SYNTHESIS AND APPLICATION OF NOVEL BIOCIDAL MATERIALS

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SYNTHESIS AND APPLICATION OF NOVEL BIOCIDAL MATERIALS

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SYNTHESIS AND APPLICATION OF NOVEL BIOCIDAL MATERIALS

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Changyun Zhu, the daughter of Kangren Zhu and Shunhua Zhu, was born in Changsha, Hunan Province, China, in 1979. She received her Bachelor of Science degree and Master's degree in Chemistry from Jilin University in July 2001 and July 2004, respectively. She entered the Graduate School at Auburn University in September 2004, where she worked under the supervision of Dr. S. D. Worley. She married Yifei Wang, son of Huiming Wang and Huirong Pang, in 2004.

THESIS ABSTRACT

SYNTHESIS AND APPLICATION OF NOVEL BIOCIDAL MATERIALS

Changyun Zhu

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For this study, a new N-halamine, 1-(2, 3-epoxypropyl)-6-methyluracil, was synthesized and was coated onto cotton fabric. The treated cotton was then activated by chlorination using Clorox bleach. The cotton swatches were found to deactivate *E.coli* in 1 min contact time and *S. aureus* in 5 min contact time and retained their biocidal activity even after 50 washing cycles. The chlorine lost was easily recovered in recharging in household bleach. This reaction is very efficient, and the reaction solution can be used directly in the finishing bath in the manufacturing process. The potential applications of this biocidal cotton fabric include use in hospitals as bed sheets and clothes for patients. This type of cotton can also be used in antimicrobial masks to stop Gram negative and Gram positive bacteria.

Another new N-halamine monomer, N-allyl-6-methylpyrimidine, was also synthesized and was copolymerized with commercial vinyl acetate (VAC) to form a

copolymer VAC-co- N-allyl-6-methylpyrimidine useful for film coating. A series of VAC-co-N-halamine copolymers,

VAC-co-3-(3'-allyl-5'-methylhydatoinyl)acetanilide,

VAC-co-5-methyl-5-(3'-propenyl)hydantoin,

VAC-co-3-allyl-6,6-dimethyl-1,3,5-triazinane-2,4-dione, and

VAC-co-5,5-dimethylhydantoin-1-ylmethylene acrylate were also synthesized. Those films had biocidal activity after treatment with Clorox bleach. The surface coated copolymer contained 2.0×10^{17} to $9.0\times10^{17}Cl^{+}$ atoms/cm^2. The films produced in this research were able to kill both Gram-positive and Gram-negative bacteria. Those polymers have many potential applications in medical devices, hygienic materials, and in the food-processing industry.

ACKNOWLEGMENTS

The author would like to take this opportunity to express her sincere thanks to her major professor, Dr. S.D. Worley, for his guidance and support. Without his tutelage, this thesis would never have been completed. The author is also grateful to her committee members, Dr. R.M. Broughton and Dr. A. Gorden, for their suggestions and discussions. Appreciation is given to her fellow group members: Dr. Liang, Dr. Akdag, Dr. Ren, Lei Kou, Hasan Kocer and Kevin Barvnes, and the assistance of Dr. T.S. Huang of the Department of Nutrition and Food Science is also appreciated for conducting antibacterials tests on the material fabricated for this research. Special thanks go to Dr. J.W. Lee of the Department of Polymer and Fiber Engineering for performing laundering washing tests.

Finally, the author would like to thank her husband, Yifei, whose constant concern, understanding and encouragement have helped her to complete this work.

Style manual or journal used American Economic Review Style Guide

Computer software used Microsoft Word 2007

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b.p.	boiling point
℃	degrees centigrade
CFU	Colony-Forming Unit
cm	centimeter
cm^{-1}	reciprocal centimeter
^{13}C NMR	carbon-13 nuclear magnetic resonance
d	doublet
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
FTIR	Fourier transform infrared spectroscopy
g	gram
PEG	polyethylene glycol
^{1}H NMR	proton nuclear magnetic resonance
hr	hour
IR	Infrared
mL	milliliter
m	multiplet

MDMH	3-methylhydroxyl-5,5-dimethylhydantoin
min	minute
Mp	Melting point
MS	Mass Spectrometry
N	normal
ppm	parts per million
QAS	Quaternary Ammonium Salts
S. aureus	*Staphylococcus aureus*
s	singlet
%	percentage
µ	micro
VAC	vinyl acetate

INTRODUCTION

Most diseases are caused by bacteria. Bacteria also spoil food, contaminate water, ruin cloth, rot wood, foul water pipes and the hulls of ships, interfere with equipment, and produce disagreeable odors in carpets and diapers, or on living organisms. [1-3] A biocide is a chemical substance that is capable of killing different forms of living organisms and is used in fields such as medicine, agriculture, naval architecture, water purification and pest control to combat the effects of bacteria. To understand how biocides act to counter bacteria, it is first necessary to examine the structure of bacteria.

The structure of a bacterial cell is shown in **Figure 1**. [4] As prokaryotes, organisms without a cell nucleus, all bacteria have a relatively simple cell structure. They also lack organelles such as amyloplasts, chromoplasts, and chloroplasts. Most bacteria are comparatively small and possess typical cell and colony morphologies. The most important bacterial structural characteristic is its cell wall, whose main purpose is to protect the interior from any outside physical force that may damage the cell. The differences in cell wall structure, which are revealed by Gram staining the bacteria cells, can be used to divide bacteria into two groups: Gram positive and Gram negative.

Gram positive bacteria, for example *S. aureus* (**Figure 2**), have a cell wall containing a thick peptidoglycan (sometimes referred to as Murein) layer and teichoic acids, which act as chelating agents, and are also involved in certain types of adherence. If flagella are present, two more rings are included in the cell wall for added support, although they may not be necessary because the thick cell wall is already sufficiently supportive. Upon staining, Gram-positive bacteria appear blue or violet under a microscope. [5]

Gram negative bacteria, such as *E. coli* (**Figure 3**), have an outer, lipopolysaccharide-containing membrane and a thin peptidoglycan layer located in the region between the outer and cytoplasmic membranes, which is called the periplasmatic space. Under a microscope, stained Gram-negative bacteria appear red or pink. [5] Gram negative bacteria have porins in the outer membrane, which act as pores for particular molecules. Furthermore, the S-layer directly attaches to the outer membrane rather than to the peptidoglycan, and the flagella have four supporting rings instead of two.

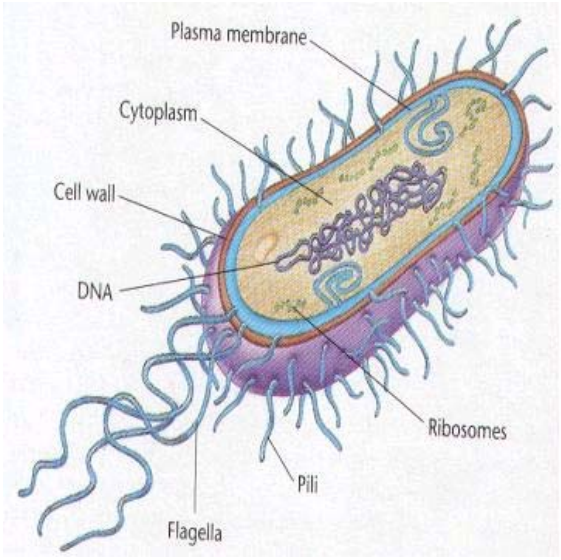


Figure 1 Structure of the Bacteria[6]

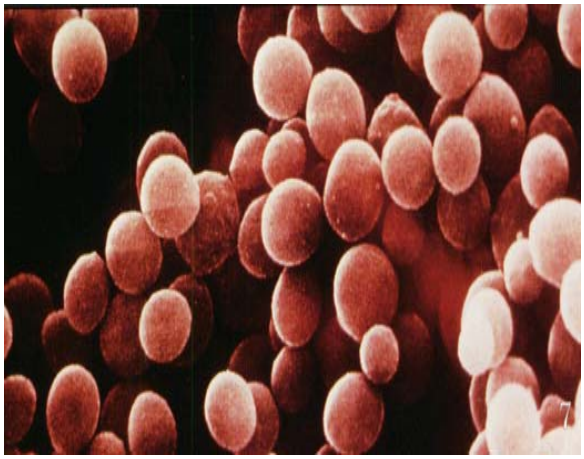


Figure 2 Shape of *S. aureus*[7]

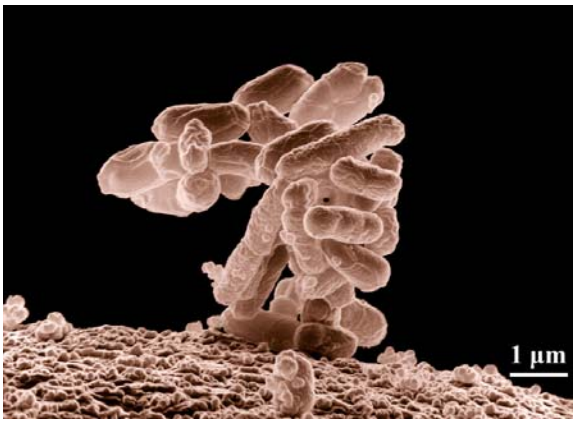


Figure 3 Shape of *E. coli*[8]

In order to kill the bacteria, a biocide first interacts with it by binding to the bacterial cell surface. Next, a change occurs at the outer layer of the bacteria that allows the biocide to penetrate the cell wall. The biocide functions as a penetrator, with the cytoplasmic membrane as its potential target. This membrane functions to maintain cell integrity and when the membrane integrity is destroyed, essential components, such as enzymes, nucleotides and nucleosides, and pentoses, can flow out of the cell. Once those cytoplasmic components leak out, the bacterial cell will eventually die.[9,10]

There are three possible kinds of interactions between a biocide and a bacteria cell. First, *physical interactions* lead to the biocides dissolving in the phospholipids in the cell wall. *Chemical interactions* occur as the biocides react with the amino, imino, amide, carboxyl, and thiol groups of the cytoplasmic constituents inside the cell such as proteins, RNA, and DNA, inhibiting the cell's biosynthesis and the replication necessary for cell growth. Finally, an *ionic interaction* occurs when biocides inhibit

cell's respiration or catabolic anabolic interaction. Because of the differences in the outer membranes of the Gram-negative and Gram-positive bacteria, their relatively thick outer membrane barrier makes Gram-negative bacteria less sensitive than Gram-positive bacteria to biocides, the latter of which are consequently easier to kill. If a biocide can kill both Gram-negative and Gram-positive bacteria, it is classified as a broad-spectrum biocide; while a 'narrow-spectrum' biocide will only be effective against a few types of bacteria.

A biocide may be a pesticide, for example fungicides, herbicides, insecticides, algicides, and rodenticides or an antimicrobial, such as germicides, antibiotics, antibacterials, antivirals, antifungals, and antiparasites. Biocides can also be added to other materials to protect them from biological infestation and growth. For example, in order to protect water sources from infestation and the growth of algae, certain types of quaternary ammonium compounds (quats) are often added to pool water or industrial water systems to act as algicides. Those biocides are antimicrobial agents that are applied particularly to inanimate objects and are known as disinfectants. These natural or synthetic chemicals are the primary focus of this study.

Some of the earliest surviving records show that the Egyptians, Chinese and Persians practiced preservation, drinking water sanitation, antisepsis for wounds and injuries, and both physical and chemical methods for mummification. At that time it was believed that epidemic diseases were spread by something in the air, so

Hippocrates lit fires of aromatic wood in the streets to drive the plague from Athens.[11]

In the 19th century, John Tyndall (1820-93) showed that although spores were resistant to boiling and could grow if they had sufficient time, their offspring could be killed by repetitive heating. This technique became known as Tyndallisation. Nicholas Appert (c. 1749-1841) demonstrated methods for preserving food during long sea voyages by sealing vegetables and fruit in glass jars and heating them.[12.13] Thus, many disinfectants have been in use for 150-200 years. Chemicals such as pitch, wine, copper, and silver were known as the earliest disinfectants. Coal tar and wood tar were the first materials used as disinfectants in the distilling industries and were used to preserve ship timber in the early 1700s.[14,15] After mercury was discovered, Arab physicians used mercuric chloride as an antiseptic and a wood preservative. Chlorine was introduced for water treatment in 1843 and is still widely used for this purpose today. Other disinfectants such as copper sulfate, zinc chloride, and sodium permanganate, acids, alkalis, sulfurs, and alcohols were introduced in the mid-1800s.[16,17]

As we enter the twenty-first century, scientists now have access to advanced techniques for studying microorganisms which should be of great assistance in the effort to develop more effective antimicrobials to solve specific problems.

Today biocides are a widespread. Chlorine is an important biocide, and Cl_2, Br_2, I_2, HOCl, NaOCl, and ClO_2 are classic halogen biocides that have been widely used for water disinfection in drinking water supplies and swimming pools because they

are both effective and inexpensive. Hydrogen peroxide, a powerful antimicrobial agent, is used in hospitals to disinfect surfaces. When mixed with colloidal silver, it causes far fewer allergic reactions than alternative disinfectants and so is often preferred in the food packaging industry to disinfect foil containers. Ozone can be used for bleaching substances and for killing bacteria, and many municipal drinking water systems now kill bacteria with ozone in place of the more traditional chlorine. Alcohol, usually ethanol or isopropanol, can be wiped over benches and skin and evaporated for rapid disinfection. Quaternary ammonium salts ("quats") (**Figure 4**), such as benzalkonium chloride, are also used as low level disinfectants. Benzalkonium chloride is deemed safe for human use, and is widely used in eyewashes, hand and face washes, mouthwashes, spermicidal creams, and in various other cleaners, sanitizers, and disinfectants.[18-21]

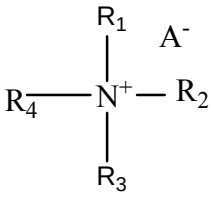
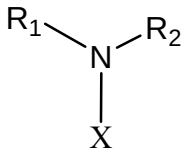


Figure 4 Structure of Quaternary Ammonium Salts

Although these biocides are widely used world wide, many suffer from serious deficiencies. In certain parts of the world, chlorine has largely been replaced because it forms some potentially hazardous byproducts. Recent studies have shown hydrogen peroxide to be toxic to growing cells as well as bacteria; its use as an antiseptic is no

longer recommended. Though ozone leaves no taste or odor in drinking water, it is an atmospheric pollutant and toxic to humans, and it is known to degrade almost all polymers and metals upon contact.

N-halamines, compounds containing one or more nitrogen-halogen covalent bonds, are classic biocides. The general structure of N-halamines is shown below.



R_1,R_2,=H, Cl, Br, inorganic group, organic group
X=Cl,Br

The halogen can be either chlorine or bromine, but is usually not iodine. R_1 and R_2 can be either inorganic groups or organic groups. If R_1 and R_2 are inorganic groups, for example phosphates or sulfates, they are called inorganic N-halamines; in the same way, if one of the R groups is an organic group such as an alkyl group or carbonyl group, it is refered to as an organic N-halamine. Some inorganic chloroamines , **1**. chloroamines, **2**. sodium N-chloroimidometaohospate, **3**. N-chlorosulfamic acids or N,N-dichlorosulfamic acid, and **4**. trichoroimidometaohophate, are shown in **Figure 5**; they are normally used as water disinfectants in place of chlorine gas.[22,23] The inorganic N-halamines do not readily react with the organic materials in water to form toxic trihalomethanes, and their cost is less than that of either chlorine dioxide or ozone. They can be used to control the taste and odor caused by organic contamination both in primary and secondary water treatment. The disadvantages of chloramines are their sensitivity to pH and

water temperature, and their potential toxicity for plants and some mammals. [26-24] Their biocidal efficiency is weaker than that of free chlorine, chlorine dioxide, or ozone in the eradication of bacteria, protozoa, and viruses.[27-33]

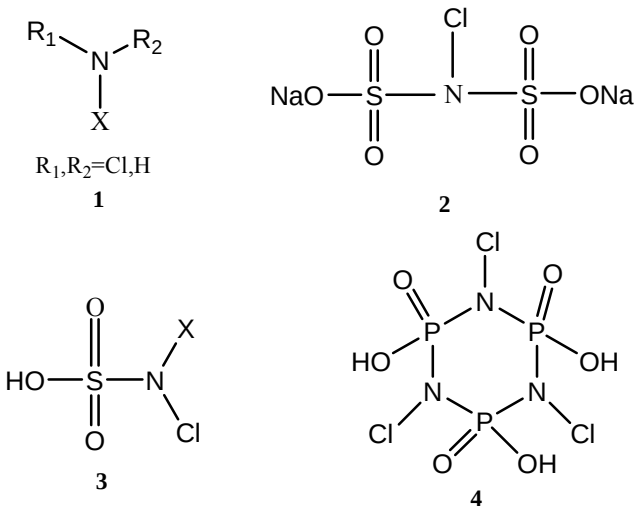
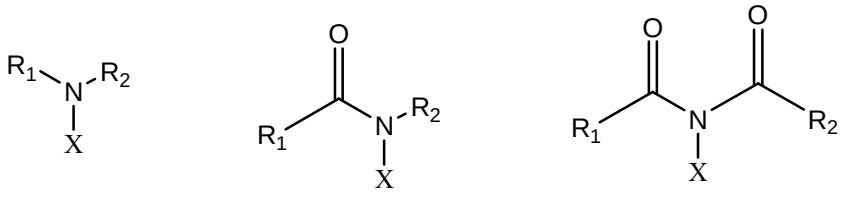


Figure 5 Structures of Inorganic Chloroamines

Organic N-halamines are much more complex than the relatively simple inorganic N-halamines. Organic N-halamines are divided into three groups, namely amines, amides, and imides, depending on the number of adjacent carbonyl groups (**Figure 6)**.



Amine Amide Imide

Figure 6 Structures of N-halamines

Their structures determine their various stabilities and also affect their biocidal efficacies. For amines, the electron donating alkyl groups adjacent to the nitrogen makes the N-X relatively stable, preventing the easy release of "free halogen". In contrast, amides contain an electron withdrawing carbonyl group that replaces an electron donating alkyl group, making the nitrogen more susceptible to the release of halogen than in amines. For imides, the N-X bond is adjacent to the two carbonyl groups. After the release of X^+, the anion is stabilized by these two electron withdrawing groups via a resonance mechanism, so N-X is relatively unstable, with ready release of the halogen compared to either amines or amides. The structure of the N-halamines implies that if an α-hydrogen is adjacent to the nitrogen, dehydrohalogenation could occur, resulting in the formation of a C=N bond. Many acyclic N-halamines decompose rapidly in the presence of light and heat, but once the halogen is lost, they have no biocidal activity. Additionally, due to the different atomic sizes of Cl, Br, and I, the stability of the N-halamine is also affected by the choice of halogen; smaller atoms will have greater bond overlap between the halogen and the nitrogen, resulting in a more stable N-X bond. Thus, the order of the stability of N-X is N-Cl >N-Br >N-I. Consequently, most stable N-halamines are cyclic structures, with no α-hydrogens.

