Antimicrobial Polymers for Coating Cotton and Other Surfaces

by

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Abstract

Antimicrobial N-halamine compounds were designed, synthesized and applied onto various materials to investigate and develop several properties of the N-halamine based technologies. Throughout the Master's degree, two independent projects were undertaken.

In the first project, there were three different commercially available N-halamine antimicrobial chemicals applied to cotton fabric. The purpose of this study was to finish cotton fabric with N-(hydroxymethyl)acrylamide (NMA) by etherification, and subsequently grafting different N-halamine monomers by vinyl copolymerization. Thus, a multifunctional coating compromising permanent press and antimicrobials will be created on cotton substrates without further processing. In this regard, first of all, N-(hydroxymethyl)acrylamide (NMA) was padded on cotton fabric, and then methacrylamide or acrylamide was grafted on the NMA treated fabric. The antimicrobial activities, stabilities, wrinkle recovery angles, and ultraviolet (UV) light resistances of different derivatives were investigated.

In the second project, N-tertbuylacrylamide monomer was copolymerized with 3-(trimethoxysilyl)propyl methacrylate and also grafted onto N-(hydroxymethyl)acrylamide (NMA). The purpose of this study was comparison of copolymer and grafted NTBA on biocidal properties. The antimicrobial activities, stabilities, wrinkle recovery angles, and ultraviolet (UV) light resistances of different derivatives were investigated.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction:

Life on Earth began early in our planet's history with microscopic organisms. Even though there were no humans, microbial life has shaped our atmosphere, our geology, and the energy cycles of all ecosystems.[1] Throughout history, human beings have had a hidden partnership with microscopic organisms ranging from food production and preservation to mining for precious minerals.[3] For instance, ancient records show that some of old country societies practiced preservation, drinking water sanitation, antisepsis for wounds and injuries and both physical and chemical mummification even though human beings were unaware of the existence of microscopic organisms during most of our history.[1-3]

Studies on these organisms really started with an Englishman, Robert Hook who reported seeing microbes under the microscope in 1655.[2] However, he could not see bacteria because of the poor quality of lenses. Then Anton van Leeuwenhoek further developed the microscope, and he was the first to see and describe bacteria in 1658. In the late1800s, the germ theory of disease was enunciated by Pasteur and proved by Koch.[4]

Throughout the Golden Age of Microbiology, numerous different infectious diseases were identified and microbial diseases were discovered during that period. The ability to stop epidemics by interrupting the spread of microorganisms opened the new science of Microbiology.[4] As a result, scientists began to devote time to searching for drugs that would kill

these disease-causing bacteria. After World War II, the antibiotics were introduced as a medicine. Diseases such as, meningitis, syphilis, and many other diseases declined because the use of antibiotics.[7]

In the early history, people led a nomadic life. Their first priorities were fresh food and clean water, so they travelled to find livable places. During that time, people developed different techniques to eliminate harmful microorganisms. For instance, Nicholas Appert discovered a method for preserving and keeping food fresh during long sea voyages by sealing fruits and vegetables in glass bottles and heating them.[5-6] The Persians put silver in milk cans in order to prevent spoilage.[3] Moreover in the 18th and 19th century, numerous disinfectants were developed. For instance, they used pitch, wine, copper, and silver as chemicals to heal wounds and for food preservation. Another discovery, chlorine, was made in 1744. It was first used for water treatment in1843 and it is still used for this purpose today. In the mid. 1800s, sodium permanganate, copper sulfate, acids, alkalis, sulfurs, and alcohols were introduced as disinfectants.[3]

The 19th century, people changed their life style and started to live in crowded cities during the industrial revolution. There were major changes in transportation. People could move one place to another in a short period of time and that resulted in spreading of infectious diseases. It caused an increase in the number of deaths due to spreading of disease in the 1900s. The discovery of antibiotics and improvements in sanitation minimized the incidence of meningitis, tuberculosis, and many other diseases.[8]

Throughout years, increasing numbers of people on earth, and spreading infectious diseases along with technological improvements enhanced the importance of antimicrobial materials.

1.2 Structures and Inactivation Mechanism of Bacterial Cells

Bacteria are a large group of unicellular organisms. They are distributed in every habitat on Earth, growing in soil, water, deep in the Earth's crust, and in the live bodies of plants and animals. They have a wide range of shapes ranging from spheres to rods and spirals. For instance, Staphylococcus appears as grape like clusters and is shown in Figure 1.1[9-10], and Escherichia coli appear rod shaped, as shown in Figure 1.2.[11]

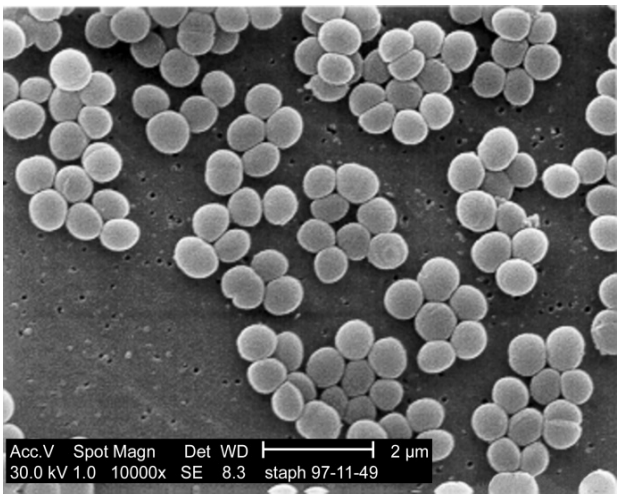


Figure1.1 Staphylococcus

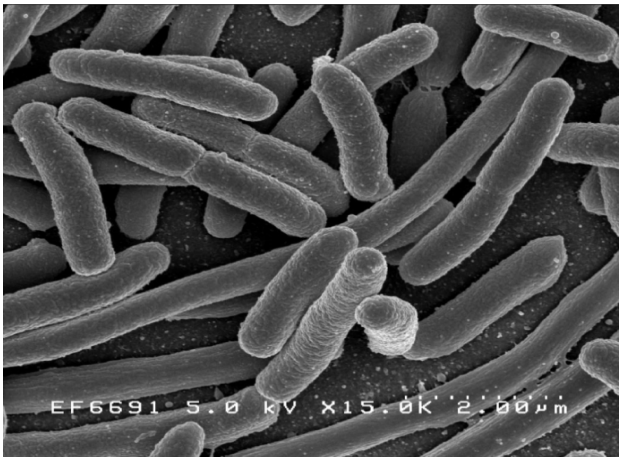


Figure1.2 Escherichia coli

A Danish physician Christian Gram invented the gram-staining method which is used to classify and identify bacteria based on their chemical and physical properties in 1884.[12] Bacteria can be separated mainly two categories. Gram positive bacteria's cell walls have a high affinity for the violet Gram stain because of high amount of peptidoglycan in the cell wall and retain it even through the alcohol rinse. After the process, they appear purple to brown. On the other hand, Gram-negative bacteria have a very low affinity for the violet stain. When they are counterstained with safranine, they appear bright pink to red.[13-14]

Gram-positive and Gram-negative bacteria have some similarities and differrences between each other which are shown in Figure 1.3. They have inner organels, nucleiod, ribosome, inner membrane, cell wall. The major difference between two the two cell types is amount of peptidoglycan in the cell wall. Gram-positive cell walls are five times as rich in peptidoglycan as Gram-negative cell walls, so Gram positive bacteria have relatively thicker cell wall than Gram-negative bacteria. Gram-negative bacteria have outer membrane which is made of lipopolysaccharide, so they are generally more resistant to antibacterial agents than Gram-positive bacteria. Moreover, Gram-negative bacteria have pores in the outer membrane, which act as entry channels for particular molecules.[13]

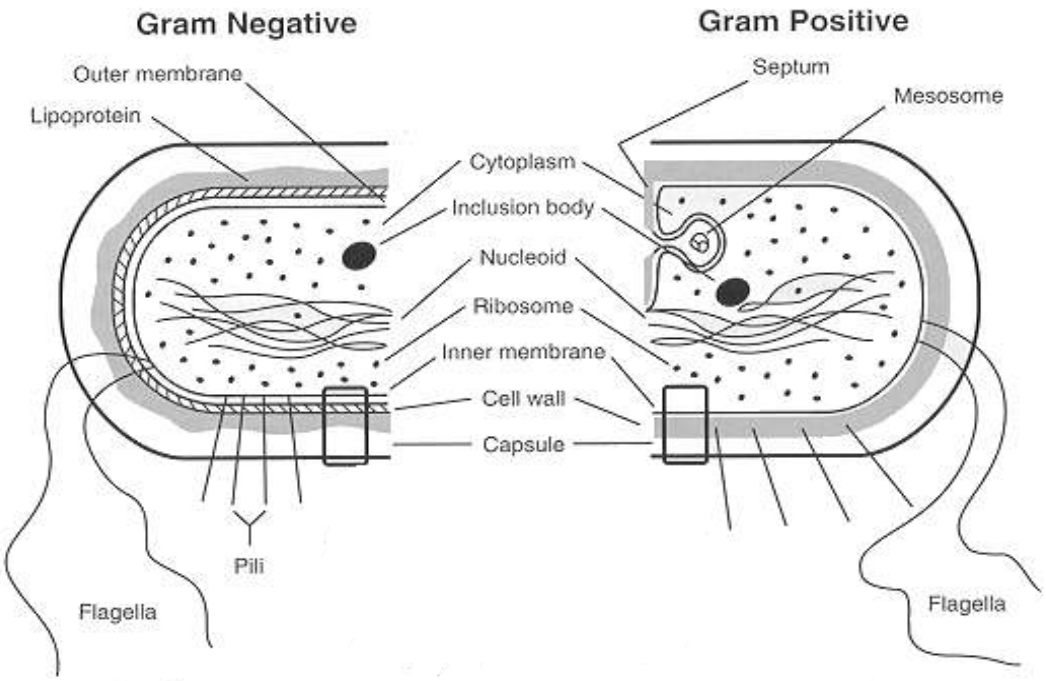


Figure1.3 Structures of the two types of bacteria

In order to get more successful results and inactivate all bacteria, it becomes necessary to understand the mechanisms of biocidal actions. The interaction between biocide and bacteria includes the following steps: 1-Binding and accumulation on bacteria cell wall, 2-Changing of the outer layer, 3-Penetration of the outer membrane and cell wall, 4-The interaction with target sites.[15]

The penetration of biocide into bacteria is influenced by several factors which include, temperature, environmental pH, and the presence of organic matter, all of which can affect on activity of an antimicrobial agent. In general, target regions on bacteria are cell wall, cytoplasmic membrane and cytoplasm constitutions.[18-19]

For inactivation of bacteria, there are two different kinds of interaction between biocide and bacteria:

1-) Physical Interaction: In this mechanism, the cytoplasmic membrane is the potential target. Biocides which destroy target's membrane integrity cause leaking of essential components such as enzymes, nucletides, and nucleosides. Leakages of cytoplasmic components of bacteria will cause death.[16-17]

2-) Chemical Interaction: The interaction occurs between biocide and cytoplasmic constituents such as proteins, DNA, and RNA which inhibits the bacteria's biosynthesis and replication for cell growth.[17-20]

Gram-negative bacteria have additional outer membrane than gram-positive bacteria which makes Gram-negative bacteria less sensitive than Gram-positive bacteria. If a biocide can kill both of them, it is classified as a broad spectrum biocide. In our research, we try to produce broad spectrum antimicrobial products.

1.3 Biocide

People sometimes refer to the chemicals used to kill bacteria as biocides, and other times as antimicrobials. However a biocide is a chemical substance capable of killing living organisms whereas an antimicrobial is a substance that kills or inhibits the growing of microorganisms.[21]

Biocides such as copper or silver were used by Persians to prevent their milk and water from spoiling as early as 450 BC even though they were not aware of microorganisms.[3] However, as we enter the twenty-first century, people use several different biocides to solve specific problems. In addition scientists now have advanced techniques for studying microorganisms. However growing concern about contamination due to microorganisms, emerging new types of

microorganisms such as SARS, bird flu, swine flu, and increasing bacterial resistance to existing agents make the antimicrobial field still important.[21]

In recent years, biocides have been used in several applications such as food packaging, medicine, and textiles.[22] Any of these applications require from the following characteristics: an acceptable price, high efficiency on wide range of bacteria, fast killing speed, no undesirable smell, and environmental friendliness. The desired characteristics of a biocide can change according to area of usage. For instance, in hospital or health related public areas, people want a biocide to kill in a short period of time, be cheap, and inactivate all microorganisms.

The most common biocides include biguanides, quaternary ammonium salts, peroxides, alcohols, heavy metals and halogens. Each of these antimicrobial agents has different mechanism to inactivate microorgnaism, so they have different limitations.[23-24-25]

Biguanides

The first reported synthesis of biguanide was by Rathke in 1879, but biguanides possessing antimicrobial activity was first discovered by IG Farben who observed antiprotozoal activity for N'-aryl biguanides in1933. Biguanides are one of the cationic biocides and their mechanism of inactivating microorganism is by damaging the cell membrane. One of the most common biguanide is Chlorhexidine (CHX) which has a strong affinity for binding to many surfaces and leaves a residue of antimicrobial activity.[16-26-27] Clinical studies showed that salt of chlorhexidine is effective for preservatives and disinfectants in a wide variety of external pharmaceutical applications for acute and chronic wounds, medicated soaps, and hand washes. Chlorhexidine was also shown to have potent antiplaque activity when it was used in a mouthwash. However microorganims can become resistance to CHX.[27]

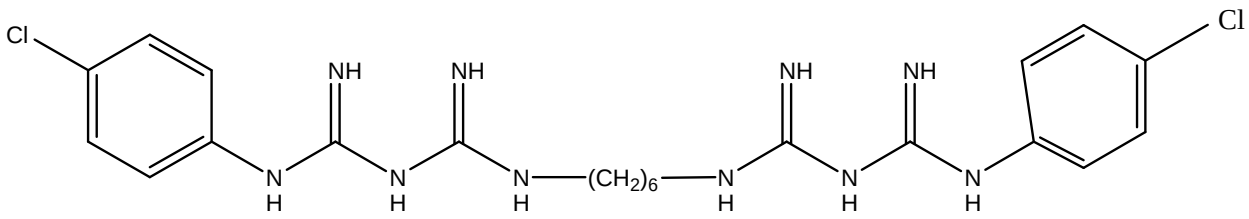


Figure1.4 Structure of CHX

Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) such as cetylpyridinium chloride (CPC), benzalkoium chloride, and dodecyltrimethyl ammonium bromide (DTAB) are examples of cationic biocides.[28] The target is the inner membrane of the microorganisms by ionic interaction with phospholipids components in the cytoplasm membrane, thereby resulting membrane distortion. The inactivation mechanism of the bacteria is electrostatic interaction between the positively charged N^+ site and the negatively charged cell wall which is relatively slow mechanism.[33] QACs show the best antimicrobial activity when they have at least one long hydrocarbon chain of eight to nineteen carbon atoms substituted at the nitrogen.[29-30] QACs are not effective against Gram-negative bacteria and spores and some other bacteria.[31-32] QACs are utilized many applications such as industrial disinfection, cosmetic industry, and household cleaners.

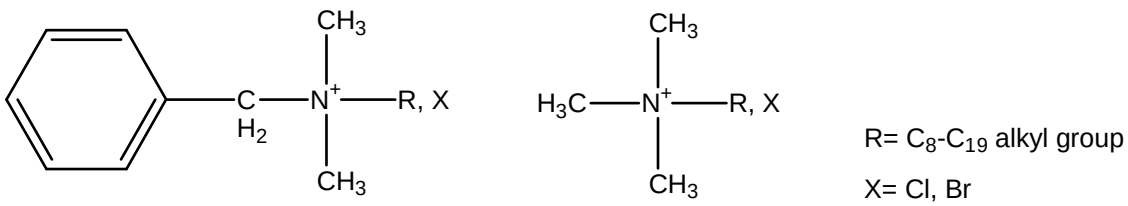


Figure1.5 Structure of Quaternary Ammonium Salts

Peroxides and other forms of oxygen

Peroxides are powerful antimicrobial agents that rapidly destroy bacteria, fungi, and spores.[34-36] The most common peroxygens are hydrogen peroxide (H_2O_2), ozone (O_3), and peracetic acid (CH_3COOOH).[35] They disrupt enzymes and proteins by oxidizing thiol groups. They are used as surface cleaners, wound cleaners, surface disinfectants and for sterilization applications. Hydrogen peroxide is a simple oxidizing agent which is used as a room disinfectant. The disadvantages of peroxides are that readily vaporized under high temperatures, and they are decomposed into oxygen and water, so they are environmental friendly.

Alcohols

The alcohols have long been regarded as effective surface disinfectants. However, their low boiling point restricts them for long-term biocidal activity. Alcohols such as ethanol, isapropyl alcohol at 60-70% concentration are quite effective.[34] Their inactivation mechanism is that of disrupting of bacteria cells membrane. Alcohols are generally not effective against bacterial spores, viruses, and resistant fungi.[34-35] They are widely used by hospitals, biological laboratories, and restaurants.

Heavy Metals

Heavy metals such as silver, copper, mercury, zinc, arsenic, antimony, and their salts have toxicity to most living forms. Metal ions complex with proteins and precipitate proteins. They inactivate bacteria's cell cytoplasm and cleave disulphide bonds within proteins. Heavy metals offer a beneficially broad antimicrobial efficacy at relatively low cost. However, most are toxic to humans. Silver and copper has been used against bacteria in applications such as protecting milk or water from spoilage; however, their inactivation rate is very slow. Mercuric chloride is

very effective against bacterial spores, but its use was abandoned due to environmental problems. Recent research projects showed that long term exposure could induce bacterial resistance to metal salts.[37]

Halogens

Halogen biocides such as Cl_2, Br_2, I_2, HOCl, NaCl, and ClO_2, are strong antimicrobial agents. Halogen compounds react with the amino groups in proteins.[35] They have high affinity for protoplasm, where they oxidize proteins and interfere with vital metabolic reactions. Halogens compounds are inexpensive biocides and have bactericidal, sporicidal, and fungicidal activity.[38]

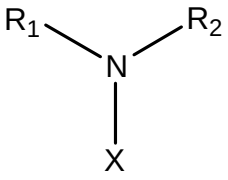
Chlorine has been the predominant biocidal agent for water disinfection and in swimming pools. Stabilized halogen biocides have been shown to more effectively destroy biofilms than free halogens.[39] Hypochlorous acid (HOCl) is a weak acid which forms when chlorine dissolves in water. Hypochlorous acid exists in neurtal form, HOCl, at below pH 7.6, and exists in hypochlorite ion, OCl^- at above pH 7.6. Both HOCl and OCl^- are considered as free chlorine. HOCl is more effective against microorganisms than OCl^-.[38] Inorganic chloride such as sodium hypochlorite solution (bleach) is one of the most widely used household antimicrobial cleaners. It is generally used as a disinfectant. However, chlorine is a toxic disinfectant.[40] In addition chlorinated hydrocarbons and oxidized organic compounds, which are produced after using chlorine, may be toxic and carcinogenic.

There are many different types of antimicrobial agents commercially available in industry. Each of them is considered according to their cost, durability, inactivation rate, environmental issues, etc. In addition, people's expectation from antimicrobial agent changes according to application area. For example, people's expectation for an antimicrobial agent is high-level effectiveness

against bacteria, viruses, and bacterial spores in hospitals or health related public areas. On the other hand, people do not look for high-level antimicrobial property from some basic types of clothes, such as underwear, or socks. As a result, each of antimicrobial agents is considered according to application area. The best antimicrobial agent should be inexpensive, effective on wide range of microorganisms, environmental friendly, durable and reusable.

1.4 N-Halamines

N-halamine materials are very effective biocides which have been used for the last few decades. An N-halamine is a compound which contains covalent bonds between nitrogen and halogen. N-halamine biocides show several characteristic properties including long term stability, environmental friendliness, non-toxicity to humans, biocidal function against broad range of microorganisms, and regenerability properties upon exposure to washing cycles.[41-42] The general structure of N-halamines is shown in Figure1.6



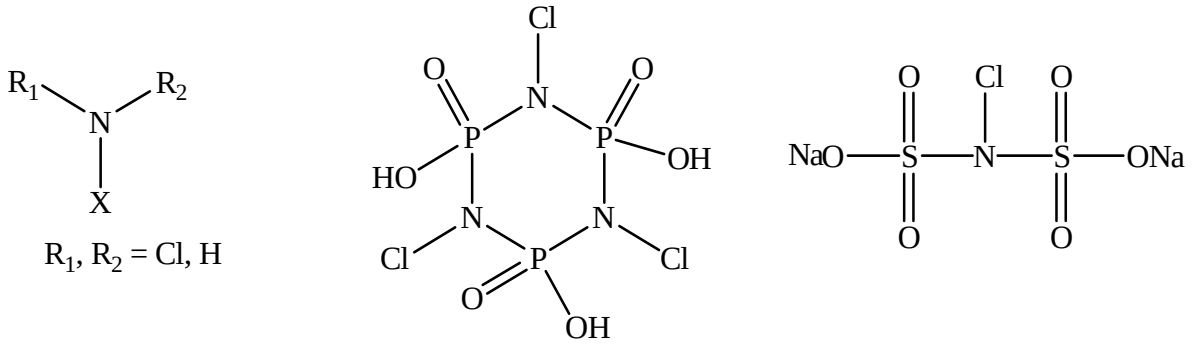
R_1, R_2 = H, Cl, Br, Organic group, and Inorganic group

X = Cl, Br

Figure1.6 The general structure of N-halamine

There are two different N-halamine compounds according to the R_1 and R_2 groups they contain. When these R_1 and R_2 groups are inorganic groups (phosphate, sulfate) or both hydrogen and halogens, they are called inorganic N-halamines. They can be used for controlling taste and odor which is caused by contaminated organic materials. The disadvantages of inorganic N-halamines

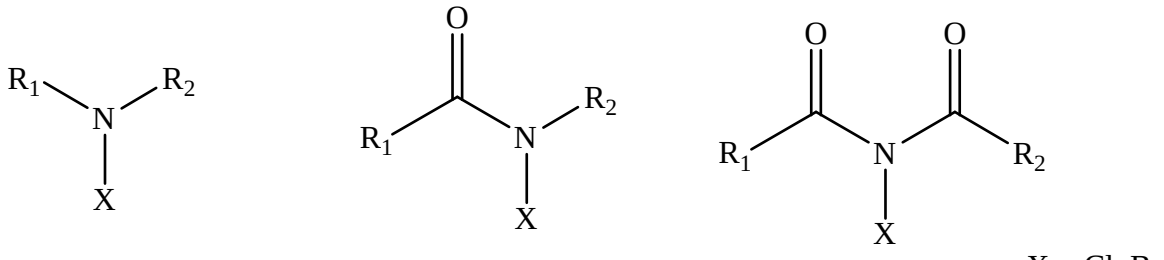
are sensitivity to pH and water temperature, and they are not environmental friendly.[43] Some of inorganic N-halamine compounds are shown in Figure 1.7.



1 2 3

Figure1.7 Structures of some inorganic N-halamines

If one of the R groups is an organic group (alkyl group, carbonyl group), it is called organic N-halamine. There are three different types of organic N-halamine structures: amines, amides, and imides as shown in Figure 1.8. They have different stabilities and biocidal efficacies between each other.[44]



Amine Amide Imide

Figure1.8 Structures of N-halamines

In the amine structure of N-halamines, the electron donating groups (i.e. alkyl) adjacent to the nitrogen strengthens the N-X bond. The halogen is held tightly, so releasing halogen from N-halamine is limited. On the other hand, two electron withdrawing carbonyl groups next to N-X bond on the imide structure makes the weakest N-X covalent bond. In other words, the N-X bond tends to release halogen because of destabilization of the carbonyl groups. The amide structures of N-halamines have both electron withdrawing groups and electron donating groups at the same time, so the releasing of halogen on N-X is intermediate between amine and imide. Consequently, the stabilities towards dissociation of N-X bonds of N-halamines are in the order amine > amide > imide, while antimicrobial activity is vice versa (imide > amide > amine). Therefore, amide structures of N-halamines are generally the most preferred one for providing good stability and effective antimicrobial property.[44]

The other factor that affects stability of bonding forced between halogen and nitrogen is the type of halogen. The strongest N-halamine bond is the one which overlaps between nitrogen and halogen. Among the halogens (except fluorine), chlorine is the smallest one, so the bond between nitrogen and chlorine overlaps well. When the size of halogens increases, the stability of the bond decreases, so the order of the stability in halogens is N-Cl > N-Br > N-I.[34]

A disadvantage of some of N-halamine chemicals is dehydrohalogenation which occurs if there is an α hydrogen in an amine or amide structure of the N-halamine; the halogen in the N-X bond can undergo dehydrohalogenation with the adjacent α-hydrogen to form C=N bonds as is shown in Figure 1.9.

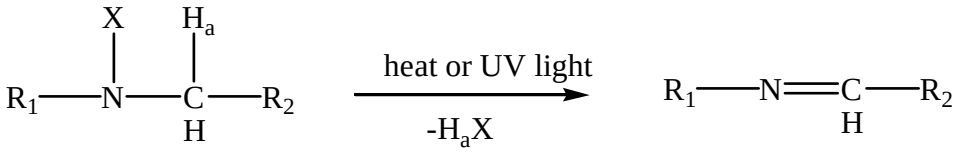


Figure1.9 Dehydrohalogenation mechanism

This process can be promoted by, bases, UV light and heat. After losing the halogen through dehydrohalogenation, the nitrogen cannot bond halogens again, and it loses its biocidal property. In order to avoid the defect of dehydrohalogenation, cyclic N-halamines are preferred. They are very effective against broad spectrum of microorganisms and have a long shelf life. The Worley group at Auburn University has focused on synthesis and development of N-halamine derivatives that have been used for industrial applications since the 1980s. Some of cyclic N-halamines are shown in Figure 1.10.

For X=H, 1: 5,5-dimethyl-2,4-imidazolidinedione (5,5-dimethylhydantoin), 2: 4,4,5,5-tetramethyl-1,33-imidazolidin-2-one, 3: 4,4-dimethyl-2-oxazolidinone, 4: 2,4,6-trihydroxy-1,3,5-triazine (cyanuric acid), 5: 6,6-dimethyl- 1,3,5-triazine-2,4-dione, 6: 3,3,6,6-tetramethyl-2,5-piperazinedione, 7: 2,5-pyrrolidinedione (succinimide), 8: 2,2,6,6-tetramethylpiperidine, 9: 2,2,4,4-tetramethyl-1,3-oxazolidine, 10: 1,3,5-triazine-2,4,6-triamine (melamine), 11: 3a,6a-dimethyltetrahydroimidazo[4,5- d]imidazole-2,5(1H,3H)-dione, 12: 7,7,9,9-tetramethyl-1,3,6-triazospiro[4,5]decane-2,4-dione.

