SYNTHESIS AND APPLICATIONS OF NOVEL ANTIMICROBIAL

POLYMERIC MATERIALS

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SYNTHESIS AND APPLICATIONS OF NOVEL ANTIMICROBIAL POLYMERIC MATERIALS

Jaewoong Lee

A Dissertation

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SYNTHESIS AND APPLICATIONS OF NOVEL ANTIMICROBIAL

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Jaewoong Lee

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VITA

Jaewoong Lee, son of Yongjang Lee and Chunki Kim, was born on November 24, 1971, in Andong, Republic of Korea. He was graduated from Yeungnam University in Textile Engineering Department in 1997. While employed as an assistant in Kyungpook National University in Polymer Science Department, he attended the master program in Textile Materials and Chemistry Major at Yeungnam University in August 1999 and received the degree of Master of Science in August 2001. He entered Graduate School at Auburn University, in August, 2003 where he worked under the supervision of Dr. Roy M. Broughton. He married Eunjin Heo, daughter of Taewon Heo and Youngsuk Kim, on November 9, 1997.

DISSERTATION ABSTRACT

SYNTHESIS AND APPLICATIONS OF NOVEL ANTIMICROBIAL POLYMERIC MATERIALS

Jaewoong Lee

Doctor of Philosophy, August 7, 2006
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Antimicrobial precursors, especially *N*-halamine precursors, were developed and synthesized. After being applied onto polymers and fibers, the durability, rechargeability and antibacterial efficacy of the *N*-halamine-treated polymers and fibers were evaluated. An *s*-triazine-based reactive group was synthesized and used for attaching *N*-halamine precursor onto cotton fabrics, and crosslinking agents were employed for antimicrobial finishing. An antimicrobial acrylic fiber and *m*-aramid/cellulose composite fiber were invented and prepared using the dry-jet wet spinning process, and physical properties, as well as antimicrobial efficacy, were investigated.

After synthesizing and characterizing an *s*-triazine-based *N*-halamine precursor, dichloro-*m*-aminophenylhydantoinyl-*s*-triazine (DAPHT), this was applied to cotton fabrics through a reactive dyeing process. The optimum condition through the dyeing process was investigated. Due to the lower dyeing temperature (~30ºC), the application allows mild processing conditions. Since even after 50 standard-washing cycles the recharged chlorine-recovery was over 60%, the durability of the material is sufficient for practical application. Bacterial efficacy tests resulted in inactivation of both Gram-positive and Gram-negative bacteria within 30 min with 6 log reductions.

To apply *m*-aminophenyl hydantoin (MAPH) onto cotton fabric, polycarboxylic acids provided good connection between MAPH and cotton cellulose. Through one finishing process, the MAPH treated cotton fabrics have durable press properties as well as antimicrobial activities. The chlorinated BTCA/MAPH treated cotton fabric provided inactivation against Gram-positive and Gram-negative bacteria within 1 min of contact time.

An antimicrobial-acrylic fiber precursor (PAN/PSH composite fiber) was produced by dry-jet wet spinning. The acrylic fiber possessed durable and rechargeable antimicrobial properties up to 50 standard washing cycles, and the chlorinated acrylic fibers inactivated *S. aureus* (Gram-positive bacteria) within 30 min.

Cellulose and *m*-aramid were dissolved in an ionic liquid, and dry-jet wet spinning was used to prepare composite fibers which could be rendered antimicrobial

through exposure to chlorine bleach. Chlorination of the aramid nitrogen produced antimicrobial properties which were retained over 50 standard-washing cycles. Cellulose/*m*-aramid blends showed a much higher chlorination level than the pure *m*-aramid fiber. Up to 10% (based on the weight of cellulose) *m*-aramid composite fibers did not produce any decrease in mechanical properties. The chlorinated fibers inactivated both *E. coli* O157:H7 (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) within 5 min with 6 log reductions.

As medical textiles and biomaterials for healthcare, *N*-halamine treated polymers and fibers have potential for commercial use and through this research, novel and variable applications are suggested.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Inactivation of microorganisms causing odor as well as contagious diseases is the goal of antimicrobial agents. Numerous materials have been developed over to the years as mankind has moved toward this goal. The recent concern over pandemics and terrorism has prompted increased emphasis in antimicrobial materials. Thus, it is valuable to understand antimicrobial materials as well as the inactivation mechanism.

There are three major concepts for biocidal functions, described in terms of the strength of the treatment: sanitization, disinfection and sterilization. Sanitization is defined as the process to kill or inactivate microorganisms up to the level that permits a safety for public health. It requires reducing microorganisms in the environment by significant numbers, but it is not a mandatory to eliminate all of microorganisms [1]. Disinfection applies to the process to destroy or eliminate fungi and bacteria but not necessarily their spores. The three levels of disinfection and disinfectants are suggested with specific intended uses [1-3]:

Low-level; Quaternary ammonium compounds and 70-90% isopropyl alcohol

Medium-level; Phenolic compounds and 70-90% ethyl alcohol

High-level; Sodium hypochlorite (1000 ppm) and 8% formaldehyde

Sterilization is the eliminating or killing of all forms of microorganisms including their spores. Spores are the most difficult to be destroyed of all forms of microorganisms. Hence, sterilization is the best function to inactivate micro-organisms. Conventional sterilization systems include three methods: Steam under pressure (autoclaves), dry heat (ovens) and chemical [1, 4]. Non-conventional and more recent methods include plasma and gamma irradiation [4, 5]. Ethylene oxide (ETO) gas or chemicals such as peracetic acid and glutaraldehyde are typical chemical sterilization agents. ETO gas is also very toxic unlike heat and steam, and in addition, residues of ETO in contact with water create ethylene glycol, which is also toxic. Even though heat is non-toxic, during repeated exposure to high temperature of dry heat, decomposition of fabrics or polymers can occur, and steam does not effectively penetrate specimens when they are stuck [1].

Unless disinfectants or sterilizers remain on specimens after disinfection or sterilization, the risk of recontamination still exists [6, 7]. Thus, shelf life may be maintained by impermeable packaging. In case of antimicrobial clothing material, retention and reusability of antimicrobial functions after washing are a primary concern. With all agents, safety to the environment is important. For personal safety against contagious microorganisms, antimicrobial functions should be required at least at the disinfection level.

1.2 Structure and Inactivation Mechanism of Bacterial Cells

Bacteria, are unicellular organisms, and can be separated into two categories: gram negative and gram positive. Gram-negative is identified by absorption and retention of the Gram stain (Gram's Iodine, a kind of mordant dye). Gram-positive colorless after exposure to the Gram stain followed by washing with alcohols. Due to the outer membrane of Gram-negative bacteria (Figure 1), they are normally less sensitive to biocides. A biocide, which inactivates both types of bacteria, can be defined as a broad-spectrum biocide. *E. coli* and *S. aureus* are conveniently handled and are frequently used to represent Gram-negative and Gram-positive, respectively.

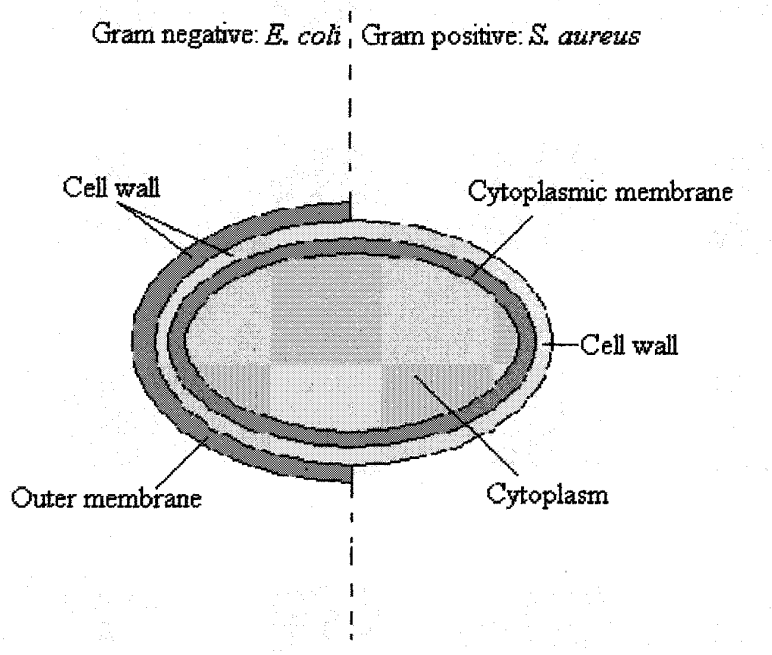


Figure 1-1 *Structure of the Two Types of Bacteria*

The process of interaction between biocide and cell occurs in the following sequence

1. Attachment to or uptake of biocide by cell
2. Penetration of biocide to target(s)
3. Concentration of biocide at target(s)
4. Damage to target(s)
5. Inactivation of the bacteria

The interaction of a biocide with bacteria induces an initial binding of a biocide to the bacterial cell surface. A deformation of the bacterial outer layer may allow a biocide to permeate the cell and reach target(s) [8-10]

Some cells may recover from metabolic injury after removing or neutralization of the biocide [11]. This is referred to as a bacteriostatic action. Bactericidal action is caused when the damage is to vital cellular structure or function and is irreversible. The penetration of biocide into cells is influenced by several factors affecting biocide chemistry and/or microbial physicochemistry. For example, bactericidal activity is influenced by pH. Weak acids are most active at pHs below their pKa, where they are less dissociated. Cationic surfactants have their most powerful biocidal activity at pHs which ensure the surface negative charge of cells [12]. In general, the target regions of cells are the cell wall, cytoplasmic membrane and cytoplasm. Damaging events at each target region are well defined and summarized below.

Cell wall: Structural/functional changes; disrupts of wall components; initiation of autolysis

Cytoplasmic membrane: Loss of structural organization and integrity, selective increase in permeability to protons and other ions, inhibition of membrane-bound enzymes

Cytoplasm: Inhibition of cytoplasmic enzymes; interaction with functional biomolecules,

coagulation and precipitation of cytoplasmic constituents

The most available target region is the cytoplasmic membrane, due to its fundamental metabolic and structural role within the cell. It also provides large surface area for interaction with biocides. The damage of the membrane integrity may result in the leakage of enzymes, nucleotides and nucleosides, and sugars. After leaking of vital components, the cell will die. A wide range of biocides in different chemical classes will damage cytoplsamic membrane by a variety of different mechanisms [8].

A few species of bacteria have the ability to produce highly resistant structures known as endospores (or simply spores) to help them survive through tough conditions. A bacterial spore is a complex entity, being composed of several different layers (Figure 2), resistant to inactivation by variety of chemical and physical agents.

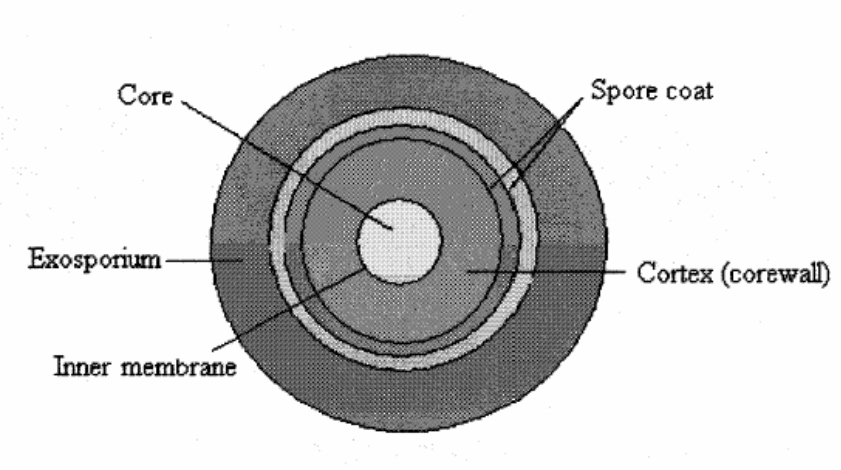


Figure 1-2 *Structure of Bacterial Spore*

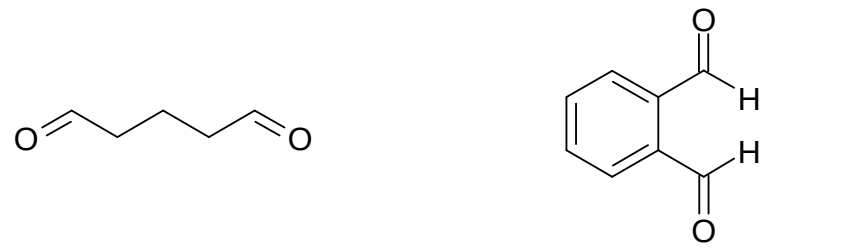
Bacterial spores basically consist of spore core, cortex and spore coats. A spore has an outermost layer known as, exosporium. Beneath the exosporium, there is the spore coat, which includes the layer(s) of modified peptidoglycans. Due to limited permeability of the spore coat, the spore has high resistance to chemical disinfection, heat, radiation and drying. The cortex exists within the spore coat. The cortex has the normal, but small cell wall and cellular constituents. The resistance of spores against solvents is much higher than that of bacterial cells. Some spores can survive at high concentration of ethanol solutions and even withstand boiling in hydrochloric acid for 30 minutes. The spore presents several sites at which interaction with an antibacterial agent is possible, e.g., the inner and outer spore coats, cortex, spore membranes, and core. However, it is also obvious that the spore has barriers, which limit biocide penetration [13].

1.3 Biocides

Numerous biocides are active against bacteria. Normally, aldehydes, cationic agents, alcohols, peroxygens, phenols and chlorinated phenols, metal ions and halogens (in a variety of oxidation states including *N*-halamines) are being used as biocides [9, 10, 14-17].

Aldehydes inhibit the metabolism and replication cycles of proteins, RNA and DNA by alkylating with the amino, imino, amide, carbonyl and thiol groups. They can also harm some cell/spore wall constituents and may have effectiveness against fungi and yeast. Vapor of formaldehyde has been used as a sanitizer for poultry, and farm animal housing facilities and non-food contact surfaces. Formaldehyde has been used for preserving dead bodies: however, it is a carcinogen and is persistent in the environment. Strains of formaldehyde-

resistant *E. coli* and *Serratia marcescences* are known [18]. Glutaraldehyde (GTA) acts as cross-linking agent on amino groups in bacteria proteins [19]. Ortho-phthalaldehyde (OPA), an aromatic dialdehyde, has been investigated with Gram-positive bacteria and Gram-negative bacteria, and has shown less effectiveness in cross-linking than GTA [20].

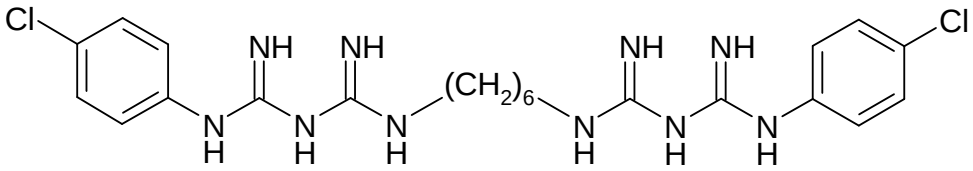


Glutaraldehyde (GTA) *o*-phthalaldehyde (OPA)

Figure 1-3 *Structure of Glutaraldehyde and o-phthalaldehyde*

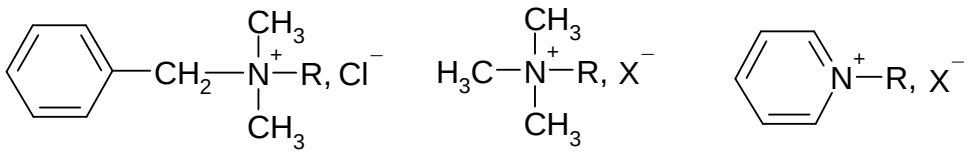
Cationic biocides induce severe membrane damage in various kinds of microorganisms, including Gram-positive and Gram-negative bacteria. Chlorohexidine (CHX) and quaternary ammonium compounds (QACs) are good examples of cationic biocides [21]. CHX leaves a residual antimicrobial function after application to a variety of materials. Antimicrobial soaps, mouthwash and antiseptic hand-gels are common applications of CHX. After repeated exposure under CHX, microorganisms may develop resistance [22]. Biguanides such as CHX and alexidine may be applied in a polymeric form and the polymeric biguanides have been studied. Their mechanism of attack has been shown to result noticeable damage on the inner membrane of *E. coli* [23]. QACs such as benzalkoium chloride, dodecyltrimethyl ammonium bromide (DTAB) and cetylpyridinium chloride (CPC), destroy outer membrane surfaces on many pathogens. The key feature

of antimicrobial QACs is at least one long hydrocarbon chain substituted at the nitrogen. Normally the range of the chain is from C_8H_{17} to $C_{19}H_{39}$, with a best activity around $C_{14}H_{29}$ [24]. The suggested mechanism is electrostatic interaction between N^+ in QACs and negatively charged cell surface increases a defect in the bilayer allowing the hydrophobic chain to permeate into the cell wall, which produces disruption. QACs are a kind of ionic surfactants and act as disinfectants and detergents. However, QACs are only weakly effective against Gram-negative bacteria and are ineffective against spores [22, 25].



Chlorohexidine (CHX)

Figure 1-4 *Structure of Chlorohexidine*



R= C_8-C_{19} alkyl group
X= Cl or Br

Figure 1-5 *Structure of Quaternary Ammonium Salts*

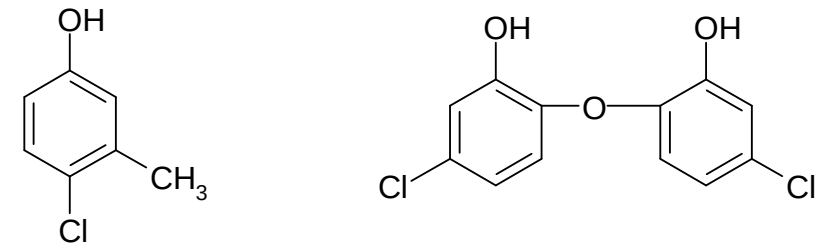
Alcohols usually have biocidal activity. They show rapid bactericidal properties, even in some cases, acid-fast bacteria. Alcohols can make hydrogen bonds with proteins/enzymes, denature them and render them inactive. However, they are ineffective to bacterial spores even at high concentrations. Ethanol and isopropanol disrupt membranes of bacteria cells. Ethanol inhibits DNA, RNA, protein and peptidoglycan synthesis in *E. coli* [26]. Because absolute alcohols (without water) can not denature bacteria as well as when water is included, a solution of around 70% iso-propanol or ethanol is typically used. These are widely used by hospitals, biological laboratories, in antiseptic gels and hand decontamination products. Due to volatility, they can only supply relatively short-period protection with no residue [27].

Peroxygens are strong biocides. The major peroxygens are hydrogen peroxide (H_2O_2), peracetic acid (CH_3COOOH) and ozone (O_3). They disrupt enzymes and proteins by oxidizing thiol groups. Because of depolymerization of collagen or gelatin, they are used against spores, and they also destroy biofilm with oxygen bubbles assisting the penetration of the active agent. Hydrogen peroxide is a straightforward oxidizing agent. The hydroxyl radical (•OH), which oxidizes thiol groups in target microorganisms, is the main feature of peroxy-biocides. Peracetic acid is the most powerful peroxygen, and it is bactericidal, fungicidal and sporicidal. The free radical oxidation of enzymes and protein thiol groups is believed as its major action. Hydrogen peroxide and peracetic acid are readily vaporized under hot air condition, and they are also decomposed into oxygen and water, which are harmless to environment.

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

Ozone is a powerful bactericidal, sporicidal and fungicidal agent; however, ozone does not remain in water long enough to supply a residual protection against latent contamination. In addition, ozone is very harmful to humans and decomposes polymers and corrodes metals on contact [28].

Phenols and chlorinated phenols are used for disinfectants or preservatives. Phenol induces effective loss of intracellular components from bacteria, and germination is inactivated by low phenol concentration. However, phenols are ineffective against bacterial spores even at higher concentrations [26]. Chlorocresol and triclosan are good examples of chlorinated phenols. They readily permeate into a phospholipid bilayer, then membrane integrity of bacteria is disturbed. They promote leakage and intracellular coagulation of bacteria. Even though triclosan inactivates bacteria on contact, the effectiveness is not very good. Triclosan is basically bacteriostatic or fungistatic in action. Chlorinated phenols are predominantly applied for sanitation of floors, garbage cans, toilet facilities and several surfaces. Due to relatively non-corrosive properties, triclosan has been used as the active ingredient for antimicrobial soaps, deodorants, body-wash and antiseptic hand-gels. However, triclosan resistance has been found in *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) [22].



Chlorocresol Triclosan

Figure 1-6 *Structure of Chlorinated Phenols*

Heavy metals such as Ag, Hg, Zn, As, Cu, Sb and their salts, or organomercury and organosilver compounds have toxicity to living organisms. Metal ions complex with proteins and may precipitate proteins after cleavage of disulphide bonds within proteins. Thus, the configuration of the enzymes and proteins to bind to DNA are inhibited. Silver and mercury are not effective against spores, but may be effective in preventing bacterial growth from the spores. Since heavy metal salts are inexpensive and biocidal, they have been applied as the active ingredient in anti-fouling formulations. Mercuric chloride is sporicidal, but applications of mercuric chloride are inhibited due to environmental problems, and some research has suggested that repeated exposure induced bacteria resistance to metal salts. For instance, silver-resistant Pseudomonas has been reported [29].