In general, biocides aim to kill bacteria while protecting the humans. An ideal biocide provides complete sterilization over a broad-spectrum, with no harm to other

forms of life, and is also inexpensive, non-corrosive, and long-lived. Unfortunately, at present such ideal biocides do not exist. Many biocides are only able to achieve partial sterilization, and bacteria are also highly resistant to many disinfectants. All biocides are considered potentially harmful to humans or animals and should be treated with appropriate care. Among the currently available biocides, N-halamines stand out due to their many advantages. They leach small amounts of "free chlorine" into water and they are relatively stable. They also have the long shelf-life.[34-37] As a biocide, the most important advantage which attraction interest is that N-halamines can be regenerated even as they lose their chlorine after many applications. (**Figure7**).

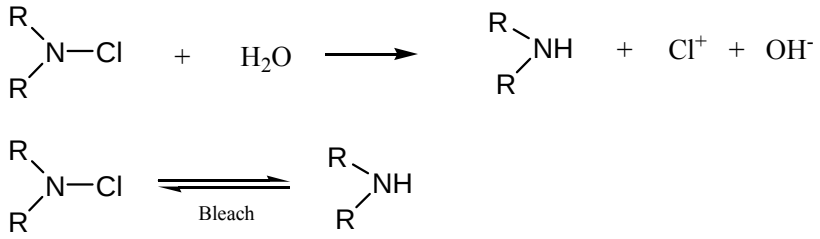
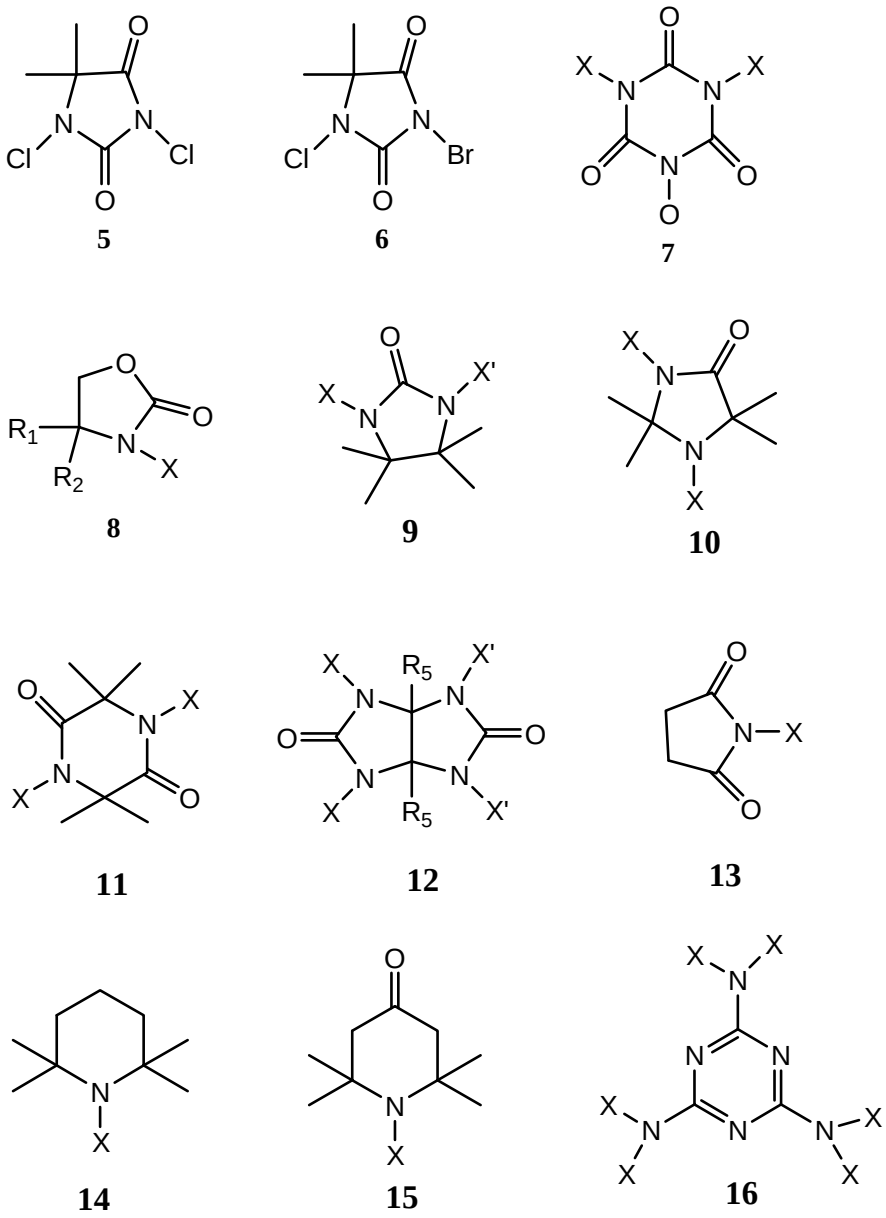


Figure 7 Recharging Reaction of N-halamine

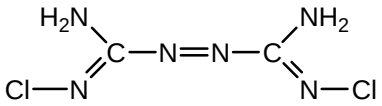
In Worley's group at Auburn University, a great deal of work has focused on the synthesis and testing of novel N-halamine biocidal compounds that can be used to meet industrial and commercial needs. During the 1980s and early 1990s, several new cyclic N-halamines were synthesized, primarily for water disinfection purposes (**Figure 8**). They include 1, 3-dichloro-5,5-dimethylhydantoin (**5**), 1-bromo-3-chloro-5,5-dimethylhydantoin (**6**), chlorinated isocyanurate (**7**), 3-halo-4,4-dimethyl-2-oxazolidinones (**8**), 1,3-dihalotetramethyl-2-imidazolidinones

(**9**), and 1,3-dihalo-2,2,5,5- tetramethylimidazolindin-4-ones[TMIO derivatives] (**10**). Those N-halamine derivatives are known to be stable in aqueous solution as well as in dry storage. Other cyclic N-halamines developed include 1,4-dihalo-2,25,5-tetramethyl-3,6-piperazinedione (**11**), haloglycourils (**12**), N-halosuccinimides (**13**), halogenated piperdines (**14**), halogenated piperidones (**15**), and halogenated melamine (**16**). There are also several acyclic organic N-halamines, including N,N'-dichloroazodicarbonamidine (Chloroazodin)(**17**), and many numerous derivatives of alpha-amino acids (**18-20**)(**Figure 9**).



X,X'= Cl, Br, H, Na

Figure 8 Structures of Cyclic Organic N-halamine



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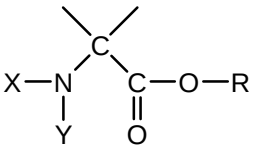
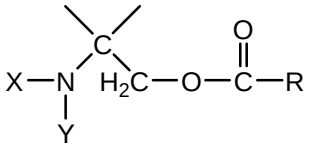
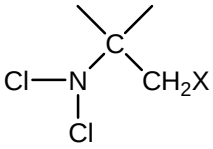




X= H or Cl, Y=Cl, R= alkyl,groups

18 **19**



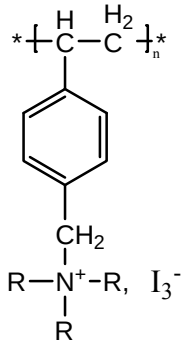
X=COOH, CH_2COOH, $CO_2(CH_2)_2COOH$, $CO_2(CH_2)_3COOH$

20

Figure 9 Structures of Acyclic Organic N-halamines

In an attempt to extend the use of N-halamine chemistry, polymers have also been introduced as biocides for water disinfection. Biocidal polymers can be made by grafting biocidal moieties onto polymer backbones, for example, by modifying commercial polymeric resins, or by polymerization of monomers containing biocidal moieties. The higher the N-halamine content in a polymer, the higher its biocidal activity. However, it is important to note that the addition of too many biocidal moieties will change the polymers' properties. Hence, a delicate balance must be maintained between these two often conflicting sets of demands.

In the early 1980s, Lambert and coworkers[38,39] developed the quaternary ammonium compound (poly quat) as a polymer biocide. The resin-triiodide combination (**Figure 10**) is water insoluble and can release biocidal iodine into water. It has been shown to kill Gram-positive and Gram-negative bacteria, as well as RNA and DNA viruses, in water.



R=alkyl group

Figure 10 Structure of the Triiodide Anionic-Exchange Resins

In the past, the only commercially available contact polymer biocides were polymeric quaternary ammonium compounds (quats), polymeric phosphonium compounds, and halogenated sulfonamides.[40-42] These compounds effectively inactivated a broad range of organisms, but suffered from many drawbacks. As antimicrobial filters, the quats and phosphonium compounds easily leach into water because they partially dissolve in it, so the biocidal activity of the surface cannot be regenerated once exhausted. The halogenated sulfonamides can be water insoluble, but they are pH sensitive and release high concentrations of free halogen into water resulting in the formation of toxic by-products such as trihalomethanes. The costs of quats and phosphonium compounds are also relatively high.

In an effort to produce new classes of polymeric contact biocides, several reproducible biocidal *N*-halamine polymers have been prepared by Worley's group. For example, Sun and coworkers[43-49] synthesized poly(1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin (poly Ⅰ)and poly (1,3,5-trichloro-6-methyl-6-(4-vinylphenyl)-1,3,5- triazine-2,4-dione) (poly-CTD) (**Figure 11**). Poly Ⅰ can be loaded with as much as 23% chlorine by weight. In addition to its ability to kill both *E. coli* and *S.aureus* within 1 second of contact time, it also works on fungi and rotavirus in seconds of contact time. Chen et al. used the cross-linked poly(styrene-co-divinylbenzene) to prepare polymeric beads and chlorinate them in a bleach solution, demonstrating that the chlorine loading of such beads can be controlled by carefully adjusting the pH of the bleach solution.[50] The

tests showed that at pH≤7, Cl^+=20%, at pH 8.0-8.5, Cl^+=17%, at pH 8.6, Cl^+=14%, and at pH≥12.5,Cl^+=10%. This controlled chlorine loading is useful for a wide range of applications.

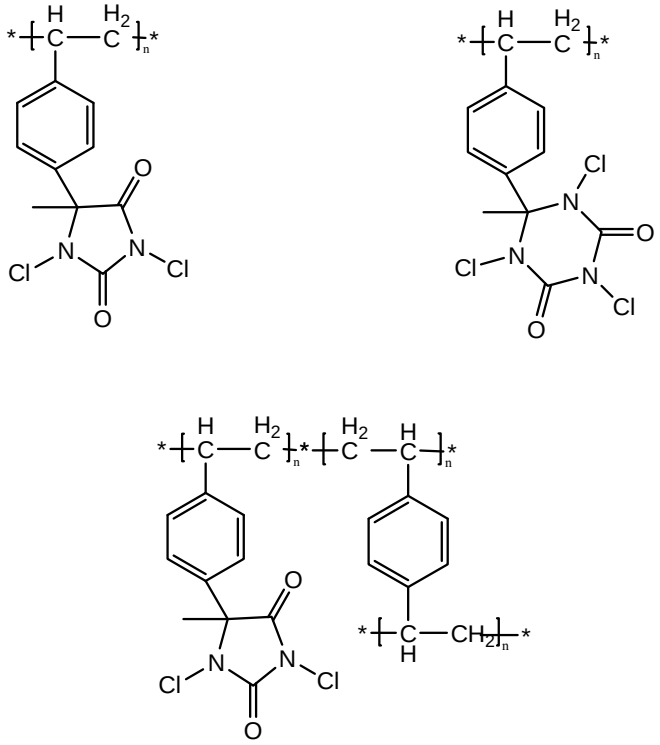


Figure 11 Structures of Poly Ⅰ and Poly-CTD

Chen et al. also prepared poly Ⅱ beads (**Figure 12**) [51]. Here crosslinked chloromethylated polystyrene beads were reacted with potassium salts of hydantoin and imidazolidinone to form new biocidal polymers, which were then chlorinated or brominated, respectively, to form polymers **21a-b, 22** and **23**. **21a** and **23** can load about 6.0% chlorine and can kill *E. coli* and *S. aureus* in 3 seconds or less contact time; the brominated polymer beads **21b** also inactivated both bacteria in less than or

equal to 1 second, while **22** was able to effect a very significant decrease in both species of bacteria. The sterically hindered amine in **22** limited the weight percentage of chlorination to 2.85%. Also, for **22**, which is the imidazolidinone derivative, the oxidative chlorine moiety is bonded to the amine nitrogen, whereas for **21a-b** and **23,** it is bonded to the amide nitrogen. The N-Cl bond is weaker for the amide nitrogen than for the amine nitrogen, so the amine N-Cl releases the oxidative chlorine less easily. However, **22** was noticeably more stable. These biocidal polymer beads can be used in the disinfection of water and moist air flowing though cartridge filters containing them.

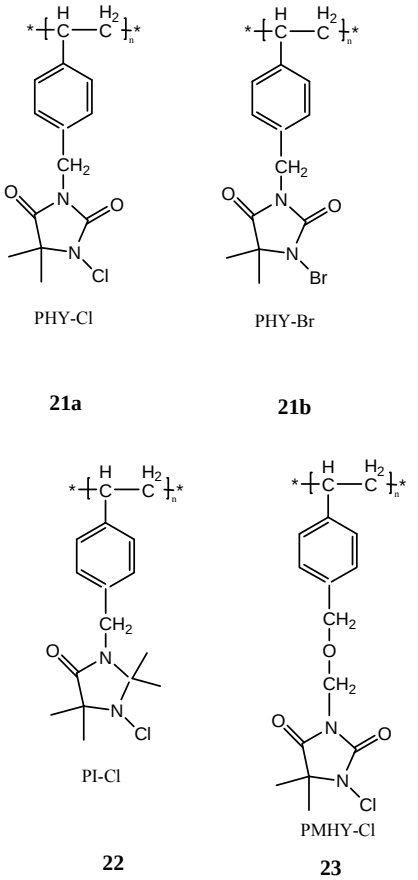


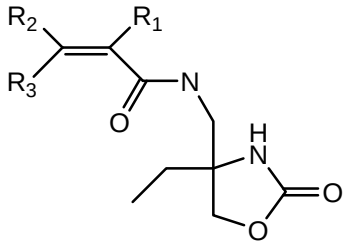
Figure 12 Structures of Other Polymer Beads

In fact, these polystyrenes containing the *N*-halamine moiety are powerful and useful broad-spectrum polymer biocides. No significant amount of chlorine has been observed to leach into water in any case, which indicates that the mode of action is direct contact with the organisms. These polymers are stable for a long time, over a year at room temperature, and inactivate a broad spectrum of organisms in a short contact time (only a few seconds). Their biocidal activities, once exhausted, are easily regenerated by flowing an aqueous solution of free halogen over them. These polymers have shown considerable commercial promise for water and air filtration systems, and are inexpensive to produce. But since polystyrenes are not useful for coatings, their application as contact disinfectants is limited.

Surface disinfectantion is a huge area that is waiting to be developed. Since the 1970s, organotin polymers have been used to protect ships' hulls from marine organisms. In contact with sea water, tributyltin compounds are slowly released from the surface of the organotin coating. Tributyltin compounds have broad spectrum biocidal properties, which can inhibit the growth of marine organisms on a surface; however these types of compounds are widely considered by environmentalists to be toxic to marine life.

Pittman and coworkers[52] synthesized polymers by copolymerizing small molecule fungicides containing hydroxyl groups with vinyl acetate or ethyl acrylate. The resulting polymers can be made into films, which have been shown to inhibit the growth of microorganisms.

In order to develop more useful surface disinfectants, Eknoian synthesized several members of a new class of monomers[53-57](**Figure 13**). These monomers were polymerized with various commercially-available monomers such as acrylonitrile, vinyl acetate, vinyl chloride, and styrene to form the copolymers. The resulting copolymers were emulsified in water to produce coatings which were suitable for use as contact disinfectants on surfaces and gave 6-log reductions against *S. aureus* within 30 min contact time. (**Figure 14**).



23a: R_1=R_2=R_3=H
23b: R_1=R_3=H R_2=CH_3
23c: R_1=R_2=H R_3=CH_3
23d: R_1=H R_2=R_3=CH_3

Figure 13 Structure of Monomers

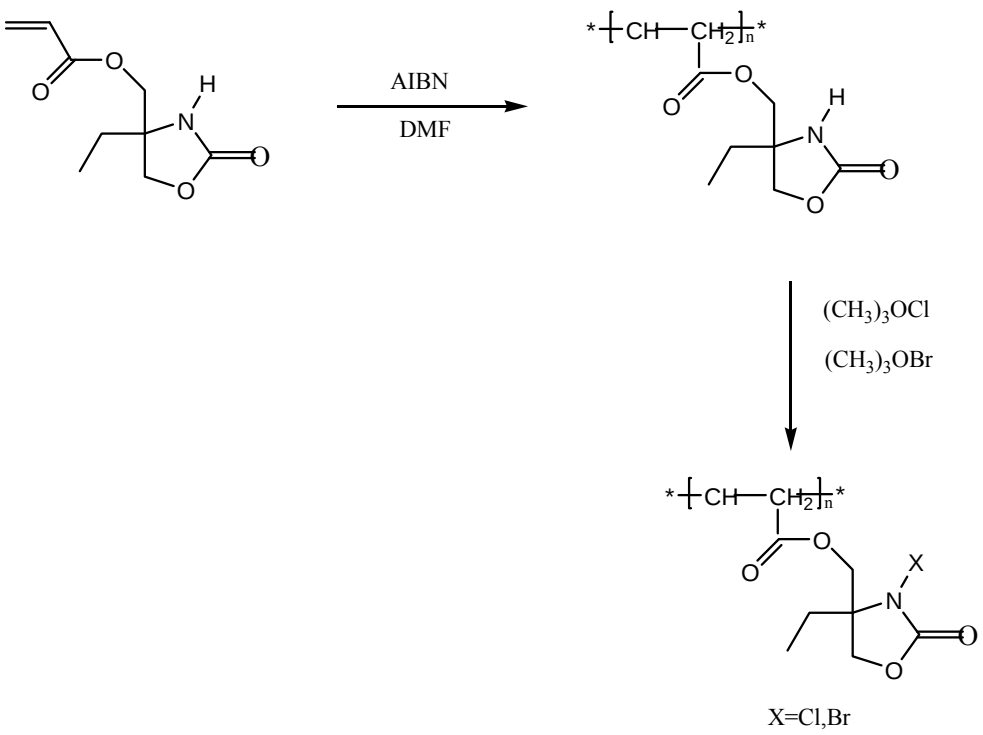


Figure 14 Monomeric *N*-halamine Becomes Biocidal Polymer

Li synthesized a series of acrylic monomers containing the hydantoin ring (**Figure 15**) .[58] These monomers can polymerize via free radical polymerization and can also be copolymerized with acrylonitrile, methacrylate, ethyl acrylate, and butyl acrylate. These copolymers were tested and found to kill *E. coli* and *S. aureus*.

