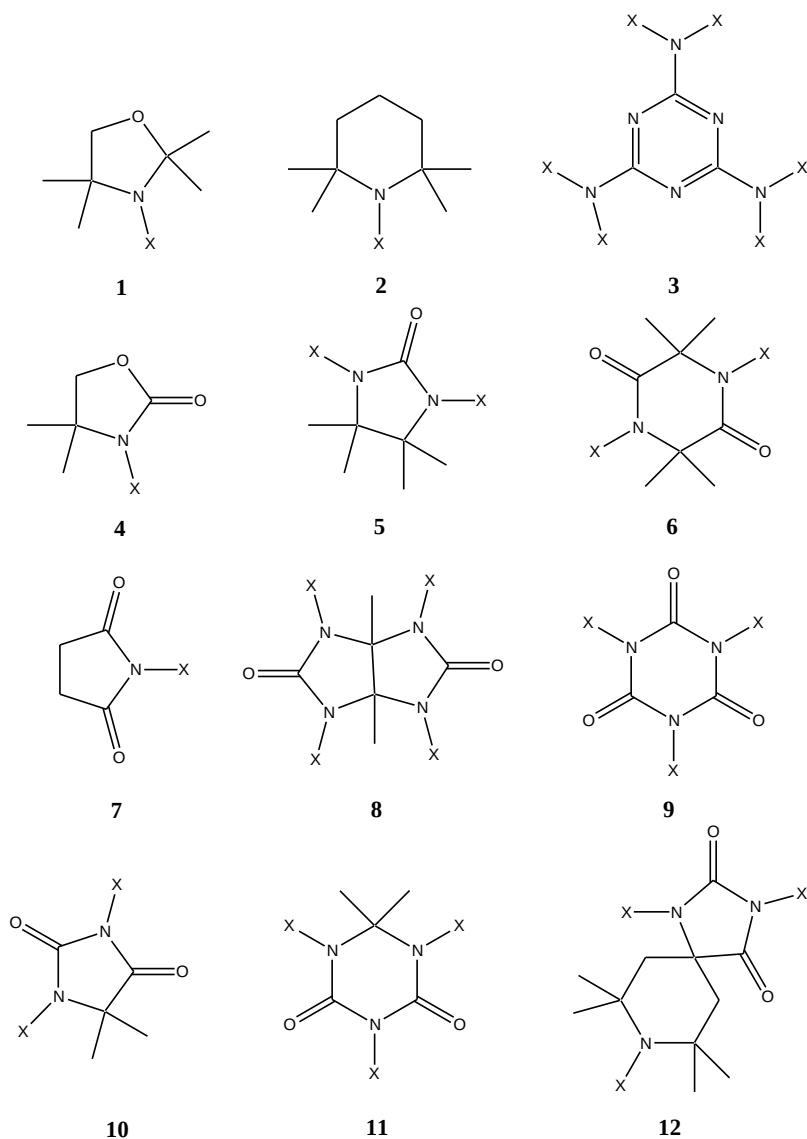


Figure 1.7. Structure of heterocyclic N-halamines. (X = H, Cl, or Br)

For X = H ; **1**: 2,2,4,4-tetramethyl-1,3-oxazolidine, **2**: 2,2,6,6-tetramethylpiperidine, **3**: 1,3,5-triazine-2,4,6-triamine (melamine), **4**: 4,4-dimethyl-2-oxazolidinone, **5**: 4,4,5,5-tetramethyl-1,3-imidazolidin-2-one, **6**: 3,3,6,6-tetramethyl-2,5-piperazinedione, **7**: 2,5-pyrrolidinedione (succinimide), **8**: 3a,6a-dimethyltetrahydroimidazo[4,5-d]imidazole-2,5(1H,3H)-dione, **9**: 2,4,6-trihydroxy-1,3,5-triazine (cyanuric acid), **10**: 5,5-dimethyl-2,4-imidazolidinedione (5,5-dimethylhydantoin), **11**: 6,6-dimethyl-1,3,5-triazine-2,4-dione, **12**: 7,7,9,9-tetramethyl-1,3,6-triazospiro[4,5]decane-2,4-dione.

The N-halamine bond is formed by reaction of an amine, imine, amide, or imide with halogen (X_2), hypohalous acid (HOX), or hypohalite (XO^-).³⁸ However, chloro and bromo derivatives are of commercial importance. N-halamines are generally halogenated in neutral to slightly acidic solutions. The basicities of N-halamines are in the order of amine>amide>imide, and therefore the rate of halogenation is in the same order amine>amide>imide.³⁸

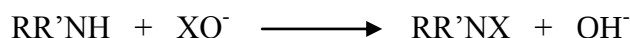
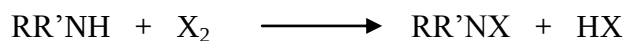


Figure 1.8. Halogenation of substituted N-halamine precursors.³⁸

One of the unique characteristic of N-halamine compounds is that they can be recharged with oxidative chlorine by exposure to dilute chlorine bleach, hopefully under conditions that occur during typical laundering. The reversible chlorination and reaction with bacteria is pictured in Figure 1.9.³¹ The “stored” chlorine is released in response contact with microorganisms.

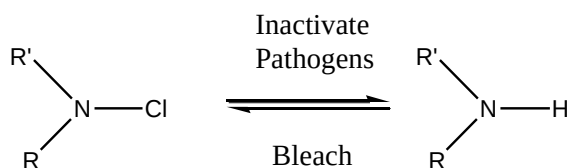


Figure 1.9. Regenerable property of N-halamines.

1.5 N-Halamine-based Polymeric Materials

In general, N-halamine-based polymeric materials could be obtained in two ways;

- (1) N-halamine precursors are bound to polymers with chemical bonding, or
- (2) N-halamine polymers can be formed from N-halamine precursors.

1.5.1 N-Halamine Coatings

N-halamine precursors can be covalently bound to substrates via various tethering groups such as siloxanes, epoxides, and hydroxyls. First, the N-halamine moiety attached to tethering group and then bound to material via tethering functionality. Figure 1.10 shows the different attachment methods of 5,5-dimethylhydantoin onto OH containing surfaces such as cellulose. 5,5-dimethylhydantoin has been used in industrial water treatment and in sanitizing spas since 1950s, and found many other applications after introducing the tethering groups.

Alkoxy silane (siloxane) is a very useful surface coupling agent and numerous N-halamine precursors have been successfully attached to them.⁴⁰ Hy-sil (Figure 1.10(A)) is produced by the reaction of hydantoin salt and 3-chloropropyltriethoxysilane. Worley et. al.⁴¹ first described the synthesis of Hy-sil which is now a commercially available product. Numerous studies have shown that Hy-sil could provide antimicrobial property on various materials such as cellulose, polyester, silica gel and paint.⁴²⁻⁴⁴

Another useful surface coupling agent is epoxides and they have been investigated for biocidal applications. Porret⁴⁵ synthesized Hy-epox (Figure 1.10(B)) to improve the flow properties of polyglycidyl ethers. However, Liang et al.⁴⁶ first described the use of

Hy-epox in biocidal materials. Hy-epox is highly water soluble and very suitable for commercial applications by eliminating organic solvent usage during the coating process.

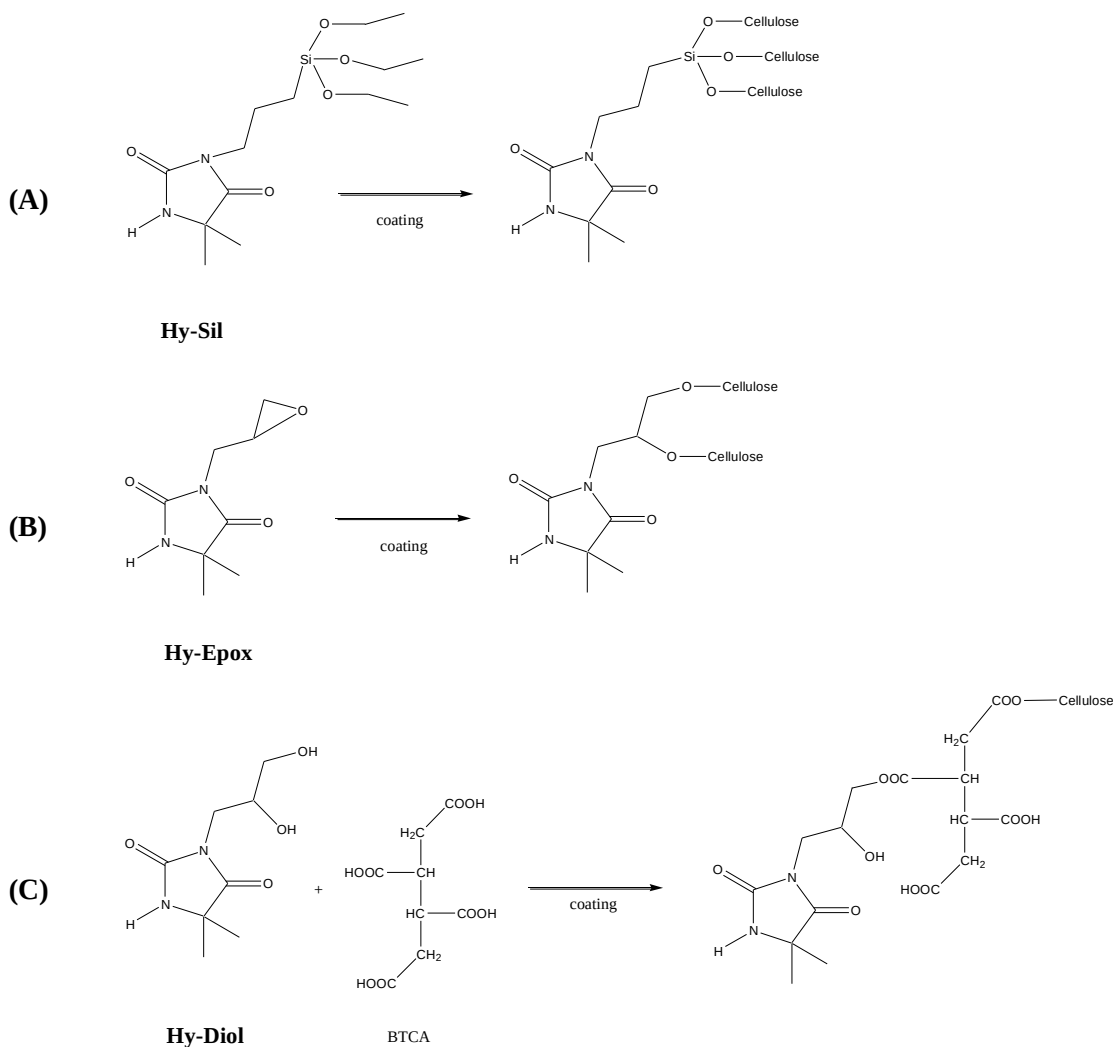


Figure 1.10. Attachment of hydantoin ring onto cellulose via various tethering groups; triethoxysilylpropyl (a), glycidyl (b), and 2,3-dihydroxypropyl (c).

Recently, Ren et. al.⁴⁷ described the synthesis of Hy-diol (Figure 1.10(C)), containing two hydroxyl tethering groups. Hy-diol coated onto cellulose with the assistance of a cross-linking agent 1,2,3,4-butanetetracarboxylic acid (BTCA) method which was determined previously by using amine (instead of diol) functionality.⁴⁸ The stabilities of

N-halamine coating towards repeated laundry washing and UV light exposure were excellent. The coating inactivated both Gram-negative and Gram-positive bacteria within very short contact times. BTCA was being used to provide permanent press property in cotton fabrics, therefore when this molecule was mixed with N-halamine and applied as a finish to cotton, both antimicrobial and permanent press effects were observed.

Numerous N-halamines were attached to polymers such as cellulose, polyamide, and polyester via grafting technology. Lin et al.^{49,50} chemically bonded the hydantoin ring, 3-hydroxymethyl-5,5-dimethylhydantoin (MH), onto previously functionalized polyamide and polyester fabrics as described in Figure 1.11. For the polyamide, first they formed a hydroxymethyl functional group at the amide nitrogen of the polymer via formaldehyde solution, and then reacted with MH. For the polyester, first they treated the fabric with ammonium hydroxide solution to form amide end groups due to alkaline hydrolysis of ester bonds, and then attached the hydantoin moiety (MH) onto the polymer. Modified polyamide fabrics showed excellent antimicrobial property after chlorination, total inactivation of 7 log of *E. coli* and *S. aureus* within 10 min, whereas the modified polyester fabric had relatively low biocidal performance, 7 log reduction of total 9.1 log *S. aureus* within 30 min.

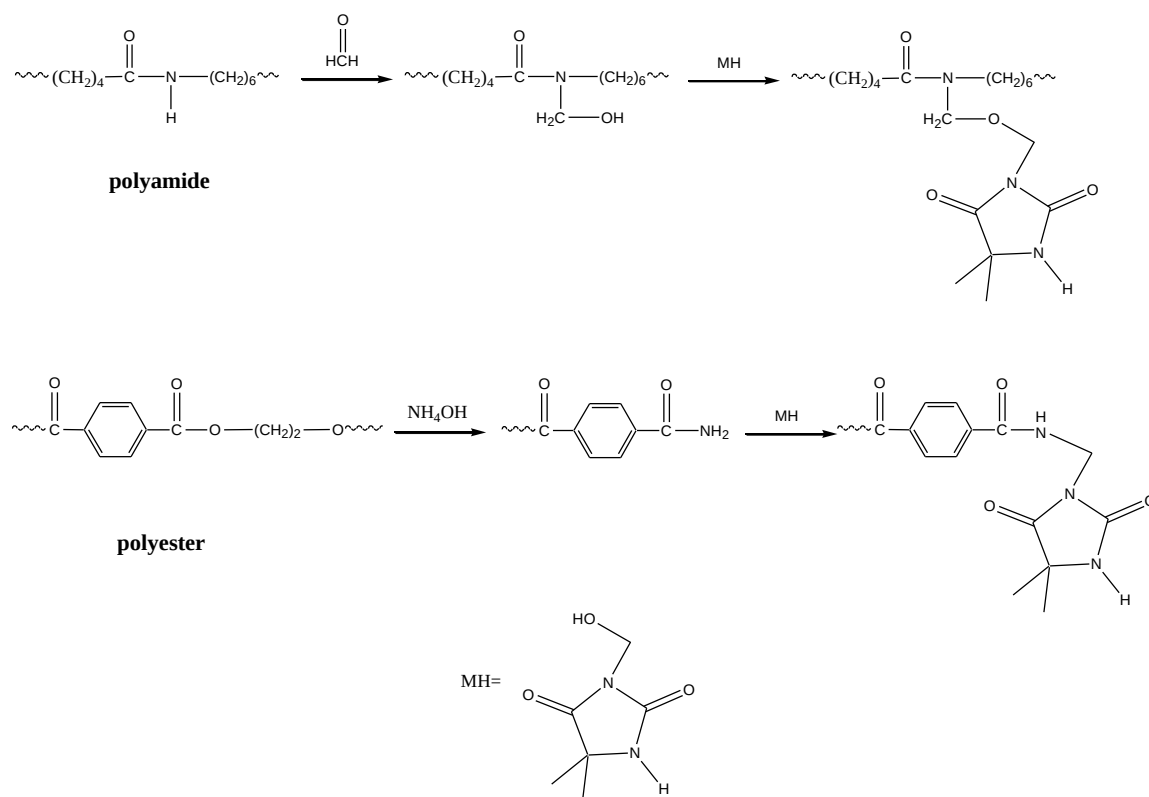


Figure 1.11. Hydatoin ring grafting onto polyamide and polyester.

For many polymeric materials, antimicrobial treatment is more difficult because the polymer is chemically inert, making the covalent binding impossible, or the covalent binding reaction deteriorates the physical properties of the polymer.

1.5.2 N-Halamine Polymers

The N-halamine chemistry was advanced towards synthesis of N-halamine polymers during the past decade, due to great commercial potential in water disinfection and biocidal materials. The consumption of polymeric N-halamine materials is expected to increase during the current decade. The N-halamine polymers can be prepared mainly in four ways; (1) a polymerizable moiety such as vinyl, or allyl group can be used to functionalize an N-halamine compound followed by polymerization, (2) a commercial

polymer can be converted into an N-halamine polymer via chemical modification, (3) a commercial polymer itself could have N-H functionality which can be converted to N-X, and (4) an N-halamine compound could simply be blended with a commercial polymer.³⁸

In the first method, N-halamine compounds are functionalized to provide N-halamine monomers and then polymerized. The most common polymerization technique has been used is addition polymerization via monomer molecules having unsaturated bonds such as vinyl bonds (-C=C-). Sun and coworkers⁵¹ synthesized a novel cyclic N-halamine monomer, 3-allyl-5,5-dimethylhydantoin (Mon 1), for further polymerization of Poly 1 as described in Figure 1.12. In addition, Mon 1 was copolymerized with various other monomers such as acrylonitrile, vinyl acetate, and methyl methacrylate.

The polymerization of homopolymer Poly 1 was difficult due to the radical “autoinhibition” of the allylic structure, the radicals are stabilized due to two equivalent allyl resonance structures. However, as commonly known in free radical vinyl polymerization mechanism, the effect of the substituent on the polarity of vinyl moiety is extremely important for the reactivity. In this regards, Sun and coworkers⁵² successfully synthesized 3-(4'-vinylbenzyl)-5,5-dimethylhydantoin (Mon 2), homopolymer Poly 2, and its copolymers with vinyl acetate, acrylonitrile and methyl methacrylate, under mild conditions. With respect to the Mon 1, higher reactivity and better monomer compatibility were achieved due to the existence of a phenyl ring. Halogenated Poly 2 and its copolymers were found stable and very effective antimicrobial materials. Similar approaches were used to synthesize N-halamine polymers via using substituted vinyl groups such as acryloyl ($\text{H}_2\text{C=CH-C=O-}$), and acrylate ($\text{H}_2\text{C=CH-C=O-O-}$).^{53,54}

The synthesized copolymers can also be applied to various surfaces as thin films by dissolving them in organic solvents followed by removal of the solvent.

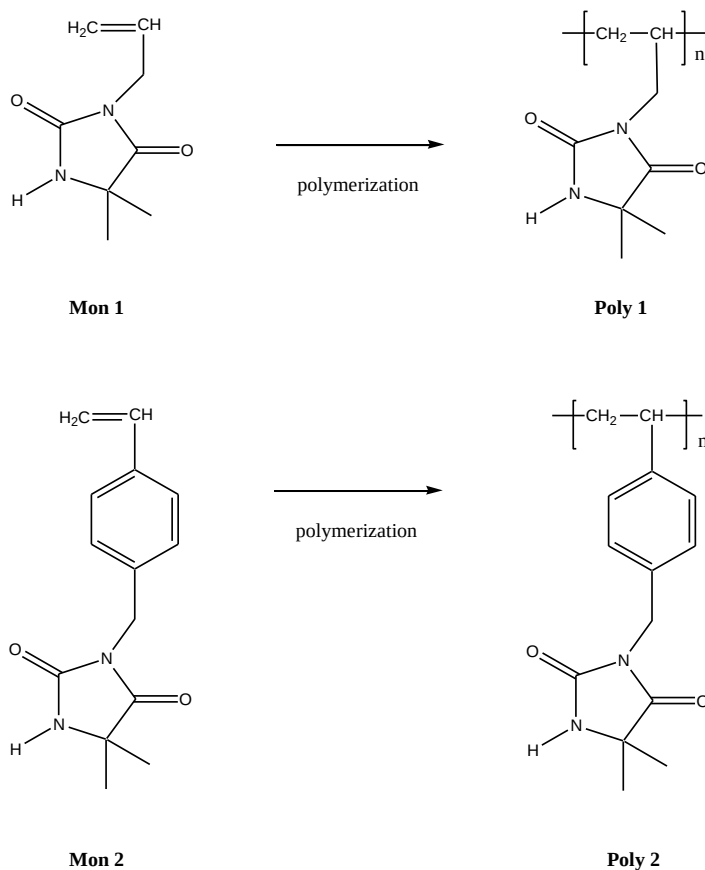


Figure 1.12. Structure of hydantoin-containing vinyl monomers and their homopolymers.

Ren et al. polymerized Mon 2 on the surface of polyester fibers through admicellar polymerization.⁵⁵ The polymer films were very stable on the fibers towards chlorination, washing, and UVA light exposure. The treated fibers completely inactivated Gram-positive and Gram-negative bacteria between 10 to 30 min of contact time after chlorination.⁵⁵

The second method to construct N-halamine polymers is conversion of commercial polymers into N-halamine materials with modification. Sun and Worley⁵⁶ successfully converted polystyrene into poly(5-methyl-5-(4'-vinylphenyl)-hydantoin) (Poly 3) as shown in Figure 1.13. In the first step, polystyrene was converted into poly(4-vinylacetophenone) with the Friedel-Crafts acylation, then in the second step, hydantoin ring was formed around the ketone moiety via the Bucherer-Berg reaction.

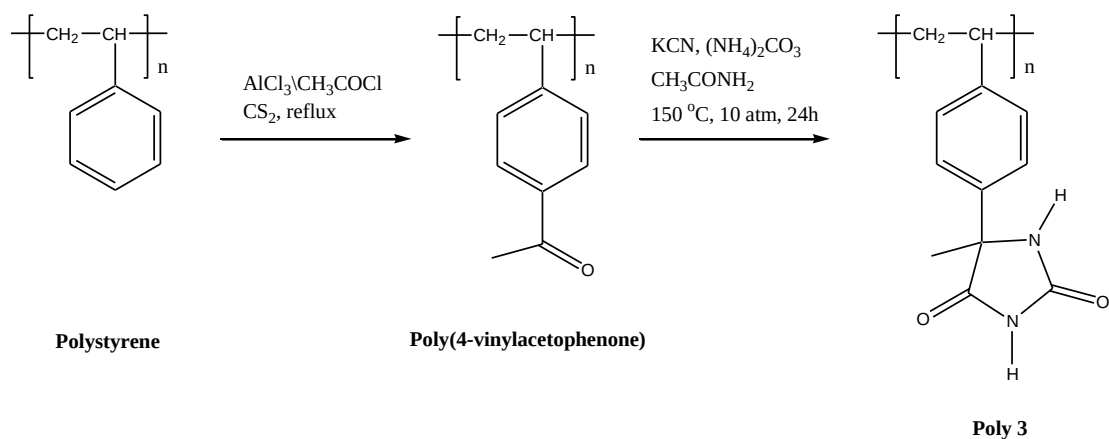


Figure 1.13. Synthesis of poly(5-methyl-5-(4'-vinylphenyl)-hydantoin).⁵⁶

Poly 3 was in the form of granular amorphous solids and interestingly suffered from limitations due to irregular size distribution. Chen further studied Poly 3 to provide more uniform and microporous beads.⁵⁷ The halogenated Poly 3 inactivated various bacteria, fungi, and rotavirus in only seconds of contact time in flowing water. Brominated Poly 3 showed relatively better performance than chlorinated Poly 3 due to weaker N-Br bond compared to N-Cl bond.⁵⁷ Poly 3 can be chlorinated up to chlorine loadings around 20 wt% (the theoretical value is 24.9 wt%) and the chlorinated polymer releases less than 0.1 mg/L free chlorine into flowing water.⁵⁷ The polymer is commercially used for water

disinfection in developing countries and in antimicrobial nonwoven fabrics used in personal care products.

The third approach to N-halamine polymers is the direct chlorination of commercial polymers containing N-H functionality. The simplicity of this approach is very attractive for commercial applications. Wayman et al. first reported direct chlorination of polymeric amines, amides, and imides to turn them into N-Halamines for effective chlorine exchange resins used in chemical synthesis.⁵⁸ One of the most important properties that should be considered for N-H moiety containing polymers is the absence of α -hydrogen in the structure which might reduce the stability and refreshability of the N-X moiety.

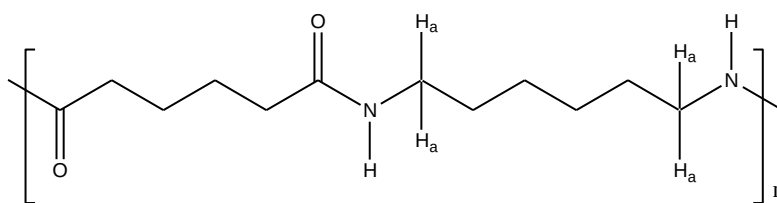
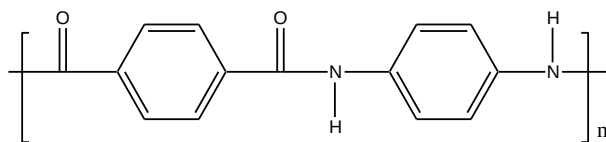


Figure 1.14. Structure of an aliphatic polyamide (PA 6.6) containing α -hydrogens.

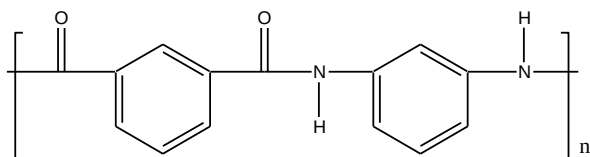
^a H_a: alpha hydrogen.

Most of the polyamides and polyurethanes include α -hydrogen in their structures, whereas some aromatic polyamides do not as shown in Figure 1.15. In this regard, Sun and co-workers have pointed out that poly(m-phenyleneisophthalamide) (meta-aramid) and poly(p-phenyleneterephthalamide) (para-aramid) could be excellent candidates for N-halamine polymers. Despite the similarity in their structures, chlorinated meta-aramid fabrics provided excellent antimicrobial activity against a broad spectrum of microorganisms while para-aramid fabric seriously decomposed after chlorination.⁵⁹ Akdag et al. described the decomposition mechanism of para-aramid upon chlorination.⁶⁰

Luo et al.⁶¹ coated para-aramid fabric with polymethacrylamide and provided antimicrobial property towards various microorganisms without compromising the original mechanical performance of the polymer.



poly(p-phenyleneterephthalamide)



poly(m-phenyleneisophthalamide)

Figure 1.15. Commercial polymers containing N-H functionality.

^a H_a: alpha hydrogen

Sandstrom et al. reported that chlorination has no significant effect on meta-aramid properties over time and the N-Cl bond is relatively stable towards washing, UV-light exposure, radiant heat exposure and storage.⁶² The results of the study suggested that the direct halogenation of commercial meta-aramid fabric can be used for durable antimicrobial applications.

The last method to enhance N-halamine materials is incorporation of N-halamine compounds into polymers. This method may suffer from the leaching potential of N-halamine compounds from the polymer over time. In addition, the N-halamine compounds generally form domains in the polymer matrix and stay embedded in

structure. However, migration of the additives to the surface is needed for sufficient antimicrobial property, especially for hydrophobic polymers.

1.6 N-Halamine based Antimicrobial Textiles

Numerous antimicrobial textiles have been developed after Lister first demonstrated the relationship between fibrous materials and disease in 1867.⁶³ In general, an ideal antimicrobial textile material requires these features: (1) rapid and total inactivation of a broad spectrum of microorganisms; (2) nonselective and nonmutable to pathogens; (3) nontoxic to higher life forms; (4) durable to repeated washes; (5) stable until needed; and (6) being active or regenerable over the life span of fabric.⁶³

N-halamines have been proved to be effective against broad spectrum of microorganisms.^{32-34,64} N-halamines inactivate microorganisms by an oxidation mechanism rather than biological functions, therefore, bacteria cannot get resistance to N-halamine structures.²⁶ N-halamines are not toxic and are being used widely in swimming pools, disinfection of water, etc. Most of the N-halamine compounds are applied onto textiles via covalent bonding and generally can survive up to 50 machine washing cycles.

N-halamines are regenerable because the halogenation and inactivation mechanisms are reversible redox reactions. The outstanding antimicrobial property of N-halamine based textiles is the regenerability upon simple treatment with household bleach.

Textile materials consist of fibrous structures that are very susceptible to contamination by various microorganisms. Moreover, some of the pathogenic microorganisms can survive longer than 90 days on the materials, and could be dispersed into the air or

transferred to the surrounding environments through direct or indirect contact.⁶⁵

Therefore, textile materials can be described as vehicles for the transport (spread) of pathogens and are important sources of cross-infections such as nosocomial infections. Antimicrobial textiles can limit the survival of microorganism to an extent that can be beneficial in controlling infectious disease transmission. The antimicrobial property is applied to various textile materials such as gowns, masks, uniforms, bedding materials, drapes, pillows, mattresses, dishcloths, etc. in institutional and household-settings.

Rising concerns about infectious diseases are promoting the use of new antimicrobial textiles. Since preventing emerging and re-emerging infectious diseases is cheaper and healthier than curing them, control of microbial contaminations on fibrous materials is required. Antimicrobial coatings can be applied onto fabrics with a simple pad-dry-cure process. Moreover, the coating compounds can be added into general finishing processes with other chemical agents. On the other hand, the antimicrobial agents can be incorporated into fibers as additives. The additives must be heat stable and uniformly mixed with the molten polymer, in dry melt spinning. The antimicrobial additives generally form domains in the fiber and stay inside the polymer which hinders the biocidal action. For dry or wet spinning, the polymer and antimicrobial agents are first dissolved in a common solvent. After the extrusion, the composite fiber is solidified by removal of the solvent. In this method, high molecular weight additives would be expected to exhibit better performance due to leaching out potential of low molecular weight additives from the fiber. In general, antimicrobial coatings onto materials might be more efficient and lower cost compared to the antimicrobial additives. The coating procedure introduces the antimicrobial property only at the surface of the materials, while

the additives are dispersed throughout entire polymer. Since the inactivation of bacteria occurs only at the surface of the materials, embedded additives do not contribute into the antimicrobial activity. Linear N-halamine polymers could be dissolved in a solvent and then applied onto fabrics to enhance thin biocidal films on the fiber surface.

Textile materials are considered as solid waste after consumed. The amount of solid waste generated by health care facilities has been increasing due to increasing usage of disposable materials. The waste can be incinerated or hauled to landfills. Incineration of all waste is impractical, and disposing without sanitizing can introduce disease organisms to the landfills, such as disposable linens.²⁵ In this regard, use of regenerable antimicrobial materials (i.e. N-halamines) in health care facilities become very advantageous.

1.7 Research Projects

There are five independent projects covered in the study. In the first two projects several derivatives of commercial Hy-sil were synthesized to improve the several properties of the coating. In the first project, five novel derivatives of Hy-sil were synthesized and then coated onto cotton fabric. The antibacterial activities, stabilities, and ultraviolet (UV) light resistances of the different derivatives were compared to attempt to ascertain the influence of electronic, steric, and hydrophobic/hydrophilic effects for the N-halamine biocidal materials. In the second project, two novel derivatives of Hy-sil were synthesized and then coated onto cotton fabric. The antibacterial activities and washing stabilities of the coated fabrics were evaluated. Especially, the stabilities toward UV light

exposure of the derivatives were compared and their degradation mechanisms were investigated via various spectroscopy techniques. In the third project, a previously declared problem, the decomposition of para-aramid polymer when treated with bleach was investigated. Four mimics, representing the acid and the amine parts, of the para-aramid and meta-aramid polymers were synthesized. The degradation mechanism of para-aramid was stated. In the fourth project, efforts were employed to provide antimicrobial property on commercial products. In the first part, a commercial nanofilter was treated with two N-halamine coatings to provide contact biocidal property. The treatment conditions were optimized considering the commercial manufacturing. The antibacterial activities and filtration efficiencies were evaluated after the treatments. In the second part, a commercial fiber containing N-H functionality was treated with acid and bleach to provide antimicrobial property. The antibacterial activity, UV light stability, and storage stability of the treated fibers were evaluated. In the fifth project; cellulose, starch and a commercial polymer containing N-H functionality were dissolved in a common solvent and then extruded into fibers. The physical properties and water absorbency characteristics of the composite fibers were evaluated. The antibacterial activities and UV light resistances of the chlorinated composite fibers were examined.

1.8 References

- [1] Tierno, P.M. *The Secret Life of Germs*. **2001**, New York, Atria Books.
- [2] Binder, S.; Levitt, A.M.; Sacks, J.J.; Hughes, J.M. Emerging Infectious Diseases: Public Health Issues for the 21st Century. *Science*. **1999**, 284, 1311-1313.
- [3] For example see: Hambraeus, A. Infection Control from a Global Perspective. *J. Hosp. Infect.* **2006**, 64, 217-223.
- [4] For example see: (a) Harbartha, S.; Saxa, H.; Gastmeier, P. The Preventable Proportion of Nosocomial Infections: an Overview of Published Reports. *J. Hosp. Infect.* **2003**, 54, 258–266. (b) Hambraeus, A. Transfer of *Staphylococcus aureus* Via Nurses' Uniforms. *J. Hyg.* **1973**, 71, 799-814. (c) Hedin, G; Hambraeus, A. Multiply Antibiotic-resistant *Staphylococcus epidermidis* in Patients, Staff and Environment - a One-week Survey in a Bone Marrow Transplant Unit. *J. Hosp. Infect.* **1991**, 17, 95-106. (d) Scott, E.; Bloomfield, S. F. The Survival and Transfer of Microbial Contamination Via Cloths, Hands and Utensils. *J. Appl. Bacter.* **1990**, 68, 271-278.
- [5] For example see: (a) Hardy, K. J.; Oppenheim, B. A.; Gossain, S.; Gao, F.; Hawkey, P. M. A Study of the Relationship Between Environmental Contamination with Methicillin-resistant *Staphylococcus aureus* (MRSA) and Patients' Acquisition of MRSA. *Infect. Cont. Hosp. Epidem.* **2006**, 27, 127-132. (b) Neely, A. N.; Maley, M. P. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastics. *J. Clinic. Microbiol.* **2000**, 724-726. (c) Boyce, J. M.; Potter-Bynoe, G.; Chenevert, C.; King, T. Environmental Contamination Due to Methicillin-resistant *Staphylococcus aureus*: Possible Infection Control Implications. *Infect. Cont. Hosp. Epidem.* **1997**, 18, 622-627.

- [6] Williams, J.F.; Suess, J.C.; Cooper, M.M; Santiago, J.I.; Chen, T.Y. Mackenzie, C.D.; Fleiger, C. Antimicrobial Functionality of Healthcare Textiles: Current Needs, Options, and Characterization of N halamine-Based Finishes. *Res. J. Text. Apparel*. **2006**, 4, 1-11.
- [7] http://en.wikipedia.org/wiki/Clostridium_difficile. accessed 05/12/2009.
- [8] <http://www.medpagetoday.com/MeetingCoverage/IDSA/6935>. accessed 05/12/2009.
- [9] Russell, A. D. Bacterial Resistance to Disinfectants: Present Knowledge and Future Problems. *J. Hosp. Infec.* **1999**, 43, S57-S68.
- [10] Russell, A.D. Bacterial Spores and Chemical Sporicidal Agents. *Clin. Microbiol. Rev.* **1990**, 3, 99-119.
- [11] Van Asselt, A.; Te Giffel, M. Pathogen Resistance and Adaptation to Disinfectants and Sanitizers. *Underst. Pathogen Beh.* **2005**, 484-506.
- [12] Wu, Rong. Preparation, Bioactivity, and Application of Novel Biocidal Materials. Dissertation. **2004**, Auburn, Alabama.
- [13] Kou, L. Preparation and Application of Regenerable N-halamine Biocidal Materials. Dissertation. **2009**, Auburn, Alabama.
- [14] Denyer, S.P.; Stewart, A.B. Mechanism of Action of Disinfectants. *Int. Biodet. Biodeg.* **1998**, 41, 261-268.
- [15] Maillard, J.Y. Bacterial Target Sites for Biocide Action. *J. Appl. Microbiol. Symp. Suppl.* **2002**, 92, 16S-27S.

- [16] Haas C. N.; Engelbrecht R. S. Physiological Alterations of Vegetative Microorganisms Resulting from Aqueous Chlorination. *J. Wat. Pollut. Control Fed.* **1980**, 52, 1976-1989.
- [17] Jacangelo, J. G.; Olivieri, V. P.; Kawata, K. Oxidation of Sulfhydryl Groups by Monochloramine. *Water Res.* **1987**, 21, 1339-1344.
- [18] Mara, D.; Horan, N.J. Handbook of Water and Wastewater Microbiology. **2003**, Academic Press. p665-669.
- [19] <http://en.wikipedia.org/wiki/Antimicrobial>. accessed 05/12/2009.
- [20] Russell, A.D. J. Introduction of Biocides into Clinical Practice and the Impact on Antibiotic-resistant Bacteria. *Appl. Microbiol. Symp. Suppl.* **2002**, 92, 121S-135S.
- [21] Bloomfield, S.F. Chlorhexidine and Iodine Formulations. Handbook of Disinfectants and Antiseptics. **1996**, New York, Marcel Dekker, Inc. p133-158.
- [22] Sauvet, G.; Fortuniak, W.; Kazmierski, K.; Chojnowski, J. Amphiphilic Block and Statistical Siloxane Copolymers with Antimicrobial Activity. *J. Polym. Sci. Part A Polym. Chem.* **2003**, 41, 2939-2948.
- [23] Holah, J.T.; Taylor, J.H.; Dawson, D.J.; Hall, K.E. Biocide Use in the Food Industry and the Disinfectant Resistance of Persistent Strains of *Listeria Monocytogenes* and *Escherichia coli*. *J. Appl. Microbiol. Symp. Suppl.* **2002**, 92, 111S-120S.
- [24] Fichet, G.; Comoy, E., Duval, C.; Antloga, K.; Dehen, C.; Charbonnier, A.; McDonnell, G.; Brown, P.; Lasmezas, C.; Deslys, J.P. Novel Methods for Disinfection of Prion-Contaminated Medical Devices. *Lancet.* **2004**, 364, 521-526.

- [25] Block, S.S. Disinfection, Sterilization, and Preservation. **1983**, Philadelphia. Lea & Febiger.
- [26] <http://www.cfsan.fda.gov/~dms/a2z-b.html>. accessed 05/10/2009.
- [27] <http://www.agion-tech.com/Technology.aspx?id=156>. accessed 05/12/2009.
- [28] Green, P.E.; Stumpf, P.K. The Mode of Action of Chlorine. *J. Am. Water Works Assoc.* **1946**, 38, 1301-1305.
- [29] National Academy of Science “Drinking Water and Health, Vol. &, Disinfectants and Disinfection By-products”. **1987**, Washington, D.C. National Academy Press.
- [30] Worley S.D.; William, D.E. Water Disinfectants. *CRC Crit. Rev. Environ. Control.* **1998**, 18, 133-175.
- [31] Broughton, R.M.; Worley, S.D.; Liang, J.; Barnes, K.; Lee, J.; Akdag, A.; Cho, U.; Huang, T.S. Antimicrobial Cellulose Using Halamines. Beltwide Cotton Conferences. New Orleans, LA. **2007**, 2029-2039.
- [32] Worley, S. D.; Sun, G. Biocidal Polymers. *Trends Polym. Sci.* **1996**, 4, 364-370.
- [33] Kenawy, E. R.; Worley, S. D.; Broughton, R. The Chemistry and Applications of Antimicrobial Polymers; A State of the Art Review. *Biomacromolecules.* **2007**, 8, 1359-1384.
- [34] Chen, Z; Sun, Y. Y. N-halamine-based Antimicrobial Additives for Polymers: Preparation, Characterization, and Antimicrobial Activity. *Ind. Eng. Chem. Res.* **2006**, 45, 2634-2640.

- [35] Akdag, A.; Okur, S.; McKee, M.L.; Worley, S.D. The Stabilities of N-Cl Bonds in Biocidal Materials. *J. Chem. Theory Comput.* **2006**, 2, 879-884.
- [36] Qian, L.; Sun, G. Durable and Regenerable Antimicrobial Textiles: Synthesis and Applications of 3-methylol-2,2,5,5-tetramethyl-imidazolidin-4-one (MTMIO). *J. Appl. Polym. Sci.* **2003**, 89, 2418-2425.
- [37] Worley, S.D. Patent WO9929678. **1999**.
- [38] N-halamines. Kirk-Othmer Encyclopedia of Chemical Technology. Fifth Ed. **2005**, New York. John Wiley & Sons. Vol.13, p 98-122.
- [39] Kaminski, J.J.; Bodor, N.; Higuchi, T. N-halo Derivatives III: Stabilization of Nitrogen-chlorine Bond in N-chloroamino Acid Derivatives. *J. Pharm. Sci.* **1976**, 65, 553-557.
- [40] Liang, J.; Barnes, K.; Akdag, A.; Worley, S.D.; Lee, J.; Broughton, R.M.; Huang, T.S. Improved Antimicrobial Siloxane. *Ind. Eng. Chem. Res.* **2007**, 46, 1861-1866.
- [41] Worley, S.D.; Chen, Y.; Wang, J.W.; Wu, R.; Li, Y. N-halamine Siloxanes for Use in Biocidal Coatings and Materials. Patent WO03106466. **2003**.
- [42] Worley S.D.; Chen, Y.; Wang, J-W.; Wu, R.; Cho, U.; Broughton, R.M.; Kim, J.; Wei, C.I.; Williams, J.F.; Chen, J.; Li, Y. Novel N-halamine Siloxane Monomers and Polymers for Preparing Biocidal Coatings. *Surf. Coat. Int. Part B: Coat. Trans.* **2005**, 88, 93-99.

- [43] Liang, J., Owens, J.R.; Huang, T.S.; Worley, S.D. Biocidal Hydantoinylsiloxane Polymers. IV. N-halamine Siloxane-Functionalized Silica Gel. *J. Appl. Sci.* **2006**, 101, 3448-3454.
- [44] Ren, X.; Kocer, H.B.; Kou, L.; Worley, S.D.; Broughton, R.M.; Tzou, Y.M.; Huang, T.S. Antimicrobial Polyester. *J. Appl. Polym. Sci.* **2008**, 109, 2756-2761.
- [45] Porret, D. Heterocyclic N-glycidyl Compounds. Patent CH488729. **1970**.
- [46] Worley, S.D.; Liang, J.; Chen, Y. Broughton, R.M.; Wang, J-W.; Wu, R.; Cho, U.; Lee, J. Barnes, K. Biocidal N-halamine Epoxides. Patent WO06099567. **2006**.
- [47] Ren, X. Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Huang, T.S. Rechargeable Biocidal Cellulose: Synthesis and Application of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione. *Carbohydrate Polym.* **2009**, 75, 683-687.
- [48] Lee, J.; Broughton, R.M.; Akdag, A.; Worley, S. D.; Huang, T.S. Antimicrobial Fibers Created via Polycarboxylic Acid Durable Press Finishing. *Textile Res. J.* **2007**, 77, 604-611.
- [49] Lin, J.; Winkelman, C.; Worley, S.D.; Broughton, R.M.; Williams, J.F. Antimicrobial Treatment of Nylon. *J. Appl. Polym. Sci.* **2001**, 81, 943-947.
- [50] Lin, J.; Winkelmann, C.; Worley, S.D.; Kim, J.; Wei, C.I.; Cho, U.; Broughton, R.M.; Santiago, J.I., Williams, J.F. Biocidal Polyester. *J. Appl. Polym. Sci.* **2001**, 85, 177-182.

- [51] Sun, Y.; Sun, G. Novel Regenerable N-halamine Polymeric Biocides. I. Synthesis, Characterization, and Antibacterial Activity of Hydantoin-containing Polymers. *J. Appl. Polym. Sci.* **2001**, 80, 2460-2467.
- [52] Sun, Y.; Sun, G. Durable and Refreshable Polymeric N-Halamine Biocides Containing 3-(4'-vinylbenzyl)-5,5-dimethylhydantoin. *J. Polym. Sci. Part A: Polym. Chem.* **2001**, 39, 3348-3355.
- [53] Li, Y.-J.; Worley, S.D. Biocidal Copolymers of N-haloacyloxymethylhydantoin. *J. Bioact. Compat. Polym.* **2001**, 16, 493-506.
- [54] Sun, Y.Y.; Chen, T.Y.; Worley, S.D.; Sun, G. Novel Refreshable N-halamine Polymeric Biocides Containing Imidazolidin-4-one Derivatives. *J. Polym. Sci. Part A: Polym. Chem.* **2001**, 39, 3073-3084.
- [55] Ren, X.; Kou, L.; Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Tzou, Y.M.; Huang, T.S. Antimicrobial Modification of Polyester by Admicellar Polymerization. *J. Biomed. Mat. Res. Part B: Appl. Biomater.* **2009**, 89, 475-480.
- [56] Sun, G.; Wheatley, W.B. Worley, S.D. A New Cyclic N-halamine Biocidal Polymer. *Ind. Eng. Chem. Res.* **1994**, 33, 168-170.
- [57] Chen, Y.; Worley, S.D.; Kim, J.; Wei, C.I.; Chen, T.Y.; Suess, J.; Kawai, H.; Williams, J.F. Biocidal Polystyrenehydantoin Beads. 2. Control of Chlorine Loading. *Ind. Eng. Chem. Res.* **2003**, 42, 5715-5720.
- [58] Wayman, M.; Salamat, H.; Dewar, E.J. Chlorine Exchange Resins. *Can. J. Chem. Eng.* **1968**, 46, 282-287.

- [59] Sun, Y.; Sun, G. Novel Refreshable N-halamine Polymeric Biocides: N-Chlorination of Aromatic Polyamides. *Ind. Eng. Chem. Res.* **2004**, 43, 5015-5020.
- [60] Akdag, A.; Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Webb, T.R.; Bray, T.H. Why Does Kevlar Decompose, while Nomex Does Not, When Treated with Aqueous Chlorine Solutions? *J. Phys. Chem. B.* **2007**, 111, 5581-5586.
- [61] Luo, J.; Sun, Y. Acyclic N-halamine Coated Kevlar Fabric Materials: Preparation and Biocidal Functions. *Ind. Eng. Chem. Res.* **2008**, 47, 5291-5297.
- [62] Sandstrom, A.; Sun, G. Durability of Biocidal Nomex Fabrics for Multi-functional Firefighter Uniforms. *Res. J. Text. Apparel.* **2006**, 10, 13-17.
- [63] Sun, G.; Worley, S.D.; Chemistry of Durable and Regenerable Biocidal Textiles. *J. Chem. Edu.* **2005**, 82, 60-64.
- [64] Sun, G.; Xu, X. Durable and Regenerable Antibacterial Finishing of Fabrics: Biocidal Properties. *Text. Chem. Colorist.* **1998**, 30, 26-30.
- [65] Chen, Z.; Luo, J.; Sun, Y. Biocidal Efficacy, Biofilm-controlling Function, and Controlled Release Effect of Chloromelamine-based Bioresponsive Fibrous Materials. *Biomaterials.* **2007**, 28, 1597-1609.

CHAPTER 2

EFFECT OF ALKYL DERIVATIZATION ON SEVERAL PROPERTIES OF N-HALAMINE ANTIMICROBIAL SILOXANE COATINGS

2.1 Introduction

Healthcare associated infections are an increasingly important problem for medical facilities in spite of expanding infection control efforts.¹⁻⁸ The transmission of the microorganisms through contamination of surfaces in the environment among patients and healthcare workers could be minimized by deactivating them on the contaminated surface within sufficiently brief time intervals. Quaternary ammonium salts,⁹⁻¹¹ metal ions,¹²⁻¹³ and cyclic N-halamine compounds¹⁴⁻²⁰ are currently used in manufacturing numerous biocidal materials. Among these, materials incorporated with N-halamine functional groups have been demonstrated to be the most efficacious due to their long-term stabilities, non-toxicity to humans, biocidal functions against a broad range of microorganisms, fast biocidal activity, and regenerable properties upon exposure to chlorine bleach in washing cycles.¹⁴⁻²⁰ Briefly, N-halamine precursors are covalently bonded to various surfaces and then converted into N-halamine materials through a halogenation process, as shown for cellulose in Figure 2.1.^{16-19,21}

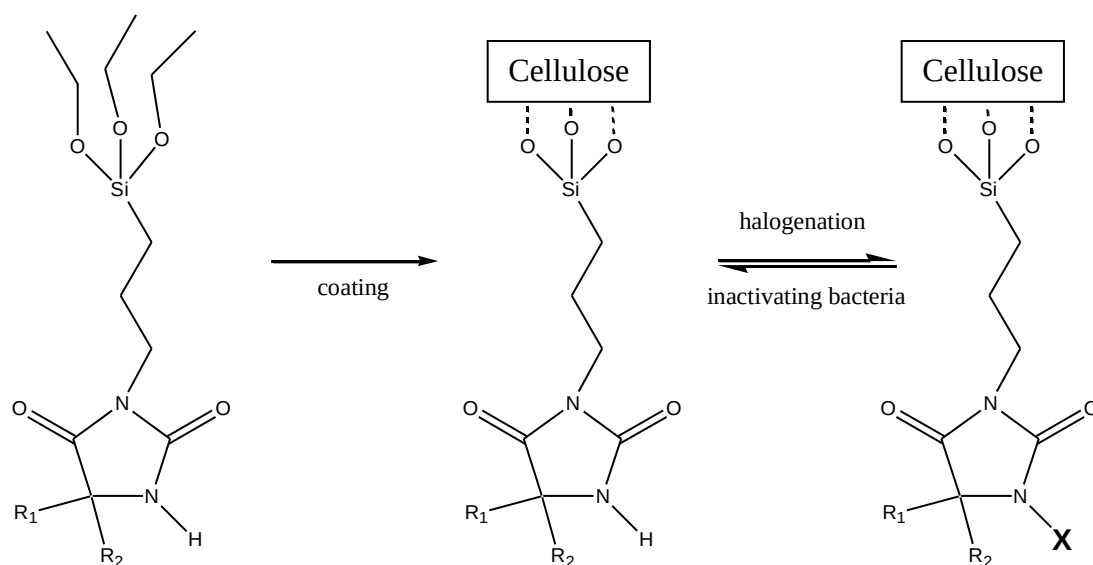


Figure 2.1. The preparation of antimicrobial coatings. (X = Cl, Br)

The excellent stability of N-halamine materials is provided by electron-donating alkyl groups substituted onto the heterocyclic rings adjacent to the oxidative N-X moieties which strengthen the N-X covalent bond, hindering the release of oxidative free-halogen into aqueous solution, which is not necessary for biocidal activity.²² The stability of the N-halamine moieties can generally be enhanced by increasing the hydrophobicity of the material and steric hindrance around the N-X moiety which reduce the rate of hydrolysis of the N-X moieties.²³

The stable combined N-halamines serve as contact biocides which transfer oxidative halogen to a microbial cell upon direct contact with the cell. The rate of this process depends upon the N-X bond strength and the susceptibility of the target site on the microbial cell. Since lethal target sites are found within the cell, the biocidal agent must first interact with cell wall or outer membrane to reach target sites.²⁴ Gram-negative bacteria are generally more resistant to biocides than are Gram-positive ones because of

an extra layer (outer membrane) that is surrounding the cell wall, composed of polysaccharides, proteins, and phospholipids.²⁴ The outer membrane (OM) has a significant moderating influence on the penetration of both hydrophilic and hydrophobic molecules.²⁵⁻²⁶ The OM can restrict the uptake of biocides²⁷ and limit the concentration of active biocide which can reach the target site(s).²⁸ The OM of Gram-negative bacteria performs as a molecular filter for hydrophilic compounds. Transport phenomena in vivo and through membranes has been demonstrated to be dependent on lipophilic (hydrophobic) contributions.²⁹ Reorganization of phospholipid in the OM allows the penetration of hydrophobic molecules by dissolution and diffusion in the lipid.³⁰⁻³¹

The importance of lipophilicity in the structure-activity relationship has been recognized for many years. Increasingly demanding antimicrobial applications require more sophisticated use of biocidal systems. For electrophilic agents, including the active-halogen compounds, reactivity within the cell is dependent upon low steric hindrance and strong electron withdrawing capability. On the other hand, more lipophilic character may be needed to transport the active biocide through the OM of the cell. Quaternary ammonium salt biocides (quats) require a lengthy alkyl group (C12-C16) on the cationic nitrogen to increase lipophilicity for penetration of the cell OM; the cell then is inactivated by leakage of critical components from the inner cell layers.^{15,32-34}

N-halamine biocides require penetration of the OM by oxidative halogen (Cl^+) which then destroys the cell by oxidation of target sites on the enzymes in the cell.