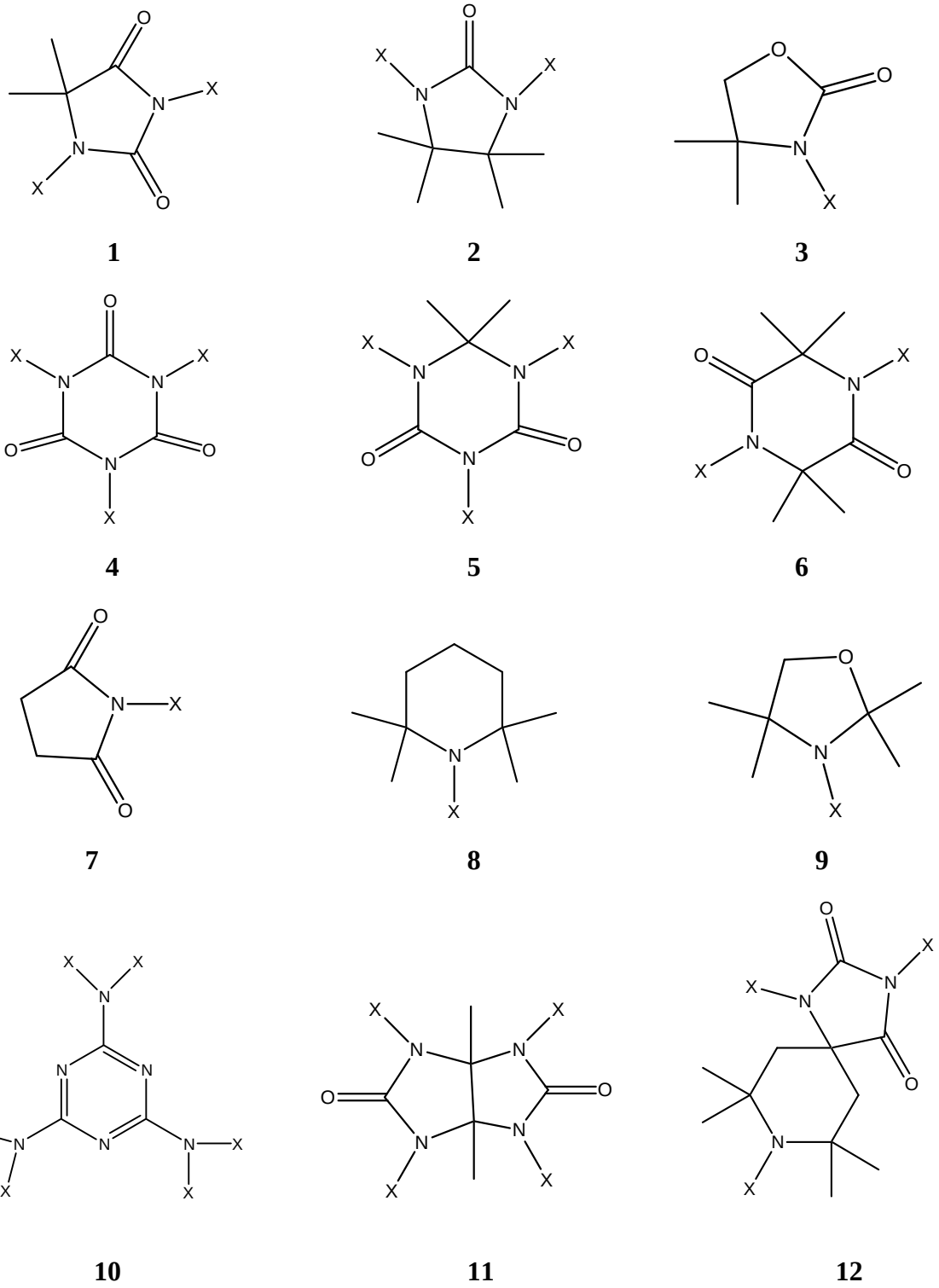


Figure1.10 Structure of heterocyclic N-halamines. (X = H, Cl, or Br)

One of the most outstanding features of N-halamines is rechargeability. Even if they lose the chlorine after interaction with microorganisms, they can be recharged through a simple halogenation reaction with sodium hypochlorite solution or household bleach. It is a reversible reaction which is shown in Figure 11.

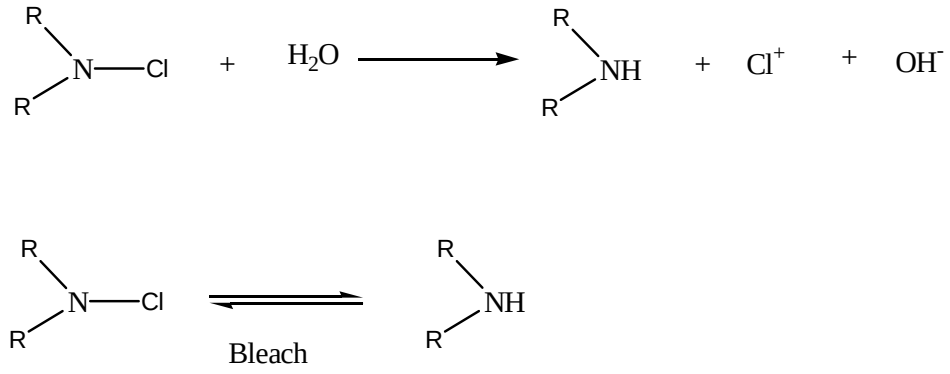


Figure1.11 Recharging Reaction of N-halamine

1.5 N-N-Halamine Based Polymeric Materials

The first attempt to use of N-halamine chemistry with polymers was introduced as a biocide for water disinfection. There are two different ways to make N-halamine biocidal polymers:

1- Polymerization of a biocidal monomer by itself or with other monomers

2- Grafting biocidal moieties onto polymer backbone.

In the first method, N-halamine compounds have functional groups which allow polymerization with the assistance of polymerization initiators. The most common method is addition polymerization via monomers that have unsaturated bonds in their structures such as a vinyl group.[50] For this method, 2,2-azobisisobutyronitrile (AIBN), hydrogen peroxide, and potassium persulfate are used to initiate the polymerization reaction.

In Figure 1.12 and 1.13 the polymerization process was demonstrated using the N-halamine monomers. After halogenations of Poly I and Poly II, they have been shown to inactivate various bacteria, fungi, and viruses within short periods of time. They can be regenerated with exposure to household bleach (sodium hypochlorite solution). In addition they show the best biocidal property for water treatment. These polymers are being used commercially in numerous applications in developing countries.[51]

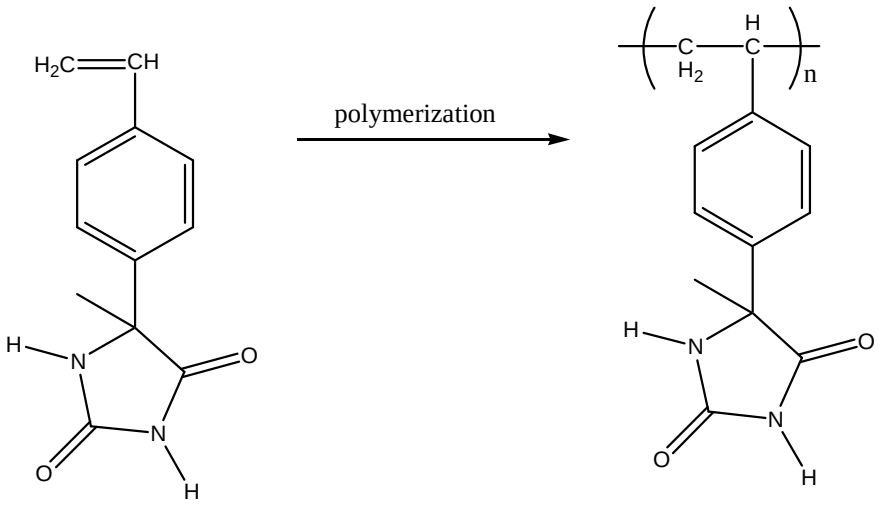


Figure 1.12 Structure of Poly 1 monomer and its homopolymer

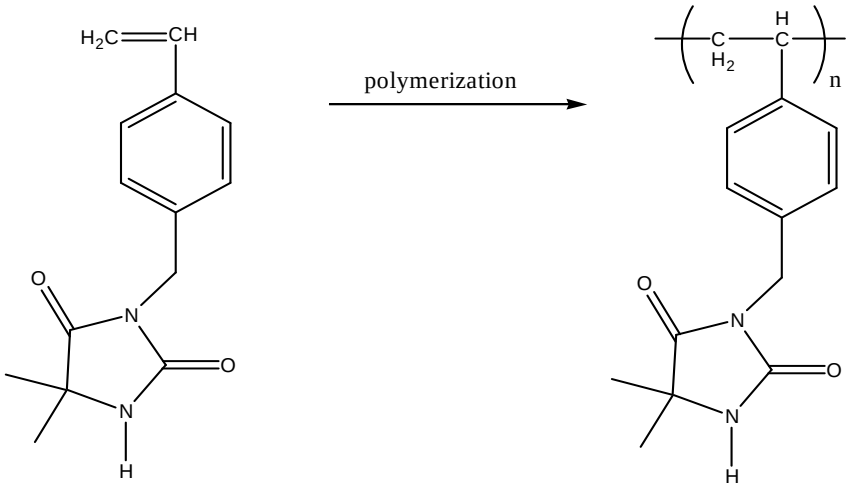


Figure 1.13 Structure of Poly 2 monomer and its homopolymer

There are several types of N-halamine precursors which are commercially available in industry. Some of them have no available chemical group to bind onto a substrate. Therefore, to attach these kinds of N-halamine precursors to a substrate requires tethering groups such as siloxanes, epoxides, and hydroxyls. Figure 1.14, 1,15, and 1.16 show several attachment methods of 5,5 dimethylhydantoin onto cotton fabric.[34]

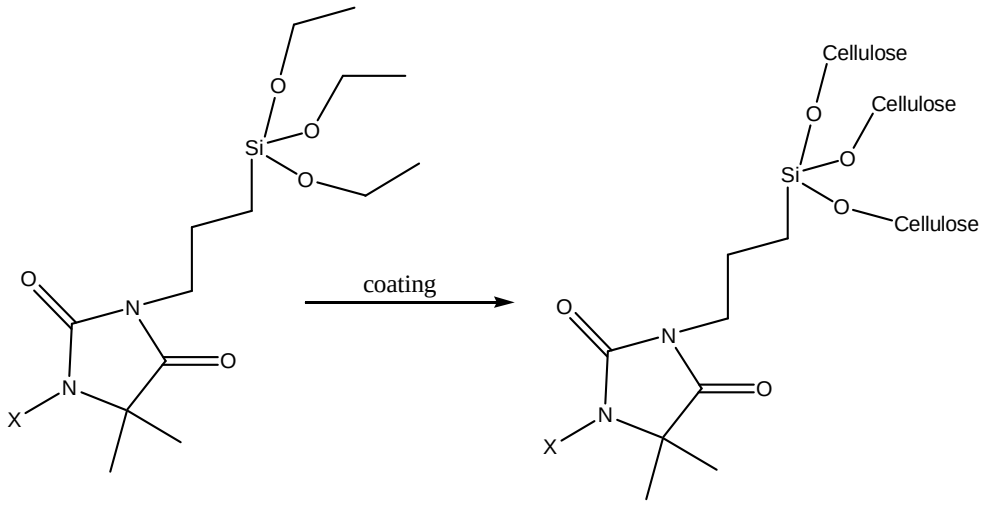


Figure1.14 The attachment of hydantoin siloxane onto cellulose

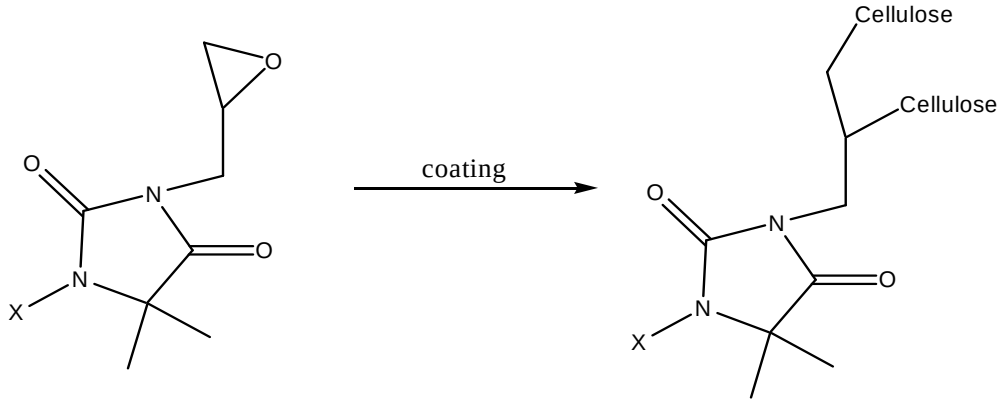


Figure1.15 The attachment of hydantoin epoxide onto cellulose

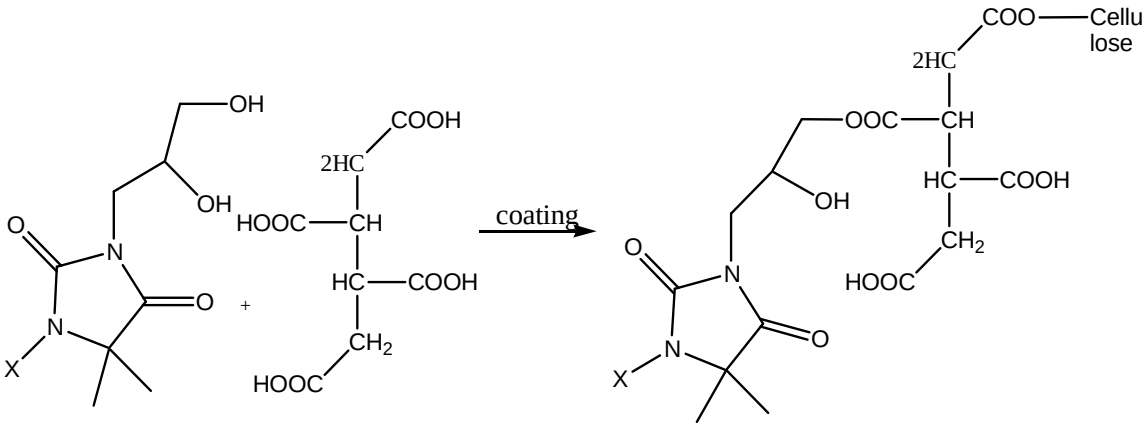
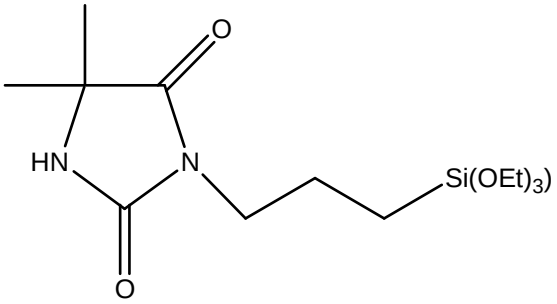
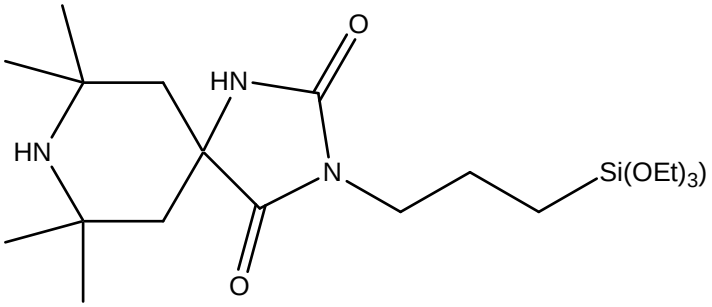


Figure1.16 The attachment of hydantoin diol onto cellulose via BTCA

Alkoxy silane (siloxane) is an outstanding tethering group for bonding various organic groups onto substrates.[49] Numerous N-halamine precursors have been attached onto different substrates by using this coupling agent that is shown in Figure 17. Synthesis of Hy-sil (Figure 1.17 (a)) was carried out by the reaction of 5,5-dialkylhydantoin salt and 3-chloropropoyltriethoxysilane (BA-1). In addition, synthesis of TTDD Siloxane (Figure 1.17 (b)) was carried out 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4,5]decane-2,4-dione salt 3-chloropropoyltriethoxysilane. Both N-halamines have been shown to provide antimicrobial properties on various materials such as polyester, silica gel, cellulose and paint.[34]



A) BA-1



B) TTDD Siloxane

Figure 1.17 Structures of N-halamine siloxanes

1.6 N-Halamine Based Antimicrobial Textile Materials

The importance of textiles started with the first human being. They used textile materials to cover their body. In the Stone Age most clothing was made of leather or fur. By the Bronze Age, people had learned to spin yarn and to weave clothes, and in the Middle Age, textile clothing was common and everybody had several clothes to wear.[45] As we enter the twenty-first century, textile materials are used for several significant purposes besides clothing such as filtering, protecting from fire and environmental conditions etc.

Textile materials are a vehicle which allows transferring of microorganisms and spreading infectious diseases. Textile materials consist of fibrous structures that are very susceptible to contamination by various microorganisms. In addition, some of pathogenic microorganisms can survive more than 90 days on the materials, and could be dispersed into the air and spread to other people or materials by direct or indirect contact.[47] Therefore, antimicrobial textiles can prevent spreading or transferring from clothes to people or other textiles.

N-halamine antimicrobial textiles have an ability to inactivate Gram-positive and Gram-negative bacteria successfully in a short period of time. As an antimicrobial textile, one of the most important advantages which attracts interest is that N-halamine based antimicrobial textiles can be regenerated even they lose their chlorine after microbial exposure, because N-halamines can be recharged through simple exposure to diluted household bleach. Therefore, N-halamine based antimicrobial textiles can be used every place for a long period time. They can still the active after 50 machines washing cycles, and they are not toxic.[34]

N-halamine based antimicrobial textile products have numerous outstanding properties which make them favorable. Some of the application areas are military, hospital, and health related

public areas. In addition, various N-halamine compounds have been used as effective antimicrobial compounds for treating cotton, nylon, polyester, so they can be applied to different textile materials such as sheets, pillows, uniforms, bedding materials, hospital clothes, gowns, socks, shirts etc. In addition, N-halamine based antimicrobial products are applied in military applications such as for tents and army uniforms.[35]

Several millions of textile materials are discarded as waste every year. In addition the percentage of disposable materials has been increasing over the years. All of these textile materials are potentially hazardous for the environment and also human health. In order to reduce this risk, regenerable antimicrobial materials (i.e. N-halamines) can become advantageous for upcoming health problems.

1.7 Research Projects:

Two independent projects have been studied during the master's degree. In the first project, we examined a commercially available chemical which was previously used as a conventional durable press (DP) finishing agent for cellulosic textiles. The objective of this project was producing stable and an effective against broad-spectrum of microorganism antimicrobial fabric, and simultaneously to improve the crease recovery of the cotton fabric. First of all, N-(hydroxymethyl)acrylamide (NMA) was coated on cotton fabric, and then methacrylamide or acrylamide was grafted on NMA coated cotton fabric. The antimicrobial activities, stabilities, wrinkle recovery angle, and ultraviolet (UV) light resistances of different derivatives were tested and compared. In the second project, the objective was to synthesize an N-halamine precursor that is stable, rechargeable and effective against Gram-positive and Gram-negative bacteria. In this regard N-tertbutylacrylamide (NTBA) was copolymerized with 3-

chloropropyltriethoxysilane. Since the chlorine loading is almost zero when it was chlorinated at pH 7 or 11, and toxic chlorine gas is produced when chlorination pH decreases to 4.0 or lower than 4.0, synthesis of NTBA-siloxane is not commercially favorable. Then it was grafted onto NMA finished cotton fabric. Then the antimicrobial activities, stabilities, wrinkle recovery angles, and ultraviolet (UV) light resistances of different derivatives were investigated.

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CHAPTER 2

NMA AS A MULTIFUNCTIONAL FINISH TO COTTON AND A TETHER FOR GRAFTING AND MONOMERS

2.1 Introduction:

Demands for a healthy life style make hospitals and health related public areas the most significant places. On the other hand the transmission of the microorganisms via air and fabrics from patients to staff could make these places potential source of infectious, because of the probability of the spread of these microorganisms which are found there in high concentration. In order to reduce the risk of transmission from contaminated surface to the environment and workers or patients, the microorganisms must be inactivated in short period of time. This suggests a great need for antimicrobial textiles that are able to protect against all pathogenic microorganisms.[20-21] There are numerous different antimicrobials, such as quaternary ammonium salts[10], peroxides[11], heavy metals[12], and N-halamines[13] used as a biocide. Among these antimicrobial agents, N-halamines biocides are the best effective biocides due to their long term stabilities, effectiveness of broad range of microorganisms, non-toxicity to humans, deactivation microorganisms in short period of time, and recharge ability.[13-15] N-halamines which have at least one of nitrogen-halogen bond in the structure.

N-halamine precursors can be covalently bound to various surfaces via tethering groups such as siloxane, epoxides, and hydroxyls,[14-16-17] and also some of N-halamine chemicals have some functional groups that can bind to some surfaces and undergo polymerization with other unsaturated monomers. These N-halamine precursors converted into the N-halamine materials through halogenations process which is shown in Figure 2.1.

There are three types of organic N-halamine structures: amines, amides, and imides.[10-14] In the amine structures of N-halamines, electron donating group adjacent to the nitrogen strengthen the N-X bond, so the bond has the lowest dissociation constant and is the most stable of the N-halamines. The imide structure of N-halamines has highest dissociation constant and is the least stable, because of two electron withdrawing carbonyl groups next to the N-X bond. Halogen on amides has an intermediate stability,[18] so amide structures of N-halamines are generally the most preferred one for providing good stability and effective antimicrobial properties.

Textile finishing is a method which includes chemical and mechanical processes to improve the acceptability of the product.[19] Conventional durable press is one of them and also is one of the most preferred ones.[7-19] In Durable press (DP) finishing, padding and curing forms cross links. Padding of curing forms cross links between cellulose molecules and allows to cotton to retain its flat, unwrinkled state, particularly through the drying cycle after washings. This allows the fabric to come from the dryer with an ironed appearance. Conventional durable press finishing of cotton results in fabrics with reduced physical properties such as abrasion resistance and strength.[6-7] In this study, N-halamine methyl substituent used for biocidal activity and to increase durable press hopefully without any further decrease physical properties (shown in Figure 2.1). The antimicrobial effectiveness against broad range of microorganisms, stabilities, UV

resistance, recharge ability, and wrinkle recovery angle investigated and the results were examined.

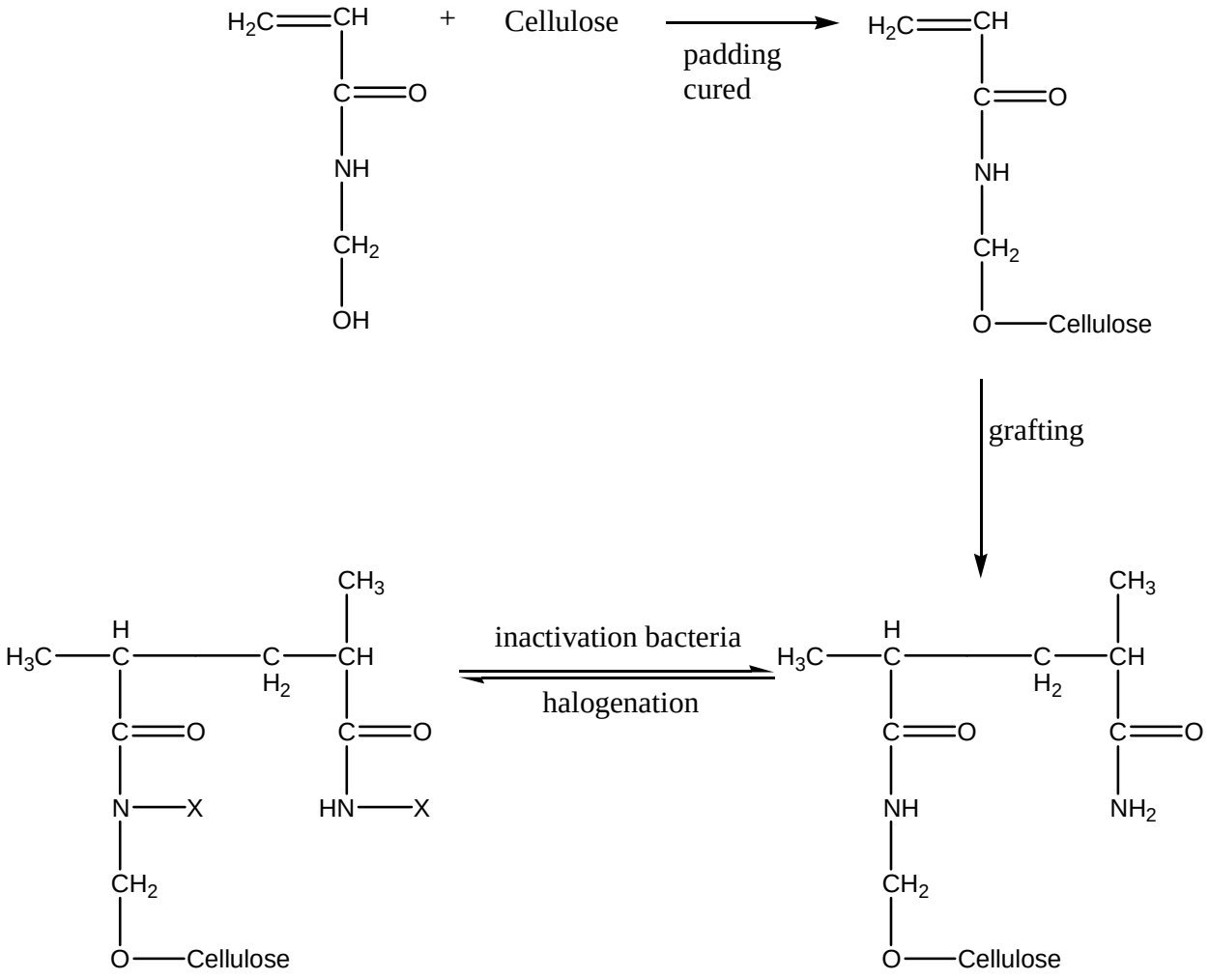


Figure 2.1 The preparation of antimicrobial padding and coating onto cotton fabric. (X= Cl. Br)

2.2. Experimental

Materials:

All chemicals were purchased from the Sigma Aldrich Chemical Company, and Fisher Scientific Chemicals. The fabric used was Style 400 Bleached 100% Cotton print Cloth, (Test fabrics, West Pittston, PA). Test fabrics, West Pittston, PA. The household bleach was Clorox brand (Clorox, Inc., Oakland, CA). The bacteria used were Staphylococcus aureus ATCC 6538 and Escherichia coli O157:H7 ATCC 43895 (American Type Culture Collection, Rockville, MD)

Instruments:

The FT-IR data were obtained with Nicolet 6700 FT-IR. UV light stabilities were measured with Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA). Scanning Electron Microscope pictures were taken with a model Zeiss Evo 50VP.