Halogen biocides such as Cl_2, Br_2, I_2, HOCl, HOBr, NaOCl, and ClO_2 are powerful antimicrobial agents. Halogen compounds readily halogenate amino groups in proteins. Since chlorine can oxidize the sulphydryl group of triosephosphoric dehyrogenase, the enzyme that supports the oxidation of triosephosphoric acid to phosphoglyceric acid, enzymatic activity is destroyed [30]. Halogen compounds, especially hypochlorites, are inexpensive and broad spectrum biocides. They are widely used and powerful

oxidizing agents having bactericidal, sporicidal and fungicidal activity. Halogen compounds are referred to as multi-target reactors: acting on cell walls and the amino groups in proteins. Chlorine dioxide has been shown to have activity against bacteria, fungi, protozoa and algae [31]. In water systems and swimming pools, chlorine has been the predominant biocidal agent. Stabilized halogen biocides such as monochloroamine have been shown to more effectively penetrate and destroy biofilms than free halogen such as free chlorine [32]. Hypochlorous acid (HOCl) is the reaction product of chlorine and water.

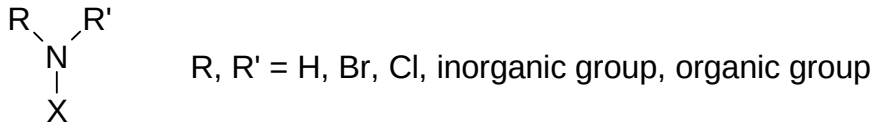
$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

$$HOCl \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} H^+ + OCl^-$$

The pKa value of hypochlorous acid is 7.6 at room temperature [33]. Hence, most hypochlorous acid could reside in its neutral form, HOCl at pH<7.6. At pH> 7.6, most hypochlorous acid resides as the hypochlorite ion, OCl^-. Both HOCl and OCl^- are considered as “free” chlorine. HOCl is known to be more effective against microorganisms, and is 150~300 times more antimicrobial than OCl^-. Even if halogen compounds are powerful biocides, chlorine is still corrosive. In addition, chlorinated hydrocarbons such as trichloromethane and oxidized organic compounds are produced after using chlorine, and those products may be toxic. Indeed, trihalomethanes are suspected carcinogens [34-37].

1.4 *N*-Halamines

N-halamine materials have debuted as antimicrobial agents over the last few decades. An *N*-halamine is a compound which has covalent bonding between nitrogen and halogen. In general, the halogen is bromine or chlorine (seldom iodine) and can be released into aqueous media as “positive halogen” from the compounds. The outstanding feature of the compounds is a relative stability and very small amount of released halogen over a long time period. The general structure of *N*-halamine is shown below.



In an *N*-halamine, R and R′ can be an organic group (alkyl group, carbonyl group), inorganic group (phosphate, sulfate), hydrogen, or halogens. When R or R′ is an inorganic group, or both are hydrogen or halogens, it is considered as an inorganic *N*-halamine. Some inorganic *N*-halamine structures are depicted in Figure 1-7.

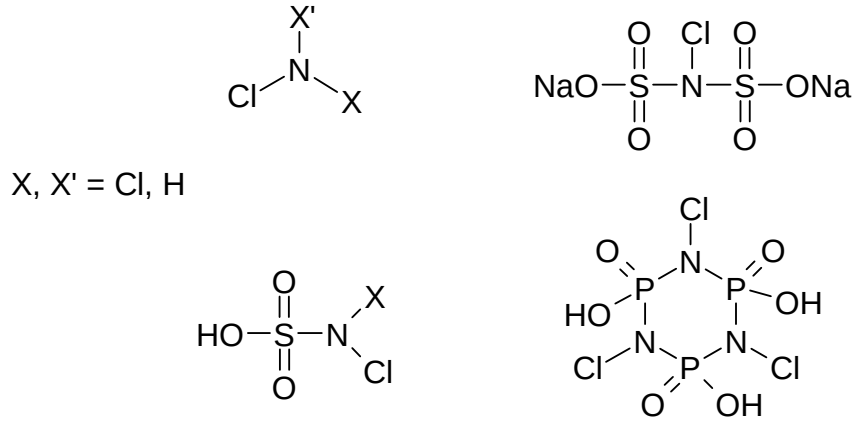
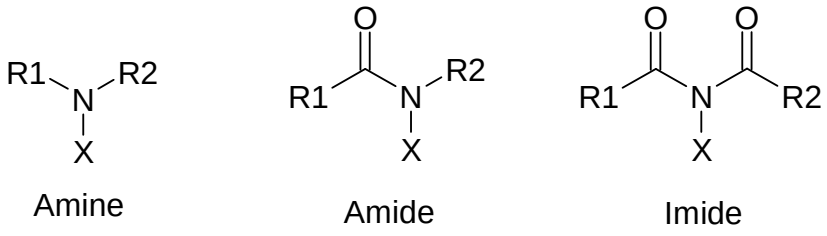


Figure 1-7 *Structures of Inorganic N-halamine Compounds*

If one of R groups is an organic group, it is considered as an organic *N*-halamine, and major structures are amines, amides and imides. The type of organic *N*-halamine determines stability of halogen and biocidal efficacy.



In the amine case, an electron donating alkyl group adjacent to the nitrogen stabilizes the N–X bond, unlike the imide group which has two electron withdrawing carbonyl groups beside the N–X bond. The halogen in an amine should be most stable among them, which means the “free chlorine” released from the *N*-halamine is limited and held tightly. Thus, the N–X in amines may have biocidal activity for an extended time.

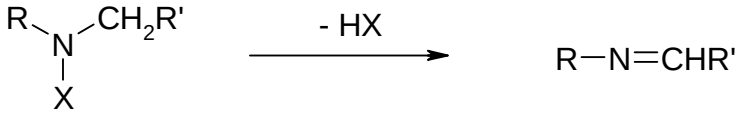
In the imide case, due to destabilization of two carbonyl groups, the N–X bond tends to release halogen rapidly. In other words, instead of the N–X bond, N^- is preferred for the stability. This can provide rapid biocidal activity.

In the amide case, it has both electron withdrawing group and electron donating group at the same time. In consequence, the stability and the releasing rate of halogen are intermediate between amine and imide. Consequently, the amine has the lowest dissociation constant and is the most stable. Conversely the imide has the highest dissociation constant and is least stable [38].

One other factor for the stability of the bonding force between halogen and nitrogen is the type of halogen. The greater the bond overlap between halogen and nitrogen, the

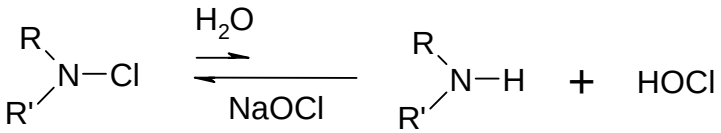
stronger the *N*-halamine bond. Among halogens except fluorine, chlorine is the smallest one. Hence, chlorine bonds with nitrogen with greatest overlap. The order of the stability in terms of the identity of halogen is N–Cl > N–Br > N–I. In the same formulae in an *N*-halamine, N–Br has been shown to have a faster biocidal activity than N–Cl.

If there is an α-hydrogen in an amine or amide, the halogen in the N–X bond can undergo dehydrohalogenation with the adjacent α-hydrogen to form C=N bonds.



In general, UV light and heat can promote this kind of reaction. After losing the halogen through dehydrohalogenation, it would not be an *N*-halamine biocide anymore. To avoid the defect of dehydrohalogenation, nitrogen in a heterocyclic structure is preferred; hence, a cyclic organic *N*-halamine biocide without α-hydrogen could be the best choice. To date Worley's group at Auburn University have synthesized various cyclic *N*-halamine precursors which are depicted in Figure 1-8.

As a biocide, refreshability is one of the most outstanding features of *N*-halamines. Even if it loses the halogen after repeated application, halogen can be recharged through simple halogenation with sodium hypochlorite solution or household bleach to recover its biocidal efficacy, as depicted in the following scheme:



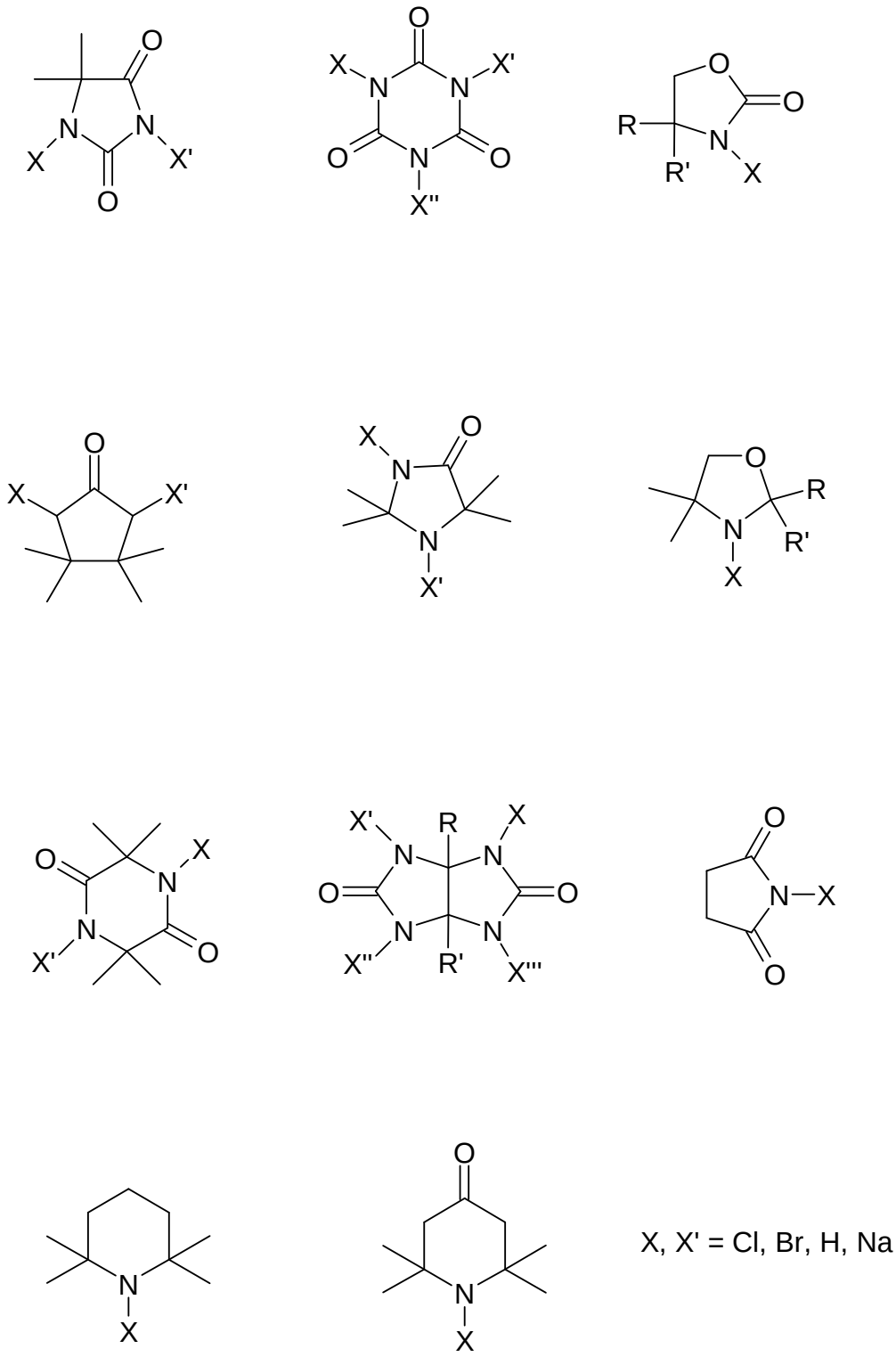


Figure 1-8 *Structures of Cyclic Organic N-halamines*

As discussed, cationic biocides, alcohols, phenols, chlorinated phenols, and metal ions have biocidal efficacy for most Gram-positive and some Gram-negative bacteria, whereas *N*-halamines, aldehydes and peroxygens are broad spectrum bactericides and sporicides. The refreshability and broad spectrum and sporicidal activity to microorganisms make cyclic *N*-halamines versatile biocides. To date, most works for cyclic *N*-halamine biocides have been devoted to water purification [39-41]. The applications of cyclic *N*-halamine for polymers and fibers have tremendous potential which has not been completely exploited.

1.5 Antimicrobial Textiles

Antimicrobial textiles are one of important and growing sectors in the textile world. Compared with conventional textiles, their antimicrobial properties broaden the application area, enhance the product value, and upgrade the service quality of textile products. Applications can be found in apparel, medical and healthcare, housing and decorative, automotive, construction, marine, and military and space, etc.

A discussion of how antimicrobial compounds function can be found in references 42-44. Selective toxicity is the desirable mode of action, and selectivity is often the deciding factor in choice of antimicrobial agent (which agent does the least harm to the life forms other than the target of the antimicrobial?). The term ecotoxicity is sometimes used to describe the effect of a discarded agent on the environment. Particular concerns are the beneficial insects, birds, and organisms at the lower end of the aquatic food chain. There is

also concern about the depletion of beneficial microorganisms, in specific local environments.

Selectivity is often a two edged sword. An antimicrobial that is very selective usually acts by blocking a specific metabolic pathway. Microorganisms can often find a way around a specific metabolic bottleneck. Indeed, the natural population of a microorganism may naturally contain strains which conduct specific metabolic reactions by different mechanisms. The antimicrobial in these cases simply provides a selection mechanism which increases the population of the unaffected (resistant) microbe.

Even though many antimicrobial chemicals can be selected, the number of antimicrobials used on textiles is very limited because of the challenges in making a textile product which is antimicrobial include the following:

Selecting an agent that will kill the undesirable microbe;

Attaching the antimicrobial agent to the textile in at least a semipermanent manner;

Insuring that the attachment to the textile does not inhibit the antimicrobial activity;

Insuring a durable effect or easy regeneration (excessive persistence is not desirable);

Insuring that the product is not excessively toxic to humans or the environment (EPA);

Insuring that microbial life does not develop immunity to the agent;

Demonstrating the antimicrobial effect to regulators and institutional legal advisors; and

Retention of other desirable fabric properties, depending on the use.

Initially the industry largely ignored the toxicity problems and did not mind using agents which were toxic to most life forms. The result was (for example) a rot resistant

cotton tentage product used by the military. The cotton fabric was treated with a mixture of chlorinated waxes, antimony and copper salts, and the fabric became resistant to water, fire and microorganisms. Among the early treatments used to impart microbial resistance in cellulosic fabrics are [45, 46]:

Cadmium, copper, chromium, mercury, tin, and zinc salts or organometallic compounds;

Phenols and various phenol derivatives;

Ammonium and phosphonium compounds;

Amino-formaldehyde resins;

Various tars and creosote compounds; and

Chemical modification of cellulose (makes cellulose indigestible to microorganisms).

Resistance to attack from microorganisms is not always the result of antimicrobial activity, and lack of microbial growth does not always indicate good biocidal activity. In particular, most synthetic textiles inherently resist attack by microorganisms, but are generally not inherently biocidal. Further, ability to inhibit growth of microorganisms does not reveal how fast, or even whether the microorganism is destroyed. (Those products that do not allow bacterial growth, but do not kill the organisms are called bacteriostatic.) With natural fibers, however, there is a significant overlap of treatments which both protect the textile and attack the microorganisms. All of the treatments which have broad-spectrum antimicrobial activity also provide some measure of protection for the textile itself. Antimicrobial compounds, some of which have been proposed for antimicrobial textiles, include:

Quaternary ammonium compounds

Chitosan

Metals and cations of heavy metals; Copper, silver, mercury, zinc, etc

Oxidizing agents; Compounds which release atomic oxygen or OH radicals

Compounds which release halogen atoms or cations

1.5.1 Quaternary Ammonium Compounds

Most of quaternary ammonium compounds for antimicrobial textiles are quaternary ammonium salts (QAS), and they, especially containing 12-18 carbons, have the best antimicrobial activity. For cellulose application, in general, the hydroxyl group of cellulose has been modified to prepare strong ionic interaction with QAS. A dichloro-*s*-triazinyl reactive group, which has a reactive sulfonate, was applied on cotton fabric. A natural quaternary ammonium cationic colorant, Berberine, was reacted with this sulfonate, the solubilizing group. The treated cotton fabrics showed an antibacterial property against *S. aureus* [47, 48].

Cationic dyes, which include QAS, were prepared. Specifically, anthraquinone derivatives were connected to QAS to make antimicrobial cationic dyes. *N*,*N*-dimethylbutylamine, *N*,*N*-dimethyloctylamine, or *N*,*N*-dimethyldodecylamine was applied as QAS parts. Those antimicrobial cationic dyes were used for dyeing acrylic fabrics, and the cationic dyed fabrics exhibited antibacterial efficacy against *E. coli* and *S. aureus* [49, 50]. Conversely, acid dyes were employed to combine QAS onto nylon fabrics. Most acid azo dyes also have sulfonate groups; thus, acid dyed nylon 66 and nylon 6 fabrics were

readily treated with QAS and durable antimicrobial nylon fabrics were prepared [51, 52]. Carboxylic acid end groups of nylon were converted to carboxylate anions with base to allow ionic interaction with cationic antimicrobial QAS. This interaction provided durable antimicrobial functions. Bulky QAS showed low exhaustion ratio and poor durability [53]. Antimicrobial wool fabrics were prepared through ionic interactions between carboxylate groups in wool protein and, QAS or cetylpyridium chloride (CPC). The antimicrobial functions of CPC survived repeated washing with better durability [54]. A quaternary ammonium surfactant, *N*-dodecyl-*N*,*N*-dimethylglycine cysteamine hydrochloride (DABM), was used for wool fabrics. The DABM treated wool fabrics showed antimicrobial activity against *B. pumilus* [55].

1.5.2 Chitosan

Because of non-toxicity to humans, ready degradation into the environment, and abundance next to cellulose, chitosan is a wide-researched material for textile finishing. Chitosan is the derivative of chitin and is prepared through the deacetylation of chitin. It is assumed that positively charged chitosan interacts with the negatively charged components of microorganisms. This may cause inactivation of microorganisms. Considerable research of chitosan applications is ongoing for antimicrobial textiles.

The antimicrobial activities dependent on different molecular weight were studied. Similar degrees of deacetylation of chitosan were used, and the treated cotton fabrics were prepared by a pad-dry-cure method [56]. Immobilizing chitosan or chitosan derivatives onto fabrics is the key to enhance durability. For this reason, corssliking agents were

employed. Butanetetracarboxylic acid (BTCA) and Arcofix NEC (low formaldehyde content) were used with chitosan to prepare a durable press finishing and antimicrobial properties at the same time. Both treated fabrics had antimicrobial activity against Gram-positive and Gram-negative bacteria [57]. Chito-oligosaccharides were applied on cotton fabrics with dimethylol dihydroxyethyleneurea (DMDHEU) as a crosslinking agent. The treated cotton fabrics showed good durability and antimicrobial activity after twenty washing cycles [58]. Citric acid (CA) and chitosan were also used for durable press and antimicrobial finishing for cotton fabrics [59]. *N*-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride (HTCC), a water-soluble chitosan quaternary ammonium derivative, was applied onto cotton fabrics with DMDHEU, BTCA and CA [60]. Methyltrimethoxysilane and γ-glycidoxypropyltrimethoxysilane were used with chitosan as flexible couplers for cotton fabrics. A pad-dry-cure process was used and chitosan-silane mixed solution showed improved antimicrobial activity and durability without significant loss of tensile strength [61].

Another method to apply chitosan or chitosan derivatives onto fabrics is by the modification of chitosan and chitosan derivatives. Thus, fiber-reactive chitosan derivatives can be readily applied to fabrics. A fiber-reactive chitosan derivative, *O*-acrylamidomethyl-*N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (NMA-HTCC), was applied to cotton fabrics, and the antimicrobial activity against *S. aureus* remained after 50 washings [62]. A fiber-reactive chitosan-cyanuric chloride (CHI-CNC) was used to enhance the durability of antimicrobial activity on cotton fabrics [63].

Blending of chitosan with other fiber forming material can produce antimicrobial

fibers. Specifically, chitosan antimicrobial fibers were prepared through chitosan/cellulose blending system. Chitosan, which was dispersed (not dissolved) in cellulose solution in dimethylacetamide (DMAc) with LiCl, was spun by wet-spinning [64].

1.5.3 Metal and Metal Salts

Except for silver and copper, others of the less toxic heavy metals exert a weak antimicrobial activity or toxicity to the environment. Thus, the major concern of metal or metal salts for antimicrobial textiles is silver and copper. The mechanism of metal or metal salts to inactivate microorganisms is due to the fact that most metal ions can combine with electron donor groups such as sulfur, oxygen, or nitrogen. Hence, in biological systems, thiols, carboxylates, phosphates, hydroxyl, amines, imidazoles and indoles can combine with the metal ions [65]. As a result, metal ions can inhibit the metabolism of microorganisms.