It is conceivable that this process could be accentuated by the presence of lipophilic alkyl groups as for quats. However, Chen and Sun have shown that increasing the alkyl chain length at the 3 position on the hydantoin ring of water soluble hydantoin derivatives

actually slows the rate of disinfection for both Gram-negative and Gram-positive bacteria, although they attribute this to solubility problems with the longer alkyl chains causing less contact with cell OM's than for the shorter alkyl chains.²⁰

In this study a series of hydantoinyl siloxanes containing variation in alkyl substitution at the 5 position of the hydantoin ring (shown as R₁ and R₂ in Figure 2.1) were prepared and bonded to cellulose. The antibacterial activities, stabilities, and UV resistance of the different derivatives were compared to attempt to determine the influence of electronic, steric, and hydrophobic/hydrophilic effects for the N-halamine biocidal materials.

2.2 Experimental Section

General Procedure for the Synthesis of 5,5-dialkylhydantoin Siloxanes.

The appropriate 5,5-dialkylhydantoin (DAH) was prepared by reaction of the necessary dialkyl ketone, ammonium carbonate, and potassium cyanide (Aldrich Chemical Company, Milwaukee, WI) in a 1:2:6 molar ratio in a water/ethanol (1:1 by volume) solvent mixture in a pressure autoclave at 90 °C for 8 h. After evaporation of the ethanol, the crude products were isolated by exposure to dilute HCl and filtration. Products were confirmed by ¹H and ¹³C NMR; yields ranged from 89 to 97% by weight.

The triethoxysilylpropyl hydantoin derivatives were prepared according to a general procedure outlined previously.³⁵ The sodium salts of the DAHs were prepared by mixing the appropriate DAH with an equimolar quantity of NaOH in ethanol and heating at reflux for about 10 min. After evaporation of the solvent, the sodium salt of a DAH was dried in a vacuum oven at 50 °C for 3 d. The anhydrous salt was dissolved in

N,N-dimethyl formamide (DMF) at 95 °C, and then an equimolar amount of 3-chloropropyltriethoxysilane (Aldrich Chemical Company, Milwaukee, WI) was added to the solution. The resulting mixture was stirred for 8 h. The NaCl produced in the reaction was removed by filtration, and DMF was removed at reduced pressure. The DAH monomer was separated from its oligomer by dissolving the resulting viscous residue in hexane and removing the hexane after separation. The structures of the 3-triethoxysilylpropyl-5-alkyl-5-alkylhydantoin derivatives were confirmed by ^1H and ^{13}C NMR; yields ranged from 87 to 93% by weight.

Spectral Data for the Synthesized Compounds.

(i) 3-triethoxysilylpropyl-5,5-dimethylhydantoin (Methyl-methyl). Methyl-methyl oligomer was obtained as a colorless viscous liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.58 (t, J = 8.50, 2H), 1.19 (t, J=7.00, 9H), 1.40 (s, 6H), 1.70 (p, J = 7.94, 2H), 3.45 (t, J =7.38, 2H), 3.78 (q, J = 7.00, 6H), 6.95 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 177.53, 156.98, 61.80, 58.43, 40.97, 24.91, 21.57, 18.18, 7.57.

(ii) 3-triethoxysilylpropyl-5-methyl-5-propylhydantoin (Methyl-propyl). Methyl-propyl was obtained as a slightly yellow liquid. ^1H NMR (CDCl_3 , 400 MHz) δ 0.60 (t, J = 8.60, 2H), 0.90 (t, J = 7.20, 3H), 1.21 (m, 11H), 1.38 (s, 3H), 1.72 (m, 4H), 3.46 (t, J = 7.42, 2H), 3.79 (q, J=6.93, 6H), 6.68 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 177.16, 157.45, 61.75, 58.23, 40.89, 39.87, 23.83, 21.58, 18.12, 16.81, 13.78, 7.61.

(iii) 3-triethoxysilylpropyl-5-methyl-5-pentylhydantoin (Methyl-pentyl). Methyl-pentyl was obtained as a slightly yellow liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.61 (t, $J = 8.50$, 2H), 0.86 (t, $J = 6.25$, 3H), 1.22(m, 15H), 1.40 (s, 3H), 1.72 (m, 4H), 3.47 (t, $J = 7.37$, 2H), 3.80 (q, $J = 7.00$, 6H), 6.44 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 177.01, 157.25, 61.80, 58.37, 41.04, 37.78, 31.50, 23.99, 23.11, 22.32, 21.63, 18.23, 13.84, 7.69.

(iv) 3-triethoxysilylpropyl-5-heptyl-5-methylhydantoin (Methyl-heptyl). Methyl-heptyl was obtained as a slightly yellow liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.61 (t, $J = 8.50$, 2H), 0.87 (t, $J = 6.38$, 3H), 1.22 (m, 19H), 1.40 (s, 3H), 1.73 (m, 4H), 3.48 (t, $J = 7.37$, 2H), 3.80 (q, $J = 7.00$, 6H), 6.31 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 177.00, 157.21, 61.78, 58.32, 41.04, 37.84, 31.68, 29.36, 29, 24.01, 23.49, 22.54, 21.63, 18.23, 13.98, 7.69.

(v) 3-triethoxysilylpropyl-5,5-diethylhydantoin (Ethyl-ethyl). Ethyl-ethyl was obtained as a colorless liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.62 (t, $J = 8.5$, 2H), 0.85 (t, $J = 7.41$, 6H), 1.21 (t, $J = 7.01$, 9H), 1.69 (m, 4H), 1.82 (m, 2H), 3.43 (t, $J = 7.31$, 2H), 3.80 (q, $J = 7.02$, 6H), 6.44 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 176.37, 158.00, 66.29, 58.32, 41.05, 29.77, 21.72, 18.25, 8.29, 7.72.

(vi) 3-triethoxysilylpropyl-5,5-dibutylhydantoin (Butyl-butyl). Butyl-butyl was obtained as a colorless liquid ^1H NMR (CDCl_3 , 250 MHz) δ 0.62 (t, $J = 8.54$, 2H), 0.87 (t, $J = 6.96$, 6H), 1.21 (m, 17H), 1.70 (m, 6H), 3.47 (t, $J = 7.40$, 2H), 3.81 (q, $J = 7.00$, 6H), 6.31 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 176.56, 157.76, 65.34, 58.33, 41.05, 36.86, 25.35, 22.57, 21.67, 18.26, 13.83, 7.74.

Coating Procedure. In this work the objective was to prepare coatings with as nearly as possible equal chlorine loadings to remove chlorine loading as a variable. This was best achieved by using the oligomeric fraction of the dimethyl derivative, but the monomeric fraction of the other derivatives. Precursor siloxane monomers and oligomers were first dissolved in an ethanol/water mixture (1:1 by weight) at concentrations ranging from 2 to 4 weight percent so as to subsequently obtain chlorine loadings between 0.25 to 0.30%. The mixture was stirred for 15 min to produce a uniform solution. Cotton swatches (Style 400 Bleached 100% Cotton Print Cloth from Testfabrics, Inc., West Pittston, PA) were soaked in the solution for 15 min and then cured at 95 °C for 1 h. Then the swatches were soaked in a 0.5% detergent solution for 15 min, rinsed several times with water, and dried at room temperature. The coating solution containing the dissolved siloxane derivatives weighed 50 g, and the cotton swatches were cut into areas of 50 in² each.

Chlorination Procedure. The treated fabrics were chlorinated by soaking in 10% household bleach (0.6% sodium hypochlorite) at pH 7 (adjusted with 6 N HCl) for 1 h. After rinsing with tap and distilled water, the swatches were then dried at 45 °C for 1 h to remove any unbonded chlorine from the material.

FTIR Confirmation of Siloxane Derivative Bonding. FTIR spectra of the coated fabrics confirmed that the N-halamine precursors bonded to the cotton fabric, as a band at ca. 1770 cm⁻¹ was detected, which can be assigned to the carbonyl band of the amide structure in the hydantoin moieties. After the treatment with bleach, this band shifted to ca. 1780 cm⁻¹ indicating disruption of N-H---O=C hydrogen bonding as conversion of N-H to N-Cl occurred (see supporting information).

Analytical Titration. The chlorine concentration loaded onto the samples was determined by the iodometric/thiosulfate titration method. The Cl^+ % on the samples was calculated by the following formula;

$$\text{Cl}^+\% = (\text{N} \times \text{V} \times 35.45) / (2 \times \text{W}) \times 100\% \quad (1)$$

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

Biocidal Efficacy Testing. Gram-negative *Escherichia coli* (*E. coli*) is generally more resistant to chlorine disinfection than other organisms of vegetative bacteria, so it was selected as the test organism for determining the effectiveness of disinfection by the chlorinated hydantoinyl siloxane derivatives. Coated fabric swatches were challenged with *E. coli* O157:H7 (ATCC 43895) bacterial suspensions in pH 7 phosphate buffer solution (100 mM). 25 μ l of the bacterial suspensions were added in the center of a 1 in. square fabric swatch, and a second identical swatch was laid on the first swatch. A sterile weight was used to ensure sufficient contact of the swatches with the inoculums.

The contact times for the swatches with the bacteria were 1, 5, 10, and 30 min. At those contact times the fabric swatches were quenched with 0.02 N sodium thiosulfate solution to remove any oxidative chlorine which could cause extended disinfection. Serial dilutions of the solutions contacting the surfaces were plated on Trypticase agar, incubated for 24 h at 37 °C, and colony counts were made to determine the presence of viable bacteria. Unchlorinated control samples were treated in the same manner.

Washing Testing. The stability and rechargeability of chlorine on the samples were evaluated by using a standard washing test according to AATCC Test Method 61.

The cotton samples were washed for the equivalents of 5, 10, 25, and 50 machine washes in a Launder-Ometer. The Cl^+ % loadings of the samples were determined after the washings (with or without prechlorination and after rechlorination) by the titration procedure outlined above.

UV Light Stability Testing. UV light stabilities of the bound chlorine and the hydantoinyl siloxane coating on the derivatized cotton fabric were measured by using an Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA).

The samples were placed in the UV (Type A, 315-400 nm) chamber for times in the range of 1 to 6 h. After a specific time of exposure to UV irradiation, the samples were removed from the UV chamber and titrated, or rechlorinated and titrated.

2.3 Results and Discussion

Coating Procedure. In general, the biocidal characteristics of a coated fabric depend on the structure of the coating compound and the concentration of the biocidal sites.¹⁵

However, for N-halamine compounds an increase of Cl^+ active sites may cause an increase in hydrophobicity of the surface coating which actually may lead to a poorer biocidal performance due to a decrease in contact with microbial cells.¹⁸ For this reason an attempt was made to keep the Cl^+ active site concentration at a constant level for all of the derivatives. This was not a simple task in this work as different derivatives required different precursor bath concentrations to coat the fabric swatches so as to give a constant

final chlorine loading. An attempt was made to load all of the swatches at a concentration level of about 0.30% by using different concentrations of the various monomers in the coating solutions, except for 3-triethoxysilylpropyl-5,5-dimethylhydantoinylsilane (Methyl-methyl), for which the oligomer fraction of the Methyl-methyl was necessarily used in order to achieve a chlorine loading of about 0.3%. A loading of only 0.1% could be obtained using the monomer of Methyl-methyl. As expected, it was observed that more hydrophobic structures, having less affinity for water, could be coated at lower precursor bath concentrations. An approximately linear relationship was observed between the compound concentration in the coating solution and the amount of N-halamine precursor which could be coated onto cotton as shown in Figure 2.2.

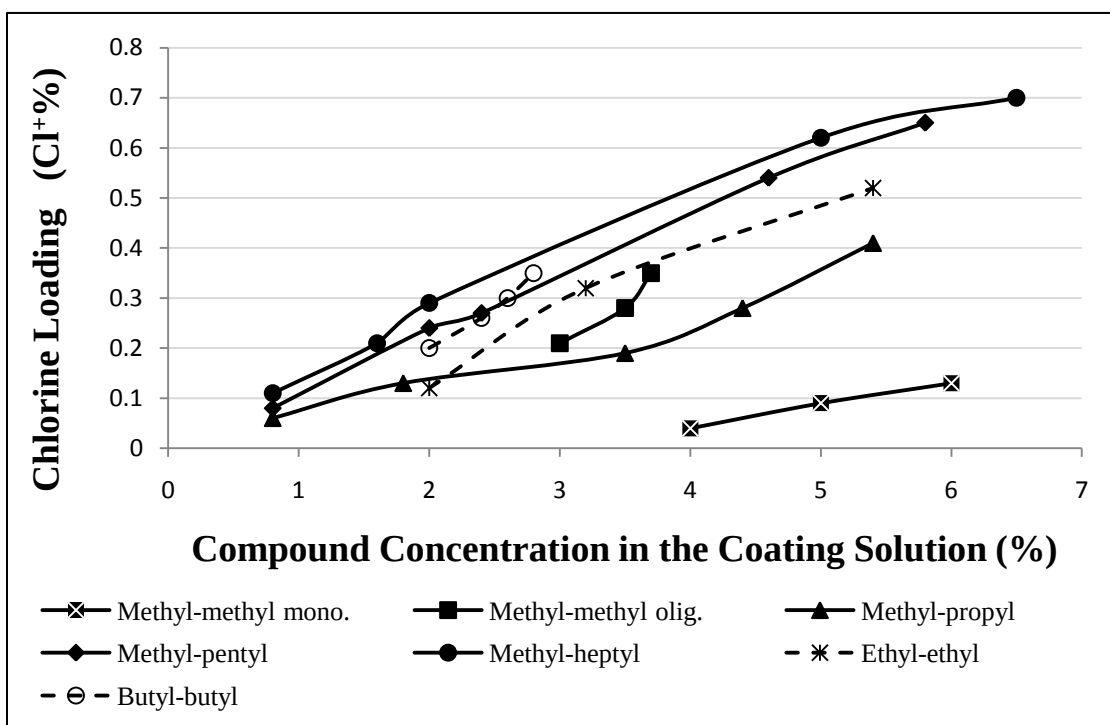


Figure 2.2. Chlorine loadings ($\text{Cl}^+\%$) on cotton at different concentrations of the coating solution.

It proved impossible to achieve all coatings with a loading of exactly 0.30% Cl⁺, although we were able to attain this goal to within about +/- 0.03%. Past work in these laboratories has demonstrated that changes of 0.03% in chlorine loading do not materially affect the performance of a given N-halamine biocide, so any differences observed among the derivatives in this work should not be attributed to slightly different chlorine loadings on the swatches.

Antimicrobial Efficacies. The treated cotton swatches were challenged with *E. coli* O157:H7 at concentrations between 10⁶ to 10⁸ CFU. The unchlorinated control samples provided only about 0.10 log reductions, due to the adhesion of bacteria to the cotton swatches, within 30 min contact time intervals when a cell density of 6.03x10⁶ CFU was used in the challenge (Table 2.1). All of the chlorinated treated samples showed excellent antimicrobial activity. It was found that all *E. coli* O157:H7 bacteria were inactivated (6.78 logs) by the treated swatches in the contact interval of 1 min, except Methyl-heptyl-Cl, which has the longest alkyl chain. Methyl-heptyl-Cl inactivated the bacteria in a contact time interval of 1 to 5 min. Since it is possible that the 6.82 log reduction could have been attained within as little as 61 sec for Methyl-heptyl-Cl, the data suggest that there may be little or no effect on the inactivation rate introduced by varying the alkyl substitution pattern at the 5 position of the hydantoin ring for the surface bound siloxane N-halamines.

Table 2.1. Biocidal Test I.

Dialkyl derivative	Contact time (min)	Bacterial reduction	
		%	log
Methyl-methyl	30	28.9	0.07
Methyl-propyl	30	26.8	0.15
Methyl-pentyl	30	21.8	0.12
Methyl-heptyl	30	24.0	0.13
Methyl-methyl-Cl	1	100.0	6.78
	5	100.0	6.78
	10	100.0	6.78
	30	100.0	6.78
Methyl-propyl-Cl	1	100.0	6.78
	5	100.0	6.78
	10	100.0	6.78
	30	100.0	6.78
Methyl-pentyl-Cl	1	100.0	6.78
	5	100.0	6.78
	10	100.0	6.78
	30	100.0	6.78
Methyl-heptyl-Cl	1	99.9	5.86
	5	100.0	6.78
	10	100.0	6.78
	30	100.0	6.78

^a Microorganism: *E. coli* O157:H7. Total bacteria: 6.03x10⁶ CFU/sample (6.78 logs).

^b Chlorine loadings on the coated swatches were 0.27, 0.30, 0.34 and 0.33%, respectively.

^c The error in the measured Cl⁺ weight percentage values was ±0.01.

Since the rapid inactivation rates for a challenge of 6.03×10^6 CFU cell density failed to yield a significant difference among the various derivatives, it was decided to challenge samples with a higher cell density (5.33×10^7 CFU), the results of which are shown in Table 2.2. It was observed that longer alkyl chains lead to slightly lower inactivation rates in biocidal Test II. Methyl-methyl-Cl and Methyl-propyl-Cl provided a total inactivation within 5 min, while Methyl-pentyl-Cl to Butyl-butyl-Cl required 30 min contact which was the same trend reported by Chen and Sun²⁰ for their studies of alkyl derivatization at position 3 on the hydantoin ring. However, the same trend was not reproduced in Test III when the same bacterial concentration was employed. The chlorinated samples with the exception of Methyl-propyl-Cl and Methyl-heptyl-Cl inactivated all of the bacteria within 1 min. The lack of reproducibility between Tests II and III illustrate the difficulties in performing biocidal tests on antimicrobial coatings. We attribute this to lack of uniform coatings on the swatches, ie. if a given swatch has a small island free of coating, and thus Cl^+ , a few bacteria may survive for a longer contact time until migration occurs to a chlorinated site. We conclude that the results of this study indicate little or no significant differences in the biocidal efficacies of dialkyl derivatives when the derivatization is performed at position 5 of the hydantoin ring for the hydantoinylsiloxane coatings. We also conclude that all of the derivatives produced extremely effective biocidal coatings.

Table 2.2. Biocidal Tests II and III.

Dialkyl derivative / Chlorine loading (Cl ⁺ %) Test II - Test III	Contact Time (min)	Test II		Test III	
		Bacterial reduction		Bacterial reduction	
		%	log	%	log
Methyl-methyl-Cl	1	99.9	4.42	100.0	7.73
0.32 – 0.29	5	100.0	7.73	100.0	7.73
	10	100.0	7.73	100.0	7.73
	30	100.0	7.73	100.0	7.73
Methyl-propyl-Cl	1	99.9	5.60	99.9	4.42
0.33 – 0.30	5	100.0	7.73	99.9	5.60
	10	100.0	7.73	100.0	7.73
	30	100.0	7.73	100.0	7.73
Methyl-pentyl-Cl	1	99.9	4.58	100.0	7.73
0.33 – 0.29	5	99.9	4.82	100.0	7.73
	10	99.9	5.20	100.0	7.73
	30	100.0	7.73	100.0	7.73
Methyl-heptyl-Cl	1	99.9	4.49	99.9	4.45
0.35 – 0.31	5	99.9	5.60	99.9	5.90
	10	99.9	5.90	100.0	7.73
	30	100.0	7.73	100.0	7.73
Ethyl-ethyl-Cl	1	99.9	4.54	100.0	7.73
0.29 – 0.27	5	99.9	4.86	100.0	7.73
	10	99.9	4.70	100.0	7.73
	30	100.0	7.64 ^a	100.0	7.73
Butyl-butyl-Cl	1	99.9	4.70	100.0	7.73
0.28 – 0.29	5	99.9	4.91	100.0	7.73
	10	99.9	5.12	100.0	7.73
	30	100.0	7.64 ^a	100.0	7.73

^a Microorganism: *E. coli* O157:H7. Total bacteria: 5.33 x 10⁷ CFU/sample (7.73 log).

^b Control unchlorinated swatches exposed to 4.40 x 10⁷ CFU/sample (7.64 logs) for Ethyl-ethyl and Butyl-butyl provided 0.31 and 0.16 log reductions, respectively.

Stabilities toward Washing and UV Light Exposure. Data pertaining to machine washing of coated fabric swatches are presented in Table 2.3. Three types of washing experiments were performed: prechlorinated coatings at the concentration levels indicated at 0 machine washes in Table 2.3 (X), prechlorinated and rechlorinated after a given number of machine washes (Y), and unchlorinated until after a given number of machine washes (Z). Both monomeric and oligomeric coatings are partially lost upon successive washing. Several observations can be made pertaining to the data in Table 2.3. First, the greatest loss of Cl^+ and/or coating occurred during the first launderometer washing cycle (equivalent to 5 machine washes) in all cases; the losses between 5 and 50 washes were small. Clearly not all siloxane molecules are equally bound to the cotton. The decline in Cl^+ values of X and Z are indicative of the loss of both chlorine and coating. A comparison of Y and Z values indicates that the loss of coating was substantial. Second, prechlorination did not significantly protect the coatings from hydrolyses from the surfaces of the fibers as can be seen from the small differences between Y and Z values. We have noted previously in these laboratories that prechlorination generally increases the hydrophobicity of surfaces bound to N-halamines and thus protects the coatings to some extent from hydrolyses;¹⁹ however, that work addressed surfaces with substantially higher initial chlorine loadings. Third, all of the surfaces retained at least 0.03% chlorine loading after the equivalent of 50 machine washes which is adequate for an antimicrobial effect.²¹ Fourth, just as for the biocidal efficacy studies addressed above, there was no real significant difference between the several derivatives in the washing tests.

Table 2.3. Stability toward washing of cotton coated with derivatized hydantoinyl siloxanes (Cl⁺% remaining).

Machine washes ^a	Methyl-methyl			Methyl-propyl			Methyl-pentyl		
	X ^b	Y ^b	Z ^b	X	Y	Z	X	Y	Z
0	0.27	0.27		0.30	0.30		0.28	0.28	
5	0.09	0.14	0.05	0.08	0.09	0.05	0.09	0.12	0.06
10	0.09	0.09	0.04	0.04	0.05	0.03	0.08	0.07	0.04
25	0.07	0.09	0.03	0.04	0.04	0.03	0.04	0.06	0.04
50	0.03	0.06	0.03	0.03	0.03	0.02	0.03	0.04	0.03

Machine washes ^a	Methyl-heptyl			Ethyl-ethyl			Butyl-butyl		
	X ^b	Y ^b	Z ^b	X	Y	Z	X	Y	Z
0	0.30	0.30		0.25	0.25		0.27	0.27	
5	0.13	0.16	0.13	0.08	0.09	0.05	0.13	0.16	0.09
10	0.12	0.13	0.13	0.07	0.06	0.03	0.12	0.16	0.09
25	0.07	0.11	0.09	0.03	0.05	0.03	0.08	0.13	0.06
50	0.06	0.08	0.07	0.01	0.03	0.02	0.07	0.10	0.04

^a A washing cycle is equivalent to 5 machine washes in AATCC Test Method 61.

^b X: Chlorinated before washing, Y: Chlorinated before washing and rechlorinated after washing, Z: Unchlorinated before washing, but chlorinated after washing.

^c The error in the measured Cl⁺ weight percentage values was ± 0.01 .

The UV light stability of the N-Cl bond of the synthesized N-halamine siloxane derivatives did not exhibit any distinctive difference among the several derivatized coatings (Table 2.4). Clearly there was some decomposition of the coatings over the six hour exposure in the UV chamber as evidenced by the lower Cl⁺ loadings after

rechlorination after the six hour exposure. However, the primary decomposition process was dissociation of the N-Cl bonds. The stability was quite remarkable given that a six hour exposure in the UV chamber was equivalent to the direct midday summer sunlight.

Table 2.4. Stability toward UV light exposure of cotton coated with derivatized hydantoinyl siloxanes (Cl⁺% remaining).

Time of exposure (h)	Methyl -methyl	Methyl -propyl	Methyl -pentyl	Methyl -heptyl	Ethyl -ethyl	Butyl -butyl
0	0.30	0.28	0.32	0.33	0.26	0.27
1	0.18	0.15	0.15	0.16	0.10	0.14
2	0.13	0.12	0.10	0.12	0.07	0.11
3	0.12	0.09	0.07	0.08	0.05	0.08
6	0.05	0.06	0.03	0.03	0.03	0.03
Rechlorination	0.21	0.20	0.23	0.25	0.17	0.24

Potential Factors Affecting Nitrogen-Halogen Bond Lability. There are several factors which could influence the nitrogen-halogen bond lability upon alkyl derivatization at position 5 of the hydantoin ring which, in turn, would influence the rate of disinfection of microbes since the direct transfer of Cl⁺ from the N-halamine moiety to the microbial cell is thought to be the rate-determining step in the disinfection process for stable N-halamine compounds. (1) Alkyl groups are electron donors, with their donating ability increasing with chain length; this could strengthen the N-Cl bond by increasing its polarity, thus reducing the rate of Cl⁺ transfer to microbial cells and thus reducing the rate of disinfection. (2) Increasing the chain length of alkyl groups at position 5 of the hydantoin ring could cause steric hindrance to the approach of microbial cells, thus

reducing the rate of disinfection. (3) Increasing the length of alkyl groups on the N-halamine moiety should increase the hydrophobicity of the N-halamine moiety; this could cause more intimate contact of the N-Cl moiety with the cells, and an increase in rate of disinfection, but it could also cause less wetting of the entire surface, thereby reducing the contact with the cells and lowering the rate of disinfection.¹⁸ The ¹H NMR data for the unchlorinated derivatives indicated that as the chain length of the alkyl groups at position 5 of the hydantoin ring increased, the signal for the amide proton at position 1 shifted to higher field, indicative of increased electron density about the amide nitrogen. This illustrated that factor (1) above was operative. Factors (2) and (3) are opposed to one another, as the cells may be either held close to the N-Cl site through the hydrophobicity effect, or repelled from the site due to steric hindrance and reduced surface wetting. The work of Chen and Sun showed that extending the alkyl chain length at position 3 (the imide nitrogen) of the hydantoin ring significantly caused a reduction in the rate of disinfection.²⁰ Factors (1) and (2) would not be expected to be operable for substitution at position 3 due to distance in bonding and space from the amide N-Cl moiety. Therefore, in the work of Chen and Sun factor (3) provides a reasonable explanation, ie. poorer surface wetting due to an increase in hydrophobicity; again the substitution is presumably too far away from the disinfection site for a beneficial hydrophobicity effect to occur. In the current work, variation in alkyl derivatization at position 5 of the hydantoin ring of the surface bonded siloxanes causes no observable significant differences in disinfection rate, stability to washing, or stability to dissociation under UV irradiation. We must conclude that all three factors noted above are operable and fortuitously cancel out one another. Since the dimethyl derivative is the least

expensive to produce, and is equal to the other derivatives in disinfection ability, it clearly should be the derivative of choice for use as a surface biocide.

2.4 Conclusions

A series of 3-triethoxysilylpropylhydantoin derivatives were synthesized with variation of alkyl substitution at the 5 position of the hydantoin moiety. The N-halamine precursor compounds were coated onto cotton and converted into surface-bound N-halamines by exposure to household bleach. There was no observable significant differences in disinfection rate, stability to washing, or stability to dissociation under UV irradiation noted for the several derivatives. This led to the conclusion that a complex mix of electronic, steric, and hydrophobicity effects are operable for these hydantoinyl siloxane derivatives. It is recommended that the dimethyl derivative should be the one of choice for a potential application because of it being the least expensive to prepare.

2.5 References

- [1] Hambraeus, A. Infection Control from a Global Perspective. *J. Hosp. Infect.* **2006**, 64, 217-223.
- [2] Hardy, K. J.; Oppenheim, B. A.; Gossain, S.; Gao, F.; Hawkey, P. M. A Study of the Relationship Between Environmental Contamination with Methicillin-resistant *Staphylococcus aureus* (MRSA) and Patients' Acquisition of MRSA. *Infect. Cont. Hosp. Epidem.* **2006**, 27, 127-132.
- [3] Neely, A. N.; Maley, M. P. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastics. *J. Clinic. Microbiol.* **2000**, 724-726.
- [4] Harbartha, S.; Saxa, H.; Gastmeier, P. The Preventable Proportion of Nosocomial Infections: an Overview of Published Reports. *J. Hosp. Infect.* **2003**, 54, 258–266.
- [5] Hambraeus, A. Transfer of *Staphylococcus aureus* Via Nurses' Uniforms. *J. Hyg.* **1973**, 71, 799-814.
- [6] Hedin, G; Hambraeus, A. Multiply Antibiotic-resistant *Staphylococcus epidermidis* in Patients, Staff and Environment - a One-week Survey in a Bone Marrow Transplant Unit. *J. Hosp. Infect.* **1991**, 17, 95-106.
- [7] Scott, E.; Bloomfield, S. F. The Survival and Transfer of Microbial Contamination Via Cloths, Hands and Utensils. *J. Appl. Bacter.* **1990**, 68, 271-278.
- [8] Boyce, J. M.; Potter-Bynoe, G.; Chenevert, C.; King, T. Environmental Contamination Due to Methicillin-resistant *Staphylococcus aureus*: Possible Infection Control Implications. *Infect. Cont. Hosp. Epidem.* **1997**, 18, 622-627.

- [9] For example see: Isquith, A. J.; Abbot, A.; Walters, P. A. Surface-bonded Antimicrobial Activity of an Organosilicone Quaternary Ammonium Chloride. *Appl. Microbiol.* **1972**, 24, 859-86.
- [10] For example see: Lambert, J. L.; Fina, G. T. Preparation and Properties of Triiodide, Pentaiodide-, and Heptaoidide-quaternary Ammonium Strong Base Anion-exchange Resin Disinfectants. *Ind. Eng. Chem. Prod. Res. Dev.* **1980**, 79, 256-258.
- [11] For example see: Sauvet, G.; Fortuniak, W.; Kazmierski, K.; Chojnowski, J. Amphiphilic Block and Statistical Siloxane Copolymers with Antimicrobial Activity. *J. Polym. Sci. Part A: Polym. Chem.* **2003**, 41, 2939–2948.
- [12] For example see: Stobiea, N.; Duffya, B.; McCormackb, D. E.; Colreavya, J.; Hidalgo, M.; McHalec, P; Hinderd, S. J. Prevention of Staphylococcus epidermidis Biofilm Formation Using a Low-temperature Processed Silver-doped Phenyltriethoxysilane Sol–gel Coating. *Biomaterials.* **2008**, 29, 963–969.
- [13] For example see: Nagar, R. Structural and Microbial Studies of Some Transition Metal Complexes. *J. Inorg. Biochem.* **1989**, 37, 193-200.
- [14] Liang, J.; Chen, Y.; Barnes, K.; Wu, R.; Worley, S.D.; Huang, T.S. N-halamine/quat Siloxane Copolymers for Use in Biocidal Coatings. *Biomaterials.* **2006**, 27, 2495-2501.
- [15] Worley, S. D.; Sun, G. Biocidal Polymers. *Trends Polym. Sci.* **1996**, 4, 364-370.
- [16] Kenawy, E. R.; Worley, S. D.; Broughton, R. The Chemistry and Applications of Antimicrobial Polymers; A State of the Art Review. *Biomacromolecules.* **2007**, 8, 1359-1384.

- [17] Sun, Y. Y.; Chen, T.; Worley, S. D.; Sun, G. Novel Refreshable N-Halamine Polymeric Biocides Containing Imidazolidin-4-one Derivatives. *J. Polym. Sci. Part A: Polym. Chem.* **2001**, 39, 3073–3084.
- [18] Makal, U.; Wood, L.; Ohman, D. E.; Wynne, K. J.. Polyurethane Biocidal Polymeric Surface Modifiers. *Biomaterials.* **2006**, 27, 1316-1326.
- [19] Worley, S. D.; Chen, Y.; Wang, J. W.; Wu, R.; Cho, U.; Broughton, R. M.; Kim, J.; Wei, C .I.; Williams, J .F.; Chen, J.; Li, Y. Novel N-halamine Siloxane Monomers and Polymers for Preparing Biocidal Coatings. *Surf. Coat. Int. Part B: Coat. Trans.* **2005**, 88, 93-100.
- [20] Chen, Z; Sun, Y. Y. N-Halamine-based Antimicrobial Additives for Polymers: Preparation, Characterization, and Antimicrobial Activity. *Ind. Eng. Chem. Res.* **2006**, 45, 2634-2640.
- [21] Liang, J.; Wu, R.; Wang, J. W.; Barnes, K.; Worley, S. D.; Cho, U.; Lee, J.; Broughton, R. M.; Huang, T. S. N-halamine Biocidal Coatings. *J. Ind. Microbiol. Biotechnol.* **2007**, 34, 157-163.
- [22] Williams, D. E.; Elder, E. D.; Worley, S. D. Is Free Halogen Necessary for Disinfection? *Appl. Environ. Microbiol.* **1988**, 54, 2583-2585.
- [23] Akdag, A.; Okur, S.; McKee, M. L.; Worley, S .D. The Stabilities of N-Cl Bonds in Biocidal Materials. *J. Chem. Theory Comput.* **2006**, 2, 879-884.
- [24] Russell, A. D.; Chopra, I. Understanding Antibacterial Action and Resistance. Second Ed. **1996**, Chichester, Ellis Horwood.

- [25] Denyer, S. P. Mechanisms of Action of Antibacterial Biocides. *Int. Biomet. & Biodeg.* **1995**, 36, 221-245.
- [26] Denyer, S. P.; Stewart, G. S. A. B. Mechanisms of Action of Disinfectants. *Int. Biomet. & Biodeg.* **1998**, 41, 261-268.
- [27] Russell, A. D. Mechanisms of Bacterial Insusceptibility to Biocides. *Am. J. Infect. Cont.* **2001**, 29, 259-261.
- [28] Russell, A. D. Bacterial Resistance to Disinfectants: Present Knowledge and Future Problems. *J. Hosp. Infec.* **1999**, 43, S57-S68.
- [29] Scholl, S.; Koch, A.; Henning, D.; Kempter, G.; Kleinpeter, E. The Influence of Structure and Lipophilicity of Hydantoin Derivatives on Anticonvulsant Activity. *Struc. Chem.* **1999**, 10, 355-366.
- [30] Block, S. S. Disinfection, Sterilization and Preservation. Third Ed. **1983**, Philadelphia, Lea & Febiger, pp.743-745.
- [31] Nakae, T. Outer Membrane of Salmonella. Isolation of Protein Complex that Produces Transmembrane Channels. *J. Biol. Chem.* **1976**, 251, 2176-2178.
- [32] Shirai, A.; Sumitomo, T.; Yoshida, M.; Kaimura, T.; Nagamune, H.; Maeda, T.; Kourai, H. Synthesis and Biological Properties of Gemini Quaternary Ammonium Compounds, 5,5'-[2,2'-(α,ω -Polymethylenedicarbonyldioxy)diethyl]bis-(3-alkyl-4-methylthiazolium iodide) and Its Brominated Analog. *Chem. Pharm. Bull.* **2006**, 54, 639-645.

- [33] Liu, W.; Liu, X.; Knaebel, D.; Luck, L.; Li, Y. Synthesis and Antibacterial Evaluation of Novel Water-soluble Organic peroxides. *Antimicrobial Agents & Chemo.* **1998**, 42, 911-915.
- [34] Jia, Z.; Shen, D.; Xu, W. Synthesis and Antibacterial Activities of Quaternary Ammonium Salt of Chitosan. *Carbohy. Res.* **2001**, 333, 1-6.
- [35] Berger, A. Hydantoinylsilanes, US Patent 4,412,078, **1983**.

2.6 Supporting Information

The Supporting Information includes additional information about chlorine loadings of synthesized compounds at different concentrations, NMR spectra of the compounds, and FT-IR spectra of coated fabrics.

Table S.2.1. Chlorine loadings (Cl^+ %) for the coating solutions at different concentrations.

Compound concentration (%)	Methyl-methyl	Methyl-methyl ^a	Methyl-propyl	Methyl-pentyl	Methyl-heptyl	Ethyl-ethyl	Butyl-butyl
0.8			0.06	0.08	0.11		
1.6					0.21		
1.8			0.13				
2				0.24	0.29	0.12	0.20
2.4				0.27			0.26
2.6							0.30
2.8							0.35
3.0		0.21					
3.2						0.32	
3.5		0.28	0.19				
3.7		0.35					
4.0	0.04						
4.4			0.28				
4.6				0.54			
5.0	0.09				0.62		
5.4			0.41			0.52	
5.8				0.65			
6.0	0.13						
6.5					0.70		

^a Oligomer.

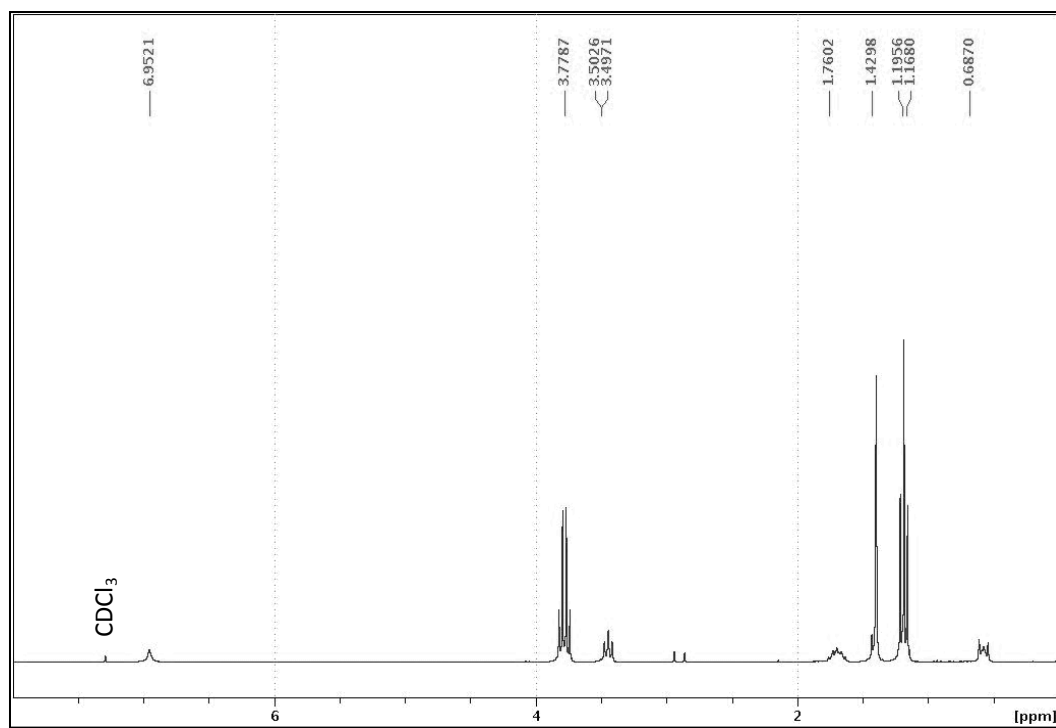


Figure S.2.1. The ¹H NMR spectra of 5,5-dimethylhydantoin siloxane.

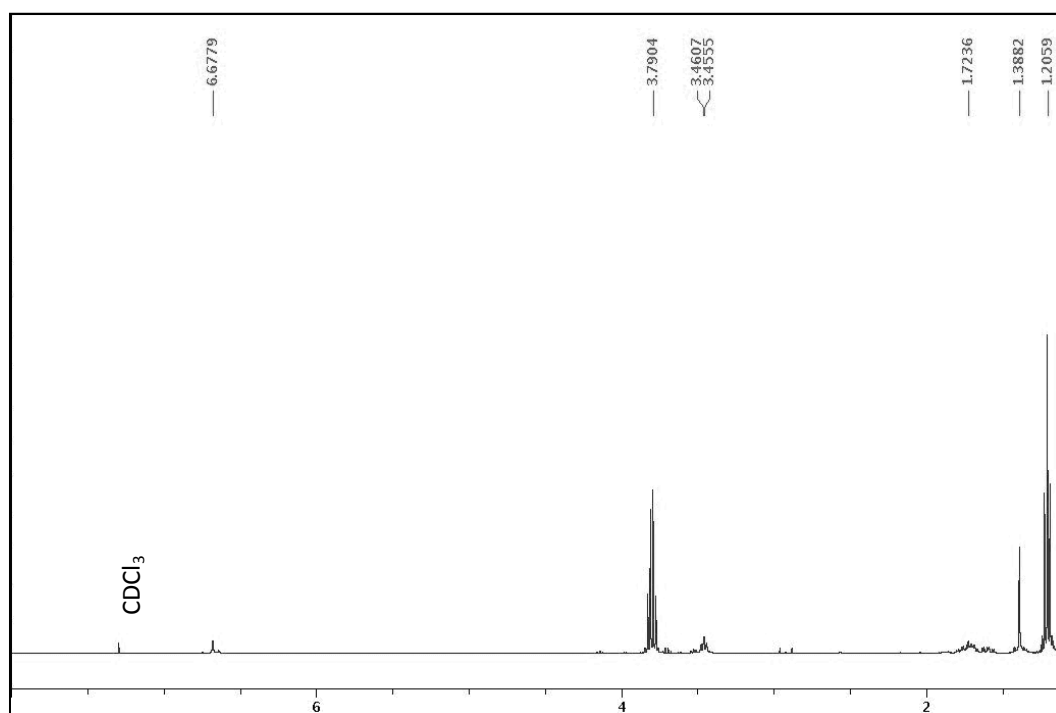


Figure S.2.2. The ¹H NMR spectra of 5-methyl-5-propylhydantoin siloxane.

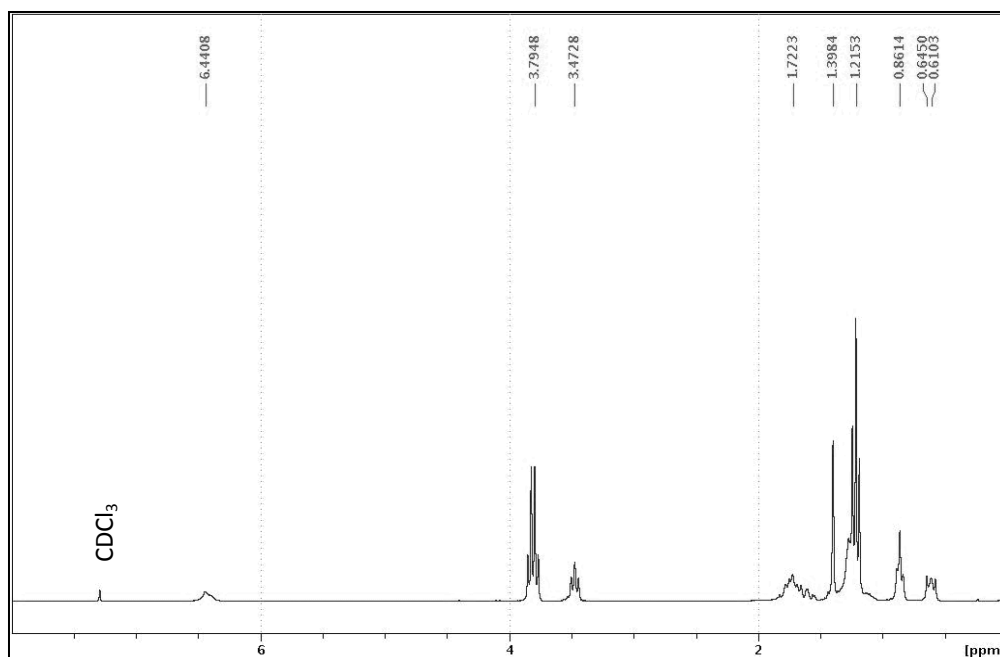


Figure S.2.3. The ¹H NMR spectra of 5-methyl-5-pentylhydantoin siloxane.

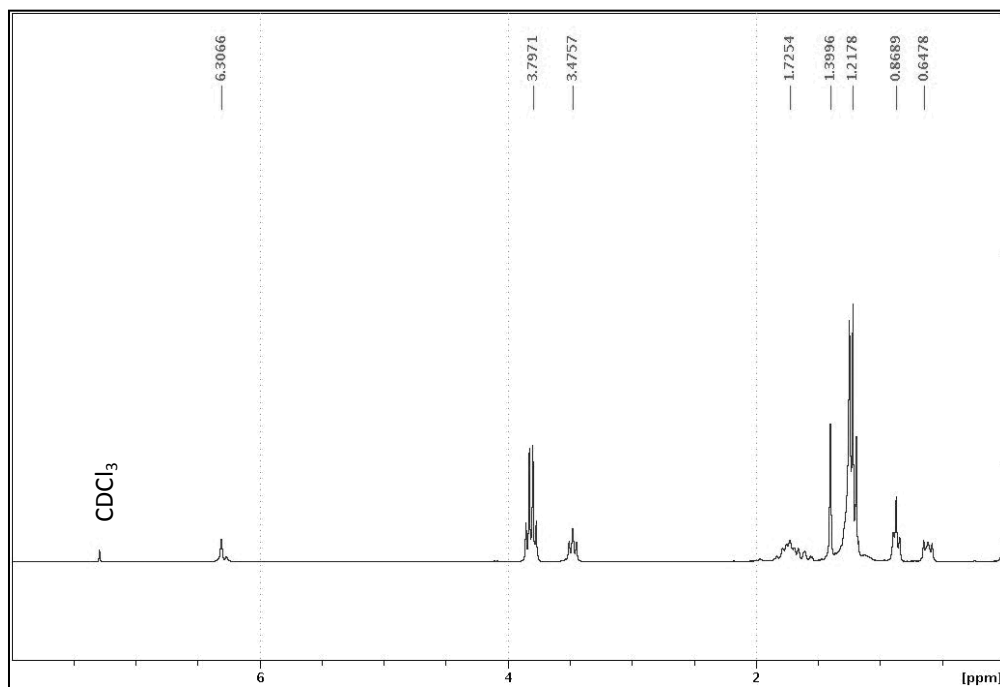


Figure S.2.4. The ¹H NMR spectra of 5-heptyl-5-methylhydantoin siloxane.

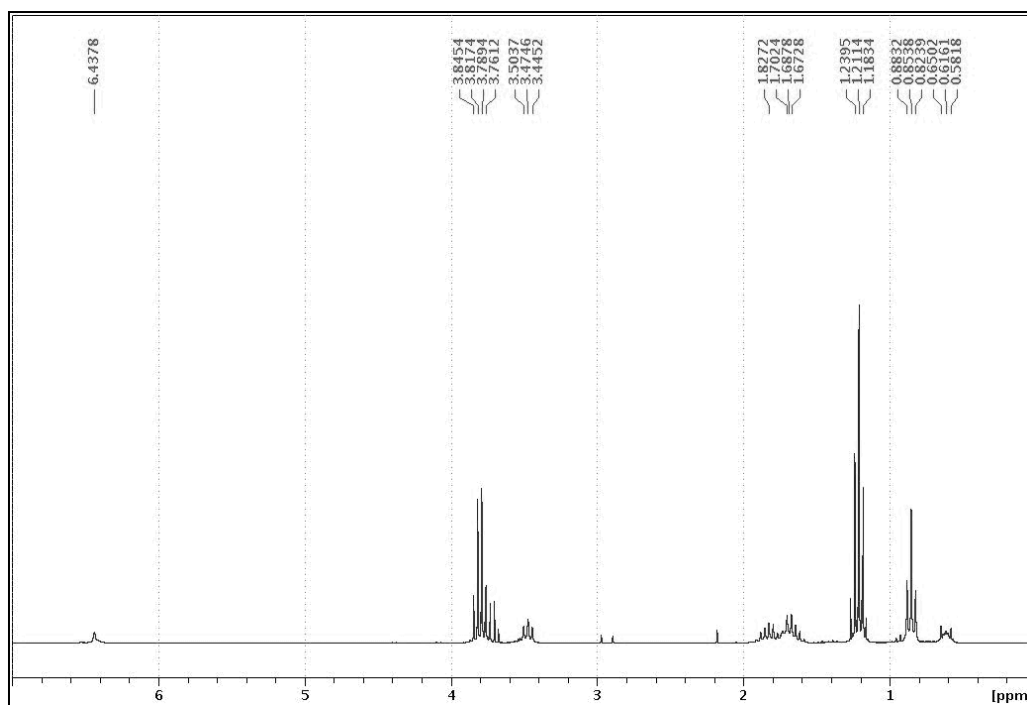


Figure S.2.5. The ^1H NMR spectra of 5,5-diethylhydantoin siloxane.