Coating Procedure:

In this study, three different commercially available monomers that might be converted to N-halamines were used: N-(hydroxymethyl) acrylamide (NMA), methacrylamide (MA), and acrylamide (AM). The monomers in various concentrations were padded and coated onto the cotton fabric. There were two different necessary approached tried to complete the process. The first step was padding N-(hydroxymethyl)acrylamide onto cotton swatches. In this process, the first N-(hydroxymethyl) acrylamide monomer and the catalyst, magnesium chloride, were dissolved in water at concentrations 5 and 1 percent respectively and the mixture was stirred 15

minutes to produce a uniform solution. After cotton swatches were immersed in solution three times and squeezed with roller, they were dried at 90^{o}C for 5 min. and cured at 175^{o}C for 3 min.[7] Then the swatches were soaked and washed vigorously in a 0.5% detergent solution for 15 min., rinsed with water several times to remove any weakly bonded or unbonded compounds, and then dried at 45^{o}C for 1 hour. The second step was grafting methacrylamide or acrylamide onto N-(hydroxymethyl)acrylamide coated cotton swatches. In this process, methacrylamide or acrylamide monomer and the initiator, potassium persulfate, were dissolved in water at concentrations 5 and 1 percent respectively and the mixture was stirred for 15 minutes to produce a uniform solution. Then cotton swatches were immersed in solution for three times and squeezed with roller. Then the swatches were dried at 60^{o}C for 10 min. and cured at 120^{o}C for 5 min.[22] Then cotton swatches were washed vigorously in a 0.5% detergent solution for 15 min., rinsed with water several times to remove weakly bounded or unbounded chemicals, and dried at 45^{o}C for 1 hour.

Chlorination Procedure:

The treated cotton swatches were chlorinated by soaking in a 10% aqueous solution of NaOCl household bleach (0.6% sodium hypochlorite) at pH 7 (adjusted with 6N HCl) at ambient temperature for 1 hour. The chlorinated swatches were washed with distilled and tap water and dried at 45^{o}C for 1 hour to remove any unbonded free chlorine from the material. The loading of bound chlorine on the swatches was determined by an analytical titration procedure.

Analytical Titration Procedure:

The chlorine concentration on the treated samples was measured by a standard iodometric/thiosulfate titration method. In this procedure, about 0.2g of coated and chlorinated

cotton swatch material was suspended in 50 mL of 0.1 N acetic acid solution. After addition of 0.25 g KI, and starch as an indicator, the solution was stirred for 45 min. to 1 hour. Then the solution was titrated with 0.00375N sodium thiosulfate until the blue color disappeared at the end point. The Cl^+% on the samples were calculated from the equation below:

$$Cl^+\% = [N \times V \times 35.45 / (2 \times W)] \times 100\% \quad (1)$$

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

Biocidal Efficacy Testing:

One inch square of chlorinated and unchlorinated treated cotton samples were challenged with Gram-positive Staphylococcus aureus ATCC 6538 and a second cotton sample was challenged with Gram-negative Escherichia coli (E. coli) O157:H7 ATCC 43895 for antimicrobial efficacy analyses by using a modified AATCC Test Method 100-1999. A bacterial suspension (25µL), having a known concentration of colony forming units in pH 7 phosphate buffer solution was added to the center of one inch square cotton swatch and a second identical swatch was sandwiched over the first swatch. A sterile weight was used to insure sufficient contact of the swatches with the bacteria suspension. After the contact times of 1, 5, 30, and 60 min., the samples were quenched with 5.0 mL of sterile 0.02N sodium thiosulfate solution to remove any oxidative chlorine. Serial dilutions of the quenched samples solution were placed on Trypticase agar. The plates were incubated at 37^{o}C for 24 h and then counted to determine the number of viable bacteria that had remained after exposure to the fabric.

Washing Testing:

Laundering tests were performed to measure the stability and rechargeability of chlorine on coated cotton swatches by using standard washing test of American Association of Textile Chemists and Colorist (AATCC) Test Method 61. All types of coated swatches were washed for the equivalent of 5, 10, 25, and 50 machine washing cycles. The chlorine loading of the samples after the washing, after rechlorination, and unchlorinated ones after chlorination were determined by the titration procedure which was described above. The samples were then titrated as previously described to determine the chlorine content.

UV Light Stability Testing:

UV light stabilities of chlorinated NMA, MA grafted onto NMA, and AM grafted onto NMA coated cotton swatches were measured in an Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA). The samples were placed in the UV (Type A, 315-400nm) chamber for times in the range of 1 to 72 hours. After specific time of exposure, samples were removed from the UV chamber and titrated, or rechlorinated and titrated.

Wrinkle Recovery Angle Testing:

Wrinkle recovery angle (WRA), warp (W) + filling (F) of the treated cotton fabrics was evaluated by using AATC test method 66-1998, Option 2. According to this test method, treated samples were prepared cut 40 x 15 mm folded and 500 $\pm$ 5 g of weight was loaded on the folded specimen for 5 min. The fabric on one side of the fold is held while the other is released and allowed to unfold with its orientation maintained vertically downward to eliminate the effect of

gravity on unfolding. The angle to which the fabric unfolds within 5min is called the its wrinkle recovery angle.

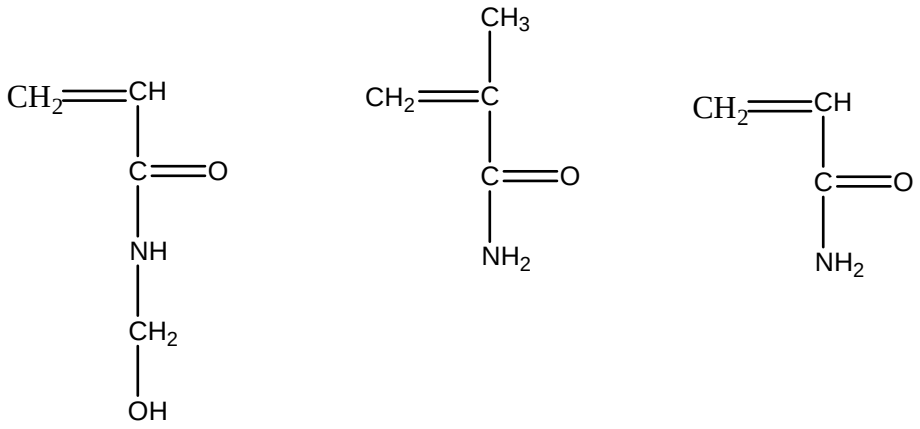
2.3 Results and Discussion:

Coating Procedure:

Biocidal efficacy of coating depends on biocide's chemical structure (amine, amide or imide) and its N-halamine content[1]. Higher N-H bond concentration in the structure results higher chlorine or bromine loading. However, too many halogen atoms may decrease the hydrophilic nature of compounds that are coated onto fabric[3]. Since it is the active Cl^+ inactivating microorganism, higher chlorine loading should improve biocidal efficacy; but in contrast, it is sometimes reduced because high chlorine loading increases surface hydrophobicity of cotton, resulting poor contact with the suspension of microorganisms.[2]

N-(hydroxymethyl)acrylamide (NMA) can be used as a cross-linker to provide wrinkle free or permanent press[4]. NMA has two functional groups, N-methylol, and vinyl that can be controlled to undergo etherification with cellulosic hydroxyl group and vinyl polymerization, respectively. Although, it has also an amide functional group for biocidal activity, the chlorinated form of NMA is expected to undergo dehydrohalogenation reaction forming C=N bond since there is an α hydrogen adjacent to amide group[5]. The C=N- has no replaceable H, so cannot be recharged. The purpose of this study is to react or modify cotton fabric with NMA by etherification, and then grafting different N-halamine monomers onto NMA treated fabric by vinyl copolymerization. Thus, a multifunctional finishing process permanent press and antimicrobial functionality onto cotton fabric is gained in single process. In this regard, either acrylamide or

methacylamide were grafted onto NMA finished cotton fabric. The structures of these three monomers are shown in Figure 2.2.



a) N-(Hydroxymethyl)acrylamide b Metharcylamide c) Acrylamide

Figure 2.2 Chemical structure of N-halamine precursors used in this project

NMA is padded onto cotton by dipping the sample into NMA solution, squeezing between the rotating rollers and curing at specific temperature. A detailed description of the process can be found in the experimental section 2.2.3. Firstly, effect of NMA concentration on chlorine loading was studied and as was expected, chlorine loading increases linearly when NMA concentration increases, shown in Figure 2.3.

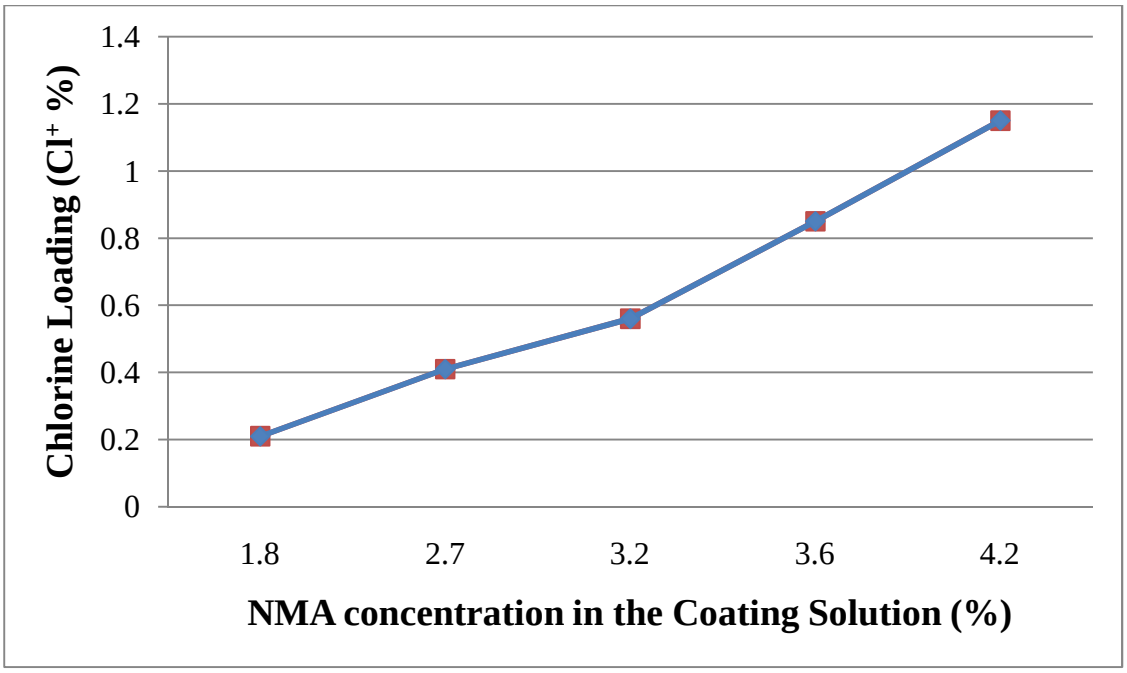


Figure 2.3 Chlorine loading (Cl^+ %) versus of NMA content of cotton fabric

Secondly, in order to see the effect of dehydrohalogenation, chlorinated NMA padded cotton swatches were dried in an oven at different temperatures for 1 hour. It is reported that dehydrohalogenation reaction can be promoted by UV light and heat[6], so it is expected to lose chlorine by increasing temperature. As it can be seen form Figure 2.4, chlorine loading decreasing by increasing drying temperature. However, the loss of chlorine by heat is not a fast process and at this point it is hard to determine if this loss is because of dehydrohalogenation reaction or just breaking of N-Cl bond. In order to address the reason of this chlorine loss, samples were rechlorinated after drying step, and then dried at standard temperature (45^{o}C). The chlorine loading increased to the initial amount, so the loss of chlorine by heat was just breaking N-Cl bond, not dehydrohalogenation. The results of UV exposure are described in detail later, but there was not 100% rechargeability. At this might indicate dehdrohalogenation, we continued with the experiments to minimize α hydrogen in the structure.

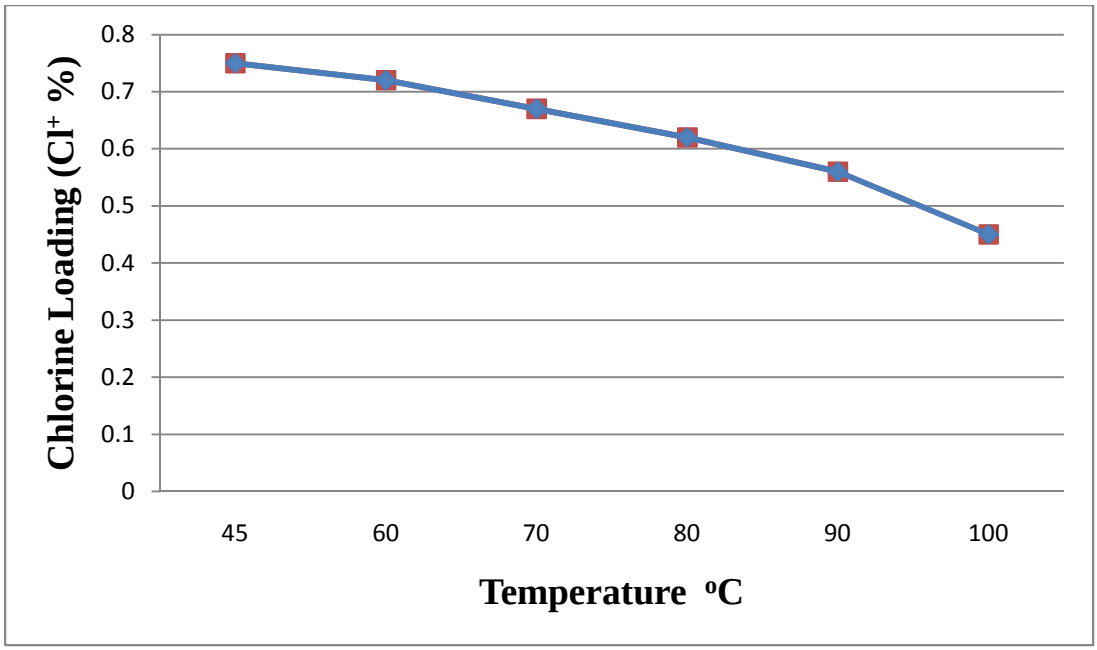


Figure 2.4 Chlorine loading (Cl^+ %) of NMA padded cotton fabric dried at different temperatures for 1 hour after chlorination

The second step for this study is grafting methacrylamide (MA) or acrylamide (AM) onto N-(hydroxymethyl)acrylamide (NMA) finished cotton fabric. MA or AM is grafted onto NMA finished cotton by dipping the sample into MA or AM solution, squeezing between the rotating rollers and curing at specific temperature. A detailed description of the process can be found in the experimental section 2.2.3. The results are shown in Table 2.1.

Table 2.1 Chlorine loadings of MA or AM grafted onto NMA coated cotton fabrics

Samples	NMA Content on fabric* (%)	MA Content on fabric* (%)	AM Content on fabric* (%)	Measured [Cl+]%	Theoritical [Cl+]%
2.5% NMA + MgCl26H2O	2.8	0	0	0.52	0.73
5% NMA + MgCl26H2O	3.12	0	0	0.76	0.81
(2.5% NMA + MgCl26H2O) + (5% MA + PPS)	2.81	3.12	0	0.41	2.17
(2.5% NMA + MgCl26H2O) + (10% MA + PPS)	2.78	4.7	0	0.45	2.88
(5% NMA + MgCl26H2O) + (5% MA + PPS)	3.22	3.21	0	0.44	2.32
(5% NMA + MgCl26H2O) + (10% MA + PPS)	3.42	4.71	0	0.47	3.06
(2.5% NMA + MgCl26H2O) + (5% AM + PPS)	2.88	0	3.34	0.51	2.42
(2.5% NMA + MgCl26H2O) + (10% AM + PPS)	2.82	0	4.83	0.57	3.15

* Measured by weight gain.

After grafting MA or AM onto NMA finished cotton fabric, the chlorine loading was unexpectedly lower than the loading before grafting. Theoretically, Cl can make a bond with all the nitrogen in the copolymer structure but experimentally chlorine loading is one fourth of this theoretical calculation.

FT-IR Confirmation:

FT-IR spectra of the treated cotton fabrics confirmed that N-halamine precursors bonded to cotton fabric. The band at ca. 1662cm^{-1}, 1630cm^{-1} and 1540cm^{-1} were detected which can be assigned amide structure of NMA padded cotton fabric. After grafting MA or AM, two detected band ca.1662cm^{-1} and1630cm^{-1} overlapped one strong single band at ca. 1658cm^{-1}, and also the band at 1540cm^{-1} was detected which were assigned to amide structure of NMA+MA and NMA+AM (in Supporting Information).

Antibacterial Efficacy

Chlorinated NMA, NMA+MA, and NMA+AM treated cotton fabrics were challenged with Gram-positive bacteria, S. aureus, and Gram-negative, E. coli O157:H7. Table2.2 shows that chlorination version all three types of treatment samples (NMA, NMA+MA, and NMA+AM) inactivated both Gram-positive and Gram-negative bacteria with 7 log reduction in 5 min contact time. Moreover, just NMA padded cotton fabrics inactivated both species in 1 min contact time. NMA+MA treated fabrics inactivated just Gram-positive and Gram-negative bacteria in 1 min and 5 min contact time, respectively. NMA+AM treated fabrics inactivated both bacteria in 5 min contact time. The short time effectiveness against both bacteria was in the order of NMA, NMA+MA, and NMA+AM. Chlorinated NMA+MA, and NMA+AM coated fabrics were less rapidly effective against Gram-negative bacteria with a 0.10 log reduction within 1 min contact time. However, all three types chlorinated fabrics showed excellent antimicrobial efficacy against both bacteria in 5 min contact time. Unchlorinated treated cotton fabrics served as controls which provided only around 1.3 log reduction for Gram-positive and around 0.10 log reduction for Gram-negative after 30 min contact time.

Table 2.2 Biocidal efficacy against microorganisms

Samples	Contact time (min)	Escherichia colli O157:H7[a]		Staphylococcus aureus[b]	
		Bacterial Reduction		Bacterial Reduction	
		% Reduction	Log Reduction	% Reduction	Log Reduction
NMA-control	60	3	0.015	94.47	1.26
NMA + MA-control	60	23.3	0.12	95.08	1.31
NMA + AM-control	60	30.07	0.16	96	1.40
NMA - Cl	1	100	7.99	100	7.97
	5	100	7.99	100	7.97
	30	100	7.99	100	7.97
	60	100	7.99	100	7.97
NMA + MA-Cl	1	25.56	0.13	100	7.97
	5	100	7.99	100	7.97
	30	100	7.99	100	7.97
	60	100	7.99	100	7.97
NMA +AM-Cl	1	21.04	0.11	99.99	5.04
	5	100	7.99	100	7.97
	30	100	7.99	100	7.97
	60	100	7.99	100	7.97

[a] Microorganism: E. coli O157:H7. Total bacteria: 9.67×10^7 CFU/sample (7.99 logs).

[b] Microorganism: S. aureus. Total bacteria: 9.33×10^7 CFU/sample (7.97 logs).

[c] Chlorine loadings on the coated swatches were 0.78, 0.45, 0.49 respectively.

[d] The error in the measured Cl+ weight percentage values was ±0.01.

Durability and Rechargeability toward Washing Test:

Rechargeability is a significant property for N-halamine biocides. Durability is also the important feature, because a stable N-halamine bound enhances the shelf life of biocidal property. Through the laundering test, rechargeablitiy and durability of NMA, NMA + MA, and NMA + AM treated cotton fabrics were evaluated and results are summarized in Table 2.3 and 2.4. Washing tests were performed at 5, 10, 25, and 50 equivalent washing cycles for swatches including prechlorinated and unchlorinated coatings. In the Table 2.3 and 2.4, the X column represents the Cl^+ concentration of prechlorinated samples after each washing cycle (the initial chlorine loading is shown as 0 washing cycles). The Y Column represents the Cl^+ concentration of prechlorinated washed and then rechlorinated samples after given number of each washing cycles. The Z column represents the Cl^+ concentration of unchlorinated washed samples which were chlorinated after given number of each washing cycles.

The data for washing stability test for just NMA padded samples showed that after 5 washing cycles, a substantial amount of Cl^+ were lost. The amount of Cl^+ on the fabric between 5 to 50 cycle washes is gradually decreased and after 50 washing cycle, there was little Cl^+ observed on padded fabric. The Cl^+ values of Y showed that the loss of halamine precursor was not excessive. The previous studies indicated that prechlorination increases the hydrophobicity of the surfaces and it protects the coating from hydrolysis. A comparison of X and Z column for NMA padded sample indicated that some NMA chemical was lost from 5 to 50 washing cycle. When methacrylamide or acrylamide was grafted on NMA padded cotton fabrics, the results were considerably different.

The X column for NMA+MA, and NMA+AM coated fabrics showed the amount of Cl^+ decreased around 60% after 5 cycles washing. After 5 to 50 cycles, Cl^+ gradually decreased and when 50 washing cycle was reached, there was still some Cl^+ observed on coated fabric. The Y column values of NMA+MA coated cotton fabric showed that after 5 cycle washing, the amount of Cl^+ was actually higher than the beginning of the amount Cl^+ and after 50 washing cycle, it was almost the same as beginning Cl^+ ratio. The Y column values of NMA+AM coated cotton fabric showed that after 5 cycles washing, the amount of Cl^+ was a little bit higher than the beginning amount of Cl^+, but after 5 cycles, the amount of Cl^+ was getting lower than the beginning Cl^+ ratio. To date, no increase on chlorine loading has been reported previously compared to initial chlorine ratio of sample. A possible reason for this unexpected result might be that the MA forms a film that is difficult to penetrate and that washing abrades the film making to NMA sites more accessible to bleach. Loss of both NMA and MA from the surface is responsible for the decrease in chlorine ratio between 5 to 50 cycles.

Table 2.3 Durability and rechargeavility of NMA padded cotton fabric

Washing Cycles	**X[a]**	**Y[b]**	**Z[c]**
0	0.76	0.76	
5	0.32	0.61	0.73
10	0.21	0.6	0.7
25	0.16	0.56	0.68
50	0.09	0.5	0.65

[a] X : Chlorinated before washing

[b] Y : Chlorinated before washing and rechlorinated after washing

[c] Z : Unchlorinated during washing, but chlorinated after washing

Table 2.4 Durability and rechargeavility of NMA + MA and NMA + AM treated cotton fabrics

Washing Cycles	NMA + MA			NMA + AM		
	X[a]	Y[b]	Z[c]	X[a]	Y[b]	Z[c]
0	0.4	0.4		0.47	0.47	
5	0.16	0.53	0.4	0.19	0.49	0.44
10	0.11	0.52	0.39	0.14	0.48	0.4
25	0.08	0.49	0.36	0.08	0.42	0.38
50	0.05	0.44	0.33	0.05	0.41	0.37

[a] X : Chlorinated before washing

[b] Y : Chlorinated before washing and rechlorinated after washing

[c] Z : Unchlorinated during washing, but chlorinated after washing

Wrinkle Recovery Angle Test

Conventional durable-press (DP) is one of the finishing processes that cause greatly reduced strength and abrasion resistance of cotton fabric, so studies previous focused on improvement of both, DP, strength, and abrasion resistance properties by forming of network polymeric system onto cotton fabric[7-8-9]. N-(hydroxymethyl)acrylamide (NMA) monomer is commonly used in paints, adhesives, and textile applications where wrinkle resistance is desirable, because NMA has two functional groups, N-methylol and acrylic, can be controlled independently to undergo etherification (with cellulosic hydroxyl group) and acrylic polymerization[7-8]. Therefore, the objective of this study was further evaluating the importance and effectiveness of fiber

penetration and the sequence of polymerization (fixation) and cellulose cross-linking. The schematic configuration is shown in Figure 2.5.