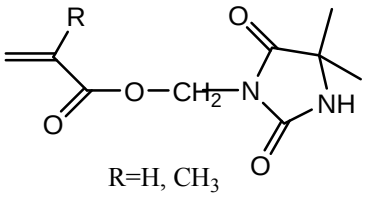


Figure 15 Structure of Acrylic Monomers

Moon *et al*. synthesized a polymer biocide containing phosphonium salts.[59] The new polyester found contained phosphonium biocides, which were incorporated into the polymer as counter ions of sodium sulfonate moieties. A film made of this polyester can be used to guard against *E. coli* and *S. aureus*. In Moon's research, polymers with azole moieties via free radical polymerization were also prepared (**Figure 16**). Some benzimidazole derivatives have been known to possess antibacterial and antifungal activity due to their ability to inhibit the cytochrome P-450 monooxygenase. This enzyme is a key enzyme in fungal ergosterol biosynthesis. Polymers with (benzimidazol-2-yl)thio groups and (5-methyl-1,3,4-thiadiazol-2-yl)thio groups were synthesized, and a shaken flask test showed that these two polymers also possessed antimicrobial activity.

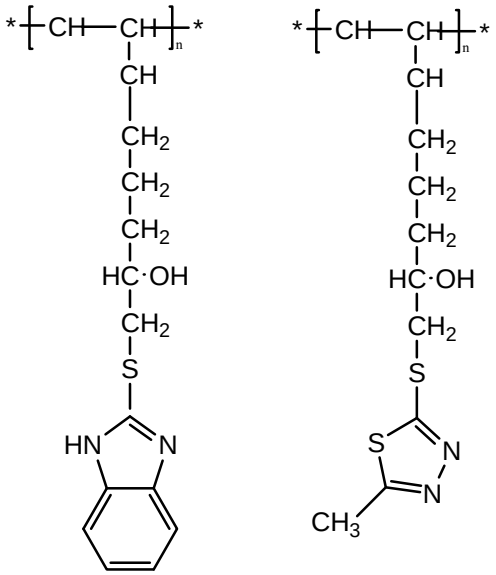


Figure 16 Structure of Polymers with Azole Moieties

Sauvet and coworkers prepared biocidal polyurethane films via the reaction of an aliphatic triisocyanate with hydroxytelechelic polybutadiene (**Figure 17**).[60] The films were shown to be a broad-spectrum biocide whose activity was dependent on the concentration of QAS attached. However, the biocidal activity decreased slowly in water as the QAS slowly broke down.

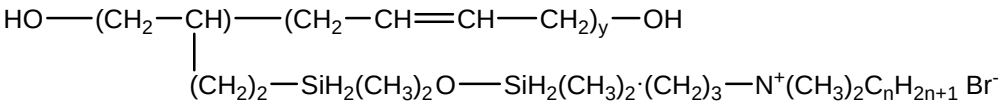


Figure 17 Structure of a Hydroxytelechelic Polybutadiene Polyurethane Film Containing Quaternary Ammonium Salt Group

Sanvet and coworkers also synthesized polysiloxanes with 3-(alkyldimethylamino)- propyl pendent groups by quaternization of n-octyldimethylamine or n-dodecyldimethylamine with linear polysiloxane containing 3-chloropropyl groups and/or 3-bromopropyl groups attached to silicon atoms (**Figure 18**).[61,62] These QAS-containing polysiloxanes showed bactericidal activity against *E coli* by contact, and the biocidal activity remained stable even after one month of immersion in water.

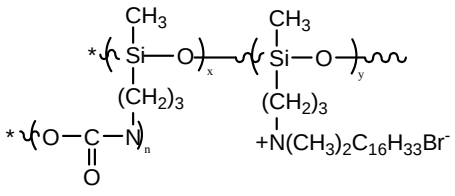


Figure 18 Structure of Polysiloxane-Urethane

Wang prepared biocidal polyurethanes by grafting the silicone of the quaternary ammonium salts to a modified polyurethane backbone (**Figure 19**).[63] These polymers are potentially a good source of films and fabrics. It was showed that both the films and finished fabrics can inactive *S. aureus* in a short contact time.

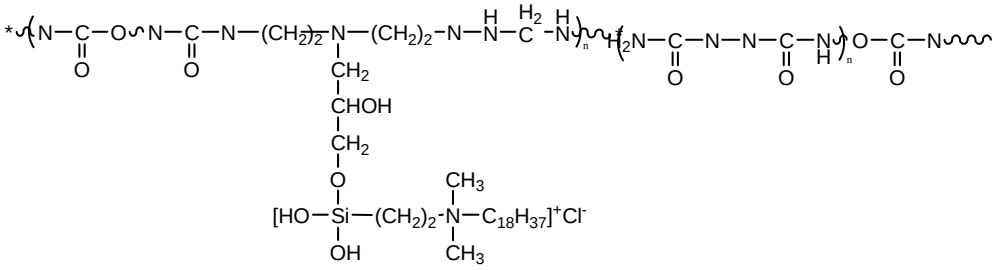
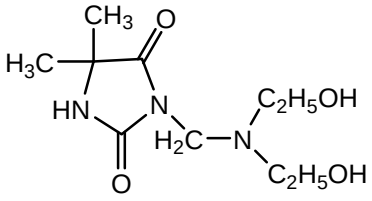


Figure 19 Structure of Quaternary Ammonium Polyurethane

A novel N-halamine-containing diol, 5,5-dimethyl-3-(N,N-di- β -hydroxylethyl-aminomethyl) hydantoin, **24**, has also been prepared (**Figure 20**).[64] This H-halamine monomer can be copolymerized with commercial isocyanate and water-borne acrylic polyol to form a polyurethane. Because the polyurethane can be coated on many surfaces, this copolymer has proved to be a useful biocidal coating. Experiments have shown that a coating of this copolymer on a wall retained its biocidal activity for more than 6 months. This biocidal coating will be useful in many areas, such as in medical facilities, food preparation areas, and the prevention of biofouling in aqueous and humid environments.



24

Figure 20 Structure of Diol

Most synthetic fibers are polymers. When bacteria attack cloth they cause physicochemical degradation such as discoloration, mechanical strength loss, and foul odor generation. They also adversely affect human health. To overcome such problems, polymers containing N-halamines can be used to form antimicrobial fibers. Kim *et al*. found that N-(2-hydroxyl)propyl- 3-trimethylammonium chitosan chloride was a good N-halamine compound. If this compound can be combined with fibers fibers can gain antibacterial properties. So they treated cotton fabrics using N-(2-hydroxyl)propyl- 3-trimethylammonium chitosan chloride and polycarboxylic acid (HTCC) (**Figure 21**). The antimicrobial cotton provided effective protection against *S. aureus*., *K. pneumoniae*, and *E. coli*.[65]

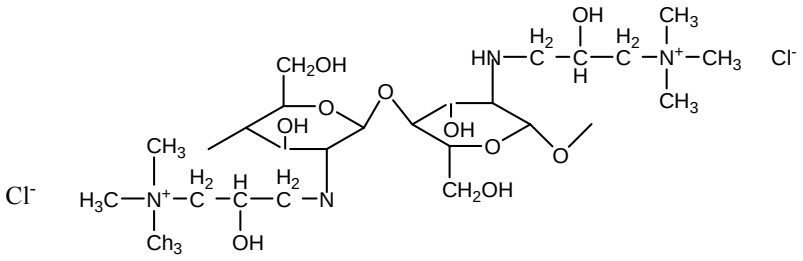


Figure 21 Structure of HTCC

A novel cyclic-amine monomer, 3-allyl-5,5-dimethylhydatoin (ADMH), was prepared by Sun and Sun, who then grafted the ADMH onto textiles (**Figure 22**)[66]. After the fabrics were exposed to chlorine, the hydantoin rings in the grafted samples could be transformed into N-halamines. They exhibited powerful, durable, and reproducible antibacterial activity against both Gram-positive and Gram-negative bacteria.

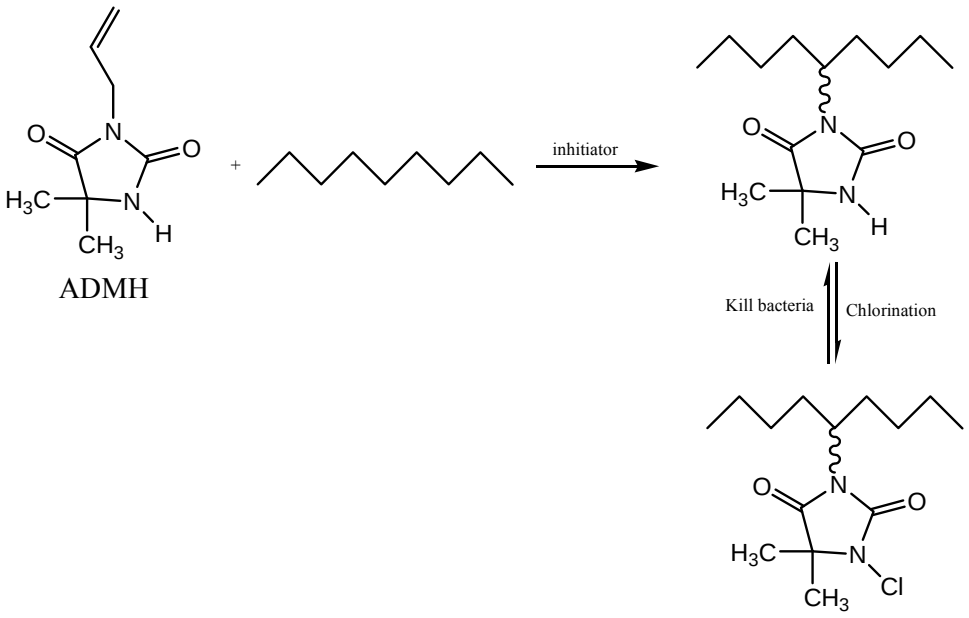


Figure 22 ADMH Grafting Copolymerization and Chlorination on the Synthetic Fabric

Sun and Sun studied the ionic interaction between reactive groups on the polymers and the dye interactions involved in the acidic or basic dyeing of fibers. Anionic carboxylic end groups of polyamides caused ionic interactions to occur with

the cationic quaternary ammonium salts in the chemical finishing of nylon fabrics, producing durable antimicrobial functions (**Figure 23**).[67] However, this approach is sensitive to both pH and temperature. Even with the disadvantages, this method is useful for the textile finishing industry due to simple and practical.

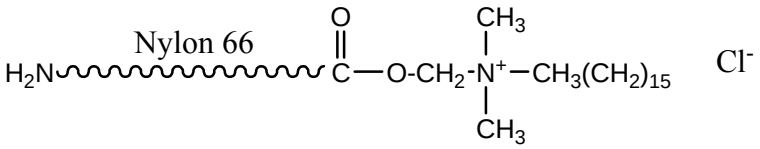


Figure 23 Antimicrobial Nylon 66

Lin *et al*. modified polyester fabrics by introducing 3-hydroymethyl-5,5-dimethylhydantoin (MDMH) into the polyester.[68] Here, polyester reacted with ammonium hydroxide, after which a portion of ammonia in the ester was reacted with MDMH to form an amide. Thus, the hydantoin moiety was chemically bonded to the surface of the polyester (**Figure 24**). After being exposed to bleach, the modified fabrics were shown to be biocides against both *S. aureus* and *E. coli.*

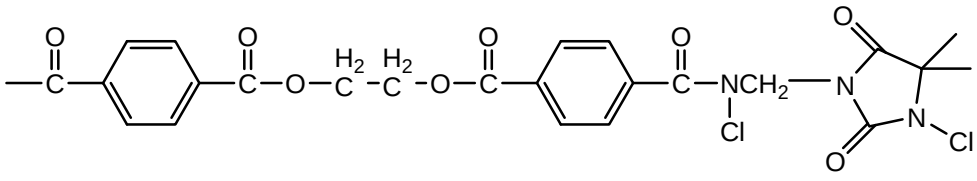


Figure 24 Structure of Antimicrobial Polyester

Chen et al.[69,70] prepared BA-1, 3-(3'-triethoxysilylpropyl)-5,5-dimethylhydantoin and a series of BA-1 derivatives (**Figure 25, 26**). He also synthesized 1,3,8-trichloro-7,7,9,9- tetramethyl-1,3,8-triazaspiro[4,5]decane-2,4-dione (TTDD) and TTDD siloxane (**Figure 27**). After these new compounds were loaded with chlorine, they exhibited high activities in killing both *E.coli* and *S.aureus* and are quite stable in dry storage at room temperature for several months. They can be coated onto various materials, such as cotton, glass, paper, and sand. The potential applications of this antimicrobial cotton include its use for bed sheets and cloth in hospital and laboratory, as well as in odor control. The treated cotton gauze may also be used to make antimicrobial masks to stop infective pathogen trans/cross-contamination, while treated paper may be used as air filters or envelopes. Sand coated with BA-1 can be used as a disinfectant in a filter for wastewater or municipal water treatment and has been shown to completely inactivate both *E. coli* and *S. aureus* in as little as 1 min contact time.

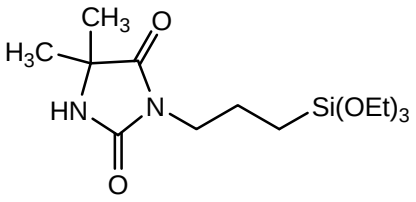


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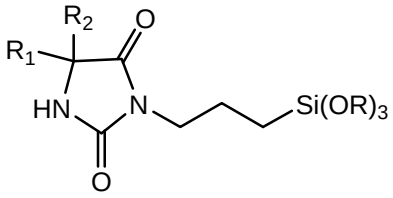
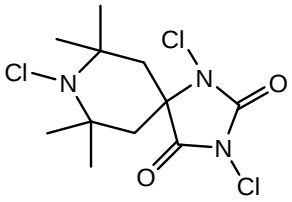
Figure 25 Structure of BA-1



26: R1,R2=CH_3, R=CH_3
27: R1=CH_3,R2=C_3H_7, R=C_2H_5
28: R1=CH_3,R2=$C_5H_{11,}$ R=C_2H_5
29: R1=CH_3,R2=C_6H_{13}, R=C_2H_5
30: R1=CH_3,R2=C_7H_{15}, R=C_2H_5
31a: R1=CH_3,R2=C_6H_5, R=C_2H_5
32a: R1=CH_3,R2=p-$CH_3C_6H_4$, R=C_2H_5
33a: R1=CH_3,R2=p-tbutylC_6H_4, R=C_2H_5
34: R1,R2=C_6H_5, R=CH_3

Figure 26 Structure of BA-1 Derivatives



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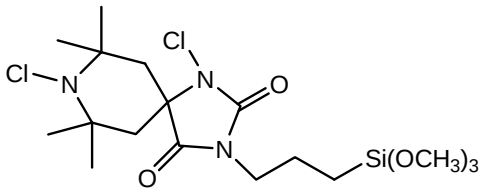
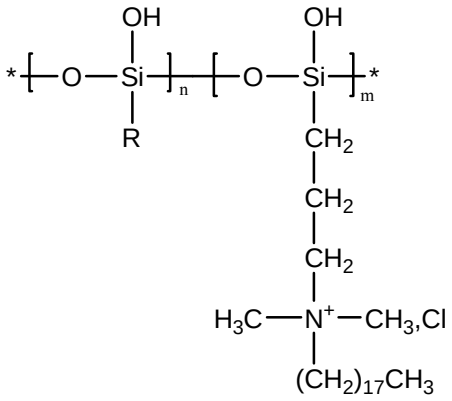


36

Figure 27 Structure of Chlorinated TTDD and TTDD Siloxane

Furthermore, the same group prepared several quanternary amino N-halamine polysiloxanes by copolymerization of TTDD siloxanes, or BA-1' with DC-7500 (**Figure 28**). The resulting copolymers inactivated *S. aureus* and *E. coli* completely in 15 min of contact time.

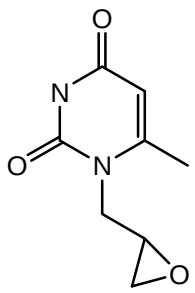


37: R= 3-propyl-5,5-dimethlhydantoin
38: R= 3-propylTTDD

Figure 28 Structure of Dual Functional Polysiloxanes

Reseach projects:

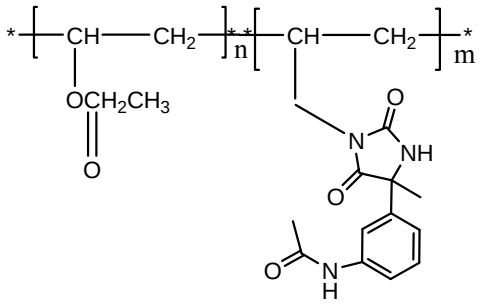
Worley's group has continued to develop new N-halamines. In this thesis work a new N-halamine 1-(2, 3-epoxypropyl)-6-methyluracil was synthesized (**Figure 29**) and then coated onto cotton. Its laundering durability was tested using a washing trial. The shelf-life times were measured, and its antimicrobial activity was also tested. The effect of curing temperature was investigated.



39

Figure 29 Structure of 1-(2,3-epoxypropyl)-6-methyluracil

Another project was to synthesize a series of new N-halamine copolymers VAC-co-3-(3'-allyl-5'-methylhydatoinyl)acetanilide (**40**), VAC-co-5-methyl-5-(3'-propenyl)hydantoin (**41**), VAC-co-3-allyl-6,6-dimethyl-1,3,5-triazinane-2,4-dione (**42**), VAC-co-5,5-dimethylhydantoin-1-ylmethylene acrylate (**43**), VAC-co-1-allyl-6-methylpyrimidine (**44**), (**Figure 30**). Those copolymers can be made into films that have biocidal properties. The shelf-life times were measured and their antimicrobial activities were also tested.



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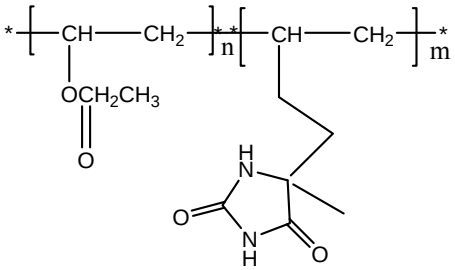

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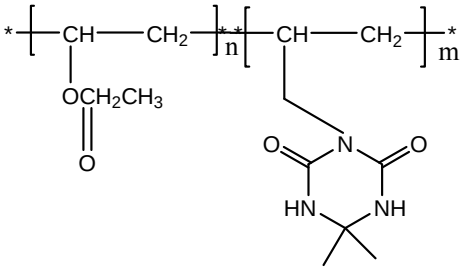

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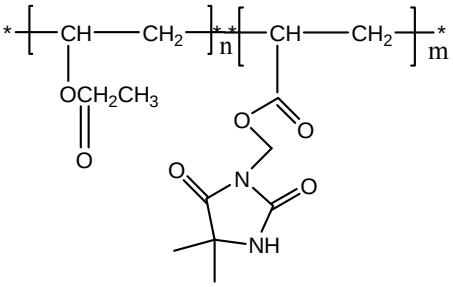

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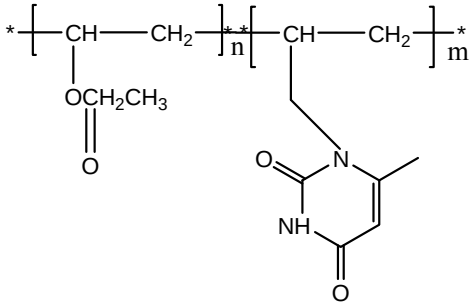


44

Figure 30 Structures of VAC-co-N-halamine copolymers

EXPERIMENTAL

Materials and Instrumentation

All chemicals and solvents were purchased either from Fisher Scientific or Aldrich and used directly. The bleached 100% cotton fabric was from Testfabrics Inc., West Pittston, PA.