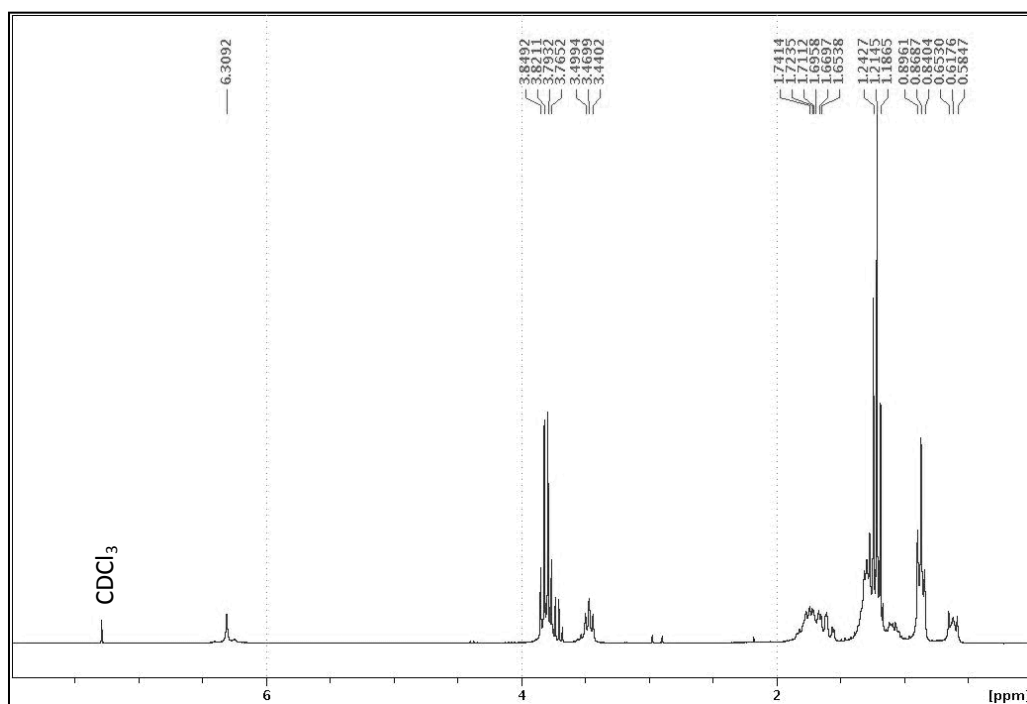


Figure S.2.6. The ^1H NMR spectra of 5,5-dibutylhydantoin siloxane.

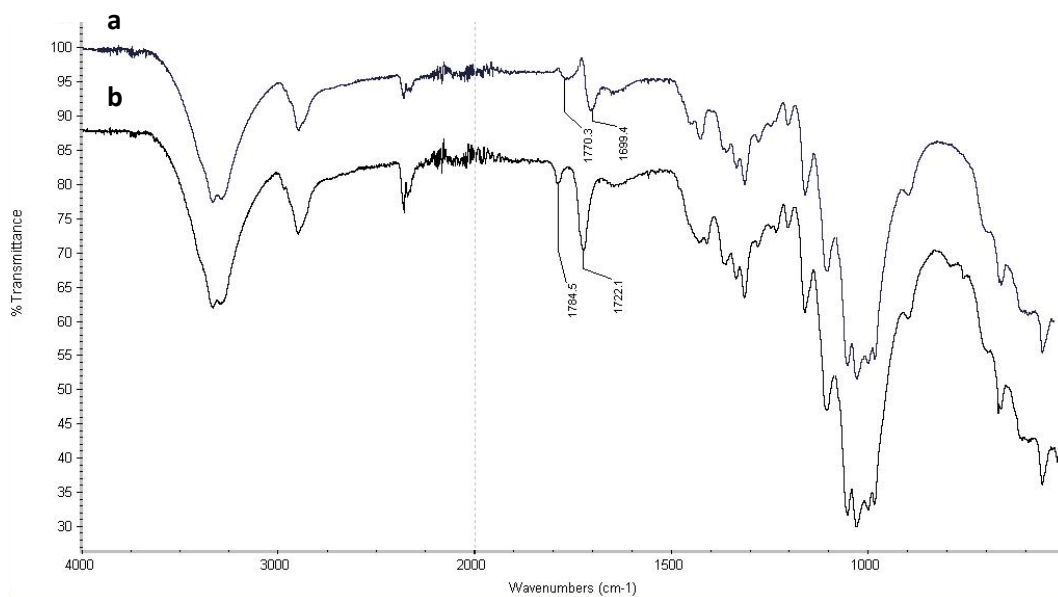


Figure S.2.7. FT-IR spectra of 5,5-dimethylhydantoin siloxane (methyl-methyl) coated cotton fabric before (a) and after (b) chlorination.

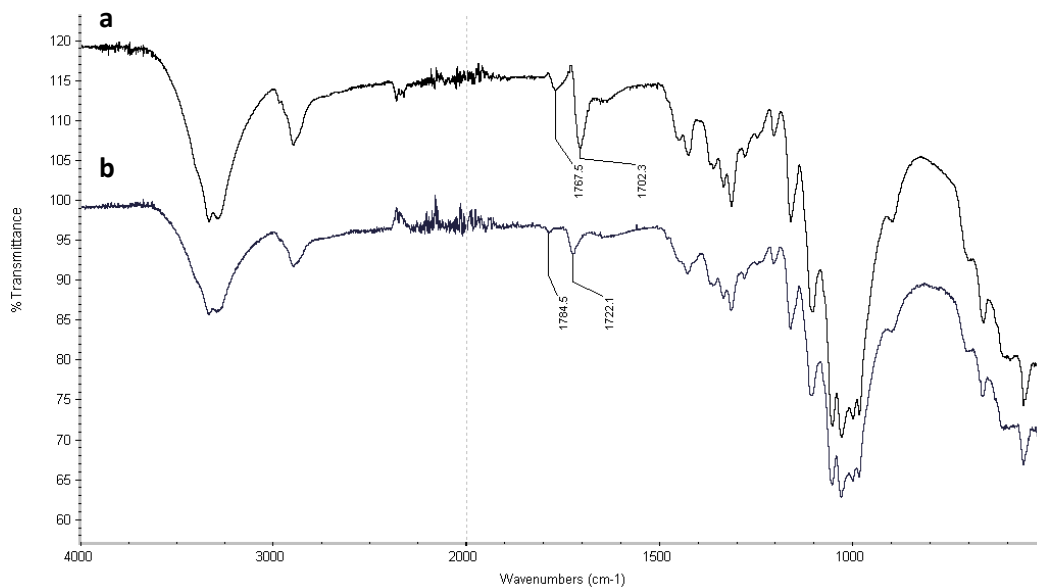


Figure S.2.8. FT-IR spectra of 5-methyl-5-propylhydantoin siloxane (methyl-propyl) coated cotton fabric before (a) and after (b) chlorination.

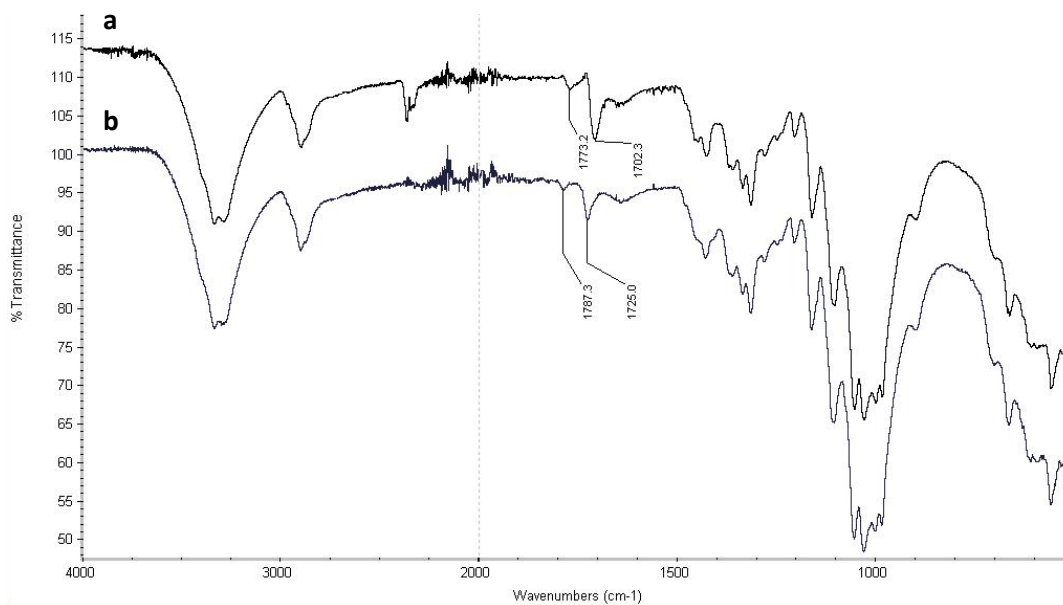


Figure S.2.9. FT-IR spectra of 5-methyl-5-pentylhydantoin siloxane (methyl-pentyl) coated cotton fabric before (a) and after (b) chlorination.

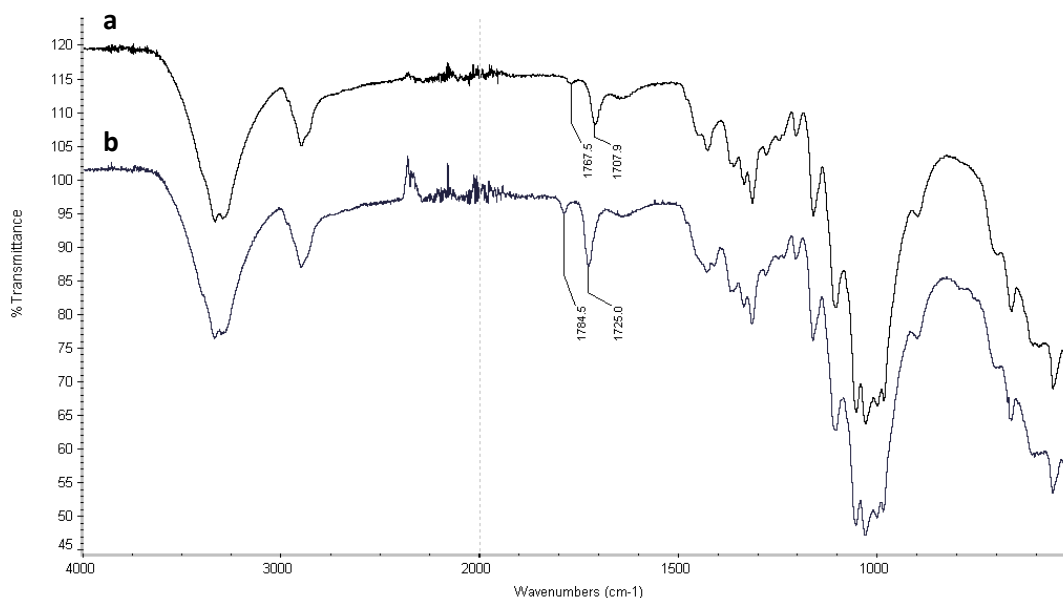


Figure S.2.10. FT-IR spectra of 5-heptyl-5-methylhydantoin siloxane (methyl-heptyl) coated cotton fabric before (a) and after (b) chlorination.

CHAPTER 3

EFFECT OF PHENYL DERIVATIZATION ON SEVERAL PROPERTIES OF N-HALAMINE ANTIMICROBIAL SILOXANE COATINGS

3.1 Introduction

N-halamine compounds are currently used in the manufacturing of numerous biocidal materials.¹⁻⁷ Briefly, N-halamine precursors are covalently bonded to various surfaces and then converted into N-halamine materials through a halogenation process.³⁻⁸ They have been demonstrated to be one of the most efficacious biocidal agents due to their long-term stabilities, non-toxicity to humans, biocidal functions against a broad range of microorganisms, and regenerable properties upon exposure to washing cycles.¹⁻⁷

Since the kinetics of inactivation of the microorganisms depend on the nature of the N-Cl moiety,⁹ various heterocyclic rings were designed containing amine, amide, or imide groups.^{10,11} Due to their differences in chemical environment, they have different stabilities, towards dissociation of the N-Cl moiety, in the order amine>amide>imide halamine, while the antimicrobial activity is in reverse order. Generally, amide N-halamines are preferred for providing both good stability and strong biocidal activity.¹² Among various N-halamine compounds, heterocyclic structures such as hydantoins, oxazolidinones and imidazolidinones have been studied intensively to achieve higher

stability compared to aliphatic structures.¹³ N-halamine agents can be incorporated into substrates as an additive or can be covalently bound to substrates via various tethering groups (siloxanes, diols, etc.).^{1,6,7} In this regard, several N-halamine moieties have been successfully attached onto various polymers by using alkoxysilanes.^{10,11,14} These N-halamine precursors exhibited superior biocidal activity and washing durability. However, in addition to fast breakage of N-Cl bonds after exposure to UV light, the precursors could not be regenerated back to their initial chlorine loadings. UV light stability is one of the most important requirements for the materials subjected to sunlight frequently (eg. army uniforms). The N-Cl bond is very sensitive to UV light and needs protection from exposure.¹⁴ Thus, N-halamines are unstable under UV light.¹⁵ In general, only unsaturated organic molecules absorb at wavelengths greater than 220nm.^{16-18,20,21} Therefore, the principal structures used in UV blockers are the more stable aromatic molecules. This general structure allows the molecule to absorb high-energy UV rays and release the energy as lower-energy rays.

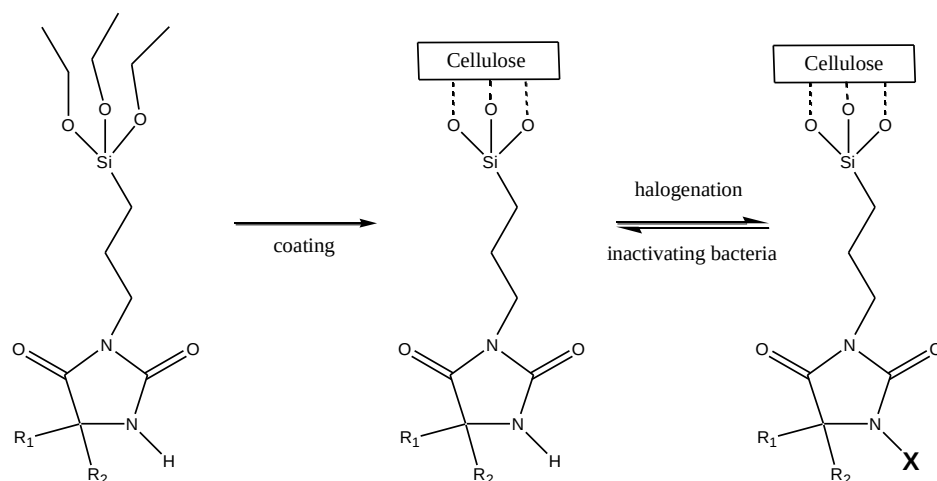


Figure 3.1. The preparation of antimicrobial coatings. (X = Cl, Br)

The stability of N-halamine materials is provided by electron-donating groups (alkyl, phenyl, etc.) substituted onto the heterocyclic rings adjacent to the oxidative N-X moieties which strengthen the N-X covalent bond.^{9,19} In this regard, phenyl group substitution onto the carbon next to the N-X moiety might increase the stability of the N-X covalent bond and can contribute the absorption of UV light. The UV light blocking property of phenyl substitution on various compositions was investigated.^{20,21}

In this regard, N-halamine polymers containing aromatic rings on their structures have been found remarkably resistant to UV exposure because aromatic structures can shield and protect N-Cl moieties by contributing to the absorption of UV irradiation.²²

In addition, although the hydantoin ring itself does not present any medical activity, many 5-phenyl substituted hydantoins have shown interesting biological properties which might be necessary in biocidal activity.^{25,26,41}

The hydantoin ring system has been intensively studied since it was discovered in 1861²³ and has been studied by our research group for the last two decades. Increasingly demanding antimicrobial applications require more sophisticated use of biocidal systems. In this study a series of hydantoinyl siloxanes (SHS) containing variations in methyl and phenyl group substitution at the 5 position of the hydantoin ring (shown as R₁ and R₂ in Figure 3.1) were prepared and bonded to cellulose. The antibacterial activities, stabilities, and UV resistance of the different derivatives were compared to attempt to ascertain the influence of electronic, steric, and hydrophobic/hydrophilic effects for the N-halamine biocidal materials. The mimics of the hydantoinyl siloxanes (SHSm) were prepared to investigate the stability of the N-Cl bond and the degradation mechanism during UV light exposure.

3.2 Experimental Section

General Procedure for the Synthesis of 5-substituted-hydantoin Derivatives (SH) and Their Siloxanes (SHS). 5,5-dimethylhydantoin and 5,5-diphenylhydantoin were purchased from Sigma Aldrich Co. (Milwaukee, WI) and used without any further purification. 5-methyl-5-phenylhydantoin was prepared by the Bucherer-Berg reaction described previously in section 2.2. The product was confirmed by ^1H and ^{13}C NMR; the melting point was 192.8 °C, and the yield was 85% by weight.

The triethoxysilylpropyl hydantoin derivatives (SHSs) were prepared according to a general procedure outlined previously in section 2.2. The structures of SHSs were confirmed by ^1H and ^{13}C NMR; yields ranged from 92 to 97 % by weight.

Spectral Data for the Synthesized SHSs.

(i) 3-triethoxysilylpropyl-5,5-dimethylhydantoin (MM). MM was obtained as a colorless viscous liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.58 (t, J = 8.50, 2H), 1.19 (t, J =7.00, 9H), 1.40 (s, 6H), 1.70 (p, J = 7.94, 2H), 3.45 (t, J = 7.38, 2H), 3.78 (q, J = 7.00, 6H), 6.95 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 177.53, 156.98, 61.80, 58.43, 40.97, 24.91, 21.57, 18.18, 7.57.

(ii) 3-triethoxysilylpropyl-5-methyl-5-phenylhydantoin (MP). MP was obtained as a colorless viscous liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.54 (t, J = 8.25, 2H), 1.15 (t, J = 7.00, 9H), 1.67 (p, J = 8.25, 2H), 1.77 (s, 3H), 3.44 (t, J = 7.25, 2H), 3.73 (q, J =7.00, 6H), 7.31 (m, 3H), 7.51 (d, J = 6.75, 2H), 7.84 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 175.46, 157.40, 139.01, 128.73, 128.19, 125.22, 63.51, 58.33, 41.13, 25.68, 21.52, 18.21, 7.42.

(iii) 3-triethoxysilylpropyl-5,5-diphenylhydantoin (PP). PP was obtained as a slightly yellow liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.60 (t, J = 8.50, 2H), 1.20 (t, J = 7.00, 9H), 1.77 (p, J = 7.75, 2H), 3.57 (t, J = 7.25, 2H), 3.78 (q, J = 7.00, 6H), 7.38 (m, 10H), 7.94 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 173.43, 157.16, 139.36, 128.74, 128.40, 126.83, 69.98, 58.33, 41.46, 21.61, 18.33, 7.52.

General Procedure for the Synthesis of 3-butyl-5-substituted hydantoin derivatives (SHSm). The potassium salts of the hydantoin derivatives were prepared as described in the general procedure for the synthesis of hydantoin siloxanes. The anhydrous salt was dissolved in *N,N*-dimethyl formamide (DMF) at 95 °C, and then an equimolar amount of 1-bromobutane (J.T. Baker Chemical Co.) was added to the solution. The resulting mixture was stirred for 3 h.²⁴ The KBr produced in the reaction was removed by filtration, and DMF was removed at reduced pressure. The residual mixture was dissolved in chloroform, and then the solid impurities (salt) were filtered. The chloroform was removed at reduced pressure. The structures of the 3-butyl-5-substituted hydantoin derivatives were confirmed by ^1H and ^{13}C NMR; yields ranged from 90 to 95 wt% .

(iv) 3-butyl-5,5-dimethylhydantoin (MMm). MMm was obtained as a colorless viscous liquid. ^1H NMR (CDCl_3 , 250 MHz) δ 0.94 (t, J =7.25, 3H), 1.33 (sext, J =8.00, 2H), 1.44 (s, 6H), 1.60 (pent, J =7.25, 2H), 3.49 (t, J =7.25, 2H), 6.70 (s, 1H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 13.60, 19.82, 24.87, 30.09, 38.22, 58.56, 157.09, 177.73.

(v) 3-butyl-5-methyl-5-phenylhydantoin (MPm). MPm was obtained as a white powder, melting point: 89.8 °C. ¹H NMR (CDCl₃, 250 MHz) δ 0.90 (t, J=7.25, 3H), 1.29 (sext, J=7.50, 2H), 1.58 (pent, J=7.25, 2H), 1.82 (s, 3H), 3.49 (t, J=7.25, 2H), 6.82 (s, 1H), 7.29-7.44 (m, 3H), 7.51 (d, J=6.50, 2H). ¹³C NMR (CDCl₃, 63 MHz) δ 13.62, 19.86, 25.64, 30.07, 38.61, 63.51, 125.23, 128.41, 128.88, 138.79, 157.16, 175.39.

(vi) 3-butyl-5,5-diphenylhydantoin (PPm). PPm was obtained as a white powder, melting point: 129.1 °C. ¹H NMR (CDCl₃, 250 MHz) δ 0.90 (t, J=7.25, 3H), 1.31 (sext, J=8.00, 2H), 1.61 (pent, J=7.00, 2H), 3.55 (t, J=7.25, 2H), 7.24 (s, 1H), 7.29-7.53 (s, 10H). ¹³C NMR (CDCl₃, 63 MHz) δ 13.62, 19.89, 30.08, 38.83, 70.01, 126.83, 128.50, 128.80, 139.29, 156.99, 173.38.

General procedure for the synthesis of 3-butyl-1-chloro-5-substituted hydantoin derivatives (SHSm-Cl). SHSm and trichloroisocyanuric acid (molar ratio, 1:3) were

dissolved in acetone and stirred for 1 h at room temperature.²⁴ The acetone was removed and hexane was added to the mixture. The insoluble solids were filtered, and then the hexane was evaporated. SHSm-Cl's were obtained as powders. The active chlorine content of the compounds was determined by a modified iodometric/thiosulfate titration. SHSm-Cl (around 0.05 g) was suspended in a solution of 90 mL ethanol and 10 mL 0.1N acetic acid. After addition of 0.2 g KI, the mixture was titrated with 0.0375N sodium thiosulfate until the yellow color disappeared at the end point. The weight percent Cl⁺ on the samples was calculated by the following formula;

$$\text{Cl}^+\% = (\text{N} \times \text{V} \times 35.45) / (2 \times \text{W}) \times 100\% \quad (1)$$

where $\text{Cl}^+(\%)$ is the weight percent of oxidative chlorine on the samples, N and V are the normality (equiv/L) and volume (L) of the titrant sodium thiosulfate, respectively, and W is the weight of the sample in g.

(vii) 3-butyl-1-chloro-5,5-dimethylhydantoin (MMm-Cl). MMm-Cl was obtained as white crystals, melting point: 44.1 °C (lit.²⁴ 45.4 °C). $\text{Cl}^+\%=16.0$, theoretical value is 16.2. ^1H NMR (CDCl_3 , 250 MHz) δ 0.93 (t, $J=7.50$, 3H), 1.32 (sext, $J=7.75$, 2H), 1.46 (s, 6H), 1.61 (pent, $J=7.50$, 2H), 3.56 (t, $J=7.25$, 2H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 13.57, 19.79, 22.20, 29.96, 39.47, 65.71, 154.71, 174.35.

(viii) 3-butyl-1-chloro-5-methyl-5-phenylhydantoin (MPm-Cl). MPm-Cl was obtained as a white powder, melting point: 80 °C. $\text{Cl}^+\%=12.5$, theoretical value is 12.6. ^1H NMR (CDCl_3 , 250 MHz) δ 0.92 (t, $J=7.25$, 3H), 1.31 (sext, $J=7.75$, 2H), 1.62 (pent, $J=7.25$, 2H), 1.90 (s, 3H), 3.59 (t, $J=7.25$, 2H), 7.32-7.55 (m, 5H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 13.57, 19.79, 21.48, 29.94, 39.82, 70.36, 125.96, 129.07, 129.09, 135.32, 155.07, 172.54.

(ix) 3-butyl-1-chloro-5,5-diphenylhydantoin (PPm-Cl). PPm-Cl was obtained as a white powder, melting point: 83.1 °C. $\text{Cl}^+\%=10$, theoretical value is 10.3. ^1H NMR (CDCl_3 , 250 MHz) δ 0.94 (t, $J=7.50$, 3H), 1.35 (sext, $J=7.50$, 2H), 1.69 (pent, $J=7.00$, 2H), 3.68 (t, $J=7.25$, 2H), 7.26-7.37 (m, 4H), 7.37-7.56 (m, 6H). ^{13}C NMR (CDCl_3 , 63 MHz) δ 13.58, 19.89, 30.01, 40.21, 76.83, 128.34, 128.69, 129.33, 135.67, 154.64, 171.23.

Characterization. The NMR spectra were obtained using a Bruker 250 MHz spectrometer; ^1H and ^{13}C spectra were recorded with 16 and 1024 scans, respectively. The IR data were obtained with a Nicolet 6700 FT-IR spectrometer ATR (Attenuated Total Reflectance) accessory, recorded with 32 scans at 2 cm^{-1} resolution. Thermal data were obtained using a DSC Q2000 TA Instruments at a heating and cooling rate of $10\text{ }^\circ\text{C}/\text{min}$ under N_2 atmosphere.

Coating Procedure. In this work the objective was to prepare coatings with as nearly as possible equal chlorine loadings to remove chlorine loading as a variable. Precursor siloxane monomers were first dissolved in an ethanol/water mixture (1:1 by weight) at concentrations ranging from 3 to 15 weight percent so as to subsequently obtain chlorine loadings between 0.25 to 0.30%. The mixture was stirred for 15 min to produce a uniform solution. Cotton swatches (Style 400 Bleached 100% Cotton Print Cloth from Testfabrics, Inc., West Pittston, PA) were soaked in the solution for 15 min and then cured at $95\text{ }^\circ\text{C}$ for 1 h. Then the swatches were soaked in a 0.5% detergent solution for 15 min, rinsed several times with water, and dried at room temperature. The coating solution containing the dissolved siloxane derivatives weighed 50 g, and the cotton swatches were cut into areas of 50 in^2 each.

Chlorination Procedure. The treated fabrics were chlorinated by soaking in 10% household bleach (0.6% sodium hypochlorite) at pH 7 (adjusted with 6 N HCl) for 1 h. After rinsing with tap and distilled water, the swatches were then dried at $45\text{ }^\circ\text{C}$ for 1 h to remove any unbonded chlorine from the material.

FTIR Confirmation of Siloxane Derivative Bonding. FTIR spectra of the coated fabrics confirmed that the N-halamine precursors bonded to the cotton fabric, as a band at ca. 1770 cm^{-1} was detected, which can be assigned to the carbonyl band of the amide structure in the hydantoin moieties. After the treatment with bleach, this band shifted to ca. 1780 cm^{-1} indicating disruption of N-H---O=C hydrogen bonding as conversion of N-H to N-Cl occurred.

Analytical Titration. The chlorine concentration loaded onto the samples was determined by the iodometric/thiosulfate titration method. The weight percent Cl^+ was determined from Equation 1.

Biocidal Efficacy Testing. Gram-negative *Escherichia coli* (*E. coli*) is generally more resistant to chlorine disinfection than other organisms of vegetative bacteria, so it was selected as the test organism for determining the effectiveness of disinfection by the chlorinated hydantoinyl siloxane derivatives. Coated fabric swatches were challenged with *E. coli* O157:H7 (ATCC 43895) bacterial suspensions in pH 7 phosphate buffer solution (100 mM). 25 μl of the bacterial suspensions were added in the center of a 1 in. square fabric swatch, and a second identical swatch was laid on the first swatch. A sterile weight was used to ensure sufficient contact of the swatches with the inoculums. The contact times for the swatches with the bacteria were 1, 5, 10, and 30 min. At those contact times the fabric swatches were quenched with 0.02 N sodium thiosulfate solution to remove any oxidative chlorine which could cause extended disinfection. Serial dilutions of the solutions contacting the surfaces were plated on Trypticase agar,

incubated for 24 h at 37 °C, and colony counts were made to determine the presence of viable bacteria. Unchlorinated control samples were treated in the same manner.

Washing Testing. The stability and rechargeability of chlorine on the samples were evaluated by using a standard washing test according to AATCC Test Method 61.

The cotton samples were washed for the equivalents of 5, 10, 25, and 50 machine washes in a Launder-Ometer. The Cl^+ % loadings of the samples after the washings with or without prechlorination and after rechlorination were determined by the titration procedure outlined above.

UV Light Stability Testing. UV light stability of the bound chlorine and the hydantoinyl siloxane coatings on the derivatized cotton fabric were measured by using an Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA).

The samples were placed in the UV (Type A, 315-400 nm) chamber for times in the range of 1 to 10 h. After a specific time of exposure to UV irradiation, the samples were removed from the UV chamber and titrated, or rechlorinated and titrated. UV light irradiation of the chlorinated mimics (SHSm-Cl_s) was managed in the UV chamber. The chlorinated mimics were exposed to UV light for 24 h and then immediately analyzed by NMR. The temperature was 37.6 °C, and the relative humidity was 17% during the UV irradiation.

3.3 Results and Discussion

Coating Procedure. The biocidal characteristics of a coated fabric mainly depend on the structure of the coating compound and the concentration of the biocidal sites.² In this regard, an attempt was made to keep the Cl^+ active site concentration on the fabric at a constant level, about 0.30% of Cl^+ loading, for all of the SHS derivatized cotton fabrics. Past work in these laboratories has demonstrated that changes of 0.03% in chlorine loading do not materially affect the performance of a given N-halamine biocide,¹⁴ so any differences observed among the derivatives in this work should not be attributed to slightly different chlorine loadings on the swatches. The concentration of the coating solution was 8 wt% for the MM derivative while it was 3 wt% for the MP and PP derivatives, because phenyl substitution reduces the water solubility of the compounds.

Antimicrobial Efficacies. The treated cotton swatches were challenged with *E. coli* O157:H7 at concentrations between 10^8 to 10^9 CFU (colony-forming units), as summarized in Table 3.1. The unchlorinated control samples provided only about 0.10 log reductions, due to the adhesion of bacteria to the cotton swatches, within 30 min contact time intervals. All of the chlorinated treated samples showed excellent antimicrobial activity through three independent biocidal tests. PP-Cl and MP-Cl provided a total inactivation within 5 min, while MM-Cl required 10 min contact, in Test I. The phenyl substituted derivatives (PP-Cl and MP-Cl) exhibited the same trend, relatively higher inactivation rate, in Test II and Test III.

Table 3.1. Biocidal Tests.

Hydantoin derivative	Contact time (min)	Bacterial reduction					
		Test I		Test II		Test III	
		%	log	%	log	%	log
MM	30	42.6	0.24	5.5	0.03	6.8	0.03
MP	30	23.4	0.12	22.7	0.11	1.0	0.01
PP	30	29.8	0.15	9.8	0.05	3.9	0.02
MM-Cl	1	99.9	4.7	99.9	5.29	99.9	4.42
	5	99.9	5.29	100.0	8.19	99.9	5.33
	10	100.0	8.32	100.0	8.19	100.0	8.36
	30	100.0	8.32	100.0	8.19	100.0	8.36
MP-Cl	1	99.9	5.89	100.0	8.19	99.9	5.42
	5	100.0	8.32	100.0	8.19	99.9	5.54
	10	100.0	8.32	100.0	8.19	100.0	8.36
	30	100.0	8.32	100.0	8.19	100.0	8.36
PP-Cl	1	99.9	6.02	100.0	8.19	99.9	4.89
	5	100.0	8.32	100.0	8.19	100.0	8.36
	10	100.0	8.32	100.0	8.19	100.0	8.36
	30	100.0	8.32	100.0	8.19	100.0	8.36

^a Microorganism: *E. coli* O157:H7. Total bacteria: 2.10×10^8 (8.32 logs), 1.56×10^8 (8.19 logs) and 2.30×10^8 (8.362 logs) for Test I, Test II and Test III, respectively.

^b Test I: Chlorine loadings on the coated swatches were 0.31, 0.32, and 0.29 %, respectively. Test II: Chlorine loadings on the coated swatches were 0.28, 0.33, and 0.31 %, respectively. Test III: Chlorine loadings on the coated swatches were 0.31, 0.33, and 0.29 %, respectively.

We conclude that the results of this study indicate slight differences in the biocidal efficacies of the derivatives when the derivatization (phenyl group) is performed at position 5 of the hydantoin ring for the hydantoinylsiloxane coatings. The phenyl substituted derivatives inactivated relatively faster than the dimethyl derivative. We also conclude that all of the derivatives produced extremely effective biocidal coatings.

Stability toward Washing. The stabilities toward machine washing of coated fabric swatches are presented in Table 3.2. Three types of washing experiments were performed - prechlorinated coatings at the concentration levels indicated at 0 machine washes in Table 3.2 (X), prechlorinated and rechlorinated after a given number of machine washes (Y), and unchlorinated until after a given number of machine washes (Z). All coatings are partially lost upon successive washing. Several observations can be made pertaining to the data in Table 3.2. First, the greatest loss of Cl^+ and/or coating occurred during the first washing cycle (equivalent to 5 machine washes) in all cases; the losses between 5 and 50 washes were small. The decline in Cl^+ values of X and Z are indicative of the loss of coating and chlorine. A comparison of Y and Z values indicates that the loss of coating was substantial. Second, prechlorination protected the coatings from hydrolyses from the surfaces of the fibers as can be seen from the differences between Y and Z values.

We have noted previously in these laboratories that prechlorination generally increases the hydrophobicity of surfaces bound to N-halamines and thus protects the coatings to some extent from hydrolyses.⁶ Third, all of the surfaces retained at least 0.03% chlorine loading after the equivalent of 50 machine washes which is adequate for an antimicrobial effect.⁸ Fourth, just as for the biocidal efficacy studies addressed above, there are some differences between the several derivatives in the washing tests. Phenyl substituted

derivatives lost the bound chlorine faster than the methyl substituted derivatives (Table 3.2 (X)). Moreover, this rate is not a result of the dissociation of tethering groups (siloxane) from cotton because rechlorination of the derivatives provided similar chlorine loadings for all derivatives (Table 3.2 (Y)). Lastly, unchlorinated phenyl derivatives (Z) are slightly more resistant toward washing cycles compared to methyl substituted derivatives, most likely because of the more hydrophobic coating character.

Table 3.2. Stability toward washing of cotton coated with derivatized hydantoinyl siloxanes (Cl⁺% remaining).

	MM			MP			PP		
Machine washes^b	X ^a	Y ^a	Z ^a	X	Y	Z	X	Y	Z
0	0.39	0.39		0.38	0.38		0.41	0.41	
5	0.21	0.24	0.07	0.12	0.17	0.10	0.12	0.19	0.17
10	0.16	0.21	0.05	0.06	0.12	0.07	0.09	0.18	0.16
25	0.11	0.13	0.03	0.03	0.08	0.05	0.06	0.14	0.14
50	0.08	0.09	0.03	0.01	0.05	0.04	0.01	0.07	0.06

^a X: Chlorinated before washing, Y: Chlorinated before washing and rechlorinated after washing, Z: Unchlorinated before washing, but chlorinated after washing.

^b A washing cycle is equivalent to 5 machine washes in AATCC Test Method 61.

^c The error in the measured Cl⁺ weight percentage values was ± 0.01 .

Stability toward Ultraviolet Light Irradiation. The UV light stability of the N-Cl bond of the synthesized N-halamine siloxane derivatives exhibited distinctive differences among the several derivatized coatings (Figure 3.2). Phenyl substituted derivatives, PP-Cl and MP-Cl, lost bound chlorine faster than methyl substituted (MM-Cl) derivative. Clearly, there was some decomposition (around 40%) of the coatings over the six hour exposure as evidenced by the low Cl^+ loadings after rechlorination. In this regard, the primary decomposition process was the dissociation of N-Cl bonds. The stability was quite remarkable given that a six hour exposure in the UV chamber was equivalent to the same time in direct midday summer sunlight.

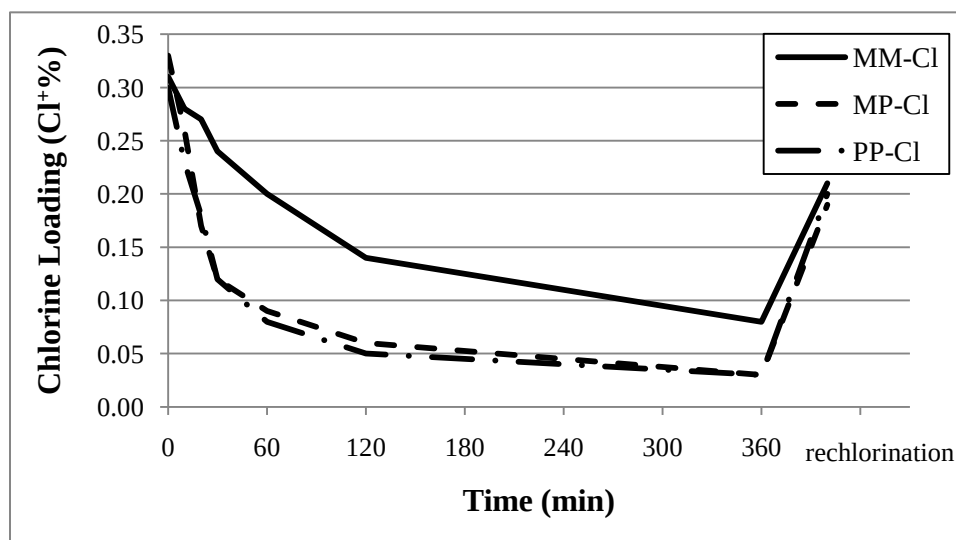


Figure 3.2. Stability toward UV light exposure of cotton coated with derivatized hydantoinyl siloxanes (Cl^+ % remaining).

The decomposition of the siloxane derivatives was investigated further by an additional experiment. The halogenated siloxane derivatives might decompose further under UV light compared to unhalogenated derivatives. In this regard, two set of samples; SHS

coated and SHS coated and then chlorinated, (coated onto cotton) were exposed to UV light to investigate the stability of the coatings (Table 3.3). Prechlorinated samples lost their rechargability gradually and the greatest loss of coating occurred during the first 10 h of UV light exposure. The UV light exposed samples were rechlorinated and then further exposed to UV light for several times.

Table 3.3. Stability toward repeated UV light exposure of cotton coated with derivatized hydantoinyl siloxanes (Cl⁺% remaining).

Time of exposure (h)	MM		PP	
	Prechlorinated	Unchlorinated	Prechlorinated	Unchlorinated
	0.34	0	0.29	0
10	0.03		0.04	
Rechlorination 1	0.22	0.33	0.17	0.27
10	0.06		0.04	
Rechlorination 2	0.18	0.27	0.10	0.27
10	0.07		0.03	
Rechlorination 3	0.16	0.26	0.07	0.28
10	0.07		0.02	
Rechlorination 4	0.11	0.26	0.06	0.27
10	0.06		0.02	
Rechlorination 5	0.11	0.27	0.06	0.28

Both prechlorinated MM and PP exhibited inefficient performance towards UV light exposure. However, unchlorinated samples can be chlorinated up to around initial chlorine loadings after exposed to UV light. Specifically, unchlorinated PP derivative exhibited better performance than MM derivative. The presence of chlorine on nitrogen

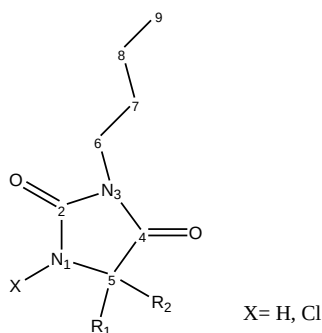
in the ring seems leading to more rapid ring degradation. We conclude that chlorinated hydantoin ring might decompose after the dissociation of the chlorine atom, and the tethering groups (Si-O-cell bonds) are not responsible for the loss of the coatings.

Potential Factors Affecting Nitrogen-Halogen Bond Lability. There are several factors which could influence the nitrogen-halogen bond lability upon phenyl derivatization at position 5 of the hydantoin ring which, in turn, would influence the rate of disinfection of microbes, stability towards washing, and UV light irradiation.

(1) Phenyl groups are better electron donors than methyl groups, and thus phenyl substitution should strengthen the N-Cl bond by increasing its polarity. (2) Phenyl groups at position 5 of the hydantoin ring could cause steric hindrance to the approach of microbial cells, thus reducing the rate of disinfection. However, the disinfection rate of phenyl substituted derivatives was higher implying that the N-Cl bond became weaker after phenyl substitution. Additionally, UV-light stability and washing tests also suggested weaker N-Cl bonds as in the biocidal tests. Thus, neither electronic effects nor steric hindrance are the primary mechanism influencing the N-Cl bond dissociation. (3) Phenyl rings are more lipophilic than methyl groups²⁵ and can contribute to the transport of the biocide through the bacteria's outer membrane consisting of phospholipids and thus increase the rate of disinfection or bioactivity.²⁶⁻²⁸ (4) An intramolecular through-space interaction can occur between the π -electrons of the aromatic ring and the hydrogen of the N₁ amide,²⁹⁻³¹ and this non-bonded interaction would be expected to be stronger after replacing the hydrogen with a chlorine (after the chlorination) atom having anisotropic electron density distribution.²⁹ The chlorine atom also might influence the electron distribution in the hetero hydantoin ring.³²

(5) N-Cl bond dissociation might be aided by π -complex formation between the chlorine atom and the π -electrons of the neighboring ring (phenyl substituent), and a stronger complex bond could make N-Cl dissociation much easier.³³ The chlorine atom also can provide its p-electrons to the phenyl ring when it is photo excited, so this process could further increase the bond strength of the π -complex,³³ due to the increased polarity of the amide N-Cl bond, leading to more-ionic character in the former and more-rapid dissociation by hydrolysis.⁹ (6) A less stable molecule conformation could occur due to non-bonded attraction between the aromatic ring and N-Cl moiety.^{16,30,34} The interaction between the chlorine atom and the π -cloud of the aromatic ring has an important role on the conformation of the overall molecule.³⁴

In this regard, mimics of all siloxane derivatives were synthesized, by attaching butyl groups onto the imide nitrogen (N₃) simulating the siloxane groups, to explore the structures (Figure 3.3).



	MMm	MPm	PPm
R₁	methyl	methyl	phenyl
R₂	methyl	phenyl	phenyl

Figure 3.3. Structure of the synthesized mimics.

The ¹H NMR data for the unchlorinated and chlorinated mimics gave further necessary information about the N₁-X bond (X=H, Cl) environment and the N-Cl bond dissociation rate (Figure 3.4). The most significant differences were found in the chemical shifts of

the N₁-H hydrogen among the unchlorinated mimics (MMm, MPm, and PPm), and phenyl hydrogens among unchlorinated and chlorinated phenyl substituted mimics (MPm, MPm-Cl, PPm, and PPm-Cl). Acetone-d₆ was used as solvent to detect the protic N₁-H hydrogen on the mimics for two reasons; the solvent CDCl₃ largely can shift the proton position due to inter-molecular hydrogen bonding,³⁵ and the characteristic CHCl₃ solvent residual peak at 7.27 ppm overlaps with the aromatic hydrogen signals in this region. For the unchlorinated mimics, instead of shifting to higher field due to the better electron donor substituents (phenyl),¹⁴ the N₁-H hydrogen in the phenyl substituted mimics resonated at lower fields 8.43 ppm, and 7.84 ppm, for PPm and MPm, respectively, than their counterpart in the MMm at 7.29 ppm. This might be due to the proximity of the N₁-H moiety to the benzene ring (substituted phenyl moiety).³⁶

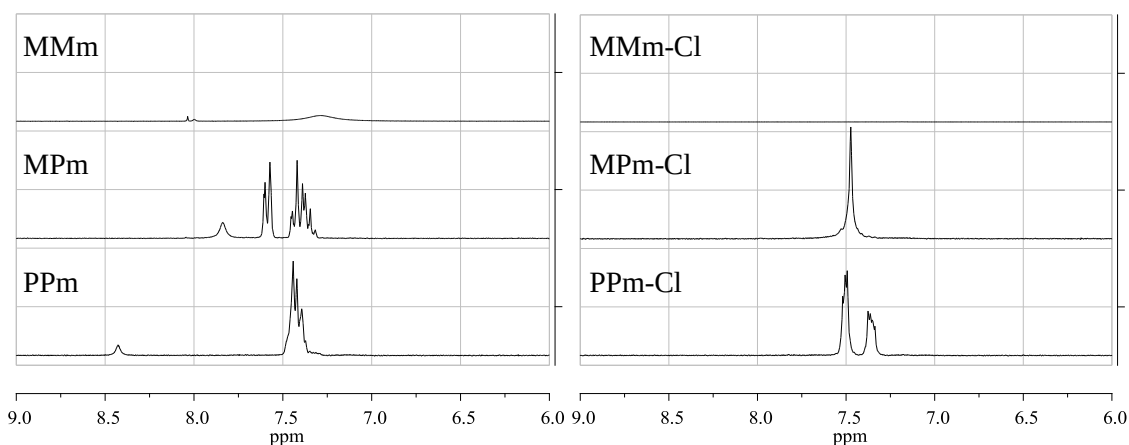


Figure 3.4. ¹H NMR spectra of the mimics before and after chlorination.(solv.acetone-d₆)

For the chlorinated mimics, the chlorinated phenyl substituted mimics (MPm-Cl and PPm-Cl) exhibited shifts in the signals of phenyl group hydrogens (aromatic) in the region between 7.3 - 7.6 ppm. This finding strongly suggests that the environment of the

aromatic hydrogens changed after chlorination, Figure 3.4. For the MPm derivative, the doublet at low field around 7.58 ppm was assigned to the aromatic ortho hydrogens (H_o), and the integrated area of the signal is two-fifths of the total area of the curve in this region of the spectrum. Coupling between the aromatic hydrogens prevented the identification of the meta and para protons signals, a multiplet around 7.42 ppm. The ortho phenyl hydrogens shifted to higher field, while the meta and para hydrogens shifted to lower field after chlorination and became a single signal at 7.47 ppm. For the PPm derivative, the aromatic hydrogens exhibited a multiplet between 7.35-7.50 ppm. The signals for some of the aromatic protons shifted to higher field while the others shifted to downfield after chlorination, as in the MPm derivative. For PPm-Cl, the group of signals at higher field (around 7.36 ppm) can be assigned to the ortho hydrogens since they occupy the theoretical value of four-tenths of the area of the curve. The meta and para hydrogen signals overlap at lower field (around 7.50 ppm), and the effect at the meta and para protons would be negligibly small. The difference in the chemical shifts between the signals indicates compositions of different conformational structures.³⁷

The ortho phenyl protons shifted to higher field possibly due to $CH\cdots HC$ repulsion, and similar shifts are reported in the literature for biphenyl-like molecules due to inter-annular phenyl conjugation.³⁸

Fujiwara et. al. investigated 5-benzyl-substituted hydantoins; the benzyl group was found remarkably folded over the hydantoin ring due to the strong dipole-dipole interaction between hydantoin and the π -electrons of the benzyl group; obviously, repulsion due to steric crowding is more than over compensated.^{29,30} However, in our case folding of the phenyl substituent is geometrically impossible over the hydantoin ring, and the

phenyl group encounters steric crowding (Figure 3.5).³⁰ The steric effect of cyclic moieties (phenyl group) at the 5 position of the hydantoin ring can cause the N₁-Cl moiety to be more labile than the methyl substituted derivatives.⁹

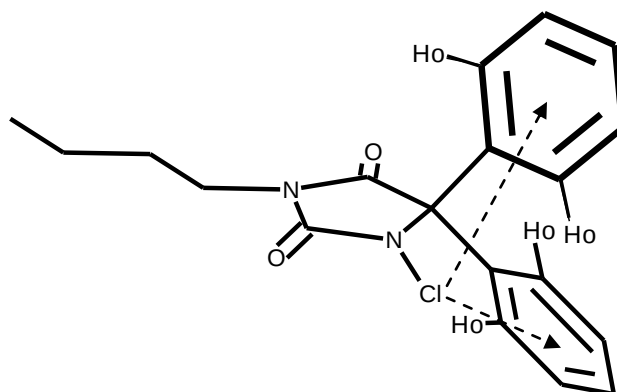


Figure 3.5. Representation of the steric effects.

^a Dotted lines represent trough-space interactions.

Structural configurations of 5-phenyl substituted hydantoins were studied in the literature; for 5,5-diphenylhydantoin, both phenyl groups were reported orthogonal, and intramolecular interaction between the aromatic protons and carbonyl oxygen restrained their orientation.^{39,40} In 5,5-diphenylhydantoin, the phenyl substituents were found twisted from the plane of the planar hydantoin ring by 104-114°; the two benzene rings are also twisted with respect to each other 90-105°.^{41,42} For the 5-methyl-5-phenylhydantoin, the twist of the ring from the hydantoin plane was reported smaller but still remarkable, 117-120°.⁴³ Prasanna et al. observed that the chlorine points towards the center of the aromatic ring resulting in an anti conformation of a similar molecule.³⁴ While the N₁-H hydrogen in phenyl substituted mimics cannot prevent rotation of the phenyl rings, the chlorine atom could force the phenyl rings to be twisted to lie in their

shielding region.⁴⁰ This case can cause rotation, about the C5-phenyl bond, of the substituted phenyls into a less stable conformation and result in shielded aromatic ortho hydrogen signals.^{44,45} On the other hand, the anisotropic effect of the 4-carbonyl group should be considered which can shield the phenyl ortho hydrogens (H_o), and this effect on the more distant meta and para protons would be much smaller.⁴⁰

DSC and FTIR Analysis. The DSC thermograms of all chlorinated mimics, Figure 3.6, exhibited an exothermic peak representing the N-Cl bond dissociation. The peak maximum was 221°C, 196 °C, and 199 °C for MMm-Cl, MPm-Cl, and PPm-Cl, respectively, suggesting the N-Cl bond become weaker due to phenyl substitution at the 5 position of the hydantoin ring.