Layers of silver, copper, gold, platinum and platinum/rhodium (90/10) were deposited on SiO_2 fabrics using magnetron sputtering. Antimicrobial activity of copper was most effective against bacteria and fungi. Silver was also effective against bacteria, but the effectiveness was limited against fungi [66]. A protein fiber, Bombyx mori silk were modified by tannic acid (TA) or ethylenediaminetetraacetic acid (EDTA) dianhydride followed by the absorption of Cu^{2+} and Ag^{+}. The reaction of silk with EDTA–dianhydride enhanced the capacity of the fiber to absorb and bind metal cations. All metal containing silk exerted significant antimicrobial activity [67]. Cotton fabrics were modified with succinic anhydride to attach metal salt ions such as Cu^{2+}, Fe^{2+}, Fe^{3+} and Zn^{2+}. The

antimicrobial activity of Cu^{2+} treated fabric against *Escherichia coli* was the most effective [68]. After functionalizing the polyester-polyamide fabric surfaces by RF-plasma or vacuum-UV, the fabrics are immersed in solutions with different concentrations of $AgNO_3$ solution. The Ag clusters were deposited on the two polymer components of the fabric but having widely different sizes. The antimicrobial activity of the fabric was effective against *E. coli* [69]. Nano-emulsion of chitosan/silver oxide composite was prepared using ultrasound. The antimicrobial activity of the treated cotton fabric was durable and effective [70]. Nano-sized Ag colloids were used for antimicrobial activity on polyethylene/polypropylene (PE/PP) nonwovens. A dip-pad-dry method was employed, and the 10 ppm of Ag colloids treated nonwovens showed a complete inactivation against Gram-positive and Gram-negative bacteria [71]. Nano-sized silver (Ag) powder was mixed with polypropylene (PP) chip for conjugate spinning with PP. The PP/Ag nanocomposite fibers were melt-spun by co-extrusion, and the antimicrobial activity against Gram-positive and Gram-negative bacteria was effective when PP/Ag master-batches were used as the sheath [72].

1.5.4 Photocatalyst

The photocatalytic activity of titanium dioxide (titania) allows a thin layer coating of the material to exert self-cleaning and antimicrobial properties. Exposure to UV radiation results in the production of the (•OH) radical which is a strong oxidizing agent. Due to its abundance in the world, low cost, and outstanding stability, titanium dioxide could be the representative of photocatalysts. To date most of photocatalyst applications are

devoted to wall paper, or other furniture, plastic, or interior surfaces. Photocatalysts have recently been tried for antimicrobial textiles.

Thin film coating of TiO_2 was prepared on polyacrylonitrile (PAN) fibers through a dip-coating method [73]. Nanoparticles of TiO_2 added into polyester gave antimicrobial polyester fibers when extruded through melt spinning. Dipping fabric into a TiO_2 nanoparticle solution also provided a photocatalytic antibacterial textile [74]. A TiO_2-SiO_2 complex of two different sizes (90 and 30 nm) was added to the spinning solution of rayon fiber. The antimicrobial activity showed that 30 nm TiO_2-SiO_2 complex fibers had better effectiveness than 90 nm fibers [75].

1.5.5 *N*-Halamine Based Antimicrobial Textiles

N-halamines are compounds which have at least one nitrogen and halogen covalent bond within the structure. After repeated exposure to microorganisms or releasing antimicrobial functions, *N*-halamines can be recharged through simple exposure to diluted household bleach or halogen releasing agents; thus the biocidal properties of *N*-halamines could be retained indefinitely [76]. The antimicrobial mechanism of *N*-halamines against microorganisms is that the positive charged halogen transfers from the *N*-halamines to the proper reacting site of microorganisms. The reaction directly inhibits and inactivates the metabolism, and destruction of microorganisms is promoted [77, 78]. Thus, *N*-halamines are regenerable and are strong biocides against microorganisms. Various *N*-halamine compounds have been used as an effective antimicrobial compounds for treating cotton, polyester, nylon, etc.

The antimicrobial activity of cellulose with incorporation of *N*-halamine has been studied. An *N*-halamine precursor, 1,3-dimethylol-5,5-dimethylhydantoin (DMDMH) was used for the chemical finishing process with cotton fabrics. Theoretically, DMDMH is a crosslinking agent for cellulose, and DMDMH treated cotton fabric should have no available site for chlorination. In practice, however, after some loss of formaldehyde during the treatment process, the DMDMH treated cotton fabric was exposed to chlorine bleach and was turned into antimicrobial textiles [79]. A similar finishing process was employed for 3-methylol-2,2,5,5-tetramethylimidazolidin-4-one (MTMIO) and monomethylol-5,5-dimethylhydantoin (MDMH), which have one methylol group to connect to cellulose. The MTMIO and MDMH treated cotton fabrics had durable and rechargeable antimicrobial activity [80, 81]. Siloxanes have been used as an *N*-halamine precursor coupler due to versatile bonding properties on most of surfaces. For instance, an antimicrobial cotton fabric was produced using 3-trihyroxysilylpropyl-5,5-dimethylhydantoin (SPH), and the chlorinated fabrics showed a complete 5.7 log reduction against *S. aureus* and *E. coli* in 30-120 min contact-time. Other *N*-halamine siloxane monomer precursors, which are 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)imidazolidin-2,4-dione] and 3-[3-triethoxysilylpropyl-7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (TSTTDD), and a polymer precursor, poly[3-(7,7,9,9-tetramethyl-1,3,8-

triazaspiro[4.5]decane-2,4-dion-3-yl)propylhydroxy siloxane] (PTTDDS) were applied onto cotton fabrics [82, 83]. Grafting is another method to incorporate *N*-halamine precursors on textiles. An *N*-halamine precursor, 3-allyl-5,5-dimethylhydantoin (ADMH) was grafted on cotton fabrics [84]. Acrylamide (AM), methacrylamide (MAM) and tert-Bu

acrylamide (TBAM) were also grafted onto cotton fabrics followed by chlorination [85]. A dyeing process was used for application of an *N*-halamine precursor on cotton fabrics. An *s*-triazine based *N*-heterocycle, dichloro-*m*-aminophenyl hydantoinyl-*s*-triazine (DAPHT), which could be rendered antimicrobial through exposure to diluted chlorine bleach, was synthesized. Dyeing technology, particularly reactive dyeing, was used to apply the *N*-halamine precursor onto cotton fabric. The DAPHT treated cotton fabric resulted in durable and rechargeable antimicrobial properties for up to 50 standard washing cycles [86].

Synthetic fibers have been also challenged to apply *N*-halamine precursors. Polyester fabrics were modified with ammonium hydroxide solution to impart *N*-halamine moieties, and then the treated polyester fabrics were chlorinated [87]. A cyclic-amine monomer, 3-allyl-5,5-dimethylhydantoin (ADMH) was grafted on polyethylene (PE), polypropylene (PP), acrylic, polyester/polyamide (PET/PA) and polyamide 66 fabrics with triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TATAT). The treated fabrics exerted antimicrobial activity against E. coli [88]. Antimicrobial Nylon fabrics were prepared. After attaching a hydroxymethyl functional group on Nylon 66, *N*-halamine precursors were imparted. The chlorinated fabrics showed 7 log reductions against Gram-positive and Gram-negative bacteria in 30 min contact-time [89].

A wet-spinning method was used to prepare *N*-halamine antimicrobial fibers. Polystyrene hydantoin (PSH), which was an *N*-halamine precursor, and polyacrylonitrile (PAN) were blended in dimethyl acetamide (DMAc). The PSH/PAN blended fibers were spun through a dry-jet wet spinning process. The *N*-halamine fibers showed durable and regenerable antimicrobial activity after recharging up to 50 standard washing cycles

[90]. A copolymer, polyacrylonitrile-co-3-allyl-5,5-dimethylhydantoin was blended with PAN in a NaSCN aqueous solution. A wet spinning process was used, and the chlorinated blended fibers had antimicrobial ability [91].

Fiber forming polymers, which are also *N*-halamine precursors, were introduced [92]. They have advantages such as higher durability and no additional finishing.

1.5.6 Commercial Products

The considerable commercial products for antimicrobial textiles are growing. In spite of the risk that claims of antimicrobial activity might invite litigation, the number of antibacterial products has grown dramatically over the last couple of years. Sometimes the products are offered under the guise of freshness or odor control, but successful EPA/FDA registration of several products has allowed the antimicrobial claims to be advertised, and most large fiber producers now have these specialty products in their inventory. The antimicrobial products currently available in the textile/fiber market (or known by the authors to be in commercial development) include the following:

Interface Inc. developed an organic substituted ammonium phosphate sold under the name Intersept®, and used by Interface in its carpet products [93, 94].

Dow-Corning produced a series of quaternary amines which could be fixed to a surface via silane chemistry [95-97]. Aegis Environments (Midland Michigan), a spin-off company, has developed the technology under the trade name Microprobe Shield®. The product is used in a wide cross-section of the textile industry – nonwovens, wipes, medical wear, socks, athletic apparel, uniforms, floor mats, ceiling tiles etc. Familiar companies

using this technology include: Dr. Scholls®, Brillo®, Odor Eaters®, Franklin Sports, Burlington, Kaiser Roth, BBA, Precision Fabrics, Russell, US Gypsum (ceiling tiles), and others.

Bioshield, has modified the Dow chemistry in their laboratories [98-100] and has a number of companies using their technology. They claim control of dust mites via control of the fungus dust mites eat. With three recent patents and EPA registrations, Bioshield is beginning to capture some textile/nonwovens applications (Burlington House, Milyon, and others). They also offer cleaning and fabric freshening products for home use.

Triclosan appears in the patent literature as early as 1976. It is produced by Ciba and most promoted under the name Microban® (Microban Products Co., Huntersville, NC, U. S. A.). It is available in a variety of fiber and textile products [101]. It is easily the most widely known of the antimicrobial products. Most often it is incorporated into the fiber. Fibers using triclosan are available from Synthetic Industries (olefin), Sterling Fibers (acrylic), and Cydsa (acrylic) as well as from several other suppliers of acetate, olefin, and acrylic fibers.

Healthshield® Agion® appears to be a rapidly developing technology which has significant development momentum. The technology is a silver based inorganic ion exchange material and has signed on Foss, DuPont (tooth brushes), Smith and Nephew, Taconic (conveyor belting), and a variety of shoe companies, medical fabricators, metal fabricators, surface coating, and film producers. While one might question the safety of releasing silver ions, apparently the concentration is small, and the product has FDA (food

contact) and NSF (National Sanitation Foundation) approval (food and beverage contact) [102, 103].

Biguanides [104, 105] were developed for textile applications by Zeneca, (now Avecia®) and are available under the trade name Reputex®. The textile treatment works well on cellulosic fibers and is available in fiber form from Acordis® and in cotton fabric from Kendall Health Care®. A number of other manufacturers are developing products with Reputex as a fabric finishing agent. Thomson Research Associates (Toronto, Canada) sells a variety of antimicrobials under the name Ultra-fresh®. Their formulated products include iodine releasers (eg. diiodomethyl-p-tolyl sulfone), organotin compounds, isothiazalonones, quaternary ammonium compounds, and triclosan. Textile products using the Ultra-fresh® technology are available from Avondale, Charlescraft, American Textile, Spenco, Rockland and others.

Chitosan is derived from chitin, a major component in crustacean shells. Chitosan has some level of antimicrobial activity, and fibers made from chitosan are available in the marketplace. Coatings of chitosan on conventional fibers or films appear to be a more realistic prospect for development of this material. Because it does not provoke an immunological response, chitosan has been suggested for bandages, sutures and other items placed in the human body [106]. Purity will certainly be an issue in these applications.

Most *N*-halamine based antimicrobial textile applications are household articles such as socks, shirts and towels. In addition to household products, interests from military applications such as tents and army uniforms have been developing because of powerful biodcidal effects. Sheets and pillowcases containing HaloShield® (*N*-halamine

compounds made by Vanson HaloSource (Seattle, WA)) treated bed-linen products are now available from Medline Industries for use in hospitals, nursing homes and managed care facilities in the U.S., and more companies are joining to develop *N*-halamine based antimicrobial textiles; Miliken & Company (Spartanburg, SC, U. S. A.), and Ecolab Inc. (St. Paul, MN, U. S. A.) etc.

We probably should make some comment about testing. Most published data for textiles and fibers are still generated by placing a fabric on an inoculated nutrient agar plate and measuring the inhibition zone [107]. This procedure depends on antimicrobial diffusion in the agar and reveals little about the speed nor whether the action is bacteriocidal or bacteriostatic. We much prefer the process of soaking the textile with inoculum for varying time periods and washing out and growing the residual microorganisms [108]. Even this test requires a "neutralizing" solution, which may not be available. We also use a test that recirculates innoculum through a treated textile "filter" plug, with serial plating of small samples at various time intervals.

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CHAPTER 2

ANTIMICROBIAL FIBERS VIA DYEING PROCESS: SYNTHESIS AND APPLICATION OF *s*-TRIAZINE BASED NOVEL *N*-HALAMINE BIOCIDE

2.1 Introduction

Hospitals serve patients who are susceptible to viruses or bacteria, but these places can also be a source of infection. For hospital supplies, particularly polymers and fibers, sterilization is needed, and is particularly important for reusable supplies. Even though dry heat and steam sterilization offer inexpensive and simple ways to kill microorganisms, fabrics and plastics suffer from repeated exposures to heat [1]. Even after sterilization, the hospital materials still have risk of recontamination [2], and microorganisms may survive on hospital materials for several days [3, 4].

Considerable research is ongoing for inactivation of microorganisms on polymers and fibers [5-8]. Particularly *N*-halamine biocides have been attractive to researchers due to rechargeability and broad spectrum biocidal properties [9, 10]. *N*-halamines refer to organic and inorganic compounds which contain nitrogen-halogen covalent bonds. In *N*-halamines, electron-donating groups retard the release of halogen, and electron-withdrawing groups enhance it. Thus, halogen-nitrogen bonding in amines shows the lowest dissociation constant and is the most stable, whereas halogen-nitrogen

bonding in imides provides the highest dissociation constant and is the least stable. Halogen in amides has an intermediate stability between amine and imide structures [11].

To date, most *N*-halamine applications on polymers and fibers have involved drying and curing processes; however, curing treatment requires high temperatures, around 160^{o}C [11a, 12], thus requiring energy which could be saved. To provide chemical attachment while minimizing energy usage, a novel approach has been employed herein. Initially an *N*-halamine precursor is bound to a triazine-based dyeing bridge, which then reacts with cellulose to yield antimicrobial cellulosic fibers (following exposure to household bleach). This method is advantageous due to its low processing temperature (~30^{o}C). In this research, a novel *s*-triazine-based *N*-halamine will be described, and dyeing procedures for the attachment of *N*-halamine precursors in or on polymeric materials will also be discussed (see Figure 2-1).

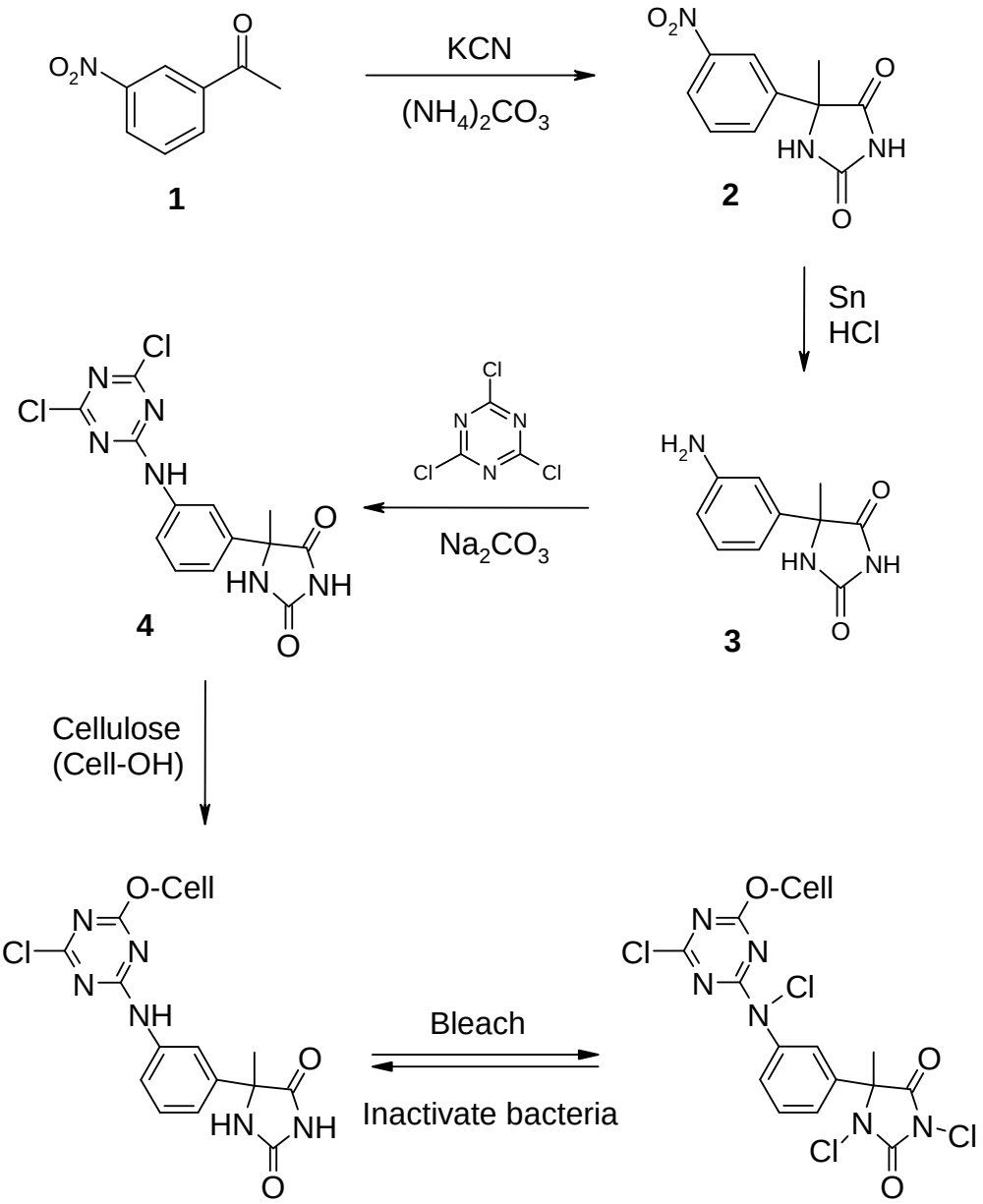

Figure 2-1 *Synthesis and application scheme of DAPHT on cotton fabrics*

2.2 Experimental

All solvents and reagents were purchased from Aldrich Chemical Co. or Fisher Scientific Co., unless otherwise stated, and were of reagent grade, and were used without further purification. A Bruker AV-400 (400 MHz) spectrophotometer was used to record ^{1}H and ^{13}C NMR spectra. Melting points (mp) were evaluated by a Mel-Temp melting

point apparatus. A Simadzu IR Pestige-21 was used to observe Fourier transform infrared (FT-IR) spectra of the samples. Thin KBr pellets containing the samples were prepared for FT-IR.

2.2.1 5-Methyl-5-(3-nitrophenyl)hydantoin (*m* -NPH) (2)

A total of 19.54 g (0.3 mol) of potassium cyanide and 57.65 g (0.6 mol) of ammonium carbonate in 100 mL water were added to 24.78 g (0.15 mol) of *m*-nitroacetophenone and 100 mL of ethanol in a 500-mL round bottom flask. The reaction mixture was heated to 80^{o}C under stirring for 8 h. The reaction mixture was cooled to ambient temperature. A 600 mL portion of 10% hydrochloric acid solution was slowly added to the reaction mixture until the pH became 6. The product was filtered and washed with distilled water. A total of 30.26 g of deep yellowish solid was obtained (Yield: 85.8%, Mp: 175^{o}C). The product exhibited the following spectral data: ^{1}H NMR (DMSO-d6) δ 1.71 (s, 3H), 7.73 (t, 1H, *J* = 8.0 Hz), 7.99 (m, 1H), 8.21 (m, 1H), 8.31 (t, 1H, *J* = 2.0 Hz), 8.85 (s, 1H), 10.98 (s, 1H); ^{13}C NMR (DMSO-d6) δ 25.6, 63.7, 119.9, 122.9, 130.3, 132.3, 142.1, 147.8, 156.1, 176.2; FT-IR (KBr) 1252, 1354, 1410, 1522, 1722, 1782, 3084, 3188 cm^{-1}.

2.2.2 5-Methyl-5-(3-aminophenyl)hydantoin (*m*-APH) (3)

A total of 23.51 g (0.1 mol) of ***m*-NPH** and 26 g (0.2 mol) of tin were stirred in 125 mL of ethanol and 100 mL of hydrochloric acid solution in a 500-mL round bottom flask; the resulting solution was heated to 105^{o}C for 6 h. Potassium hydroxide solution 10% was added to the reaction mixture up to pH 6. White solid was removed by filtration. The

solution was evaporated under lower pressure, followed by washing with ethanol and filtering. Pale brown crystals (18.50 g) were collected by evaporation of the ethanol solution (Yield: 90.2%, Mp: 77^{o}C). The product exhibited the following spectral data: ^{1}H NMR (DMSO-d6) δ 1.59 (s, 3H), 5.18 (s, 2H), 6.50 (m, 1H), 6.57 (m, 1H), 6.67 (t, 1H, *J* = 2.0 Hz), 7.00 (t, 1H, *J* = 7.9 Hz), 8.46 (s, 1H), 10.66 (s, 1H); ^{13}C NMR (DMSO-d6) δ 24.4, 63.9, 110.8, 112.7, 113.3, 128.9, 140.5, 148.8, 156.3, 177.2; FT-IR (KBr) 1261, 1408, 1605, 1717, 1771, 3237, 3343 cm^{-1}.