Infrared (IR) spectra were recorded on a Nicolet 5PC FT-IR Spectrometer by either using KBr pellets, or making neat thin films on a window. ^{1}H and ^{13}C NMR spectra in solution were obtained on a BRUKER AVANCE 400 MHz or 250 MHz spectrophotometer operating at 20℃. The NMR solvents dimethyl sulfoxide (DMSO-d_6) and acetone-d_6 were obtained from Aldrich. Chemical shifts were reported in parts per million (ppm) from tetramethylsilane (TMS) on the δ scale. Melting points were measured on a Mel-Temp^{TM} melting point apparatus which were not corrected. Thin-layer-chromatography (TLC) was performed by using silica-coated plates which purchased from Whatman.

A. **Synthesis of 1-(2,3-epoxypropyl)-6-methyluracil (39)**

2.00g (0.0158 mole) 2,4-dihydroxy-6-methylpyrimidine, 1.46g (0.0158 mole) epichlorohydrin and 1g (0.0158 mole) potassium hydroxide were added to 20 ml water in a 100 ml flask. The resulting mixture was stirred at room temperature overnight. Ethyl acetate was then used to extract the reaction solution, after which the ethyl acetate was removed under reduced pressure, and the residue subjected to T.L.C. Pure **39** was obtained. Mp 177-178℃; ^{1}H NMR (DMSO) δ 2.023 (s, 3H), δ 3.358-3.983 (m, 5H), δ 5.460 (s, 1H), δ 11.114 (s, 1H); ^{13}C NMR (DMSO) δ 18.51, 68.13, 98.81, 151.78, 152.16, 163.53; IR (KBr) 3493, 1728, 1639, 1618 cm^{-1}.

B. Synthesis of copolymer VAC-co-N-halamine copolymers

VAC-co-3-(3'-allyl-5'-methylhydatoinyl)acetanilide copolymer (40)

2.00 g vinyl acetate and 0.2 g 3-(3'-allyl-5'-methylhydatoinyl)acetanilide were added into 5 mL Methanol (mole ratio: 40:1). N_2 gas was used to remove O_2 in the solution. After adding 0.008 g AIBN, the mixture was stirred at 78℃ for 3 h. The reaction solution was then poured into 100mL H_2O. The resulting precipitate was separated out and dried by evaporation. The copolymer was obtained. IR (KBr) 2977, 1728 cm^{-1}.

VAC-co-5-methyl-5-(3'-propenyl)-hydantoin copolymer (41)

3.30g vinyl acetate and 0.2 g 5-methyl-5-(3'-propenyl)-hydantoin were added into 5 mL Methanol (mole ratio: 40:1). N_2 gas was used to remove O_2 in the solution. After adding 0.008 g AIBN, the mixture was stirred at 85℃ for 2 h. The reaction solution was then poured into 100 mL H_2O. The resulting precipitate was separated out and dried by evaporation. The copolymer was obtained. IR (KBr) 2924, 1727 cm^{-1}.

VAC-co-3-allyl-6,6-dimethyl-1,3,5-triazinane-2,4-dione copolymer (42)

1.54 g vinyl acetate and 0.1 g 3-allyl-6,6-dimethyl-1,3,5-triazinane-2,4-dione were added into 5 mL DMF(mole ratio: 40:1). N_2 gas was used to remove O_2 in the solution. After adding 0.008 g AIBN, the mixture was stirred at 70℃ for 3 h. The reaction solution was then poured into 100 mL H_2O. The resulting precipitate was separated out and dried by evaporation. The copolymer was obtained. IR (KBr) 2989, 1729 cm^{-1}.

VAC-co-5,5-dimethylhydantoin-1-ylmethylene acrylate copolymer (43)

2.60 g vinyl acetate and 0.2 g 5,5-dimethylhydantoin-1-ylmethylene acrylate were

added into 5 mL Methanol (mole ratio: 40:1). N_2 gas was used to remove O_2 in the solution. After adding 0.008 g AIBN, the mixture was stirred at 85℃ for 4 h. The reaction solution was then poured into 100 mL H_2O. The resulting precipitate was separated out and dried by evaporation. The copolymer was obtained. IR (KBr) 2977, 1728 cm^{-1}.

Synthesis of 1-allyl-6-methyluracil (44a)

2.00g (0.0158mole) 2,4-dihydroxy-6-methylpyrimidine, 1.90 g (0.0158 mole) allyl bromide and 2.18 g (0.0158mole) K_2CO_3 were added to 40 ml DMF in a 100 ml flask. The resulting mixture was stirred at 75℃ overnight. The reaction mixture was then poured into 100 mL of water and 200 mL of chloroform were used to extract the mixture four times, after which the chloroform was evaporated. A light yellow solid was obtained. Ethanol was used to recrystallize the solid and finally, a white needle solid, **44a**, was obtained. Mp: 170℃-172℃; ^{1}H NMR δ2.183 (s, 1H), δ 4.386-4.407 (m, 2H), δ 4.994-5.040 (d, 1H), δ5.134-5.163, (d, 1H), δ5.510 (s, 1H), 5.724-6.073 (m,1H), 11.209 (s, 1H); ^{13}C NMR δ 19.32, 45.49, 101.52, 116.17, 133.74, 151.85, 154.73, 162.95; IR (KBr) 3388, 1708,1660, 1604 cm^{-1}.

VAC-co-1-allyl-6-methyluracil copolymer (44) 19.00 g vinyl acetate，1.00 g of 1-allyl-6-methyluracil and 0.1g 2,2'-azobisisobutyronitrile (AIBN) were added into 20 mL DMF. N_2 gas was used to remove O_2 in the solution. The mixture was stirred at 78℃ for 4.5 h. The reaction solution was then poured into 100 mL H_2O. The resulting precipitate was separated out and dried by evaporation to obtain the copolymer. IR (KBr) 3132, 1710, 1660 cm^{-1}.

C. Biocidal Test

Preparation of Finishing Bath

Bath 1 for Cotton Swatches: An appropriate amount of the N-halamine epoxide compounds was dissolved in water or 0.1 N of sodium hydroxide solution. The bath was stirred for 30 min.

Bath 2 for Transparent Slide Swatches: An appropriate amount of the copolymers was dissolved in acetone solution. The bath was stirred for 30 min.

Preparation of Biocidal Cotton

Style 400 Bleached 100% Cotton print Cloth (Testfabrics, Inc.) was cut into small swatches that were then soaked in the bath 1 solution for 15 min and air dried at room temperature for 10 min, followed by curing at 175℃ for 30 min. The cured swatches were soaked in a 0.5% liquid detergent for 15 min, rinsed with distiled water, and then dried. Next the swatches were soaked in a 10% household bleach solution for 1 h to load the chlorine onto the cotton surface, after which they were rinsed 5 times with tap water and finally dried at 45-50℃ for 1 h. The swatches were stored in a refrigerator at 0℃until use for analytical and microbiological characterization.

Preparation of Biocidal Film

The poly N-halamine was dissolved in anhydrous acetone (bath 2). Transparency slides were cut into 25 square centimeter (cm^2) pieces, and 0.5 mL of bath 2 solution was spread on the surface of the square slide. The treated slides were dried at room

temperature overnight. During this period, the film formed on the surface of the slides. The films were loaded with chlorine by soaking in a 10% household bleach solution for 1 h after which the films were rinsed 5 times with tap water. Finally, the films were dried at 45-50℃ for 1 h and stored in a refrigerator at 0℃ until use for analytical and microbiological characterization.

Titration Test

An iodometric/thiosulfate titration test was used to determine the total Cl^+ content on the cotton/film swatches treated with the N-halamine compounds.

For cotton swatches: In a 250 mL flask, about 0.25 g of potassium iodide was dissolved in 50 mL of distilled water. About 0.15 g of N-halamine treated sample was cut into tiny pieces and added to the flask. To each of these pieces were added 1 mL of 4N acetic acid and 1 mL 1% starch solution. A buret was filled with standardized 0.00375 M sodium thiosulfate solution and dripped into the flask until the sample solution turned from a blue color to a colorless endpoint, and the solution remained colorless for 1 min. This procedure was usually repeated 3 times, and the average titration volume was used for calculations.

For transparecy films: the films were titrated by a refined method. In a 250 mL flask, about 0.25 g of potassium iodide was dissolved in 10 mL of 0.1 N acetic acid and 90 mL absolute enthanol. About 1 inch square N-halamine treated transparency film was cut into tiny pieces and added to the flask. A buret was filled with standardized

0.00375 M sodium thiosulfate solution and dripped into the flask until the sample solution turned from a yellow color to a colorless endpoint and the solution remained colorless for 1 min. This procedure was usually repeated 3 times, and the average titration volume was used for calculations.

Preparation of Bacteria Solution:

Staphyloccus aureus (S. aureus)-ATCC 6538 and *Escherichia coli (E. coli)* O157: H7-ATCC 43895 were grown on auger plates in an incubator 24 h before testing. The bacteria solution was prepared by gently rubbing a sterile cotton swab across an agar plate containing *Staphyloccus aureus (S. aureus)*-ATCC 6538 and/or *Escherichia coli (E. coli)* O157: H7-ATCC 43895. The bacteria were introduced into PBS solution by the swab brushing the wall of the tube. A spectrophotometer was used to measure the CFU (colony forming units) of bacteria in the solution. The bacterial solution was diluted to a 10^6-10^7 CFU/mL concentration of the bacteria. Tryticase soy agar plates (TSA) were prepared for use by dividing them into 6 equal sections on the bottom of the plates with a black marker. The sections were labeled from 0 to 5 (referring to the 100-106 dilution factors used), with section 5 being the untreated section, and the plates themselves were labeled with their sample ID.

Bacterial Efficacies of Surfaces (Swatch Test)

Several 50 mL centrifuge tubes, each containing 5 mL of sterile solution of 0.02 N sodium thiosulfate were prepared. Treated cotton surfaces were cut into 1 inch squares, and 25 μL of bacterial suspension were sandwiched between two squares. After various contact times, the surfaces were quenched by vortexing for 2 min in 50 mL centrifuge tubes. Serial dilutions of the solutions containing the surfaces were made. For example, a dilution of 10^1 was made by pipetting 100 μL of quenched bacterial-containing solution (referred to as 10^0) into a sterile culture tube containing 900 μL of PBS and vortexing briefly; dilution of 10^2 was made by pipetting 100 μL of the above solution (10^1) into a sterile culture tube containing 900 μL of PBS and vortexing briefly, and so on up to a dilution of 10^5. Three 25 μL aliquots of each dilution were plated on the appropriate section of the dried surface of TSA agar in a previously prepared Petri dish at the designated contact times. After incubation for 24 h at 37℃, colony counts were conducted to quantify the viable bacteria. The bactericidal properties of each sample were determined in terms of a log reduction. The colonies were counted at the lowest dilution factor at which counting was possible.

D. Calculation Equations

The percentage of active chlorine was calculated using the following equations:

For cotton swatches treated with biocide on both sides

$$[Cl^{+}\%] = \frac{\text{Titrant Volume (mL)} \times \text{Normality of Titrant} \times 35.45\ \text{(g/mole)}}{2000 \times \text{Sample Weight (g)}} \times 100\%$$

For material with one side of surface treated with biocide (e.g. film on transparent slides)

$$[Cl^{+}\ \text{atoms/cm}^2] = \frac{\text{Titrant Volume (mL)} \times \text{Normality of Titrant} \times 6.02 \times 10^{23}}{2000 \times \text{Sample Area (cm}^2\text{)}}$$

E. Application of Biocidal Materials

Preparation of antimicrobial cotton with 1-(2,3-epoxypropyl)-6-methyluracil

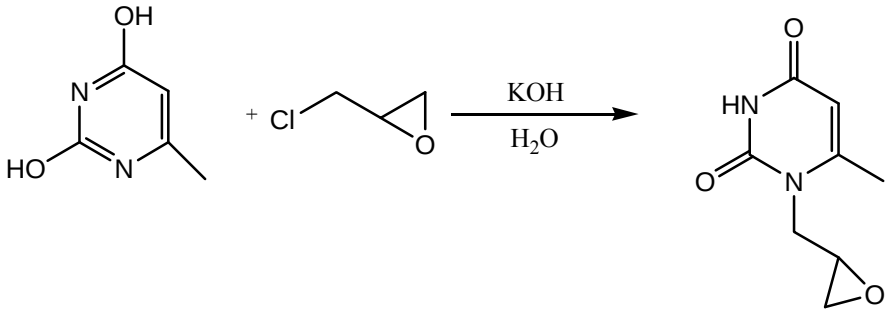
1g **39** was dissolved in 19 g 0.1 N of sodium hydroxide solution to act as the bath solution. Swatches of Style 400 Bleached 100% Cotton print Cloth (Testfabrics, Inc.) were soaked in the bath solution prepared above for 15 min. After drying in air at room ambient temperature for 10 min, the swatches were cured at 175℃ for 30 min. They were then soaked in a 0.5% aqueous liquid detergent for 15 min, rinsed with tap water, and dried to constant weight. The swatches were loaded with chlorine by soaking in a 10% Household solution at 1g swatches per 100 mL for 1 h. The swatches were rinsed 5 times with tap water and finally dried at 45-50℃ for 1 h. The swatches were stored in a refrigerator at 0℃ until use for analytical and microbiological characterization.

Preparation of antimicrobial film with copolymer

(VAC-co-N-halamine copolymer): 1 g copolymer VAC-co-N-halamine was dissolved in 9 g acetone. The transparency slides were cut into 25 cm^2 squares. Then the 0.5 mL acetone solution was spread on the whole square slide. The treated slide samples were dried at room temperature overnight then loaded with chlorine by soaking in a 10% Household solution for 1 h. The films were rinsed 5 times with diionied water and finally dried at 45-50℃ for 1 h. The films were stored in a refrigerator at 0℃ until need for analytical and microbiological characterization.

RESULTS AND DISCUSSION

Synthesis and Characterization of 1-(2,3-epoxypropyl)-6-methyluracil



Scheme 1 Synthesis of 1-(2,3-epoxypropyl)-6-methyluracil

We want to develop the product applicable to meet industry and commercial needs. The important thing is low cost. This reaction is easy to perform at very low cost. 2,4-dihydroxy-6-methylpyrimidine is not soluble in water, but when KOH is added, it becomes a salt which can then be dissolved in water. After epichlorohydrin was added into the salt solution, the substitution reaction took place at position N-1. However, examining the structure of the pyrimidine ring reveals two positions, N-1 and N-3, at which the substitution reaction can occur. This issue has already been widely studied, although most studies have focused on the alkylaltion of uracils,

including pyrimidine.

Research has shown that the products of the alkylation of uracils normally consists of a mixture of both N-1 and N-3 monoalkylated products and N-1, N-3 dialkylated products, with a variable ratio of the three products. This balance can be affected by the ratio of the alkylation agent to the uracil ring, with the N-1 position being more active than N-3 because the proton residing on N-1 is more acidic than that at N-3. Consequently, the dissociation sequence of N-H is in the order of N-1, followed by N-3. It was pointed out that it is impossible to obtain 3-alkyluracils by direct alkylation of uracils. To solve this problem, a blocking group can be added to the N-1 position, but the N-1 position product can also be obtained by controlling the ratio of the alkylation agent. In the reaction used for this research, the ratio of pyrimidine and the alkylation agent, epichlorohydrin, is 1 to 1, thus favoring the N-1 position product.[71]

The structure of **39** was confirmed by ^{1}H and ^{13}C NMR, as well as FT-IR (**Figure 31-33**). **Figure 31** shows the proton NMR spectrum of **39**. The peak assignments are clearly illustrated on the spectrum. The single peak in the region of δ 2.02 ppm is assigned to the three hydrogens of the methyl group; the multiple peaks in the region of δ 3.36-3.98 ppm are attributed to the epoxypropyl group; the single peak at δ 5.46 ppm is attributed to the hydrogen on the pyrimidine ring; and the single peak at δ 11.11 ppm is assigned to an imide proton.

Figure **32** shows the C^{13} NMR spectrum of **39**. The peak at δ18.51 ppm is assigned the carbon of the methyl group; the peaks at δ 43.50, 48.59, 68.13 are

attributed to the carbons of the epoxypropyl group; the peak at δ 98.81 ppm is attributed to the carbon of the double bond on the uracil ring; the peaks at δ 151.78 and 163.53 ppm are assigned to the carbonyl carbons; and the peak at δ 152.16 ppm is attributed to the carbon attached to the methyl group.

Figure 33 shows the FT-IR spectrum of **39**. The absorption band at 3493 cm^{-1} represents the N-H bond vibrational mode. The C-H stretching modes occur at 2956 and 2945 cm^{-1}. The absorption band at 1728 cm^{-1} represents the carbonyl group.

The FTIR spectra of cotton, cotton + **39**, and cotton + **39** + Cl are shown in **Figure 34**. Cotton + **39** refers to the cotton swatch loaded with **39**, and cotton + **39** + Cl refers to the cotton swatch with **39** loaded with chlorine. The trace shows a small peak appearing at 1746 cm^{-1} for the cotton loaded by **39**, which is characteristic of the carbonyl group.