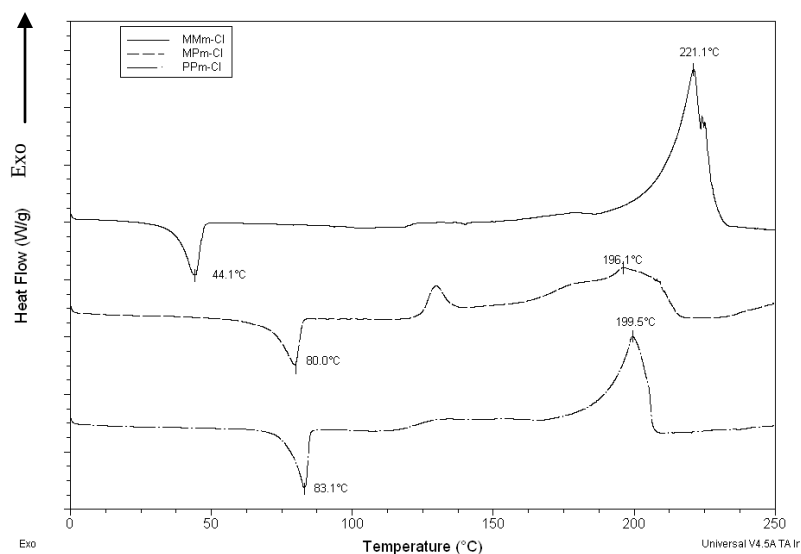


Figure 3.6. The DSC thermograms of the chlorinated mimics.

FTIR spectra of the mimics were also interesting to study, Figure 3.7. The N-H stretching band is at 3293 cm^{-1} for MMm, and the band occurred at relatively lower frequency 3254 cm^{-1} and 3167 cm^{-1} for MPm and PPm, respectively. Thus, the shift by about 40 cm^{-1} in MPm and 125 cm^{-1} in PPm with reference to the absorption at 3293 cm^{-1} exhibited by MMm might suggest the existence of an interaction between the free N-H moiety and the aromatic ring (phenyl group).⁴⁶ The second band at 3173 cm^{-1} appearing in the MPm spectra might correspond to the N-H $\cdots\pi$ interaction, and this band is more obvious in the PPm spectra at 3092 cm^{-1} .⁴⁷

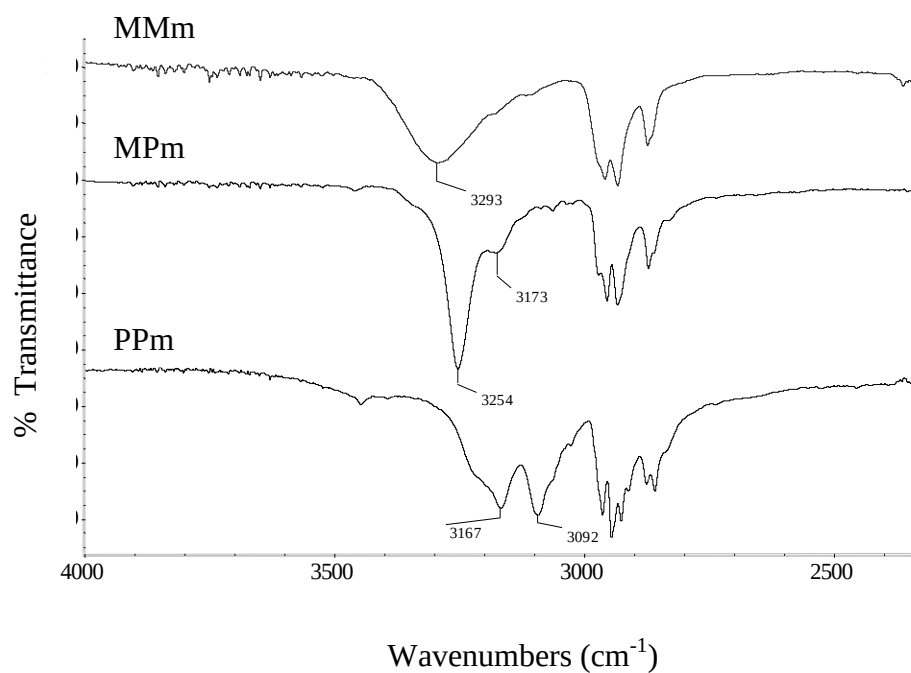


Figure 3.7. FTIR spectra of the mimics.

Decomposition under UV Light. The chlorinated mimics were irradiated with UV light for 24 h to investigate (with NMR spectroscopy) the structural changes after the decomposition. UV light exposure in 315-400nm range is considered most effective for organic compounds.²² N-halamine structures are sensitive to UV light because of the weak N-Cl bond. UV light irradiation can cause the N-Cl bond disruption and/or structural decomposition.^{15,22}

In general, the photolysis of N-halamines results in homolytic cleavage of the N-X bond (X=Cl, Br) involving radical or ionic cleavage pathways.⁴⁸ For the radical intermediates, Neal et al.⁴⁹ reported a rearrangement of the chlorine atom from the nitrogen atom into the acyl chain of an N-chloramide under UV irradiation which might explain the degradation mechanism of the chlorinated mimics. N-halamides having three or more methylene units in their acyl side chain give rise to the Hoffmann-Loeffler-Freytag rearrangement under UV irradiation: the halogen atom migrates to the amidyl radical, Figure 3.8.⁴⁹⁻⁵² The high selectivity for halogenation of C-4 in the acyl chain requires an intramolecular hydrogen shift (1,5-hydrogen shift from carbon to nitrogen) involving a six-atom transition state, in some cases a 1,6 hydrogen transfer was observed with low yields.^{49,52,53}

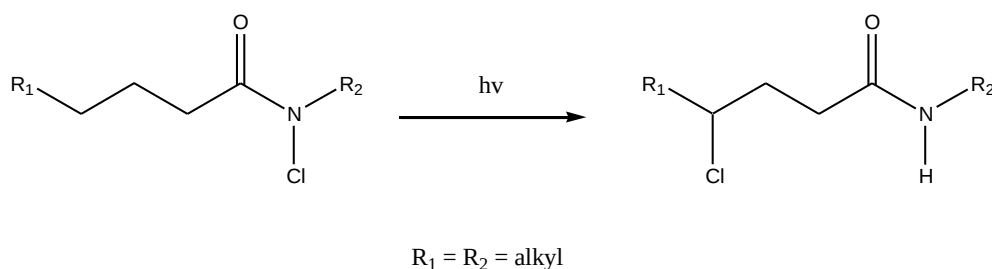


Figure 3.8. Intramolecular photorearrangement of N-halamides (1,5-hydrogen transfer).⁵¹

The N-Cl photolytic cleavage results in a free amidyl radical (Figure 3.9 (A)).

The intramolecular photorearrangement resulted from the transfer of the hydrogen atom in the acyl side-chain of the amido-radical. The amido-radical can be represented by the three resonance structures A, B, and C. The hydrogen transfer occurs to the oxygen (C) as well as to the nitrogen atom (A, B).⁵⁰

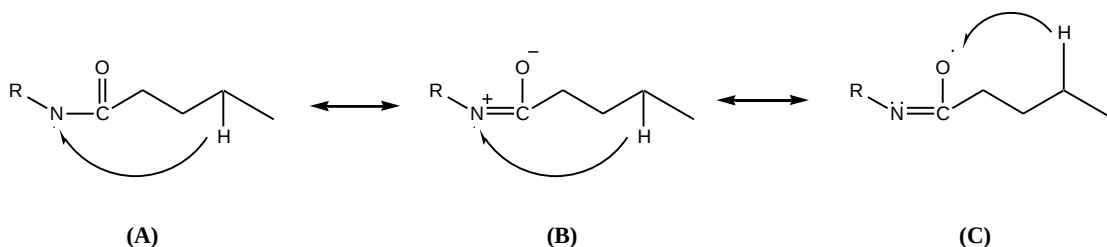


Figure 3.9. The hydrogen transfer onto amidyl-radical intermediate.⁵⁰

In this regard, a similar intramolecular photo rearrangement can occur on the 3-alkyl substituted hydantoin rings. The chlorine atom can migrate onto the alkyl chain at C7 or C8 positions (Figure 3.10) giving products A and B, respectively. Product A and Product B are not very stable and would further decompose through alpha dehydrohalogenation process.⁵⁴

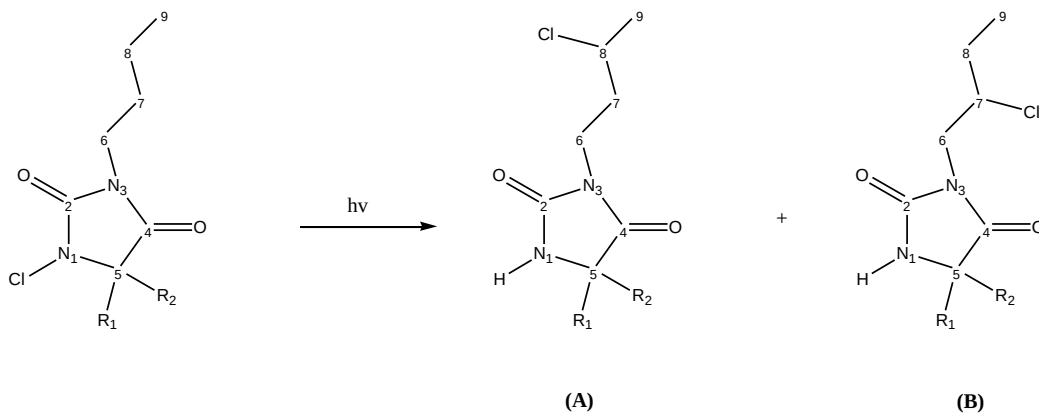


Figure 3.10. The photo rearrangement on 3-alkyl-substituted-1-chlorohydantoin.

Figure 3.11 shows the ^1H NMR spectra of PPm and PPm-Cl exposed to UV light for 24 h. There are not any ^1H NMR signals that can be assigned to PPm-Cl in the UV irradiated spectra indicating that all N-Cl bonds were broken, therefore a comparison between PPm and PPm-Cl irradiated samples would be more proper for structural changes. There are obviously several additional signals after UV irradiation suggesting there might be some structural changes on the chlorinated mimic during the irradiation. First, the ratio between the three methyl hydrogens of C9 at 0.91 ppm to ten aromatic hydrogens of phenyl groups at 7.36 ppm of PPm reduced to 3/25 after irradiation, indicating that there are some structural changes on the attached n-butyl group at the 3 position of the hydantoin ring, or loss of the butyl group.

The most intense additional signal is a doublet at 1.50 ppm which can be assigned the Product A (Figure 3.11(B)) methyl group hydrogens (A9). The signal at 3.97 ppm can be assigned to the Product A C8 hydrogen (HC-Cl) while the bands at 2.04 ppm and 3.73 ppm can be assigned to Product A C7 and C6 hydrogens, respectively. There is a small amount of Product B in the UV irradiated sample for which the triplet at 1.06 ppm can be assigned for the methyl hydrogens (B9) of Product B. Other hydrogen atoms of the Product B are difficult to make an interpretation because of the low amount of Product B in the mixture.

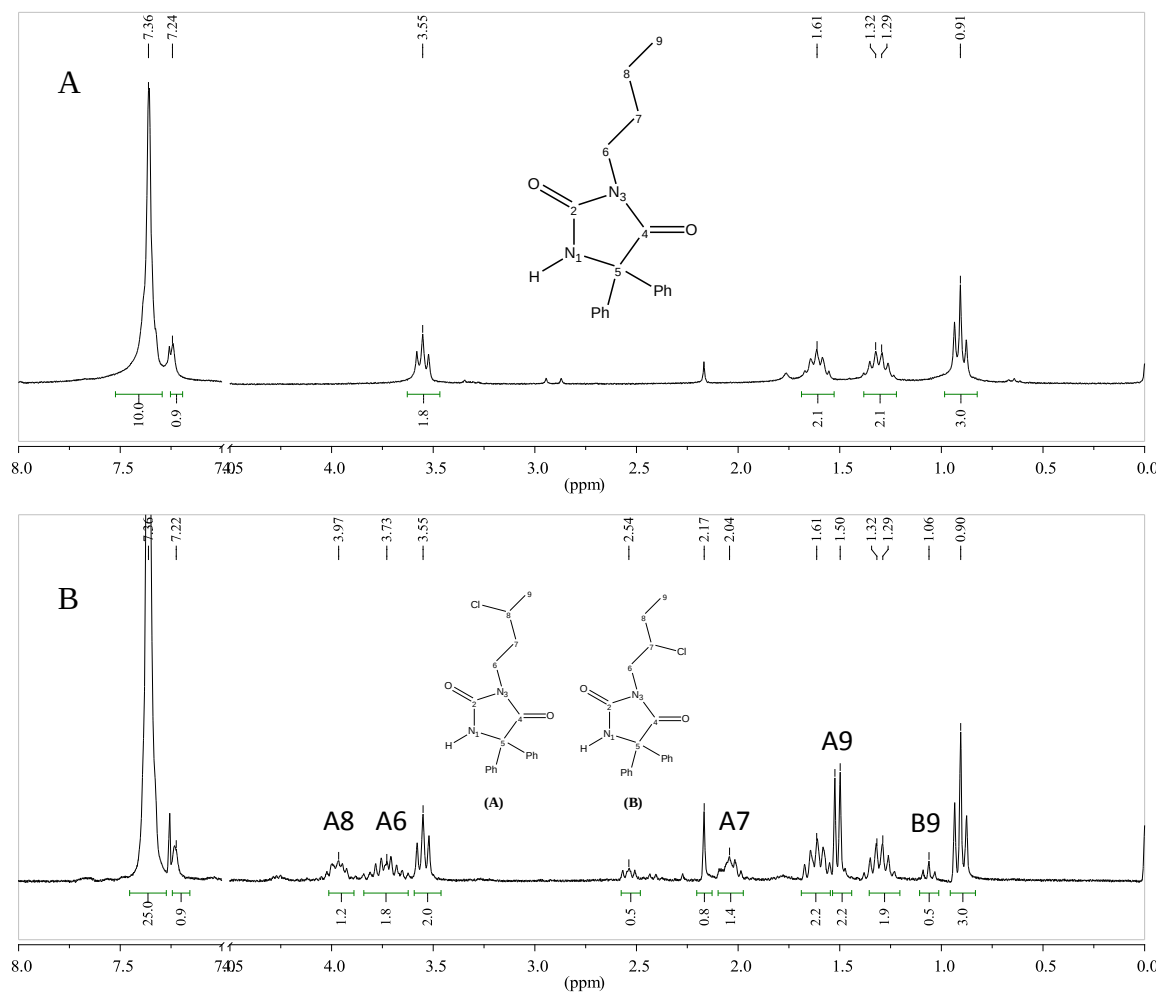


Figure 3.11. The ^1H NMR spectra of PPM (a), and UV light irradiated PPM-Cl (b).

Figure 3.12, shows the ^{13}C NMR spectra of PPM and PPM-Cl exposed to UV light for 24 h. There are four additional signals observed in the UV irradiated sample. C8 of the Product A (HC-Cl) had a signal at 55.6 ppm. Other carbon atoms of the Product A are observed at 25.2, 36.7, and 39.1 ppm which can be assigned to C9, C7, and C6, respectively. Product B carbon atoms could not be observed because of the low concentration of the compound in the mixture.

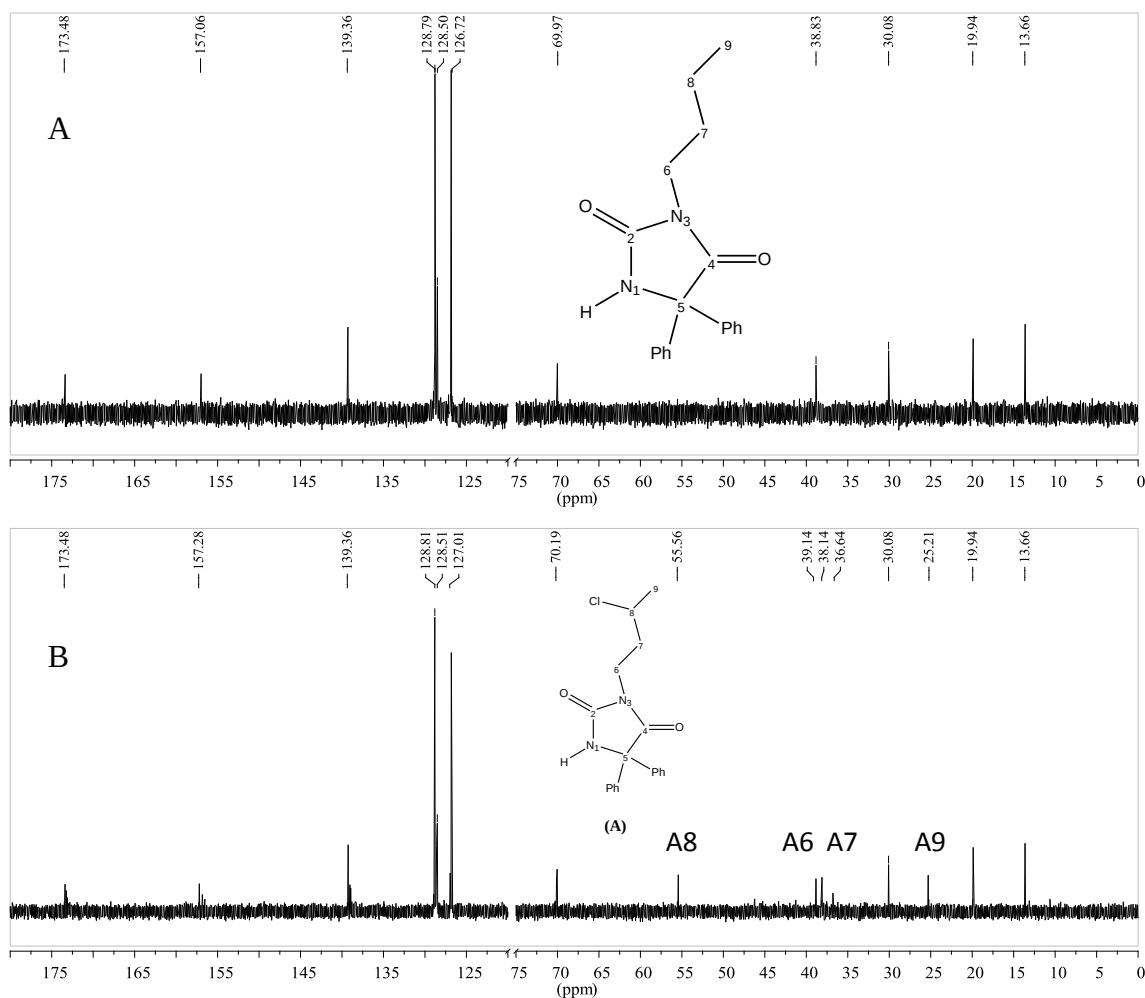


Figure 3.12. The ^{13}C NMR spectra of PPM (a), and UV light irradiated PPM-Cl (b).

Figure 3.13, shows the ^1H NMR spectra of MMm and MMm-Cl exposed to UV light for 24 h. The signals belonging to MMm in the irradiated sample spectra have very low intensity compared to PPM spectra discussed above, indicating that UV degradation of MMm-Cl is higher compared to PPM-Cl. Again, the ratio between the three methyl hydrogens (C9) at 0.94 ppm to six methyl hydrogens (at 5 position of the hydantoin ring) at 1.44 ppm was reduced to 3/37 indicating that there are some structural changes on the attached n-butyl group at the 3 position of the hydantoin ring, or loss of the butyl group.

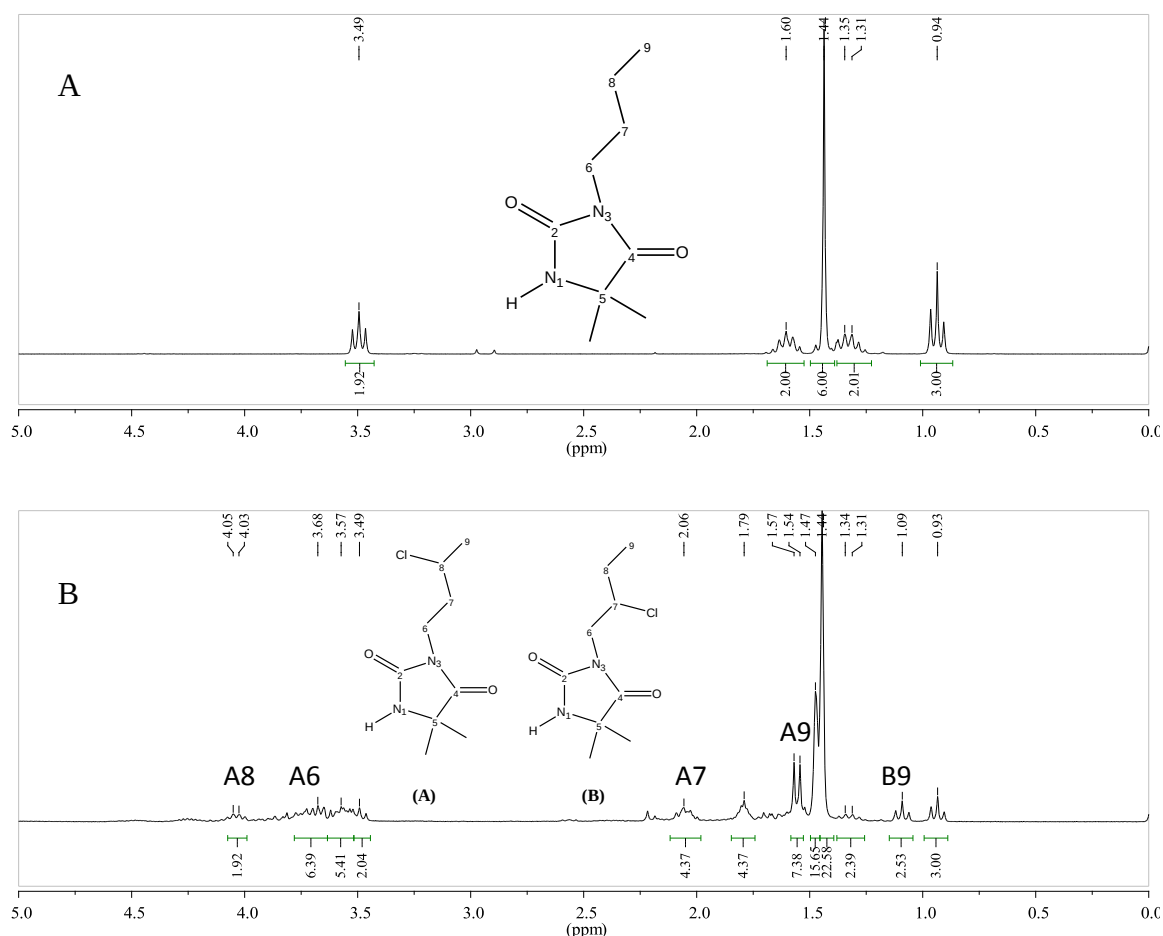


Figure 3.13. The ^1H NMR spectra of MMm (a), and UV light irradiated MMm-Cl (b).

The most intense additional signal is a doublet at 1.55 ppm which can be assigned the Product A (Figure 3.13(B)) methyl group hydrogens (C9). Other additional signals belonging to Product A are difficult to identify; however some of the signals are identified on the Figure 3.13. There is some Product B in the UV irradiated sample to which the triplet at 1.09 ppm can be assigned for the methyl hydrogens of Product B (C9).

Figure 3.14, shows the ^{13}C NMR spectra of MMm and MMm-Cl exposed to UV light for 24 h. There are several additional signals observed in the UV irradiated sample. C8 of the Product A (HC-Cl) had a signal at 55.8 ppm. As observed in the PPm-Cl irradiated sample, resonances for other carbon atoms of the Product A are observed at 25.5, 36.3, and 38.1 ppm which can be assigned to C9, C7, and C6, respectively. Product B carbon atoms were more obvious in the MMm-Cl irradiated sample compared to the PPm-Cl irradiated sample. A resonance for the methyl carbon atom of the Product B (C9) was observed at 10.7 ppm, while B8, B7, and B6 carbon atom signals were observed at 28.7, 60.5, and 44.2 ppm, respectively.

Similar signals were observed for MPm-Cl after UV irradiation, however they will not be discussed here.

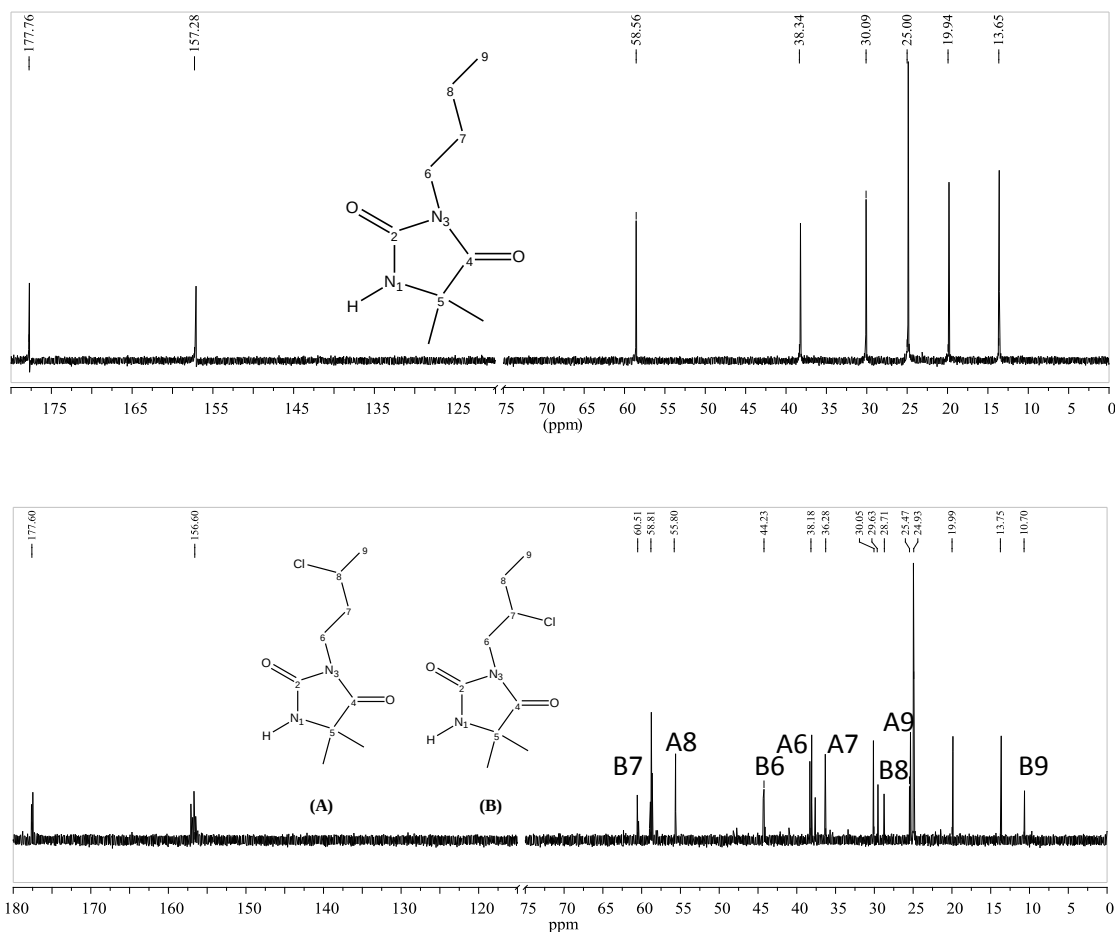


Figure 3.14. The ^{13}C NMR spectra of MMm (a), and UV light irradiated MMm-Cl (b).

3.4 Conclusions

A series of 3-triethoxysilylpropylhydantoin derivatives were synthesized with variation of methyl and phenyl substitution at the 5 position of the hydantoin moiety.

The derivatives were coated onto cotton fabric and then treated with bleach to provide an antimicrobial property. The antibacterial activities, stabilities, and UV light resistance of the derivatives were compared. The disinfection rates of phenyl substituted derivatives were slightly higher implying that the N-Cl bond become weaker after phenyl

substitution. Additionally, UV-light stability and washing tests also suggested a weaker N-Cl bond as in the biocidal tests. The mimics of the derivatives were synthesized to investigate the stability of the N-Cl bond. The DSC experiments showed that the N-Cl bond was weaker, ie. lower N-Cl bond breakage exotherm peak maximum, in the phenyl substituted derivatives. The NMR and FTIR results suggested an interaction between the N-X moiety and the π -electrons of the substituted phenyl groups which could influence the N-X bond lability. The UV light stability of the siloxane coatings was investigated. Chlorinated coatings decomposed, while unchlorinated coatings did not exhibit any decomposition. The decomposition mechanism of the chlorinated siloxane coatings was explained by an intramolecular photorearrangement reaction (Hoffmann-Loeffler-Freytag rearrangement). The study enlightened the future of N-halamine siloxane coating technology with respect to UV light stability.

3.5 References

- [1] Liang, J.; Chen, Y.; Barnes, K.; Wu, R.; Worley, S.D.; Huang, T.S. N-halamine/quat Siloxane Copolymers for Use in Biocidal Coatings. *Biomaterials*. **2006**, 27, 2495-2501.
- [2] Worley, S. D.; Sun, G. Biocidal Polymers. *Trends Polym. Sci.* **1996**, 4, 364-370.
- [3] Kenawy, E. R.; Worley, S. D.; Broughton, R. The Chemistry and Applications of Antimicrobial Polymers; A State of the Art Review. *Biomacromolecules*. **2007**, 8, 1359-1384.
- [4] Sun, Y. Y.; Chen, T.; Worley, S. D.; Sun, G. Novel Refreshable N-Halamine Polymeric Biocides Containing Imidazolidin-4-one Derivatives. *J. Polym. Sci. Part A: Polym. Chem.* **2001**, 39, 3073–3084.
- [5] Makal, U.; Wood, L.; Ohman, D. E.; Wynne, K. J. Polyurethane Biocidal Polymeric Surface Modifiers. *Biomaterials*. **2006**, 27, 1316-1326.
- [6] Worley, S. D.; Chen, Y.; Wang, J. W.; Wu, R.; Cho, U.; Broughton, R. M.; Kim, J.; Wei, C .I.; Williams, J .F.; Chen, J.; Li, Y. Novel N-halamine Siloxane Monomers and Polymers for Preparing Biocidal Coatings. *Surf. Coat. Int. Part B: Coat. Trans.* **2005**, 88, 93-100.
- [7] Chen, Z; Sun, Y. Y. N-Halamine-based Antimicrobial Additives for Polymers: Preparation, Characterization, and Antimicrobial Activity. *Ind. Eng. Chem. Res.* **2006**, 45, 2634-2640.

- [8] Liang, J.; Wu, R.; Wang, J. W.; Barnes, K.; Worley, S. D.; Cho, U.; Lee, J.; Broughton, R. M.; Huang, T. S. N-halamine Biocidal Coatings. *J. Ind. Microbiol. Biotechnol.* **2007**, 34, 157-163.
- [9] Akdag, A.; Okur, S.; McKee, M. L.; Worley, S. D. The Stabilities of N-Cl Bonds in Biocidal Materials. *J. Chem. Theory Comput.* **2006**, 2, 879-884.
- [10] For example see: Liang, J.; Barnes, K.; Akdag, A.; Worley, S.D.; Lee, J.; Broughton, R.M.; Huang, T.S. Improved Antimicrobial Siloxane. *Ind. Eng. Chem. Res.* **2007**, 46, 1861-1866.
- [11] Ren, X.; Kou, L.; Liang, J.; Worley, S.D.; Tzou, Y.M.; Huang, T.S. Antimicrobial Efficacy and Light Stability of N-Halamine Siloxanes bound to cotton. *Cellulose.* **2008**, 15, 593-598.
- [12] Worley, S.D.; Williams, D.E. Halamine Water Disinfectants. *CRC Crit. Rev. Environ. Control.* **1998**, 18, 133-175.
- [13] Worley, S.D. Patent WO9929678. **1999**.
- [14] 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.
- [15] Metcalf, W.S. The Absorption Spectra of Mono-, Di-, and Tri-Chloroamines and Some Aliphatic Derivatives. *J. Chem. Soc.* **1942**, 148-50.
- [16] Koutchma, T.N.; Forney, L.J.; Moraru, C.I. Ultraviolet Light in Food Technology. **2009**. CRC Press.

- [17] Karukstis, K.K; Van Hecke, G.R. Chemistry Connections. **2003**. Academic Press. p.163.
- [18] Groves, M.J. Pharmaceutical Biotechnology. **2005**. CRC Press. p.148.
- [19] Williams, D. E.; Elder, E. D.; Worley, S. D. Is Free Halogen Necessary for Disinfection? *Appl. Environ. Microbiol.* **1988**, 54, 2583-2585.
- [20] Kishioka, T; Sakamoto, R.; Hiroi, Y.; Maruyama, D. Antireflecting Film for Semiconductor Containing Condensation Type Polymer. Patent EP 1,757,986 A1, **2005**.
- [21] Kawasaki, T.K.; Miyoshi, T.; Takehera, M. Method of Stabilizing a UVB Absorbing Compound, a Stabilized UV Absorber, and a Cosmetic Composition Containing the Same. Patent US 5,000,945, **1991**.
- [22] Sandstrom, A.; Sun, G. Durability of Biocidal Nomex Fabrics for Multi-functional Firefighter Uniforms. *Res. J. Textile Appl.* **2006**, 10, 13-18.
- [23] Baeyer, A. *Liebigs Ann Chem.* **1861**, 117, 178.
- [24] Chen, Z.; Sun, Y.Y. N-halamine-Based Antimicrobial Additives for Polymers: Preparation, Characterization, and Antimicrobial Activity. *Ind. Eng. Chem. Res.* **2006**, 45, 2634-2640.
- [25] Tiedje, K.E.; Weaver, D.F. Deducing the Bioactive Face of Hydantoin Anticonvulsant Drugs Using NMR Spectroscopy. *Can. J. Neurol. Sci.* **2008**, 35, 232-236.
- [26] Scholl, S.; Koch, A.; Henning, D.; Kempter, G.; Kleinpeter, E. The Influence of Structure and Lipophilicity of Hydantoin Derivatives on Anticonvulsant Activity. *Struc. Chem.* **1999**, 10, 355-366.

- [27] Block, S. S. Disinfection, Sterilization and Preservation. Third Ed. **1983**. Philadelphia, Lea & Febiger, pp.743-745.
- [28] Nakae, T. Outer Membrane of Salmonella. Isolation of Protein Complex that Produces Transmembrane Channels. *J. Biol. Chem.* **1976**, 251, 2176-2178.
- [29] Fujiwara, H.; Bose, A.K; Manhas, M.S.; Van der veen, J.M. Non-bonded aromatic-amide attraction in 5-benzyl-3-arylhydantoin. *J. Chem. Soc. Perkin Trans.II.* **1979**, 653-658.
- [30] Fujiwara, H.; Van der veen, J.M. An X-ray Study of the aromatic ring-dipole interaction in hydantoin crystals. *J. Chem. Soc. Perkin Trans.II.* **1979**, 659-663.
- [31] Robinson, R.; Jencks, W.P. The Effect of Concentrated Salt Solutions on the Activity Coefficient of Acetyltetraglycine Ethyl Ester. *J. Am. Chem. Soc.* **1965**, 87, 2470-2472.
- [32] Colebrook, L.; Giles, H.G.; Granata, A.; Icli, S. Restricted Internal Rotation in 1-Arylhyaantoin, #-Arylhyaantoin, and 3-Aryl-2-Thiohyantoin: Reversal of the Effective Size of Methyl and Chlorine. *Can. J. Chem.* **1973**, 51, 3635-3639.
- [33] Lee, G.H.; Park, Y.T. A Simple Huckel Approach to Intramolecular Photocyclization Reaction of N-(2-Chlorbenzyl)-Pyridinium, N-(Benzyl)-2-Chloropyridinium, and N-(2-Chlorobenzyl)-2-Chloropyridinium Salts. *Bull. Korean Chem Soc.* **1994**, 15, 857.
- [34] Prasanna, M.D.; Guru Row, T.N. C-Halogen $\cdots\pi$ Interactions and Their Influence on Molecular Conformation and Crystal Packing: a Database Study. *Cryst. Eng.* **2000**, 3, 135-154.

- [35] Abraham, R.J.; Byrne, J.J; Griffiths, L.; Perez, M. ^1H Chemical Shifts in NMR: Part 23, the Effect of Dimethyl Sulfoxide versus Chloroform solvent on ^1H Chemical Shifts. *Mag. Res. Chem.* **2006**, 44, 491-509.
- [36] Kleinpeter, E. The Structure of Hydantoins in Solution and in the Solid. *Struct. Chem.* **1997**, 8, 161-173.
- [37] Lehman, P.A.; Jorgensen, E.C. Thyroxine Analogs – XIII NMR Evidendence for Hindered Rotation in Phenyl Ethers. *Tetrahedron.* **1965**, 21, 363-3.
- [38] Palazzi, A.; Stagni, S.; Bordoni, S.; Monari, M.; Selva, S. Interannular Conjugation in New Iron(II) 5-Aryl Tetrazolate Complexes. *Organometallics.* **2002**, 21, 3774-3781.
- [39] Michaux, C.; Muccioli, G.; Lambert, D.; Wouters, J. Binding Mode of New (Thio)hydatoins Inhibitors of Fatty Acid Amide Hyrolase: Comparison with Two Original Compounds, OL-92 and JP104. *Bioorg. Med. Chem. Let.* **2006**, 16, 4772-4776.
- [40] Tan, S.F.; Ang, K.P., Fong, Y.F. (Z)- and (E)-5-Arylmethylenehydantoins: Spectroscopic Properties and Configuration Assignment. *J. Chem. Soc. Perkin Trans. II.* **1986**, 1941-1944.
- [41] Camerman, A.; Camerman, N. The Stereochemical Basis of Anticonvulsant Drug Action. I. The Crystal and Molecular Structure of Diphenylhydantoin, a Noncentrosymmetric Structure Solved by Centric Symbolic Addition. *Acta. Cryst. B.* **1971**, 27, 2205-2211.

- [42] Koch, M.H.J.; Germain, G.; Declercq, J.P. (+)-5-p-Hydroxyphenyl-5-phenylhydantoin Camphor-10-sulphonate Ethyl Acetate. *Acta Cryst. B.* **1975**, 31, 2547-2549.
- [43] Kleinpeter, E.; Heydenreich, M.; Kalder, L.; Koch, A.; Henning, D.; Kempter, G.; Benassi, R.; Taddei, F. NMR Spectroscopic and Theoretical Structural Analysis of 5,5-disubstituted Hydantoins in Solution. *J. Mol. Struct.* **1997**, 403, 111-122.
- [44] Verma, S.M.; Singh, N.B. A Study of Conformation About the Aryl C-N Bonds in N-Aryl Imides by Dynamic N.M.R. Spectroscopy. *Aust. J. Chem.* **1976**, 29, 295.
- [45] Falle, H.R.; Adam, F.C. A Paramagnetic Resonance Study of Hindered Diarylmethyl Radicals and Related Compounds. *Can. J Chem.* **1966**, 44, 1387.
- [46] Jimnez, A.I.; Cativiela, C.; Cataln, J.G.; Prez, J.J.; Aubry, A.; Pars, M.; Marraud, M. Influence of Side Chain Restriction and $\text{NH}\cdots\pi$ Interaction on the β -Turn Folding Modes of Dipeptides Incorporating Phenylalanine Cyclohexane Derivatives. *J. Am. Chem. Soc.* **2000**, 122, 5811-5821.
- [47] For example see: (a) Nikolic, A.; Petrovic, S; Antonovic, D.; Gobor, L. N-H $\cdots\pi$ hydrogen bonding: FTIR study of N-butylpropionamides-aromatic donor systems. *J. Mol. Struct.* **1997**, 408/409, 355. (b) Stefov, V.; Pejov, Lj.; Soptrajanov, B. The Influence of N-H $\cdots\pi$ Hydrogen Bonding on the Anharmonicity of the $\nu(\text{N-H})$ Mode and Orientation Dynamics of Nearly Continuously Solvated Indole. *J. Mol. Struct.* **2000**, 555, 363.
- [48] Droin, A.; Lessard, J. Ring Contraction of N-clorolactams, a Novel Rearrangement. *Tetrahedron Lett.* **2006**, 47, 4285-4288.

- [49] Neale, R.S., Marcus, N.L., Schepers, R.G. The Chemistry of Nitrogen Radicals. IV. The Rearrangement of N-Halamides and the Synthesis of Iminolactones. *J. Am. Chem. Soc.* **1966**, 88, 3051-3053.
- [50] Chow, Y.L.; Joseph, T.C. Selectivity of Intramolecular Hydrogen Transfer in the Free Amino-Radical. *Chem. Commun.* **1969**, 490-491.
- [51] Koval, I.V. N-Halo Reagents. Synthesis and Reactions of N-Halocarboxamides. *Russ. J. Org. Chem.* **2001**, 37, 327-346.
- [52] Johnson, R.A.; Greene, F.D. Chlorination with N-Chloro Amides. I. Inter- and Intramolecular Chlorination. *J. Org. Chem.* **1975**, 40, 2186-2192.
- [53] Beckwith, A.L.J.; Goodrich, J.E. Free-Radical Rearrangement of N-Chloro-Amides: A Synthesis of Lactones. *Aust. J. Chem.* **1965**, 18, 747-757.
- [54] Kaminski, J.J.; Bodor, N.; Higuchi, T. N-halo Derivatives III: Stabilization of Nitrogen-chlorine Bond in N-chloroamino Acid Derivatives. *J. Pharm. Sci.* **1976**, 65, 4, 553-557.
- [55] Avendano, C.; Menendez, J.C. Hydantoin and Its Derivatives. Kirk-Othmer Encyclop. Chem. Tech. **2000**.
- [56] Chruma, J.J.; Liu, L.; Zhou, W.; Breslow, R. Hydrophobic and Electronic Factors in the Design of Dialkyglycine Decarboxylase Mimics. *Bioorg. Med. Chem.* **2005**, 13, 5873-5883.

[57] Garcia, M.J.; Azerad, R. Production of Ring-Substituted D-Phenylglycines by Microbial or Enzymatic Hydrolysis/Deracemisation of the Corresponding DL-Hydantoins. *Tetrahedron: Assym.* **1997**, 8, 85-92.

[58] Parman, T.; Chen, G.; Wells, P.G. Free Radical Intermediates of Phenytoin and Related Teratogens. *J. Biol. Chem.* **1998**, 273, 25079-25088.

3.6 Supporting Information

The Supporting Information includes additional information about chlorine loadings of synthesized compounds at different concentrations, NMR, and FTIR spectra of the compounds.

Table S.3.1. Chlorine loadings (Cl^+ %) for the coating solutions at different concentrations.

Compound concentration (%)	MM	MP	PP
3.0		0.32	0.31
3.2		0.37	0.35
3.4			0.40
6.0	0.13		
8.0	0.29		
9.0	0.39		
15.0	0.55		

Table S.3.2. Stability toward UV light exposure of cotton coated with derivatized hydantoinyl siloxanes (Cl^+ % remaining).

Time (min)	MM-Cl	MP-Cl	PP-Cl
0	0.31	0.33	0.30
10	0.28	0.26	0.23
20	0.27	0.17	0.18
30	0.24	0.12	0.12
60	0.20	0.09	0.08
120	0.14	0.06	0.05
360	0.08	0.03	0.03
rechlorination	0.21	0.19	0.20

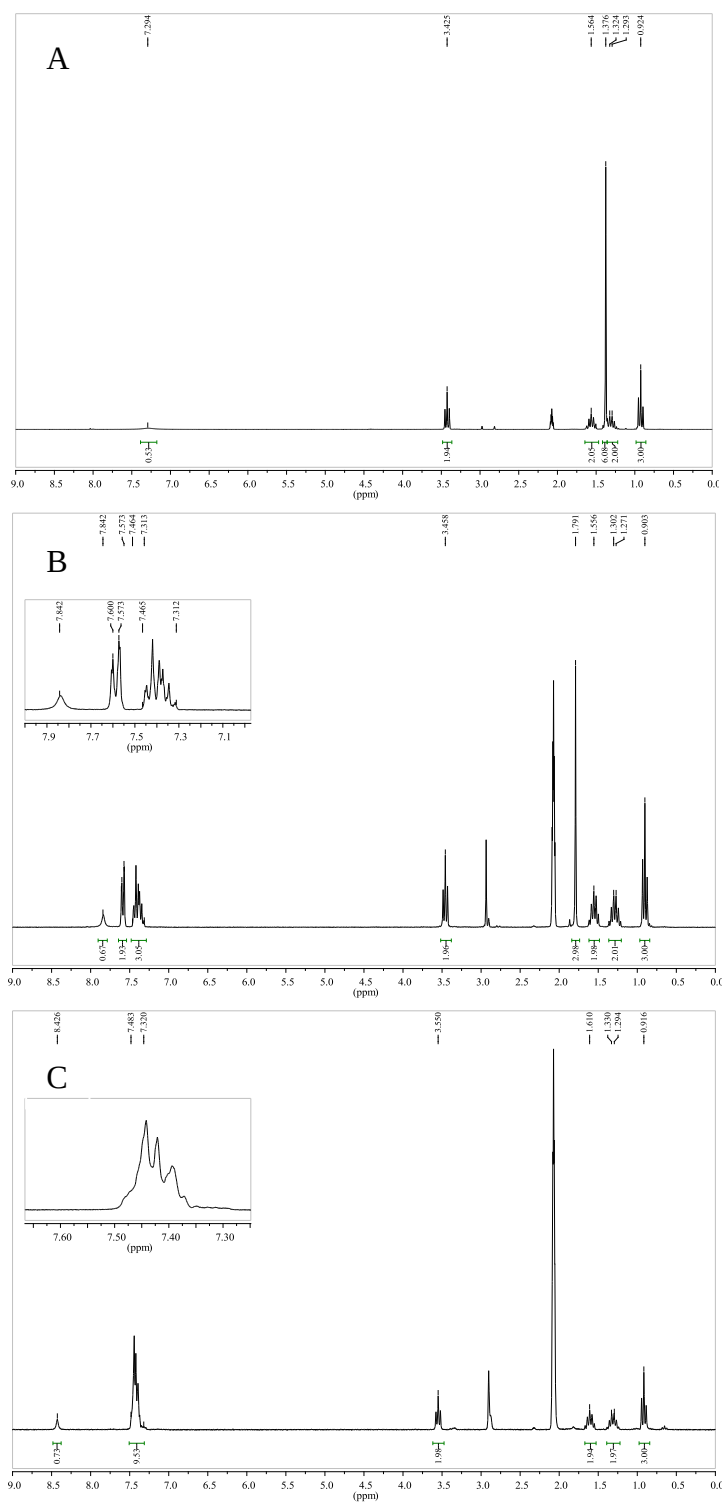


Figure S.3.1. ^1H NMR Spectra of MMm (a), MPm (b), and PPm (c). (s: acetone- d_6 .)