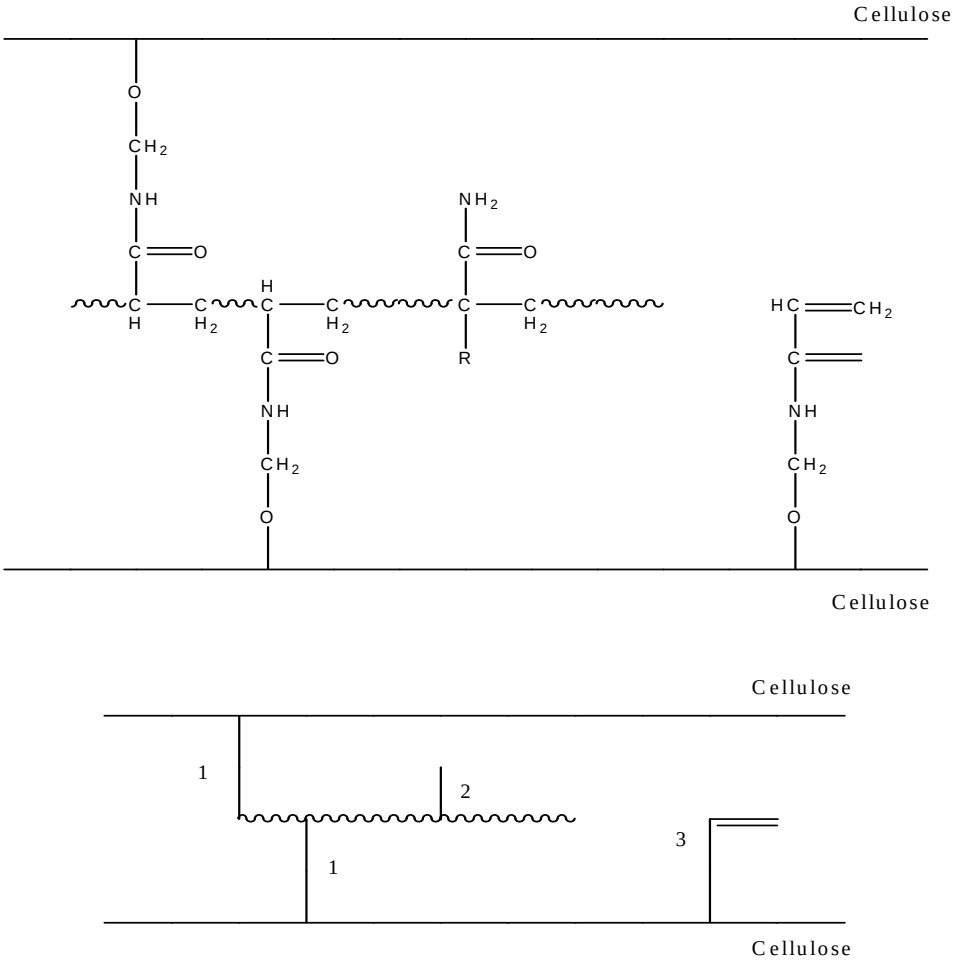


Figure 2.5 Schematic representation of structural units NMA and MA. Structural units are derived from (1) polymerization and etherification, (2) polymerization, (3) etherification only.

The effect of NMA, NMA+MA, and NMA+AM coated fabrics on crease recovery angle was tested. A detailed description of the process can be found in the experimental section 2.2.9. The results showed in Table2.5 that when NMA undergo etherification with cellulosic hydroxyl group, there was some improvement (about 30^{o}) on wrinkle recovery angle whereas there was no further improvement when NMA undergo etherification with cellulosic hydroxyl group and

polymerization. In addition, NMA concentration did not affect the wrinkle recovery angle. However, when MA or AM polymerized onto NMA finished cotton fabric (grafting onto NMA), the results significantly improved. According to the wrinkle or crease recovery angle results, grafting vinyl monomer onto NMA finished cotton fabric most probably make a network structure, so it gives better wrinkle recovery angle than just undergoing etherification and polymerizing NMA coated cotton fabric.

Table 2.5 Wrinkle Recovery Angle Test Results

Samples	(W + F)[a]	Measured [Cl+]%
Control Sample[b]	165	
5% NMA	220	0.74
Control Sample[c]	225	0.73
10% NMA	235	1.12
Control Sample[d]	240	1.12
5%NMA + 5%MA	285	0.45
5%NMA + 5%AM	280	0.51

(W + F)[a] : Total value for Warp and Filling wrinkle recovery angle

Control Sample[b] : Wrinkle Recovery Angle of just pure cotton

Control Sample[c] : Wrinkle Recovery Angle of 5% NMA which undergo etherification with cellulosic hydroxyl group and polymerized.

Control Sample[d] : Wrinkle Recovery Angle of 10% NMA which undergo etherification with cellulosic hydroxyl group and polymerized.

UV Light Stability Test

The stability of the chlorinated NMA, NMA+MA, and NMA+AM coated fabrics under UV irradiation was investigated. The biocidal efficacy is related with N-Cl bond, so the UV stability of the N-Cl bond of the coated N-halamine precursors is presented in Table2.6. All the samples (NMA, NMA + MA, NMA + AM) were exposed UV for 24 hours, and then they were rechlorinated. After rechlorination, samples were exposed to the UV an additional 24 hours, and then they were rechlorinated again. This procedure was repeated four times. It was observed that NMA padded cotton fabrics decomposed faster than NMA+MA, and NMA+AM. There was 45% decomposition after 24 hours UV irradiation. Decomposition percentage became relatively constant after 72 h UV irradiation. In addition, the UV stability data proved that there is no substantial dehydrohalogenation type decomposition in the structure, so dehydrohalogenation does not affect the biocidal efficiency for NMA padded samples.

The UV stability of the NMA+MA and NMA+AM coated fabrics was complicated, because after rechlorination of UV light exposed samples, the chlorine loading increased than the initial chlorine loading. Each of samples were exposed UV light for 24 hours, and then they were rechlorinated. After rechlorination, they were further exposed UV light for 24 hours and rechlorinated again. This process was repeated four times. For each of rechlorination, the chlorine loading was higher than the initial chlorine loading. However, after reaching 96 hours, the chlorine loading did not increase than initial chlorine loading after rechlorination of UV light exposed samples.

Table 2.6 UV light stability test

Time of exposure (h)	NMA		NMA + MA		NMA + AM	
	Measured [Cl+]%	**Rchlrn [Cl+]%**	Measured [Cl+]%	**Rechlrn [Cl+]%**	Measured [Cl+]%	**Rechlrn [Cl+]%**
0	0.72		0.48		0.48	
12	0.35		0.29		0.37	
24	0.21		0.21		0.35	
24-Rechlorination		0.43		0.64		0.56
36	0.2		0.36		0.38	
48	0.13		0.32		0.32	
48- Rechlorination		0.37		0.7		0.51
60	0.16		0.32		0.34	
72	0.09		0.21		0.14	
72-Rechlorination		0.19		0.48		0.44
84	0.13		0.25		0.19	
96	0.1		0.19		0.13	
96-rechlorination		0.19		0.41		0.32
Control Sample[a]	0.68		0.51		0.5	
Control Sample[b]	0.66		0.46		0.46	
Control Sample[c]	0.62		0.5		0.45	
Control Sample[d]	0.62		0.51		0.44	

Control Sample[a] : Chlorination of unchlorinated 24 UV light exposure sample

Control Sample[b] : Chlorination of unchlorinated 48 UV light exposure sample

Control Sample[c] : Chlorination of unchlorinated 72 UV light exposure sample

Control Sample[d] : Chlorination of unchlorinated 96 UV light exposure sample

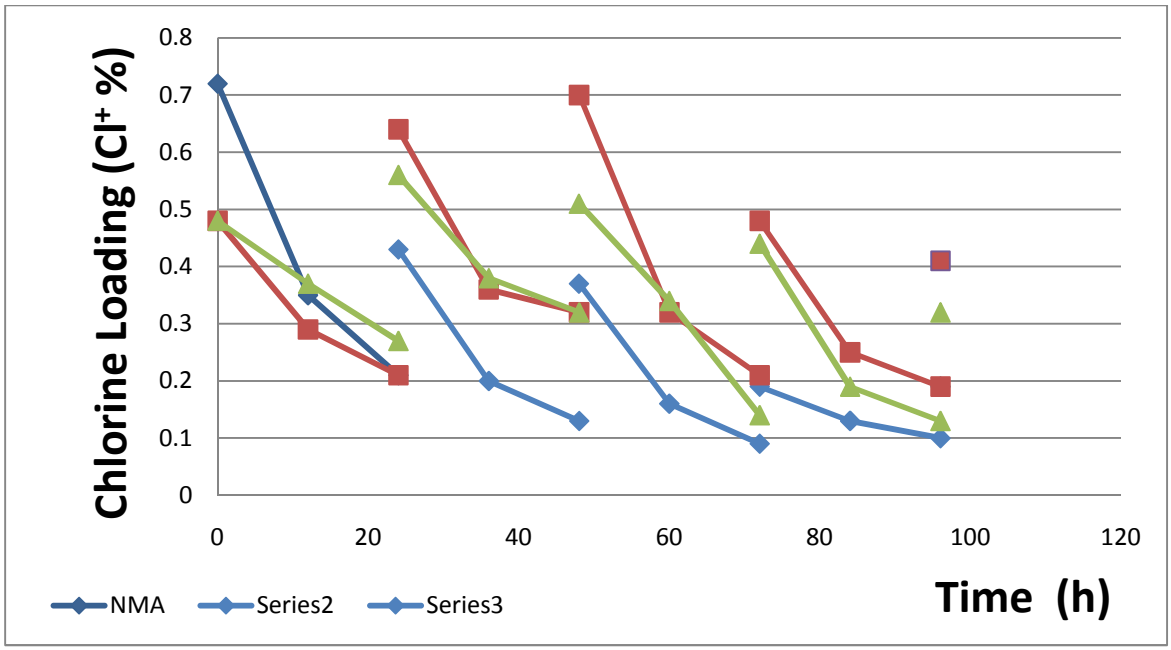


Figure 2.6 UV light stability test of NMA, NMA + MA, and NMA + AM coated samples for four days

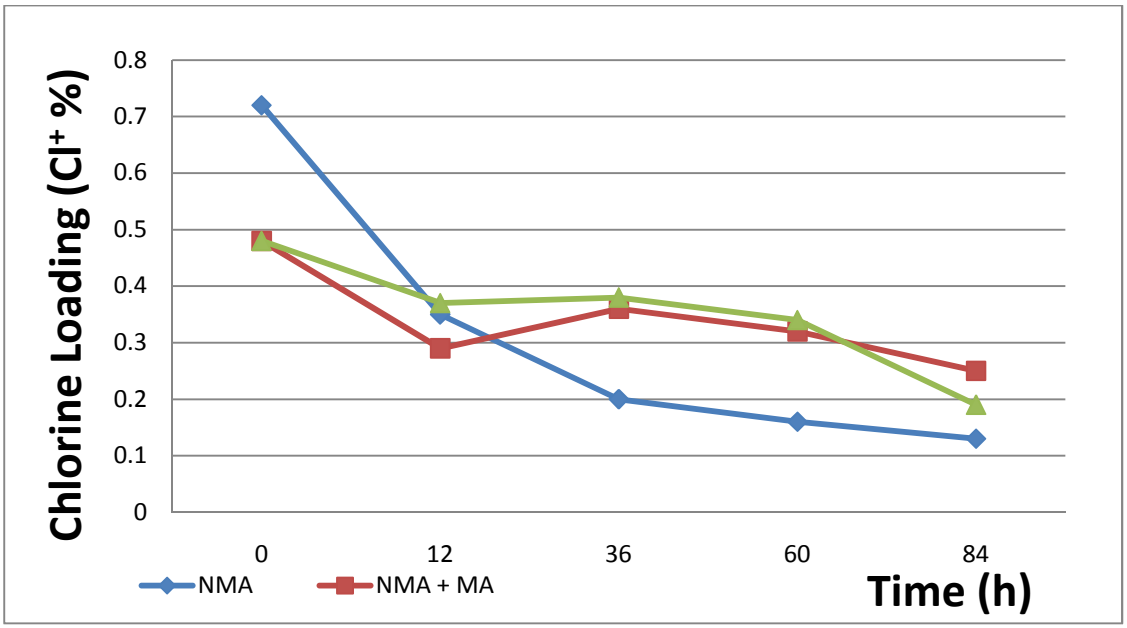


Figure 2.7 UV light stability of NMA, NMA + MA, and NMA + AM coated samples for each 12 hours

A Potential Reason Affecting the Increasing of Chlorine Loadings after Rechlorination of UV Exposed NMA+MA and NMA+AM coated fabrics.

In the beginning, a possible hypothesis was created in order to explain unexpected low chlorine content after grafting MA or AM. According to this hypothesis, the reason of decreasing chlorine loading after grafting MA or AM onto NMA finished cotton fabric is that NMA is such a small molecule and can go through etherification reaction not only surface of cotton fiber, but also inside of cotton fiber. Therefore, the reason of increasing chlorine loading after rechlorination of UV light exposed NMA+MA and NMA+AM coated fabrics is that decomposition of MA or AM surface coating allowed bleach to reach NMA inside the fiber. In order to show evidence for this hypothesis, Scanning Electron Microscope (SEM) pictures of UV exposed NMA, NMA+MA, and NMA+AM coated cotton fabrics were taken. These pictures are shown in Figure X. The decomposition of MA and AM was detected after 24 and 48 hours UV light exposed samples on the SEM pictures. The UV decomposition of NMA+MA coated cotton fabric was mor easily observed than others. For NMA and NMA+AM coated cotton fabrics, it was observed that there were some cracks which supported the decomposition hypothesis.

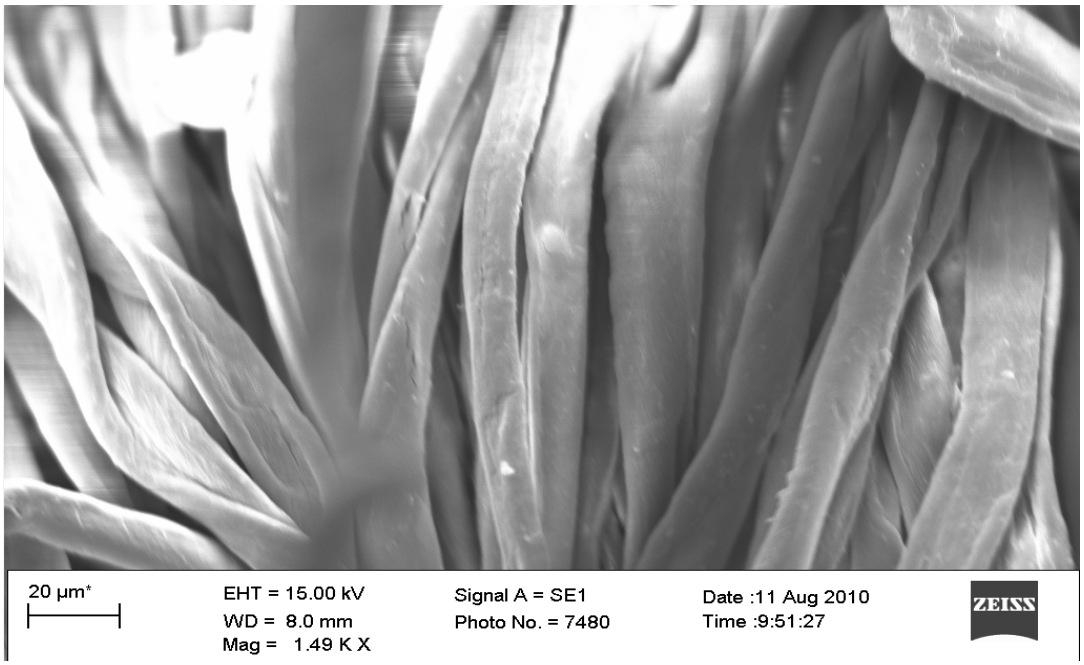


Figure 2.8 SEM picture of pure cotton fabric

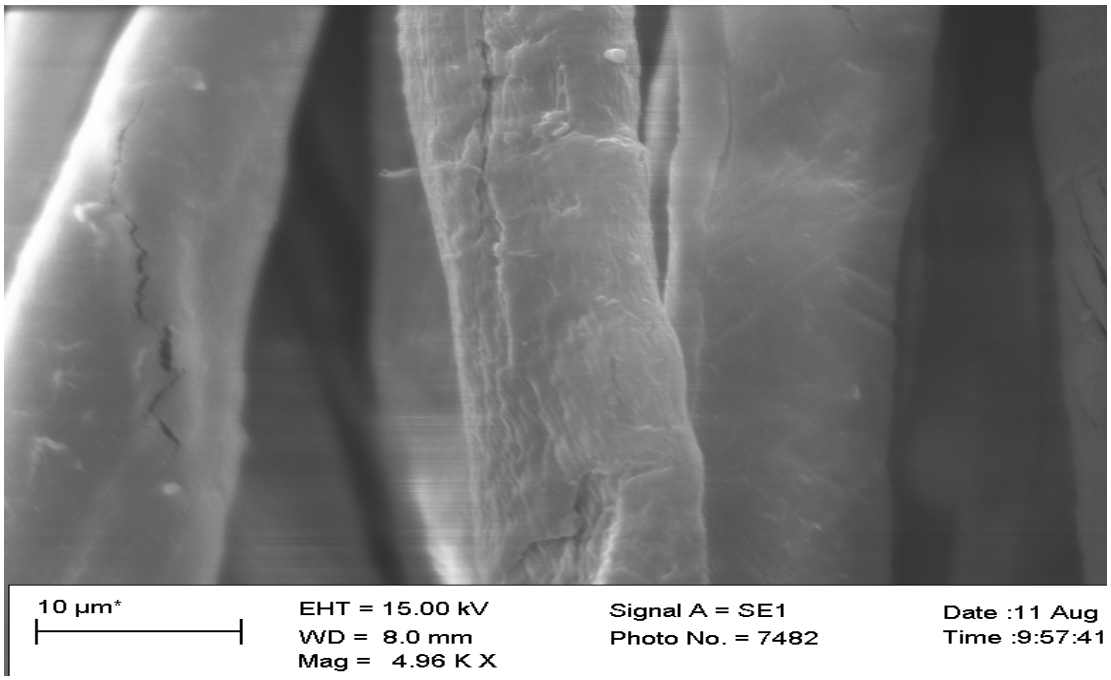


Figure 2.9 SEM picture of pure cotton fabric at higher magnification

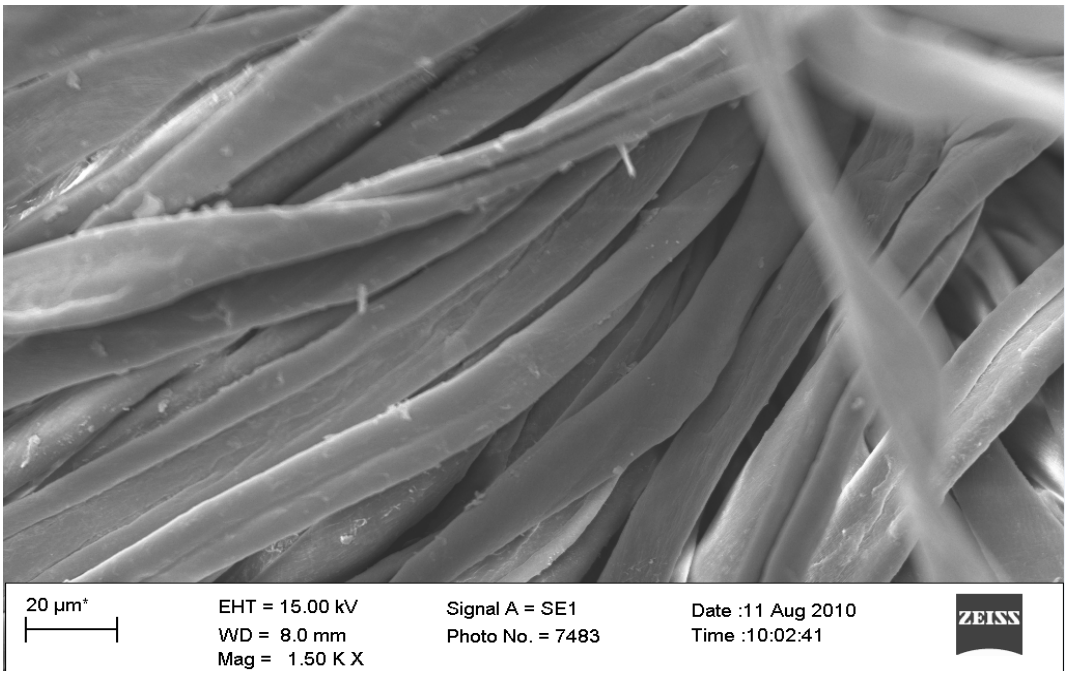


Figure 2.10 SEM picture of 24 hours UV light exposed pure cotton fabric

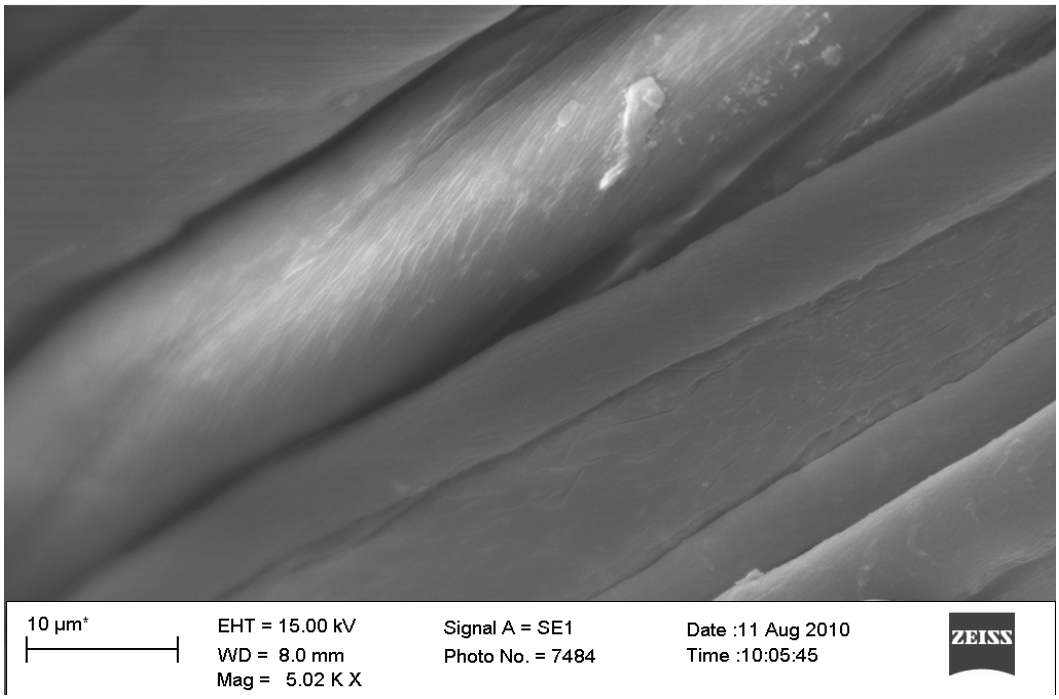


Figure 2.11 SEM picture of 24 hours UV light exposed pure cotton fabric at higher magnification

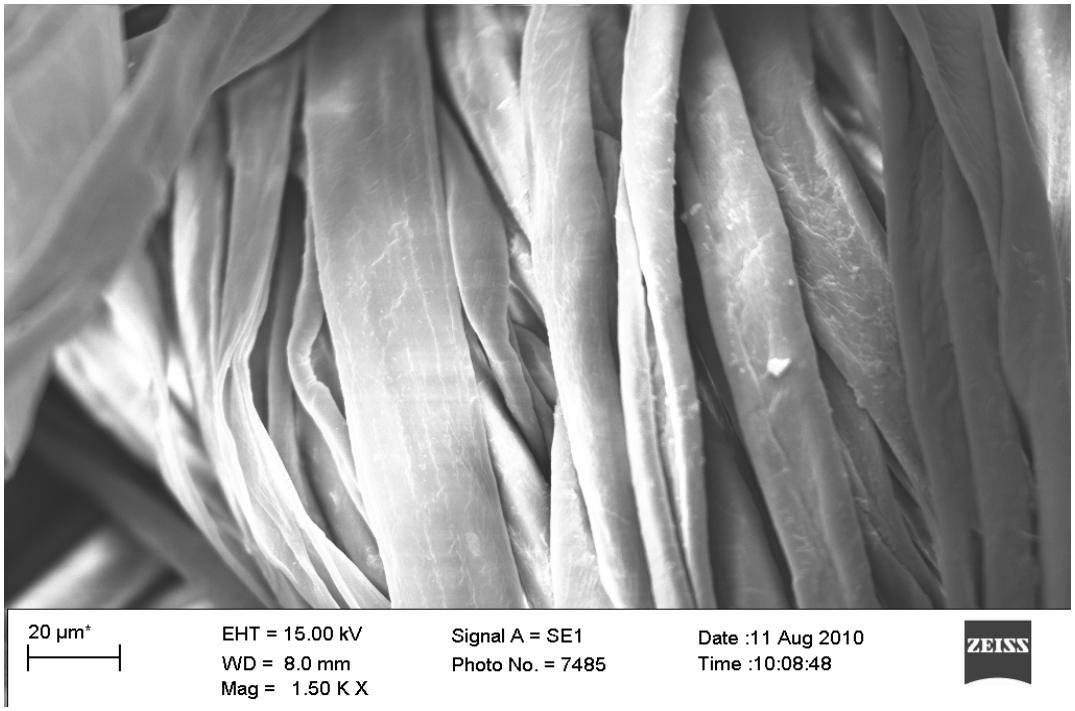


Figure 2.12 SEM picture of 48 hours UV light exposed pure cotton fabric

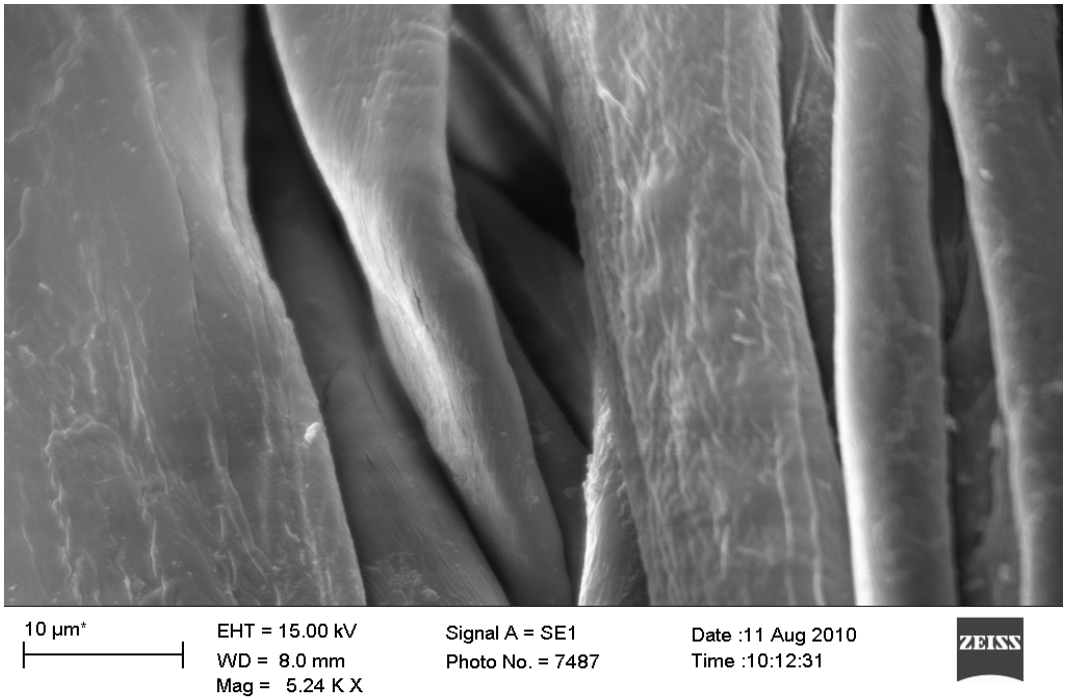


Figure 2.13 SEM picture of 48 hours UV light exposed pure cotton fabric at higher magnification