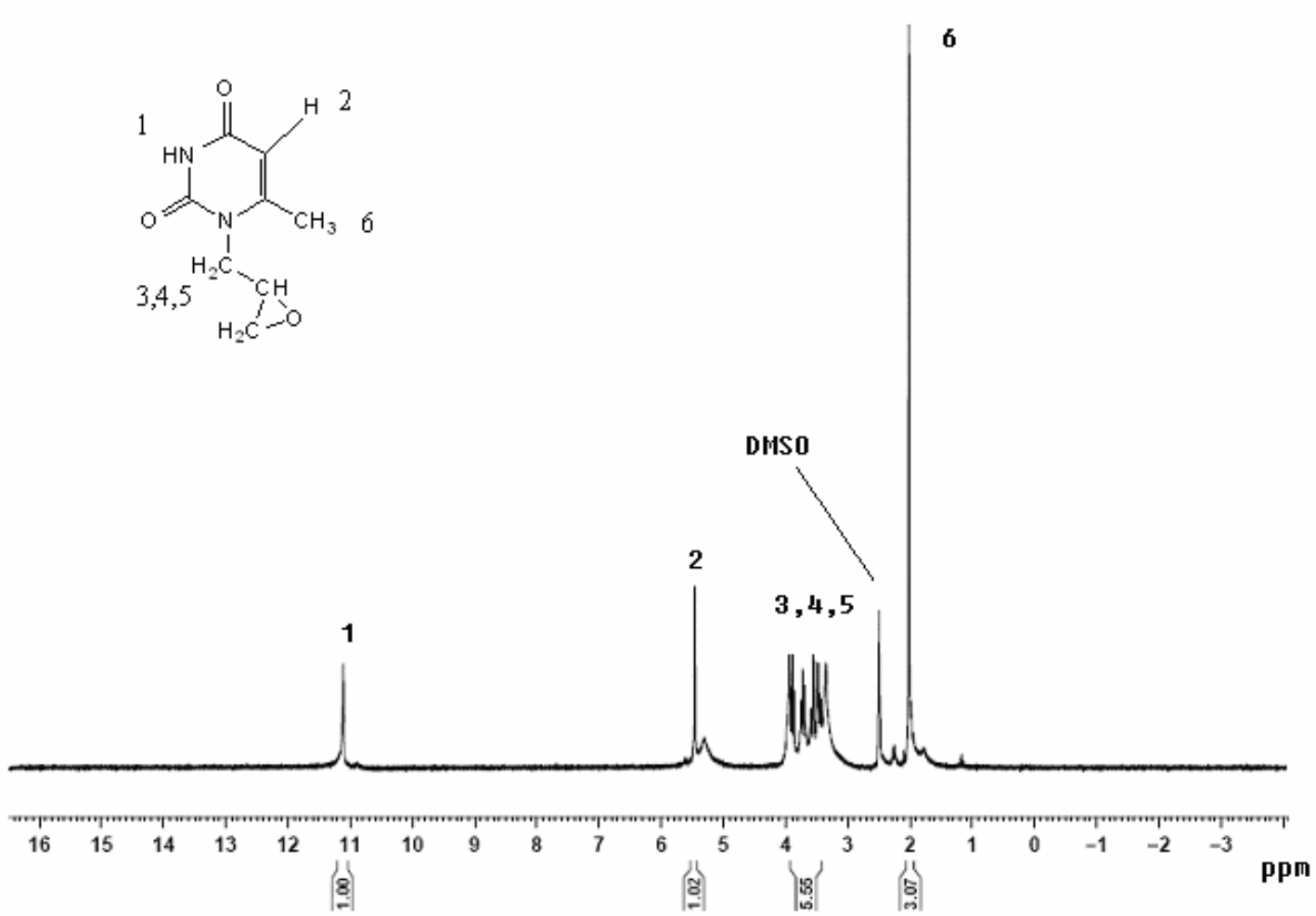


Figure 31 ^{1}H NMR of **39**

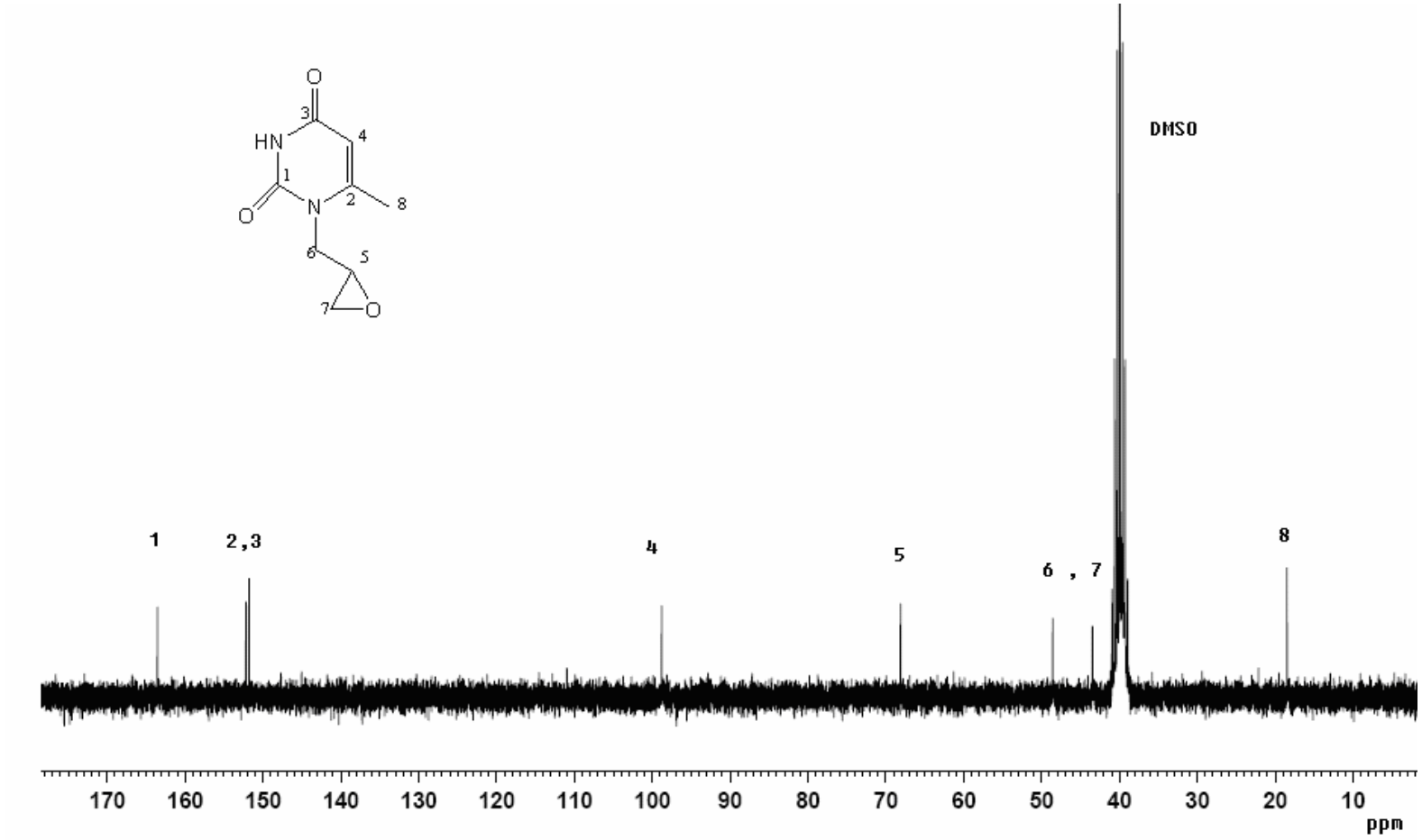


Figure 32 ^{13}C NMR of **39**

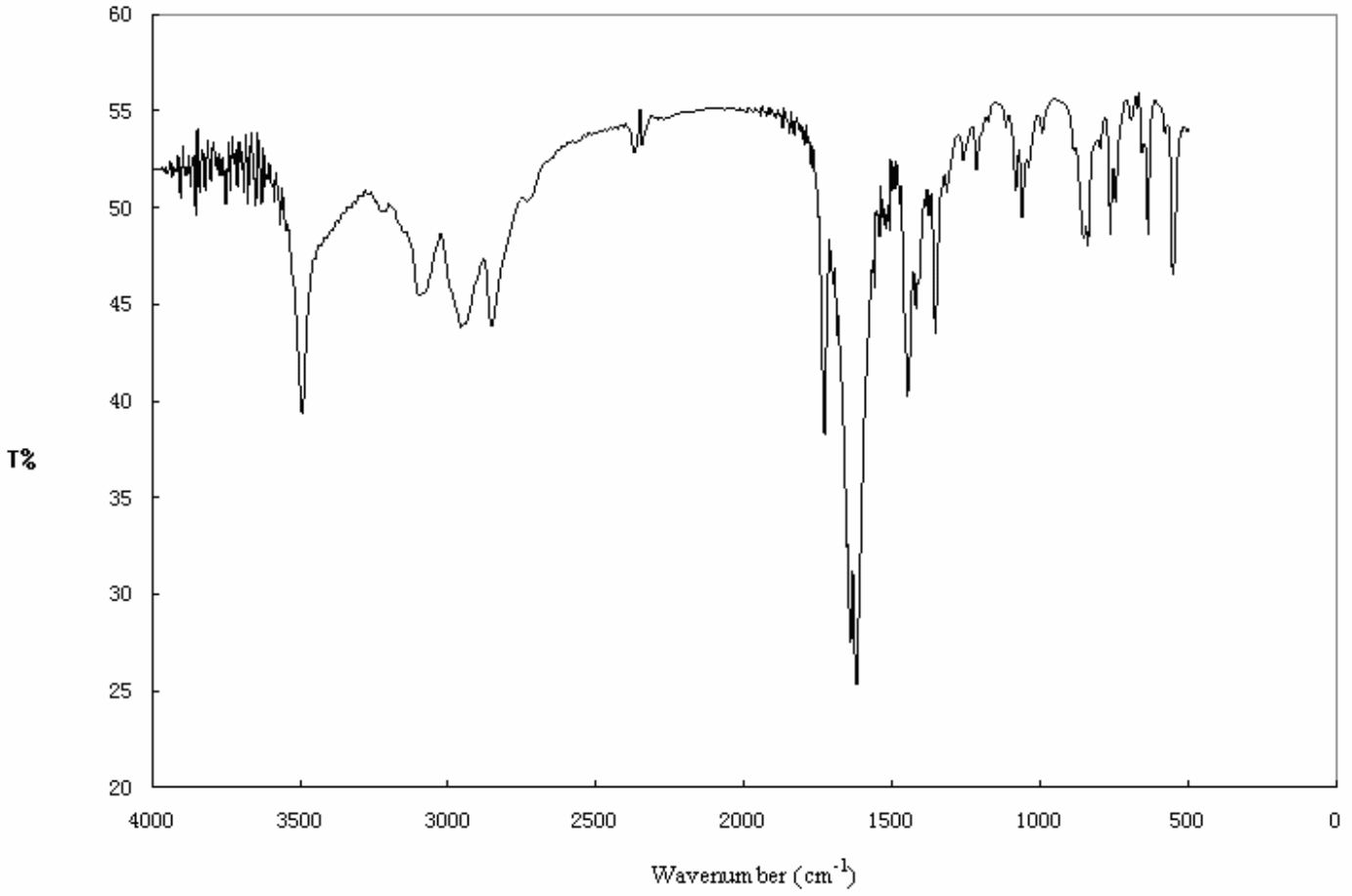


Figure 33 FTIR of **39**

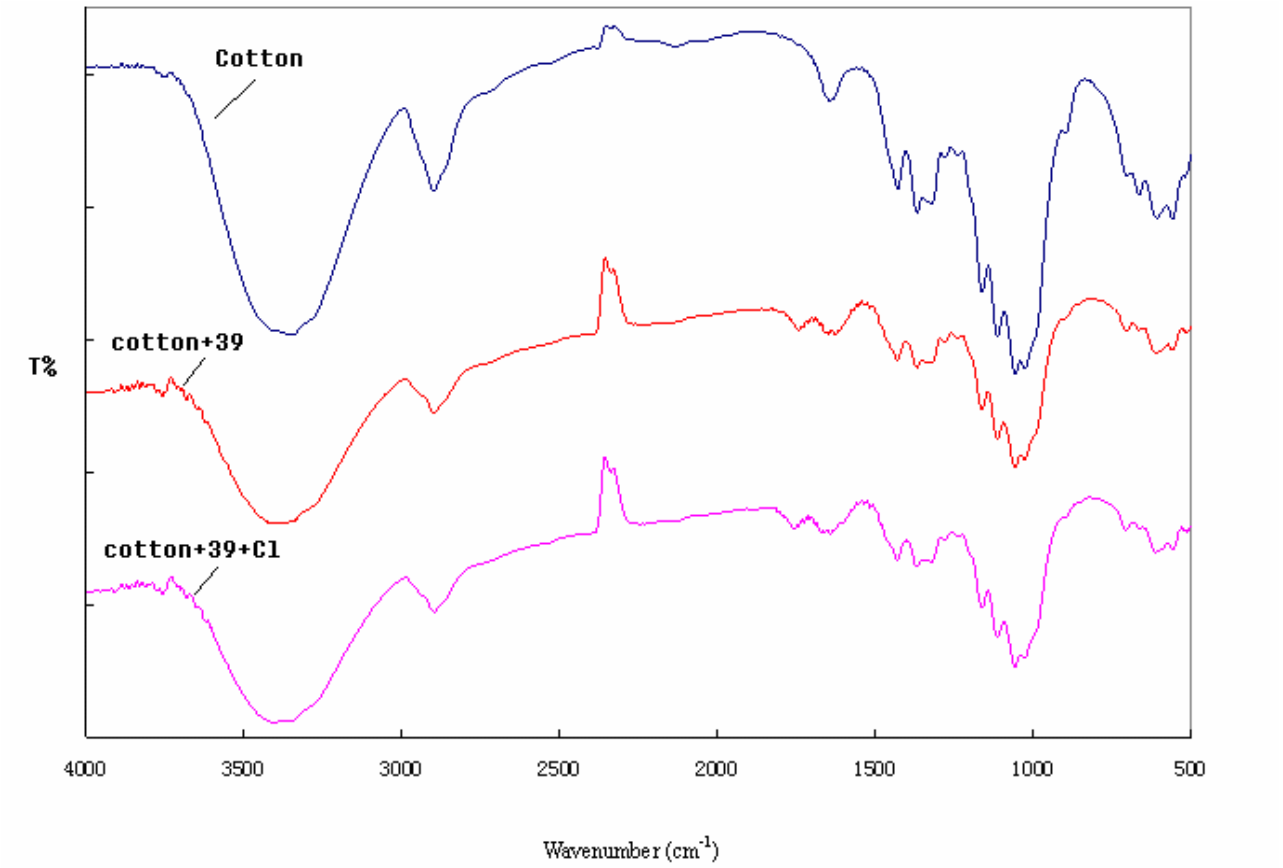


Figure 34 FTIR of 39 Cotton

Application of 1-(2,3-epoxypropyl)-6methyluracil

The cotton swatches were treated with **39**. The cotton swatches were soaked in a finishing bath prepared by dissolving 1 g **39** in 19 g 0.1 N sodium hydroxide solution. The cotton swatches were then cured at 175℃ for 30 min. After curing, the treated cotton swatches were activated by soaking in a 10% aqueous solution of Household bleach buffered to pH 7. The initial chlorine contents were determined by iodometric/thiosufate titration.

The Effects of Temperature, Concentration and pH Value. (**Tables 1.1, 1.2 1.3**)

1. The effect of curing temperature

Here, 10% Household bleach solution was used and the pH value was 7.

Table 1.1 Effect of Curing Time at 95℃, then 145℃

entry	Curing Temperature(℃)	Cl^+%
1	95℃ 20 min, 145℃ 20 min	0.0475
2	95℃ 20 min, 145℃ 30 min	0.0693
3	95℃ 20 min, 145℃ 40 min	0.0821
4	95℃ 20 min, 145℃ 50 min	0.0928
5	95℃ 20 min, 145℃ 60 min	0.1234

Table 1.2 Effect of Curing Time at 145℃

Entry	Curing Temperature(℃)	Cl^+%
1	145℃ 20 min	0.0390
2	145℃ 30 min	0.0721
3	145℃ 40 min	0.0920
4	145℃ 60 min	0.1105
5	145℃ 90 min	0.1484

Table 1.3 Effect of Curing Time at 175℃

Entry	Curing Temperature(℃)	Cl^+%
1	175℃ 10 min	0.0632
2	175℃ 20 min	0.1534
3	175℃ 30 min	0.2073
4	175℃ 40 min	0.2355
5	175℃ 50 min	0.2419

The higher temperature and the long curing time lead to higher chlorine loading. At the same time, they also affect the quality of the cotton by making the cotton brittle and losing toughness. The temperature based on these results, which was therefore chosen for the remaining tests, was 175℃ for 30 min.

2. The effects of concentration (**Table 2**)

Different concentrations of bath solutions were prepared.

10% Household bleach solution was used, and the pH value was 7. The curing temperature was 175℃ for 30 min.

Table 2 The Effect of Concentration of Bath Solution

Entry	Concentration of bath solution (%)	Cl^+%
1	1.0%	0.039
2	2.5%	0.092
3	5.0%	0.196
4	Reaction solution (theoretically 10%)	0.233

Not unexpectedly, higher concentrations led to higher chlorine loading. When a 5.0% solution was prepared, the pure **39** was not dissolved in watercompletely, but could be dissolved in 0.1 N sodium hydroxide solution. When a 7.5% solution was prepared, **39** could not be dissolved in 0.1 N, or even higher concentrations of sodium hydroxide solution. It is worth noting that the reaction solution can be used directly as a bath solution without any further purification.

3. The effect of pH value (**Table 3**)

10% Household bleach solution was used. The curing temperature was 175℃ for 30 min.

Table 3 The Effect of pH Value

Concentration of bath solution (%)	Cl^+%, pH=7	Cl^+%, No pH adjustment
1%	0.0392	0.0079
5%	0.197	0.049
Reaction solution	0.230	0.099

The pH value greatly affected the chlorine loading. The pH value of the original 10% Household bleach solution was about 10 to 11, and it was expected that the more competitive reactions would dominate in basic conditions. For example, the proton on nitrogen may be removed to form a salt. When the pH value of the bleach solution is 7, more HOCl is present in the solution, which makes the formation of the N-Cl bond easier.

Shelf-life of Antibacterial Functions

The stability of the antibacterial function coated onto the cotton under dry storage conditions was investigated. The antibacterial functions were measured as a function of the retention of N-halamines on cotton by weight percentage of oxidative chlorine. It has been established in this laboratory that the loading of oxidative chlorine and the types of N-Cl bonds determine the biocidal efficacy. The chlorine content was titrated after varying periods of storage. **Table 4** presents the results for cotton gauze coated with **39**. The result indicated that the chlorine content was stable under these conditions. It was important to note that the chlorine content can be fully regenerated at any time by simply recharging with bleach. This is because although some of the chlorine may be lost during storage, the pyrimidine units remain bonded to the cotton.

Table 4: Shelf-life of Antibacterial Function

Days	% Cl^+ Retention	% Cl^+ Recharge
0	0.169	----
5	0.162	---
10	0.145	----
20	0.115	0.168
30	0.096	0.160
45	0.076	0.148

Laundering Durability of Antibacterial Function

The laundering durability of the antibacterial function was assessed using standard laundering washing tests (AATCC test method 124). Three sets of washing tests were conducted at 5, 10, 25, and 50 equivalent washing cycles for swatches including both unchlorinated and prechlorinated coatings. After washing, the unchlorinated swatches from each cycle were charged with 10% bleach (pH 7) (group1); half of the prechlorinated swatches from each washing cycle were recharged with chlorine (group 3), and the remaining prechlorinated swatches were used as is (group 2). The chlorine contents of each group of samples were measured using iodometric/thiosulfate titration, and the results of the evaluations are listed in **Table 5**.

Table 5: Laundering Durability of Antibacterial Function

Entry	Washing Cycle	% Cl^+ Retention		
		Group 1 unchlorinated	Group 2 prechlorinated	Group 3 prechlorinated recharged
1	0	0	0.182	0.182
2	5	0.178	0.118	0.180
3	10	0.170	0.071	0.173
4	25	0.147	0.010	0.167
5	50	0.102	0	0.128

Antibacterial Properties

The treated cotton was also evaluated for biocidal efficacy. Cotton swatches after chlorinaion were challenged with bacteria, and the number of surviving bacteria (in CFU) were determined. Unchlorinated treated cotton and native cotton served as controls. Inactivation of the microorganism was considered to be 100% for the materials when no colonies were detected in the thiosufate-quenched aliquots. The results for *E. coli* and *S. aureus* inactivation are presented in **Table 6** and **Table 7**, respectively. Cotton swatches treated with compound **39** showed a complete inactivation of *E. coli* with a 6 log-reduction in 1 min contact time, and a complete inactivation of *S. aureus* with a 6 log-reduction in 5 min contact time.