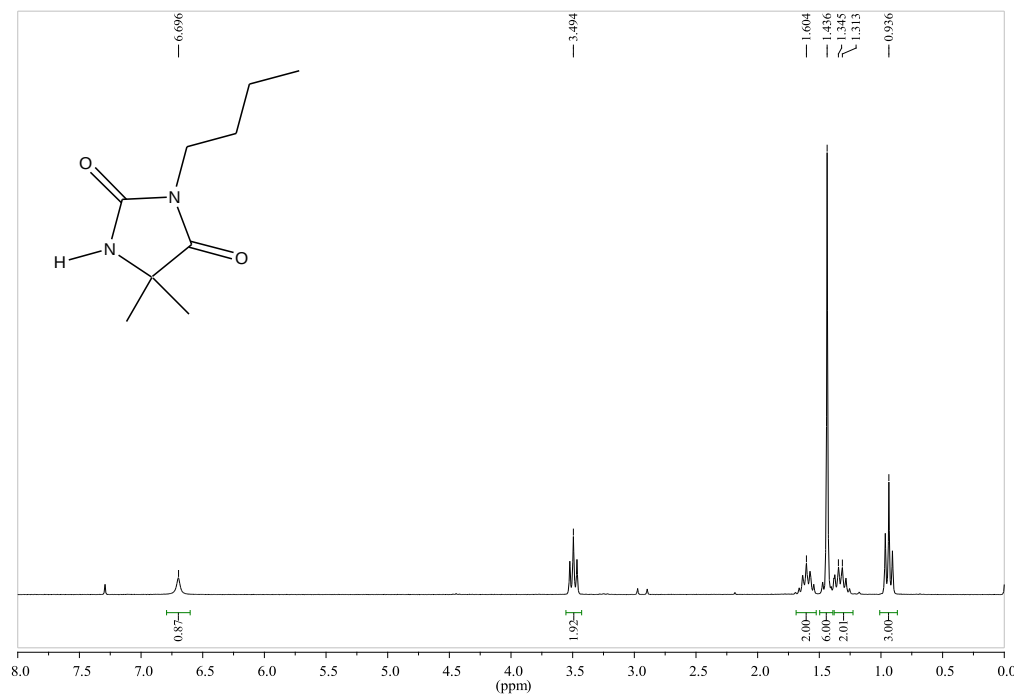


Figure S.3.2. ^1H -NMR spectra of MMm. (solvent: CDCl₃)

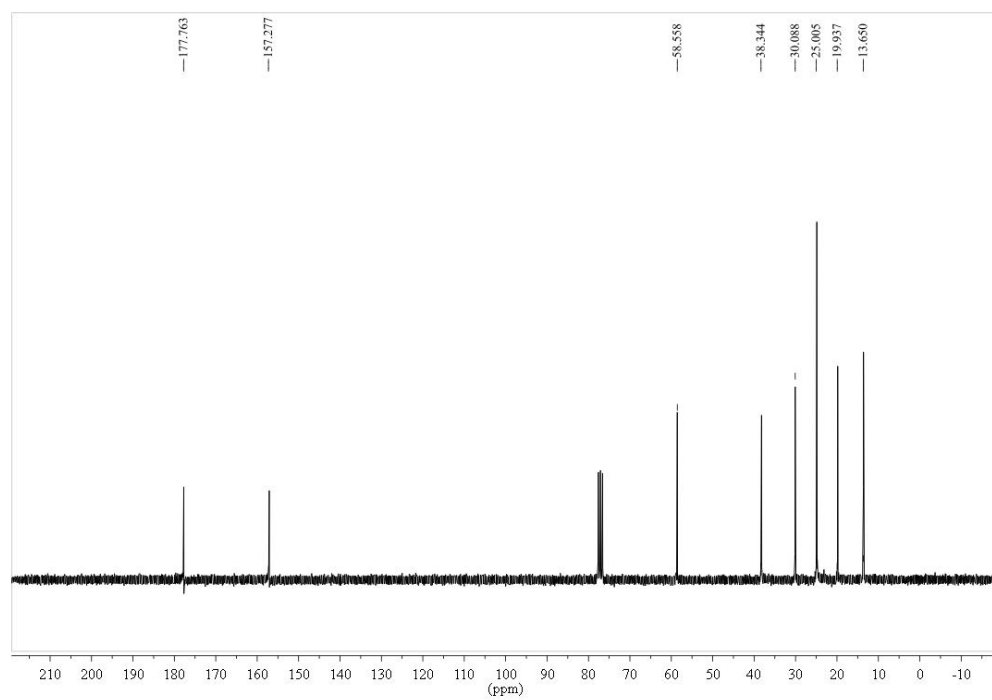


Figure S.3.3. ^{13}C -NMR spectra of MMm. (solvent: CDCl₃)

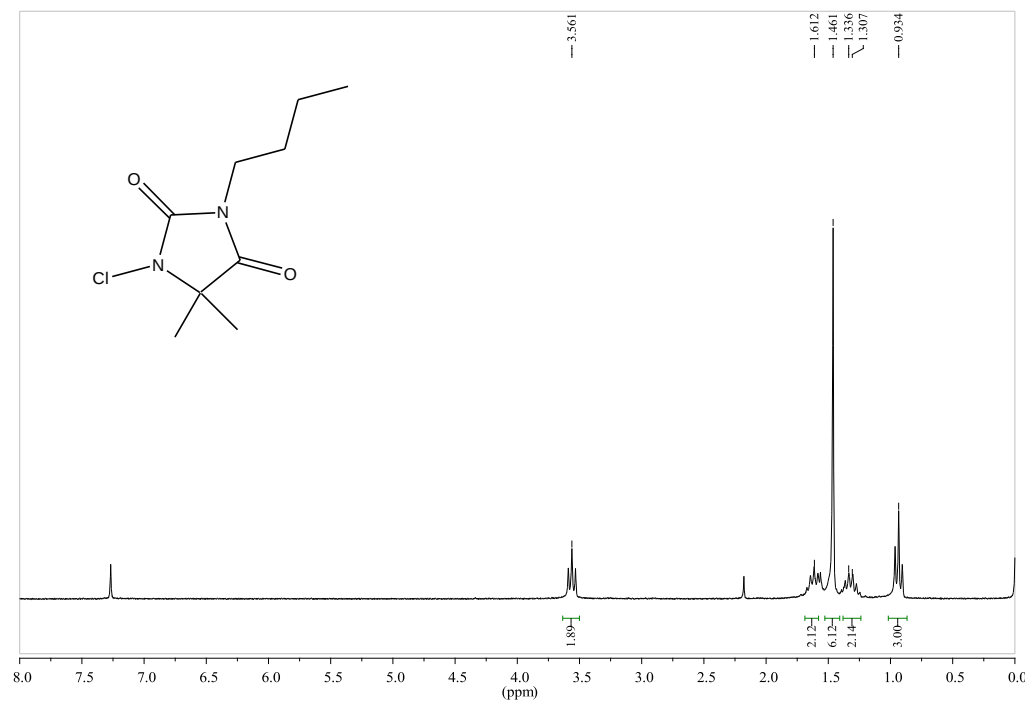


Figure S.3.4. ¹H-NMR spectra of MMm-Cl. (solvent: CDCl₃)

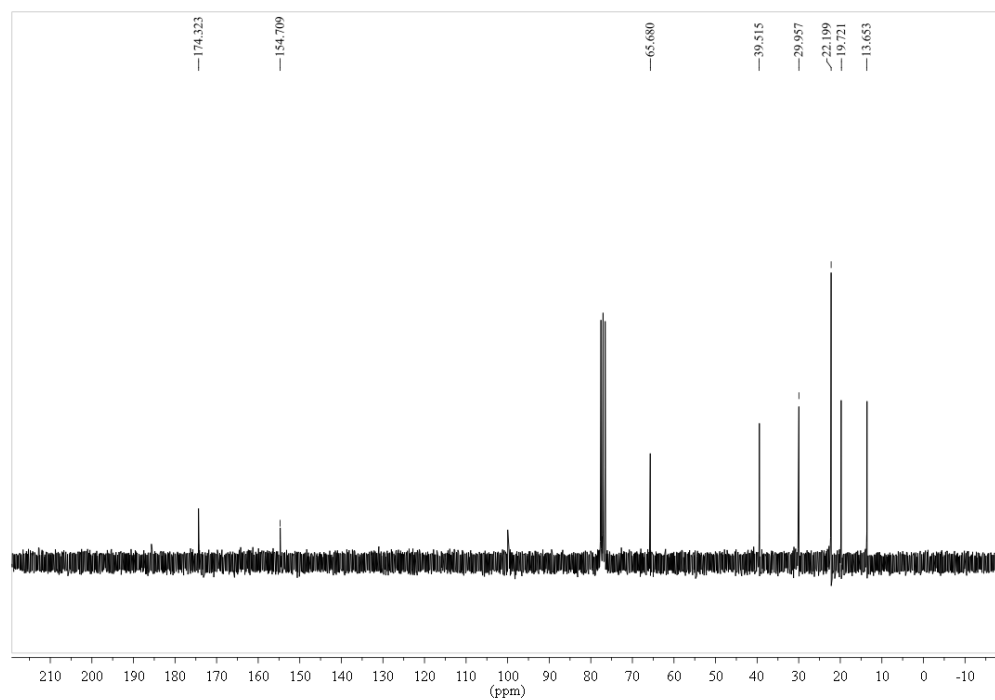


Figure S.3.5. ¹³C-NMR spectra of MMm-Cl. (solvent: CDCl₃)

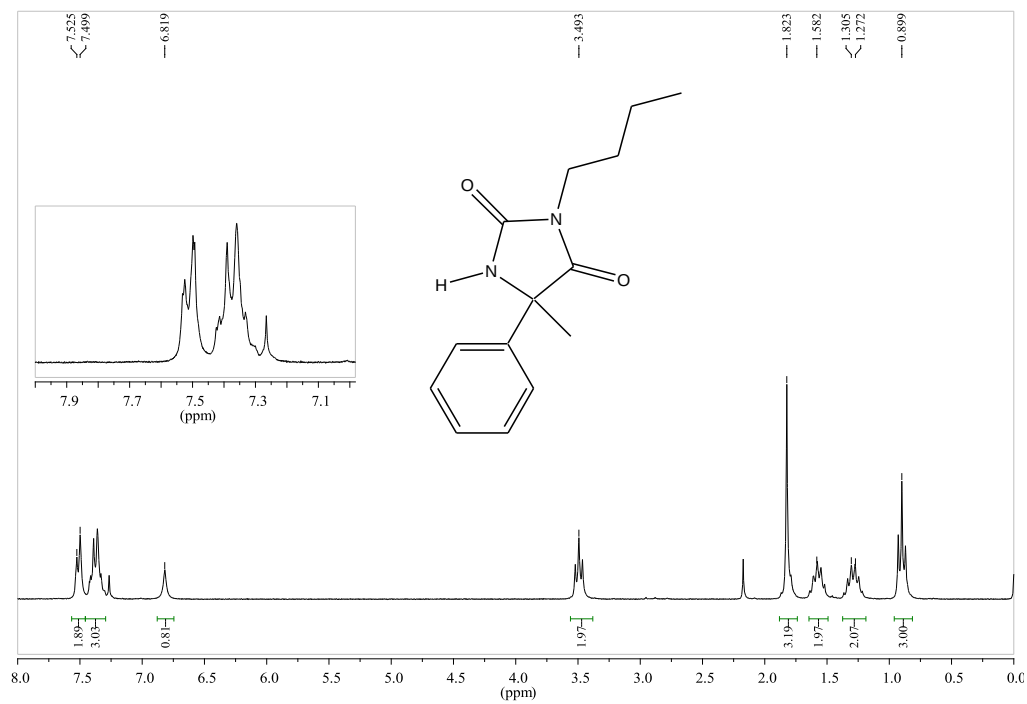


Figure S.3.6. ¹H-NMR spectra of MPm. (solvent: CDCl₃)

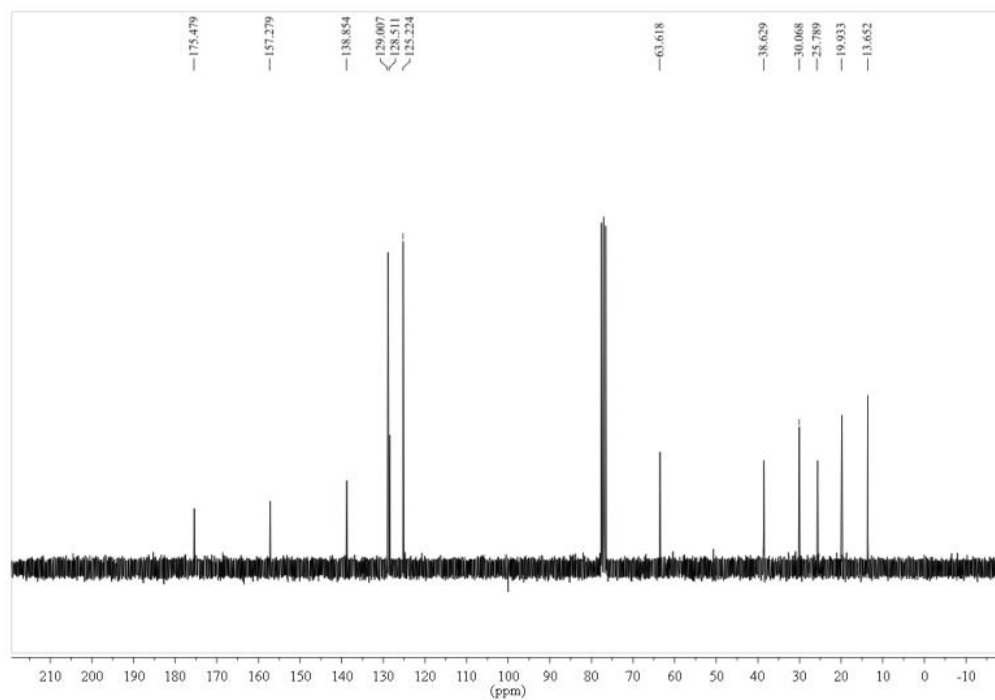


Figure S.3.7. ¹³C-NMR spectra of MPm. (solvent: CDCl₃)

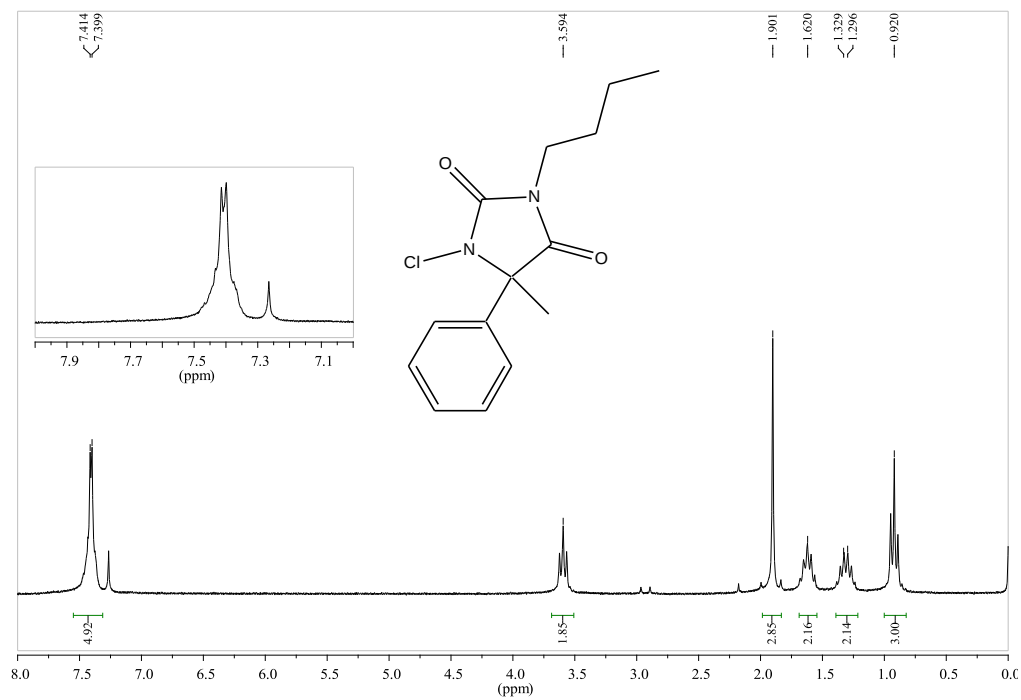


Figure S.3.8. ¹H-NMR spectra of MPm-Cl. (solvent: CDCl₃)

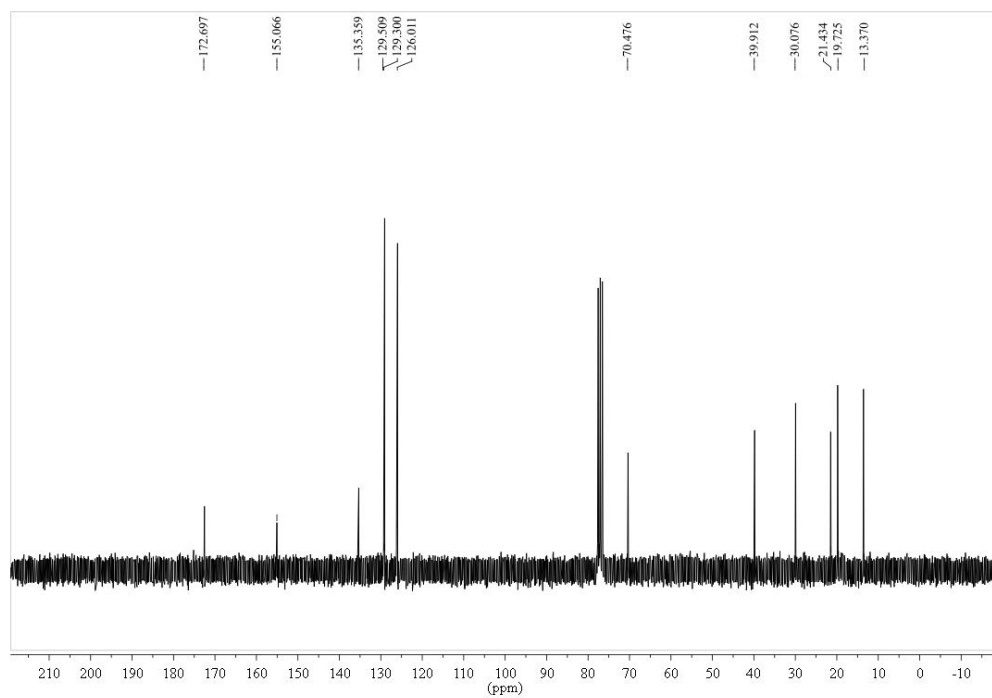


Figure S.3.9. ¹³C-NMR spectra of MPm-Cl. (solvent: CDCl₃)

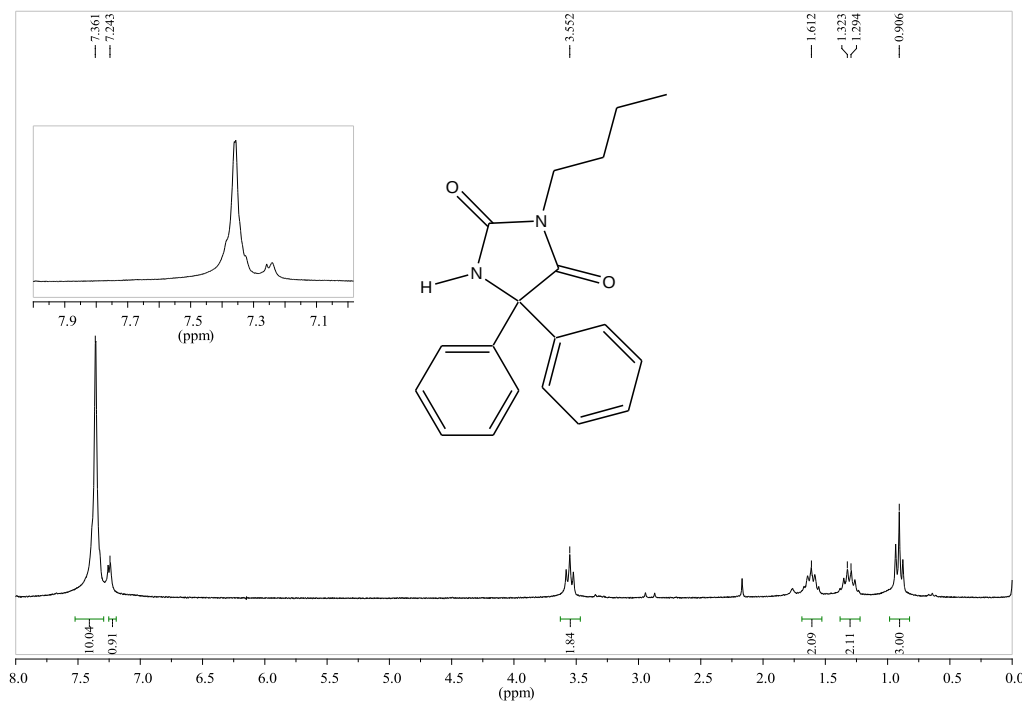


Figure S.3.10. ^1H -NMR spectra of Ppm. (solvent: CDCl_3)

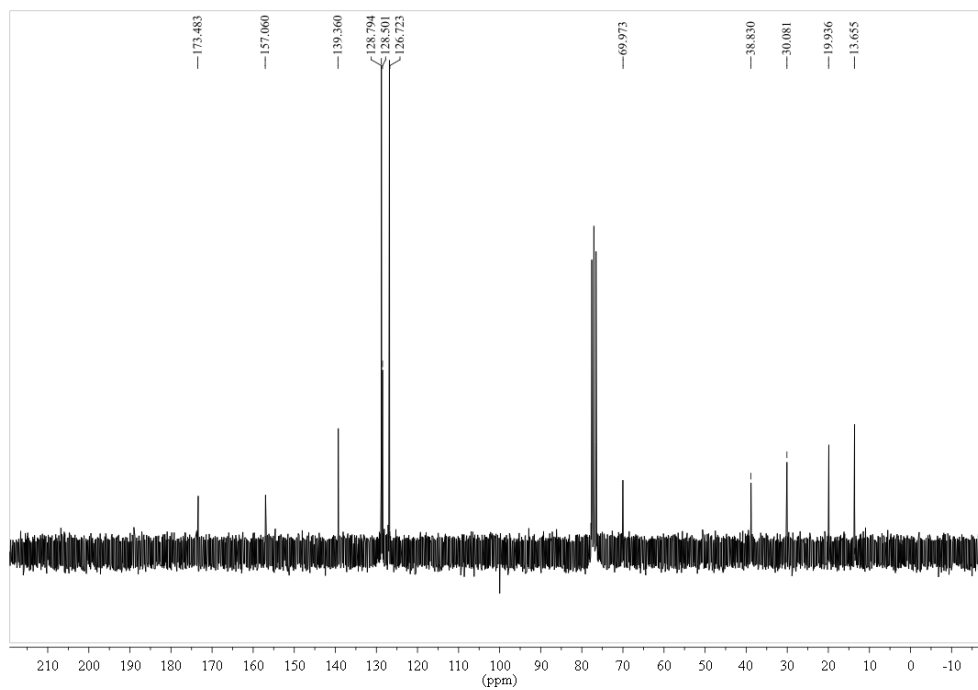


Figure S.3.11. ^{13}C -NMR spectra of Ppm. (solvent: CDCl_3)

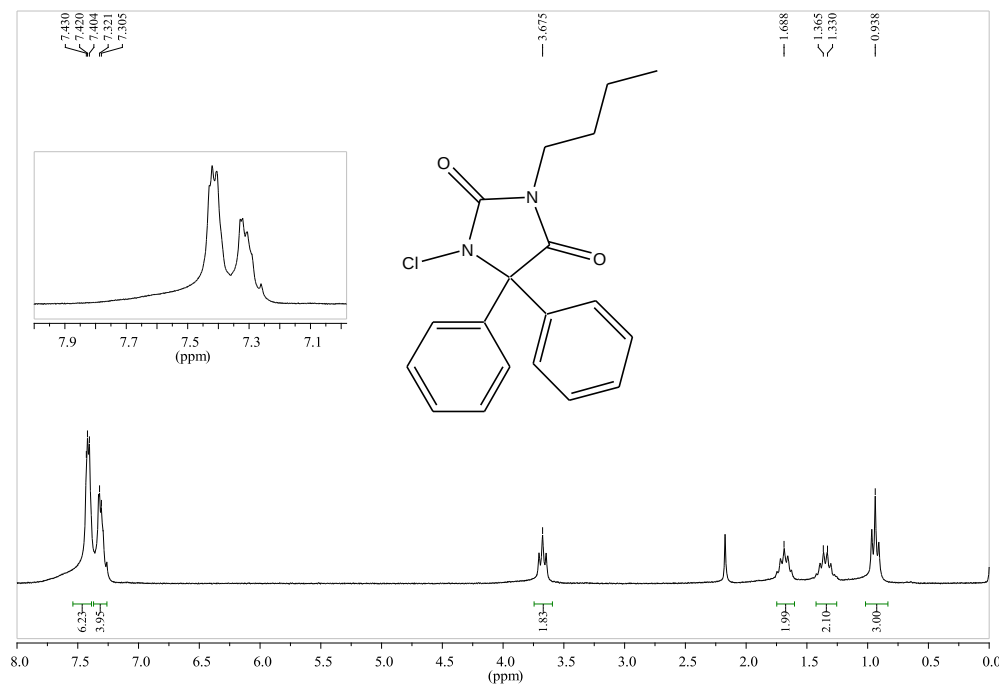


Figure S.3.12. ¹H-NMR spectra of Ppm-Cl. (solvent: CDCl₃)

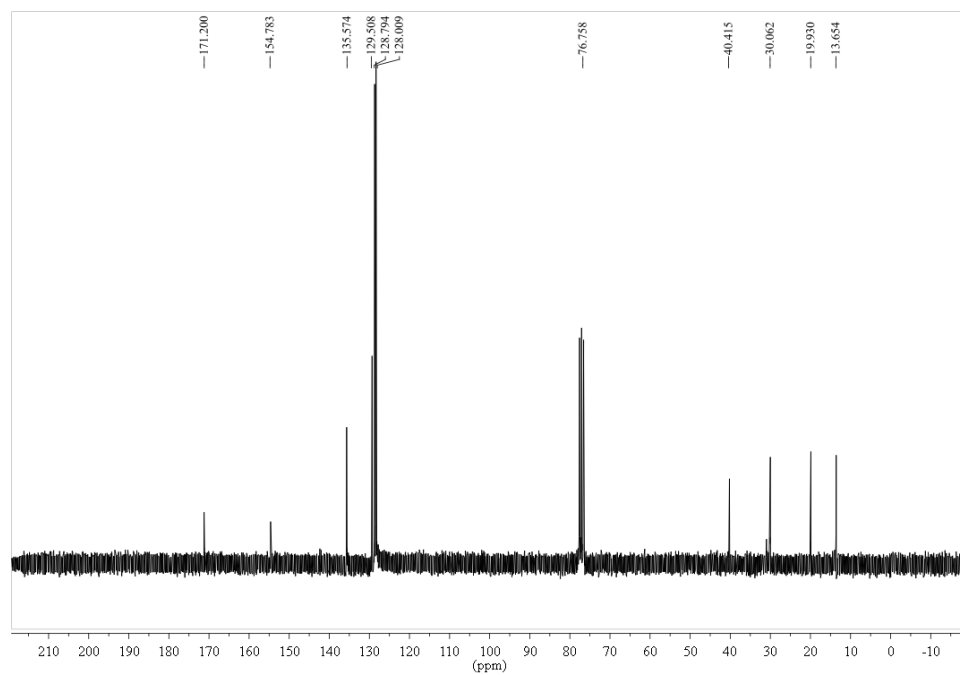


Figure S.3.13. ¹³C-NMR spectra of Ppm-Cl. (solvent: CDCl₃)

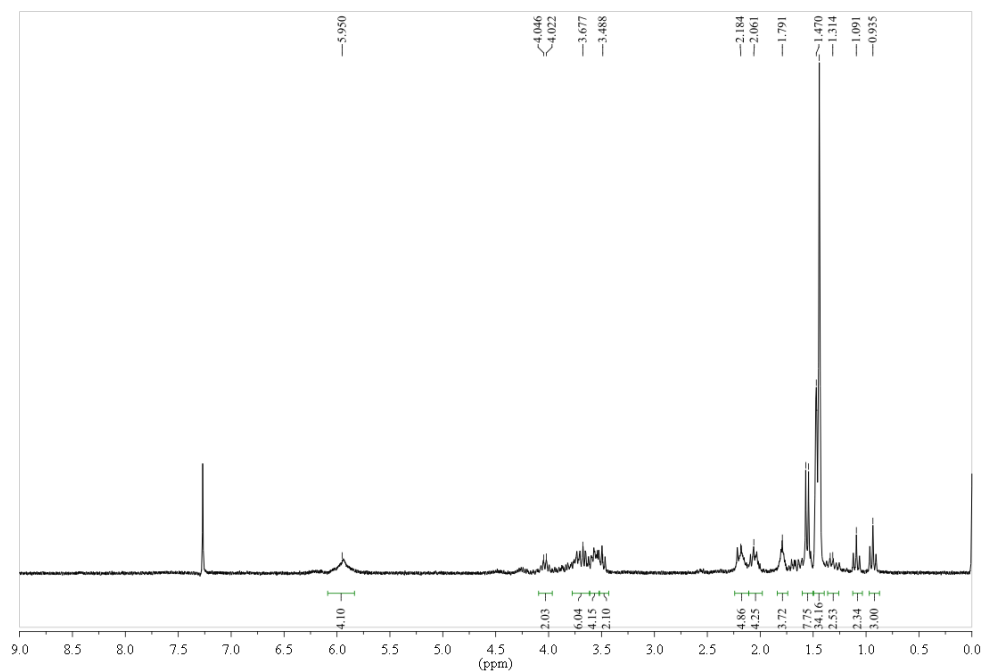


Figure S.3.14. ¹H-NMR spectra of UV irradiated MMm-Cl. (solvent: CDCl₃)

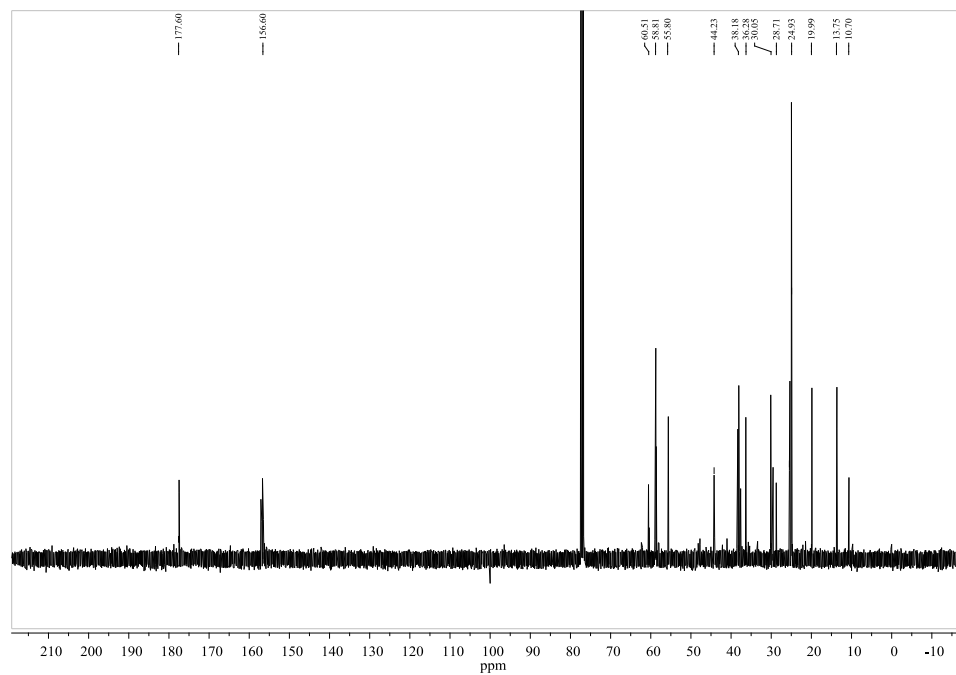


Figure S.3.15. ¹³C-NMR spectra of UV irradiated MMm-Cl. (solvent: CDCl₃)

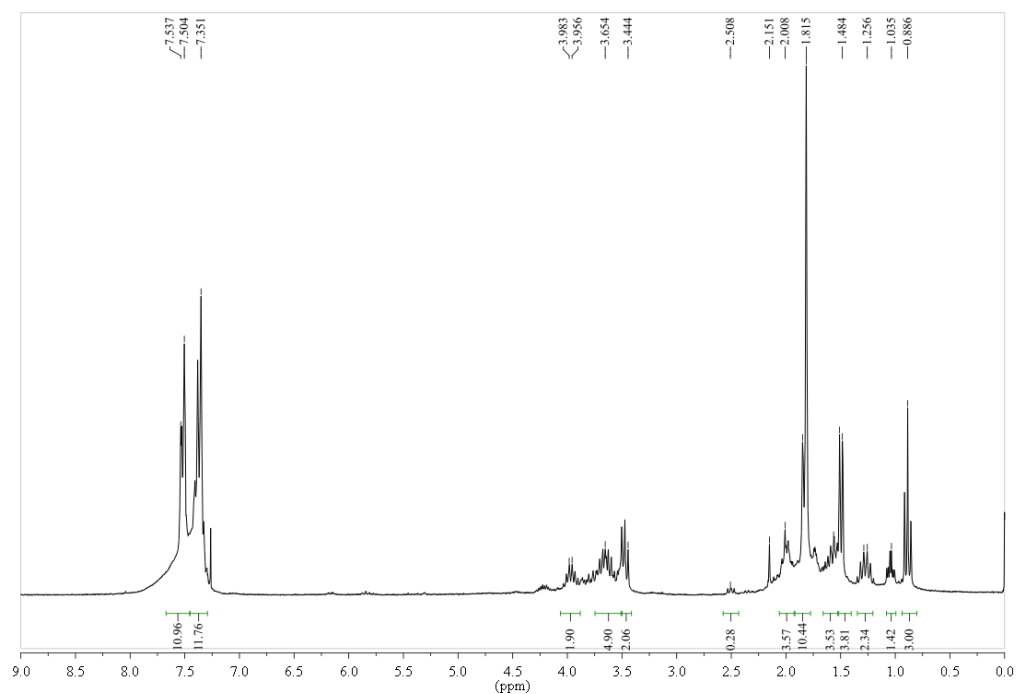


Figure S.3.16. ¹H-NMR spectra of UV irradiated MPm-Cl. (solvent: CDCl₃)

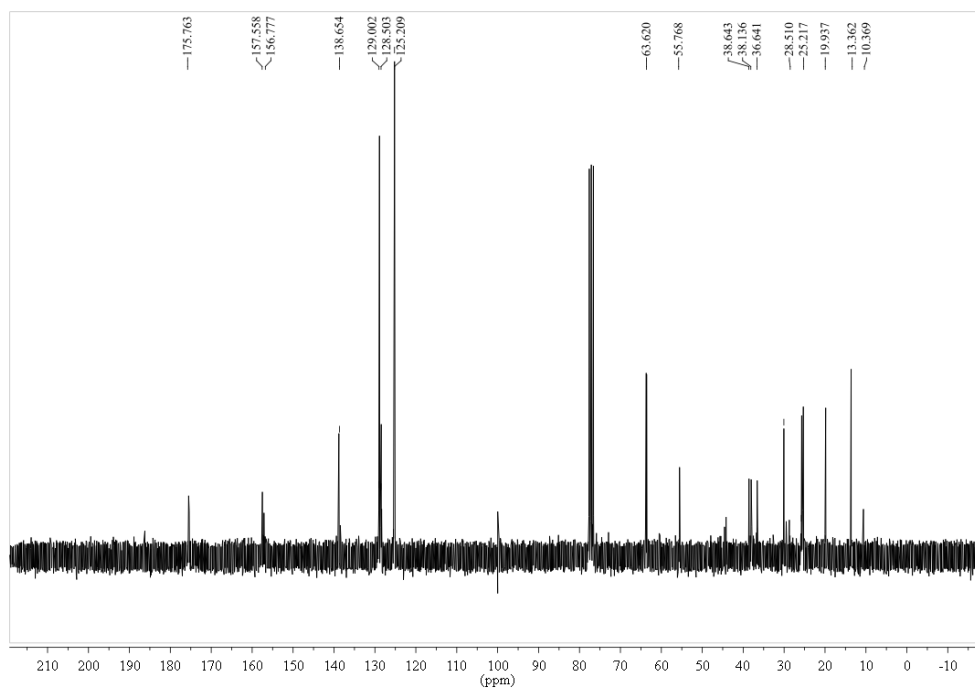


Figure S.3.17. ¹³C-NMR spectra of UV irradiated MPm-Cl. (solvent: CDCl₃)

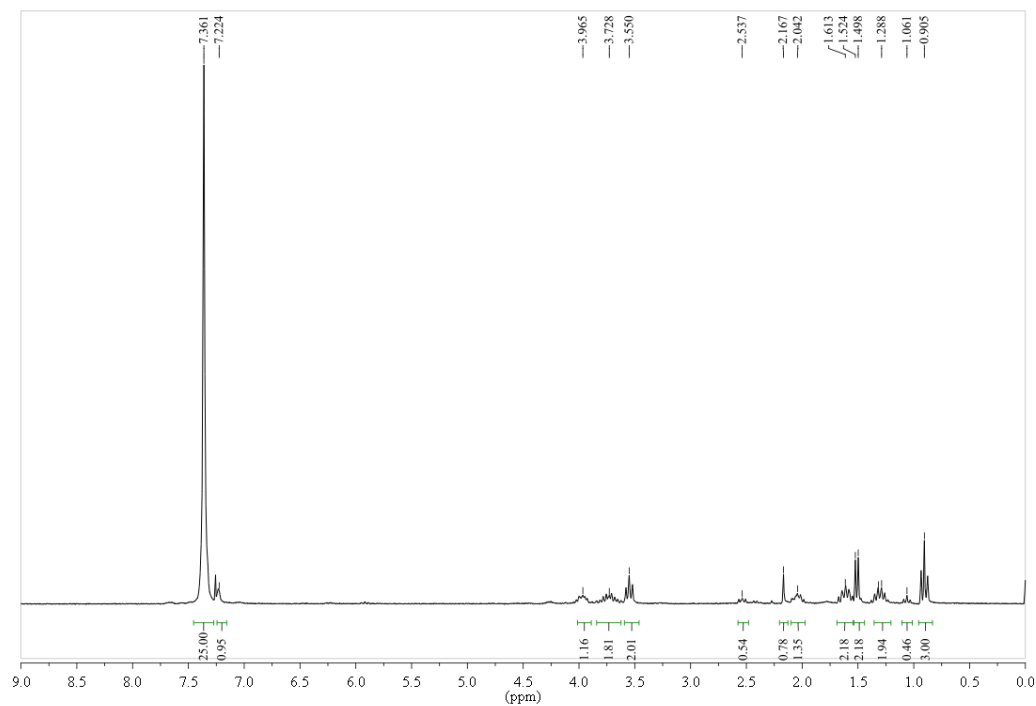


Figure S.3.18. ^1H -NMR spectra of UV irradiated PPM-Cl. (solvent: CDCl_3)

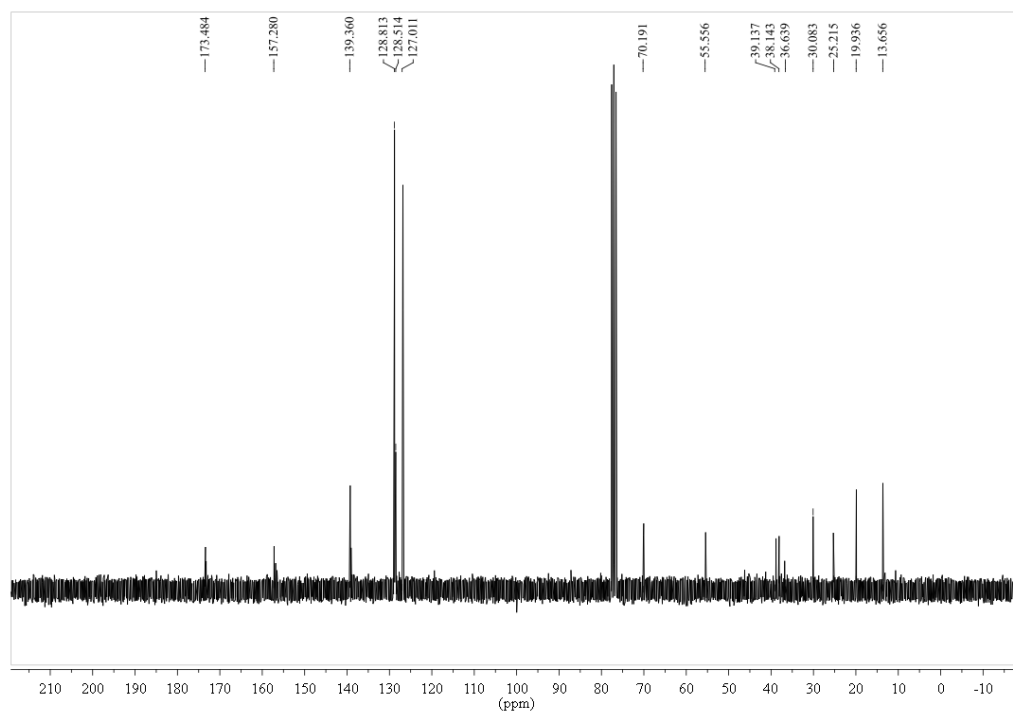


Figure S.3.19. ^{13}C -NMR spectra of UV irradiated PPM-Cl. (solvent: CDCl_3)

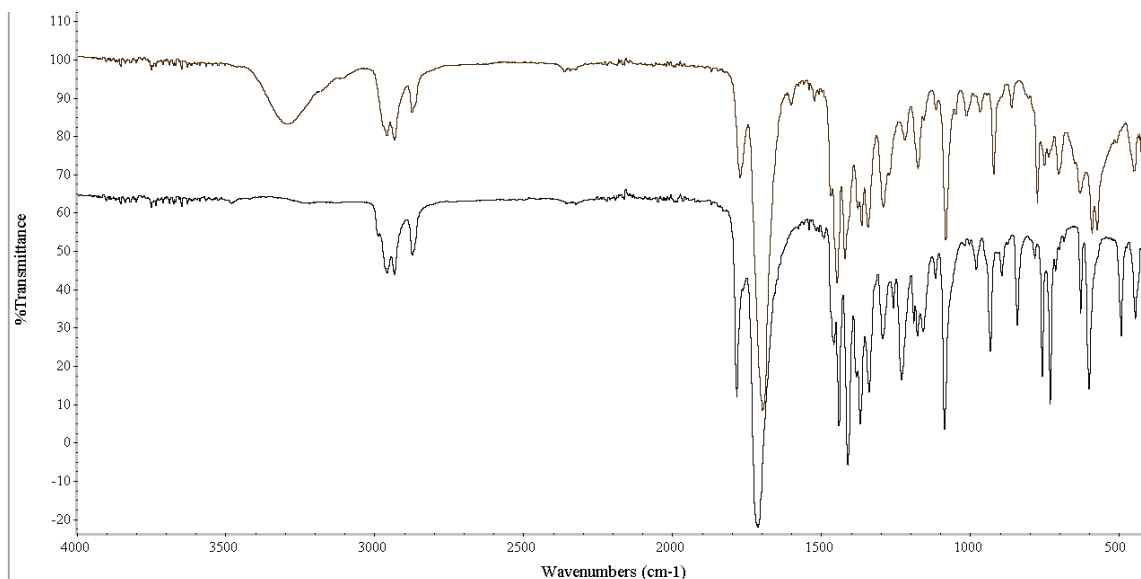


Figure S.3.20. FT-IR spectra of MMm (a) and MMm-Cl (b).

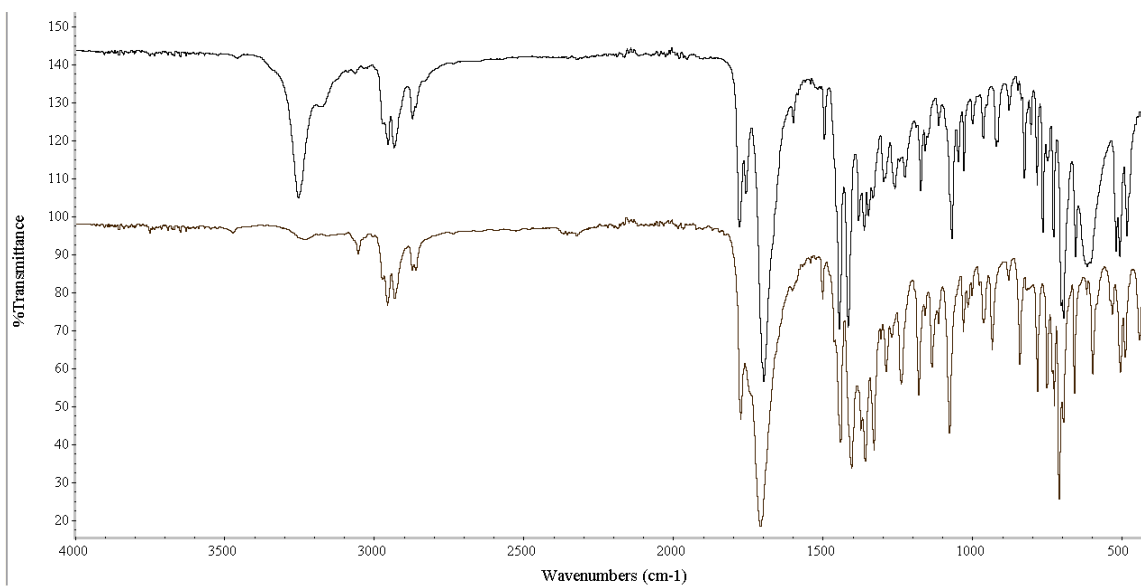


Figure S.3.21. FT-IR spectra of MPm (a) and MPm-Cl (b).

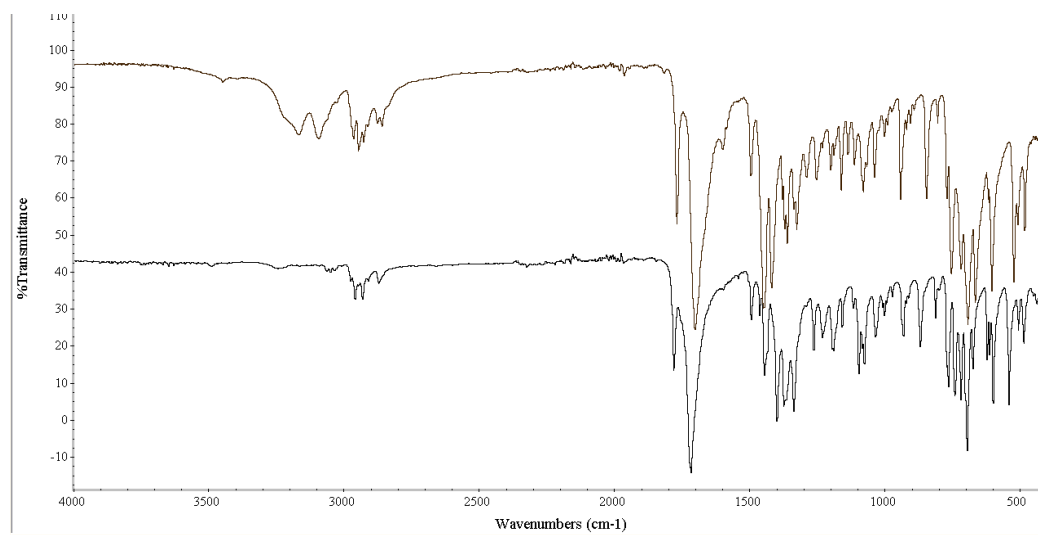


Figure S.3.22. FT-IR spectra of PPM (a) and PPM-Cl (b).

CHAPTER 4

WHY DOES KEVLAR DECOMPOSE, WHILE NOMEX DOES NOT, WHEN TREATED WITH AQUEOUS CHLORINE SOLUTIONS?

4.1 Introduction

N-Halamine chemistry has been a fruitful area of research since the late 1970s.¹

These compounds contain at least one nitrogen-halogen bond, where halogen generally refers to chlorine and bromine. The halogens have Pauling electronegativities (3.2 for Cl and 3.0 for Br) which are comparable to that for nitrogen (3.0); this renders halogens on N-halamines partially positively charged, thus oxidative.² The most important practical application for N-halamine compounds has been directed toward inactivation of pathogens. In other words, stable N-halamines are effective oxidizing agents that can oxidize the molecules on cell surfaces which are vital for cell survival.³

Therefore, N-halamine chemistry has proved to be important in the development of effective antimicrobial compounds.¹⁻⁴

Incorporation of N-halamines into polymeric materials has provided a new avenue of research.⁴ Such polymers can be used in a wide variety of applications, such as in textiles, coating materials, paints, water disinfectants, etc.

Recent biological security threats have stimulated the exploration of new efficient ways to generate N-halamines. Sun and co-workers have pointed out that Nomex and Kevlar could be excellent candidates for incorporation of N-halamine functionality because their amide groups do not contain any α -hydrogens (e.g. explicitly shown hydrogen on R_2N-CH_2R are α -hydrogens), unlike Nylon derivatives (Figure 2).⁵ That is, dehydrohalogenation cannot occur as a mechanism for the loss of biocidal efficacy. They have found that Nomex can be chlorinated sufficiently to inactivate microbial pathogens. On the other hand, they found that Kevlar, upon chlorination, decomposed.

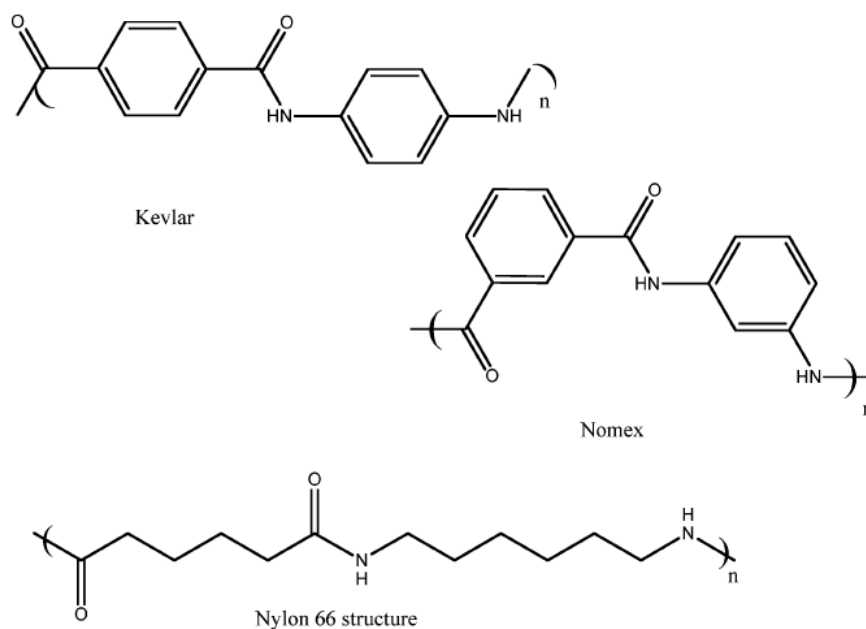


Figure 4.1. Structure of Kevlar, Nomex, and Nylon 66.

The chlorination of Nomex has been explored further by Sun and Broughton.⁶ It was found that low chlorination loadings ($Cl^+/amide$) were due to the crystallinity of the Nomex. Therefore, low crystalline Nomex or Nomex blends were employed in order to generate high chlorination loadings. Since chlorination occurs on the surface of the fibers,

it is understandable that later studies provided high chlorine percentage with increased surface area.⁶ In these studies, the question to be addressed is: Why is Kevlar decomposed, but Nomex is chlorinated without decomposition, upon treatment with hypochlorous acid? The answer to this question could provide enlightenment to stimulate exploration of N-halamine chemistry for high-performance polymers. Sun and co-workers suggested that the decomposition of Kevlar upon attempted chlorination is due to the hydrolysis of the amide structure.⁵ This work will attempt to address the matter through a study of four compounds which mimic portions of the Kevlar and Nomex structure.

The mimics were subjected to chlorination at various conditions with use of household bleach. The crystal structure of the chlorinated Nomex mimic was solved, and it was compared to a previously solved crystal structure of unchlorinated Nomex mimic. The crystal structure of the Kevlar mimic has been reported. On the basis of the information obtained, an explanation concerning the title question will be offered.

4.2 Experimental Section

General Procedure for KM1 and NM1 Synthesis (See Figure 4.2). To a solution of aniline (2 mol equiv) in freshly distilled THF was added terephthaloyl chloride for KM1 or isophthaloyl chloride for NM1 (1 mol equiv). The mixture was stirred at room temperature for 4 h, and then the mixture was filtered and the resulting solid was washed with water and then with cold ethanol. The solids were dried in air to provide the corresponding materials.

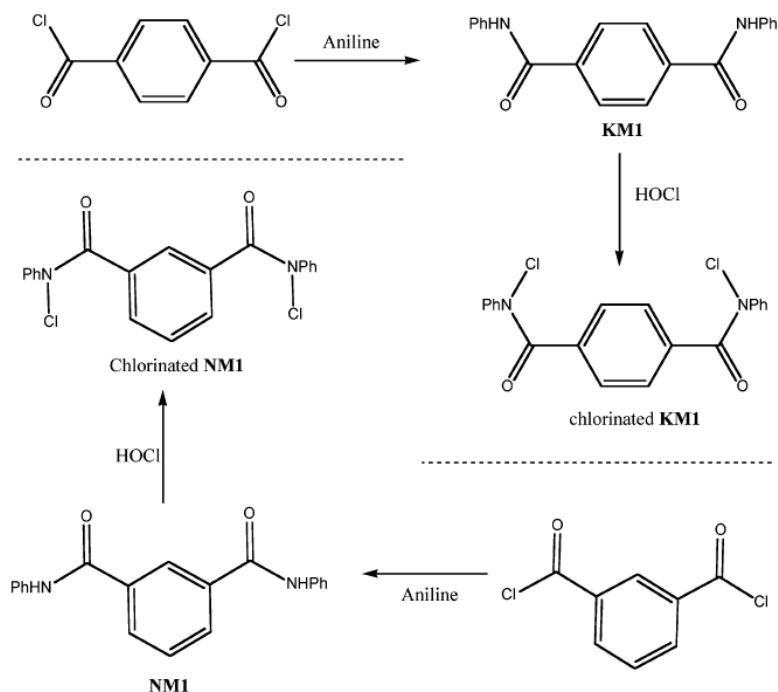


Figure 4.2. Synthesis of KM1, NM1, and their chlorinated derivatives.