2.2.3 Dichloro-*m*-aminophenylhydantoinyl-*s*-triazine (DAPHT) (4)

A total of 9.22 g (0.05 mol) of 2,4,6-trichloro-1,3,5-triazine were added to 100 mL of acetone in a 500 mL round bottom flask. The solution was cooled to 0^{o}C under stirring in an ice bath for 10 min. A total of 10.26 g (0.05 mol) of ***m*-APH** were added to 25 mL of acetone and 25 mL of water, and this mixture was slowly poured into the *s*-triazine solution. The reaction mixture was stirred for another 10 min in an ice bath, and then 5.3 g (0.05 mol) of sodium carbonate were added. The reaction mixture was cooled to 0^{o}C under stirring for 3 h. The crude product was collected via filtration and washing with ice water. The resulting solid was extracted from ethyl ether to give a white solid (Yield: 61.0%, Mp: 284^{o}C). The product exhibited the following spectral data: ^{1}H NMR (DMSO-d6) δ 1.66 (s, 3H), 7.27 (m, 1H), 7.43 (t, 1H, *J* = 8.0 Hz), 7.59 (m, 1H), 7.73(t, 1H, *J* = 1.9 Hz), 8.61 (d, 1H, *J* = 1.5 Hz), 10.81 (s, 1H), 11.79 (s, 1H); ^{13}C NMR (DMSO-d6) δ 24.9, 64.3, 119.4, 121.6, 122.5, 129.5, 137.5, 141.3, 156.7, 164.4, 169.3, 177.2; FT-IR (KBr) 1227, 1456, 1551, 1719, 1755, 3277, 3321 cm^{-1}.

2.2.4 Application of *N*-halamine Precursor on Cotton Fabrics

Bleached cotton fabrics (Style #493, weight: 151 gm/m^2) were supplied by Testfabrics, Inc. (West Pittston, PA). After scouring and rinsing, the dried cotton fabric was treated with varied concentrations of neutral salt (Na_2SO_4, 0-200 g/L), base (NaOH, 0-3 %) and **DAPHT** (1-50% owf) under various conditions at 30-80^{o}C for 10-240 min to determine the optimum condition of dyeing. Unfixed **DAPHT**s were removed by boiling the samples in water.

2.2.5 Chlorination and Titration

A commercial 6% sodium hypochlorite solution was used to chlorinate the fabrics (diluted to 3,300 ppm of the commercial strength with distilled water) at pH 7 to produce biocidal materials. After soaking the fabric in the solution at ambient temperature for 60 min, and rinsing with a large excess of distilled water, the samples were dried at 45ºC for 2 h to remove any unbonded chlorine.

An iodometric/thiosulfate titration procedure was used to analyze oxidative chlorine content. The [Cl^+]% in the sample was calculated with the following equation:

$$[Cl^+]\% = (V \times N \times 35.45) / (W \times 2 \times 10)$$

Where [Cl^+]% is the wt % of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution, mL), N is equal to the normality of the titrant, and W is the weight of the sample (g).

2.2.6 Laundering Test

AATCC Test Method 61-2001 was used to investigate the stability of **DAPHT** chlorine in/on the fabric after home laundering. A Launder-Ometer was fitted with stainless steel cylinders (3 x 5 in). The volume of 150 mL of water, 0.15% AATCC detergent and 50 stainless steel balls were added to the cylinder and rotated for 45 min at 42± 0.5 RPM and 49ºC. These conditions are estimated to be the equivalent of 5 washing cycles in a home laundry. After detaching the cylinders, the fibers within the cylinder were rinsed with three 300 mL portions of distilled water and then air dried at ambient temperature.

2.2.7 Antimicrobial Test

Control and chlorinated fabrics were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) using a modified AATCC Test Method 100-1999. Bacterial suspensions (25 μL) made with pH 7 phosphate buffer were added to one inch square cotton swatches. A second swatch was sandwiched over the first to ensure contact between the suspension and the fabric. After contact times of 10, 30, and 60 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution. Serial dilutions of the quenched samples were made using pH 7 phosphate buffer, and they were plated on Trypticase soy agar. The plates were incubated at 37ºC for 24 h and then counted to determine the presence or absence of viable bacteria.

2.3 Results and Discussion

2.3.1 Synthesis of DAPHT

To synthesize structure **3** (Figure 2- 1), the method of reference [13] was

modified. The synthesis of **DAPHT** is composed of three steps: hydantoin ring-formation, reduction of the nitro group, and nucleophilic aromatic ring-substitution. First, the hydantoin ring was formed through a Bucherer-Berg synthesis [14, 15]. When *m*-aminoacetophenone was used instead of *m*-nitroacetophenone as a starting material to prepare *m*-aminophenylhydantoin **3**, no yield of **3** was observed. It is assumed that the nucleophile, the amino group in *m*-aminoacetophenone, probably attacked the carbonyl groups during the ring formation of hydantoin.

The reduction of *m*-nitrophenylhydantoin **2** is the key step to prepare the nucleophilic hydantoin derivatives. Due to good solubility of potassium chloride in water, after the hydroxide of tin is filtered out, the solution should be evaporated and dried, and then ethanol should be used to precipitate out the potassium chloride; otherwise the salt will remain as an impurity.

In fact, most applications of *N*-halamine precursors, especially hydantoin derivatives, on polymers and fibers have employed the imide nitrogen, which is more easily hydrolyzed than the amine nitrogen because the dissociation constant of the imide is much higher than the amide structure [11]. In addition, being involved in connection for most derivatives, the imide connection results in the loss of one available chlorination site. When the aromatic amine on *m*-aminophenylhydantoin **3** is used for connections, more stable bonding than for the imide site can be obtained. Furthermore, two more chlorination sites are available.

The *m*-aminophenyl hydantoinyl-*s*-triazine **4** is insoluble in ethyl ether unlike the reactants 2,4,6-trichloro-1,3,5-triazine and *m*-aminophenylhydantoin **3**. Therefore, the product is readily recovered from ethyl ether.

2.3.2 Optimum Conditions for Halamine Precursor Application

In practice, *procion* type dyes have *s*-triazine-based linking structures with halogen, especially chlorine. Two chlorines on *s*-triazine, *procion* cold type, result in low dyeing temperature (~30^{o}C). In like manner, due to the dichloro-triazine structure, **DAPHT** is applied on cotton fabric at lower temperature through a dyeing process.

Because cellulose does not bind chlorine, the effectiveness of the **DAPHT** concentration on cotton fabrics was measured through content of chlorine on the fabrics. The various conditions studied herein are given in Table 2-1. The results in Figure 2-2 show that the higher concentration of the neutral salt provided, the more bonds between **DAPHT** and the cotton cellulose were achieved. In fact, the major purpose of the neutral salt in the dyeing system is to decrease repulsion between the dye and fiber, thus allowing a close approach and more effective bonding [16]. After dissolving **DAPHT** in base (NaOH), the imide nitrogen has a negative charge because of deprotonation; hence, the reduced repulsion force by neutral salt leads to an enhanced reaction. Our previous work has indicated that at least 0.1% oxidative chlorine content is required for rapid inactivation of bacteria. A concentration of over 50 g/L of Na_2SO_4 should be available for biocidal application of **DAPHT**-treated-cotton.

Figure 2-3 shows the effect of base during the dye application. It is presumed that the base, sodium hydroxide, is involved in two roles, dissolving **DAPHT** and driving the nucleophilic substitution caused by cellulosate anions. It is assumed that sodium hydroxide exists predominantly to deprotonate the **DAPHT**, which results in dissolution, and to

form cellulosate anions; however, over 0.5% of sodium hydroxide may destroy the rings in the hydantoin moiety. Without base, the substitution reaction did not occur effectively because of a limited solubility of **DAPHT** and lack of nucleophiles.

Table 2-1 Optimization scheme of dyeing process

Variable	*Constant*				
	Salt (g/L)	NaOH (%)	DAPHT (%, owf)	Dyeing Temp. (^{o}C)	Dyeing time (min)
0 10 50 100 150 200	-	0.5	10	30	60
0 0.1 0.5 1.0 1.5 2.0 3.0	150	-	10	30	60
1 3 5 10 2 30 50	150	0.5	-	30	60
30 40 50 60 70 80	150	0.5	10	-	60
10 30 60 90 120 240	150	0.5	10	30	-

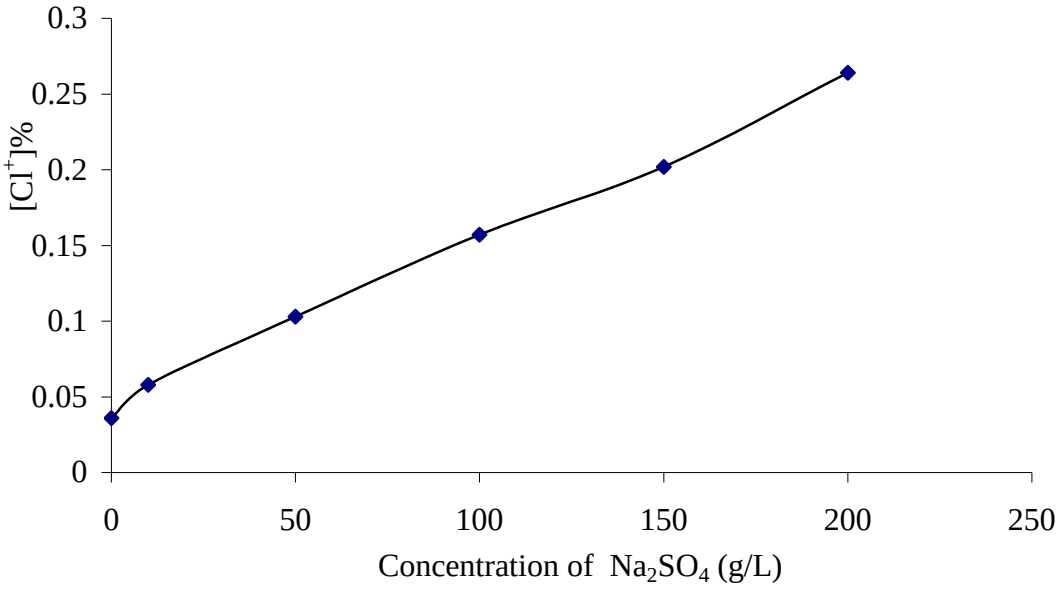

Figure 2-2 *Effect of neutral salt for the dyeing process*

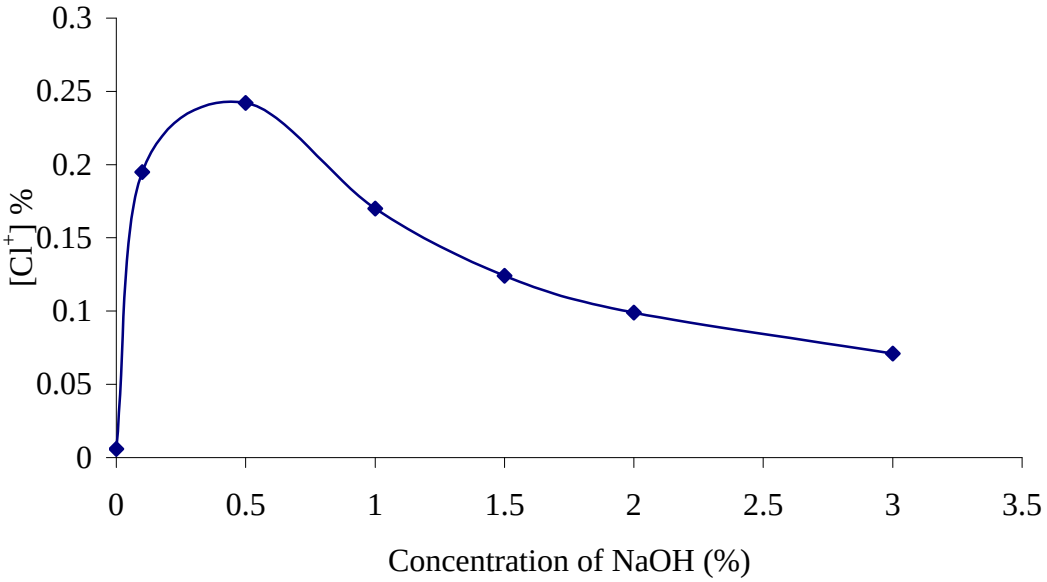

Figure 2-3 *Effect of base for the dyeing process*

Figure 2-4 shows that increasing the concentration of **DAPHT** results in a higher amount of **DAPHT** on cotton fabrics. The amount of **DAPHT** gradually increased up to 30% owf; however, at 50% owf the amount on cotton declined. In general, after the critical point, the fixation amount of reactive dye is maintained with small variation even at higher dye concentration due to the dyeing sites being occupied [17]. The results in Figure 2-4 were not expected. It is assumed that at 50% owf of **DAPHT**, the 0.5% concentration of sodium hydroxide used was not enough to form cellulosate anions of cellulose after deprotonation of **DAPHT**; thus, the effectiveness of reactivity was decreased. Because of showing over 0.2 $[Cl^+]$%, 10% owf concentration would be cost effective in the actual application for cotton fabrics.

The results in Figure 2-5 show the reactivity of the **DAPHT** at various dyeing temperatures. It is obvious that the nucleophilic aromatic substitution of dichloro-*s*-triazine derivatives was dominated by temperature. Trichloro *s*-triazine has a unique reactive temperature depending on the number of chlorines on the ring. The first, second and third chlorines are generally substituted by nucleophiles at around 0^oC, 30^oC, and 80^oC, respectively [18]. In the case of trichloro-*s*-triazine, two other chlorines on the ring attract electrons inductively; thus, the reactivity of the first target carbon is increased. In like manner, a chlorine attracts electrons in dichloro-*s*-triazine derivatives, and it enhances the reaction at 30^oC.

Conversely, at increased temperature, the reactive site suffers from hydrolysis, and the attractive force of the dichloro-*s*-triazine derivatives toward cotton is reduced [19]. This could explain the effect of temperature on **DAPHT**.

Sufficient bonding appeared in 60 min dyeing time (Figure 2-6); longer dyeing

time of **DAPHT** after 60 min will not provide more bonding to cotton. Thus, the best time for the application of **DAPHT** is about 60 min.

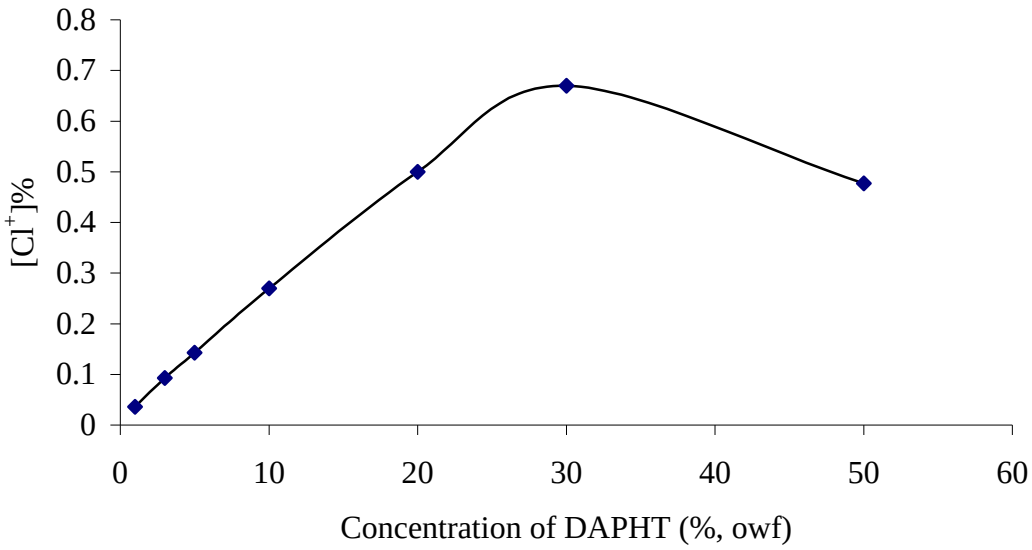

Figure 2-4 *Effect of concentration of DAPHT for the dyeing process*

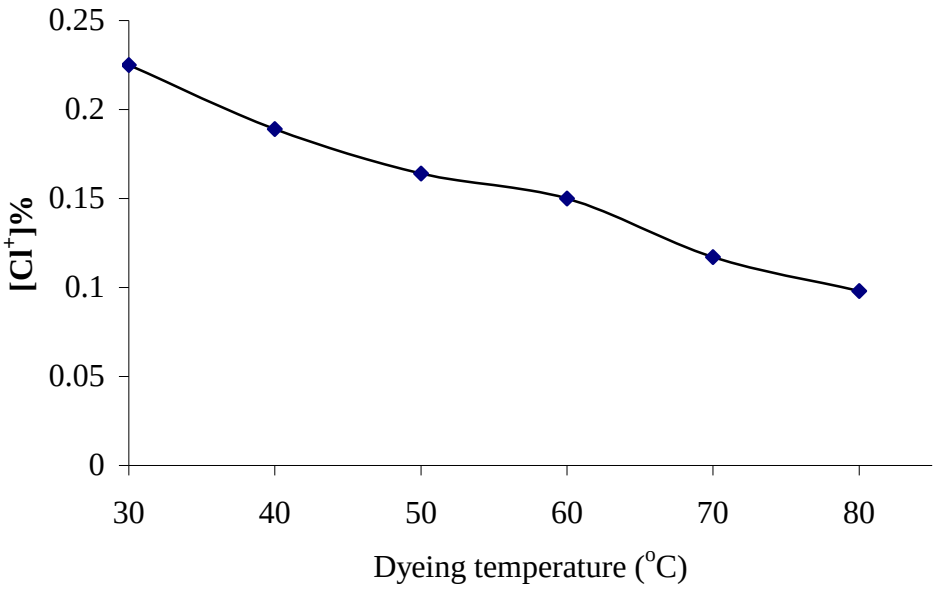

Figure 2-5 *Effect of dyeing temperature for the dyeing process*

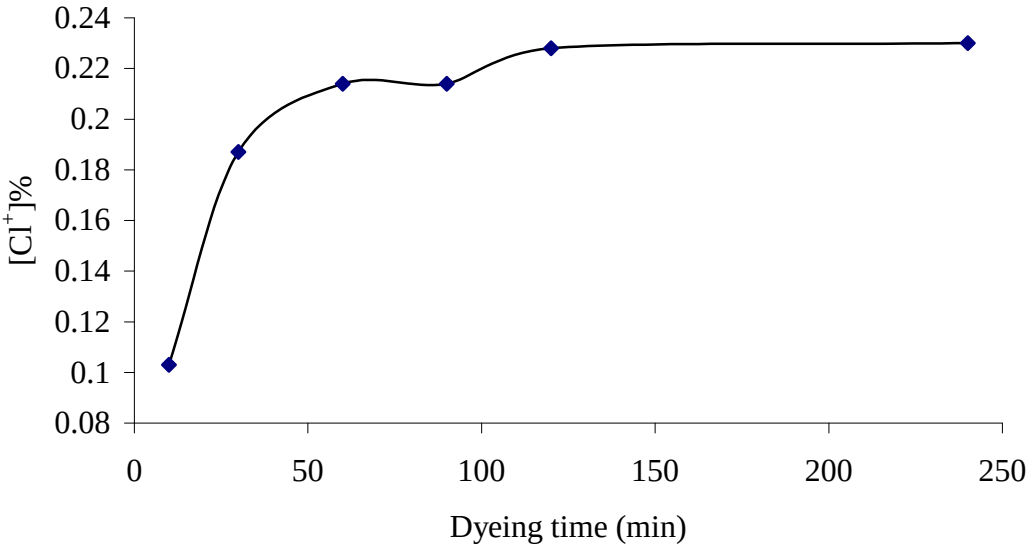

Figure 2-6 *Effect of dyeing time for the dyeing process*

The carbonyl groups in the hydantoin ring exhibit two prominent bands in the FT-IR: 1720 and 1780 cm^{-1} [12, 20]. To identify the moiety of the hydantoin ring, the FT-IR spectra of **DAPHT** on cotton fabrics were measured. The two prominent carbonyl bands in **DAPHT** treated cotton fabrics are shown at 1724 and 1770 cm^{-1} (Figure 2-7b). After chlorination, the carbonyl bands shifted to 1747 and 1805 cm^{-1} (Figure 2-7c), respectively. The band shifts correspond to the results for dichlorinated polystyrene hydantoin [21].