Table 6: Efficacies of Cotton Swatch Test Against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO. (cfu/ml)	Bacterial reduction	
			%	Log
1	1	2.67×10^5	6.29	0.028
2	5	2.53×10^5	10.98	0.050
3	10	2.40×10^5	16	0.074
4	1	2.67×10^5	6.29	0.028
5	5	2.27×10^5	20.35	0.098
6	10	1.87×10^5	34	0.183
7	1	0	100	6.155
8	5	0	100	6.155
9	10	0	100	6.155

Total bacterial load (cfu/sample): 1.43×10^6

Chlorine loading on the cotton swatch: $Cl^+\%$ = 0.201%

Sample 1-3: Cotton control

Sample 4-6: Epoxy uracil treated, but unchlorinated, cotton control

Sample 7-8: Epoxy uracil treated and chlorinated cotton

Table 7: Efficacies of Cotton Swatch Test Against *Staphylococcus aureus*

sample	Contact time (min)	Bacterial NO. (cfu/ml)	Bacterial reduction	
			%	Log
1	1	2.13×10^5	36.94	0.200
2	5	1.87×10^5	44.82	0.258
3	10	1.87×10^5	44.82	0.258
4	1	2.00×10^5	40.88	0.228
5	5	1.21×10^5	64.88	0.445
6	10	1.15×10^5	66.11	0.469
7	1	5.3×10^1	99.98	3.802
8	5	0	100	6.230
9	10	0	100	6.230

Total bacterial load (cfu/sample): 1.70×10^6

Chlorine loading on the cotton swatch: $Cl^+\%$ = 0.201%

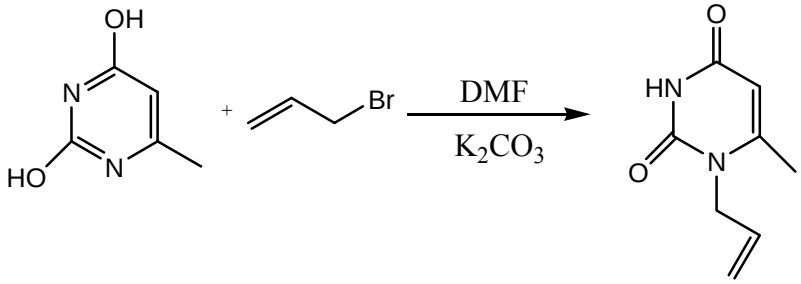
Sample 1-3: Cotton control

Sample 4-6: Treated, but unchlorinated, cotton control

Sample 7-8: Treated and chlorinated cotton

Synthesis and Characterization of the Copolymer

The objective of this synthesis was to design a polymer with biocidal functionalities. Vinyl acetate is a well characterized polymer with good flexibility and ductibility. Chlorinated 1-allyl-6-methyluracil is thought to be a useful biocidal compound. Thus, a copolymer of VAC and 1-allyl-6-methyluracil should be an effective film that also counteracts bacteria. The first step of this reaction is the synthesis of 1-allyl-6-methyluracil. 2,4-dihydroxy-6-methylpyrimidine, 1.9g (0.0158 mole), allyl bromide, and 2.1g (0.0158 mole) K_2CO_3 were added to 40 ml DMF in a 100 ml flask.



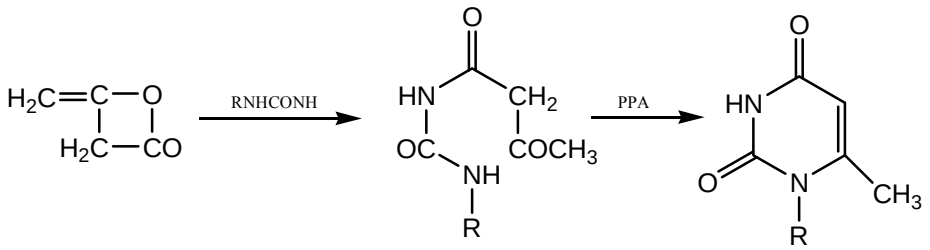
Scheme 2 Synthesis of 1-allyl-6-methyluracil

Allylation took place in the N-1 position of pyimidine ring rather than the N-3 position, as discussed previously for the synthesis of 1-(2,3-epoxypropyl)-6-methyluracil. The allylation reaction was modified by adding K_2CO_3 to the DMF solution at 75℃. The 2,4-dihydroxy-6-methylpyrimidine was not dissolved in the DMF solution, but allyl bromide and K_2CO_3 were added to the mixture. As the reaction progressed, the newly formed 1-allyl-6-methyluracil dissolved in the DMF solution. After reaction, the mixture was poured into the water

and the product extracted by CCl_4.

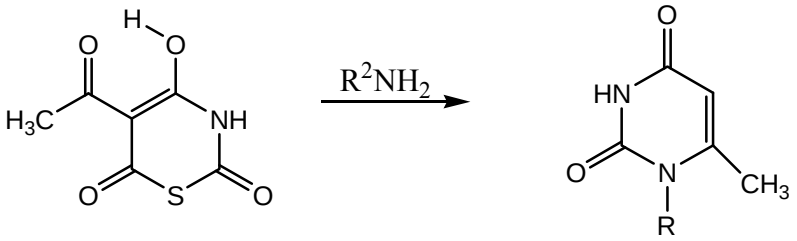
Two other methods may also be used to prepare the 1-allyl-6-methyluracil:

Synthesis of 1-allyl-6-methyluracil from ureas[72]:



R = allyl group

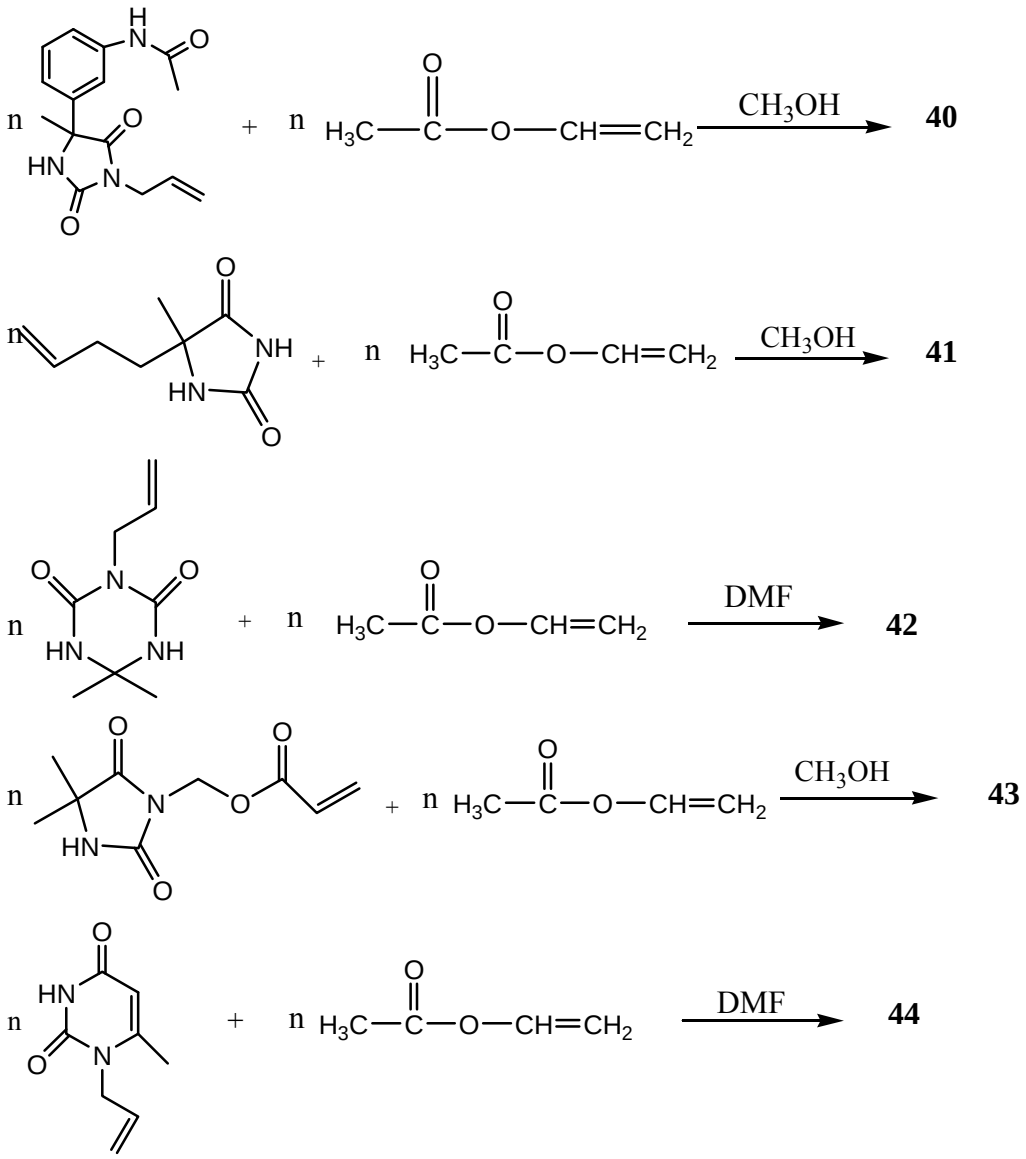
Synthesis of 1-allyl-6-methyluracil from 5-acylthiazines and primary amines[73]:



R = allyl group

Scheme 3 Other Synthetic Routes of 1-allyl-6-methyluracil

In this study, 1-allyl-6-methyluracil and other N-halamine allyl compounds were copolymerized with vinyl acetate via double bond condensation at 75-85℃ temperature for 2-4.5 h. The copolymers could then be precipitated by pouring the reaction solution into water. The copolymers were further dried under reduced pressure.



Scheme 4 Synthesis of Copolymer VAC-co-N-halamine Copolymers

^{1}H NMR, ^{13}C NMR, and FTIR were used to analyze 1-allyl-6-methyluracil and the newly formed copolymers. **Figure 35** shows the proton NMR spectrum of **44a**. The peak assignments are clearly labeled on the spectrum. For example, the single peak in the region of δ 2.18 ppm can be assigned to the three hydrogens of the methyl

group; the multiple peaks in the region of δ 4.39-4.41, 4.99-5.16, and 5.72-6.70 ppm are attributed to the allyl group; the single peak at δ 5.51 ppm is attributed to the hydrogen on the pyrimidine ring; and the single peak at δ 11.21 ppm is assigned to an imide proton.

Figure 36 shows the C^{13} NMR spectrum of **44a**. The peak at δ 19.32 ppm is assigned to the carbon of the methyl group; the peaks at δ 45.49, 116.17, and 133.74 are attributed to the carbons of the allyl group; the peak at δ 101.52 ppm is attributed to the carbon of the double bond on the pyrimidine ring; and the peaks at δ 151.85 and 162.95 ppm are assigned to the carbonyl carbons; the peak at δ154.73 ppm is attributed to the carbon attached to the methyl group.

Figure 37 shows the FTIR spectrum of **44a**. The absorption line at 3388 cm^{-1} represents the N-H bond. The C=O stretching mode bands appear at 1708 and 1660 cm^{-1}. The absorption band at 1604 cm^{-1} corresponds to the C=C bond stretch.

Figure 38 shows the FTIR spectrum of the copolymer **40**. The C=O stretching mode bands appear at 1727 cm^{-1}. No appearance of C=C bond stretching mode bands indicates that the formation of copolymer.

Figure 39 shows the FTIR spectrum of the copolymer **41**. The C=O stretching mode bands appear at 1727 cm^{-1}. No appearance of C=C bond stretching mode bands indicates that the formation of copolymer.

Figure 40 shows the FTIR spectrum of the copolymer **42**. The C=O stretching mode bands appear at 1729 cm^{-1}. No appearance of C=C bond stretching mode bands

indicates that the formation of copolymer.

Figure 41 shows the FTIR spectrum of the copolymer **43**. The C=O stretching mode bands appear at 1729 cm^{-1}. No appearance of C=C bond stretching mode bands indicates that the formation of copolymer.

Figure 42 shows the FTIR spectrum of the copolymer **44**. The absorption band at 3132 cm^{-1} corresponds to the N-H bond stretching mode. The C=O stretching mode bands appear at 1710 and 1660 cm^{-1}. No appearance of C=C bond stretching mode bands indicates that the formation of copolymer.