N,N'-Diphenylterephthalamide (KM1). ^1H NMR (DMSO- d_6 , 250 MHz) δ 10.39 (s, 2H), 8.10 (s, 4H), 7.80 (d, 4H), 7.38 (t, 4H), 7.13 (t, 2H). ^{13}C NMR (62.5 MHz) δ 165.28, 139.44, 137.93, 129.13, 128.19, 124.37, 120.95.

N,N'-Diphenylisophthalamide (NM1). ^1H NMR (DMSO- d_6 , 400 MHz) δ 10.41 (s, 2H), 8.59 (s, 1H), 8.18 (m, 2H), 7.84 (m, 4H), 7.72 (m, 1H), 7.41 (m, 4H), 7.15 (m, 2H). ^{13}C NMR (100 MHz) δ 165.57, 139.56, 135.70, 131.15, 129.17, 129.11, 127.51, 124.32, 120.89.

Preparation of p-Phenylenediamine. To mixture of p-nitroacetanilide (1.8 g, 0.010 mol) in ethanol, prepared according to a literature procedure,⁷ was added tin (2 g, 0.017 mol) and 10 mL of concentrated hydrochloric acid solution. The resulting mixture was

refluxed for 4 h. Then the solvent was removed by evaporation, and the residue was dissolved in 100 mL of water with pH adjustment of the solution to 12 by addition of 6 M NaOH solution. This solution was extracted with n-butanol (4 x 50 mL), and combined organic phases were dried over anhydrous MgSO₄. Then the mixture was filtered, and the solvent was evaporated to provide p-aminoaniline, which was used in the next reaction step without further purification. ¹H NMR (DMSO-d₆, 250 MHz) δ 6.39 (s, 4H), 4.51 (s, 4H). ¹³C NMR (66.50 MHz) δ 139.07, 116.10.

General Procedure for the Synthesis of KM2 and NM2 (See Figure 4.3). To a solution of the p-aminoaniline for KM2 or m-aminoaniline for NM2 (1 mol equiv) in freshly distilled THF was added pyridine (2 mol equiv). To this solution was added acetyl chloride (2 mol equiv) dropwise. The solution was stirred for 4 h, the solvent was evaporated, and the residue was dissolved in water and extracted with ethyl acetate. The combined organic phases were dried over anhydrous MgSO₄, which was removed by filtration. After evaporation of the solvent, the corresponding KM2 and NM2 were obtained.

N-(4-Acetylamino-phenyl)acetamide (KM2). ¹H NMR (DMSO-d₆, 250 MHz) δ 9.86 (s, 2H), 7.48 (s, 4H), 2.02 (s, 6H). ¹³C NMR (66.50 MHz) δ 168.37, 135.08, 119.81, 24.33.

N-(3-Acetylamino-phenyl)acetamide (NM2). ¹H NMR (DMSO-d₆, 250 MHz) δ 9.94 (s, 2H), 7.89 (s, 1H), 7.20 (m, 3H) 2.04 (s, 6H). ¹³C NMR (66.50 MHz) δ 168.75, 140.03, 129.20, 114.29, 110.24, 24.47.

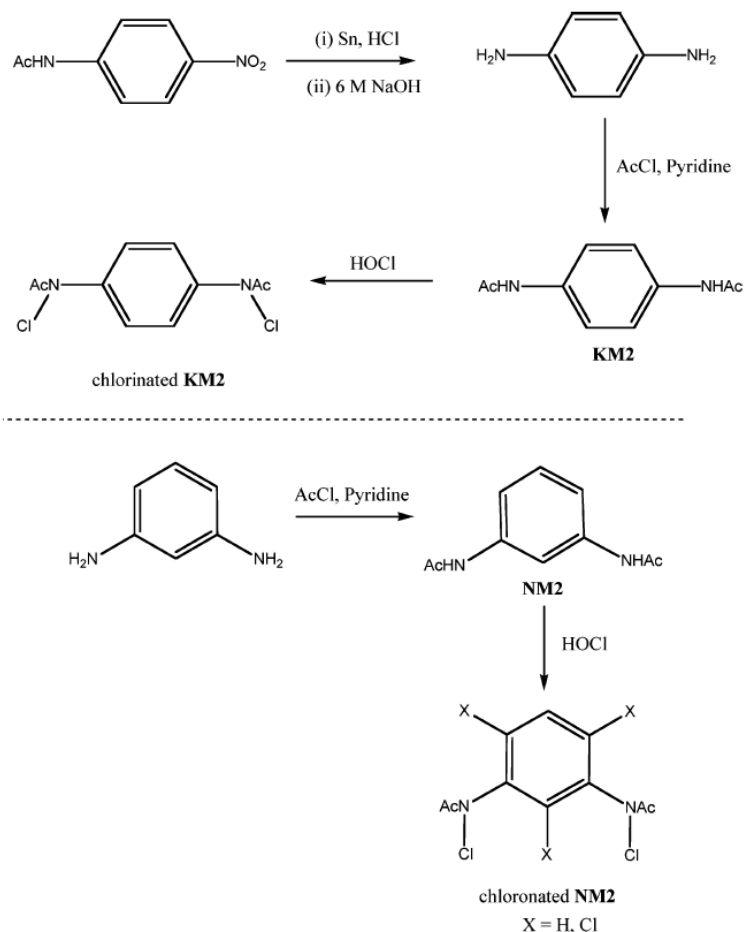


Figure 4.3. Synthesis of KM2, NM2, and their chlorinated derivatives.

Chlorination Procedure. Heterogeneous chlorination of the compounds was performed at 25 °C by using a 10% aqueous solution of commercially available household bleach, which contained 0.6% sodium hypochlorite; the pH of the solution was adjusted with 1 M HCl solution to the desired levels. The insoluble chlorinated products were removed by filtration and dried in air at ambient temperature. X-ray diffraction-quality crystals of chlorinated NM1 were obtained by recrystallization from acetone and slow evaporation. Such crystals could not be obtained by this procedure for chlorinated KM1. The NMR data for the chlorinated compounds are given below.

Chlorinated KM1. ^1H NMR (CDCl_3 , 250 MHz) δ 7.20 (m). ^{13}C NMR (66.50 MHz) δ 167.27, 143.98, 135.43, 129.58, 128.99, 128.73, 128.12.

Chlorinated NM1. ^1H NMR (CDCl_3 , 250 MHz) δ 7.20 (m). ^{13}C NMR (66.50 MHz) δ 167.18, 144.18, 133.45, 131.17, 129.83, 129.60, 128.89, 128.21, 127.97.

Chlorinated KM2. ^1H NMR (CDCl_3 , 250 MHz) δ 7.51 (s, 4H), 2.23 (s, 6H). ^{13}C NMR (66.50 MHz) δ 168.82, 143.15, 128.95, 22.46.

Chlorinated NM2 (after 20 min of chlorination). ^1H NMR (CDCl_3 , 250 MHz) δ 7.50 (m, 4H), 2.23 (s, 6H). ^{13}C NMR (66.50 MHz) δ 168.88, 143.92, 130.58, 128.14, 127.16, 22.42.

Chlorinated NM2. In this case there was not a single product (after 2 h of chlorination); therefore, the raw data for the mixture are given. ^1H NMR (CDCl_3 , 250 MHz) δ 7.50 (m), 2.23 (m). ^{13}C NMR (66.50 MHz) δ 168.97, 143.88, 142.45, 140.84, 131.56, 130.63, 130.10, 129.85, 128.20, 127.17, 22.42, 21.51.

Analytical Titration. The percentage of the oxidative chlorine as “ Cl^+ ” was determined by iodometric titration, in which KI and starch were used as reactant and indicator, respectively, and $\text{S}_2\text{O}_3^{2-}$ as a reducing agent. The final Cl^+ percent was calculated by using the following equation;

$$\text{Cl}^+ \% = (\text{N} \times \text{V} \times 35.45) / (2 \times \text{W}) \times 100\%$$

where N and V are the normality (equiv/L) and volume (L), respectively, of the $\text{Na}_2\text{S}_2\text{O}_3$ consumed in the titration, and W is the weight in grams of the sample.

Computational: All computations were performed with Gaussian03.⁸ The B3LYP/6-311+G(2d,p) level of theory was employed for all of the theoretical predictions.

4.3 Results and Discussion

The focus of this work was to answer the following question: Why does Kevlar decompose, whereas Nomex does not when treated with aqueous bleach? This question was tackled by examining mimics for portions of the polymer structures. Two sets of the mimics of Kevlar and Nomex were synthesized. The first set of mimics was synthesized to understand the role of the terephthaloyl portion of the Kevlar polymer and the isophthaloyl portion of the Nomex polymer. The second set of mimics was synthesized to address the p-aminophenylene portion of the Kevlar and the m-aminophenylene portion of the Nomex.

The synthesis of the first set of the aromatic polyamides was straightforward. One equivalent of the terephthaloyl chloride (for KM1) or isophthaloyl chloride (for NM1) was reacted with 2 equiv of aniline in THF according to a literature procedure.⁹ The air-dried KM1 and NM1 NMR spectra (¹H and ¹³C in DMSO-d₆) were consistent with the published data. Although KM1 was sparingly soluble in the NMR solvent, NM1 dissolved completely. Moreover, we have observed that the KM1 melting point (350 °C) was higher than the NM1 melting point (290 °C). This suggests that intermolecular interactions among KM1 molecules are stronger than those for the NM1 molecules. It was decided to examine the X-ray crystal structures of the mimics to see if large differences could help address the title question.

The crystal structure of KM1 was reported previously by Harkema et al.¹⁰ The structure revealed that the molecule does not have Cs symmetry (i.e., it is not planar). The terminal phenyl groups are in the same plane, but not in the plane of the middle phenylene moiety. That is, the terminal phenyl groups are 68° out of the plane of the phenylene ring. Similarly, the carbonyl groups are out of the plane of the phenylene ring by 29°. The terminal phenyl groups are out of the plane of the N-H bonds by 20°. To increase intermolecular hydrogen bond efficiency in the crystal structure, the carbonyl moieties are out of the plane to both the phenylene and terminal phenyl groups.

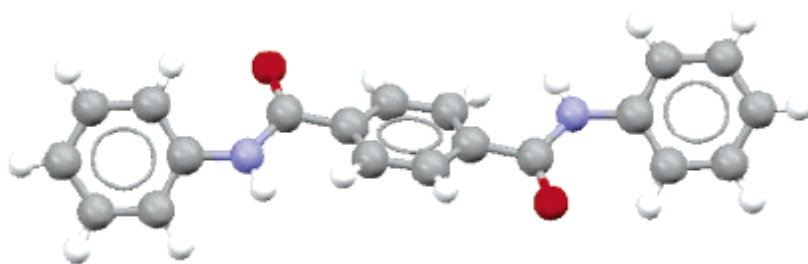


Figure 4.4. Crystal structure of KM1.

The NM1 crystal structure was reported by Malone et al.¹¹ The crystal structure showed that the molecule is not planar (i.e., it has C1 symmetry) in the solid state. The carbonyl moieties are out of the plane of the middle m-phenylene ring by 32° and 34°. Similarly, the N-H bonds are out of the phenylene ring plane by 24° and 29°. The terminal phenyl rings are not in the same plane as well, i.e., the planes are off from each other by 11°. The terminal phenyl groups are out of the plane of the adjacent N-H bonds by 26° and 34°. This conformation of the molecule in the solid state is necessitated in order to increase the efficiency of the intermolecular hydrogen bonding. There is no obvious difference in the crystal structures of the two mimics which can help answer the title

question, although the DSC data for the two clearly indicate that the crystalline interaction is stronger for KM1 (mp 350 °C) than for NM1 (mp 290 °C).

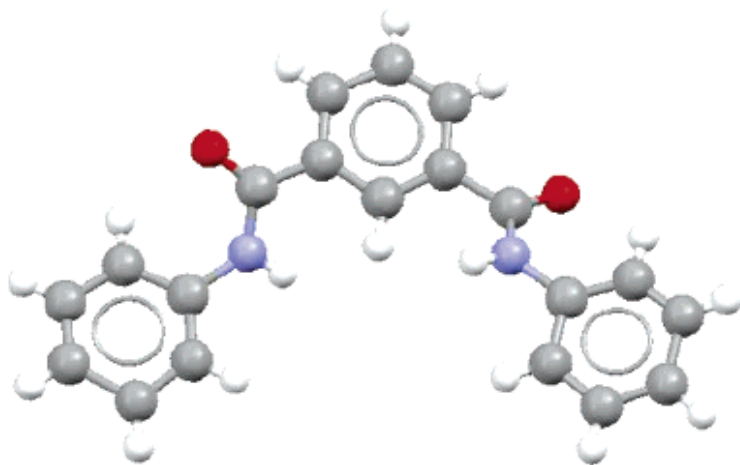


Figure 4.5. Crystal structure of NM1.

When NM1 was chlorinated by hypochlorous acid at a pH of about 8, a white solid was obtained. This solid was recrystallized in acetone by slow evaporation of the acetone. The crystal structure revealed that the terminal phenyl rings are bent over the middle of the phenylene ring. The carbonyl groups are out of the plane of the phenylene by 44° and 70°. In the crystal structure there is a close interaction between a carbonyl oxygen and one of the C-H's (para to the to C-N bond) of the terminal phenyl at a distance of 2.65 Å (the sum of van der Waals radii is 2.72 Å). There is also a π - π interaction existing between the two phenylene rings on identical molecules which are related to each other by inversion. These interactions contribute to the crystal structure formation; however, in the NM1 structure conventional hydrogen bonding contributed most to the crystal structure. The N-Cl bond length on the chlorinated NM1 was 1.70 Å. This bond length is consistent with previously calculated values.² Although a substantial difference in crystal

structure for NM1 occurs upon chlorination, there is no obvious reason for its ease of chlorination as compared to KM1 for which diffraction-quality crystals could not be obtained.

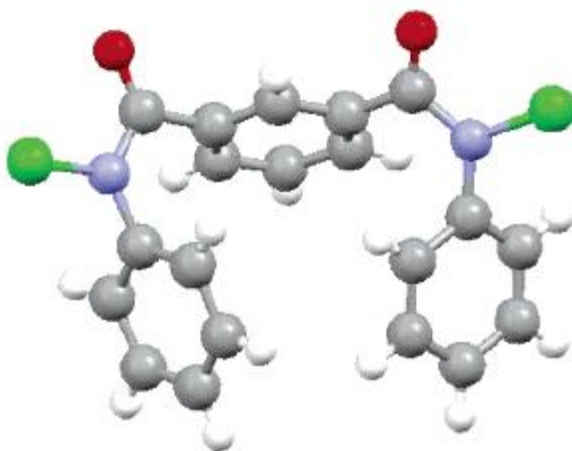


Figure 4.6. Crystal structure of chlorinated NM1.

The KM1 and the NM1 compounds can be chlorinated in dilute hypochlorous acid solutions as shown in Figure 4.7. The NM1 can be chlorinated at a higher rate than the KM1. The theoretical oxidative chlorine percent (ca. 18%) can be attained at around pH 8 for NM1. On the other hand, pH 9 was necessary for the chlorination of KM1. This is because NM1 is appreciably polar (computed dipole moment was 3.92 D), while KM1 is nonpolar (computed dipole moment was 0.001 D). The dipole moments were predicted by a single-point calculation on the crystal structures published at the B3LYP/6-311+G(2d,p) level. Dipole moments affect the ability of molecules to interact with polar solvents like water; the more polar the molecule, the greater the interaction with water. Alkaline conditions are needed for KM1 to be chlorinated efficiently due to the acidic proton on the amide moiety, which can be abstracted by hydroxide. Therefore,

the ionized KM1 specie can interact with the water and hypochlorous acid to proceed in chlorination.

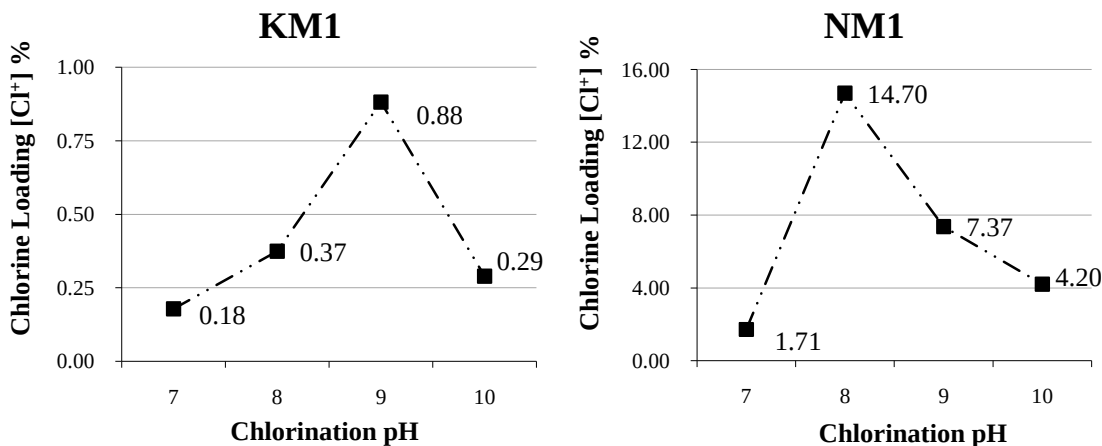


Figure 4.7. Chlorine contents of chlorinated KM1 and NM1 at various pH-values.

^a Compounds were chlorinated for 8 h.

Figure 4.8 shows the chlorine content of KM1 and NM1 after various time of chlorination. For KM1, even at ca. pH 9, the chlorination was extremely slow such that only 12% oxidative chlorine could be titrated after 140 h of chlorination. On the other hand, the NM1 could be chlorinated up to 17% in less than 24 h. Therefore, from these percent conversions over much different time periods, it can be concluded that the degree of crystallinity, which is related to the ease of intermolecular hydrogen bonding and π -stacking, can affect the rate of N-chlorination by virtue of the polarity of the mimics interacting with aqueous HOCl. Of most importance to the title question, neither of the two mimics decomposed upon chlorination.

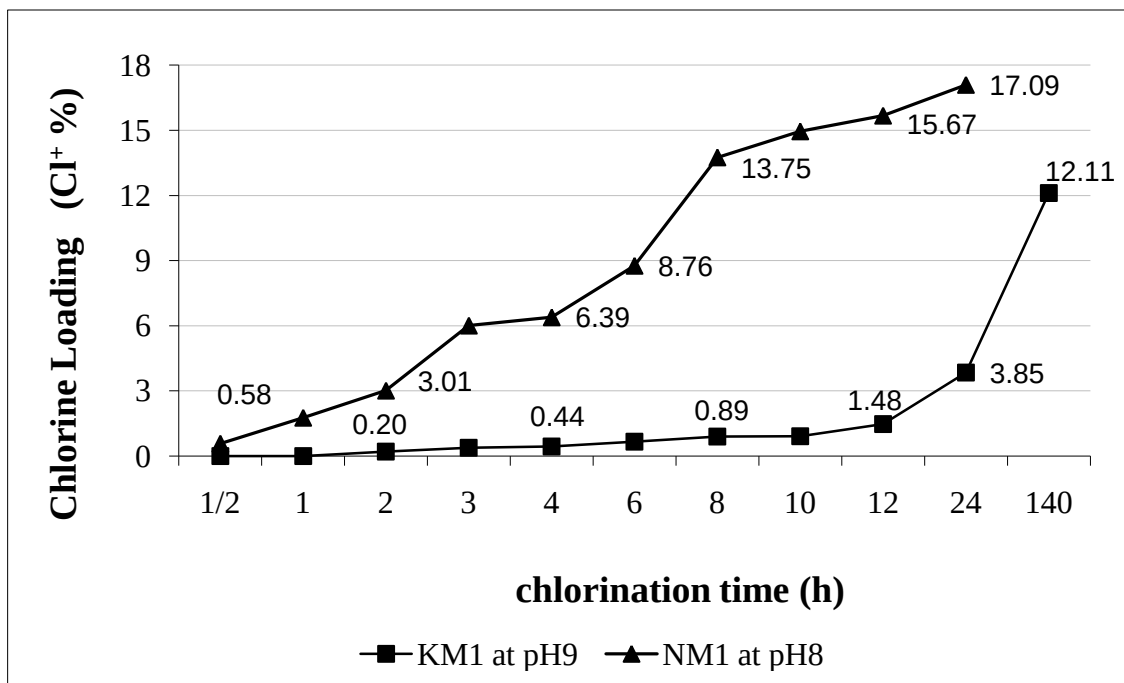


Figure 4.8. Chlorine contents of chlorinated KM1 and NM1 over chlorination time.

^a pH of the chlorination solutions were 9 and 8, for KM1 and NM1, respectively.

A DSC study of the chlorinated mimics showed that chlorinated KM1 is less thermally stable than the chlorinated NM1. There is an exothermic DSC signal around 157 °C for the chlorinated KM1, but at 191 °C for the chlorinated NM1. When the chlorinated NM1 was heated to 200 °C in vacuum for 45 min, it was observed by ¹H NMR that chlorine transferred to one of the terminal phenyl groups. This is similar to the Orton rearrangement observed for N-chloroacetanilide.¹²

In summary, the first set of mimics has suggested that the stabilities of Kevlar and Nomex are not related to the carboxyl portions of the polymers as neither decomposes during chlorination. However, the carboxyl portions dictate the rates of chlorination due to the relative contributions to the crystallinity. It was also revealed that the chlorination of NM1 forced it further out of plane to attain the observed crystal structure. Since the

perturbation is less severe for the chlorinated KM1, these mimics cannot aid our understanding of why Kevlar decomposes upon chlorination.

The second set of mimics were synthesized to simulate the effect of the p-aminoaniline and m-aminoaniline portions of the Kevlar and Nomex, respectively. The syntheses of these mimics KM2 and NM2 were accomplished by treating the corresponding diaminophenylene with acetyl chloride in THF.¹³ The NMR spectra of the compounds obtained were consistent with literature reports.¹³ The ¹H NMR spectrum of KM2 showed three signals at 9.86, 7.48, and 2.02 ppm for N-H, C-H, and CH₃, respectively. The white colored KM2 was chlorinated in a 10% bleach solution at ca. pH 7 for 20 min. The ¹H NMR spectrum of the chlorinated KM2 showed that the N-H signal disappeared, with the two signals remaining at 7.51 ppm for the aromatic C-H and 2.23 ppm for the CH₃ protons. ¹³C NMR spectra of the KM2 and chlorinated KM2 showed large shifts for the aromatic carbons: 135 → 143 ppm for the quaternary carbons, and 120 → 129 ppm for the other carbons (unchlorinated → chlorinated).

Over a time of 12 h, the solution of the chlorinated KM2 changed from colorless to red, and the NMR spectra became markedly more complex (developing ¹H NMR signals at 1.75 and 4.84 ppm), indicating the formation of decomposition products. When a drop of water was added, the solution became a vivid orange/red after 12 h. The UV spectrum of the product mixture showed λ_{max} at 2.65 nm, which was red-shifted by 10 nm from the corresponding λ_{max} in a sample of freshly chlorinated and colorless KM2. The FTIR spectrum of the sample mixture exhibited substantial losses in intensity of vibrational bands at 829 and 1565 cm⁻¹ relative to that of KM2, which are characteristic of para-

disubstituted aromatic rings. These observations lead us to propose the following mechanism: the chlorinated KM2 with contact of water loses one of the chlorine atoms to form a negative charge which, when delocalized, leads to dissociation of the other chlorine to yield a quinone-type structure (Figure 4.9). Moreover, when the chlorinated KM2 was suspended in distilled water, a red color appeared over 12 h. The resulting quinone-type structure is not stable and yields many byproducts based on literature reports.¹⁴ Under dry conditions, the chlorinated KM2 solid color turned light pink over the period of one week. In fact, the proposed structure in Figure 4.8 has been prepared by oxidation of unchlorinated KM2 with lead tetraacetate under dry conditions, and in its pure form, it was deep red with a UV λ_{max} at 2.80 nm.¹⁴

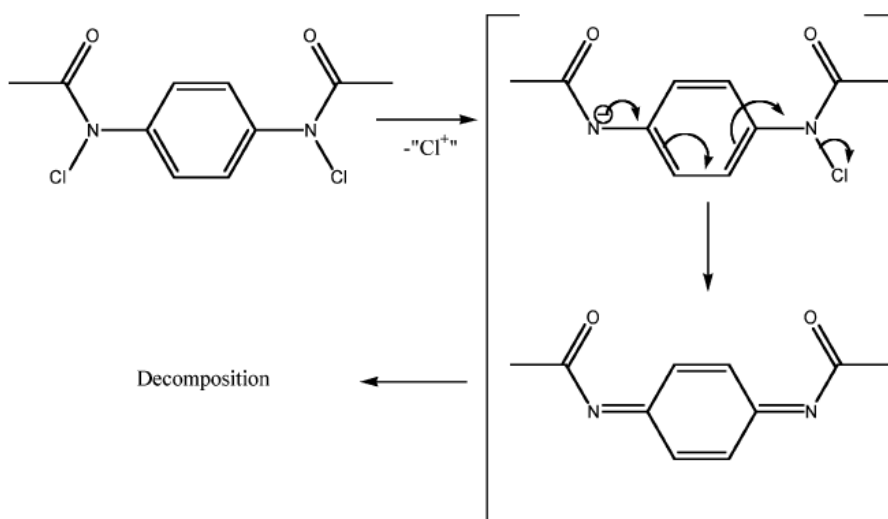


Figure 4.8. Decomposition mechanism for KM2.

NM2 was also chlorinated under the same conditions as for KM2. The reaction conditions yielded the desired product as a light yellow solid. However, chlorination did not yield a solid when the reaction time was extended to 2 h. Therefore, the chlorination

solution was extracted with ethyl acetate. The solvent was evaporated to give a yellow solid whose ^1H NMR spectrum showed that the N-H signal disappeared. However, the region for the CH_3 protons exhibited several new signals. The ^{13}C NMR spectrum of the chlorinated NM2 showed two signals for the acetyl CH_3 carbon and 10 signals for the aromatic region. This suggests that the aromatic ring is chlorinated along with the formation of the N-Cl bond because there are three chlorination sites based on ortho-para directional ability of the acetanilide moieties on the m-phenylene ring. There are no other N-Cl decomposition mechanisms possible as for KM2 except for the aromatic electrophilic substitution reactions and Orton rearrangement. On the basis of the above observation, it can be suggested that KM2 decomposes easily through oxidation of the phenylene rings to quinone-type structures and subsequent hydrolyzes. Therefore, we suggest that the p-aminoaniline moiety in the polymer Kevlar becomes a quinone-type structure upon chlorination. However, as mentioned earlier, this structure is not stable under moist conditions leading to decomposition of the polymer. On the other hand, chlorination of NM2 revealed that such a mechanism is not possible for the Nomex polymer because of its meta substitution pattern, but it can undergo an Orton rearrangement and electrophilic aromatic substitution.

4.4 Conclusions

Many N-halamine structures have been incorporated into polymers. The quest for an easily obtainable polymer containing a chlorinated amide has led to work employing aromatic polyamide structures. However, it is known that Kevlar decomposes upon

chlorination, while Nomex does not. This work has attempted to provide a rationalization for these observations. Two sets of mimics were prepared to help attack the problem. A first set of mimics was synthesized to simulate the carboxyl moiety in the Nomex and Kevlar. A second set of mimics was prepared to model the p-diaminophenylene and m-diaminophenylene units of the Kevlar and Nomex. It was shown that upon chlorination, the mimic NM1 structurally underwent a large conformational change. The NM1 crystal structure is dominated by intermolecular hydrogen bonding and π - π interaction. The chlorinated NM1 solid-state structure is dominated by C-H $\cdots$ O hydrogen bonding and π - π interaction. The same is presumably true for KM1. Chlorination of the mimics KM1 and NM1 demonstrated that crystallinity affects the rate of chlorination. However, these changes cannot explain the decomposition mechanism for Kevlar. The second set of mimics KM2 and NM2 were chlorinated by treating them with hypochlorous acid. It was observed that KM2 was decomposed over time under moist conditions through a quinone-type intermediate. Although NM2 could not undergo such a transformation, it was susceptible to aromatic electrophilic substitution reactions and Orton rearrangements. We suggest that the mechanism for decomposition of Kevlar upon chlorination is that shown in this work for the mimic KM2.

4.5 References

- [1] (a) Worley, S. D.; Wojtowicz, J. A. *Kirk-Othmer Encycl. Chem. Technol.*, 4th Ed. **2004**, 98-122. (b) Worley, S. D.; Sun, G. *Trends Polym. Sci.* (Cambridge, U.K.) **1996**, 4, 364-370.
- [2] Akdag, A.; Okur, S.; McKee, M. L.; Worley, S. D. *J. Chem. Theory Comput.* **2006**, 2, 879-884.
- [3] Kohl, H. H.; Wheatley, W. B.; Worley, S. D.; Bodor, N. *J. Pharm. Sci.* **1980**, 69, 1292-1295.
- [4] (a) Liang, J.; Chen, Y.; Barnes, K.; Wu, R.; Worley, S. D.; Huang, T.-S. *Biomaterials.* **2006**, 27, 2495-2501. (b) Sun, G.; Wheatley, W. B.; Worley, S. D. *Ind. Eng. Chem. Res.* **1994**, 33, 168-170. (c) Sun, Y.; Sun, G. *J. Appl. Polym. Sci.* **2003**, 88, 1032-1039. (d) Sun, Y.; Sun, G. *Macromolecules.* **2002**, 35, 8909-8912. (e) Makal, U.; Wood, L.; Ohman, D.; Wynne, K. J. *Biomaterials.* **2006**, 27, 1316-1326.
- [5] Sun, Y.; Sun, G. *Ind. Eng. Chem. Res.* **2004**, 43, 5015-5020.
- [6] (a) Sun, G.; Sun, Y.; Morshed, M. Abstracts of Papers; 227th National Meeting of the American Chemical Society, Anaheim, CA, March 2006; American Chemical Society: Washington, DC, **2006**; CELL-135 (b) Lee, J.; Broughton, R. M.; Worley, S. D.; Huang, T. S.; Fan, X. Abstracts of Papers; 232nd National Meeting of the American Chemical Society; San Francisco, CA, September 2006; American Chemical Society: Washington, DC, **2006**; CELL-009. (c) Sun, G.; Sandstrom, A. Abstracts of Papers; 231st National Meeting of the American Chemical Society; Atlanta, GA, March 2006; American Chemical Society: Washington, DC, **2006**; CELL-022.

- [7] Suzuki, H.; Tatsumi, A.; Ishibashi, T.; Mori, T. *J. Chem. Soc., Perkin Trans. 1*. **1995**, 339-343.
- [8] Frisch, M. J.; et al. Gaussian03, Revision D.01; Gaussian, Inc.: Wallingford, CT, **2004**.
- [9] (a) Kavallieratos, K.; Bertao, C. M.; Crabtree, R. H. *J. Org. Chem.* **1999**, 64, 1675-1683. (b) Kavallieratos, K.; de Gala, S. R.; Austin, D. J.; Crabtree, R. H. *J. Am. Chem. Soc.* **1997**, 119, 2325-2326. (c) Sellarajah, S.; Lekishvili, T.; Bowring, C.; Thompsett, A. R.; Rudyk, H.; Birkett, C. R.; Brown, D. R.; Gilbert, I. H. *J. Med. Chem.* **2004**, 47, 5515-5534.
- [10] Harkema, S.; Gaymans, R. J.; van Hummel, G. J.; Zylberlicht, D. *Acta Cryst.* **1979**, B35, 506-508.
- [11] Malone, J. F.; Murray, C. M.; Dolan, G. M.; Docherty, R.; Lavery, A. *J. Chem. Mater.* **1997**, 9, 2983-2989.
- [12] (a) King, H.; Orton, K. J. P. *J. Chem. Soc. Trans.* **1911**, 99, 1377- 1382. (b) Underwood, G. R.; Dietze, P. E. *J. Org. Chem.* **1984**, 49, 5225- 5229.
- [13] (a) De Renzi, A.; Panunzi, A.; Saporito, A.; Vitagliano, A. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1095-1098. (b) Dzierzbicka, K.; Trzonkowski P.; Sewerynek, P.; Myliwski, A. *J. Med. Chem.* **2003**, 46, 978-986.
- [14] (a) Adams, R.; Anderson, J. L. *J. Am. Chem. Soc.*, 72, 5154-5157. (b) Avdeenko, A. P.; Marchenko, I. L. *Russ. J. Org. Chem.* **2001**, 37, 822- 829.

4.6 Supporting Information

The Supporting Information includes additional information about DSC plots for KM1, NM1, and their chlorinated derivatives.

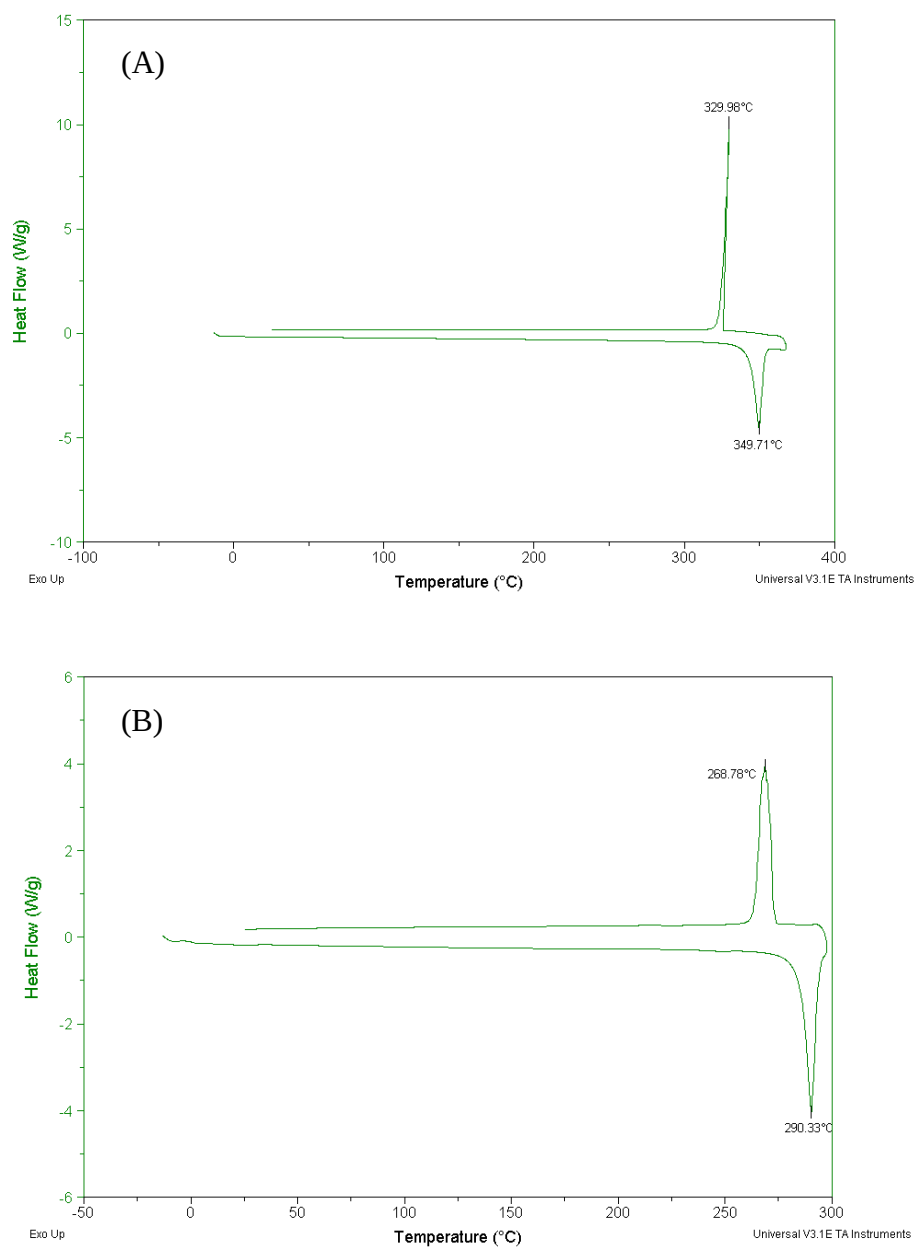


Figure S.4.1. DSC plots of KM1 (a) and NM1 (b).

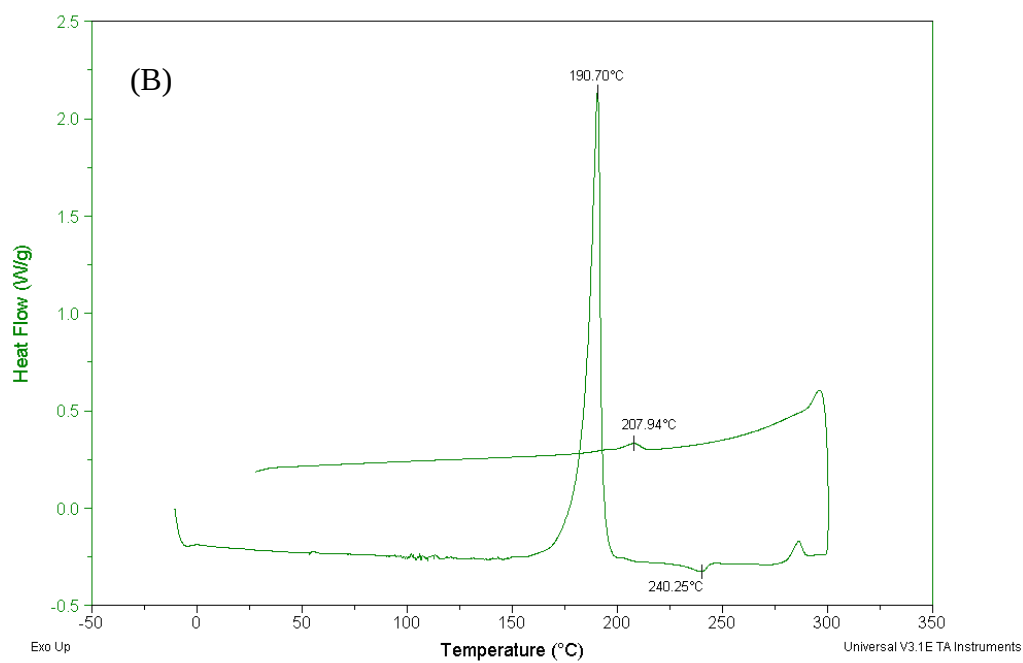
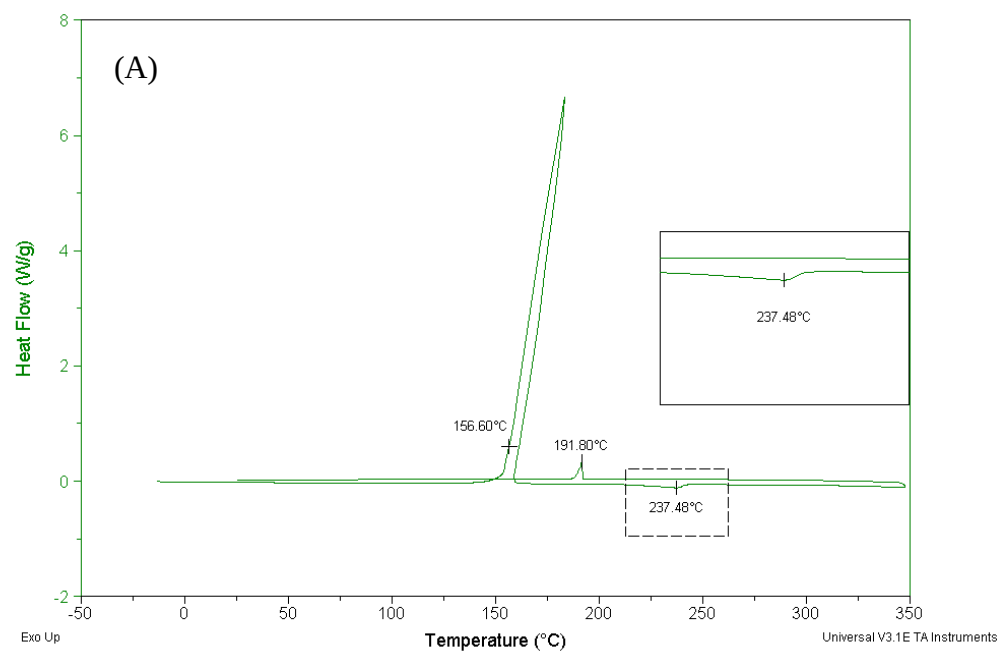


Figure S.4.2. DSC plots of chlorinated KM1 (a) and chlorinated NM1 (b).

CHAPTER 5

ANTIMICROBIAL TREATMENTS PROVIDING CONTACT BIOCIDAL PROPERTIES FOR FILTER MEDIA

5.1 N-Halamine Coated Antimicrobial Water Filters

5.1.1 Introduction

As the world population continues to increase, industries and cities continue to expand, various natural resources become unsuitable for direct use because of contamination and pollution.¹ Hence, both decontamination of waste and purification of the resources, especially fluids, became one of the most important issues for society. Among many fluids, water is the essential for the survival of all known forms of life. There are various treatment methods used for water purification and filtration methods are occasionally preferred for separation of water from hazardous substances. Filtration became more popular after new developments in material sciences (i.e. nanotechnology) allowing production of media capable of high retention of submicron particulates (i.e. bacteria, virus and DNA) at relatively high flow rates.² However, separated microorganisms can easily remain viable or even continue to grow on the filters and decrease the filtration efficiency. Thus, a contact biocidal property would be useful for such filters.

N-halamines have been shown to be efficient, direct-contact biocides.^{3,4} Various novel antimicrobial N-halamine monomers has been developed in these laboratories including N-halamine siloxanes and N-halamine epoxides.⁵⁻⁸ Both N-halamine precursors are particularly useful biocidal treatment materials applicable to variety of surfaces such as cellulose,^{5,6,9} silica gel,¹⁰ and polyurethanes.⁵ The N-halamine derivatized coatings have several advantages over other biocidal reagents. They are broad-spectrum biocidal materials effective against Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses. They act rapidly in their disinfection function, within seconds to less than 30 min of contact time. Most important advantage of N-halamines are their rechargability property upon exposure to free halogen and slow release of halogens into aqueous mediums while the conventional chlorination treatments of water might result in excessive chlorination which might then need neutralization by addition of proper agents. In this research, a commercial wood pulp-based (cellulose) water filter containing glass and alumina was coated with 3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro-[4.5]decane-2,4-dione (TTDDS) and 3-glycidyl-5,5-dimethylhydantoin (GH) to provide contact biocidal activity. Both monomers were previously synthesized and demonstrated as contact biocidal coatings.^{5,6} Their structures are shown in Figure 5.1.1. TTDDS and GH contain both N-halamine a precursor moiety and an active tethering group; siloxane and epoxide ring, respectively. TTDDS can be covalently bound to both cellulose and glass, whereas GH can be only bound to cellulose. The coated filters can provide biocidal property after chlorination with household bleach. To provide chemical attachment while minimizing energy usage, coating procedures were optimized and

adjusted for commercial applications. The treated filters have been tested for contact biocidal efficacy against Gram-negative bacteria.

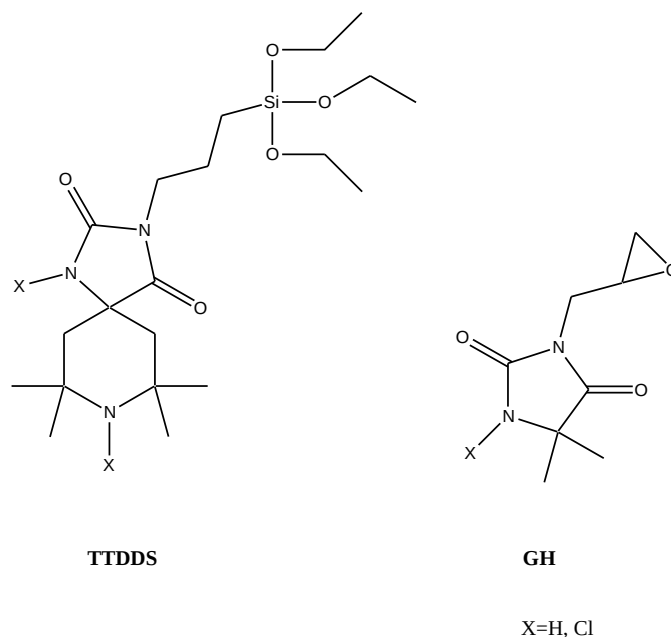


Figure 5.1.1. Structure of compounds considered in the study.

5.1.2 Experimental Section

All solvents and reagents were purchased from Aldrich Chemical Co. or Fischer Scientific Co., unless otherwise stated, were of reagent grade and used without any further purification. Water filter was provided from Argonide Corp. that is consist of wood pulp, and nano alumina fiber grafted (electroadhesively) microglass fibers.²

Synthesis of 3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro-[4.5]decane-2,4-dione. 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (TTDD) was synthesized in 99% yield by reaction of 2,2,6,6-tetramethyl-4-piperidone, potassium cyanide, and ammonium carbonate in a molar ratio of 1:2:4, respectively, in ethanol/water (1:1 v/v) at room temperature for one week.¹¹ The filtered product TTDD

has a sharp melting point at 362.8 °C as determined from DSC; the literature melting point was 360-365 °C.¹¹ TTDDS was prepared according to a general procedure outlined previously.⁵ 35.7g of solid product TTDDS has a melting point of 97.5 °C was obtained (yield, 85 wt%). IR (ATR, cm⁻¹): 3371, 3270, 2970, 2879, 1770, 1702, 1455, 1364, 1267, 1101, 1075, 953, 755, 476. ¹H NMR (CDCl₃, 250 MHz): δ 0.60 (t, 2H), 1.23 (m, 21H), 1.71 (m, 7H), 3.49 (t, 2H), 3.80 (q, 6H), 6.59 (s, 1H).

Synthesis of 3-glycidyl-5,5-dimethylhydantoin. 3-glycidyl-5,5-dimethylhydantoin was prepared according to a procedure outlined previously.⁶ The sodium salt of 5,5-dimethylhydantoin was prepared by mixing 5,5-dimethylhydantoin with an equimolar quantity of NaOH in water at ambient temperature for 10 min. The reaction was accomplished by subsequent addition of epichlorohydrin in one pot and stirred for 8 h. Following the reaction, water was removed by vacuum evaporation, and the desired product was dissolved in acetone. Then the byproduct sodium chloride was filtered and the acetone was evaporated to get the product. ¹H NMR (acetone-d₆, 250 MHz): δ 1.36 (s, 6H), 2.60 (m, 2H), 3.06 (m, 1H), 3.55 (m, 2H). To obtain clear NMR data purified product was used, however, the unpurified product was used for the study.

Instruments. The NMR spectra were obtained using a Bruker 250 MHz spectrometer, while the IR data were obtained with a Nicolet 6700 FT-IR spectrometer ATR (Attenuated Total Reflectance) accessory. Thermal data were obtained using a DSC Q2000 TA Instruments at a heating and cooling rate of 10 °C/min. Air permeability tests were managed using a TMI Densometer Model 58-03.

Chlorination and Analytical Titration Procedures. A commercial 6% sodium hypochlorite solution was used to chlorinate the filters. For the determination of oxidative chlorine (Cl^+) content onto the filter swatches, a standard iodometric/thiosulfate titration procedure was employed. The Cl^+ % on the sample was calculated with the equation below;

$$\% \text{Cl}^+ = [\text{N} \times \text{V} \times 35.45 / \text{W} \times 20]$$

where N and V are the normality (eqv/L) and volume (mL), respectively, of the sodium thiosulfate consumed in the titration, and W is the weight of the sample (g).

Antimicrobial Efficacy Testing. Control and chlorinated filters were challenged with *Escherichia coli* O157:H7 (ATCC 43895) using a “sandwich test”. 25 μL of bacterial suspension, made with pH7 buffer, was dropped in the center of a 1 in. square filter swatch, and a second identical swatch was laid on the first swatch. A sterile weight was used to ensure sufficient contact of the swatches with the inoculums. After the determined contact times, the samples were quenched with 5.0 mL of sterile 0.02N sodium thiosulfate solution to remove any oxidative chlorine that could cause extended disinfection. Serial dilutions of the solutions contacting the surfaces were plated on Trypticase agar and incubated for 24 h at 37 °C, and colony counts were made to determine the presence of viable bacteria.

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5.1.3 Optimum Conditions for N-Halamine Precursor Application

The filter media used in the study is manufactured by conventional wet-laid paper technology.² During the manufacturing process, a suspension of pulp, microglass fiber, binder and water is delivered into the paper machine. Therefore, a water soluble biocidal reagent would be dramatically cost effective by eliminating additional coating procedures. However, in the literature TTDDS has been coated onto cellulose in a bath containing typically 5.0% weight of the compound in ethanol/water (3:1 v/v) solution.⁵ In addition, ethanol is not a process-friendly solvent for commercial applications. The water solubility of TTDDS can be increased by protonating the amine hydrogen on the piperidine ring. A mixture of 2% TTDDS was titrated by 0.5N HCl to reach complete dissolution. The pH at the complete dissolution was 2.3 reasonably suitable for the filter manufacturing process. The filter swatches were soaked in the coating solution for 15 min and then cured at 95 °C for 1 h. After the curing process, the swatches were soaked in a 0.5% detergent solution for 15 min, washed with distilled water, and dried in air. Then the swatches were soaked in a 10% solution of household bleach (pH buffered to 7 with HCl) at ambient temperature for 1 h, rinsed with distilled water and dried at 45 °C for 2 hours to remove any unbounded chlorine. 0.83% Cl⁺ was detected on the swatches whereas the theoretical value was 1.15%.