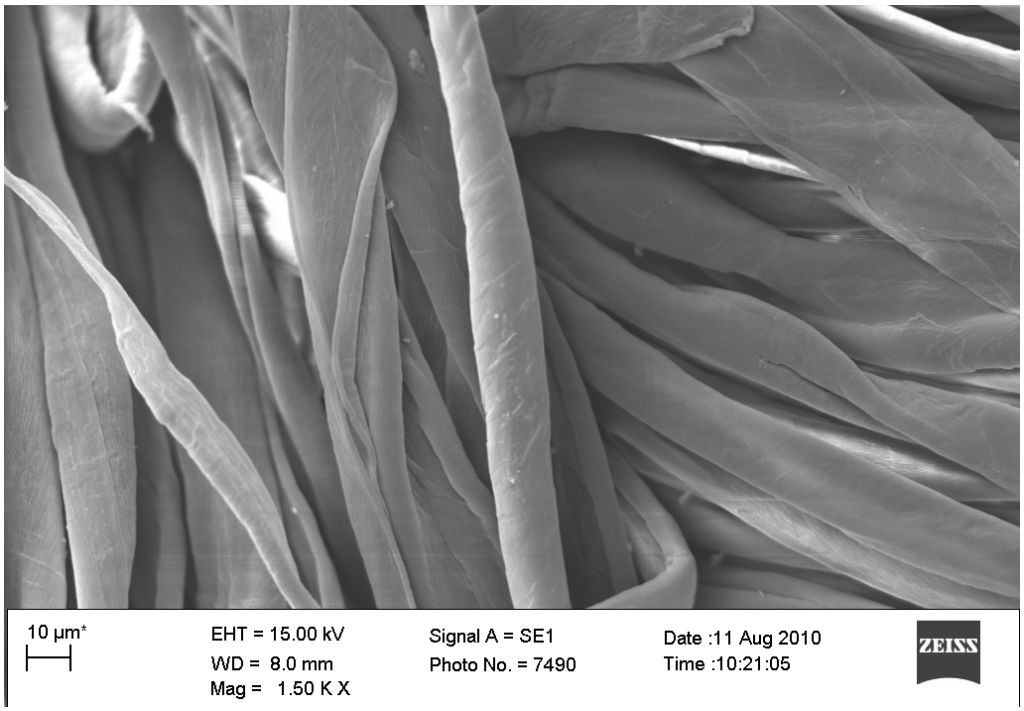


Figure 2.14 SEM picture of NMA treated cotton fabric

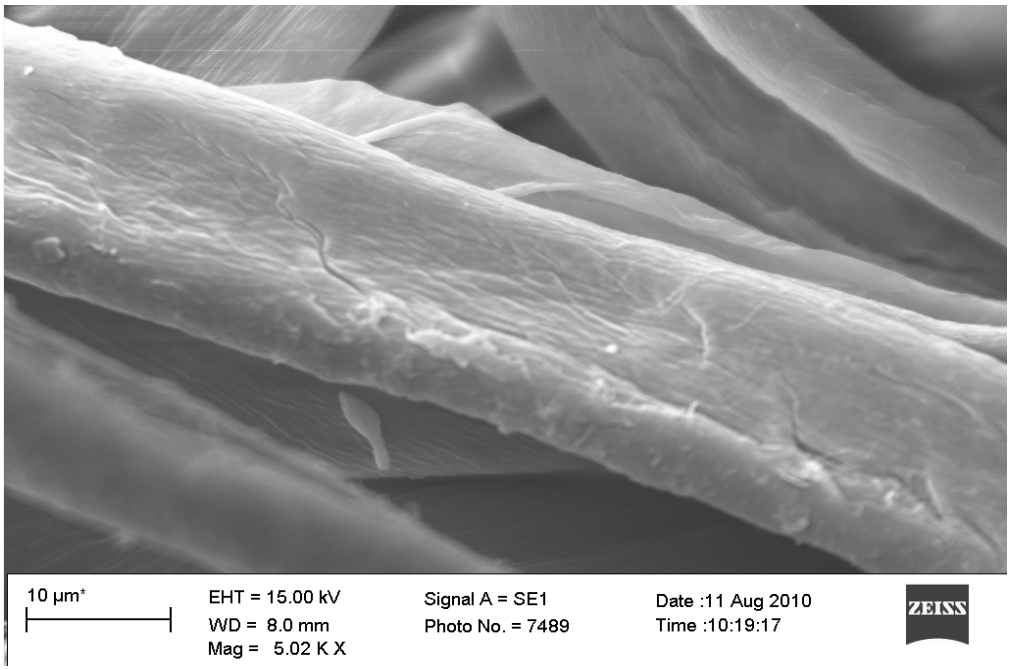


Figure 2.15 SEM picture of NMA treated cotton fabric at higher magnification

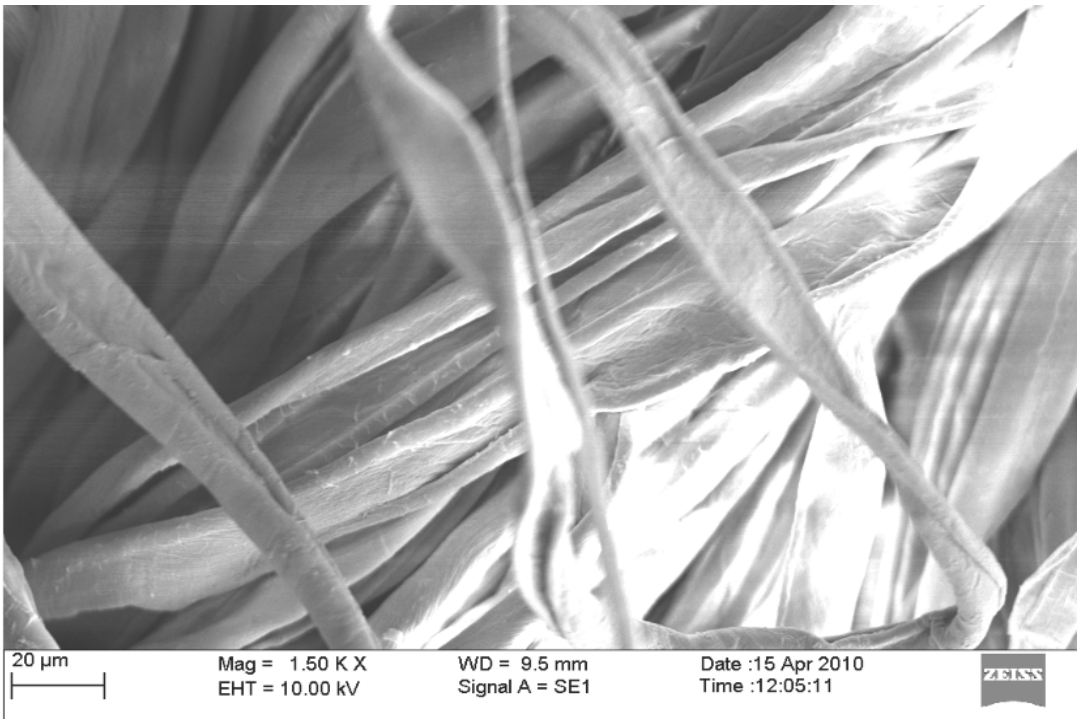


Figure 2.16 SEM picture of 24 hours UV light exposed NMA treated cotton fabric

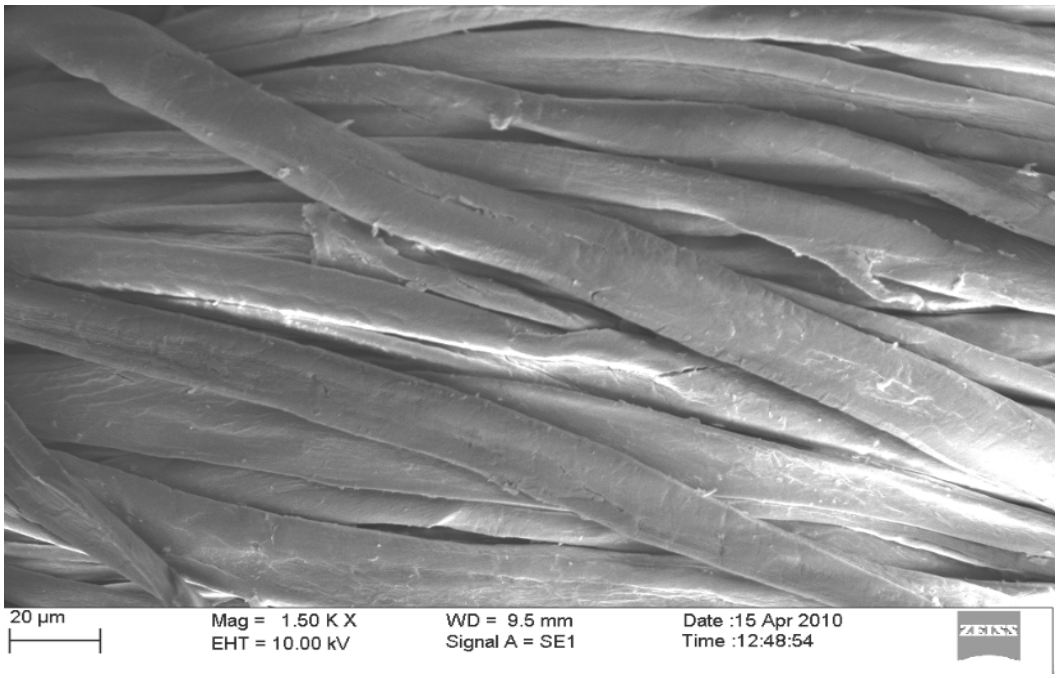


Figure 2.17 SEM picture of 48 hours UV light exposed NMA treated cotton fabric

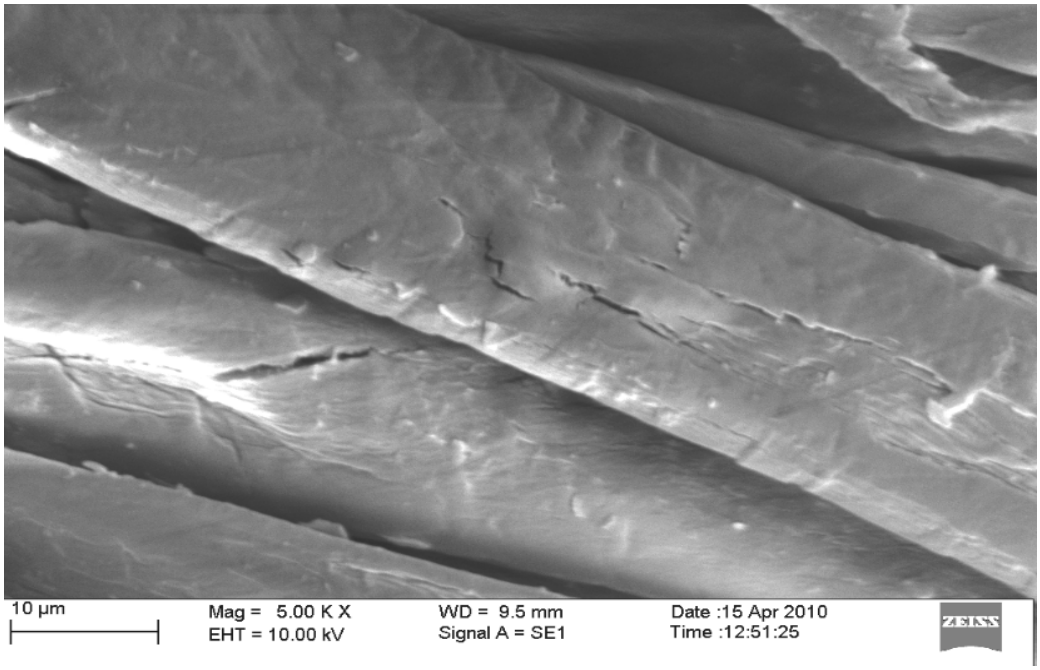


Figure 2.18 SEM picture of 48 hours UV light exposed NMA treated cotton at higher magnification

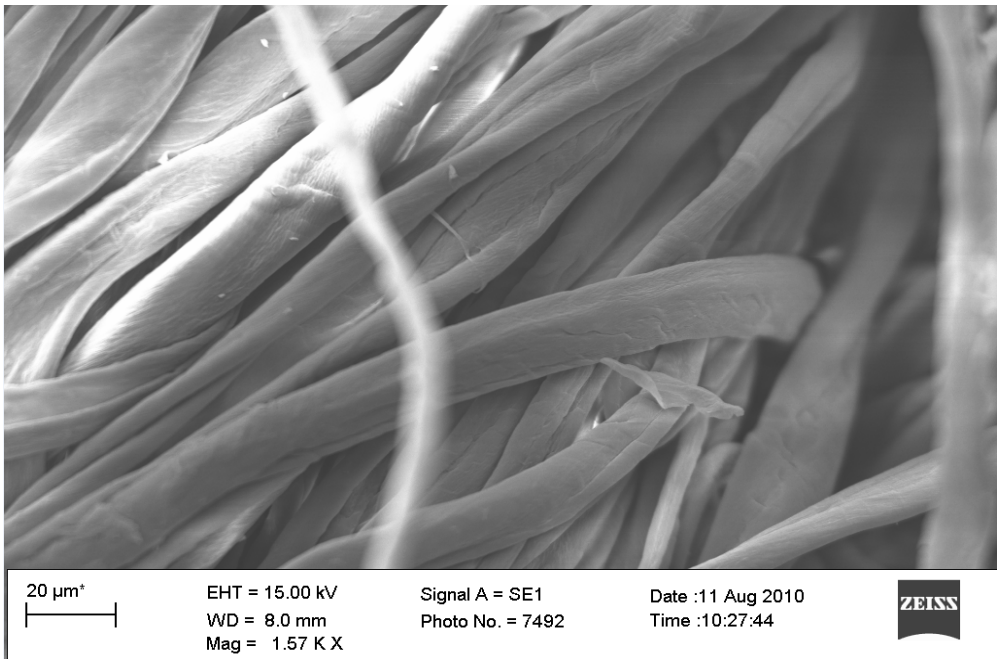


Figure 2.19 SEM picture of NMA + MA treated cotton fabric

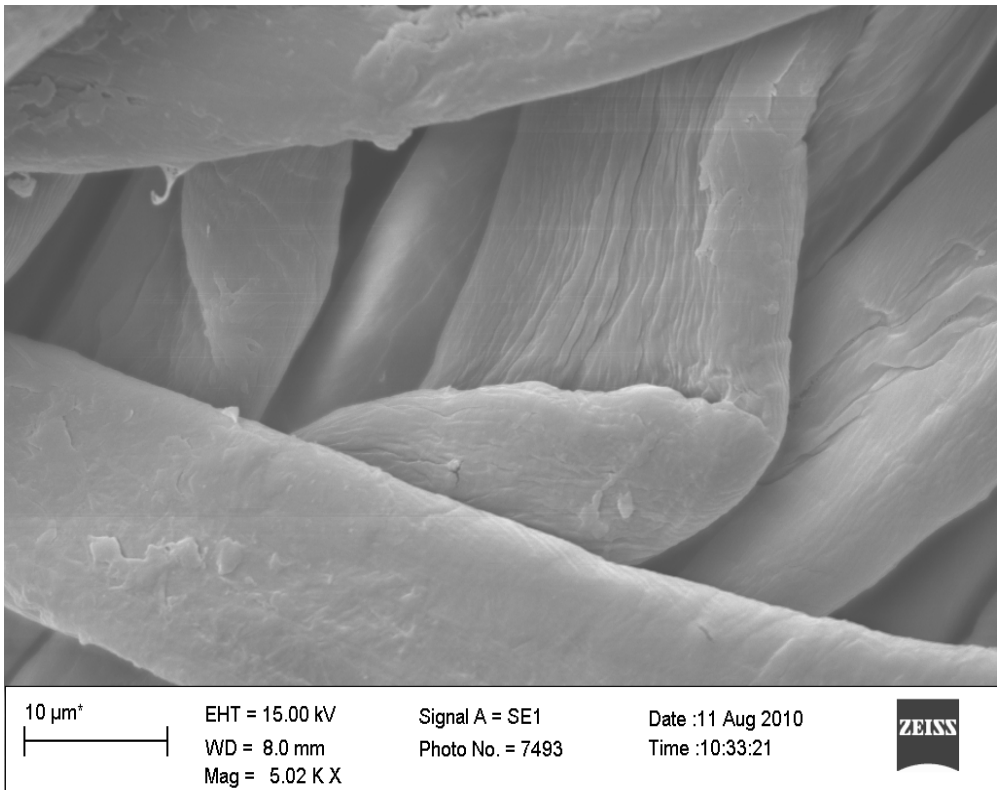


Figure 2.20 SEM picture of NMA + MA treated cotton fabric at higher magnification

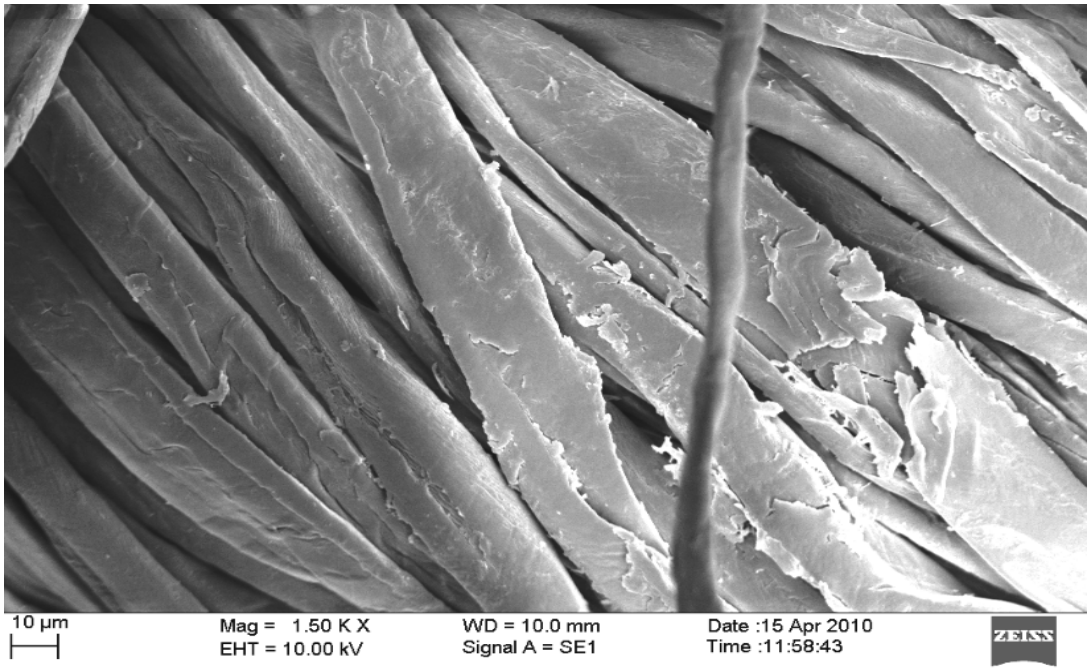


Figure 2.21 SEM picture of 24 hours UV light exposed NMA + MA treated cotton fabric

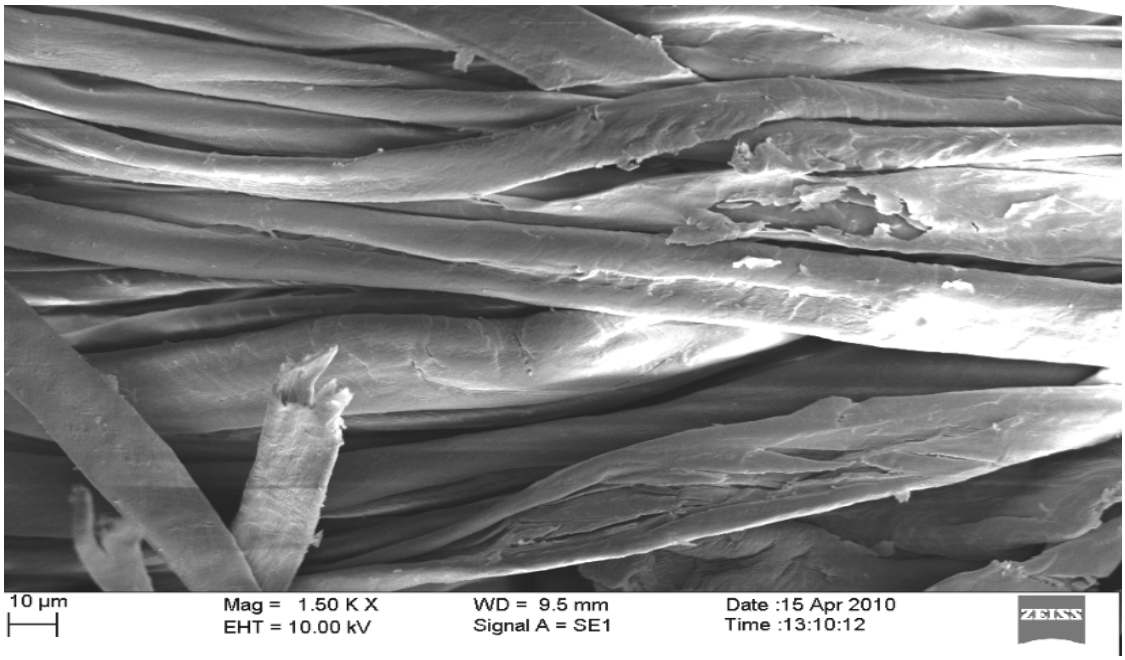


Figure 2.22 SEM picture of 48 hours UV light exposed NMA + MA treated cotton fabric

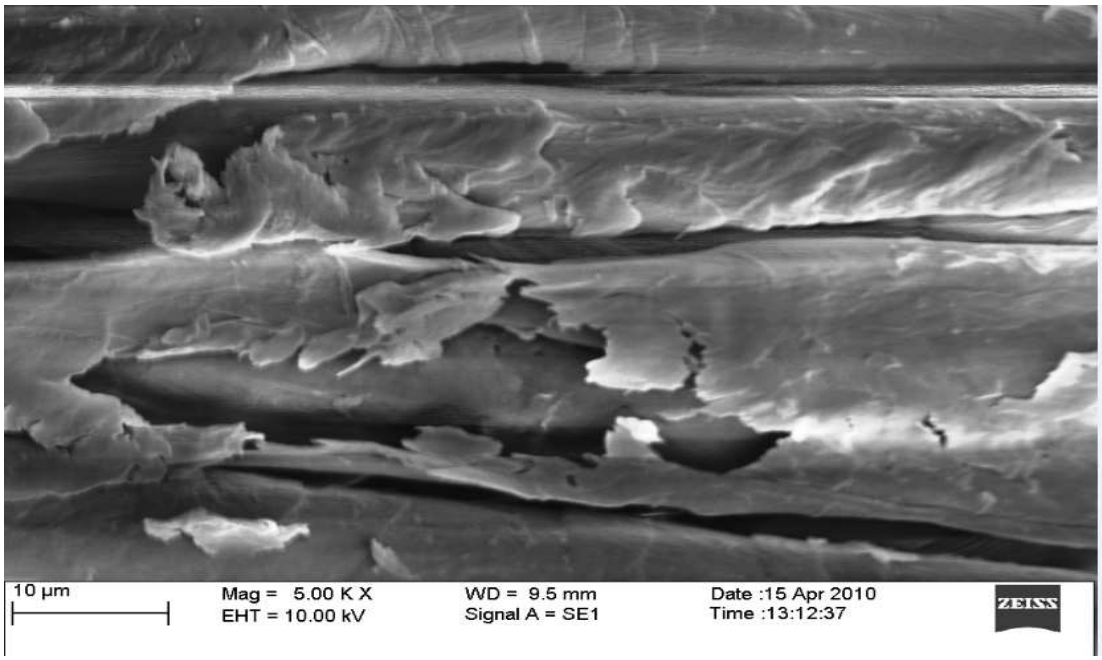


Figure 2.23 SEM picture of 48 hours UV light exposed NMA + MA treated cotton fabric at higher magnification