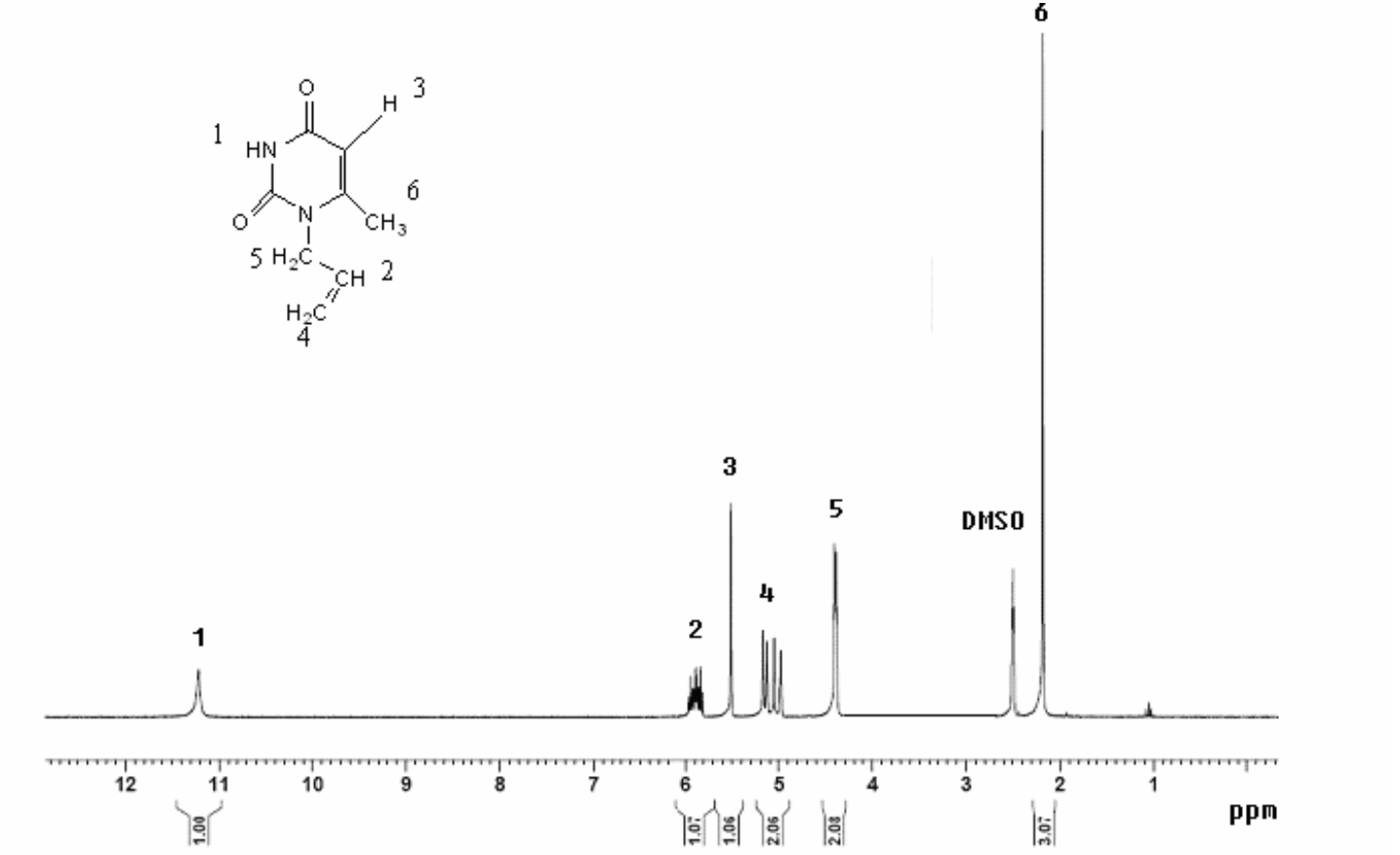


Figure 35 ^{1}H NMR of **44a**

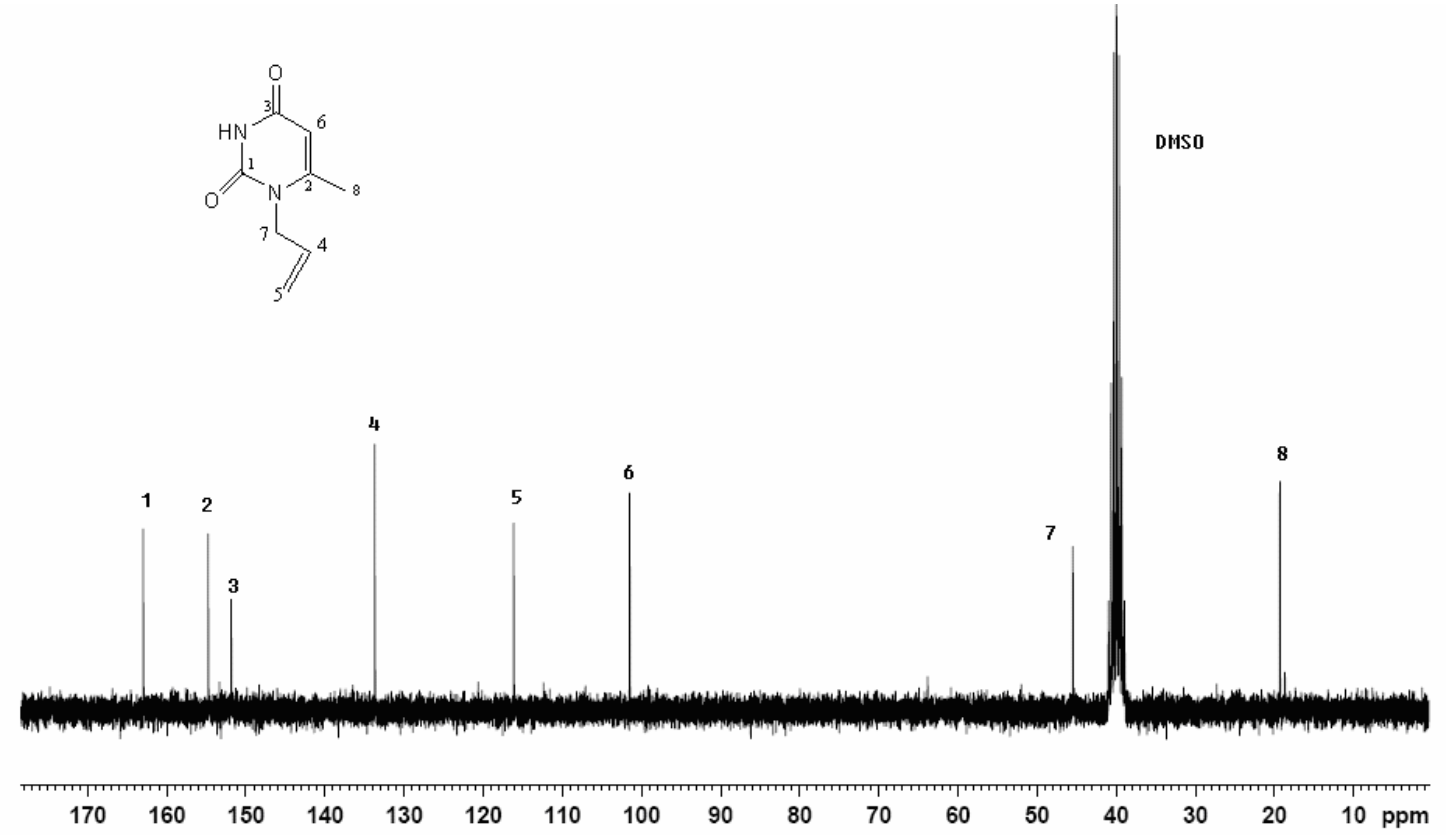


Figure 36 ^{13}C NMR of **44a**

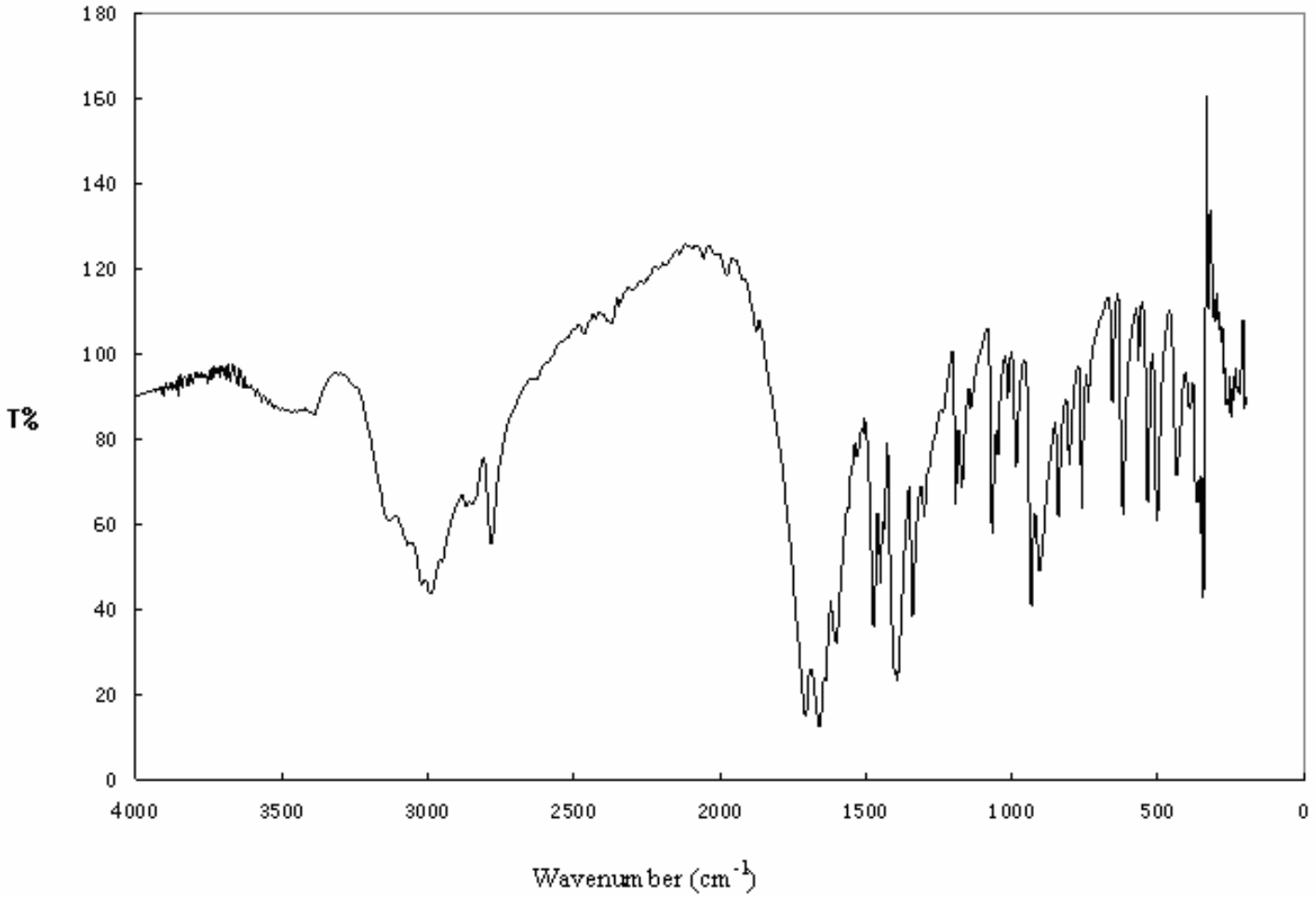


Figure 37 FTIR of **44a**

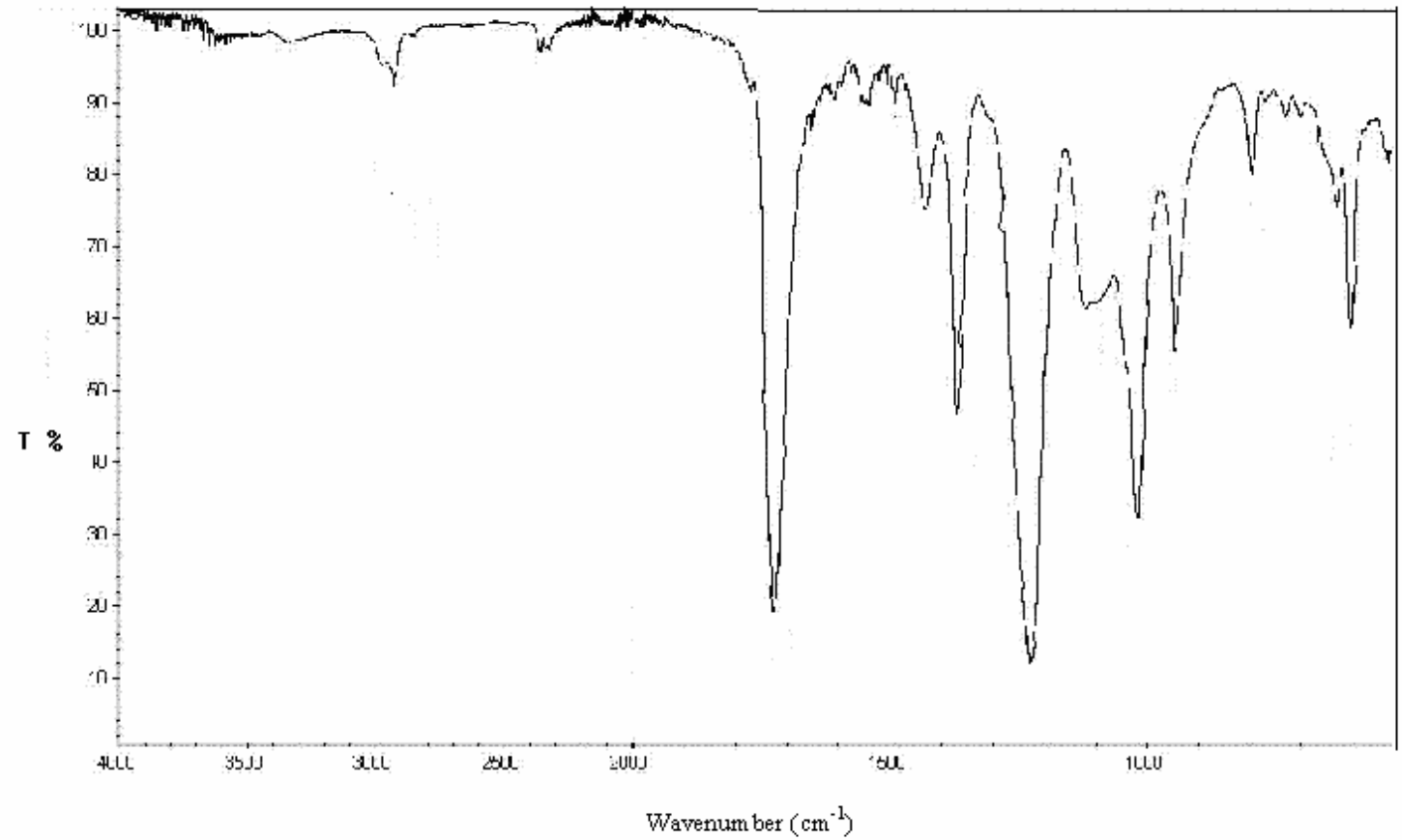


Figure 38 FTIR of Copolymer **40**

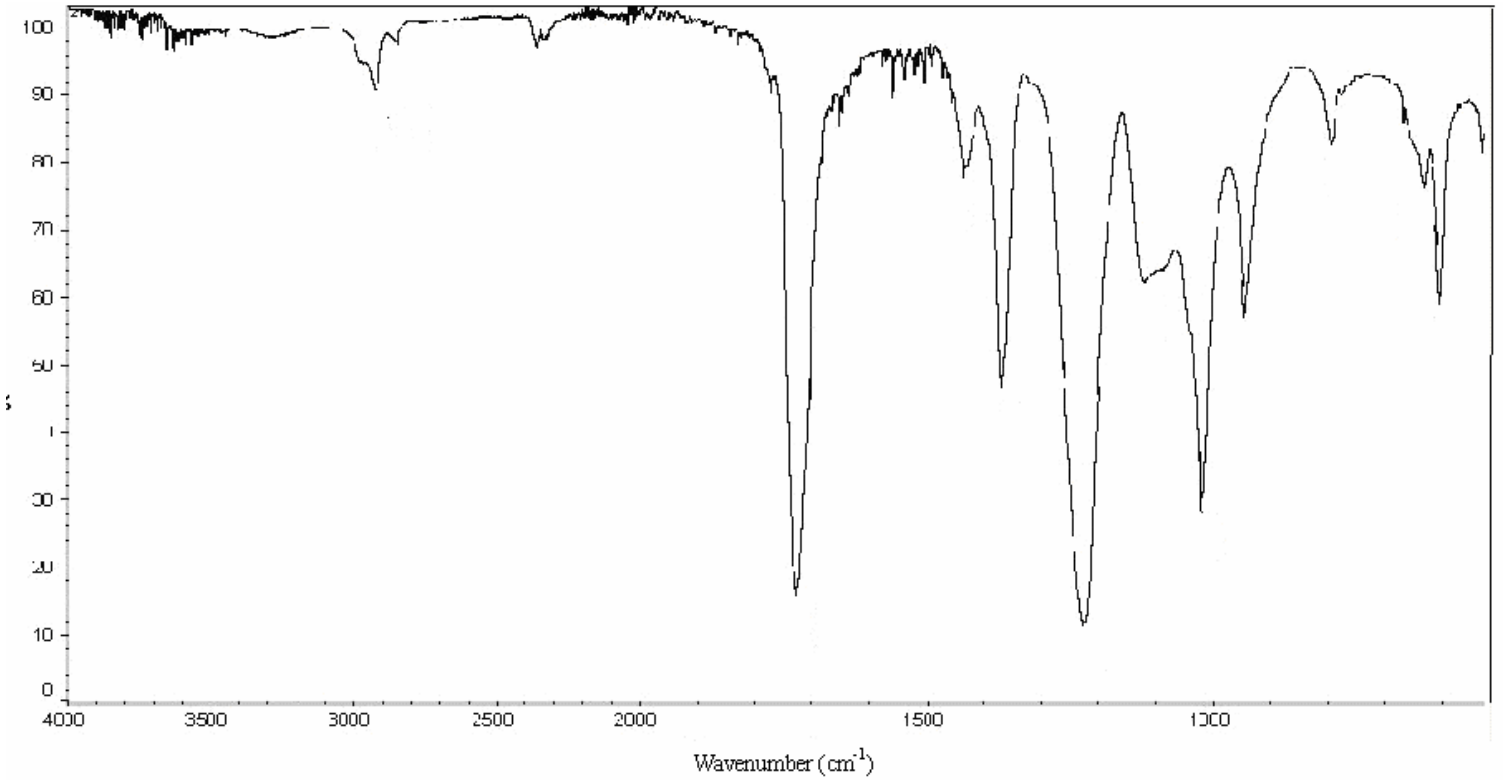


Figure 39 FTIR of Copolymer **41**

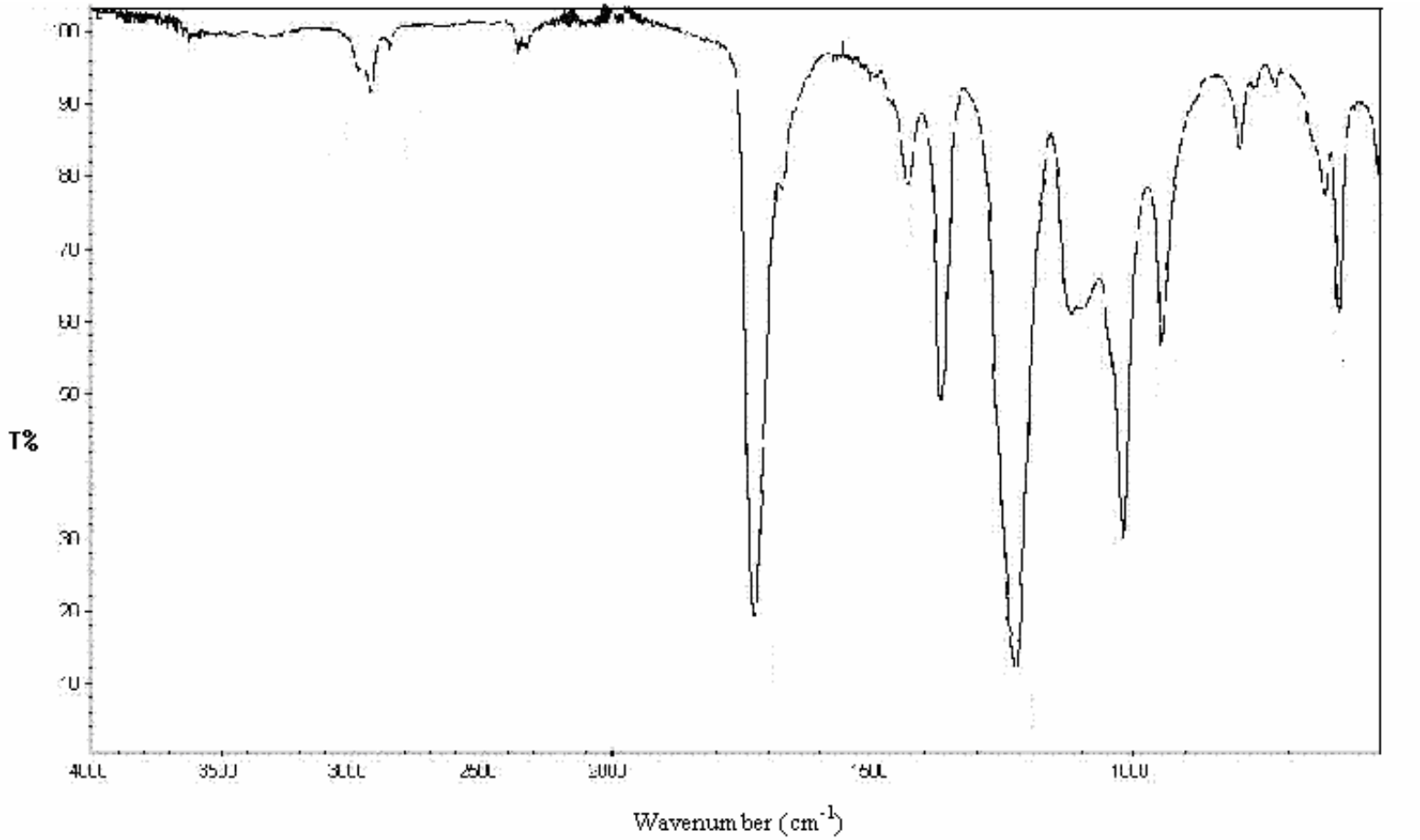


Figure 40 FTIR of Copolymer **42**

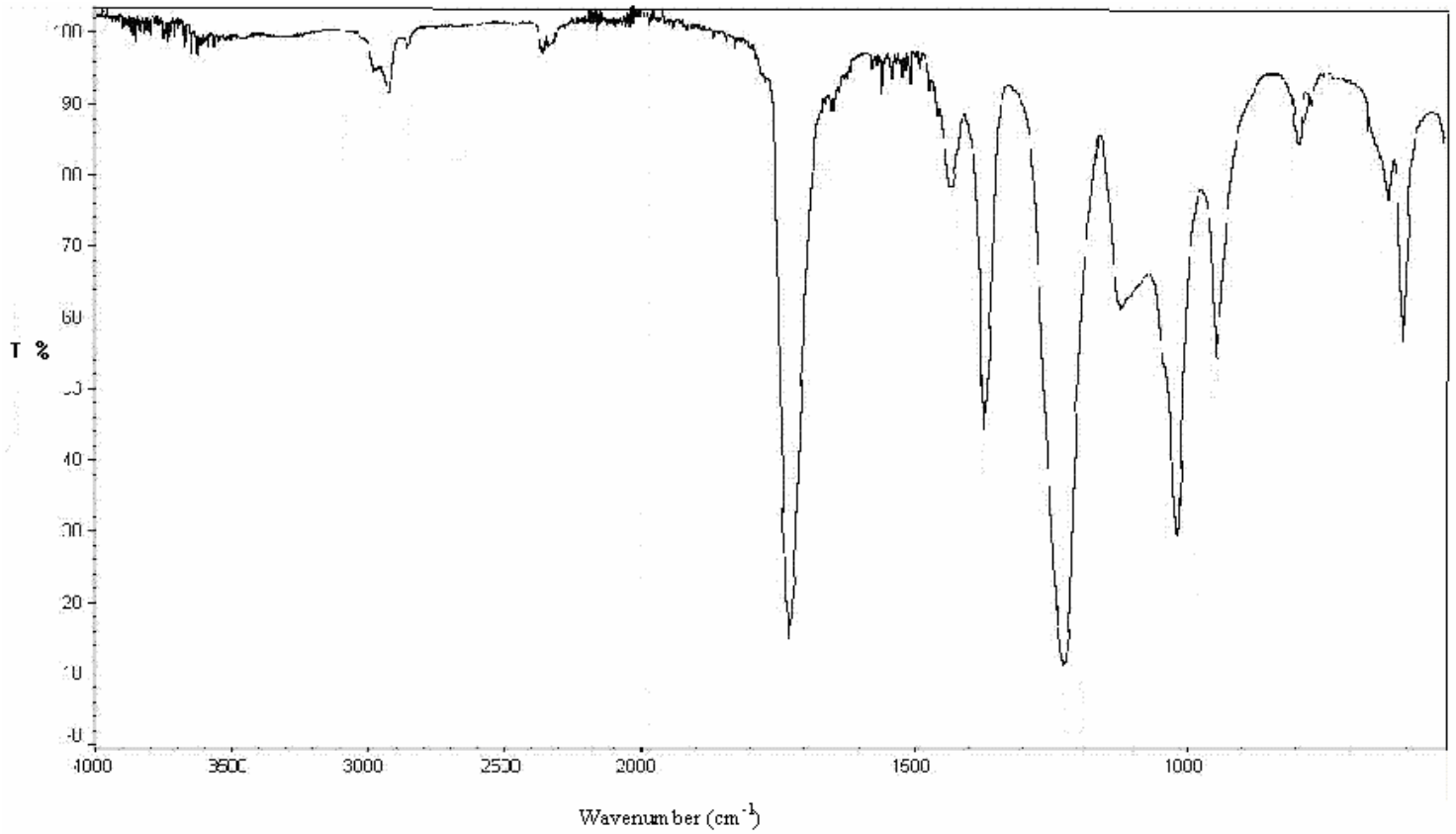


Figure 41 FTIR of Copolymer **43**

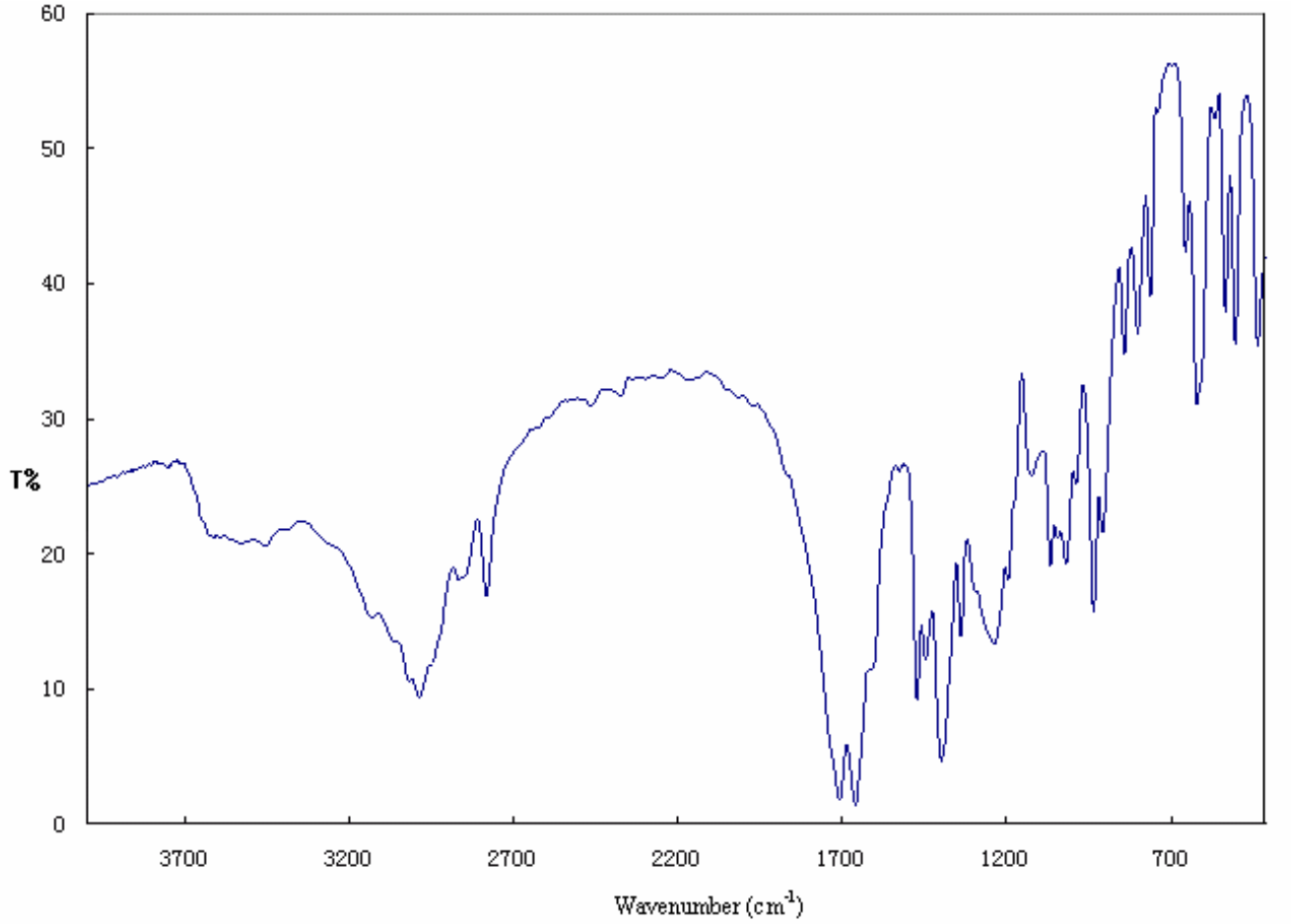


Figure 40 FTIR of Copolymer **44**

A variety of mole ratios of VAC and N-halamines were produced for this study (**Table 8, Table 9, and Table 10**). The VAC homopolymer can be made easily and has good flexibility and tractility making it an ideal material for films. The tests showed that the chlorinated films made of the poly VAC alone had no biocidal activity, but when the N-halamine was added, films made using the chlorinated copolymer were able to kill bacteria. In general, the higher the ratio of the N-halamine in the copolymer, the higher the biocidal activity is, but too much N-halamine may adversely affect the physical properties of the copolymer. Different ratios were studied to determine the conditions necessary for producing films with high biocidal activity. **Table 8** showed that the higher ratios of N-allyl-6-methylpyrimidine allowed more Cl^+ loading, but once the ratio of the VAC and N-allyl-6-methylpyrimidine dropped below 20 to 1, the copolymer was no longer a film. For **40** and **41,** as shown in **Table 9** and **table 10**, the film still can exist when the ratio is 20 to 1. The higher ratio of **40** and **41** allows more Cl^+ loading. **40** contains two amide groups, and **41** contains an imide and an amide group. Compared to **44** which only has one amide group, **40** and **41** have higher Cl^+ loading. That means increased C-N structure also allows more Cl^+ loading.

Table 8 Different ratios of VAC and 1-allyl-6-methyluracil (**44**)

	Ratio (mole)	State	Cl^+% (atom/cm^2)
VAC	**44**		Retention
1	0	Film	0
20	1	powder	--------
40	1	Film	1.985×10^{17}
80	1	Film	9.324×10^{16}

Table 9 Different ratios of VAC and 3-(3'-allyl-5'-methylhydatoinyl)acetanilide (**40**)

	Ratio (mole)	State	Cl^+% (atom/cm^2)
VAC	**40**		Retention
1	0	Film	0
20	1	Film	1.43×10^{18}
40	1	Film	9.06×10^{17}
80	1	Film	5.37×10^{17}

Table 10 Different ratios of VAC and 5-methyl-5-(3'-propenyl)hydantoin (**41**)

	Ratio (mole)	State	Cl^+% (atom/cm^2)
VAC	**41**		Retention
1	0	Film	0
20	1	Film	1.62×10^{18}
40	1	Film	9.72×10^{17}
80	1	Film	5.96×10^{17}

Antibacterial Properties

The polymer films were cast from acetone onto the slides. The films were about 0.05 mm thick and were cut into small pieces. Post-chlorination film swatches were challenged with bacteria, and the numbers of CFU of surviving bacteria were determined. The tests showed that the transparency film itself had no biocidal activity. So in this study unchlorinated film served as controls. Inactivation of the microorganism was considered to be 100% for the materials when no colonies were detected in the thiosufate-quenched aliquots. The results for *E. coli* and *S. aureus* inactivation are presented in the following **Table 11** to **Table 20**.

Table 11 Efficacies of **40** Film Tested against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO. (cfu/75µl)	Bacterial NO. (cfu/ml)	Bacterial reduction	
				%	Log
1	30	30	2.13×10^5	86.60	0.873
2	5	5	6.40×10^2	99.96	3.396
3	10	10	0	100	6.903
4	30	30	0	100	6.903

Total bacterial load (cfu/sample): 8.0×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 5.65×10^{17} Cl^+atom/cm^2

Table 12 Efficacies of **40** Film Tested against *Staphlococcus aureus*

sample	Contact time (min)	Bacterial NO. (cfu/75µl)	Bacterial NO. (cfu/ml)	Bacterial reduction	
				%	Log
1	30	48	6.40×10^5	73.20	0.572
2	5	0	0	100	7.079
3	10	0	0	100	7.079
4	30	0	0	100	7.079

Total bacterial load (cfu/sample): 1.20×10^7

Chlorine loading on the film: Cl^+ atom/cm^2 = 5.65×10^{17} Cl^+atom/cm^2

Sample 1: treated, but unchlorinated film

Sample 2-4: treated and chlorinated film

The results for *E. coli* and *S. aureus* inactivation of copolymer **40** are presented in **Table 11** and **Table 12**, respectively. The film samples treated by the chlorinated copolymer showed a complete inactivation of *E.coli* with a 6 log-reduction in 10 min contact time, and a complete inactivation of *S. aureus* with a 7 log-reduction in 5 min contact time.

Table 13 Efficacies of **41** Film Tested against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	30	17	2.27×10^4	96.65	1.474
2	5	0	1.73×10^2	100	2.861
3	10	0	0	100	5.801
4	30	0	0	100	5.801

Total bacterial load (cfu/sample): 9.67×10^5

Chlorine loading on the film: Cl^+ atom/cm^2 = 5.90×10^{17} Cl^+atom/cm^2

Table 14 Efficacies of **41** Film Tested against *Staphlococcus aureus*

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	30	40	5.33×10^4	88.97	0.958
2	5	0	0	100	6.104
3	10	0	0	100	6104
4	30	0	0	100	6.104

Total bacterial load (cfu/sample): 1.27×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 5.90×10^{17} Cl^+ atom/cm^2

Sample 1: treated, but unchlorinated film

Sample 2-4: treated and chlorinated film

The results for *E. coli* and *S. aureus* inactivation of copolymer **41** are presented in **Table 13** and **Table 14**, respectively. The film samples treated by the chlorinated copolymer showed a complete inactivation of *E.coli* with a 6 log-reduction in 10 min contact time, and a complete inactivation of *S. aureus* with a 6 log-reduction in 5 min contact time.

Table 15 Efficacies of **42** Film Tested against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	40	64	5.87×10^5	48.83	0.415
2	5	0	0	100	6.885
3	10	0	0	100	6.885
4	40	0	0	100	6.885

Total bacterial load (cfu/sample): 6.67×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 9.82×10^{17} Cl^+atom/cm^2

Table 16 Efficacies of **42** Film Tested against *Staphlococcus aureus*

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	40	61	4.09×10^6	57.74	0.374
2	5	0	0	100	6.985
3	10	0	0	100	6.985
4	40	0	0	100	6.985

Total bacterial load (cfu/sample): 9.67×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 9.82×10^{17} Cl^+atom/cm^2

Sample 1: treated, but unchlorinated film

Sample 2-4: treated and chlorinated film

The results for *E. coli* and *S. aureus* inactivation of copolymer **42** are presented in **Table 15** and **Table 16**, respectively. The film samples treated by the chlorinated copolymer showed a complete inactivation of *E.coli* with a 6 log-reduction in 5 min contact time, and a complete inactivation of *S. aureus* with a 7 log-reduction in 5 min contact time.