The curing procedure was optimized by heating the coated swatches at relatively higher temperatures for shorter periods of time (Table 5.1.1). Treatment in acidic aqueous solution showed similar results compared to the ethanol/water solution. In addition, curing at 120 °C for 5 min provided sufficient N-halamine attachment onto the swatches

for biocidal applications. The curing procedure was determined to heat the coated filters at 120 °C for 10 min, and samples for the following experiments were prepared by this procedure.

Table 5.1.1. Chlorine loadings (Cl⁺%) on filter at different curing conditions.

Coating solution	Curing time (min)	Curing temperature (°C)	
		120	140
Ethanol/Water	5	0.58	0.61
	10	0.62	0.65
Acidic Aqueous	5	0.66	0.62
	10	0.65	0.67

^a The concentration of TTDDS was 2 %wt in the coating solution.

^b The treated samples were chlorinated with 10% household bleach at pH7 for 1 h.

The treated filter swatches were chlorinated with 10% household bleach solution at various pH values for 30 min to determine the pH dependence of the chlorination process. The results in Table 5.1.2 showed the nitrogen atoms, hindered amine and amide, on TTDDS can be chlorinated without any pH adjustment (pH10.8).

Table 5.1.2. Chlorine loadings (Cl⁺ %) on filter at different chlorination conditions.

pH	7 ^a	8.2 ^b	10.8
Cl ⁺ %	0.63	0.64	0.68

^a Adjusted with HCl, ^b Buffered with NaHCO₃.

The study was extended with different bleach concentrations of the chlorination solution. 2% bleach solution provided 0.47% Cl^+ , sufficient for biocidal activity, on the attached monomer (Figure 5.1.2.(A)) and this chlorine loading can be provided within 15 min of chlorination time (Figure 5.1.2.(B)).

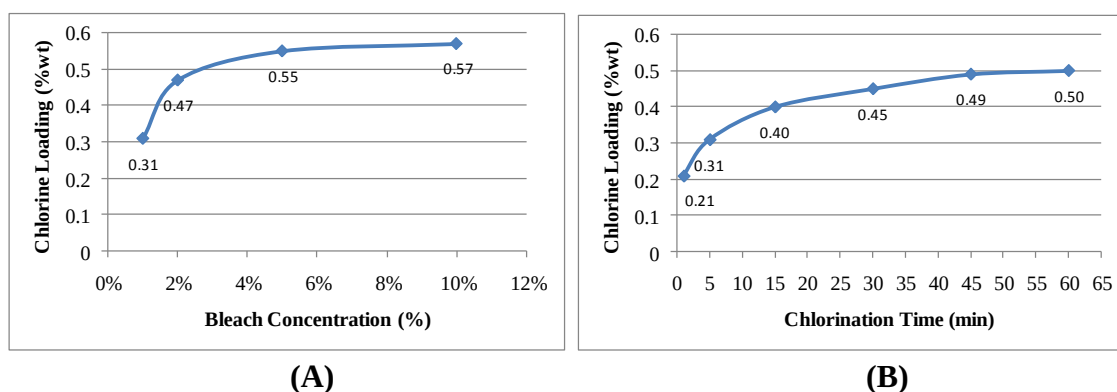


Figure 5.1.2. Chlorine loadings at different bleach concentrations of the chlorination solution (A), Chlorine loadings at 2% bleach solution for various time of chlorination process (B).

For determining the drying process after chlorination, stability of the bound chlorine was evaluated by heating the chlorinated treated filters in a conventional oven. The attached chlorine was not very stable at temperatures above 150 °C (Table 5.1.3), as previously reported.¹²

Table 5.1.3. Stability of bound chlorine on chlorinated treated filter (Cl^+ % remaining).

Heating temp. (°C)	Heating time (min)				
	0	1	5	15	30
120	0.44	0.42	0.40	0.37	0.32
150	0.44	-	0.37	0.28	0.26
170	0.44	-	0.24	0.15	0.13

The second N-halamine precursor GH is very soluble in water, requires relatively higher concentrations (4 %wt) of the compound in the coating solution to provide sufficient chlorine loadings on the surface.⁶ The curing procedure described in the literature⁶ provided low chlorine loadings on the filters after coated with 4 and 10% coating solutions (Table 5.1.4). Additionally, the binding efficiency did not increase even at 180°C for various period of time. A potential reaction mechanism of the epoxide group with water during curing process might reduce binding efficiency of the N-halamine precursor onto cellulose. Therefore, slow removal of absorbed water from the filter swatch without any heat usage might reduce the potential reaction of water with epoxide. The chlorine loadings of the filter swatches were dramatically increased after removing the absorbed water slowly by overnight drying and then curing at 180 °C. The curing procedure was determined as overnight drying subsequent heating at 180 °C for 10 min.

Table 5.1.4. Chlorine loadings on filter at various compound concentrations of the coating solution and different curing procedures.

Curing procedure	Compound concentration in coating solution (%)						
	4	10	4	4	4	4	4
temperature (°C)	95 ^a	95 ^a	180	180	overnight	overnight	overnight
time (min)	60 ^a	60 ^a	5	10	drying	drying	drying
temperature (°C)	145 ^a	145 ^a			180	180	180
time (min)	20 ^a	20 ^a			5	10	40
Chlorine loading (Cl ⁺ %)	0.10	0.12	0.09	0.11	0.17	0.20	0.55

^a The curing procedure described in the literature.⁶

5.1.4 Results and Discussion

The synthesized N-halamine precursors TTDDS and GH contain siloxane and epoxide tethering groups, respectively. TTDDS can be attached onto both cellulose and glass in the filter, whereas GH can be only attached to cellulose.^{5,6} GH is water soluble whereas TTDDS has a very limited solubility in water. TTDDS was dissolved in acidic aqueous solution because of its weak basicity at the amine nitrogen functionality. This is an advantage in that organic solvents (ethanol) can be avoided during coating and curing process and moreover, TTDDS can be coated onto the filter during the filter manufacturing process without the necessity of an additional coating process. TTDDS contains both amide and amine groups whereas GH has only an amide group. These groups were used to bind oxidative halogen to form N-halamine during the chlorination process. The FTIR spectra of the filter, filter coated with N-halamine precursor (TTDDS) before and after chlorination are shown in Figure 5.1.3. The new bands at 1765 cm^{-1} and 1699 cm^{-1} (Figure 5.1.3(B)) are the carbonyl stretching bands of N-halamine precursor. These bands shifted to higher frequency, 1778 cm^{-1} and 1712 cm^{-1} (Figure 5.1.3(C)), after the chlorination process, indicating disruption of $\text{N-H}\cdots\text{O}=\text{C}$ hydrogen bonding as conversion of N-H to N-Cl occurred. Similar FT-IR results were also observed for the second N-halamine precursor (GH).

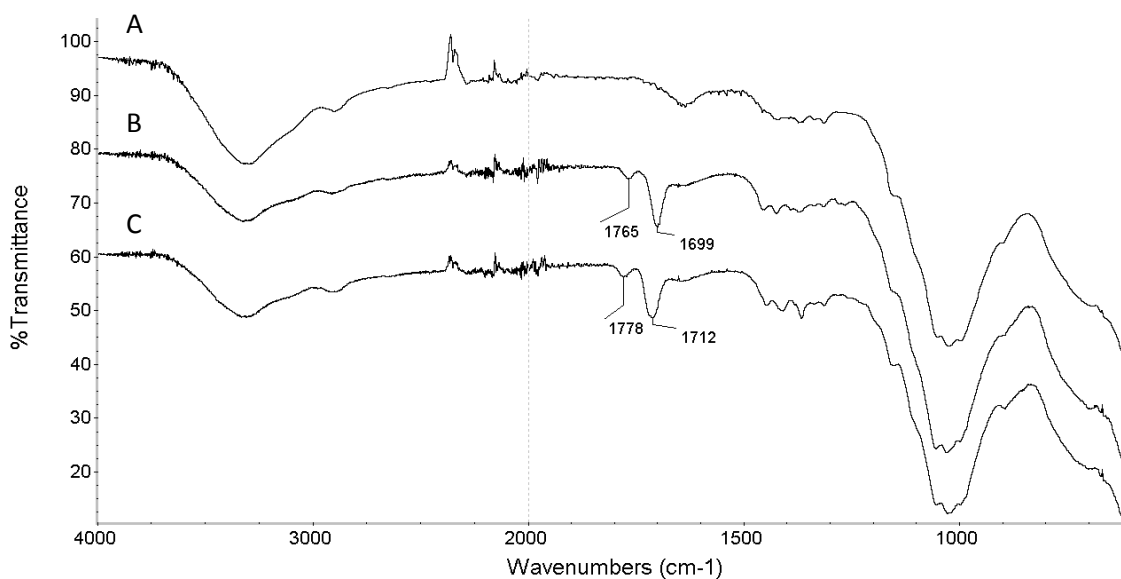


Figure 5.1.3. FTIR spectra of filter(a), filter TTDDS(b) and chlorinated filter TTDDS(c).

The treated filter swatches were challenged with *E. coli* bacteria at concentrations between 10^7 and 10^8 cfu (colony-forming units). The unchlorinated control samples, TTDDS and GH, provided about 1 to 3 log reductions, due to the adhesion of bacteria to the filter swatches (wood-pulp), within 30 min contact time intervals (Table 5.1.5).

In general, 1 to 3 log reductions for control samples are relatively high; however nano alumina grafted microglass fibers offer bacteria high surface area to adhere.

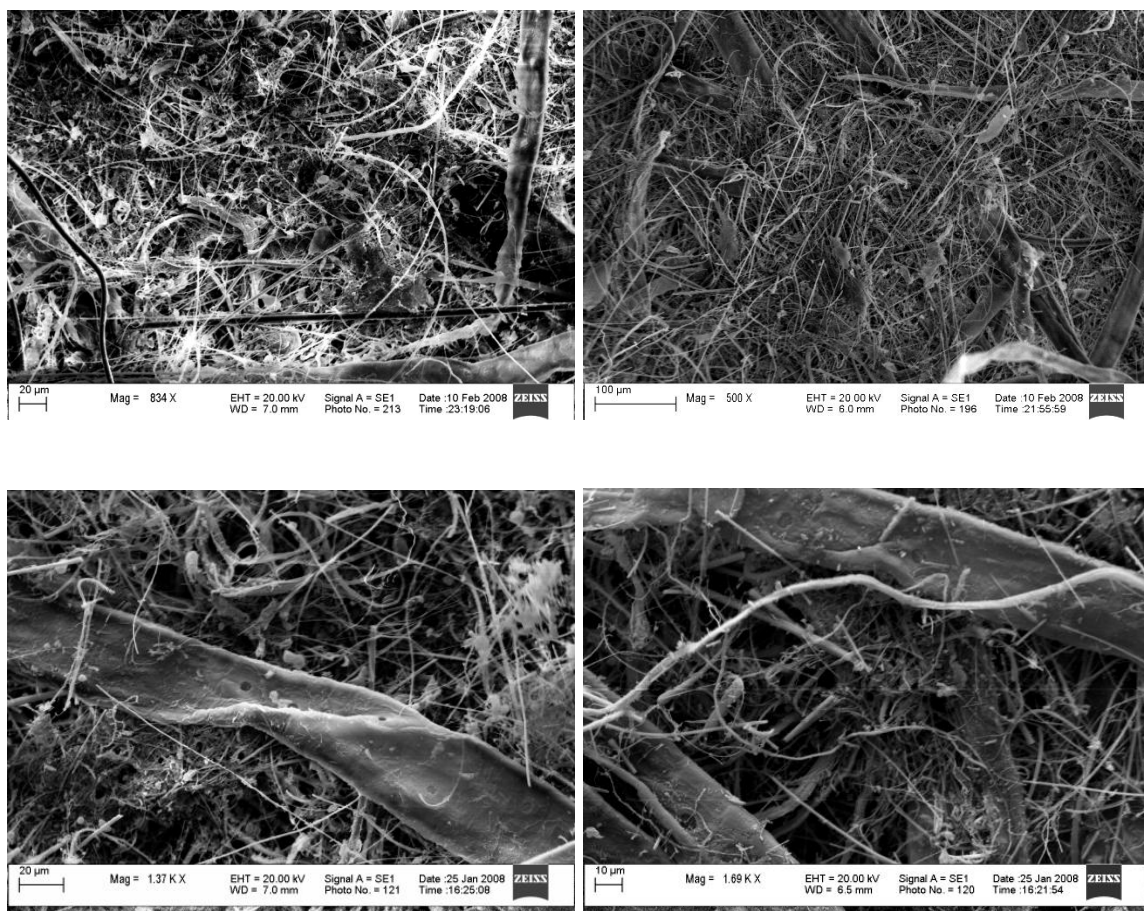
The chlorinated treated samples, TTDDS-Cl and GH-Cl showed excellent antimicrobial activity. All *E. coli* bacteria were inactivated by the treated swatches in the contact interval of 1-5 min. The inactivating rates of the chlorinated treated swatches are sufficient for filtration applications.

Table 5.1.5. Biocidal Test.

Coating material / Chlorine loading (Cl⁺%)	Contact time (min)	Bacterial reduction (log)
TTDDS	15	3.00
TTDDS-Cl	1	4.12
0.49	5	7.73
	15	7.73
GH	15	1.16
GH-Cl	1	1.76
0.21	5	7.45
	15	7.45

^a Microorganism *E. coli* O157:H7. Total bacteria: 5.33×10^7 cfu/sample (7.73 log) and 2.80×10^7 cfu/sample (7.45 log) for TTDDS and GH, respectively.

The air permeability performance of the chlorinated treated filters was 15.43 sec/100mL, and was not reduced after the treatment compared to untreated filter, 15.01 sec/100mL. SEM pictures also showed there is not any island type deposition of the N-halamine precursors on the filters (Figure 5.1.4).



(A)

(B)

Figure 5.1.4. SEM micrographs of filters before (a) and after (b) treatment with TTDDS.

5.1.5 Conclusions

Currently, filters have found more use in water purification since the new developments in material science (nanotechnology) offering separation of submicron size particles (i.e. microorganisms) from fluids without sacrificing the flow rate. However, separated living microorganisms can remain viable on filters that lack a contact biocide.

An ultrafine pore-size water filter was treated with two N-halamine precursors, and the treatments were optimized in the consideration of commercial manufacturing. The coated

filters exhibited excellent contact biocidal property after a simple treatment with household bleach. Despite the contact biocidal property, rechargeable N-halamine precursors can offer introducing low levels of halogen (chlorine) release into the filtered water over an extended period of time while the conventional water treatment methods cause unhealthy over-halogenation (excess of halogen in media).^{5,15} N-halamine coated filters could be used in various applications such as water purification and pool water treatment.

5.2 Antimicrobial Nonwoven Fabrics Composed of Melamine Formaldehyde Fibers

5.2.1 Introduction

N-halamine chemistry has been employed to provide biocidal property on fibers, fabrics, or other solid surfaces.⁵⁻⁹ However, N-halamine technology might become relatively expensive for some applications. An N-halamine is defined as a compound containing one or more nitrogen-halogen covalent bonds.⁴ Similar functional groups can be found in various commercial polymer structures in amine, amide or imide form. Therefore, chlorination of a nitrogen containing polymer might provide cost effective biocidal materials. N-chlorinations of polymers, (polyamides, polyurethanes, etc.) were extensively studied.^{13,14} Some of these polymers are available in fiber form, others as resins, and others as prepolymers which are further polymerized into a product.

Antimicrobial melamine compounds are widely known and have been used as water disinfectants and cleaning disinfectants in soluble form or as a slowly dissolving tablet in water.^{1, 15, 16} Structurally, chloromelamines belong to amine N-halamines. However,

because of the strong electron withdrawing effect of the triazine rings, their chemical environments are similar to those of amide N-halamines and their biocidal activity is expected to be between amine and amide N-halamine.¹⁷ In this regard, chloromelamine can provide both strong biocidal activity and good stability.

There is a commercial fiber, used in flame retardant applications, made from melamine formaldehyde resin. The fiber as produced has very limited ability to absorb and retain halogen/chlorine and therefore has limited biocidal activity. However, some of the formaldehyde can be hydrolyzed out and the fiber becomes more accessible to chlorine absorption (Figure 5.2.1).¹⁸ The acid treatment removes some of the methylene/methylene-oxy crosslinks between melamine units leaving exposed N-H functional groups. To accomplish this, the fibers were formed into a nonwoven fabric and then treated with acid at various conditions. The fabric was chlorinated with bleach and the stability of bound chlorine was investigated. The treated fabrics have been tested for biocidal efficacy and against a warfare stimulant.

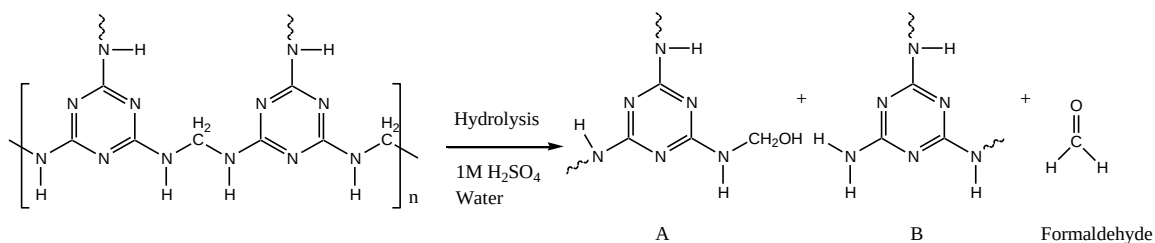


Figure 5.2.1. Hydrolysis of melamine formaldehyde resin.

5.2.2 Experimental Section

Melamine formaldehyde (MF) fibers, sold under Basofil[®] trade name, were provided by Basofil Fibers, LLC. The MF staple fibers were opened in a fiber opener/blender and vacuum deposited on a screen to form a web of fibers. The fiber webs were consolidated by needle-punching for easy handling and testing.

Acid Treatment and Chlorination Procedures. The MF nonwoven webs were hydrolyzed with 1M sulfuric acid (H_2SO_4) aqueous solution at various temperatures for various time intervals. After the treatment, samples were washed with distilled water and then kept in distilled water for 1 h, pH of the water was measured after 1 h to confirm H_2SO_4 was removed from the samples. The treated samples were air dried for one day before chlorination. The treated samples were chlorinated with 10% household bleach at pH8.2, buffered with sodium bicarbonate, for 1 h. The chlorinated treated samples were rinsed with distilled water and then dried at 45 °C for 2 h. For the determination of oxidative chlorine (Cl^+) content in the samples, a standard iodometric/thiosulfate titration procedure (described previously in section 5.1.2) was employed.

Hydrophilic N-halamine Coating Procedure. High chlorine loadings produce a hydrophobic character on the surface of materials. An N-halamine precursor, 3-triethoxysilylpropyl-5,5-dimethylhydantoin, previously synthesized and characterized (section 2.2) was used to reduce the hydrophobic surface character of the treated MF fibers. The treated MF nonwoven fabric was coated with the N-halamine precursor at 5% concentration in ethanol/water (1:1 v/v). The coated treated fabrics were cured for 1 h at 95 °C and then washed with 0.5% detergent solution for 15 min. The coated treated

fabrics were chlorinated with 10% household bleach at pH8.2 for 1 h. The chlorinated fabrics were rinsed with distilled water and then dried at 45 °C for 2 hours. The new bands in FTIR data, carbonyl stretching, at 1773 cm⁻¹ and 1696 cm⁻¹ indicate the attachment of the coating. The bands shifted to 1785 cm⁻¹ and 1708 cm⁻¹ after the chlorination. DSC plot of the chlorinated fabric exhibited only an exotherm peak at 153.60 °C due to the breakage of N-Cl bond.¹²

Warfare Stimulant Testing. The information was obtained using a CV-50W (a voltammetric analyzer) with flow injection apparatus. The 10 mM phosphate buffered saline (PBS) was used in the flow injection system. The treated fabrics were kept for 30 minutes, 90 minutes, and 24 hours in a 2.5x10⁻³ M solution of paraoxon in de-ionized water. Injections were made in 50 µL increments from each samples. The sample dimensions were 0.5 cm on a side (0.25 cm²).

5.2.3 Results and Discussion

The acid treated MF nonwoven fabrics became more accessible to chlorine absorption, as shown in Table 5.2.1. The effectiveness of the treatment increased dramatically at elevated temperature (55 °C) and for longer treatment times. A chlorine loading of 9.40% is tremendously high for an antimicrobial fibrous structure and could be useful as a long term source of Cl⁺ for antimicrobial applications. In addition, even after the severest acid treatment the fiber tensile strength decreased from 1.99 g/den to 1.28 g/den which is still sufficient for textile processing and filtration applications.

Table 5.2.1. Chlorine loadings ($\text{Cl}^+\%$) on MF nonwoven webs at different acid treatment conditions.

Treatment time (h)	Treatment temperature ($^{\circ}\text{C}$)	
	24	55
0	0.12	0.12
1	1.81	4.65
5	2.88	7.94
11	-	9.40

^a The samples were treated with 1M H_2SO_4 .

^b Chlorine loadings ($\text{Cl}^+\%$) on ground fiber was 0.28% and 15.16% for the untreated and treated (for 11h at 55 $^{\circ}\text{C}$) fibers, respectively.

SEM micrographs (Figure 5.2.2) showed the modification on the surface of MF fibers after the acid treatment. Besides increasing the number of amine groups, the treatment caused the formation of scales on the surface which allowed higher chlorine loadings due to the increased surface area.

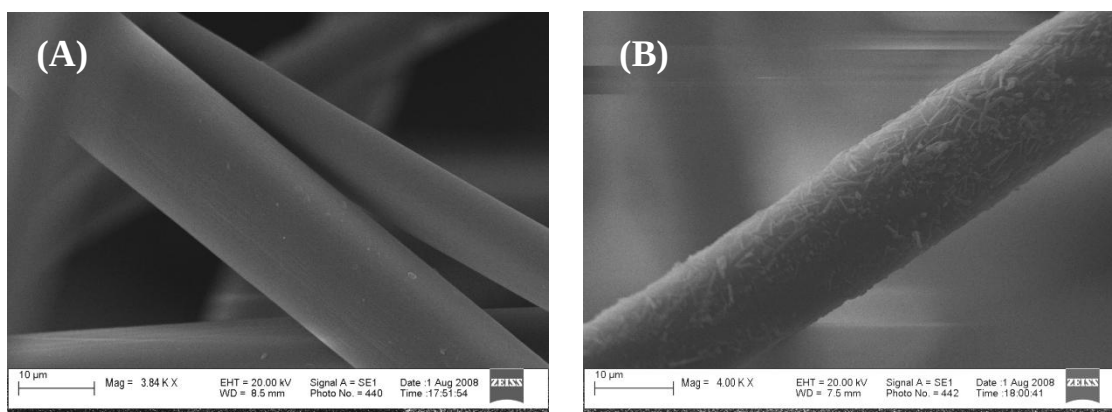


Figure 5.2.2. SEM micrographs of untreated (a) and acid treated (b) MF fibers.

The bound chlorine on MF fibers was not as stable toward UV light exposure (Table 5.2.2) as other N-halamines.¹⁹ The breakage of the N-Cl bond follows a first-order reaction rate directly proportional to the chlorine loading of the chlorinated treated MF fibers.

Table 5.2.2. Stability of bound chlorine on MF fibers toward UV light exposure (Cl⁺% remaining).

Time (hour)	Cl⁺ %	% Reduction from previous time period
0	1.81	-
1	0.77	57
2	0.34	56
3	0.15	56
4	0.08	46
5	0.04	50
6	0.02	50

The treated MF nonwoven fabrics were chlorinated at pH8.2 and exposed to stability tests. The daylight and shelf-storage stabilities of the bound chlorine were very good; shelf storage stability was superior as expected (Table 5.2.3). The chlorine remaining even after 120 days were sufficient for biocidal activity.²⁰ However, rechlorination of the 120 days aged samples could not reach the initial chlorine loadings. More acidic chlorination condition at pH7.0 (adjusted with HCl) exhibited better rechlorination results, but still not to original Cl⁺ level (11.97%).

Table 5.2.3. Stability of bound chlorine on MF fibers toward daylight exposure and shelf storage conditions (Cl⁺% remaining).

Day	Daylight storage	Shelf storage
0 (chlorination at pH8.2)	9.83	9.83
7	7.95	8.39
14	7.15	8.11
30	4.85	7.23
75	1.87	6.16
105	0.82	5.19
120	0.76	4.15
Rechlorination at pH8.2	4.48	6.40
Rechlorination at pH7.0	9.95	9.77

^a Initial chlorine loading of the treated sample was 11.97% after chlorination at pH7.0.

Chlorinated treated MF fibers lost much of their strength after aging for 120 days.

MF fibers itself and product A (Figure 5.2.1) have alpha hydrogens, the hydrogen on carbon atom next to nitrogen atom, causes alpha dehydrohalogenation reaction.³

Alpha dehydrohalogenation liberates molecular hydrochloric acid (HCl) having potential to catalyze the hydrolysis of melamine (Figure 5.2.3) and polymer degradation.

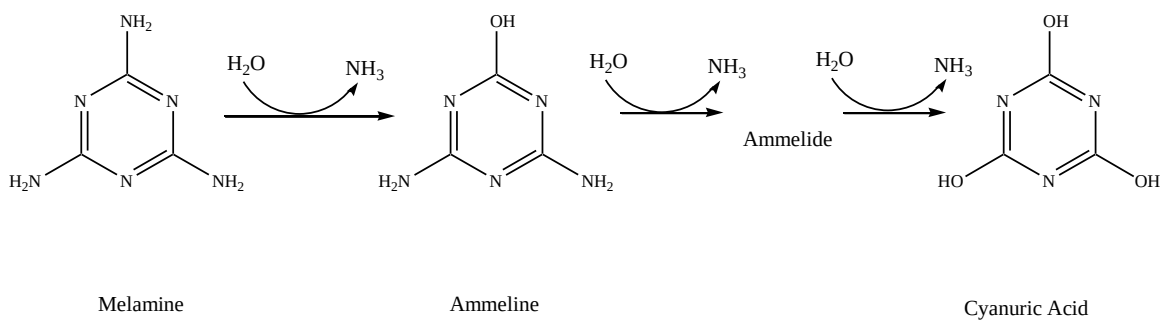


Figure 5.2.3. Hydrolysis of melamine.

The FTIR spectra of MF fiber, treated MF fiber, chlorinated treated MF fiber, and aged chlorinated treated MF fiber are shown in Figure 5.2.4. The band at 3318 cm^{-1} (Figure 5.2.4(A)) is the N-H stretching and this band broadened toward lower frequency 3188 cm^{-1} (Figure 5.2.4(B)) after the acid treatment indicating an increase in the number of hydroxyl groups. The band broadened further toward lower frequency 3119 cm^{-1} (Figure 5.2.4(D)) after aging for 120 days indicating an additional increase in the number of hydroxyl groups. The increase in the number of hydroxyl groups supports the hydrolysis of melamine.

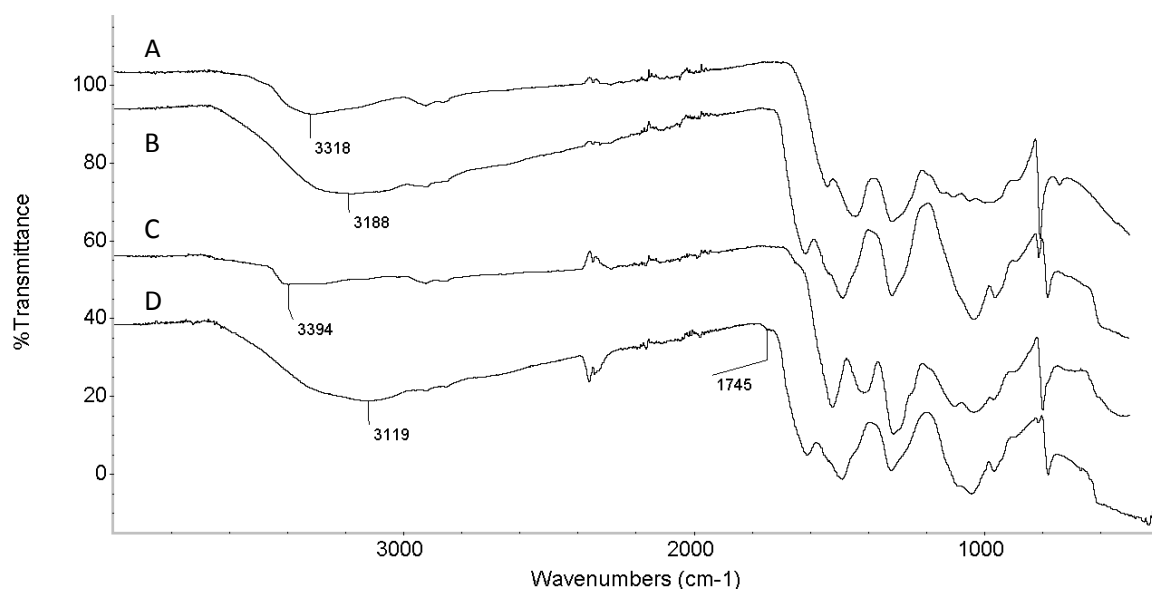


Figure 5.2.4. FTIR spectra of the MF fiber (a), the treated MF fiber (b), the chlorinated treated MF fiber (c), and the aged chlorinated treated MF fiber (d).

The new band at 1745 cm^{-1} could be carbonyl stretching of the carbonyl tautomer of ammeline or ammelide (Figure 5.2.5).^{21, 22} The nitrogen in the carbonyl tautomer can be chlorinated better at acidic conditions explaining the higher chlorine loadings at lower pH (pH7.0) for the rechlorination of the 120 days aged samples (Table 4.2.3).

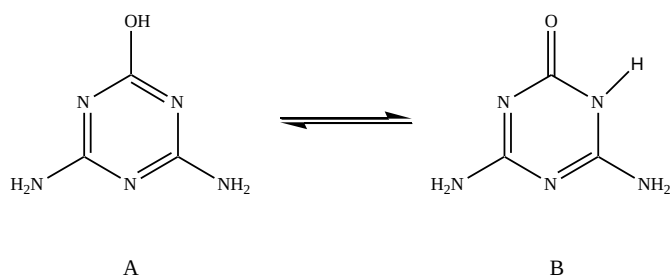


Figure 5.2.5. The hydroxyl (a) and carbonyl (b) tautomer of ammeline.

In general, the biocidal characteristic of a coated fabric depends on the concentration of the biocidal sites, N-Cl.⁴ However, for N-halamine compounds an increase in Cl⁺ active sites may cause an increase in hydrophobicity of the material, which may lead a poorer biocidal performance due to a decrease in contact with microbial cells.²³ To evaluate this hypothesis, three different samples were prepared by using the treatment conditions resulting in different chlorine loadings (Table 5.2.4). The LT sample was treated with acid for 5 min at room temperature, the MT sample was treated with acid for 11 h at 55 °C, and the MTH sample was coated with a N-halamine precursor (3-triethoxysilylpropyl-5,5-dimethylhydantoin) after the acid treatment of 11 h at 55 °C. The treated MF nonwoven fabrics were challenged with *E. coli* at concentration of 2.80×10^7 cfu (colony-forming units). The unchlorinated control samples provided only about 1 log reductions, due to the adhesion of bacteria to the swatches, within 30 min contact time intervals.

Table 5.2.4. Biocidal test I.

Sample / Chlorine loading (Cl⁺%)	Contact time (min)	Bacterial reduction (log)
LT	30	0.86
LT-Cl	5	0.96
0.98	10	2.30
	30	7.45
MT	30	0.66
MT-Cl	5	0.13
9.56	10	1.43
	30	7.45
MTH	30	0.39
MTH-Cl	5	2.42
4.46	10	5.14
	30	7.45

^a Microorganism *E. coli* O157:H7. Total bacteria: 2.80×10^7 cfu/sample (7.45 log).

All of the chlorinated treated samples showed excellent antimicrobial activity by inactivating all bacteria within 30 min. The MTH-Cl sample inactivated bacteria relatively faster than LT-Cl and MT-Cl samples. The coating compound is relatively more hydrophilic than the MF polymer, and thus increased the surface wetting characteristic of the treated MF fabric. Moreover, the surface hydrophobicity can be also reduced by using hydrophilic surface coatings such as polyethylene glycol.

SEM micrographs (Figure 5.2.6) showed the modification on the surface of MF fibers after treatment and coating procedures. There are some island type depositions of the N-halamine precursors on the fabrics.

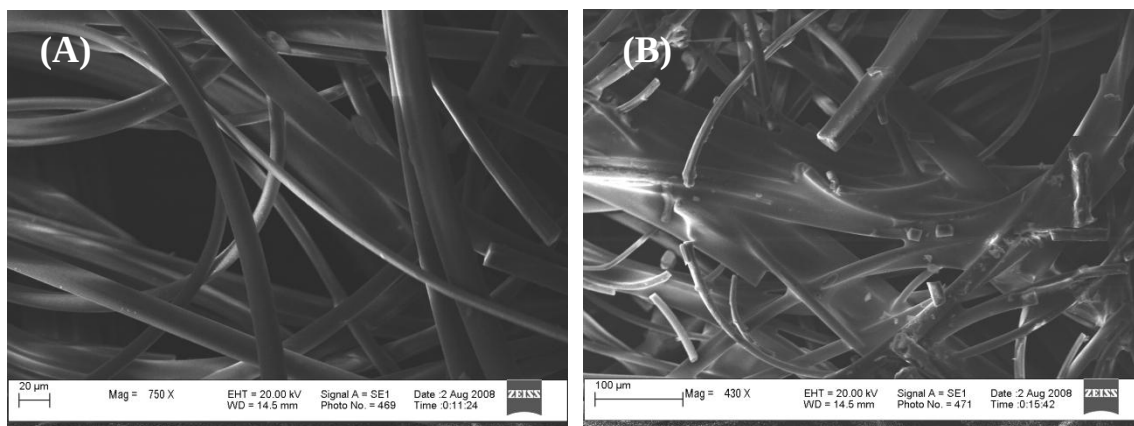


Figure 5.2.6. SEM micrographs of the coated treated MF fibers before (a) and after (b) coating.

Some N-halamine compounds are useful for the neutralization of some chemical warfare agents and some pesticides, as well as for their antimicrobial effects. The stored chlorine can easily oxidize some reactive chemicals.²⁴ In this study, MT (control), MT-Cl (chlorinated), and MTH-Cl (chlorinated hydrophilic) samples were exposed to aqueous solution of paraoxon (2.5×10^{-3} M) and then the destruction of paraoxon was monitored in the time intervals of 30 min, 90 min, and 24 hours. Paraoxon is chemically similar to the nerve agents sarin and soman²⁵ and a good stimulant in terms of its hydrophilic/hydrophobic balance.^{26,27}

Figure 5.2.7 displays the change in concentration of paraoxon after exposure to control (A), chlorinated (B) and chlorinated hydrophilic (C) samples for specific time intervals. The chlorinated (B) and the chlorinated hydrophilic samples (C) decreased the paraoxon

concentration more than the control sample (A) in all time intervals of exposure.

The chlorinated sample (B) decreased the paraoxon concentration 20%, 34%, and 60% compared to the control sample within time intervals of 30 min, 90 min and 24 hours where the chlorinated hydrophilic sample (C) decreased about 31%, 54%, and 69%. Overall the chlorinated hydrophilic sample exhibited better destruction of (or faster reaction with) the paraoxon than the chlorinated sample.

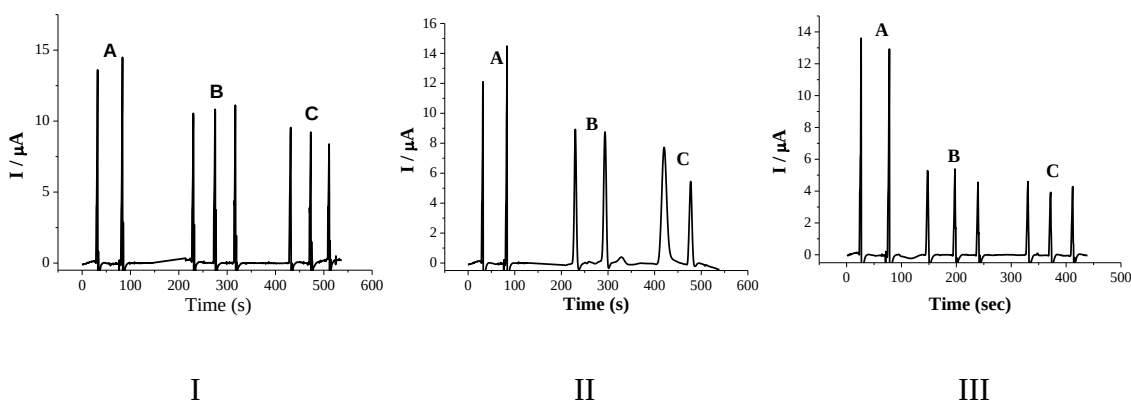


Figure 5.2.7. Current versus elution time plot for detection of paraoxon after 30 min (I), 90 min (II), and 24 hours (III).

^a A: unchlorinated(control),B: chlorinated(MT-Cl),C: hydrophilic chlorinated(MTH-Cl).

The MF fibers were successfully rendered antimicrobial. In a similar manner, chlorinated MF particles can be trapped in a filter media to make it antimicrobial; A MF resin having limited formaldehyde content (structurally similar to the hydrolyzed MF fiber) and melamine powder alone (having no formaldehyde crosslinking) were tried. The resin used for this approach is Hexio LA-15 which was subsequently ground into a powder. The MF resin powder and melamine were chlorinated; the chlorine loadings of the particles were 25.73% and 51.62%, respectively. The amounts of the particles were calculated to provide chlorine loadings of 0.5-1.0% on the filter. The calculated amount

of the chlorinated particles were dispersed in water and then filtered through a commercial water filter (described in section 5.1.2). The same particle capturing procedure was followed to prepare control samples with unchlorinated particles. The ultrafine water filter was able to hold the particles while such powdered form of the resins cannot typically be held within regular nonwoven structures.

The particle loaded filter swatches were challenged with *E. coli* at a concentration of 1.27×10^7 cfu (colony-forming units), Table 5.2.5. The unchlorinated control samples provided about 3 to 4 log reductions, due to the adhesion of bacteria to the swatches, within 30 min contact time intervals. The adhesion of the bacteria onto control samples was relatively high compare to our previous studies, however, the ultrafine structure offers tremendous amount of surface for bacteria to adhere. All of the chlorinated embedded samples showed excellent antimicrobial activity by inactivating all bacteria within 15 min of contact time.

Table 5.2.5. Biocidal test II.

Sample / Chlorine loading (Cl⁺%)	Contact time (min)	Bacterial reduction (log)
MF resin	30	4.98
MF resin-Cl	15	7.10
0.86	30	7.10
Melamine	30	3.49
Melamine-Cl	15	7.10
0.63	30	7.10

^a Microorganism *E. coli* O157:H7. Total bacteria: 1.27×10^7 cfu/sample (7.10 log).

5.2.4 Conclusions

After acid treatment, some of the formaldehyde was released to MF fiber and the fiber became more accessible to chlorine absorption (around 10% Cl^+). The initial strength loss caused by the acid treatment was less than 35% even after the severest treatment conditions. When stored for months chlorinated and dry, the fiber strength deteriorates markedly but that might be mitigated in water or by other chemical means.

This procedure allowed the production of cost efficient biocidal filters. The biocidal performance was improved by adding a hydrophilic N-halamine surface coating.

The commercial fiber meets all regulations and is used especially for flame retardant applications. In this regard, the chlorinated treated MF fibers can be used, alone or possibly mixed, in various applications such as upholstery textiles, top of the bed products, water/air filters, and disposable nonwoven fabrics. Capture of N-halamine loaded particulates by filter media might also be used to produce air filters provided the particles do not markedly reduce filter porosity.

5.3 References

- [1] Horning, D.P.; Robertson, R.E. Chlorine Resin Intended for Water Treatment. US Patent 3,948,853. **1976**.
- [2] Tepper, F.; Kaledin, L. Virus and Protein Separation Using Nano Alumina Fiber Media. Argonide Product Report. **2007**.
- [3] Kaminski, J.J.; Bodor, N.; Higuchi, T. N-halo Derivatives III: Stabilization of Nitrogen-chlorine Bond in N-chloroamino Acid Derivatives. *J. Pharm. Sci.* **1976**, 65, 4, 553-557.
- [4] Worley, S.D.; Sun, G. Biocidal Polymers. *Trends. Polym. Sci.* **1996**, 4, 364-370.
- [5] Liang, J.; Barnes, K.; Akdag, A.; Worley, S.D.; Lee, J.; Broughton, R.M.; Huang, T.S. Improved Antimicrobial Siloxane. *Ind. Eng. Chem. Res.* **2007**, 46, 1861-1866.
- [6] Liang, J.; Chen, Y.; Ren, X.; Wu, R.; Barnes, K.; Worley, S.D.; Broughton, R.M.; Cho, U.; Kocer, H.B.; Huang, T.S. Fabric Treated with Antimicrobial N-Halamine Epoxides. *Ind. Eng. Chem. Res.* **2007**, 46, 6425-6429.
- [7] Worley, S.D.; Chen, Y.; Wang, J.W.; Wu, R.; Cho, U.; Broughton, R.M.; Kim, J.; Wei, C.I.; Williams, J.F.; Chen, J. Li, Y. Novel N-halamine Siloxane Monomers and Polymers for Preparing Biocidal Treatments. *Surf. Coat. Int. Part B.* **2005**, 88, 93-99.
- [8] Worley, S.D.; Chen, Y.; Wang, J.W.; Wu, R.; Li, Y. N-halamine Siloxanes for use in Biocidal Treatments and Materials. U.S. Patent 6,969,769 B2, **2005**.

- [9] Williams, J.F.; Suess, J.; Santiago, J.; Chen, Y.; Wang, J.; Wu, R.; Worley, S.D. Antimicrobial Properties of Novel N-halamine Siloxane Treatments. *Surf. Coat. Int. Part B*. **2005**, 88, B1, 35-39.
- [10] Liang, J.; Owens, J.R.; Huang, T.S.; Worley, S.D. Biocidal Hydantoinylsiloxane Polymers. IV. N-halamine Siloxane-functionalized Silica Gel. *J. Appl. Polym. Sci.* **2006**, 101, 3448-3454.
- [11] Mailey, E.A.; Day, A.R. Synthesis of Derivatives of Alkylated and Arylated Piperidones and Piperidinols. *J. Org. Chem.* **1957**, 22, 1061-1065.
- [12] Akdag, A.; Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Webb, T.R.; Bray, T.H. Why Does Kevlar Decompose, while Nomex Does Not, When Treated with Aqueous Chlorine Solutions? *J. Phys. Chem. B*. **2007**, 111(20), 5581-5586.
- [13] For example see: (a) Wayman, M.; Salamat, H.; Dewar, E.J. Chlorine Exchange Resins. *Can. J. Chem. Eng.* **1968**, 46, 282-287.
- [14] For example see: (a) Koutinas, A.A.; Demertzis, P.G. N-Chlorination of Nylon Fabric and Polyurea-6. *J. Polym. Sci., Polym. Chem. Ed.* **1983**, 21, 335-340.
- [15] Schneider, Thomas E., Jr.; Halley, James L.; Bradley, William E., Jr. Chlorine Delivering Material for Disinfection of Water. DE 1,959,708. **1970**.
- [16] Sun, Yuyu; Chen, Zhaobin; Braun, Martha. Antimicrobial Polymers Containing Melamine Derivatives. I. Preparation and Characterization of Chloromelamine-based Cellulose. *Ind. Chem. Res.* **2005**, 44(21), 7916-7920.

- [17] Chen, Z.; Luo, J.; Sun, Y.Y. Biocidal Efficacy, Biofilm-controlling Function, and Controlled Release Effect of Chloromelamine-based Bioresponsive Fibrous Materials. *Biomaterials*. **2006**, 28, 1597-1609.
- [18] Cho, U. Novel Antimicrobial Textiles. Dissertation. **2003**, Auburn, Alabama.
- [19] 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.
- [20] Liang, J.; Wu, R.; Wang, J.; Barnes, K.; Worley, S.D.; Cho, U.; Lee, J.; Broughton, R.M.; Huang, T.S. N-halamine Biocidal Coatings. *J. Ind. Microbiol. Biotechnol.* **2007**, 34, 157-163.
- [21] Petterson, R.C.; Grzeskowiak, U.; Jules, L.H. N-Halogen Compunds. II. The N-Cl Stretching Band in Some N-Chloramides. The Structure of Trichloroisocyanuric Acid. *J. Org. Chem.* **1960**, 25(9), 1595-1598.
- [22] Newman, R.; Badger, R.M. Infrared Spectra of Cyanuric Acid and Deutero Cyanuric Acid. *J. Am. Chem. Soc.* **1952**, 74(14), 3545-3548.
- [23] Makal, U.; Wood, L.; Ohman, D.E.; Wynne, K.J. Polyurethane Biocidal Polymeric Surface Modifiers. *Biomaterials*. **2006**, 27, 1316-1326.
- [24] Akdag, A.; Liang, J.; Worley, S.D. Oxidation of Organic Sulfides by N-halamine Compounds. *Phosph. Sulf. Sil. Rel. Elem.* **2007**, 7, 1525-1533.

- [25] Hafiz., A.A.; El Awadi, M.Y.; Badawi, A.M.; Mokhar, S.M. Catalytic Destruction of Paraoxon by Metallomicelle Layers of Co(II) and Cr(III). *J. Surfact. Deterg.* **2005**, *8*, 203-206.
- [26] Moss, R.A.; Kotchevar, A.T.; Park, B.D., and Scrimin, P. Comparative Reactivities of Phosphotriesters Toward Iodoso-carboxylates in Cationic Micelles. *Langmuir*. **1996**, *12*, 2200.
- [27] Zheng, F.; Zhan, C.G.; Ornstein, R.L. Theoretical Studies of Reaction Pathways and Energy Barriers for Alkaline Hydrolysis of Phosphotriesterase Substrates Paraoxon and Related Toxic Phosphofluoridate Nerve Agents. *J. Chem. Soc., Perkin Trans.* **2001**, *12*, 2355.

CHAPTER 6

WATER ABSORPTIVE CELLULOSE/STARCH/HALS COMPOSITE FIBERS FOR BIOCIDAL APPLICATIONS

6.1 Introduction

Man made cellulose fiber is commercially produced by extrusion from different solvents. Because of the difficulty of dissolving cellulose, the processes that were developed often use chemicals that pollute the air and the process water. Molten organic salts, known as ionic liquids, are a class of solvents composed entirely of ions, and are particularly useful in dissolution of polar organic materials, even polymers, like cellulose, that are difficult to dissolve.¹⁻⁴ Ionic liquids are being intensively investigated because of their relatively environmentally friendly nature.⁴⁻⁶ It has been recently shown that cellulose fiber can be successfully extruded using ionic liquids from rayon, native cotton, and bleached cotton.¹⁻³ Having a common solvent for multiple polymeric materials leads to the consideration of making mixtures of the materials in order to form solids having properties of each component in the mixture.

Our research is concentrated on providing antimicrobial property on various polymers, especially on cellulosic substrates. The use of N-H functionality to allow halogenation of materials and production of regenerable biocidal materials is well known.⁷⁻⁹ The result is

broad-spectrum biocidal materials effective against Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses. In general, N-halamine technology can be introduced onto polymers in various ways; (1) N-halamine precursors are bound to polymers with chemical bonding,^{9,10} (2) N-halamine precursors are mixed with fiber forming polymers,¹¹ and (3) Fiber forming polymers are N-halamine precursors.^{3,35} An N-halamine is defined as a compound containing one or more nitrogen-halogen covalent bonds.¹³ Similar functional groups can be found in various commercial polymer structures in amine, amide or imide form. Since the UV light stability of cyclic N-halamines are not sufficient for long term use in sunlight,^{9,12} a polymeric N-halamine precursor having resistance towards UV light would be helpful in production of durable antimicrobial substrates. Hindered amines are used as UV light stabilizers¹⁴⁻¹⁷ and have been investigated as halamine precursors for biocidal compositions.^{11,18} The use of such materials internally in hydrophobic materials has not proved especially successful in that the compound residing internally and not on the polymer surface cannot be converted to halamine and is, therefore, inactive.¹⁹ Hindered amine light stabilizers (HALSs) can be solubilized in dilute acid and applied to the cellulose surface, however early experiments with cellulose revealed a lack of fastness to washing.¹⁹

It has been discovered that a certain hindered amine light stabilizer (herein after as HALS), as shown in Figure 6.1, is soluble in a certain ionic liquid, 1-butyl-3-methylimidazolium chloride (herein after as IL), that are used for extrusion of polymers into fibers. HALS could be added into the extrusion “dope” in small concentration and extruded into fibers. In particular, the polymeric hindered amine is less

likely to be leached or washed from the fiber due to its high molecular weight restricting the mobility.

Various studies describe the effect of hydrophilic character of the polymeric materials on biocidal property.⁹ Cellulose itself is hydrophilic, higher water absorbency of the regenerated cellulose (rayon) would be beneficial for biocidal applications due to increased wettability. It is well known that superabsorbent rayon (regenerated cellulose fiber) can be made with the inclusion of quantities of starch in the fiber.¹⁰ We found that water soluble starch is also soluble in IL, and can be a common solvent with cellulose and HALS. Besides expected better biocidal performance of the starch containing cellulose fibers, more biodegradable starch might be promising for biomedical applications.

In this study, composite fibers of cellulose, starch and HALS have been produced in various concentrations of the components using an ionic liquid in order to evaluate their physical properties and water absorption capacities. The principal objective of the study is to improve the water absorption property by introducing starch and to provide long term regenerable biocidal property by introducing HALS. The antibacterial activities and UV light resistances of the composite fibers were examined.