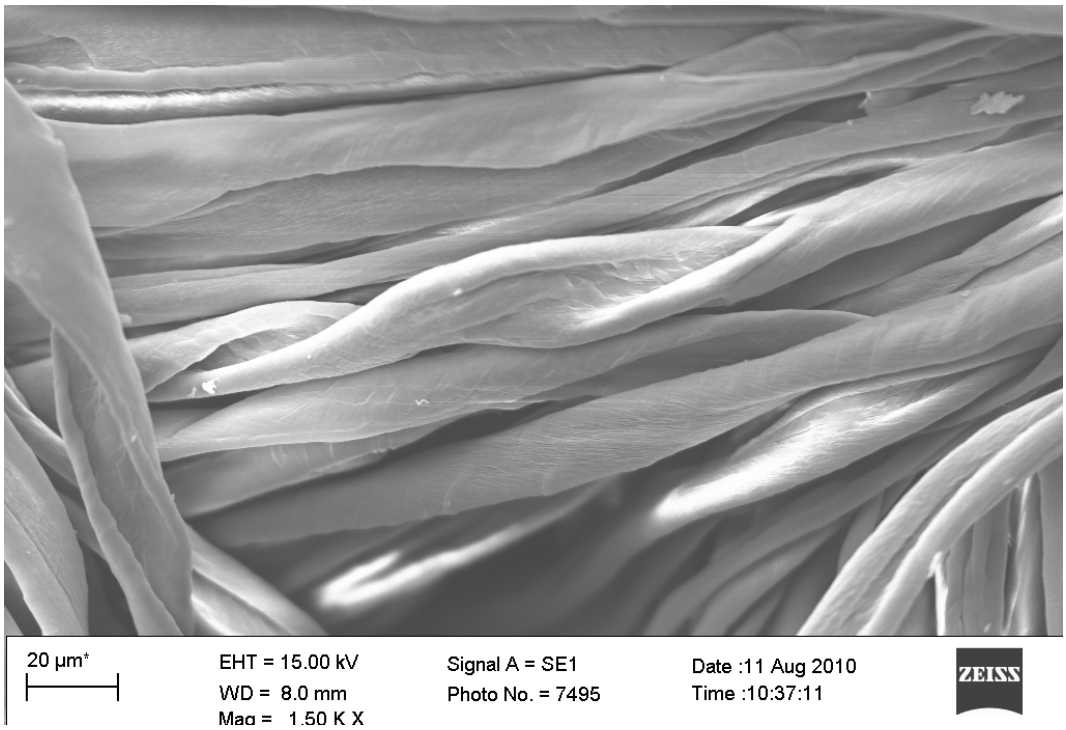


Figure 2.24 SEM picture of NMA + AM treated cotton fabric

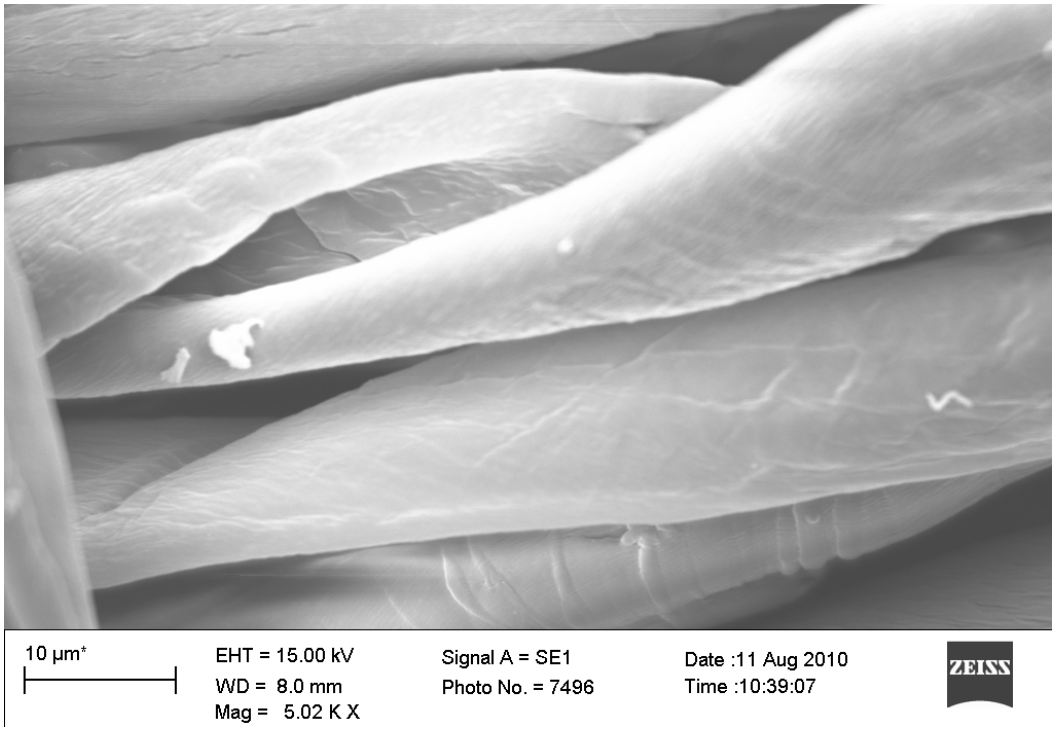


Figure 2.25 SEM picture of NMA + MA treated cotton fabric at higher magnification

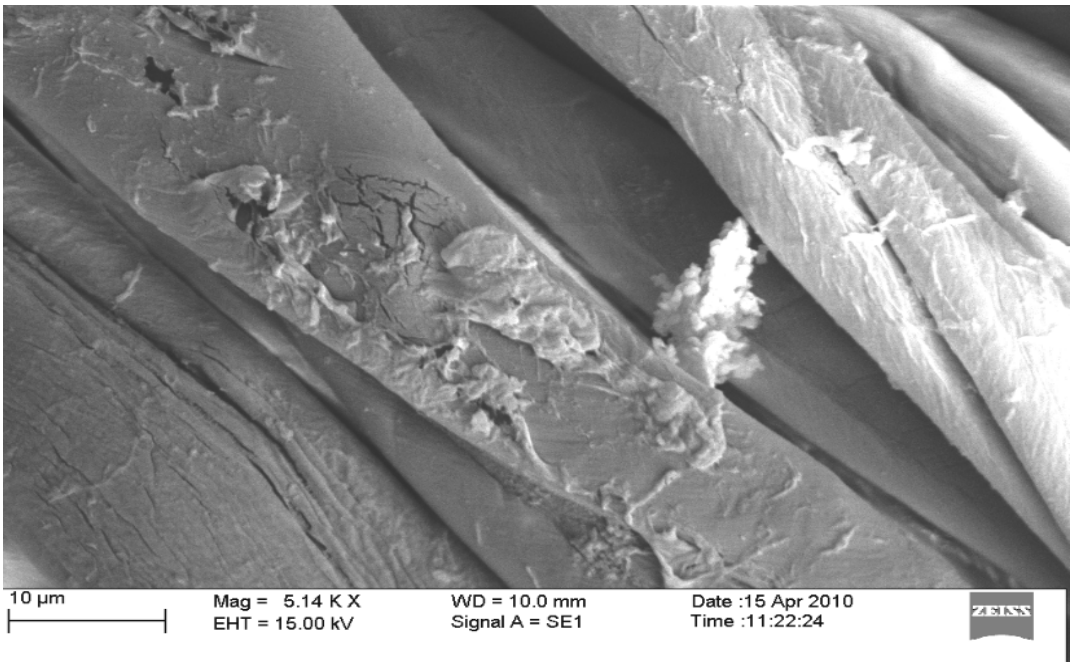


Figure 2.26 SEM picture of 24 hours UV light exposed NMA + AM treated cotton fabric at higher magnification

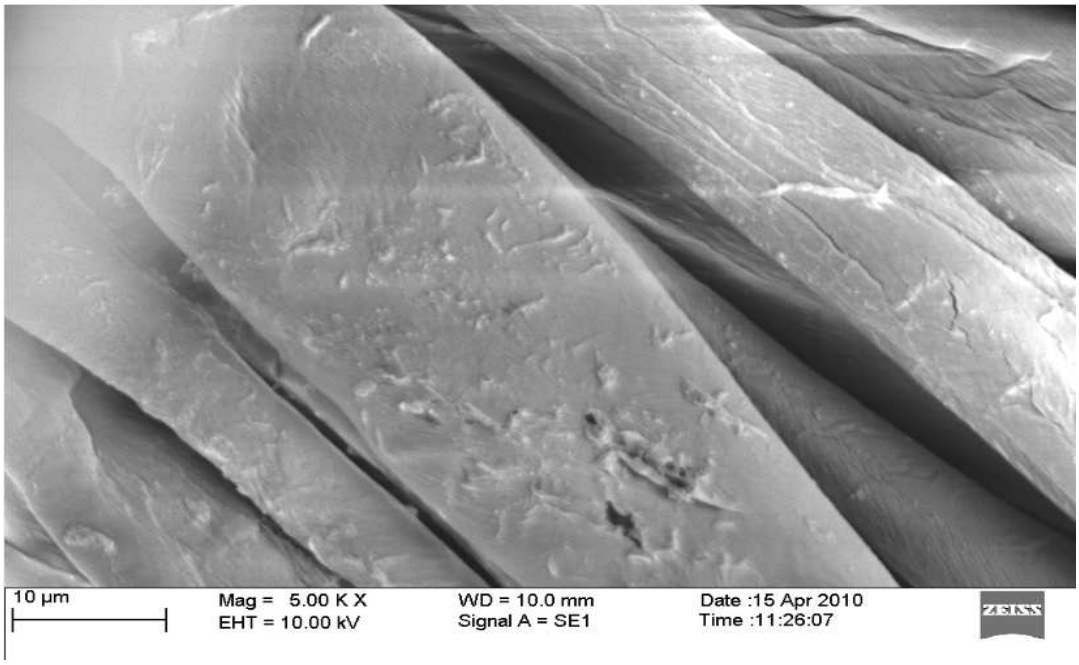


Figure 2.27 SEM picture of 24 hours UV light exposed NMA + AM treated cotton fabric at higher magnification

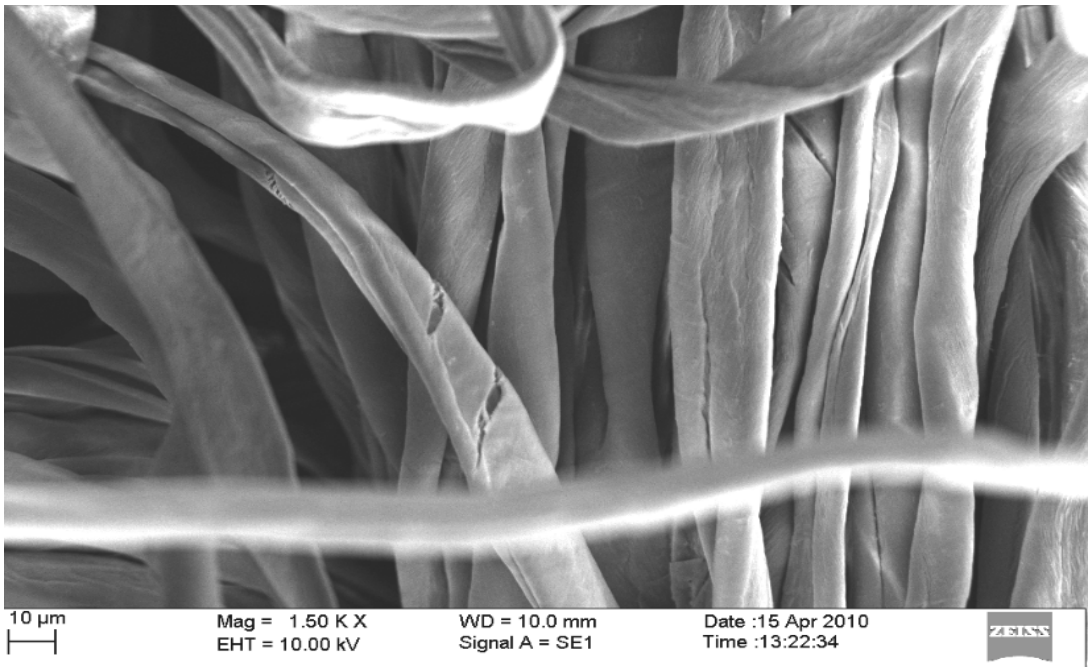


Figure 2.28 SEM picture of 48 hours UV light exposed NMA + AM treated cotton fabric

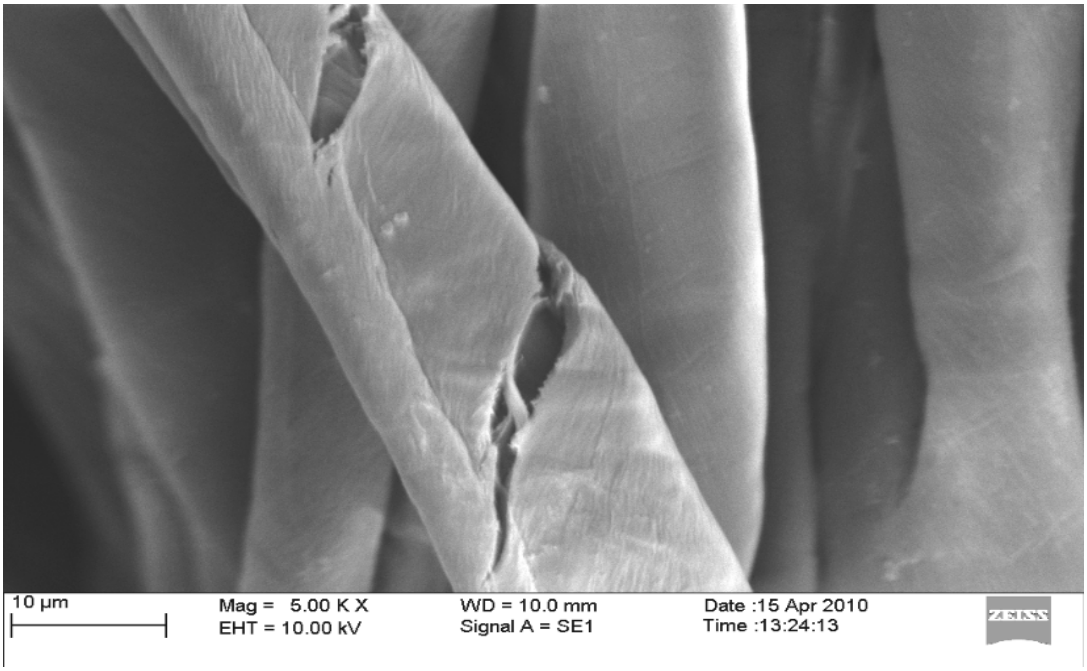


Figure 2.29 SEM picture of 48 hours UV light exposed NMA + AM treated cotton fabric at higher magnification

In addition to SEM support, the reason of increasing chlorine percent after rechlorination of UV light exposure NMA+AM and NMA+AM coated cotton fabrics was further investigated by changing the NMA, MA and AM grafting ratios. The grafting percent of MA or AM onto the NM A coated cotton fabric might affect the results. In this regard, two sets of samples: different amount of MA was grafted on constant NMA finished cotton fabrics and different amount of NMA with constant MA grafting were done. These two sets of samples were exposed UV for 24 hours, and then they were rechlorinated. After rechlorination, samples were exposed UV additional 24 hours, and then they were rechlorinated again. This procedure was repeated three times.

Table 2.7 UV light exposure of varied grafting percent MA onto constant percent NMA coated fabric

	5%NMA + 1%MA		5%NMA + 5%MA		5%NMA + 10%MA	
Time of exposure (h)	**Measured [Cl+]%**	**Rchlrn [Cl+]%**	**Measured [Cl+]%**	**Rechlrn [Cl+]%**	**Measured [Cl+]%**	**Rechlrn [Cl+]%**
0	0.37		0.42		0.45	
12	0.23		0.28		0.31	
24	0.15		0.22		0.27	
24-Rechlronation		0.33		0.52		0.6
36	0.21		0.37		0.43	
48	0.16		0.28		0.36	
48-Rechlorination		0.3		0.58		0.7
60	0.17		0.34		0.45	
72	0.11		0.23		0.31	
72-Rechlorination		0.28		0.53		0.68
Control Sample[a]	0.37		0.44		0.47	
Control Sample[b]	0.39		0.44		0.48	
Control Sample[c]	0.39		0.46		0.45	

Control Sample[a] : Chlorination of unchlorinated 24 UV light exposure sample

Control Sample[b] : Chlorination of unchlorinated 48 UV light exposure sample

Control Sample[c] : Chlorination of unchlorinated 72 UV light exposure sample

Table 2.8 UV light exposure of constant grafting percent of MA onto varied percent NMA treated fabric

	1%NMA + 5%MA		5%NMA + 5%MA		10%NMA + 5%MA	
Time of exposure (h)	**Measured [Cl+]%**	**Rchlrn [Cl+]%**	**Measured [Cl+]%**	**Rechlrn [Cl+]%**	**Measured [Cl+]%**	**Rechlrn [Cl+]%**
0	0.26		0.42		0.49	
12	0.19		0.28		0.3	
24	0.16	0.31	0.22	0.52	0.25	0.6
24-Rechlorination	0.16	0.31	0.22	0.52	0.25	0.6
36	0.2		0.37		0.45	
48	0.17	0.35	0.28	0.58	0.36	0.73
48-Rechlorination	0.17	0.35	0.28	0.58	0.36	0.73
60	0.18		0.34		0.5	
72	0.11	0.28	0.23	0.53	0.32	0.74
72-Rechlorination	0.11	0.28	0.23	0.53	0.32	0.74
Control Sample[a]	0.25		0.44		0.45	
Control Sample[b]	0.28		0.44		0.45	
Control Sample[c]	0.29		0.46		0.47	

Control Sample[a] : Chlorination of unchlorinated 24 UV light exposure sample

Control Sample[b] : Chlorination of unchlorinated 48 UV light exposure sample

Control Sample[c] : Chlorination of unchlorinated 72 UV light exposure sample

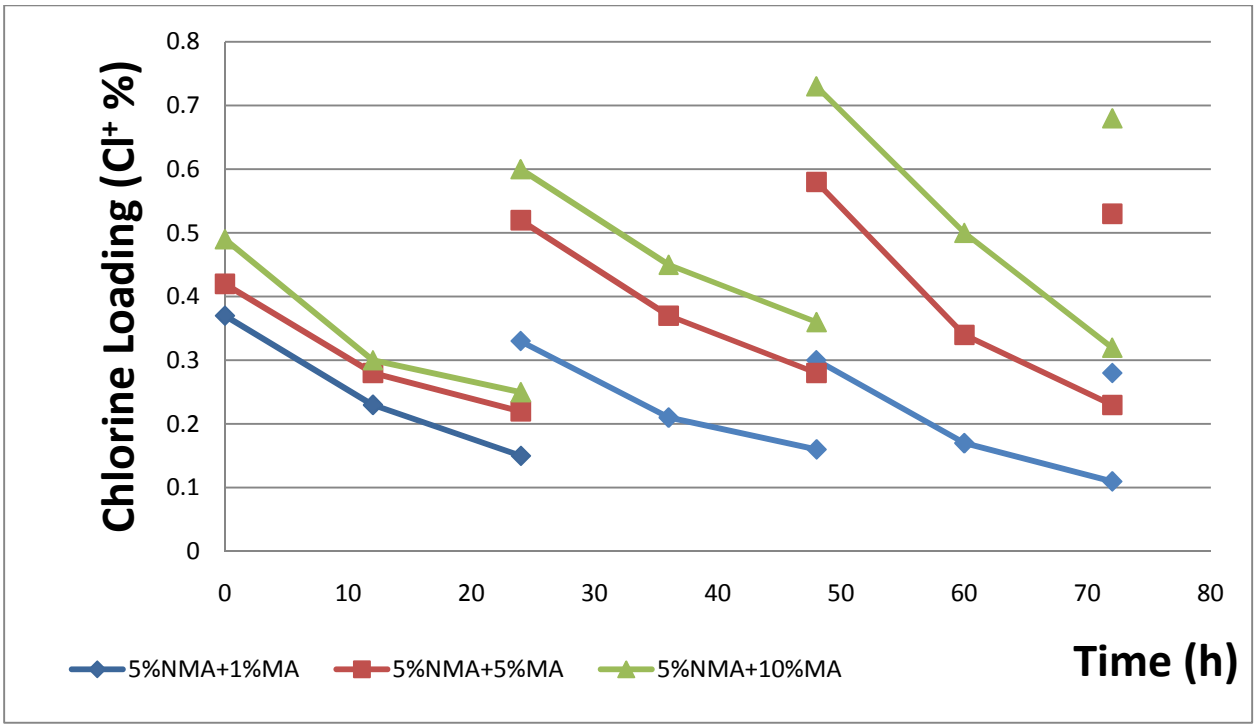


Figure 2.30 UV light stability of different amount of MA was grafted on constant NMA coated samples

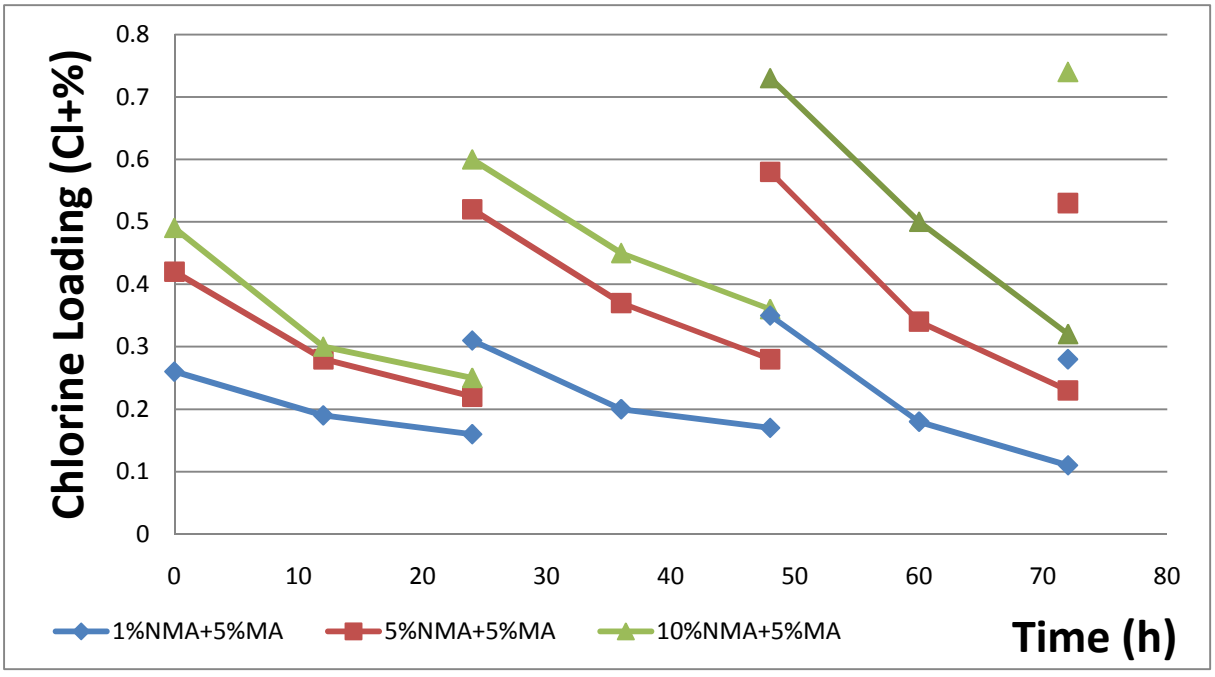


Figure 2.31 UV light stability of different amount of NMA with constant MA grafting samples

The results demonstrated that grafting percent amount affects the results, because 1%MA+5%NMA treated sample showed that the chlorine loading did not increase than initial chlorine loading after rechlorination of UV light exposure. However, when the amount of grafting MA increased, the chlorine loading reached higher ratio after rechlorination of UV light exposure. The first set of sample results showed that grafting MA percent increased in direct proportion to the increase of chlorine loading. The second set of experiment results also showed that for UV exposed samples when the amount of NMA percent increased, the chlorine loading reached higher ratio after rechlorination.

Both of experiments show that both NMA and MA percent onto treated fabrics affect the results. However, the amount of MA is more significant than amount of NMA, because lower grafting percent MA did not show any increase after rechlorination of UV light exposure. In summary, the results support our hypothesis, but they do not provide proof.

2.4 Conclusion

A series of commercially available acrylic monomers of N-halamine precursors were purchased from Sigma Aldrich Chemical and Fisher Scientific Chemical companies. First, N-(hydroxymethyl)acrylamide (NMA) was padded onto cotton fabric, and then Methacrylamide (MA) or Acrylamide (AM) grafted onto NMA padded cotton fabric. NMA, NMA+MA, and NMA+AM coated fabrics showed excellent stability to washing test, and biocidal property against Gram-positive and Gram-negative bacteria. In addition, the second functionality, conventional durable press, was gained after grafting MA and AM onto NMA padded cotton fabrics besides antimicrobial property. Interestingly, the stability to dissociation under UV irradiation results of these samples (NMA+MA and NMA+AM) were different than previous studied results, because the chorine loading of UV light exposed samples gave higher results than initial chlorine loading after rechlorination. For this reason a possible hypothesis was created in order to explain unexpected increasing this chlorine loading. It is recommended that 5% MA grafting onto 5% NMA padded fabric should be effective for a potential application because it has good permanent press besides its effective biocidal activity. Whatever the mechanism, these are the first set of experiments in our labs where UV exposure of the halamine did not reduce the chlorine retention of rechlorinated samples.

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2.6 Supporting Information

The supporting Information includes NMA, NMA+MA, and NMA+ AM treated cotton samples FT-IR spectra data.

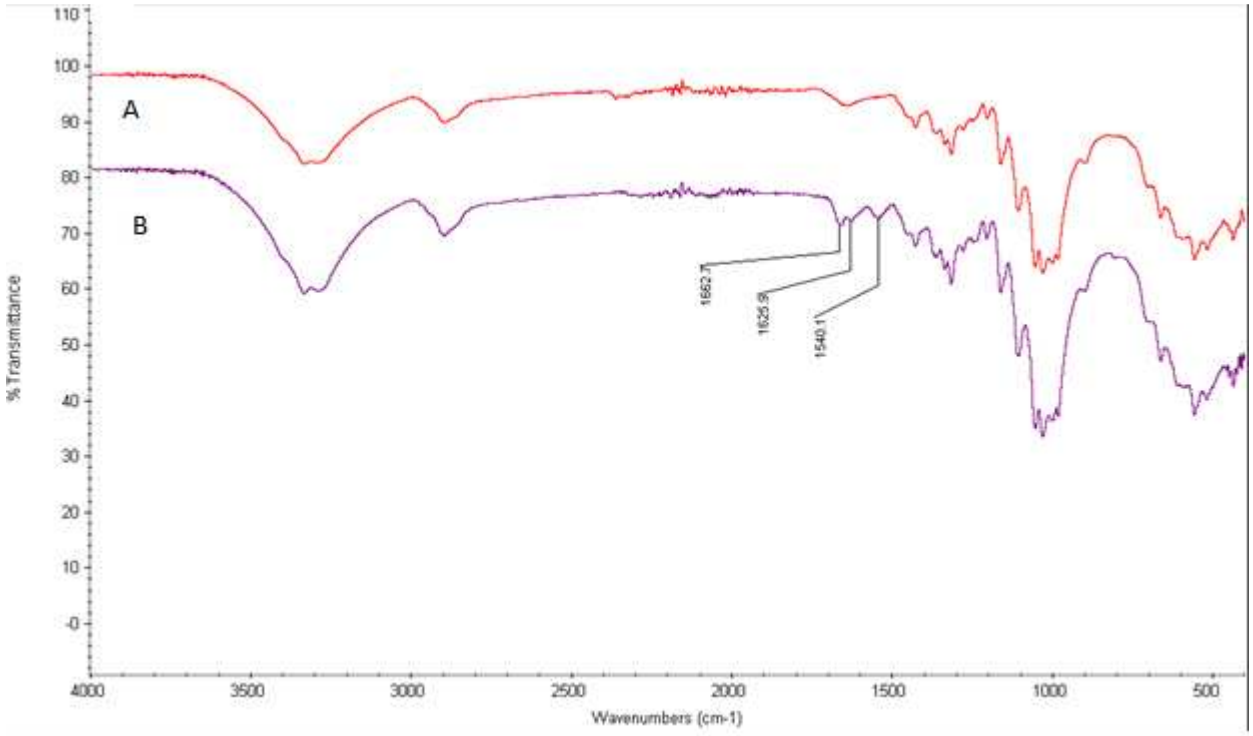


Figure S.2.1. FT-IR spectra of (A) pure cotton fabric and (B) NMA padded cotton fabric.