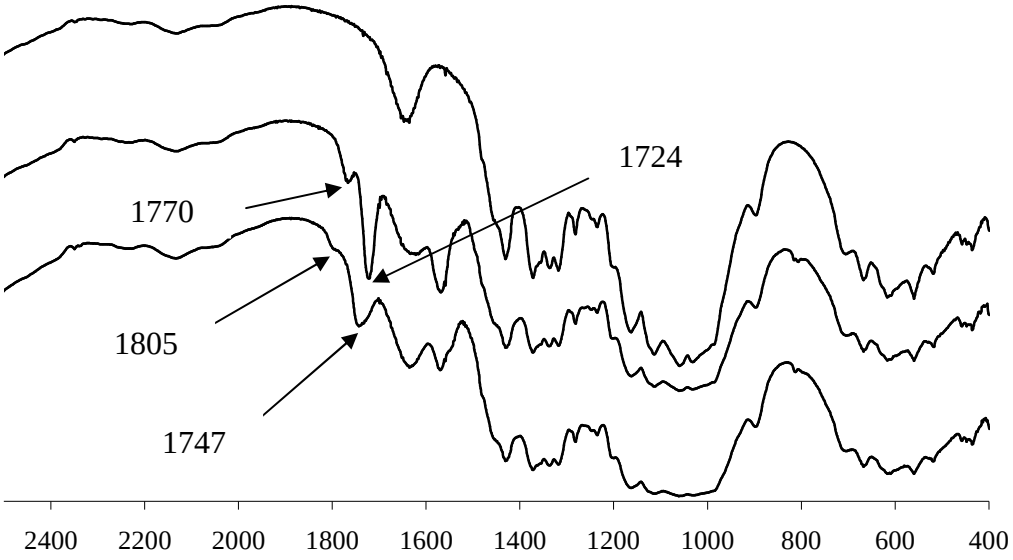

Figure 2-7 *FT-IR spectra of (a) control cotton, (b) DAPHT treated (30% owf) and (c) DAPHT treated (30% owf) with chlorination*

2.3.3 Durability and Rechargeability of the Antimicrobial Fibers

The influence of chlorine concentration on the treated fabrics was mainly affected by pH values (Table 2-2). At pH 11, the chlorine content was 0.198%; however, the content was 0.386% at pH 5, almost double the amount. The chlorine content was increased as pH was decreased. The lower the pH value in the bleach solution during chlorination, the more protons are present, which promotes the creation of Cl^+. Thus, Cl^+ directly substitutes for hydrogen on the nitrogen atom.

To evaluate the amount of **DAPHT** on fabrics from the dyeing solution, an indirect measurement is available. In Table 2-2, the highest chlorine content was gained at pH 5, and it could be hypothesized that all of the chlorine available sites were chlorinated. The reacted **DAPHT** to cotton in the dyeing solution was 50.15%. It is predicted that other **DAPHT** may be hydrolyzed in the basic condition.

Even though a lower pH value is preferred for the antimicrobial aspect, the stability of sodium hypochlorite (NaOCl) solution decreases dramatically at lower pH values, so basic conditions are attractive for practical applications. The results in Table 2-3 show that the practical chlorine solution (200 ppm) for home laundry provides enough chlorine (>0.14%) to inactivate microorganisms [22] on the treated cotton fabric. This result suggests that practical chlorination of **DAPHT** treated cotton fabrics is available through simple laundering.

Table 2-2 Effect of pH during chlorination [a]

pH of the bleach solution	$[Cl^+]$%
4	0.383
5	0.386
7	0.279
9	0.212
11	0.198

[a] **DAPHT** 10% owf treated fabrics were used and chlorine concentration of the bleach solution was 3300 ppm.

Table 2-3 Chlorination effect during standard and modified washing test

Unit: $[Cl^+]$%

Washing cycles [a]	[b]AATCC Reference Detergent (0.15%)	[c]Arm and Hammer Detergent (0.15%)
0	0	0
5	0.161	0.145
10	0.157	0.148

[a] Modified AATCC Test Method 61-2001 was used.

[b] Including 200 ppm chlorine solution, pH=10.6.

[c] Including 200 ppm chlorine solution, pH=11.1 (market available brand).

N-halamines can be recharged after exposure to microorganisms or other chemical reactions of Cl^+. Table 2-4 shows recharging effectiveness and durability of **DAPHT** treated cotton fabrics through washing cycles (AATCC Test Method 61-2001). After a period of washing cycles, the fabrics released most of Cl^+; however, recharging after 50 washing cycles recovered up to 61% of the original content, indicating that most of the chlorine precursor remained on the fabric.

In general, pre-chlorinated samples have enhanced oxidative chlorine washing fastness, due to increased hydrophobicity and reduced chance to be hydrolyzed. However, as shown in Table 2-4, the durability of pre-chlorinated fabrics is lower than that of unchlorinated fabrics for the **DAPHT** treated cotton. We focus on the carbon on the *s*-triazine ring, which is connected to the hydantoin derivative. After chlorination, the chlorine on the amino nitrogen, which is linked to the carbon, attracts electrons inductively; thus, the carbon, which already has partial positive charge, has an enhanced positive charge and may be hydrolyzed. It is assumed that increased possibility of hydrolysis results in a decreased durability of pre-chlorinated **DAPHT** fabrics.

Table 2-4 Durability and rechargeability of the fabrics

Unit: $[Cl^+]$%

Washing Cycles	Group 1 [a]	Group 2 [b]	Group 3 [c]
0	0.293	0.293	0.293
5	0.279	0.033	0.219
10	0.257	0.022	0.219
25	0.254	0.019	0.184
50	0.250	0.011	0.180

[a] Group 1: Unchlorinated samples, after each cycle were chlorinated.

[b] Group 2: Prechlorinated samples.

[c] Group 3: Prechlorinated samples, after each cycle were rechlorinated.

2.3.4 Antibacterial Efficacy against Gram-positive and Gram-negative Microorganisms

Antibacterial efficacy of the **DAPHT** treated fabrics was evaluated against Gram-positive and Gram-negative bacteria. Table 2-5 shows that chlorinated samples inactivate both bacteria with 6 log reduction in 30 min. Moreover, the same result against *S. aureus* (Gram-positive) was achieved within 10 min. However, the samples are less rapidly effective against *E. coli* (Gram-negative) with a 4 log reduction within 10 min. Due to porins, which are water-filled tubes in the outer membrane of Gram-negative bacteria, hydrophobic biocides have difficulty penetrating the membrane [23]. Because N-H bonds react to N-Cl bonds after chlorination, *N*-halamines have enhanced hydrophobicity after

chlorination; thus, bacterial efficacy against Gram-negative bacteria is generally less sensitive than for Gram-positive bacteria.

Table 2-5 Biocidal efficacy against microorganisms

Samples[a]	Contact time	*Escherichia coli* O157:H7 [b]		*Staphylococcus aureus* [c]	
		Bacterial reduction		Bacterial reduction	
		% reduction	Log reduction	% reduction	Log reduction
Unchlorinated	10 min	53.13	0.3	90.89	1.0
	30 min	69.95	0.5	92.52	1.1
	60 min	89.80	1.0	94.47	1.3
Chlorinated	10 min	99.99	4.1	100	6.3
	30 min	100	6.4	100	6.3
	60 min	100	6.4	100	6.3

[a] DAPHT 10% owf treated fabrics were used.

[b] Total bacteria: 2.43×10^6 (cfu/sample).

[c] Total bacteria: 2.06×10^6 (cfu/sample).

2.4 Conclusions

After synthesizing and characterizing, an *s*-triazine-based *N*-halamine precursor, **DAPHT**, was applied to cotton fabrics through a reactive dyeing process. The optimum conditions through the dyeing process were investigated. Due to low dyeing temperatures, the application allows mild processing conditions. Since even after 50 washing cycles the recharged chlorine-recovery was over 60%, the durability of the material is sufficient for practical application. Bacterial efficacy tests resulted in inactivation of both Gram-positive and Gram-negative bacteria in 30 min with 6 log reductions.

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CHAPTER 3

ANTIMICROBIAL FIBERS CREATED VIA POLYCARBOXYLIC ACID DURABLE PRESS FINISHING

3.1 Introduction

Enhanced probability of contaminants, such as viruses and bacteria, can increase the risk of the spread of infections/diseases between patients and staff; thus, this issue is a major priority and concern for hospitals. In order to reduce this risk and address safety issues, hospitals spend a great deal of money on disposable items, specifically on fabrics and plastics, and by the use of sterilization processes for reusable items. This methodology, however, poses some defects. Disposables create waste and leave a negative effect on the environment. In addition, incineration and landfilling, used to treat the waste, create potential hazards [1]. Sterilized items still have a risk of possible contamination due to process ineffectiveness as well as recontamination after sterilization [2,3]. In an effort to reduce waste and assure that no contamination is present, fabrics and plastics with rechargeable biocidal properties offer an attractive alternative.

To date, considerable work has been done on biocides for fabrics and polymers which will inactivate microorganisms [4-7]. *N*-halamines have been shown to be effective biocides. These are compounds which have at least one of nitrogen - halogen covalent bond in the structure. After exposure to microorganisms or reactive compounds which react with

halogen, the *N*-halamines could be recharged through simple exposure to diluted household bleach or halogen releasing agents. Thus the biocidal properties of *N*-halamines could be retained indefinitely [3]. The antimicrobial mechanism of *N*-halamines against microorganisms is considered to be that positive halogen from the *N*-halamine transfers to the proper reacting site of microorganisms and inactivates the microorganism [8,9]. The advantages are that *N*-halamines are rechargeable, and are strong biocides against microorganisms.

A crosslinking agent, dimethylol-5,5-dimethylhydantoin (DMDMH) was used for *N*-halamine antibacterial finishing [10]. Some loss of formaldehyde on DMDMH creates available halogen sites; however, formaldehyde is toxic to humans and is a probable carcinogen [11]. Considerable research has been employed to find alternative wrinkle-resistant finishing agents which do not release formaldehyde. Due to absence of formaldehyde release and their water or water/ethanol solubility, as well as good wrinkle-resistance, polycarboxylic acids have attracted attention as an alternative [11-13].

In this research, we will demonstrate:

the synthesis and characterization of an *N*-halamine, *m*-aminophenylhydantoin (*m*-APH),

the application of the compound to cotton via durable - press finishing treatment with polycarboxylic acids (Figures 3-1 and 3-2),

the efficacy of the treatment in providing durable-press fabric, and

the efficacy of the treated cotton as an antimicrobial fabric.

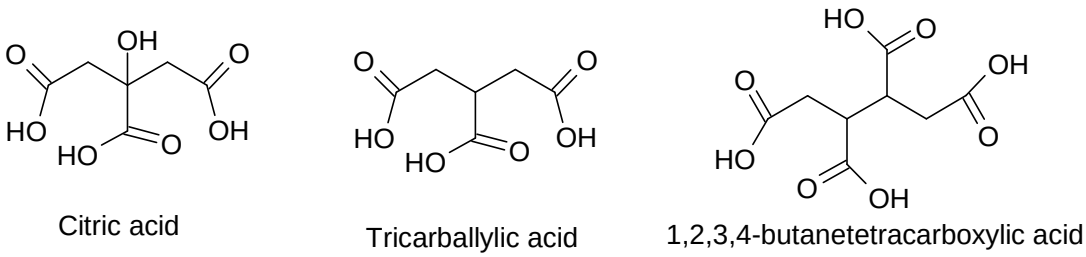

Figure 3-1 *The structure of polycarboxylic acids*

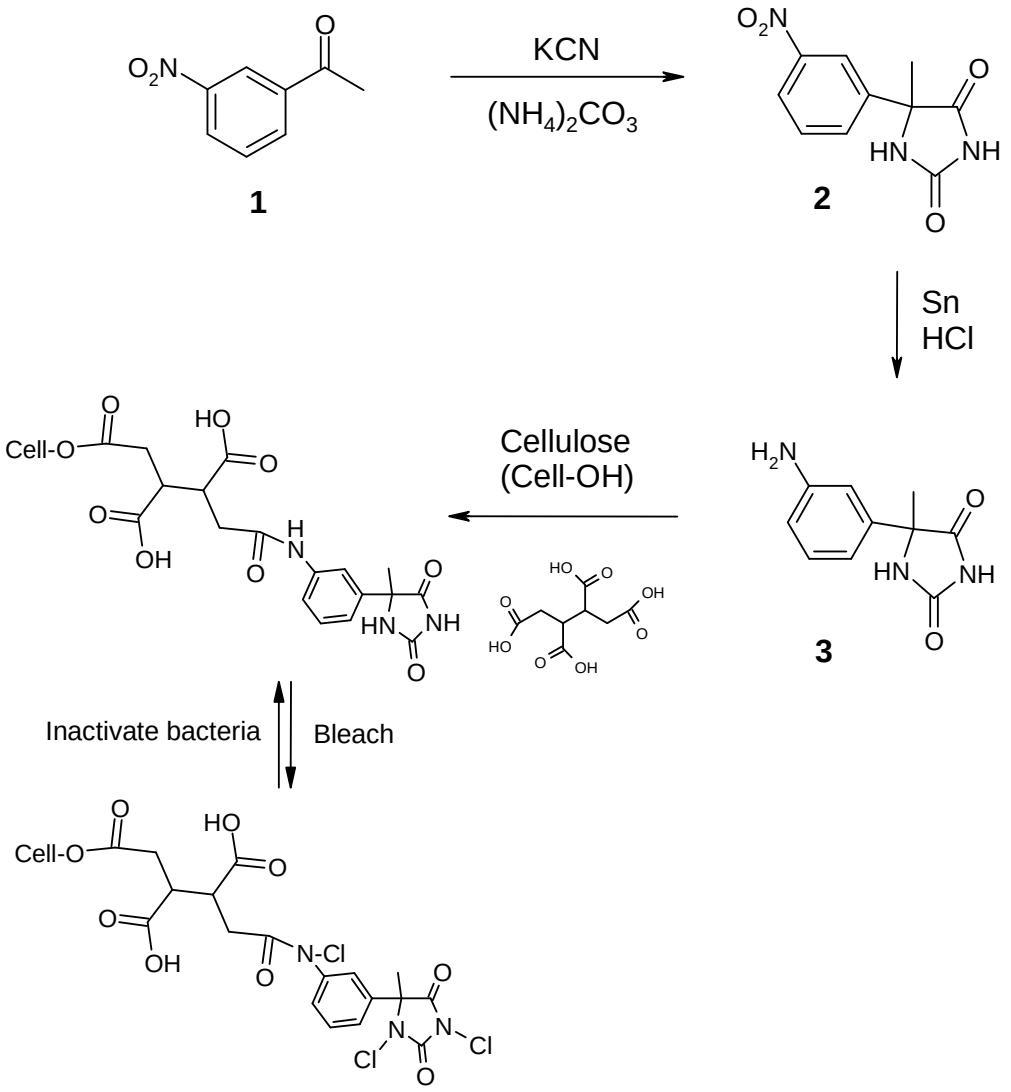

Figure 3-2 *Synthesis and one of possible application schemes of DAPHT on cotton fabrics*

3.2 Experimental

3.2.1 Materials

Citric acid, tricarballylic acid, 1,2,3,4-butanetetracarboxylic acid, *m*-nitroacetophenone, potassium cyanide, ammonium carbonate and sodium hypophosphite were purchased from Aldrich (Milwaukee, WI). Unless otherwise stated, the chemicals were of reagent grade, and used without further purification. Bleached cotton fabric (Style #493, weight: 151 gm/m^2) was supplied by Testfabrics. INC. (West Pittston, PA).

3.2.2 Instruments

^{1}H NMR and ^{13}C NMR spectra were recorded by Bruker AV-400 (400 MHz) spectrophotometer. Melting points were evaluated by a Mel-Temp melting point apparatus. FT-IR spectra were observed using a Simadzu IR Pestige-21 with potassium bromide compressed pellets which contained treated samples.

3.2.3 Synthesis of *m*-nitrophenylhydantoin (*m*-MPH) (2)

A total of 19.54 g (0.3 mol) of potassium cyanide and 57.65 g (0.6 mol) of ammonium carbonate in 100 mL water were added to 24.78 g (0.15 mol) of *m*-nitroacetophenone and 100 mL of ethanol in a 500 mL round bottom flask. The reaction mixture was heated to 80^{o}C with stirring for 8 h. The mixture was then cooled to ambient temperature. A 600 mL portion of 10% hydrochloric acid solution was slowly added to the reaction mixture to adjust down to pH 6. The product was filtered and washed with distilled water. A total of 30.26 g of deep yellowish solid was obtained (Yield: 85.8%, Mp:

175^{o}C). The product had the following spectral data: ^{1}H NMR (DMSO-d6) δ 1.71 (s, 3H), 7.73 (t, 1H, *J* = 8.0 Hz), 7.99 (m, 1H), 8.21 (m, 1H), 8.31 (t, 1H, *J* = 2.0 Hz), 8.85 (s, 1H), 10.98 (s, 1H); ^{13}C NMR (DMSO-d6) δ 25.6, 63.7, 119.9, 122.9, 130.3, 132.3, 142.1, 147.8, 156.1, 176.2; FT-IR (KBr) 1252, 1354, 1410, 1522, 1722, 1782, 3084, 3188 cm^{-1}.

3.2.4 *m*-aminophenylhydantoin (*m*-APH) (3)

A total of 23.51 g (0.1 mol) of *m*-nitrophenyl hydantoin and 26 g (0.2 mol) of Tin were stirred in 125 mL of ethanol and 100 mL of hydrochloric acid in a 500 mL round bottom flask. The resulting solution was heated to 105^{o}C for 6 h. Potassium hydroxide solution (10%) was added to the reaction mixture up to pH 6. The white solid (salt) was removed by filtration. The remaining solution was evaporated under lowered pressure resulting in a pale brown solid which was dissolved in ethanol. Pale brown crystals (18.50 g) were collected by evaporation of the ethanol solution (Yield: 90.2%, Mp: 77^{o}C). The product had the following spectral data: ^{1}H NMR (DMSO-d6) δ 1.59 (s, 3H), 5.18 (s, 2H), 6.50 (m, 1H), 6.57 (m, 1H), 6.67 (t, 1H, *J* = 2.0 Hz), 7.00 (t, 1H, *J* = 7.9 Hz), 8.46 (s, 1H), 10.66 (s, 1H); ^{13}C NMR (DMSO-d6) δ 24.4, 63.9, 110.8, 112.7, 113.3, 128.9, 140.5, 148.8, 156.3, 177.2; FT-IR (KBr) 1261, 1408, 1605, 1717, 1771, 3237, 3343 cm^{-1}.

3.2.5 Application of *m*-APH on Cotton Fabrics

After scouring and rinsing, the dried cotton fabric was treated with different polycarboxylic acids dissolved in 50/50 v/v water/ethanol, with/without sodium hypophosphite (NaH_2PO_2) as a catalyst. The soaked cotton fabrics were dried at 85 ºC for

5 min followed by curing at various temperatures for 2 min [11]. The cured fabrics were soaked in ethanol/distilled water (50/50, v/v) solution for 10 min to remove unreacted materials, followed by rinsing with distilled water.

3.2.6 Chlorination and Titration

A commercial 6% sodium hypochlorite solution was used to chlorinate the fabrics (diluted from the commercial strength with distilled water). The chlorination treatment was adjusted to pH 7 with acetic acid. After soaking the fabric in the solution at ambient temperature for 60 min, and rinsing with a large excess of distilled water, the samples were dried at 45ºC for 2 h to remove any unbound chlorine.

An iodometric/thiosulfate titration procedure was used to analyze oxidative chlorine content. The $[Cl^+]$% in the sample was calculated with the following equation:

$$[Cl^+]\ \% = (V \times N \times 35.45) / (W \times 2 \times 10)$$

where $[Cl^+]$% is the wt% of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution (mL)), N is equal to the normality of the titrant, and W is the weight of the sample (g).

3.2.7 Testing Methods

Wrinkle recovery angle (WRA) – warp (W) + filling (F) – of the treated cotton fabrics was evaluated using AATCC test method 66-1998, Option 2. Specimens were

prepared in 40 x 15 mm, and 500 ± 5 g of weight was loaded on the folded specimens for 5 min. The recorder's vertical-angle guide lines were aligned, and the recovery angles were measured

AATCC test method 61-2001 was used to perform the stability of DAPHT chlorine in/on the fabric after home laundering. A Launder-Ometer was fitted with stainless steel cylinders (3 x 5 in). The volume of 150 mL of water, 0.15% AATCC detergent and 50 stainless steel balls were added to the cylinder and rotated for 45 min at 42± 0.5 RPM and 49ºC. These conditions are estimated to be the equivalent of 5 washing cycles in a home laundry. After detaching the cylinders, the fibers within the cylinder were rinsed with three 300 mL portions of distilled water then air dried at ambient temperature.

Control and chlorinated fabrics were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 43895) using a modified AATCC Test Method 100-1999. A total of 25 μL bacterial suspensions made with pH 7 phosphate buffer were added to the fabrics. Upon doing so, another swatch was sandwiched over it to ensure contact between the suspension and the fabric. After contact times of 1, 5, and 10 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution. Serial dilutions of the quenched samples were made using pH7 phosphate buffer, and plated on Trypticase soy agar. The plates were incubated at 37ºC for 24 h and then counted to determine the presence or absence of viable bacteria.

3.3 Results and Discussion

3.3.1 *N*-Halamine Precursor with Crosslinking Agents

An *N*-halamine precursor, *m*-aminophenylhydantoin (*m*-APH) was applied on cotton fabric using polycarboxylic acids. The results in Figure 3-3 show the infrared spectra of *m*-APH and 1,2,3,4-butanetetracarboxylic acid (BTCA) treated samples. Carbonyl groups on BTCA result in a prominent band at 1732 cm^{-1}. However, because of carbonyl-band confliction between BTCA and *m*-APH, a distinguishable carbonyl band of *m*-APH did not appear on BTCA and *m*-APH treated sample (Figure 3-3, b). After subtracting the spectrum of BTCA treated cotton fabric from BTCA and *m*-APH treated cotton fabric, the carbonyl band of *m*-APH appeared at 1717 cm^{-1} (Figure 3-3, c), and this band corresponds to the carbonyl band of *m*-APH (Figure 3-3, d). It is obvious that BTCA is employed as a crosslinking agent between *m*-APH and cotton cellulose because there is no proper site for chemical bonding between *m*-APH and cotton.