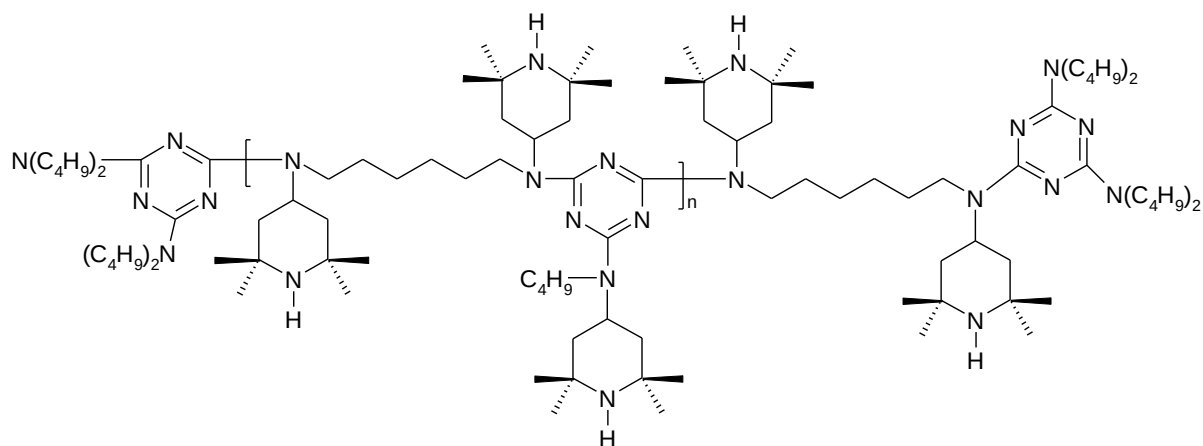


Figure 6.1. Structure of the hindered amine light stabilizer (HALS) considered in the study. (where $n = 0, 2, 4, 6, 8 \dots$)

6.2 Experimental Section

Bleached cotton cellulose, with a degree of polymerization (DP) of 1440, and a water soluble starch, Mallinckrodt Inc, was used. The cotton was ground to increase the rate of solution. The starch was dispersed in water by stirring at the boiling for 10 min. The solution was frozen and then dried in a freeze-dryer. The HALS (Chimassorb 2020[®]) was provided by Ciba Chemical Co., and synthesis of the polymer is described in the literature.²⁰ The polymer has a number average molecular weight of 2900 g/mol, polydispersity of 1.2-1.3.²⁰ The water solubility is lower than 0.5mg/L which can be considered as slightly soluble.²⁰ The ionic liquid, 1-butyl-3-methylimidazolium chloride (IL), was purchased from Aldrich Chemical Co. and used without any further purification.

Preparation of Cellulose/Starch/HALS Solution. Bleached cotton, freeze dried starch and HALS were dried at 70 °C for 12 h. Desired amounts of the compounds (wt%) were added into ionic liquid and then dissolved. For Example, 1.9 g of cellulose and 0.4 g of HALS was added into 72 g of ionic liquid and stirred for 6 h at 2500 rpm with a speed-mixer, FlackTek Inc. Then 5.7 g of starch was added to the solution and stirred for an additional 14 h at 2500 rpm. The total amount of the compounds was 5 to 10 wt% in the solution. The solution was then degassed under vacuum for 1.5 h at 100 °C to remove the air bubbles, after which, it was poured into the extruder. All polymer solutions were clear and light amber in color supporting homogenous single phase solution. Undissolved material is detected via polarized light microscopy.

Table 6.1. The composition of the extruded solutions.

Sample	Cellulose (wt%)	Starch (wt%)	HALS (wt%)	Total component in the ionic liquid (wt%)
CH ^a	96.00	0	4	4
CSH	47.50	47.50	5	5
CSSH	23.75	71.25	5	10

^a Values from previous study.⁴

^b C: Cellulose, S:Starch, H:HALS, and SS: Extra starch in the composite fiber.

Dry-jet Wet Spinning Process. The dry-jet wet spinning equipment (Figure 6.2) was composed of a piston pump (ISCO Series D), a Fischer Isotemp 3006 heating unit, a 500 µm diameter single-hole spinneret, a stepped godet with four levels (diameters were 1.33, 2.65, 3.93, 5.18 cm, for level 1, 2, 3, and 4, respectively.), a tap water

coagulation bath, and a take-up winder. The solutions were extruded at 80 °C to inhibit crystallization of IL in the extruder. The cellulose/starch/HALS-IL solutions had lower viscosity compared to cellulose-IL solutions, and could be extruded at lower temperatures (even at room temperature), however, the ionic liquid has the ability to crystallize at low temperatures. The solution was forced through a filter pack consisting of two screens having mesh size of 80 and 325 before the single hole spinneret. The air gap between the die and water was 5 cm and the length of coagulation route was around 150 – 210 cm according to used godet levels.

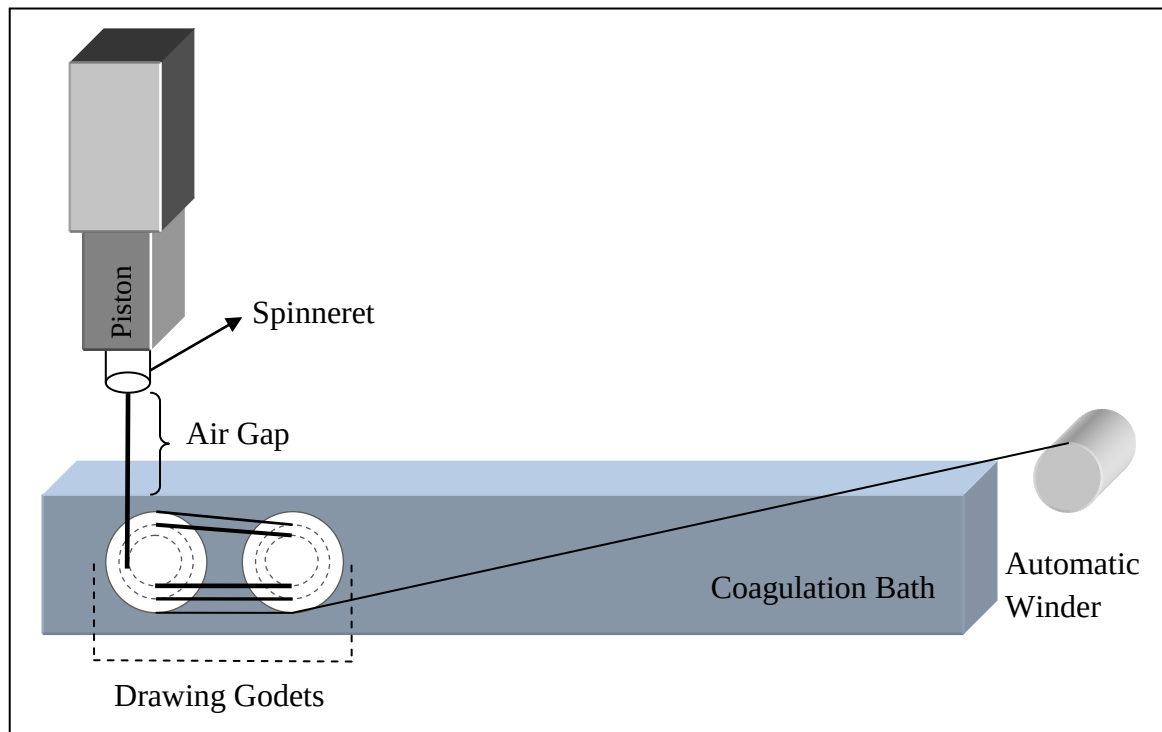


Figure 6.2. Dry-jet wet spinning process.

^a The distance between two godets is 12.6 cm and both godets have four levels with diameters of 1.33, 2.65, 3.93, 5.18 cm from inner to outside levels.

Extrusion conditions of the samples were summarized in Table 6.2. After the spinning process, the fibers were soaked in tap water at ambient temperature for 48 h to extract any residual ionic liquid and then dried at standard lab conditions (22 °C, 65% relative humidity).

Table 6.2. Extrusion conditions of the composite fibers.

Sample ID	Cellulose/Starch/HALS concentration (wt%)	Throughput (mL/min)	Godet speed (rpm)	Drawing (step levels)
CSH-1	47.50 / 47.50 / 5.00	0.20	9	1-2-3
CSH-2	47.50 / 47.50 / 5.00	0.20	15	1-2-3
CSH-3	47.50 / 47.50 / 5.00	0.15	15	1-2-3
CSH-4	47.50 / 47.50 / 5.00	0.10	15	1-2-3
CSSH-1	23.75 / 71.25 / 5.00	0.20	15	1-2-3
CSSH-2	23.75 / 71.25 / 5.00	0.20	22	1-2-3
CSSH-3	23.75 / 71.25 / 5.00	0.20	22	1-2-3-4

Instruments. The IR data were obtained with a Nicolet 6700 FT-IR spectrometer using an ATR (Attenuated Total Reflectance) accessory. Thermal data were obtained using a DSC Q2000 TA Instruments at a heating and cooling rate of 10 °C/min. UV light stabilities of the bound chlorine and composite fibers were measured by using an Accelerated Weathering Tester (The Q-panel Co., OH). The samples were placed in the UV (type A, 315-400 nm) chamber for various designated times. After a specific time of exposure to UV irradiation, the samples were removed from the UV chamber and titrated, or rechlorinated and titrated.

Chlorination of the Composite Fibers. The fibers were formed into nonwoven fabric in a wet-laid handsheet mold to make them easier to handle during the chlorination process and the following tests. The nonwoven fabrics were soaked in a 10% solution of household bleach (pH buffered to 8.2 with sodium bicarbonate) at ambient temperature for 1 h, rinsed with distilled water and dried at 45 °C for 1 h to remove any unbonded chlorine. For the determination of oxidative chlorine (Cl^+) content onto the fibers, a modified iodometric/thiosulfate titration procedure was employed.

Chlorination of HALS. To a solution of 200 mL water and 2.5 mL HCl (6 M), 1 g of HALS was added, and dissolved. After the complete dissolution of HALS, 30 mL of 6% sodium hypochlorite solution was added slowly onto the solution. The precipitated particles were collected, dried and then dissolved in 50 mL of acetone. The acetone insoluble fraction was collected and dried at 45 °C for 1h. The chlorine loading (Cl^+) was 12.64% whereas the theoretical value is 13.5% assuming the $\text{MW}_n=2900$ (section 6.6). FT-IR spectra suggested the chlorinated HALS. The DSC plot of the chlorinated HALS exhibited only an exotherm peak at 204.75 °C due to the breakage of N-Cl bond.²¹

Antimicrobial Efficacy Testing. Control and chlorinated nonwoven fabrics were challenged with *Escherichia coli* O157:H7 (ATCC 43895) and *Staphylococcus aureus* (ATCC 6538) using a “sandwich test”. 25 μL of bacterial suspension, made with pH7 buffer, was dropped in the center of a 1 in. square fabric swatch, and a second identical swatch was laid on the first swatch. A sterile weight was used to ensure sufficient contact of the swatches with the inoculums. After the determined contact times, the samples were quenched with 5.0 mL of sterile 0.02N sodium thiosulfate solution to remove any

oxidative chlorine that could cause extended disinfection. Serial dilutions of the solutions contacting the surfaces were plated on Trypticase agar and incubated for 24 h at 37 °C, and colony counts were made to determine the presence of viable bacteria.

Tensile Strength Testing. The fiber linear density (denier) was measured on a vibroscope (Vibromat M) according to ASTM D1577 (07.01). The tensile properties of the fibers were investigated with an Instron 5565 Universal Tester according to ASTM D1774-94. 20 specimens were tested for each sample. The gauge length was 15 mm and crosshead speed was 9 mm/min. The samples were conditioned at 21 °C and 65% relative humidity for 24 h before the tensile test.

6.3 Results and Discussion

Bleached cotton has a limited solubility in IL around 4.5 wt%, which makes use of IL difficult in commercial regenerated cellulose manufacturing. The water soluble starch was more soluble in IL, and allowed solving higher amount of component around 10 wt% in the fiber extrusion solution.

The composite fibers were successfully extruded into the coagulation bath and the solution filters were very clean after the extrusion process indicating that all components were extruded into the fiber structures. The composite fibers, i.e. CSSH-3, can be chlorinated up to chlorine loadings around 0.50 % Cl^+ whereas the theoretical value for complete chlorination of all N-H sites was 0.63 % showing that HALS is well trapped in cellulose/starch mixture.

The FT-IR spectra of the composite fibers are shown in Figure 6.3. The new band at 1533 cm^{-1} , for CSH and CSSH, is assigned to the C=N stretching vibration in the triazine ring^{22,23} of HALS (see Figure S.6.1 for the FT-IR spectra of HALS). Methylene ($-\text{CH}_2-$) stretching of HALS overlapped and increased the intensity of the bands around $2800\text{--}2900\text{ cm}^{-1}$.

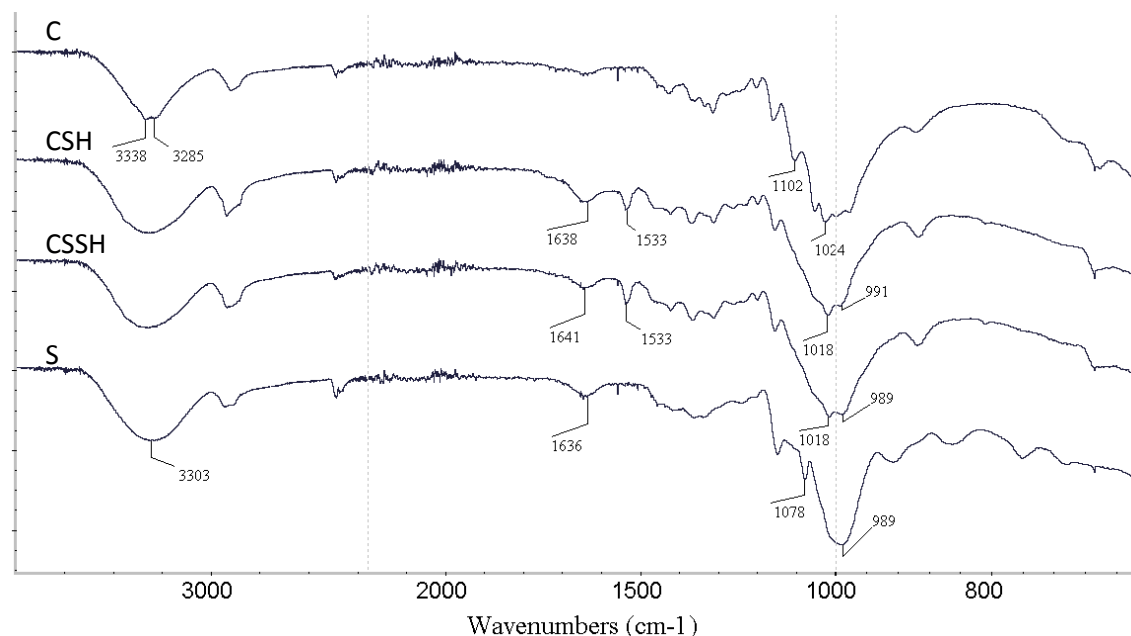


Figure 6.3. FT-IR spectra of bleached cotton (C), CSH , CSSH, and water soluble starch (S).

^a FT-IR tests were run after samples were conditioned at standard conditions for 24 h.

Due to the chemical similarities between starch (α 1,4 D-glucopyranose) and cellulose (β 1,4 D-glucopyranose) the FTIR spectra of the composite fibers were very complicated. Absorbed water in the amorphous region of the polysaccharides could be identified as a broad band at 1640 cm^{-1} .^{25,26} As the crystallinity of polysaccharides reduce, this band become stronger, therefore, less crystalline CSH and CSSH samples have more intense

peak around 1640 cm^{-1} compared to bleach cotton. This finding supports that starch/cellulose composite fibers are less crystalline compared to 100% cellulose.

There are some slight differences in the region between 1100 cm^{-1} and 900 cm^{-1} .

Bleached cotton has several peaks in this region and this can be explained by the restricted conformation of the glycosidic bonds ($\text{C}_1\text{-O-C}_4$) in cellulose due to higher crystallinity. The peaks at 1018 cm^{-1} and 989 cm^{-1} can be assigned to C-O stretching of C-O-C linkages. An increase of the absorption intensity at 990 cm^{-1} was accompanied by a sharpening of the band toward low wavenumbers, by introducing higher amount of starch. This might be the result of the relaxed conformation of the chains in more amorphous structures.²⁷ In this regard, more amorphous composite fibers exhibited more starch alike spectra by increasing starch concentration.

The initial fiber diameter, after the coagulation bath, of the starch added composite fibers were dramatically higher compared to our previous studies with cellulose. The initial diameter of CSSH (Figure 6.4(A,B)) was around $230\text{ }\mu\text{m}$ and reduced to $60\text{ }\mu\text{m}$ after water desorption. Figure 6.4(C,D) shows the optical polarizing micrographs of the sample in wet and dry state, respectively. CSSH fiber exhibits a well-oriented structure and this oriented structure was kept during drying. Interestingly, no fibrillation on the surface was observed for the well-oriented fibers, as commonly seen for lyocell fibers. The fibers were well oriented after drying procedure because they dried on the winding rolls thus prevent shrinkage. Unconstrained drying procedure resulted in poor orientation in the fibers; however, decreasing orientation did not increase the penetration and diffusion rate of water in the fiber, after drying.

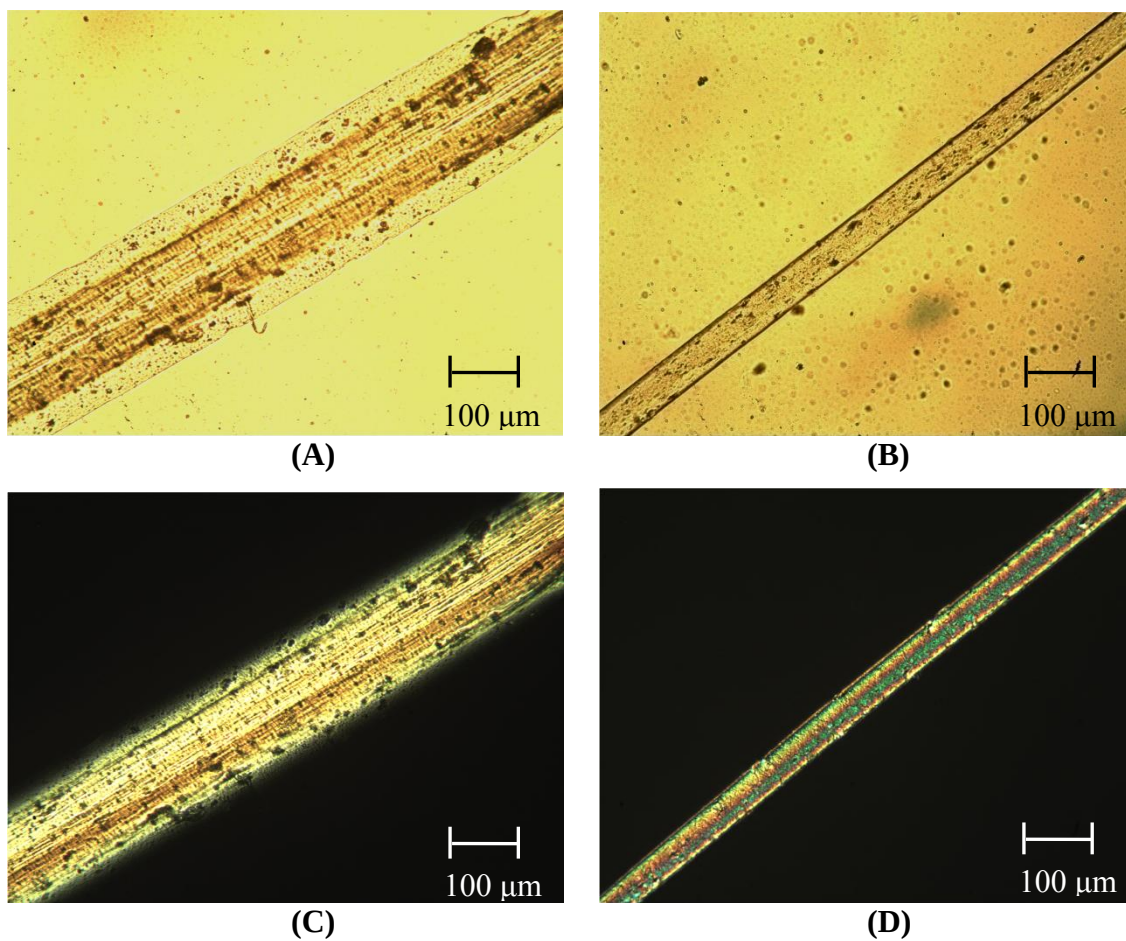


Figure 6.4. Optical micrographs of CSSH fiber before (A) and after (B) drying. Optical polarizing micrographs of CSSH fiber before (C) and after (D) drying.

Figure 6.5 shows the SEM micrographs of the surface of CSSH fiber after drying. The fibers have non-uniform fiber surface and diameter. There is crenulated fiber as is commonly seen for commercial regenerated cellulose fibers. The cross section of the composite fibers was ribbon-like in shape.

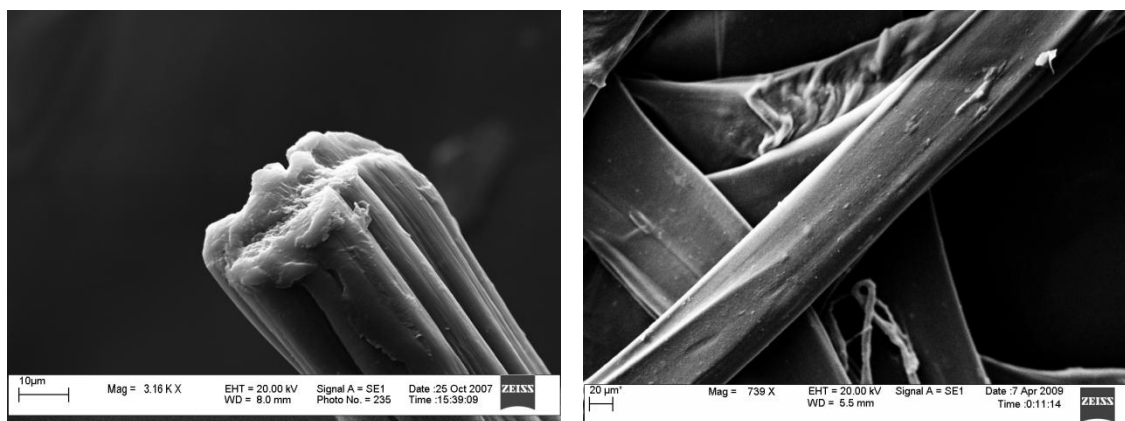


Figure 6.5. SEM micrographs of CSSH.

The results in Table 6.3 show mechanical properties of the composite fibers in terms of denier, tenacity, and strain at break. For CSH-1 and CSH-2, increasing godet speed did not change any physical property, either tenacity or strain at break, but reduced the linear density (den) as expected. Reducing extruder throughput (0.20 mL/min to 0.10 mL/min), CSH-2 to CSH-4, reduced the linear density and again did not show any significant effect on physical properties. Composite CSH fibers have tenacity around 2.4 g/den and strain at break around 8.5% which are sufficient for many textile applications where equivalent materials are used such as rayon and lyocell fibers (2.05 g/den and 3.59 g/den, respectively).³¹

CSSH-1 (having higher starch concentration) and CSH-2 samples, have equivalent extrusion parameters allowing comparisons to be made between two different samples. CSSH-1 has a tenacity value around 1.6 g/den while CSH-2 has 2.3 g/den indicating the tenacity of composite fibers reduced by increasing starch component amount. However, the tenacity of CSSH samples can be increased by increasing godet speed (CSSH-2) and further by increasing drawing ratio (CSSH-3). The strain at break for the CSSH-1 is

higher than CSH-2 because of the higher starch amount which might lower the orientation of the resultant fibers. The strain at break of the CSSH samples (CSSH-2, and CSHH-3) was reduced by increasing draw ratio and godet speed compared to CSSH-1.

Table 6.3. Mechanical properties of the composite fibers.

Sample	Throughput (mL/min)	Godet speed (rpm)	Drawing (step levels)	Denier (g/9000m)	Tenacity (g/den)	Strain at break (%)
CSH-1	0.20	9	1-2-3	38.4 ±4.3	2.4 ±0.3	8.8 ±1.9
CSH-2	0.20	15	1-2-3	28.2 ±2.8	2.3 ±0.2	8.6 ±1.9
CSH-3	0.15	15	1-2-3	20.6 ±2.6	2.6 ±0.4	8.4 ±1.2
CSH-4	0.10	15	1-2-3	16.1 ±1.7	2.5 ±0.2	8.6 ±1.3
CSSH-1	0.20	15	1-2-3	32.9 ±2.5	1.6 ±0.1	20.6 ±3.2
CSSH-2	0.20	22	1-2-3	28.9 ±1.7	1.7 ±0.1	9.5 ±1.3
CSSH-3	0.20	22	1-2-3-4	23.5 ±1.2	1.8 ±0.3	5.7 ±1.3

The physical properties of the composite fibers were sufficient in dry state for many textile processing and projected end-use applications. Figure 6.6 shows the tenacity of composite fibers in dry and wet states. The tenacity of the composite fibers reduced in wet state, as generally seen in commercial regenerated cellulose fibers. For CSH fibers, the loss in tenacity was smaller as orientation increased, from CSH-1 to CSH-3. Tenacity loss was 52% for CSSH-1 while 31% for CSH-2 (CSSH-1 and CSH-2 have equivalent

extrusion parameters) indicating higher starch content results in higher tenacity loss which might be due to higher water absorbance. The composite fibers, especially CSH fibers, have good mechanical properties in the wet state when compared with other commercial regenerated cellulose fibers such as viscose rayon and modal which have tenacity values around 1.16 g/den and 1.72 g/den in wet state.³¹

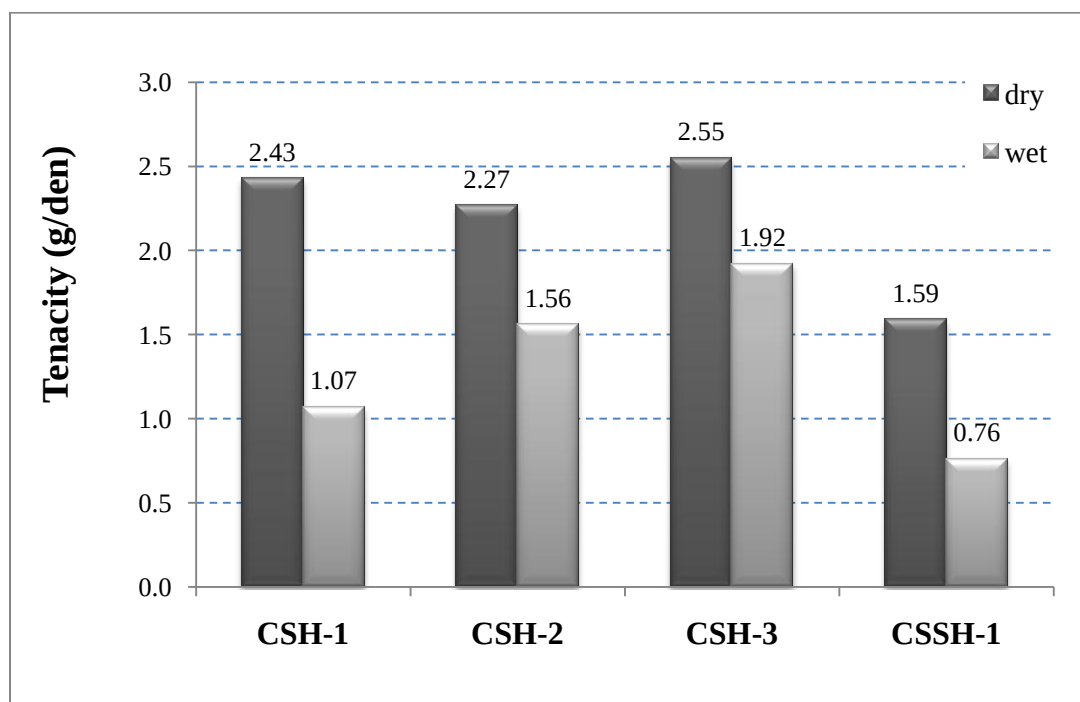


Figure 6.6. Tenacity of composite fibers in dry and wet states.

Figure 6.7 shows the variation of water absorbency of composite fibers with time.

The initial water take up of the never dried fibers was around 1500 wt% after coagulation bath; however, their water absorbency character is reduced after drying. Well oriented fiber structure might affect the expected water absorbency values. Water absorption of CSH fibers after drying is around 70 wt% and the absorption increased by increasing starch amount, for CSSH is around 140 wt%. Lower crystallinity is the likely cause of the

higher water absorbency of CSSH compared to CSH. The amount of water absorbed increases up to first 30 seconds, and then equilibrium is reached. While the water absorption is high, it is not in the range of commercial superabsorbent polymers. The drying span of the fibers were 2 min and 3 min, for CSH and CSSH, respectively.

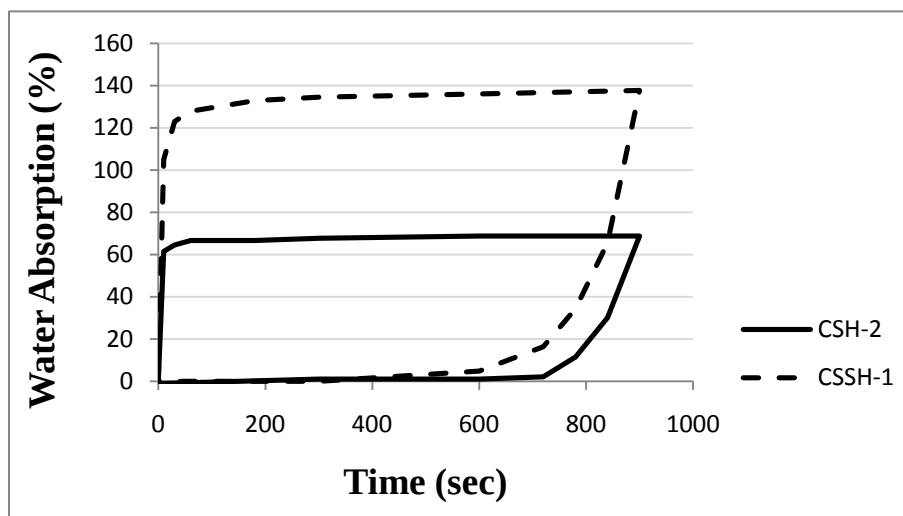


Figure 6.7. Variation of water absorbency of composite fibers with time.

One of the most promising characteristics of the composite fibers is the UV light stabilization property of HALS,³² whereas N-halamines are not usually stable towards UV light. It should be noted that the stability of bonded chlorine and the structure itself should be considered separately. The bound chlorine on composite fibers was not very stable toward UV light exposure (Table 6.4), similar to other N-halamines.⁹

The chlorinated composite fibers (CH-Cl) lost all bonded chlorine within 3 hours.

However, the chlorine loadings after rechlorinations after the UV light exposure were remarkably high, while in general N-halamines suffer from this property. HALS did not act as a UV light absorber; but rather acted to interrupt the polymer degradation mechanism.³³

Table 6.4. Stability of bound chlorine on CH fibers toward UV light exposure (Cl^+ % remaining).

Time of exposure	Cl^+ %	Rechlorination
0	0.35	
1 h	0.15	
2 h	0.11	
3 h	0.05	
4 h	0.00	0.35
1 day		0.34
15 days		0.30
30 days		0.32
45 days		0.33

UV/Vis spectra of HALS and chlorinated HALS (HALS-Cl) is shown in Figure 6.8.

The HALS exhibited a peak centered at 242 nm and the peak did not show any significant shifting after the chlorination. However, a shoulder peak, starting around 320 nm, was observed after the chlorination. This shoulder peak could influence the dissociating mechanism of the N-Cl bond³⁴ as observed in N-halamines.¹¹

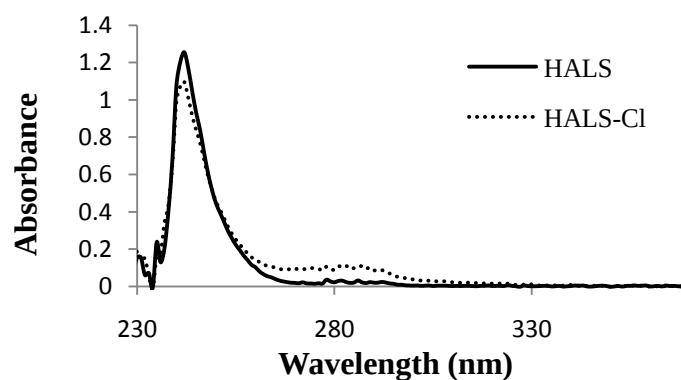


Figure 6.8. UV/Vis Spectra of HALS before and after chlorination. (solvent: THF)

Since hindered amine light stabilizers are not UV absorbers, they do not shield the polymer or N-Cl bond from UV light; however, they decompose the radical intermediates formed in the photo-oxidation process.³³ HALS' high efficiency and durability are due to a cyclic process wherein the HALS are regenerated rather than consumed during the stabilization process.³³

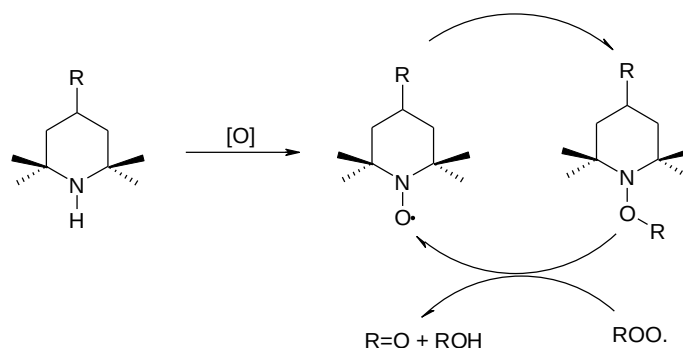


Figure 6.9. Simplified stabilization mechanism of hindered amine light stabilizers.³³

In this regard, the stability of the HALS structure, chlorinated and nonchlorinated, needs to be evaluated. Since the stability of N-halamine precursors is higher compared to N-halamines, a stability test including several rechlorinations and then exposure to UV light would give more information about the stability of the chlorinated HALS structure. The stability toward UV light of CSSH-1 composite fibers is presented in Figure 6.10. Two sets of samples were tested - prechlorinated samples were exposed to UV light and titrated and then rechlorinated and titrated, -unchlorinated samples were only chlorinated and titrated after exposed to UV light. The prechlorinated samples were rechlorinated after each UV light exposure cycle. The unchlorinated samples were very stable towards UV light; they did not show any decomposition according to chlorine loadings. The prechlorinated samples lost all bound chlorine within 6 h in every cycle. Chlorine

loadings after rechlorination for the first three cycles were around the initial chlorine loading; however they started to decrease gradually after the third cycle. Although the chlorine loadings did decrease, the loss after 6 cycles was around 27% which is remarkably good compared to various cyclic N-halamines.^{9,12}

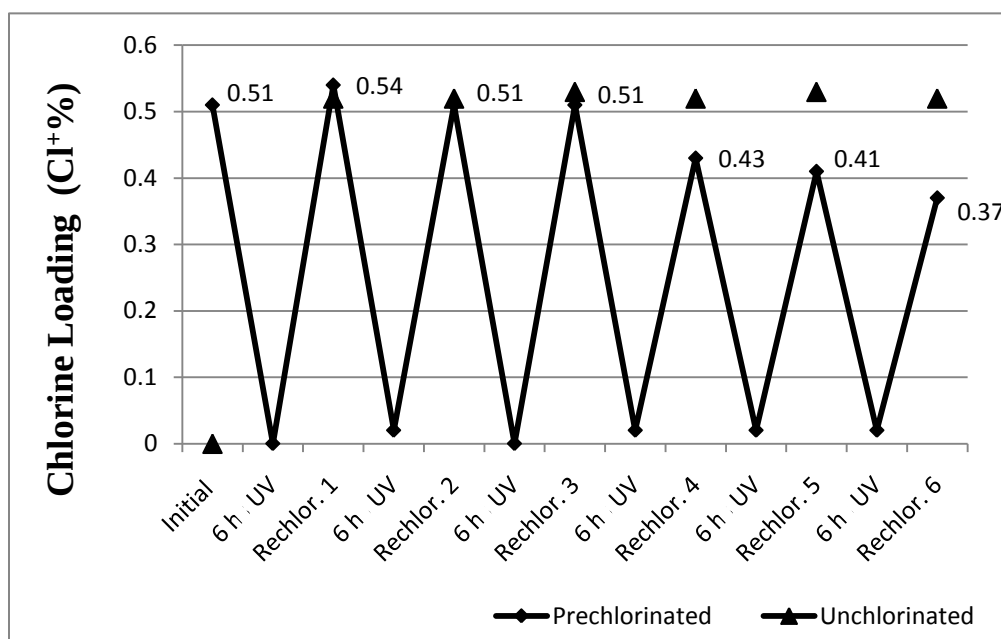


Figure 6.10. Stability of bound chlorine on CSSH fibers toward UV light exposure (Cl⁺% remaining).

^a Theoretical chlorine loading value is 0.63%.

CH fibers were challenged with *E. coli* bacteria at concentration of 7.73 log cfu (colony-forming units). The biocidal test results are shown in Table 6.5. The unchlorinated control samples provided about 0.2 log reductions, due to the adhesion of bacteria to the composite fibers, within 30 min contact time intervals. The chlorinated CH composite fibers (CH-Cl) showed excellent antimicrobial activity. All *E. coli* bacteria were inactivated by the treated fibers in the contact interval of 30 min. The inactivating rates of

the chlorinated treated fibers are superior for antimicrobial applications. On the other hand, the chlorinated CSSH fibers exhibited insufficient antimicrobial activity within 30 min due to deficient surface wettability, during the biocidal test, which results in inadequate contact of fibers with the microorganisms.

Table 6.5. Biocidal test.

Sample / Chlorine loading (Cl⁺%)	Contact time (min)	Dilution times	Bacterial No. (cfu/75μL)	Log reduction of bacteria
CH	30	1000	74	0.24
CH-Cl	10	1000	2	5.51
0.33	30	1000	0	7.73
CSSH	30	10000	20	0.07
CSSH-Cl	10	10000	17	0.14
0.52	30	10000	15	0.19

^a Microorganism *E.coli* O157:H7. Total bacteria: 5.33×10^7 cfu/sample (7.73 log).

6.4 Conclusions

A method for transformation of commercial polymers (HALS) into durable and regenerable antimicrobial polymeric materials was described. The method provides practical, cost effective and environment friendly production of cellulose based fibers having durable and regenerable antimicrobial property with lack of UV degradation. The ability to form composite fibers having reasonable strength from an ionic liquid

spinning solvent has been demonstrated. The effects noted with changes in extrusion and drawing conditions followed expected trends. The solvent used, BMIM-Cl, dissolves a variety of different polymers and shows promise for manufacture of composite fibers. The composite fibers allow desirable properties of both polymers to be retained, while producing fibers of reasonable physical properties. Oligomeric HALS was successfully trapped in the fiber structure and produced an UV light stable N-halamine for cellulosic substrates. Use of starch increased the water absorbency of the composite fibers and allowed a higher polymer content (wt%) in the ionic liquid. The composite fibers might find applications in hospital clothing, hygienic products, consumer products, and household products.

6.5 References

- [1] Kilinc-Balci, F.S.; Fan, X.; Kocer, H.B.; Broughton, R.M. Extrusion of Composite Fibers. INTC. Atlanta, GA. **2007**.
- [2] For example see: (a) Swatloski, R.; Broughton, R.M.; Sung, N.; Maxim, M.; Daly, D.; Spear, S.; Rogers, A. A Look at Ionic Liquid Generated Cellulose and Modified Cellulose Fibers. Fifteenth Molten Salts Symposium. Cancun, Mexico. October, **2006**.
(b) Farag, R.; Broughton, R.M.; Kilic-Balci, F.; Wang, W.; Swatloski, R.; Rogers, R. Development of Orientation in Extruded Cellulose Fibers, The Fiber Society, Knoxville TN, October, **2006**.
- [3] Lee, J.; Broughton, R.M.; Worley, S.D.; Huang, T.S. Antimicrobial Polymeric Materials; Cellulose and m-aramid Composite Fibers. *J. Eng. Fib. Fabr.* **2007**, 2, 25-32.
- [4] Zhang, H.; Wu, J.; Zhang, J.; He, J. 1-Allyl-3-methylimidazolium Chloride Room Temperature Ionic Liquid: A New and Powerful Nonderivatizing Solvent for Cellulose. *Macromol.* **2005**, 38, 8272-8277.
- [5] Rogers, R.D.; Seddon, K.R. Ionic Liquids; Industrial Applications to Green Chemistry; ACS Symposium Series 818; American Chemical Society, Washington, DC, **2002**.
- [6] Turner, M.B.; Spear, S.K.; Holbrey, J.D.; Daly, D.T.; Rogers, R.D. Ionic Liquid Reconstituted Cellulose Composites As Solid Support Matrices for Biocatalyst Immobilization. *Biomacromol.* **2005**, 6, 2497-2502.

- [7] Liang, J.; Chen, Y.; Ren, X.; Wu, R.; Barnes, K.; Worley, S.D.; Broughton, R.M.; Cho, U.; Kocer, H.B.; Huang, T.S. Fabric Treated with Antimicrobial N-Halamine Epoxides. *Ind. Eng. Chem. Res.* **2007**, 46, 6425-6429.
- [8] Worley, S.D.; Chen, Y.; Wang, J.W.; Wu, R.; Cho, U.; Broughton, R.M.; Kim, J.; Wei, C.I.; Williams, J.F.; Chen, J. Li, Y. Novel N-halamine Siloxane Monomers and Polymers for Preparing Biocidal Treatments. *Surf. Coat. Int. Part B.* **2005**, 88, 93-99.
- [9] For example see: 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.
- [10] For example see: Lim, K.Y.; Yoon, K.J.; Kim, B.C. Highly Absorbable Lyocell Fiber Spun from Celluloses/Hydrolyzed Starch-g-PAN Solution in NMMO Monohydrate. *Europ. Polym. J.* **2003**, 39, 2115-2120.
- [11] Chen, Z. Sun, Y.Y. N-Chloro-Hindered Amines as Multifunctional Polymer Additives. *Macromolecules.* **2005**, 38, 8116-8119.
- [12] Ren, X.; Kou, L.; Liang, J.; Worley, S.D.; Tzou, Y.M.; Huang, T.S. Antimicrobial Efficacy and Light Stability of N-Halamine Siloxanes bound to cotton. *Cellulose.* **2008**, 15, 593-598.
- [13] Worley, S.D.; Sun, G. Biocidal Polymers. *Trends. Polym. Sci.* **1996**, 4, 364-370.
- [14] Kruczala, K.; Bokria, J.G.; Shlick, S. Thermal Aging of Heterophasic Propylene-Ethylene Copolymers: Spatial and Temporal Aspects of Degradation Based on ESR, ESR Imaging, and FTIR. *Macromolecules.* **2003**, 36, 1909-1919.

- [15] Aparecida, K.; Aquino, S.; Araujo, E.S. Effects of a Hindered Amine Stabilizer (HAS) on Radiolytic and Thermal Stability of Poly(methyl methacrylate). *J. Appl. Poly. Sci.* **2008**, 110, 401-407.
- [16] Seiichi, N.; Malinee, C.; Tomoyuki, I. Radiation-induced structural changes in poly(propylene-ran-ethylene) film: effect of antioxidant 2,2,6,6-tetramethylpiperidine derivatives. *Radiat. Phys. and Chem.* **1992**, 39, 413-19.
- [17] Pauquet, J.R.; Berthelon, N. Effect Additives for Polypropylene Fibers. Improving Performances of PP Fibers and Tapes by Functional Additives. Nazareth, Belgium. **2002**.
- [18] Barnes, K.; Liang, J.; Worley, S.D.; Lee, J.; Broughton, R.M.; Huang, T.S. Modification of Silica Gel, Cellulose, and Polyurethane with a Sterically Hindered N-Halamine Moiety to produce Antimicrobial Activity. *J. Appl. Polym. Sci.* **2007**, 105, 2306-2313.
- [19] Cho, U. Novel Antimicrobial Textiles. Dissertation. **2003**, Auburn, Alabama.
- [20] Full Public Report for Chimmasorb 2020. National Industrial Chemicals Notification and Assessment Scheme. Camperdown, Australia. December, **1999**.
- [21] Akdag, A.; Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Webb, T.R.; Bray, T.H. Why Does Kevlar Decompose, while Nomex Does Not, When Treated with Aqueous Chlorine Solutions? *J. Phys. Chem. B.* **2007**, 111(20), 5581-5586.
- [22] Furutani, Y.; Kandori, H.; Kawano, M.; Nakabayashi, K.; Yoshizawa, M.; Fujita, M. In Situ Spectroscopic, Electrochemical, and Theoretical Studies of the Photoinduced

Host#Guest Electron Transfer that Precedes Unusual Host-Mediated Alkane

Photooxidation. *J. Am. Chem. Soc.* **2009**, 131, 4764-4768.

[23] Mori, K.; Kang, Z.; Wang, F.; Oishi, Y. Polymer Plating of 6-(N-Allyl-1,1,2,2-Tetrahydroperfluorodecyl)Amino-1,3,5-Triazine-2,4-Dithiol Monosodium on Aluminum in Magnetic Field and its Electrostatic Capacity. *IEEE Trans. Appl. Superconduct.* **2004**, 14, 1177-1180.

[24] Schwanninger, M.; Rodrigues, J.C.; Pereira, H.; Hinterstoisser, B. Effects of Short-Time Vibratory Ball Milling on the Shape of FT-IR spectra of Wood and Cellulose. *Vib. Spectrosc.* **2004**, 36, 23-40.

[25] Santha, N.; Sudha, K. G.; Vijaykumari, K. P.; Nayar, V. U.; Moorthy, S. N. Raman and Infrared Spectra of Starch Samples of Sweet Potato and Cassava. *Proc. Indian Acad. Sci. (Chem. Sci.)* **1990**, 102, 705-712.

[26] Kizil, R.; Irudayaraj, J.; Seetharaman, K. Characterization of Irradiated Starches by Using FT-Raman and FTIR Spectroscopy. *J. Agric. Food Chem.* **2002**, 50, 3912-3918.

[27] Caprona, I.; Roberta, P.; Colonna, P.; Broglyb, M.; Planchot, V. Starch in Rubbery and Glassy States by FTIR Spectroscopy. *Carbohydrate Poly.* **2007**, 68, 249-259.

[28] Yang, J.H.; Yu, J.G.; Ma, X.F. A Novel Plasticizer for the Preparation of Thermoplastic Starch. *Chinese Cem. Lett.* **2006**, 17, 133-136.

[29] Van Soest, J.J.G.; Tournois, H.; De Wit, D.; Vliegthart, J.F.G. Short-range Structure in (partially) Crystalline Potato Starch Determined with Attenuated Total

Reflectance Fourier-transform IR Spectroscopy. *Carbohydrate Research*. **1995**, 279, 201-214.

[30] Goodfellow, B.J.; Wilson, R.H. A Fourier Transform IR Study of the Gelation of Amylose and Amylopectin. *Biopolymers*. **1990**, 30,1183-1189.

[31] Kreze, T.; Strnad, S.; Stana-Kleinschek, K.; Ribitsch, V. Influence of Aqueous Medium on Mechanical Properties of Conventional and New Environmentally Friendly Regenerated Cellulose Fibers. *Mat. Res. Innovat.* **2001**, 4, 107-114.

[32] Holbein, P. The High Molecular Weight Hindered Amine Light Stabilizer for the New Millenium. Addcon World 98, London, UK, Rapra Technology Ltd. **1998**.

[33] <http://www.ciba.com/index/ind-index/ind-pla-effectsweoffer/ind-pla-eff-durabilityandprotection/ind-pla-eff-dur-lightstability/ind-pla-eff-dur-lightstabilizer.htm>. accessed 03/14/2009.

[34] Metcalf, W.S. The Absorption Spectra of Mono-, Di-, and Tri-chloroamines and Some Aliphatic Derivatives. *J. Chem. Soc.* **1942**, 148-50.

[35] Lee, J. Synthesis and Applications of Novel Antimicrobial Polymeric Materials. Dissertation. **2006**, Auburn, Alabama.

6.6 Supporting Information

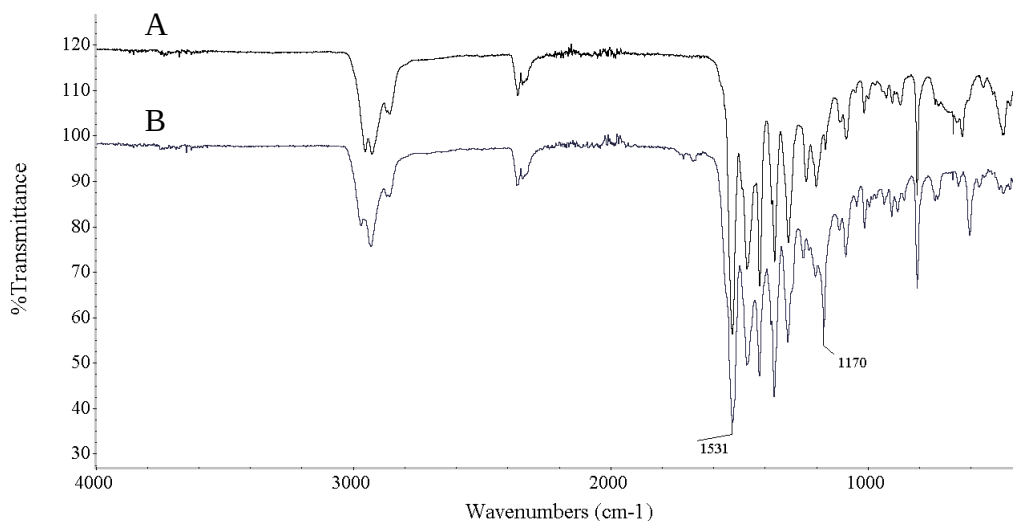


Figure 6.S.1. FTIR spectra of HALS before (A) and after (B) chlorination.

Table 6.S.1. Mechanical properties of the composite fibers in dry and wet state.

	Dry state		Wet state	
	Tenacity (g/den)	Strain at break (%)	Tenacity (g/den)	Strain at break (%)
CSH-1	2.43 ±0.33	8.76 ±1.99	1.07 ±0.12	9.28 ±1.87
CSH-2	2.27 ±0.23	8.57 ±1.99	1.56 ±0.36	7.50 ±0.93
CSH-3	2.55 ±0.36	8.40 ±1.16	1.92 ±0.36	7.15 ±0.71
CSH-4	2.47 ±0.20	8.63 ±1.31	NA	NA
CSSH-1	1.59 ±0.06	20.56 ±3.21	0.76 ±0.05	26.73 ±6.80
CSSH-2	1.73 ±0.13	9.50 ±1.30	NA	NA
CSSH-3	1.82 ±0.27	5.67 ±1.27	NA	NA