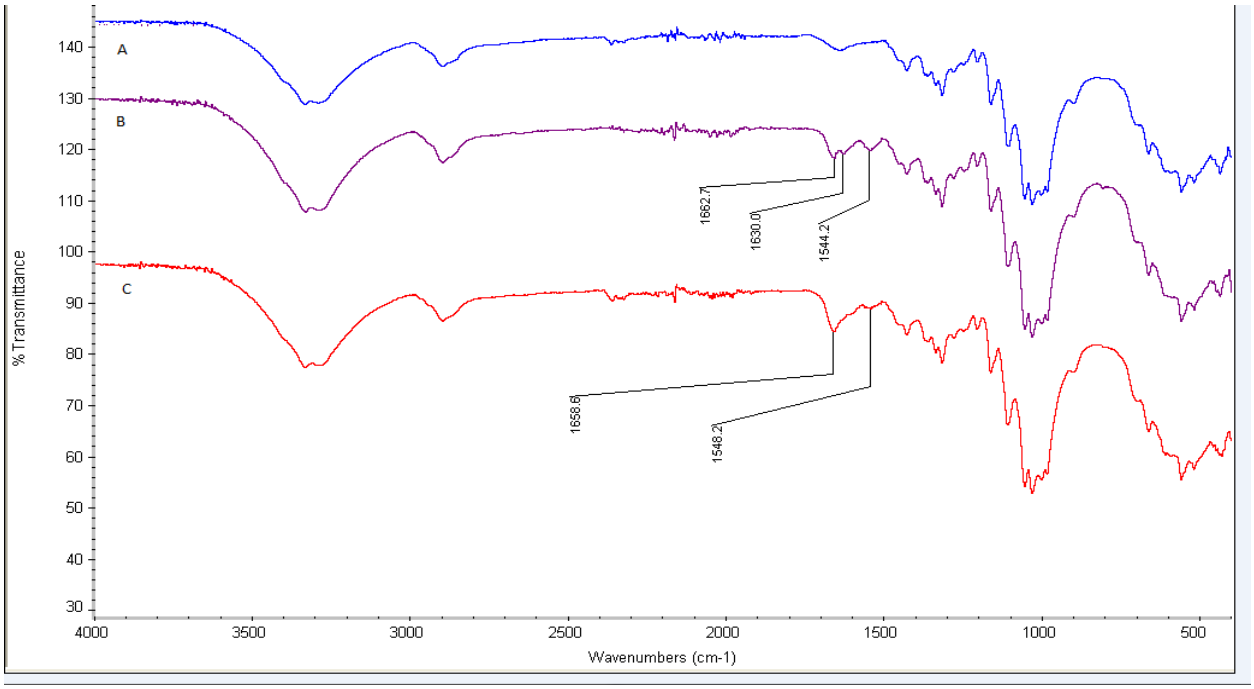


Figure S.2.2. FT-IR spectra of (A) pure cotton fabric, (B) NMA padded cotton fabric, and (C) MA grafted onto NMA finished cotton fabric

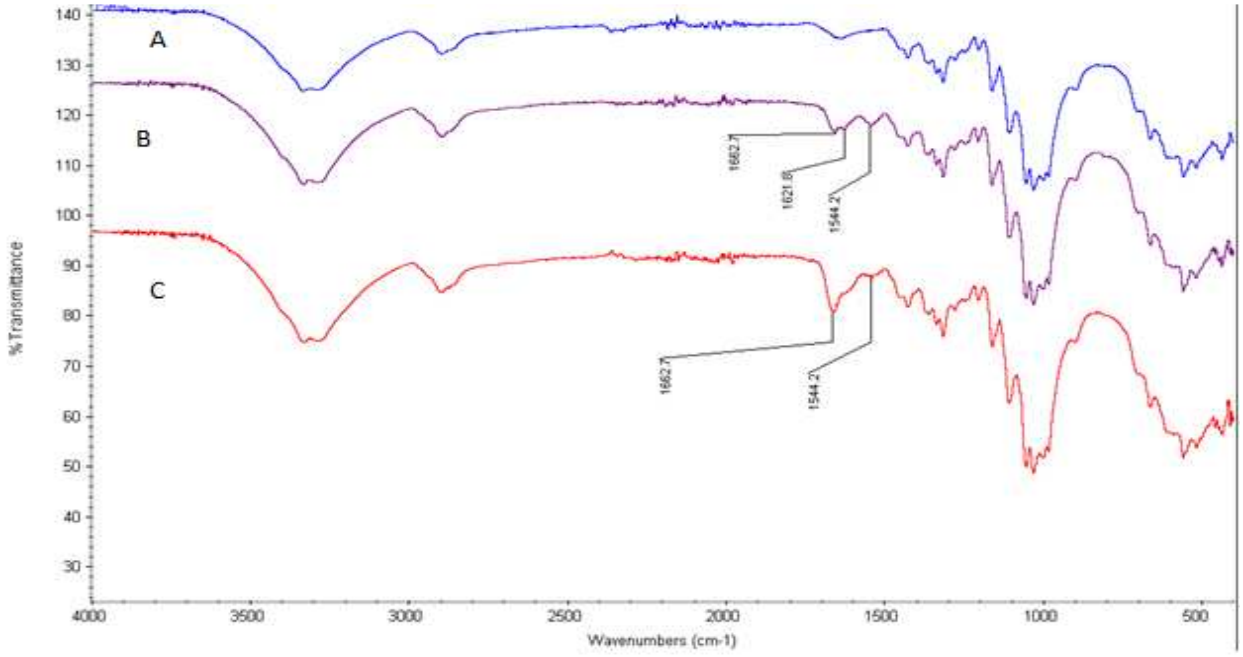


Figure S.2.3. FT-IR spectra of (A) pure cotton fabric, (B) NMA padded cotton fabric, and (C) AM grafted onto NMA finished cotton fabric

CHAPTER 3

THE BIOCAIDAL ACTIVITY OF N-TERTBUTYLACRYLAMIDE WHEN COPOLYMERIZED AND GRAFTED STRUCTURES ON FABRIC

3.1 Introduction:

Textile materials can be excellent substrate for microbial transfer, because they consist from fibrous structures and they can be easily contaminated by microorganisms. Human sweat may provide nutrients for bacterial growth.[2-3] Human skin contains numerous microorganisms even it is clean.[2-4-5] Microorganisms on textiles can cause problems such as unpleasant odors, allergic sensitization, infection and disease.[2-6] For this reason, in order to prevent transmission, growth, and spread of these microorganisms, antimicrobial materials should kill abroad spectrum microorganisms, preferable in a brief time intervals. N-halamines have demonstrated capability to inactivate various microorganisms in short period of time without causing the microorganisms to resistance.[7-8-9]

Briefly, N-halamines refer to organic and inorganic compounds, which have at least one nitrogen-halogen covalent bond in the structure. Organic N-halamines have different dissociation constants depending on the functional group: amine, amide, and imide structure. The order of dissociation constant is amine>amide>imide halamine, whereas the antimicrobial activity is vice versa. The amide structures of N-halamines are generally the most preferred ones for providing good stability and effective antimicrobial property due to intermediate stability.[10]

N-halamine precursors can be covalently bound to different surfaces via tethering groups such as siloxane, epoxides, and hydroxyls.[2-11-12] Biocidal efficacy of coating depends on concentration on biocidal sites and the structure of biocides.[13] For example some sterically hindered amide N-halamines such as N-tertbutlyacrylamide slow both in imidization and hydrolysis, so might be expected to inhibit conversion of N-H to N-Cl.[14] In this study, the sterically hindered N-substituted amide, N-tertbutlyacrylamide, was copolymerized with one of tethering group, a siloxane, and then attached onto cotton fabric (Figure 3.1).

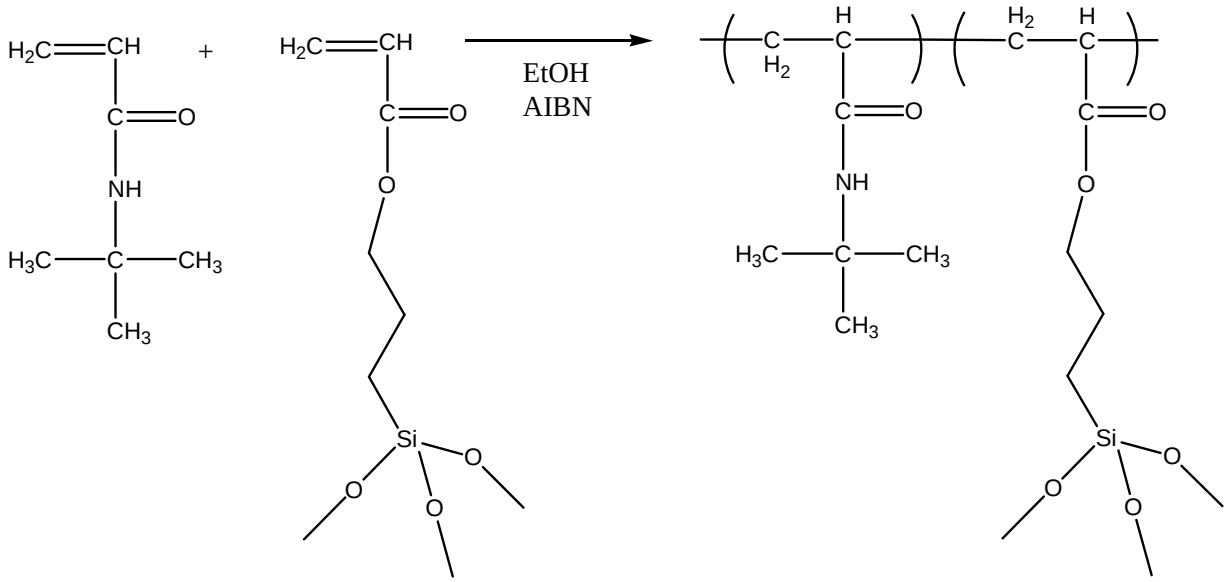


Figure3.1 The copolymer of NTBA-sil. synthesis

In addition, this monomer was grafted onto N-(hydroxymethyl)acrylamide (NMA) treated cotton fabric, as shown in Figure 3.2. The antimicrobial effectiveness against broad range of microorganisms, stabilities, UV resistance, rechargeability, and wrinkle recovery were investigated.

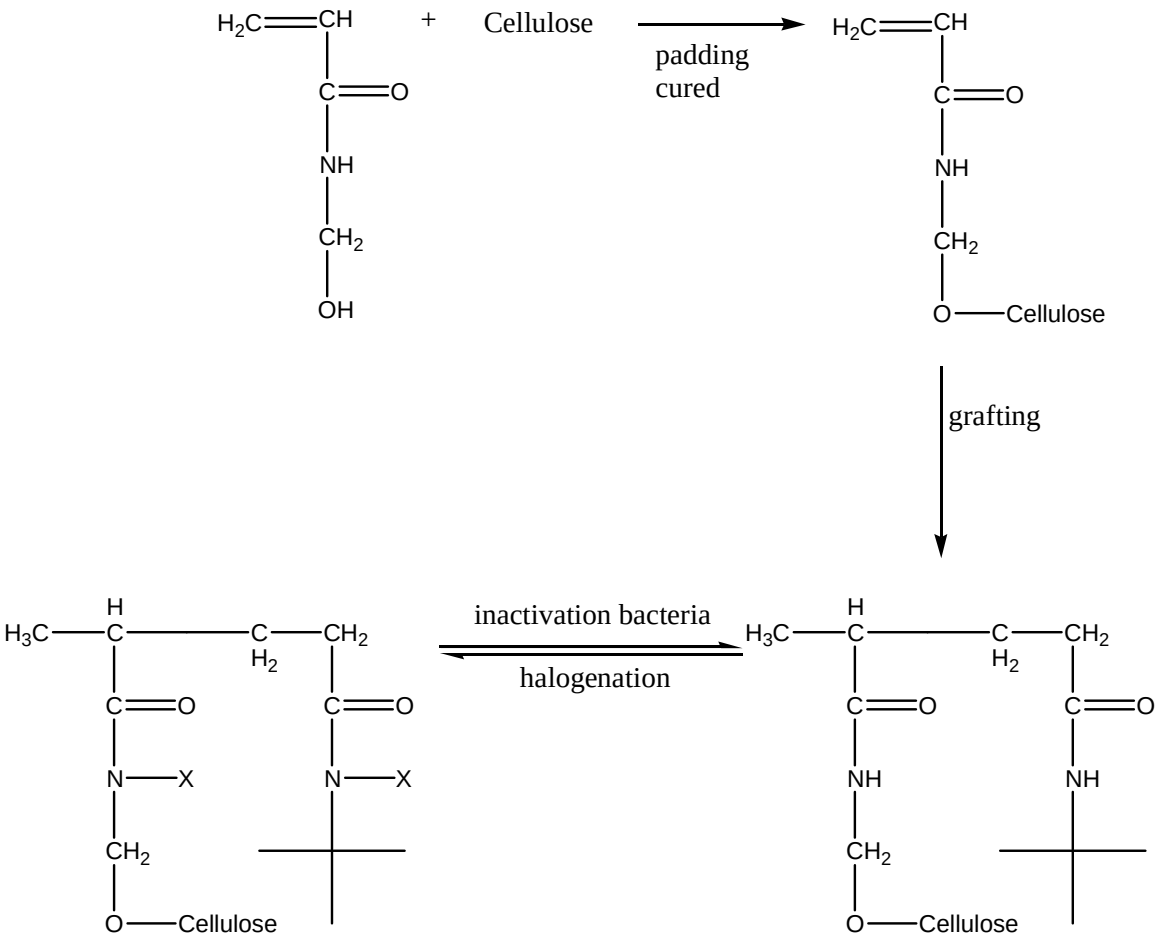


Figure 3.2 The preparation of antimicrobial coating on cotton fabric. (X= Cl. Br)

3.2. Experimental

Materials:

All chemicals were purchased from the Sigma Aldrich Chemical Company, and Fisher Scientific Chemicals. The fabric used was Style 400 bleached 100% cotton print cloth, (Test fabrics, West Pittston, PA). The household bleach was Clorox® brand (Clorox, Inc., Oakland, CA). The bacteria used were Staphylococcus aureus ATCC 6538 and Escherichia coli O157:H7 ATCC 43895 (American Type Culture Collection, Rockville, MD)

Instruments:

The FT-IR data were obtained with Nicolet 6700 FT-IR. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AV-400 (400MHz) spectrometer. UV light stabilities were measured with an Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA).

The Procedure for the Synthesis of N-tertbutylacrylamide-siloxane Copolymer:

The copolymer was synthesized by reaction of the N-tertbutylacrylamide and 3-(trimethoxysilyl)propyl methacrylate in a 10 to 1 molar ratio in 10mL ethanol solvent and the mixture was stirred for 2 hours at 80^{o}C, and then ethanol was evaporated. The product was confirmed by ^{1}H and ^{13}C NMR (see supporting information)

Preparation of NTBA Grafted onto NMA Treated Cotton Fabric:

N-tertbutylacrylamide was also grafted onto N-(hydroxymethyl)acrylamide finished cotton fabric. The first step was padding N-(hydroxymethyl)acrylamide onto cotton swatches. In this process, the N-(hydroxymethyl) acrylamide monomer and the catalyst, magnesium chloride, were dissolved in water at concentrations 5 and 1 percent respectively and the mixture was stirred for 15 minutes to produce a uniform solution. After cotton swatches were immersed in solution three times and squeezed with roller at, they were dried at 90^{o}C for 5 min. and cured at 175^{o}C for 3 min.[1] Then the swatches were soaked and washed vigorously in a 0.5% detergent solution for 15 min., and rinsed with water several times to remove any weakly bonded or unbounded compounds, and dried at 45^{o}C for 1 hour. The second step was grafting N-tertbutylacrylamide onto N-(hydroxymethyl)acrylamide coated cotton swatches. In this process, the monomer, N-tertbutylacrylamide, and the initiator, benzoylperoxide, were dissolved in ethanol solvent at concentration ratio ranging 5 and 1 percent respectively and the mixture was

stirred for 30 minutes to produce a uniform solution. NMA padded cotton swatches were soaked in the mixture, and then the mixture of beaker was sealed with aluminum foil. Then the mixture of beaker was left in oven at 70^{o}C for 15, 30, 60, and 90 min. After fabrics were squeezed with roller, they were dried at 90^{o}C for 3 min. and cured at 120^{o}C for 5min. Then cotton swatches were washed vigorously in a 0.5% detergent solution for 15 min., and rinsed with water several times to remove weakly bounded or unbounded chemicals, and dried at 45^{o}C for 1 hour. Weight gain was used as a measure of monomer grafted onto cotton.

Coating Procedure for the Copolymer of NTBA-sil:

The precursor siloxane copolymer was dissolved in ethanol at concentration ratio ranging from 3 to 5 weight percent. Then the mixture was stirred for 15 min. to produce a uniform solution. Cotton swatches were soaked in the solution for 15 min. and then cured it at 95^{o}C for 1 hour.[3] Then the swatches were soaked in a 0.5% detergent solution for 15 min., and rinsed with water several times to remove weakly bounded or unbonded chemicals, and dried at 45^{o}C for 1 hour. Weight gain was used as a measure of the coating added on the fabric.

Chlorination Procedure:

The treated cotton swatches were chlorinated by soaking in a 10% aqueous solution of NaOCl household bleach (0.6% sodium hypochlorite). The chlorination treatment was adjusted at different pH with 6N HCl at ambient temperature for 1 hour. The chlorinated swatches were washed with distilled and tap water and dried at 45^{o}C for 1 hour to remove any unbonded free chlorine from the material. The loading of bound chlorine on the swatches was determined by an analytical titration procedure.

Analytical Titration Procedure:

The determination of chlorine concentration on the treated samples was measured by a standard iodometric/thiosulfate titration method. In this procedure, about 0.2g of coated and chlorinated cotton swatch material was suspended in 50 mL of 0.1 N acetic acid solution. After addition of 0.25 g KI, and starch as an indicator, the solution was stirred for 45 min. to 1 hour. Then the solution was titrated with 0.00375N sodium thiosulfate until the blue color was disappeared at the end point. The Cl^+% on the samples were calculated from the equation below:

$$Cl^+\% = [N \times V \times 35.45 / (2 \times W)] \times 100\% \quad (1)$$

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

Biocidal Efficacy Testing:

One inch square of both chlorinated and unchlorinated treated cotton samples were challenged with Gram-positive Staphylococcus aureus ATCC 6538 and a second cotton sample was challenged with Gram-negative Escherichia coli (E. coli) O157:H7 ATCC 43895 for antimicrobial efficacy analyses by using a modified AATCC Test Method 100-1999. Bacterial suspension (25μL) having a known concentration of colony forming units in pH 7 phosphate buffer solution was added to the center of a one inch square cotton swatch and a second identical swatch was sandwiched over the first swatch. A sterile weight was used to insure sufficient contact of the swatches. After the contact times of 1, 5, 30, and 60 min., the samples were quenched with 5.0 mL of sterile 0.02N sodium thiosulfate solution to remove any oxidative chlorine. Serial dilutions of the quenched suspensions were placed on Trypticase agar. The plates

were incubated at 37^{o}C for 24 h and then counted to determine the number of viable bacteria that had remained after exposure to the fabric.

Washing Testing:

Laundering tests were performed to measure the stability and rechargeability of chlorine on coated cotton swatches by using standard washing test according to American Association of Textile Chemists and Colorist (AATCC) Test Method 61. All types of coated swatches were washed for the equivalent of 5, 10, 25, and 50 machine washing cycles. The chlorine loading of the samples after the washings, after rechlorination, and unchlorinated ones after chlorination were determined by the titration procedure which was described above.

UV Light Stability Testing:

UV light stabilities of chlorinated NTBA-siloxane copolymer, and NTBA grafting onto NMA coated cotton swatches were measured by using an Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA). The samples were placed in the UV (Type A, 315-400nm) chamber for times in the range of 1 to 72 hours. After specific time of exposure, samples were removed from the UV chamber and titrated, or rechlorinated and titrated.

Wrinkle Recovery Angle Testing:

Wrinkle recovery angle (WRA), warp (W) + filling (F) of the treated cotton fabrics was evaluated by using AATC test method 66-1998, Option 2. According to this test method, treated samples were prepared in 40 x 15 mm and 500 $\pm$ 5 g of weight loaded on the folded specimen for 5 min. The fabric on one side of the fold is hold while the other is released and allowed to unfold while its orientation is maintained vertical downward to eliminate to eliminate the effect of

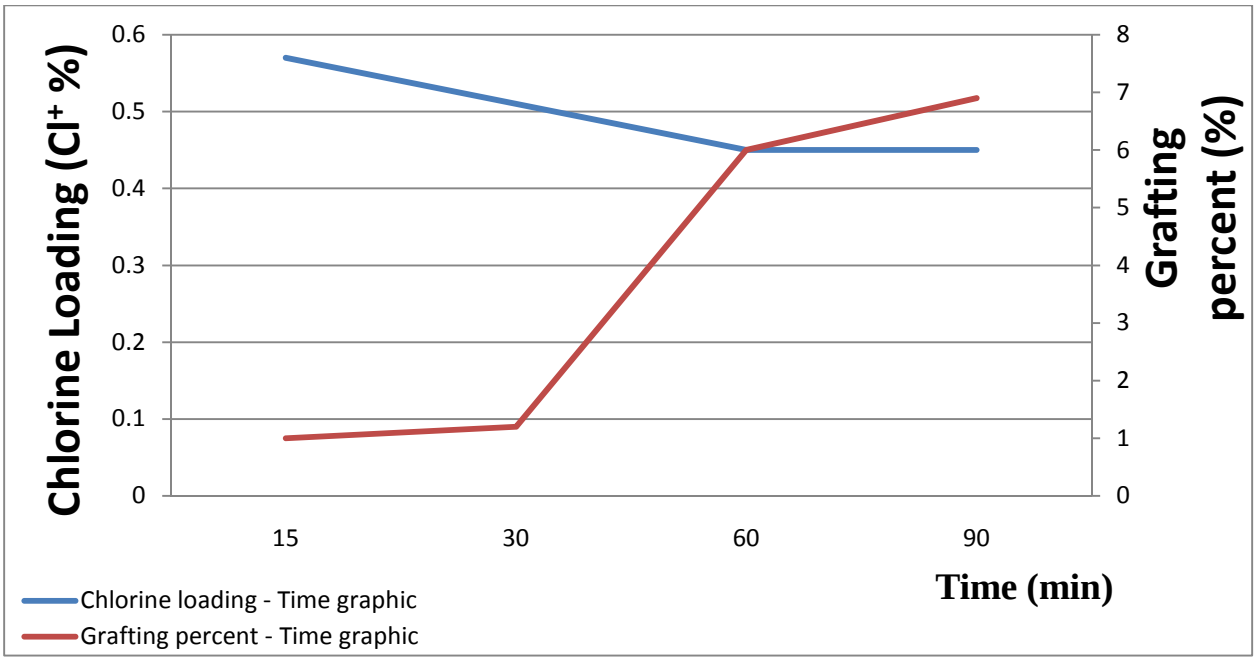
gravity or unfolding. The angle which the fabric unfolds within 5min is called the wrinkle recovery.

3.3 Results and Discussion:

Coating Procedure for Grafting NTBA onto NMA Treated Sample:

In general, biocidal efficacy of coating depends on biocides chemical structure (amine, amide or imide) and N-halamine content of molecules or polymers.[13] Higher N-H bond concentration in the structure results higher chlorine or bromine loading.[15] Since it is the active Cl^+ inactivating microorganism, higher loading should improve biocidal efficacy; but in contrast, it reduces because high chlorine loading increases surface hydrophobicity of cotton resulting poor contact with microorganism.[16]

In the beginning of this project the bulky structure of NTBA was grafted onto NMA finished cotton fabric. Firstly NMA was padded onto cotton by dipping the sample into NMA solution, squeezing between the rotating rollers and curing at specific temperature. A detailed description of the process can be found in the experimental section 3.2.4. The second step was grafting NTBA onto N-(hydroxymethyl)acrylamide (NMA) finished cotton fabric. The grafting procedure was explained in experimental part 3.2.4 more lengthily. The biocidal effect of NTBA was investigated when it was grafted onto NMA coated fabric.



. **Figure 3.3** Chlorine loading of NTBA grafted onto NMA finished cotton fabric when it was chlorinated at pH11.

The results obtained when treated cotton sample was chlorinated at pH 11, are shown in Figure 3.3. When grafting yield increased, the chlorine loading decreased. In other words, when the grafting percent was increased, the chlorine loading decreased at some point. The chlorine loading was not linear with grafting however and level off at about 0.45 Cl^+

Secondly, in order to see the effect of NMA percent on the chlorine loading percent, cotton fabric was padded with different NMA concentration, and then NTBA was grafted onto NMA finished samples. As it can be seen in Figure 3.4, there is a linear increase in chlorine loading with increasing amount of NMA percentage.

Table3.1 Chlorine loading of constant NTBA grafted onto different concentration NMA padded sample

Samples	NMA Content on Fabric (%)*	NTBA Content on Fabric (%)*	Measured [Cl+]%	Theoretical [Cl+]%
(1%NMA) + (NTBA)	1.5	6.1	0.18	1.73
(3%NMA) + (NTBA)	2.3	6.1	0.31	1.94
(5%NMA) + (NTBA)	3.1	6.1	0.45	2.15
(10%NMA) + (NTBA)	4.3	6.1	0.57	2.46

* Measured by weight gain.