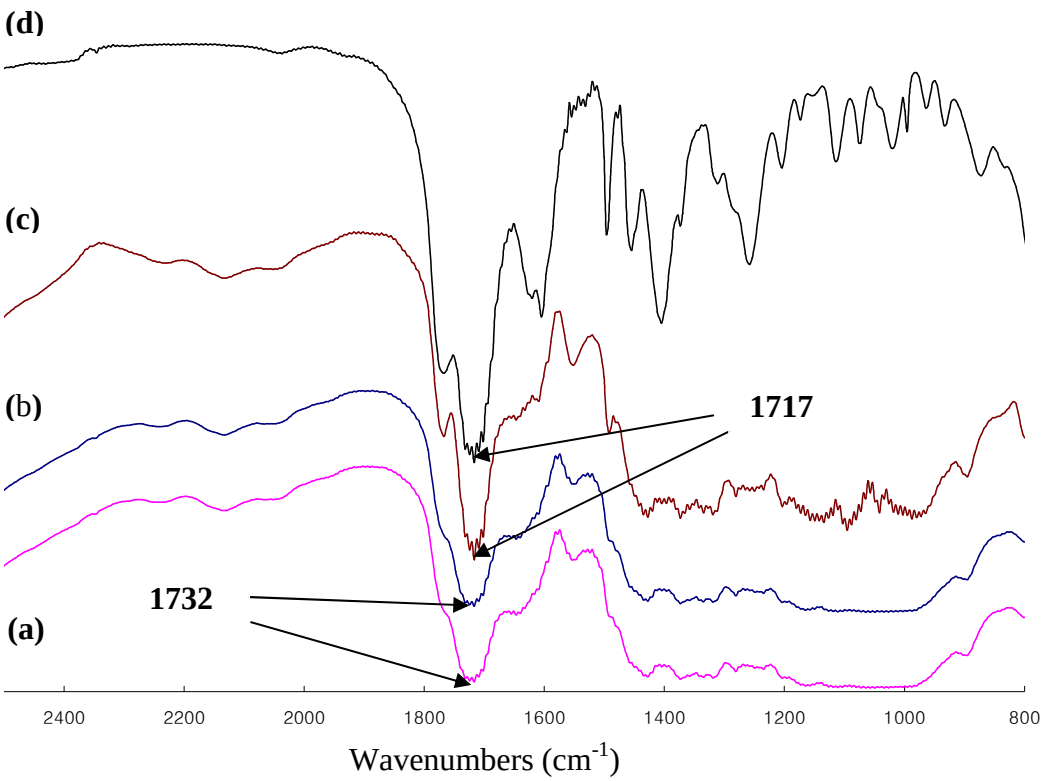

Figure 3-3 *FT-IR spectra of (a) BTCA (7.0%)/NaH_2PO_2 (4%) treated, (b) BTCA (7.0%)/NaH_2PO_2 (4%) with m-APH (6.15%) treated, (c) the different spectrum, (b) – (a), and (d) m-APH*

3.3.2 Wrinkle Recovery Angle (WRA) and Tensile Strength of Polycarboxylic Acids with *m*-APH

Polycarboxylic acids provide ester cross-linking after reacting with hydroxyl groups on cellulose chains and result in a durable press finish [11]. The *m*-APH can be applied on cotton using formation of an amide linkage with polycarboxylic acid followed by an ester linkage between the carboxylic acid and cellulose (see Figure 3-2). If ester formation (cellulose-BTCA) dominates the linkage forming reaction, *m*-APH will not be bonded to the

cotton; however, the amine on *m*-APH is a nucleophile, and amines are known as stronger nucleophiles than are hydroxyl groups [16]. It has been also revealed that the esterification mechanism of polycarboxylic acids with cellulose is completed through a cyclic anhydride intermediate [15]. Thus it is expected that the amine would react easier with the carboxylic anhydrides (inter mediates of polycarboxylic acids). It is proposed that the amidation efficacy can be measured through chlorine contents, whereas WRA could provide the efficacy of esterification. The results in Table 3-1 show that the WRA of treated samples were varied depending on curing temperatures. In addition, the higher the chlorine loading on the samples, the more the *m*-APHs are connected with cotton using polycarboxylic acids. Similarly, an increased WRA indicates enhanced cross-linking of polycarboxylic acid with cotton.

In the citric acid (CA) case, the ester cross-linking occurred effectively at 160^{o}C, unless the *m*-APH was used. Since, without increasing of WRA, chlorine content began to increase, it is assumed that the amidation formed first and then esterification had occurred. In fact, the hydroxyl group on citric acid hinders the esterification with the hydroxyl group of cellulose [12]. Therefore, the amidation may readily dominate the reaction with CA.

Tricarballylic acid (TCA) and 1,2,3,4-butanetetracarboxylic acid (BTCA) showed similar results with *m*-APH at 140^{o}C; however, particularly with TCA, the primary hydroxyl group on cotton might have enhanced compatibility to the aromatic amine on *m*-APH at higher temperature, because over 180^{o}C, the WRA – controlled by esterification – has increased, whereas chlorine content (from *m*-APH) has decreased. After esterification

(with cotton) or amidation (with *m*-APH), TCA could release another chance to make a cyclic anhydride intermediate.

BTCA, which has one more carboxylic group than TCA, could form two cyclic anhydride intermediates at the same time. Consequently, it is presumed that four carboxylic groups on BTCA more readily allowed ester and amide connections than for TCA.

In practice, BTCA might be the preferred candidate for the antimicrobial fiber because CA and TCA treated fabrics showed WRA at a treatment temperature over 200^{o}C unlike BTCA (between 160-180^{o}C). This high temperature does not make the process attractive. In addition, CA and TCA treated fabrics with *m*-APH had lower WRA than BTCA with *m*-APH.

Table 3-1 Recovery angles and chlorine content of poly carboxylic acids with/without *m*-APH (Between polycarboxylic acid and *m*-APH, equal molecular ratio was used)

Samples [a]		Curing Temperature for 2 min					
		120°C	*140°C*	*160°C*	*180°C*	*200°C*	*220°C*
Control	WRA (W+F)	119 ±2.8	118 ±1.0	117 ±1.2	120 ±0.8	119 ±2.0	119 ±2.0
	$[Cl^+]$%	0.002	0.002	0.002	0.002	0.002	0.002
Citric acid (3.5%) [b]	WRA (W+F)	137 ±2.4	141 ±1.5	212 ±2.7	277 ±2.3	263 ±3.0	242 ±2.8
Citric acid (3.5%) + *m*-APH (6.15%) [b]	WRA (W+F)	132 ±2.8	134 2.6±	130 ±2.8	147 ±1.6	188 ±2.5	190 ±3.3
	$[Cl^+]$%	0.024	0.047	0.204	0.357	0.804	1.286
Tricarballylic acid (5.3%) [b]	WRA (W+F)	124 ±1.8	158 ±2.7	205 ±2.3	240 ±3.0	281 ±2.4	263 ±5.1
Tricarballylic acid (5.3%) + *m*-APH (6.15%) [b]	WRA (W+F)	124 ±2.5	123 ±2.2	158 ±1.1	159 ±1.6	171 ±3.8	195 ±3.2
	$[Cl^+]$%	0.028	0.062	0.461	0.712	0.538	0.489
1,2,3,4-butanetetracarboxylic acid: (7.0%) [b]	WRA (W+F)	140 ±3.6	250 ±3.9	271 ±1.5	279 ±4.1	283 ±3.4	278 ±1.6
1,2,3,4-butanetetracarboxylic acid: (7.0%) + *m*-APH (6.15%) [b]	WRA (W+F)	129 ±1.6	141 ±2.7	166 ±2.7	243 ±2.7	209 ±4.1	213 ±4.6
	$[Cl^+]$%	0.026	0.139	0.970	1.492	1.523	1.531
1,2,3,4-butanetetracarboxylic acid: (7.0%) [c]	WRA (W+F)	144 ±2.6	145 ±2.3	166 ±2.0	237 ±3.5	241 ±2.0	281 ±2.3
1,2,3,4-butanetetracarboxylic acid: (7.0%) + *m*-APH (6.15%) [c]	WRA (W+F)	121 ±1.0	119 ±1.5	129 ±2.4	131 ±2.3	166 ±3.3	212 ±3.1
	$[Cl^+]$%	0.025	0.031	0.072	0.204	0.588	1.258

[a] Chlorinated at pH 7, ~3300 ppm of chlorine for 60min at ambient temperature.

[b] Sodium hypophosphite (4%) is used as a catalyst.

[c] Sodium hypophosphite (4%) is not present.

3.3.3 Durability and Rechargeability

Rechargeability of antibacterial function, after releasing the oxidative chlorine due to repeated exposures to microorganisms, is the outstanding feature of *N*-halamine biocides. In practice, durability is also an important factor because more durable *N*-halamine function enhance the longevity of the antimicrobial properties during repeated use. Through a laundering test, the durability and rechargeability of *m*-APH treated cotton fabric were evaluated and are shown in Table 3-2. For up to 10 washing cycles, chlorine on prechlorinated samples resulted in good washing fastness. This supports the stability of chlorine on the treated fabrics in practical applications. The initial loading of chlorine is over 1.0 [Cl^+]%; hence, the use of the treated materials can be suggested in medical textiles or health care areas.

Ester and amide are formed after the reaction among *m*-APH, polycarboxylic acid and cotton fabrics. Carboxylic esters may be hydrolyzed to the parent carboxylic acid and alcohol. Similar hydrolysis may occur in carboxylic amides. After 50 washing cycles, the recharged fabrics retained chlorine to over 0.15 [Cl^+]%, which is a sufficient amount of chlorine to inactivate microorganisms [14]. Considering the initial chlorine loading, however, the *m*-APH on the fabrics does not seem to be particularly stable. The pH of the AATCC detergent during washing test was around 10.5 at 49^oC (standard washing-test temperature); thus, it is assumed that the hydrolysis of ester and/or amide on the antimicrobial functions under basic conditions occurred during the washing test. Therefore, hydrolysis may reduce durability of BTCA/*m*-APH treated cotton fabrics.

The results in Figure 3-4 show the chlorination effectiveness of different

concentrations of chlorine. The BTCA/ *m*-APH treated cotton fabric after chlorination in 329 ppm chlorine solution almost reached the critical point of the 3295 ppm chlorinated fabrics in 60 min; whereas 3295 ppm chlorination fabrics approached the critical point in 30 min. The milder the chlorine bleach solution, the better for actual application because the cost of chlorination can be satisfied with lower concentration of chlorine bleach. Thus, in practical application, diluted chlorine bleach is preferable to chlorinate *N*-halamine precursors, and it is obvious that BTCA/*m*-APH treated cotton fabric could retain their antimicrobial functions with relatively low concentration of chlorine bleach solutions.

Table 3-2 Durability and rechargeability of antimicrobial functions

Unit: $[Cl^+]$%

Washing Cycles	Group 1 [a]	Group 2 [b]	Group 3 [c]
0	1.180	1.180	1.180
5	0.496	0.468	0.649
10	0.375	0.227	0.412
25	0.270	0.053	0.235
50	0.217	0.022	0.157

[a] Group 1: Unchlorinated samples, after each cycle, were chlorinated.

[b] Group 2: Prechlorinated samples.

[c] Group 3: Prechlorinated samples, after each cycle, were rechlorinated.

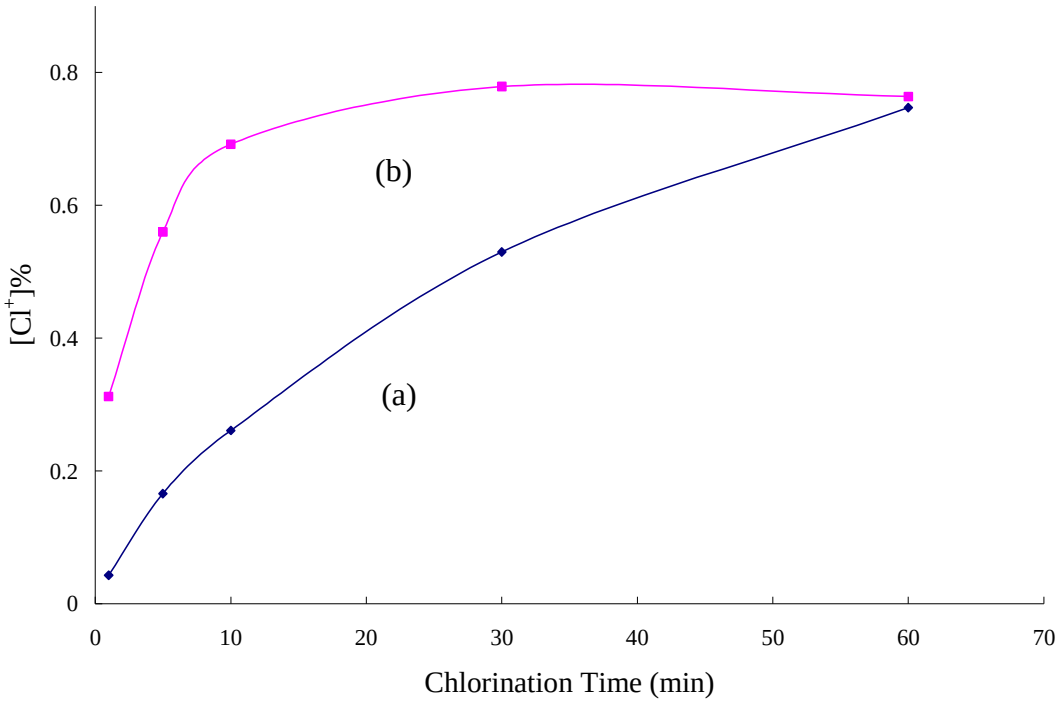

Figure 3-4 *Chlorine content on BTCA (7.0%) and m-APH (6.2%) treated samples in (a) 329 ppm (pH 10.2) of chlorine solution and (b) 3295 ppm (pH 11) of chlorine solution*

3.3.4 Antibacterial Efficacy

Gram-positive and Gram-negative bacteria were used to evaluate the biocidal efficacy against bacteria. The results in Table 3-3 show that BTCA and *m*-APH treated fabrics, which were chlorinated, revealed antimicrobial properties against Gram-negative and Gram-positive bacteria. Furthermore, in 1 min contact time, the chlorinated samples inactivated all of both bacteria.

The interesting point in the results is the biocidal efficacy of unchlorinated samples against the Gram-positive bacterium, *S. aureus*. The unchlorinated sample inactivated *S. aureus* in 10 min. Indeed, the BTCA treated samples have considerable carboxylic acids,

which remained after the ring opening reaction of cyclic anhydride intermediates. This acidic nature of BTCA treated samples may result in antimicrobial effects. In practice, several carboxylic acids have been used to preserve food as preservatives [17]. However, the biocidal efficacy of the unchlorinatd samples seemed to be limited against Gram-positive bacteria, and the effectiveness is also lower than chlorinated samples.

Table 3-3 Biocidal efficacy against microorganisms

Samples[a]	Contact time	*Escherichia coli* O157:H7 [b]		*Staphylococcus aureus* [c]	
		Bacterial reduction		Bacterial reduction	
		% reduction	Log reduction	% reduction	Log reduction
Unchlorinated	1 min	5.04	0.02	99.04	2.2
	5 min	10.31	0.05	99.37	2.5
	10 min	20.87	0.1	100	6.2
Chlorinated	1 min	100	6.1	100	6.2
	5 min	100	6.1	100	6.2
	10 min	100	6.1	100	6.2

[a] BTCA (7%) and *m*-APH (6.15%) treated fabrics were used. (Dried at 85^{o}C for 5 min, cured at 180^{o}C for 2min), [Cl^{+}]% = 1.180.

[b] Total bacteria: 1.27 x 10^{6} (cfu/sample).

[c] Total bacteria: 1.60 x 10^{6} (cfu/sample).

3.4 Conclusions

Application of *m*-APH on cotton fabric along with polycarboxylic acids provided a good connection between *m*-APH and cotton cellulose. At lower curing temperature (140~160^{o}C) with sodium hypophosphite, an esterification catalyst, *m*-APH predominated the reaction with BTCA; however, at higher temperature (200~220^{o}C) the nucleophilicity of cellulose was increased and was competitive to *m*-APH. Through one finishing process, the MAPH treated cotton fabrics have durable press properties as well as antimicrobial activities. The chlorinated BTCA/*m*-APH treated cotton fabric provided inactivation against Gram-positive and Gram-negative bacteria in 1 min contact time. The durability of BTCA/*m*-APH treated cotton fabric after washing, while perhaps adequate, was not good. It was assumed that the hydrolysis of amide and/or ester connections is the cause of the instability.

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CHAPTER 4

ANTIMICROBIAL ACRYLIC FIBER

4.1 Introduction

Inactivation of microorganisms, which cause odor as well as contagious diseases, is a desirable goal of biocides. Numerous antimicrobial materials have been developed over to the years as mankind has moved toward this goal [1a]. While it is desirable that biocidal materials should be strong inactivating agents for microorganisms, they should not be harmful to other life or the environment.

Fibers and fabrics are among the materials that are closely associated with humans, and for which antimicrobial functionality would be useful. One can easily recognize the benefit of biocidal hospital clothing and furnishings, as well as upholstery, carpets and bedding for the general public.

Work on *N*-halamines as biocidal agents has been ongoing for over two decades [1b, 2]. This work builds on the well known ability of *N*-halamine compounds such as 1,3-dihalo-5,5-dimethylhydantoin and halogenated isocyanurates to hold and stabilize chlorine in areas such as swimming pools [3]. Initially, our work produced water soluble imides, amides, and amines which were used (in halamine form) as dispersed antimicrobials for water treatment [4]. Subsequently, the nitrogen functionality was immobilized in a polymer, poly(styrenehydantoin), or PSH [5]. Over the last few years, we have attached the

halamine precursors onto a variety of materials including textile fibers [6], wall surfaces [7], glass [8], sand [9], etc. This has necessitated the attachment of a variety of reactive groups to the halamine precursor, depending on the material to which it would be attached. The attaching groups, which have proved useful, include methylol/formaldehyde [10] and siloxane [7, 11]. Much work concerning *N*-halamine biocidal textiles has also been done elsewhere [12-14]. Some work has also been done on simply mixing of halamine precursors with materials, for example during extrusion, to produce stable matrix structures, without the necessity of a covalent binding functionality. Since chemical bonding is the strongest connecting force between molecules, it should be the best way for application of antimicrobial compounds to fibers; however, as certain biocidal polymers have no appropriate functional groups for chemical bonding, blending with fiber-forming polymeric materials to make antimicrobial fibers could result in a useful alternative. This paper reports on one of these mixtures.

Poly(acrylonitrile) (PAN) is one of the standard fiber-forming materials, and it has been used as a wool substitute because it is easily made into a bulky yarn. As low molecular weight, uncrosslinked PSH and PAN are both soluble in dimethylacetamide (DMAc, normally used for the extrusion of PAN), the solution might provide a route to fibers containing both polymers. An acrylic fiber containing PSH (Figure 4-1) might be expected to allow formation of chloramines on the surface and therefore be useful as an antimicrobial fiber.

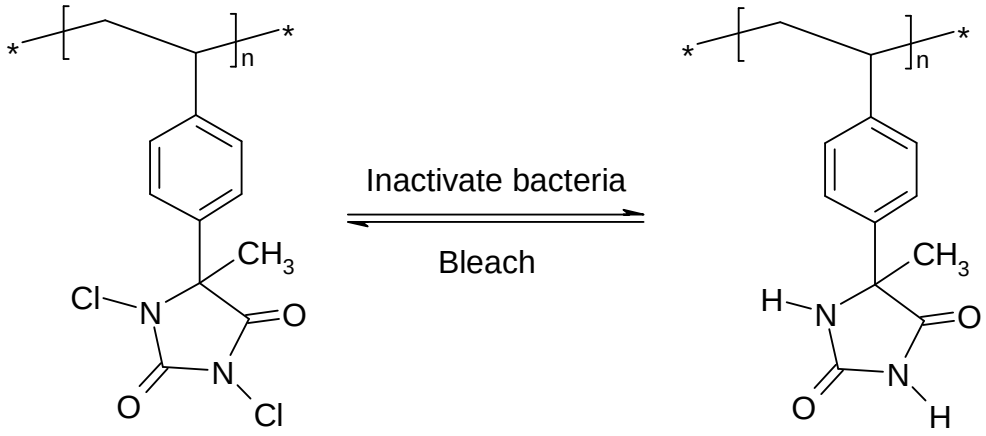

Figure 4-1 *The function of disinfection of polystyrene hydantoin*

4.2 Experimental

4.2.1 Materials

PSH used was synthesized by an established procedure [5] in a molecular weight range of 800 - 5000. The PAN was a fiber forming acrylonitrile copolymer; it was obtained from Solutia Inc. (St. Louis, MO). Dimethylacetamide was purchased from Fisher (Fair Lawn, NJ). All solvents, unless otherwise stated, were of reagent grade, and used without further purification.

4.2.2 Preparation of PAN/PSH Solution

Appropriate amounts of PSH were dissolved in 70 mL of DMAc, after which 7 g of PAN were added to the DMAc/PSH to produce a solution of approximately 10% PAN. The ratios of PAN/PSH were varied from 100/0 to 100/12 (by weight). The blended solution was stirred for 24 h at 70ºC. The solution was allowed to store for more than 2 h at

70ºC without stirring to remove air bubbles, after which it was poured into a piston wet spinning apparatus.