Table 17 Efficacies of **43** Film Tested against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	30	3	4.00×10^4	94.09	1.226
2	5	0	0	100	5.985
3	10	0	0	100	5.985
4	30	0	0	100	5.985

Total bacterial load (cfu/sample): 9.67×10^5

Chlorine loading on the film: Cl^+ atom/cm^2 = 3.31×10^{17} Cl^+atom/cm^2

Table 18 Efficacies of **43** Film Tested against *Staphlococcus aureus*

sample	Contact time (min)	Bacterial NO(cfu/75µl)	Bacterial NO(cfu/ml)	Bacterial reduction	
				%	Log
1	30	46	$6.13.00 \times 10^4$	87.32	0.897
2	5	0	0	100	6.104
3	10	0	0	100	6.104
4	30	0	0	100	6.104

Total bacterial load (cfu/sample): 1.27×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 3.31×10^{17} Cl^+ atom/cm^2

Sample 1: treated, but unchlorinated film

Sample 2-4: treated and chlorinated film

The results for *E. coli* and *S. aureus* inactivation of copolymer **43** are presented in **Table 17** and **Table 18**, respectively. The film samples treated by the chlorinated copolymer showed a complete inactivation of *E.coli* with a 6 log-reduction in 5 min contact time, and a complete inactivation of *S. aureus* with a 6 log-reduction in 5 min contact time.

Table 19 Efficacies of **44** Film Tested against *E. coli* (O157:H7)

sample	Contact time (min)	Bacterial NO. (cfu/75μl)	Bacterial NO. (cfu/ml)	Bacterial reduction	
				%	Log
1	30	41	5.47×10^4	56.60	0.362
2	5	13	1.73×10^2	100	2.861
3	15	0	0	100	5.801
4	30	0	0	100	5.801

Total bacterial load (cfu/sample): 8.33×10^5

Chlorine loading on the film: Cl^+ atom/cm^2 = 1.885×10^{17} Cl^+ atom/cm^2

Table 20 Efficacies of **44** Film Tested against *Staphlococcus aureus*

sample	Contact time (min)	Bacterial NO. (cfu/75μl)	Bacterial NO. (cfu/ml)	Bacterial reduction	
				%	Log
1	30	108	1.44×10^4	41.17	0.231
2	5	0	0	100	6.090
3	15	0	0	100	6.090
4	30	0	0	100	6.090

Total bacterial load (cfu/sample): 1.23×10^6

Chlorine loading on the film: Cl^+ atom/cm^2 = 1.885×10^{17} Cl^+ atom/cm^2

Sample 1: treated, but unchlorinated film

Sample 2-4: treated and chlorinated film

Film samples treated by copolymer **44** showed a complete inactivation of *E. coli,* with a 6 log-reduction in 15 min contact time, and a complete inactivation of *S. aureus,* with a 6 log-reduction in 5 min contact time.

Stability under Laboratory Light and UV Irradiation

The stability of antibacterial functions on chlorinated copolymers under laboratory light and UV irradiation was investigated. The biocidal efficacy was relative with two facts: the loading of Cl^+ and the types of N-Cl bond. The stability of chlorinated copolymers under laboratory light is presented in **Table 21.** It is observed that the chlorine content on the copolymers under laboratory light is quite stable and can be fully recharged with household bleach. The stability of chlorinated copolymers under UV irradiation is presented in **Table 22.** The result showed that the **40**, **41** to **43** copolymers still had some chlorine loading even after 10 days under the UV irradiation. Most chlorine can be recovered after 10 days under the UV irradiation. Both laboratory light and UV irradiation tests showed that those copolymers only dissociate the chlorine from the N-halamine bonds. The N-halamine units in those copolymers did not decompose, so that most of the chlorine could be regenerated after being exposed to laboratory light and UV irradiation.

Table 21 Stability under Laboratory Light (Cl^+ atoms/cm^2)

Time (days)	VAC-co-**40**	VAC-co-**41**	VAC-co-**42**	VAC-co-**43**	VAC-co-**44**
0	5.10×10^{17}	5.59×10^{17}	5.06×10^{17}	3.31×10^{17}	1.80×10^{17}
1	5.12×10^{17}	4.61×10^{17}	4.96×10^{17}	3.61×10^{17}	1.80×10^{17}
2	5.05×10^{17}	4.70×10^{17}	4.78×10^{17}	3.61×10^{17}	1.75×10^{17}
5	4.98×10^{17}	4.50×10^{17}	4.61×10^{17}	2.88×10^{17}	1.41×10^{17}
15	4.60×10^{17} recharge (5.09×10^{17})	3.45×10^{17} recharge (4.46×10^{17})	3.83×10^{17} Recharge (5.32×10^{17})	2.84×10^{17} recharge (3.58×10^{17})	1.04×10^{17} recharge (1.53×10^{17})
90	4.11×10^{17} recharge (5.21×10^{17})	3.76×10^{17} recharge (3.89×10^{17})	2.93 x 1017 recharge (4.02×10^{17})	2.76×10^{17} recharge (3.07×10^{17})	6.08×10^{16} recharge (1.32×10^{17})

Table 22 Stability under UV Irradiation (Cl^+ atoms/cm^2)

Time (days)	VAC-co-**40**	VAC-co-**41**	VAC-co-**42**	VAC-co-**43**	VAC-co-**44**
0	2.62×10^{17}	5.59×10^{17}	5.06×10^{17}	3.71×10^{17}	1.81×10^{17}
1	1.57×10^{17}	1.26×10^{17}	4.50×10^{16}	9.03×10^{16}	4.03×10^{16}
2	1.37×10^{17}	1.08×10^{17}	0	5.41×10^{16}	3.22×10^{16}
5	8.06×10^{16}	6.30×10^{16} Recharge (3.38×10^{17})	0 recharge (3.36×10^{17})	6.30×10^{16} recharge (2.57×10^{17})	1.67×10^{16} recharge (1.73×10^{17})
10	6.44×10^{16} recharge (2.81×10^{17})	1.8×10^{16} recharge (3.61×10^{17})	0 recharge (2.30×10^{17})	2.7×10^{16} recharge (2.75×10^{17})	0 recharge (1.69×10^{17})

CONCLUSIONS

A new N-halamine precursor, 1-(2, 3-epoxypropyl)-6-methyluracil, was synthesized in this research. This N-halamine precursor was coated on cotton, and the treated cotton was activated by chlorination with Household bleach. Treated cotton swatches inactivated *E.coli* in 1 min contact time and inactivated *S. aureus* in 5 min contact time. The cotton swatches retained biocidal activity even after 50 washing cycles. Most of the chlorine lost can be recovered by simply recharging the cloth in household bleach. This reaction is very facile, and the reaction solution can be used directly as the finishing bath. The potential applications of the biocidal cotton are in hospitals, for example in bed sheets and clothes for patients, and also to respond to potential bio-terrorism events. This kind of cotton may be particularly effective in antimicrobial masks to prevent infective pathogen trans/cross-contamination.

Another new N-halamine monomer, 1-allyl-6-methyluracil, was also synthesized and was copolymerized with commercial vinyl acetate (VAC) to form a copolymer VAC-co-1-allyl-6-methyluracil film coating. And a series of VAC-co-N-halamine copolymers,
VAC-co-3-(3'-allyl-5'-methylhydatoinyl)acetanilide,
VAC-co-5-methyl-5-(3'-propenyl)hydantoin,

VAC-co-3-allyl-6,6-dimethyl-1,3,5-triazinane-2,4-dione

VAC-co-5,5-dimethylhydantoin-1-ylmethylene acrylate, were also synthesized. Those films had biocidal activity after treatment with Household bleach. The surface coated copolymer contained 2.0×10^{17} to $9.0\times10^{17}Cl^{+}$ atoms/cm^2. The films produced in this research were able to kill both Gram-positive and Gram-negative bacteria. Those polymers have many potential applications in medical devices, hygienic materials, and in the food-processing industry.

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