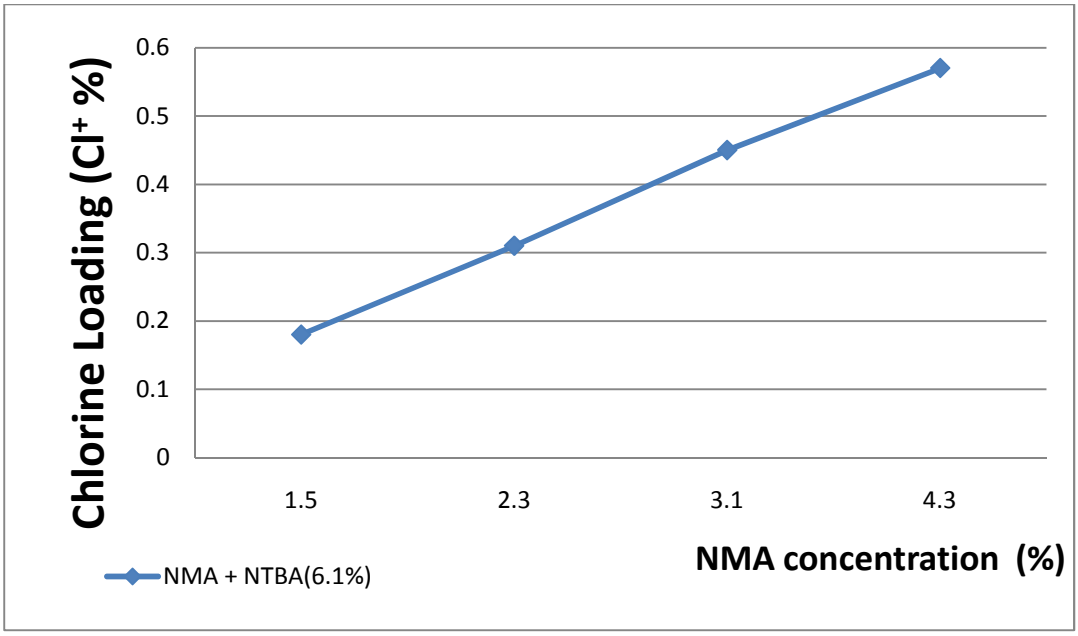


Figure 3.4 Chlorine loadings of different amount of NMA with constant NTBA grafting

The Potential Reason Affecting the Final Products of Chlorine Loading:

In order to figure out the reason of chlorine loading decrease, N-tertbutylacrylamide (NTBA) was copolymerized with 3-(trimethoxysilyl)propyl acrylate**.** After the polymerization completed, the structure of the copolymer was confirmed by FTIR and NMR spectroscopy shown in Figure S.3.1, Figure S.3.2, and Figure S.3.3 (see Supporting Information). Then, certain amount of copolymer was dissolved in ethanol and coated onto cotton fabric. A detailed description of the process can be found in experimental section 3.2.4. After attachment of NTBA-siloxane copolymer onto cotton fabric, the chlorine loading at different pH was investigated. The results are shown in Table 3.1.

Table 3.2 Chlorination at different pH of NTBA-siloxane attached cotton fabric

Samples	NTBA-sil weight gain (%)	Chlorination pH	Measured [Cl+]%	Theoretical [Cl+]%
3% NTBA-sil copolymer	5.94	11	0	0.6
5% NTBA-sil copolymer	11.73	11	0	1.18
3% NTBA-sil copolymer	5.94	7	0.09	0.6
5% NTBA-sil copolymer	11.73	7	0.06	1.18
3% NTBA-sil copolymer	5.94	4	0.54	0.6
5% NTBA-sil copolymer	11.73	4	1.02	1.18

As shown in Table 3.1, NTBA-siloxane copolymer that was attached onto cotton fabric gave no Cl^+ when chlorination was done at pH 11 and almost zero Cl^+ when chlorination was done at pH 7. However the chlorine loading increased almost theoretical chlorine loading when chlorination

was done at pH 4. This result led the conclusion that the chlorination reactions were more favorable at lower pH values. The reason of increasing Cl^+ at lower chlorination pH is that N-tertbutyl acrylamide is the bulky substituted amide nitrogen inhibited conversion of N-H to N-Cl similar to its imidization and the hydrolysis.[14] This result also explain the decreasing of chlorine loading after grafting of NTBA onto NMA treated cotton fabric.

Since the chlorine loading is almost zero when it was chlorinated at pH 7 or 11, and toxic chlorine gas is produced when chlorination pH decreases to 4.0 or lower than 4.0, use of NTBA-siloxane is not commercially favorable. In order to examine the biocidal efficacy of this monomer, it was grafted onto NMA finished cotton fabric instead of copolymerizing.

FT-IR Confirmation:

FT-IR spectra of the treated cotton fabrics confirmed that copolymer of NTBA-sil N-halamine precursor bonded to cotton fabric. The band at ca. 1536cm^{-1} were detected which can be assigned amide structure of NTBA-sil coated cotton fabric that is shown in FigureS.3.3 (see supporting information). In addition, two detected band ca.1662cm^{-1} and1625cm^{-1} overlapped one strong single band at ca. 1650$cm^{-1}$for grafting NTBA onto NMA finished cotton fabric, and also the band at 1540cm^{-1} was detected which were assigned to amide structure of NMA+NTBA that is shown in FigureS.3.4 (see supporting information).

Antibacterial Efficacy

Antimicrobial efficacy of NMA+NTBA treated cotton fabrics were challenged with Gram-positive bacteria, S. aureus, and Gram-negative, E. coli O157:H7 at concentrations between 10^7 to 10^8 CFU (colony-forming units). Table 3.3 shows that the chlorinated sample effectively inactivated gram-postitive bacteria in 5 min contact time. However gram-negative is more

durable bacteria than gram-positive bacteria because of relatively thicker outer membrane, so chlorinated samples killed gram-negative bacteria relatively longer contact time of 30 minutes. Unchlorinated samples served as controls which provided only around 0.87 log reduction for Gram-positive and around 0.13 log reduction for Gram-negative after 30 min contact time.

Table 3.3 Biocidal efficacy against microorganisms

Samples[a]	Contact time (min)	Escherichia colli O157:H7[b]		Staphylococcus aureus[c]	
		Bacterial Reduction		Bacterial Reduction	
		% Reduction	Log Reduction	% Reduction	Log Reduction
NMA + NTBA Control	30	25.56	0.13	86.60	0.87
NMA + NTBA-Cl	1	12.02	0.06	94.14	1.23
	5	75.56	0.74	100	7.51
	15	89.62	0.98	100	7.51
	30	100	7.47	100	7.51

[a] Microorganism: E. coli O157:H7. Total bacteria: 9.67×10^7 CFU/sample (7.99 logs).

[b] Microorganism: S. aureus. Total bacteria: 9.33×10^7 CFU/sample (7.97 logs).

[c] Chlorine loadings on the coated swatches were 0.78, 0.45, 0.49 respectively.

[d] The error in the measured Cl+ weight percentage values was ±0.01.

Durability and Rechargeability toward Washing Test:

Rechargeability and durability are significant properties for N-halamine biocides. Through the laundering test, rechargeablitiy and durability of NMA + NTBA treated cotton fabrics were evaluated and the result is summarized in Table 3.4. Washing tests were performed at 5, 10, 25, and 50 equivalent washing cycles for swatches including prechlorinated and unchlorinated

coatings. In the Table 3.3, the X column represents the Cl^+ concentration of prechlorinated samples after each washing cycle. The initial chlorine loading is shown as 0 washing cycles. The Y Column represents the Cl^+ concentration of prechlorinated washed and then rechlorinated samples after given number of each washing cycles. The Z column represents the Cl^+ concentration of unchlorinated washed samples which were chlorinated after given number of washing cycles.

According to the stability towards washing test data, several important results were observed. The data of stability test for NMA + NTBA treated samples showed that after 5 washing cycles, a substantial amount of Cl^+ was lost. The amount of Cl^+ lost between 5 to 50 cycle washes gradually decreased and after 50 washing cycle, there was little Cl^+ observed on coated fabric. The Cl^+ values of Y showed that the loss of coating was not excessive. A comparison of X and Z column for NMA + NTBA coated sample indicated that only a small amount of the coating was lost during 5 to 50 washing cycle. The previous studies proved that chlorination increases the hydrophobicity of the surfaces and it protects the coating from hydrolysis. It would appear that the NTBA has a similar hydrophobic effect.

Table 3.4 Durability and rechargeavility of NMA coated cotton fabric

Washing Cycles	X[a]	Y[b]	Z[c]
0	0.45	0.45	
5	0.28	0.36	0.44
10	0.21	0.31	0.46
25	0.16	0.29	0.42
50	0.11	0.25	0.39

[a] X : Chlorinated before washing

[b] Y : Chlorinated before washing and rechlorinated after washing

[c] Z : Unchlorinated during washing, but chlorinated after washing

Wrinkle Recovery Angle Test

Conventional durable-press (DP) is one of the finishing processes that cause greatly reduced strength and abrasion resistance of cotton fabric, so previous studies were focused on improvement of both, DP, strength, and abrasion resistance properties by forming of network polymeric system onto cotton fabric.[17-18-19] N-(hydroxymethyl)acrylmaide (NMA) has been used several different applications where wrinkle resistance is desirable. One of the most important properties of NMA monomer is penetration into the cotton fiber and it has two functional groups, N-methylol and vinyl, that can be controlled independently to undergo etherification (with cellulosic hydroxyl group) and vinyl polymerization.[17-18] In this regard NMA appeared to constitute a reasonable selection. The schematic configuration is shown in Figure 3.5.

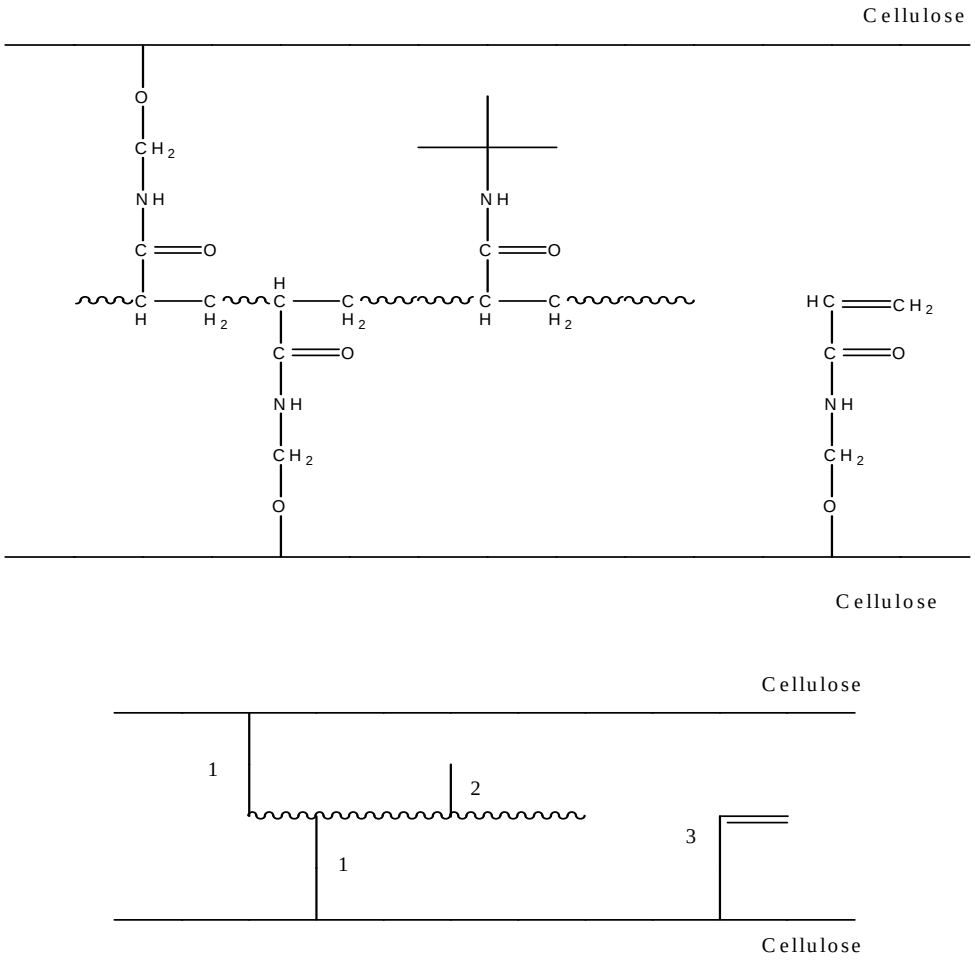


Figure 3.5 Schematic representation of structural units NMA and NTBA. Structural units are derived from (1) polymerization and etherification, (2) polymerization, (3) etherification only

The effect of NMA, NMA+NTBA treated cotton fabrics on crease recovery angle was tested. A detailed description of the process can be found in the experimental section 3.2.9. The results showed that there was not enough change between when NMA undergo etherification with cellulosic hydroxyl group, and NMA undergo both etherification and polymerization. There was not enough improvement on crease recovery angle (30^{o}). The crease recovery angle of NTBA+NMA treated cotton fabric did not enhance the wrinkle recovery angle. The reason of

lower wrinkle recovery angle result is perhaps that NTBA could not penetrate into the fiber and produce a network structure with the NMA.

Table 3.5 Wrinkle Recovery Angle Test Results

Samples	(W + F)[a]	Measured [Cl+]%
Control Sample[b]	165	-
5% NMA	220	0.74
Control Sample[c]	225	0.73
5%NMA + 10% NTBA	230	0.45

(W + F)[a] : Total value for Warp and Filling wrinkle recovery angle

Control Sample[b] : Wrinkle Recovery Angle of just pure cotton

Control Sample[c] : Wrinkle Recovery Angle of 5% NMA which undergo etherification with cellulosic hydroxyl group and polymerized.

Light Stability Test

The stability of the chlorinated NMA+NTBA coated fabric under UV irradiation was investigated. The biocidal efficacy is related with N-Cl bond, so the UV stability of the N-Cl bond of the coated N-halamine precursors is presented in Table 3.6. NMA + NTBA treated sample was exposed UV for 24 hours, and then rechlorinated. Then, the sample was exposed UV additional 24 hours, and then rechlorinated again. This procedure was repeated four times. It was observed that NMA + NTBA treated cotton fabric decomposed fast after 1 hour later. There was 30% decomposition after 1 hour, and 50% decomposition after 6 hours UV irradiation. After 6 hours later, the decomposition was getting slow and there was 60% decomposition when it was reached to 24 hours.

Table 3.6 UV light stability test

Time of exposure (h)	NMA + NTBA	
	Measured [Cl+]%	**Rechlorination [Cl+]%**
0	0.61	
1	0.43	
3	0.36	
6	0.31	
12	0.27	
24	0.25	0.42
Control Sample[a]	0.64	
48	0.17	0.29
Control Sample[b]	0.65	
72	0.15	0.28
Control Sample[c]	0.63	
96	0.11	0.16
Control Sample[d]	0.62	

Control Sample[a] : Chlorination of unchlorinated 24 UV light exposure sample

Control Sample[b] : Chlorination of unchlorinated 48 UV light exposure sample

Control Sample[c] : Chlorination of unchlorinated 72 UV light exposure sample

Control Sample[d] : Chlorination of unchlorinated 96 UV light exposure sample

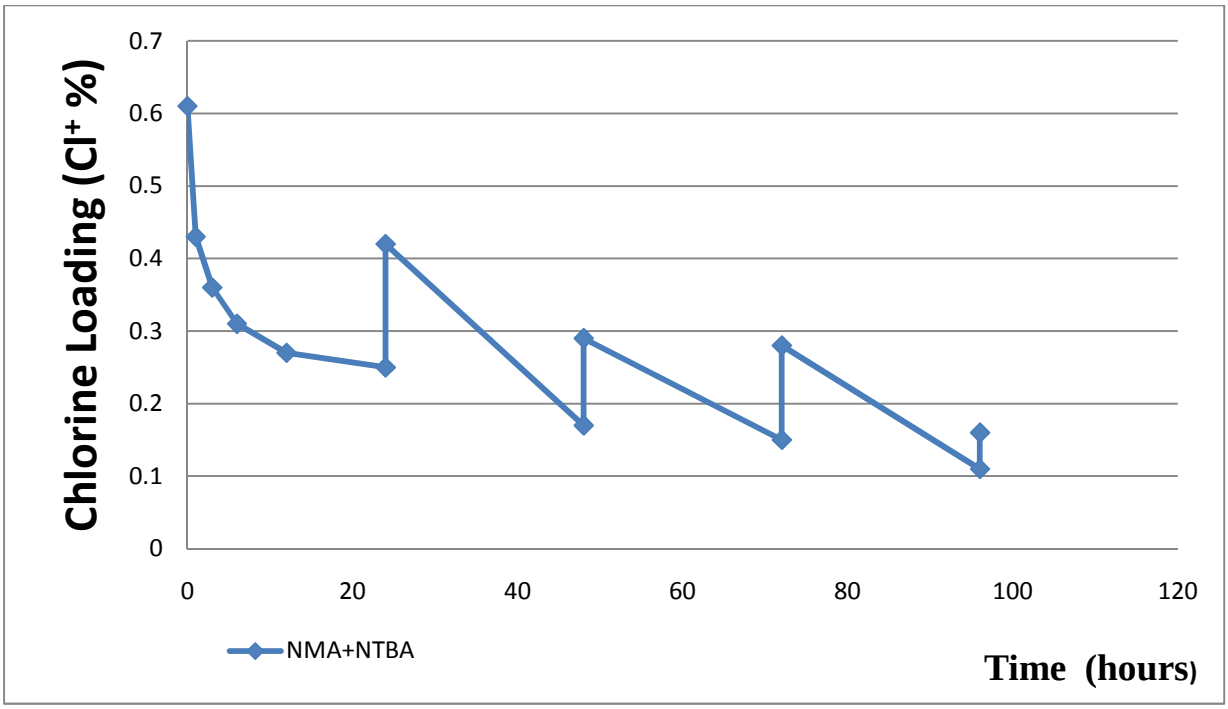


Figure 3.6 UV light stability test of NMA+NTBA treated sample for four days (The vertical line is represent rechlorination)

3.4 Conclusion

The NTBA grafting onto NMA treated sample result was the decrease in Cl^+ loading with increase in NTBA. The NTBA-sil copolymer treatment retained no Cl^+ when chlorinated at pH 11. Only when pH was lowered to pH 4 did the treated cotton retain significant amount chlorine. Clearly this monomer and its copolymers are not suitable for use as a halamine precursor in an industrial process.

3.5 References

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3.6 Supporting Information

The Supporting Information includes additional information about NMR, and FTIR spectra of the compounds.

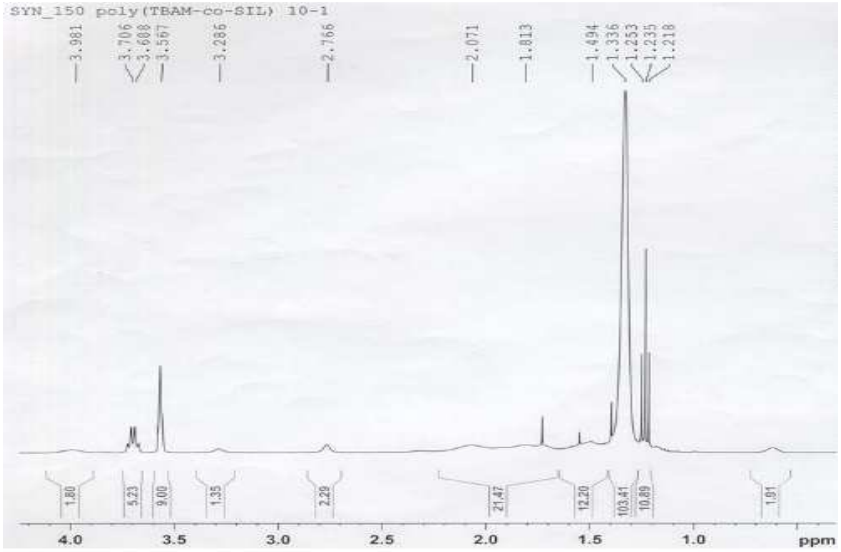

Figure S.3.1 ^{1}H NMR Spectra of NTBA-sil copolymer

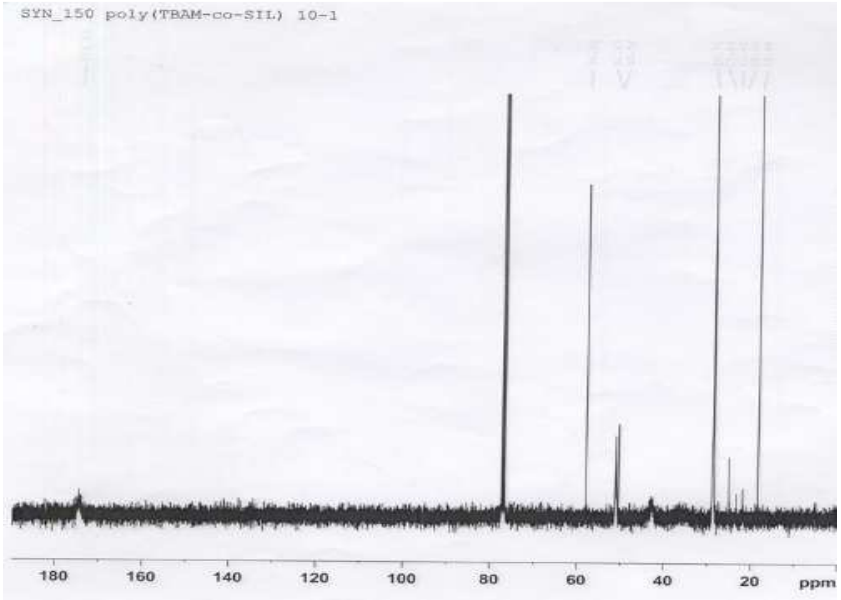

Figure S.3.2. ^{13}C NMR of NTBA-sil copolymer

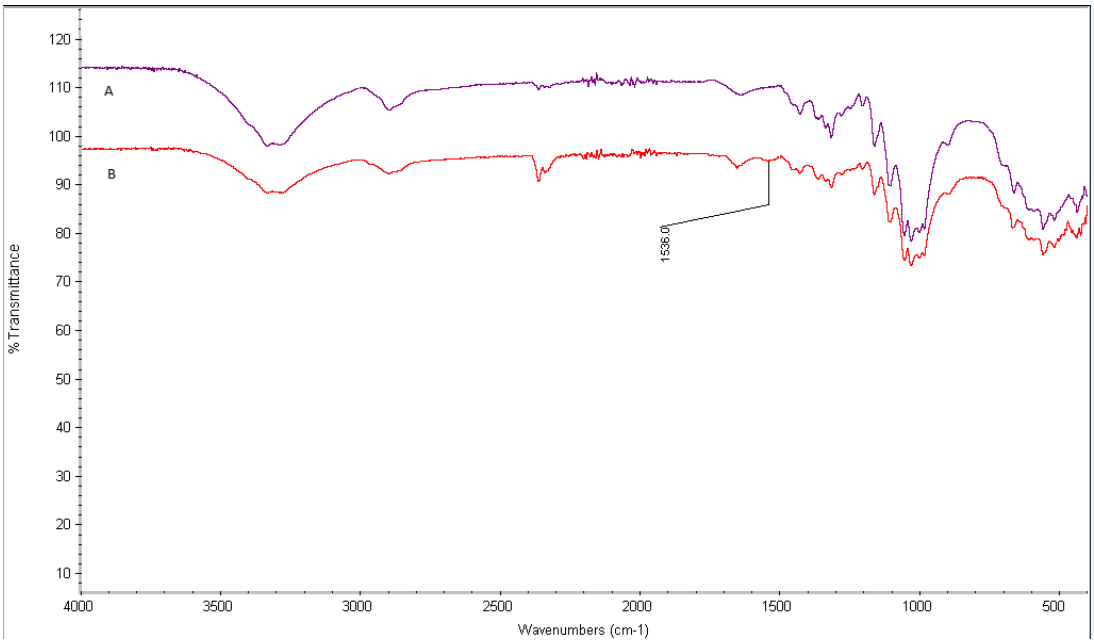

Figure S.3.3 FT-IR spectra of (A) pure cotton fabric and (B) NTBA-sil coated onto cotton fabric.

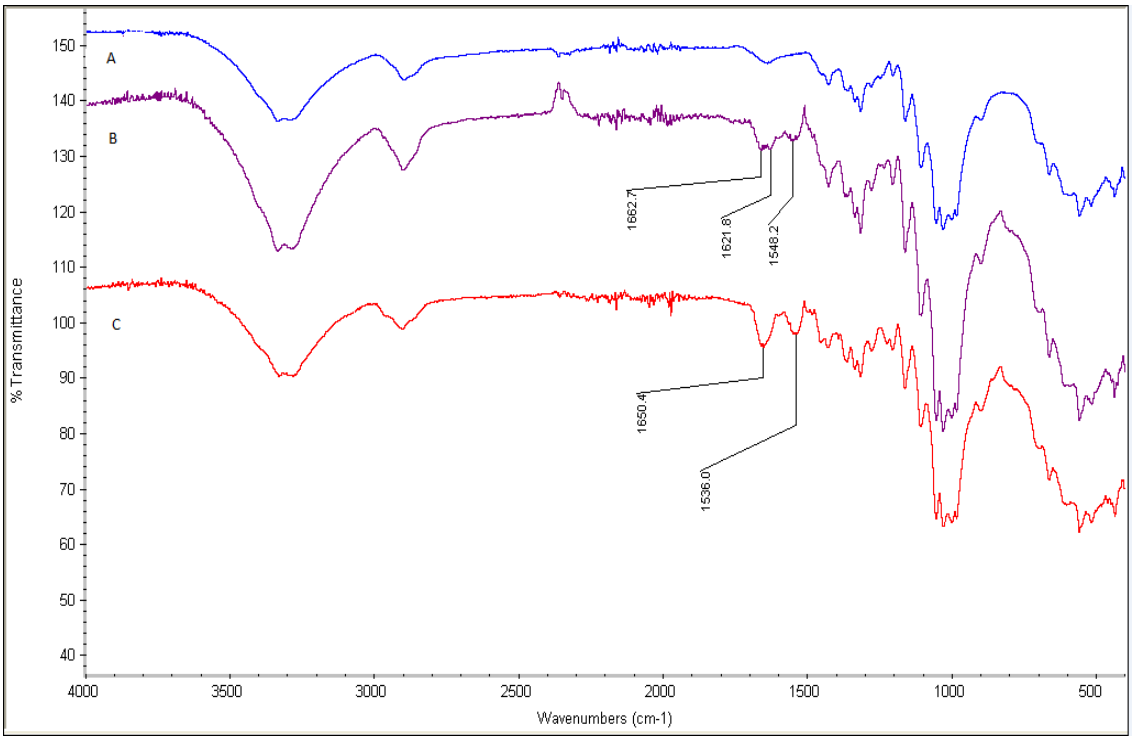

Figure S.3.4 FT-IR spectra of (A) pure cotton fabric, (B) NMA padded cotton fabric, and (C) NMA+NTBA treated onto cotton fabric