4.2.3 Wet Spinning Process

The piston pump (ISCO, Series D piston pump) was fitted with a single hole (1.25 mm diameter circular) spinneret. The set-up consisted of extruder, a coagulating bath (using tap water), a stepped godet with four levels and a take-up winder. The blended solutions in the extruder cylinder were maintained 35ºC, and forced through the spinneret fitted with a 325 mesh wire screen filter inside. Extrusion conditions were as follows:

Process:	dry-jet wet spinning
Extrusion temperature:	35ºC
Throughput:	0.16~0.64 mL/min
Coagulation bath:	tap water
Godet speed:	12.5~50 RPM
Drawing:	3 stage, steps 1, 2, 3, and 4
Draw ratio:	3.9
Take-up speed:	2.0 - 8.1 m/min

After wet spinning, the yarns were soaked in tap water at ambient temperature for 24 h to extract the solvent, which was used for spinning.

4.2.4 Measurement of Physical Properties

Tensile properties were investigated with a universal materials testing machine (Instron Model 1122) at 22ºC and 65% humidity. The data were obtained by averages of 10 tests. The length between upper and lower jaw was 25.4 mm, and the crosshead speed was 20 mm/min.

4.2.5 Chlorination and Titration

A commercial 6% sodium hypochlorite solution was used to chlorinate the fabrics (diluted to 3300 ppm of the commercial strength with distilled water) at pH 8 to produce biocidal materials. After soaking the fabric in the solution at ambient temperature for 30 min, and rinsing with a large excess of distilled water, the samples were dried at 45ºC for 2 h to remove any unbonded chlorine.

An iodometric/thiosulfate titration procedure was used to analyze oxidative chlorine content. The $[Cl^+]$% in the sample was calculated with the following equation:

$$[Cl^+]\% = (V \times N \times 35.45) / (W \times 2 \times 10)$$

Where $[Cl^+]$% is the wt % of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution, mL), N is equal to the normality of the titrant, and W is the weight of the sample (g).

4.2.6 Laundering Test

The American Association of Textile Chemists and Colorists (AATCC) Test Method 61-2001 was used to investigate the stability of PSH and chlorine in/on the fibers after home laundering. A Launder-Ometer fitted with stainless steel cylinders (3 x 5 in) including 150 mL of 0.2% AATCC detergent solution and 50 stainless steel balls was rotated for 45 min at 42± 0.5 RPM and 49ºC. These conditions are estimated to be equivalent to 5 washing cycles in a home laundry. After detaching the cylinders, the fibers inside each cylinder were rinsed with three 300 mL portions of distilled water and air dried at ambient temperature.

4.2.7 Antimicrobial Test

After making the fibers into a crude nonwoven fabric (one inch square), treated samples were challenged with *Staphylococcus aureus* (ATCC 6538) using a modified AATCC Test Method 100-1999. After applying bacteria suspensions in pH7 phosphate buffer solution to the samples, and covering with another swatch. After contact times of 30 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution. Serial dilutions of the quenched samples were made using pH 7 phosphate buffer and plated on Trypticase soy agar. The plates were incubated at 37ºC for 24 h and then counted to determine the presence or absence of viable bacteria.

4.3 Results and Discussion

4.3.1 Properties and Chlorine Content of the Fibers

FT-IR was employed to prove the existence of PSH in the composite fiber and prominent features in Figure 4-2 show that the carbonyl bands of PSH appeared at 1728 cm^{-1} and 1772 cm^{-1} (Figure 4-2, c). The PAN/PSH composite fiber exhibits the same carbonyl bands (Figure 4-2, b) with a third band at 2245 cm^{-1} corresponding to the PAN C≡N present. This is compelling evidence that the composite fiber contains both PSH and PAN.

The tensile properties of samples were measured at 65% humidity and 22^{o}C after conditioning the samples for 24 h. The results in Table 4-1 indicate that increasing the PSH content of the fiber increases the chlorine content after chlorination; however, as the PSH content increased, tenacity was decreased. It is presumed that the presence of the PSH may lower packing density of PAN resulting in a decreased tenacity. Due to PSH has a phenyl group as well as a hydantoin ring on the repeating unit, the bulky side chain could promote the disorder of PAN chains. Extrusion conditions were not, however, optimized for fiber properties.

As denier decreased, the blended acrylic released (absorbed) more chlorine. The results are indicated in Table 4-2. It is suggested that poly(acrylonitrile) has limited moisture absorption due to inherent hydrophobicity, and as a result, only PSH on the surface of the fiber is active in absorbing chlorine and inactivating bacteria. Due to that the theoretical [Cl^{+}]% of PSH is 24.87. If all of the added PSH is chlorinated, theoretical [Cl^{+}]% of PAN/PSH (100/12) should be 2.66. For the 21 denier fiber (21 g/900 m), the chlorine content was only 0.11% indicating that we have chlorinated 4.1% of the available PSH

sites. Since thinner fibers have more surface area, one would expect that thinner fibers would have greater chlorine retention and antibacterial activity at lower PSH content. The effect of surface area on absorbed chlorine is shown in Figure 4-3. Using the graph in Figure 4-3, and extrapolating to a surface area corresponding to a denier of 1, we would expect a Cl^+ content of 0.50% which corresponds to 18.80% of theoretical chlorine absorption.

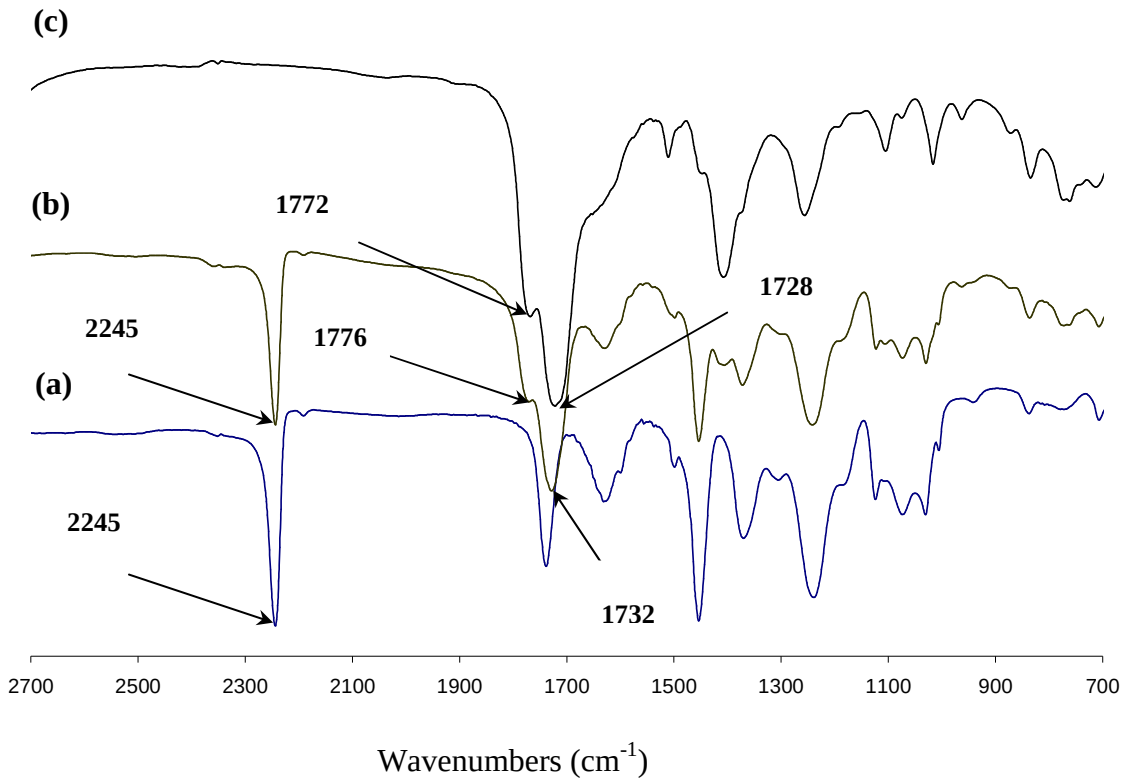

Figure 4-2 *FT-IR bands of the composite fibers of (a) PAN/PSH (100/0), (b) PAN/PSH (100/12), and (c) PSH powder*

Table 4-1 Mechanical properties and chlorine content of the acrylics(0.2 mL/min of extrusion velocity and 50 RPM of a take-up speed)

Ratio [a] *(PAN/PSH)*	*100/0*	*100/4*	*100/8*	*100/12*
$[Cl^+]$ %	0	0.01	0.05	0.07
Denier (g/9000m)	21.2 ±4.1	20.1 ±2.7	27.9 ±5.8	29.2 ±5.3
Tenacity (g/den)	1.50 ±0.16	1.33 ±0.11	0.99 ±0.05	0.92 ±0.16
Strain at break (%)	55.3 ±15.8	54.5 ±10.6	12.3 ±16.5	15.6 ±11.5

[a] PAN/PSH by weight.

Table 4-2 Variation of chlorine content of PAN/PSH(100/12) fibers with various deniers (Take-up speed: 12.5 RPM)

Extrusion velocity (mL/min)	*Denier (g/9000 m)*	*Surface area* (m^2/g)	$[Cl^+]$ %	*Atoms/cm*^{2}
0.64	398	1.57×10^{-2}	0.02	2.16×10^{16}
0.32	179	2.34×10^{-2}	0.04	2.90×10^{16}
0.16	85	3.39×10^{-2}	0.07	3.51×10^{16}
0.16 [a]	21[b]	6.86×10^{-2}	0.11	2.72×10^{16}
N/A	1	31.10×10^{-2}	0.50 [b]	2.21×10^{16}

[a] Take-up speed: 50RPM.

[b] Predicted value.

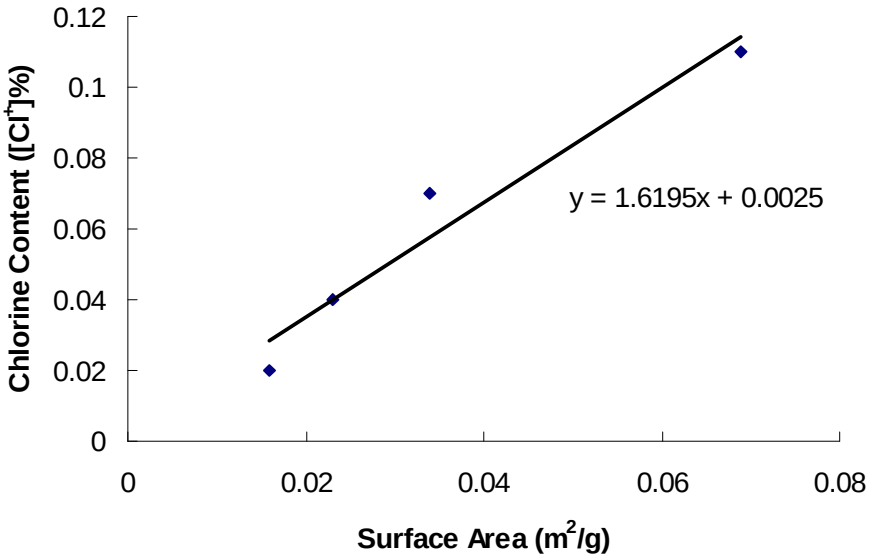

Figure 4-3 *Variation of chlorine content of PAN/PSH (100/12) fibers with various surface areas*

4.3.2 Durability, Rechargeability, and Antimicrobial Efficacies

The relative durability of chlorine to laundering and rechargeability afterwards (with Cl) was evaluated; the results appear in Table 4-3. All of blended acrylics retained little of their antimicrobial properties after 50 washes; however, after recharging, the fibers with household bleach, they regained almost all of their original chlorine content. During the washing test, the hydrophobic properties of the PAN, which is a major component, prohibited the swelling of the fibers. For this reason, the migration of the PSH out of PAN could be limited, and increased durability appeared. Even the surface of the fibers obtained chlorine after chlorination.

The fibers were formed into a nonwoven matt which upon chlorination

with 10% household bleach became antimicrobial. The results are shown in Table 4-4. From the data, most of chlorinated acrylic nonwovens caused complete inactivation of *S. aureus* in 30 min.

Table 4-3 Durability and rechargeability of the acrylics (0.16 mL/min of extrusion velocity and 50 RPM of a take-up speed)

unit: $[Cl^+]$%

Ratio [a] *(PAN/PSH)*	*100/0*	*100/4*	*100/8*	*100/12*
Chlorinated sample	0	0.03	0.07	0.11
Washing 50 cycles	0	0.01	0.01	0.05
Recharge after washing 50 cycles	0	0.04	0.07	0.10

[a] PAN/PSH by weight.

Table 4-4 Antimicrobial efficacy of PAN/PSH blended fiber against *Staphylococcus aureus*

Samples [a]		*Contact time*	*Bacterial reduction*	
		(min)	(%)	Log Reduction
Unchlorinated fibers	PAN/PSH (100/0)	30	49	0.2
	PAN/PSH (100/4)	30	32	0.1
	PAN/PSH (100/8)	30	88	0.9
	PAN/PSH (100/12)	30	99.5	2.2
Chlorinated fibers	PAN/PSH (100/0)	30	94	1.2
	PAN/PSH (100/4)	30	99.9	3.3
	PAN/PSH (100/8)	30	100	6.5
	PAN/PSH (100/12)	30	100	6.5

[a] Each sample was inoculated with 25 µl of bacterial suspension at 3 x 10^6 cfu/ml. 6 log reduction corresponds to a 99.9999% kill of the bacteria.

4.4 Conclusions

An antimicrobial-fiber precursor (PAN/PSH composite fiber) was produced by dry-jet wet spinning. Strength properties and antibacterial effectiveness of the fiber were investigated. Strength properties were somewhat decreased as more PSH was added during extrusion; however, little effort has been made to optimize extrusion conditions. As the PSH content increased, the absorbed chlorine and antibacterial effectiveness also increased. Since poly(acrylonitrile) is hydrophobic (has limited moisture absorption), it might be presumed that just PSH on the surface of the fibers is active in absorbing chlorine and inactivating bacteria. The fact that only a fraction of the theoretical chlorine absorption was observed, and the fact that absorbed chlorine is shown to increase with increasing surface

area of the fiber, supports this theory. This suggests that one should use fibers of low denier (high specific surface area) should be used to obtain a high concentration of halamine in hydrophobic fibers. This was confirmed by making very fine fibers of PAN/PSH via electrospinning. Fibers from a web of electrospun PAN/PSH (100/12) having a fiber diameter of > 2µm absorbed and retained 0.6% of Cl^+. The projected Cl^+ content of a similar fiber of 1 denier is 0.5 %.

The treatment appears stable to repeated laundering, and the chlorinated acrylic fibers inactivated *Staphylococcus aureus* (Gram-positive bacteria) within 30 min.

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CHAPTER 5

ANTIMICROBIAL POLYMERIC MATERIALS; CELLULOSE AND *m*-ARAMID COMPOSITE FIBERS

5.1 Introduction

N-halamine materials have been developed as biocides during the last few decades [1-4]. *N*-halamines refer to compounds that have at least one covalent bond between nitrogen and halogen. Due to dehydrohalogenation which can occur if an α-hydrogen is adjacent to a nitrogen-halogen bond, nitrogen in a heterocyclic structure with no α-hydrogen is preferred for *N*-halamine biocides. Of the three common *N*-halamine structures – imide, amide and amine – the amine has the lowest dissociation constant and is most stable. The imide halamine has the highest dissociation constant and is the least stable. The amide halamine has intermediate stability, and perhaps the best balance of longevity and reactivity [5]. Because they are rechargeable and are active against a spectrum of microorganisms with a rapid kill, *N*-halamines have received considerable attention [6].

To date most work for *N*-halamine antimicrobials has been devoted to water disinfection [1-3]; however, the potential applications of *N*-halamines have led some researchers recently to attach those biocides to polymers and fibers [5, 7, 8]. In general, *N*-halamine antimicrobial polymers or fibers have been prepared in three different ways.

1. *N*-halamines precursors are bound to polymers or fibers with physical/chemical bonding [5, 7, 8],

2. *N*-halamines precursors are mixed with fiber forming polymer [9], and
3. Fiber-forming polymers are *N*-halamine precursors [10].

Specifically, fiber-forming polymers, which are also N-halamine precursors, have advantages such as higher durability and no additional finishing. Indeed, aromatic polyamides, which consist of an amide group between two aromatic rings, are the representative of fiber-forming *N*-halamine precursors. Particularly, poly(*m*-phenylene isophthalamide), *m*-aramid (Nomex®) has meta substituted rings in the molecular chain unlike Kevlar®, which is para substituted. A report found that *p*-aramid fiber (Kevlar®) suffered serious degradation of physical properties after chlorination; whereas, the *m*-aramid did not suffer this problem; however, the content of chlorine on the *m*-aramid fiber (Nomex®) was limited [10], and the high Tg (~275 ºC) of the *m*-aramid fiber induces poor dyeability [11]. The amide nitrogens in *m*-aramid have no α-hydrogens; thus, if the surface area of *m*-aramid is enhanced to chlorinated, *m*-aramid should be converted to an *N*-halamine and have increased chlorine content.

Ionic liquids (IL) are solvents for chemical reactions and for polymers during wet extrusion of fibers and films. Certain ionic liquids can dissolve cellulose to form stable solutions at reasonable temperatures. The ionic solutions are relatively non-toxic and easy to handle making the IL's candidates for extruding regenerated cellulose. The ionic liquid (1-butyl-3-methylimidazolium chloride) dissolves cellulose [12] and poly(*m*-phenylene isophthalamide).

Figure 5-1 shows the function of disinfections of the chlorinated *m*-aramid, which then will simply be recharged by diluted-chlorine bleach. In this study, the composite polymer of cellulose (a natural polymer) and *m*-aramid (a synthetic polymer) will be

discussed. The physical properties, antibacterial efficacy and stability, durability and rechargeability after repeated laundering, will be also described.

It was reasoned that a composite fiber of both cellulose and *m*-aramid might provide the desirable properties of cellulose as well as the ability to create an antimicrobial halamine in the fiber.

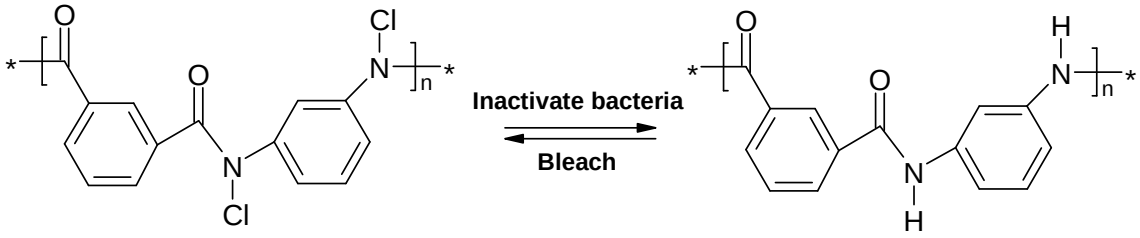


Figure 5-1 *The Scheme of the function of disinfection of m-aramid*

5.2 Experimental

5.2.1 Materials

Bleached cotton cellulose, with a degree of polymerization (DP) of 1440, was used. The *m*-aramid was a DuPont product. The ionic liquid, 1-butyl-3-methylimidazolium chloride, was purchased from Aldrich (Milwaukee, WI).

5.2.2 Instruments

The scanning electron microscope investigation was performed with a Jeol JSM-7000F (SEM). Samples were coated with gold under argon purge before observation. The wide angle X-ray diffraction (WXRD) measurement was conducted on Rigaku Miniflex x-ray diffractometer equipped with a Cu Kα radiation source.

5.2.3 Preparation of Cellulose/*m*-aramid Solution

Bleached cotton was ground and dried at 70ºC under vacuum for 8 h. 3.5 g of the cellulose was put in 100 g of 1-butyl-3-methylimidazolium chloride to produce a solution of 3.5% cellulose. The solution was then stirred for 72 h at 80ºC. Concurrently, *m*-aramid was pre-washed with ethanol followed by heating at 65 ºC, then dried. Once dried, *m*-aramid was added to the ionic liquid/cotton solution. This mixture was then stirred for 24 h at 80ºC. A total of six solutions were made with the following ratios of cotton/*m*-aramid by weight: 100/0, 100/2, 100/6, 100/10, 100/20 and 100/50. The solution was then stored under vacuum for at least 2 h at 70 ºC without stirring to remove air bubbles, after which, it was poured into the extruder.

5.2.4 Wet Spinning Process

The wet spinning apparatus (ISCO Series D, piston pump) was fitted with 0.45 mm diameter single-hole spinneret. A tap water coagulating bath and a stepped godet with four levels and a take-up winder were used. Dry-jet wet spinning was used at 50~65 ºC, with a 0.2 mL/min extrusion velocity, and an appropriate godet speed was maintained.

The solution was forced through a spin pack with a 325 mesh screen and the single hole spinneret. Extrusion conditions were as follows:

Process:	dry-jet wet spinning
Extrusion temperature:	50-65 ºC
Throughput:	0.2 mL/min
Coagulation bath:	tap water
Godet speed:	25 RPM
Drawing:	3 stage, steps 1, 2, 3, and 4
Draw ratio:	2.95 -3.89
Take-up speed:	3.09 – 4.07 m/min

After dry-jet wet spinning, the yarns were soaked in tap water at ambient temperature for 24 h to extract the solvent and then dried.

5.2.5 Measurement of Physical Properties

Tensile properties were investigated with a universal materials testing machine (Instron Model 1122) at 22 ºC and 65% of humidity. The data were obtained by averages of 10 tests. The length between upper and lower jaws was 25.4 mm, and the crosshead speed was 20 mm/min.

5.2.6 Chlorination and Titration

The fibers were chlorinated using a commercial 6% sodium hypochlorite solution (diluted to 10% of the commercial strength with distilled water) at pH 7 to produce biocidal materials. After soaking the fiber in the solution at ambient temperature for 30 min, and rinsing with distilled water, the samples were then dried at 45ºC for 2 h to remove any unbounded chlorine.

An iodometric/thiosulfate titration was used to analyze and to calculate oxidative chlorine content according to the equation:

$$[Cl^+]\% = (V \times N \times 35.45) / (W \times 2 \times 10)$$

Where $[Cl^+]\%$ is the wt% of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution, mL), N is equal to the normality of the titrant, W is the weight of the sample (g).

5.2.7 Laundering Test

AATCC Test Method 61-2001 was used to investigate the stability of *m*-aramid chlorine in/on the fibers after home laundering. A Launder-Ometer was fitted with stainless steel cylinders (3 x 5 inch). A volume of 150 mL of water, 0.15% AATCC detergent and 50 stainless steel balls were added to the cylinder which was rotated for 45 min at 42± 0.5 RPM and 49ºC. These conditions are estimated to be equivalent to 5 washing cycles in a home laundry. The fibers within the cylinder were rinsed with three 300 mL portions of

distilled water, then air dried at ambient temperature, and either titrated directly or recharged with chlorine bleach.

5.2.8 Antimicrobial Test

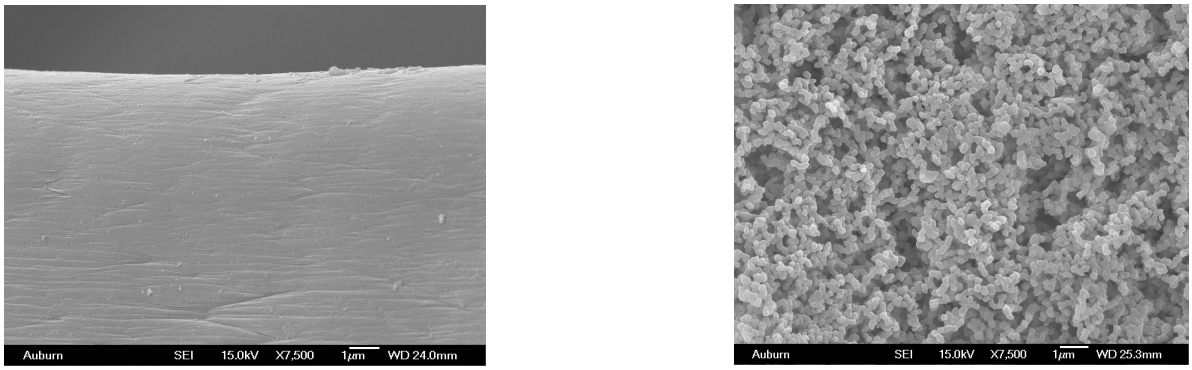
After making the fibers into a crude nonwoven fabric, control and chlorinated samples were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 43895) using a modified AATCC Test Method 100-1999. A total of 25 μL bacterial suspensions made with pH 7 phosphate buffer were added to the fabrics and another swatch was sandwiched over it to ensure contact between the suspension and the fabric. After contact times of 5 min and 30 min, the samples were quenched with 5.0 mL of sterile 0.02 N sodium thiosulfate solution. Serial dilutions of the quenched samples were made using pH 7 phosphate buffer, and plated on Trypticase soy agar. The plates were incubated at 37ºC for 24 h and then counted to determine the presence or absence of viable bacteria.

5.3 Results and Discussion

5.3.1 Coagulation of the Polymers

The dissolution mechanism of cellulose in ionic liquids is known as breaking hydrogen bonds between chains of cellulose and reforming of bonds with ionic liquids [13]. The major bonding force among *m*-aramid chains is also hydrogen bonding, which means that the possible dissolution mechanism is similar to that of cellulose. However, the cellulose film, which was coagulated in water, was transparent; whereas, *m*-aramid film, which was coagulated in water, was translucent. It is presumed that due to small

size of crystallite (< 400~700 nm, visible lay) and/or entire amorphousness, the coagulated cellulose film appears transparent. The SEM picture of *m*-aramid was shown in Figure 5-2. The coagulated *m*-aramid particles in water are aggregated to 0.8~1.0 μm in size. Thus, the size of the coagulated particles can explain the opaqueness of the coagulated *m*-aramid film.



(a) (b)

Figure 5-2 *SEM pictures of (a) surface of original m-aramid fiber, (b) surface of coagulated m-aramid film*

5.3.2 Physical Properties and Chlorination of the Composite Polymer

The results in Table 5-1 show mechanical properties of the fiber in terms of denier, tenacity, and strain at break. At lower *m*-aramid content, even up to 10% (based on weight of cellulose), denier and tenacity of the fiber are similar; however, in 100/50 (cellulose/ *m*-aramid), tenacity decreases.

A *m*-aramid film coagulated in water is shown along-side of the original *m*-aramid fiber (Figure 5-2). The coagulated film shows a very porous structure that was shown to accept 2.7% chlorine (22.9% of theoretical). The rough, porous surface caused by the

increase in reaction with chlorine (compared to original fiber – Table 5-2) shows a marked increase in surface area and accessibility. Figure 5-3 also indicates that the coagulated film has less crystalline order. The weakness of the film produced indicates that water is not a good coagulation medium, and this is bourne out by attempts to extrude the *m*-aramid into water, and perhaps by the lowered strength of the 100/50 (cellulose/*m*-aramid) composite fiber.

Chlorine loading onto the fibers varied with the concentration of *m*-aramid (Table 5-1). It can be seen that original *m*-aramid fibers have very poor chlorine loading even in high concentration of chlorine solution at high temperature (80 ^{o}C); however, coagulated *m*-aramid shows considerably enhanced chlorine loading (Table 5-2). In fact, the high glass transition temperature of commercially available *m*-aramid fiber limits dyeability [11]. Extended surface area of *m*-aramid fiber, after solvent treatment, improved the dyeing properties of basic dye [15]. Similarly, *m*-aramid in cellulose should have increased surface areas due to being divided into smaller sizes. Hence, coagulated *m*-aramid fibers exhibit increased chlorine content.

Because diluted bleach solution cannot penetrate crystal area unless a polymer is melted, higher crystallinity can be the factor for lower chlorine loading on fibers or polymers even at above Tg. Figure 5-3 shows the change of crystallinity of *m*-aramid through wide-angle x-ray diffraction (WXRD); the peaks at 2 θ = 37.37^{o} and 43.61^{o} belong to the crystalline form of *m*-aramid (Figure 5-2, a); the coagulated film revealed decreased crystallinity (Figure 5-2, b). This can also support the increased chlorine content of the coagulated *m*-aramid film; moreover, it is assumed that in the composite fiber, the *m*-

aramid exists with reduced crystallinity. (However, we do not see evidence of significant phase separation in fibers).

Table 5-1 Mechanical properties and chlorine content of the composite fibers (0.2 mL/min of extrusion velocity, 4.07 m/min of take-up speed and 3.89 of draw ratio)

Ratio (Cellulose/ m-aramid)	*100/0*	*100/2*	*100/6*	*100/10*	*100/50* [c]	*0/100* [d]
$[Cl^+]$ % [a]	0	0.230	0.696	1.141	3.771	0.022
$[Cl^+]$ % [b]	0	0.050	0.153	0.167	0.557	0.002
Denier (g/9000m)	21.9±1.8	24.2±2.6	21.9±1.7	24.0±1.5	27.1±1.1	2.1±0.4
Tenacity (g/den)	2.45±0.54	2.48±0.46	2.82±0.34	2.58±0.73	1.90±0.14	3.68±0.58
Strain at break (%)	4.7±1.7	6.0±1.7	5.8±1.9	7.6±1.6	16.2±2.2	52.6±26.4

[a] Chlorine concentration and condition of chlorination: 3300 ppm, pH 7.0 and ambient temperature for 60 min.

[b] Chlorine concentration and condition of chlorination: 3300 ppm, pH 7.0 and ambient temperature for 60 min.

[c] Take-up speed; 3.09 and draw ratio; 2.95.

[d] Original *m*-aramid fibers, no additional wet-spinning added.

Table 5-2 Chlorine content of *m*-aramid fibers and coagulated *m*-aramid film after chlorination

Unit: $[Cl^+]$%

Chlorinated temperature [a]	*Original m-aramid fiber*	*Coagulated m-aramid film*
25 °C	0.022	2.732
40 °C	0.046	3.709
60 °C	0.043	3.625
80 °C	0.086	3.950

[a] Chlorination condition: 3300 ppm of chlorine, pH 7, ambient temperature for 60 min.

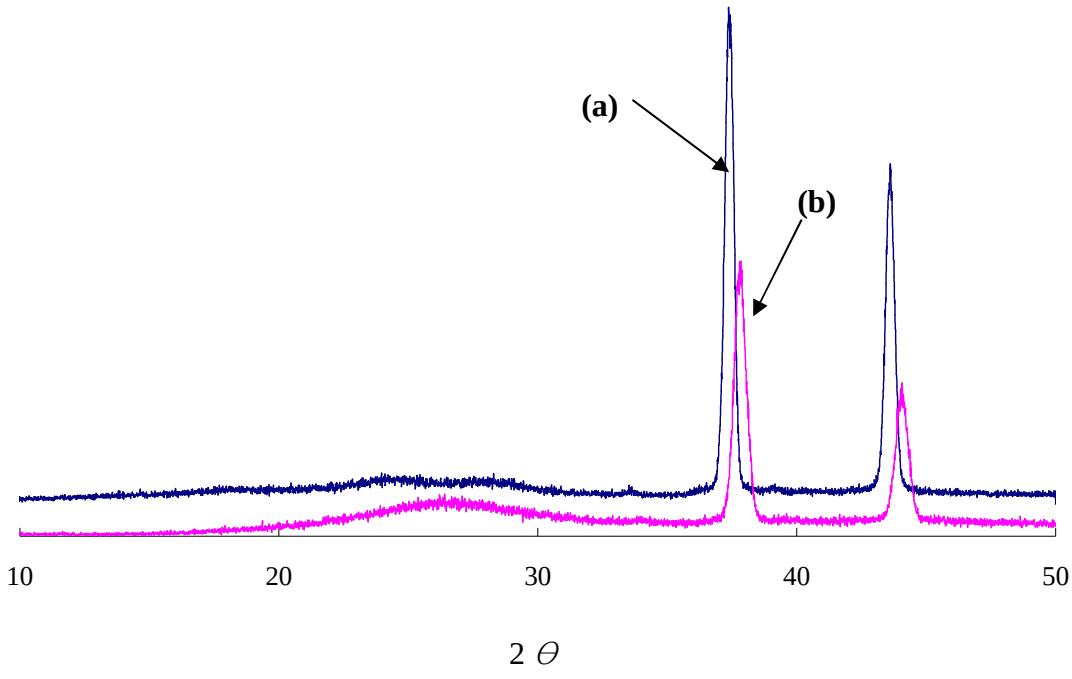


Figure 5-3 *Wide angle X-ray diffraction pattern of (a) m-aramid fiber and (b) coagulated m-aramid film*

5.3.3 Durability and Rechargeability of the Antimicrobial Fibers

Rechargebility is the factor for *N*-halamines to retain their antimicrobial functions after repeated uses. The results in Table 5-3 indicate that recharging is possible even after 50 wash cycles; the fibers recovered their original antimicrobial functions to around 70%. In fact, covalent bonding does not exist between cellulose and *m*-aramid polymer chains; bonds are due to non-covalent interactions (hydrogen bonding, van der Waals forces etc.). Therefore, it is presumed that *m*-aramid particles could possibly escape through the swollen cellulose matrix during washing. The content of *m*-aramid, which is rendered as the biocide in the composite polymer, decreased after repeated washing. Durability of chlorine on the fibers appeared quite stable. After 50 cycles, 0.14% of chlorine was retained by 6% *m*-aramid blend and 0.27% of chlorine was retained by 10% *m*-aramid blend, which is sufficient to inactivate bacteria [10].

The shelf-live of the antimicrobial fibers is shown in Figure 5-4. About 90% of the reactive chlorine is available even after 100 days. In general, the stability of nitrogen and halogen covalent bonding in *m*-aramid is dominated by the stability of negative charge on nitrogen after releasing the halogen. In the *m*-aramid formula, nitrogen in the amide group attracts the electrons from the phenyl ring inductively; however, by resonance, the amide group is the electron-donating group in the π-system. In reality, the whole situation is dominated by the resonance effect. However, since the amide groups are in the meta-position, the resonance cannot be extended to other amide groups – unlike *p*-aramid fiber (Kevlar®). Consequently, chlorine on *m*-aramid maintains antimicrobial function with quite stable durability.

Due to the increased surface area of *m*-aramid after coagulation in water, chlorine content on cellulose/*m*-aramid composite fiber after chlorination was proportionally increased with the amount of *m*-aramid. The results in Figure 5-5 show that 100% *m*-aramid film, which was coagulated in water, released relatively lower chlorine than other portions of *m*-aramid in the composite fibers. It is assumed that *m*-aramid particles in 100% coagulated *m*-aramid film (see Figure 5-2) are aggregated; thus, the chlorine solution had difficulty to access inside this cluster. However, *m*-aramid in cellulose has better dispersiveness than 100% coagulated one due to the fact of that cellulose may separate *m*-aramid particles.

Table 5-3 Durability and rechargeability of composite fibers

(0.2 mL/min of extrusion velocity and 1.04 m/min of a take-up speed)

Unit: $[Cl^+]$%

Washing cycles	*Group 1* [a]			*Group 2* [b]		
	m-aramid 2%[c]	*m*-aramid 6%[c]	*m*-aramid 10%[c]	*m*-aramid 2%[c]	*m*-aramid 6%[c]	*m*-aramid 10%[c]
0	0.230	0.696	1.141	0.230	0.696	1.141
5	0.059	0.317	0.552	0.224	0.678	1.069
10	0.045	0.260	0.446	0.217	0.659	1.017
50	0.010	0.145	0.276	0.180	0.501	0.848

[a] Group 1: Prechlorinated samples.

[b] Group 2: Prechlorinated samples were rechlorinated after each cycle.

[c] Weight based on cellulose.

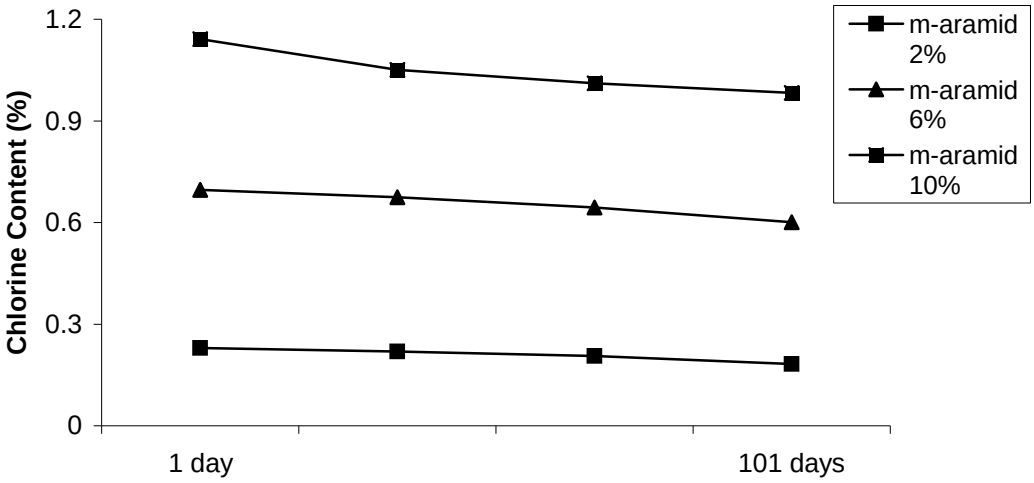


Figure 5-4 *Shelf-lives of the composite fibers*

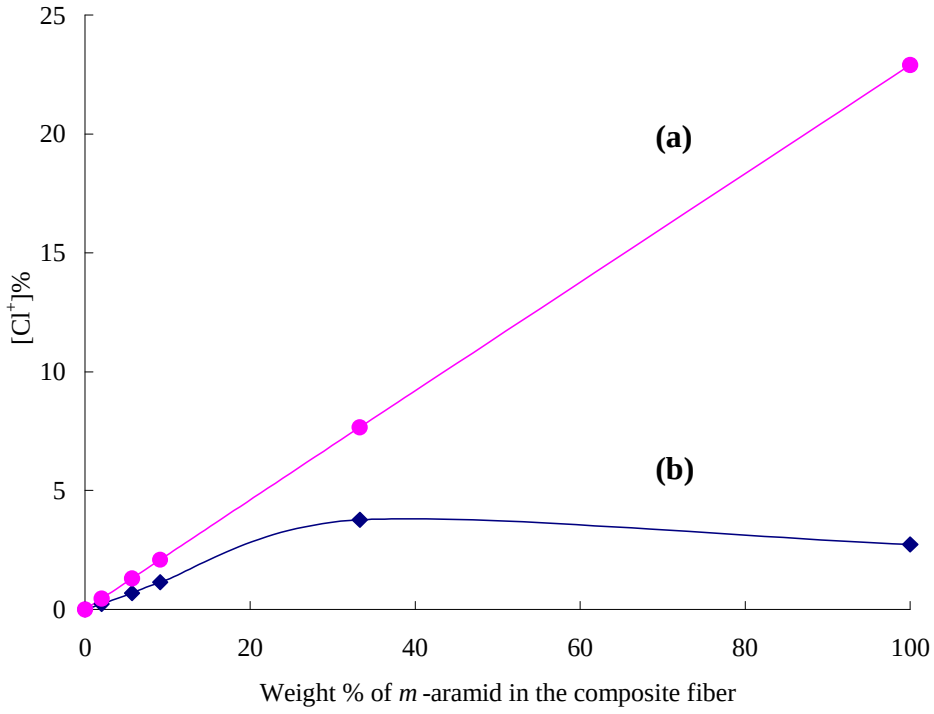


Figure 5-5 *Chlorine content of (a) theoretical and (b) actual composite fiber*

5.3.4 Antibacterial Efficacy against Gram-positive and Gram-negative Microorganisms

The results in Table 5-4 indicate that chlorinated composite fibers inactivated both Gram-positive and Gram-negative bacteria at a contact time of 30 min. More positively, 2% of *m*-aramid produced a 6 log reduction of Gram-positive bacteria at a contact time of 5 min and a 3.9 log reduction of Gram-negative bacteria at the same exposure time. A biocide, which has lipophilicity, has difficulty in penetration of the water occupied porins which are water-filled tubes in the outer membrane of Gram-negative bacteria, unlike hydrophilic biocides which have low molecular weight [16]. Due to increased hydrophobicity of *N*-halamine, chlorinated samples result in slightly less effective *m*-aramid antimicrobial function on *E. coli* (Gram-negative) than *S. aureus* (Gram-positive).

Table 5-4 Biocidal efficacy against microorganisms

Samples[a] (% *m*-aramid)		Contact time	Escherichia coli *O157:H7*[b] Bacterial reduction		Staphylococcus aureus[c] Bacterial reduction	
			% reduction	Log reduction	% reduction	Log reduction
Control	0 %	5 min	0.74	0	15.44	0.1
		30 min	31.07	0.2	71.60	0.5
	2 %	5 min	9.01	0	53.95	0.3
		30 min	42.10	0.2	70.77	0.5
	6 %	5 min	14.53	0.1	62.50	0.4
		30 min	20.04	0.1	68.84	0.5
	10 %	5 min	11.77	0.1	63.60	0.4
		30 min	44.86	0.3	65.26	0.5
Chlorinated	0 %	5 min	31.07	0.4	55.88	0.4
		30 min	78.49	0.7	92.28	1.1
	2 %	5 min	99.99	3.9	100	6.3
		30 min	100	6.4	100	6.3
	6 %	5 min	100	6.4	100	6.3
		30 min	100	6.4	100	6.3
	10 %	5 min	100	6.4	100	6.3
		30 min	100	6.4	100	6.3

[a] *m*-aramid content in the composite polymer (based on weight of cellulose).

[b] Total bacteria: 2.43×10^6 (cfu/sample).

[c] Total bacteria: 2.06×10^6 (cfu/sample).

5.4 Conclusions

An ionic liquid, 1-butyl-3-methylimidazolium chloride has allowed the production of cellulose/*m*-aramid fibers. Physical and antimicrobial properties were investigated. Stable and rechargeable cellulose/*m*-aramid composite fibers were prepared by dry-jet wet spinning of a composite polymer solution. Chlorination of the aramid nitrogen produced antimicrobial properties which were retained over 50 standard-washing cycles. Cellulose/*m*-aramid blends show a much higher chlorination level than the pure *m*-aramid fiber. Up to 10% (based on weight of cellulose) *m*-aramid composite fibers did not produce any decline in mechanical properties. The chlorinated fibers inactivated both Gram-negative (*E. coli*) and Gram-positive (*Staph. aureus*) bacteria within 5 min with 6 log reductions.

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