THE SYNTHESIS AND PRACTICAL APPLICATIONS

OF NOVEL N-HALAMINE BIOCIDES

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THE SYNTHESIS AND PRACTICAL APPLICATIONS

OF NOVEL N-HALAMINE BIOCIDES

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THE SYNTHESIS AND PRACTICAL APPLICATIONS

OF NOVEL N-HALAMINE BIOCIDES

Paul Kevin Barnes

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THE SYNTHESIS AND PRACTICAL APPLICATIONS

OF NOVEL N-HALAMINE BIOCIDES

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A novel set of *N*-halamine siloxane precursors were synthesized for the purpose of coating various substrates to determine the factors, which are the most dominant influences of the overall stability of the newly modified surfaces. Examination of both the coating, as well as the chlorine stability under varying conditions, has been performed. The number and types of bonds to the surface have been examined using several techniques to simulate the most stringent circumstances envisioned for the practical use of the newly modified materials.

The siloxane moiety used in the design of these new halamine precursors proved to be extremely versatile demonstrating the capability of attaching to a variety of surfaces under a wide range of conditions. Factors such as solvent and curing temperatures were also evaluated to emphasize the versatility of these coatings and to demonstrate the potential usefulness of these materials as future commercial products. This dissertation will discuss the following specific projects.

The first project was the design of a novel *N*-halamine siloxane precursor, 5,5'-ethylenebis[5-methyl-3-(3-tri-ethoxysilylpropyl)hydantoin], which possessed the capability of forming numerous chemical bonds to a large variety of surfaces such as cellulose. This enabled the coating to be considerably more durable than the previously developed siloxane monomer, 3-triethoxysilylpropyl-5,5-dimethylhydantoin.

The second project was to develop a series of more industrially friendly water soluble siloxane copolymers incorporating both *N*-halamine and quaternary ammonium salt moieties. The copolymers with greater portions of *N*-halmine moieties proved to provide more efficient log reductions for both Gram-positive and Gram-negative bacteria in much shorter time intervals than in the instances in which the ratios were reversed, respectively. These copolymers showed great potential for commercial applications for which an aqueous media is preferred.

Projects three and four both focused on the design of a new series of more stable *N*-halamine siloxane monomer precursors, 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethyl-piperdine, and 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethyl-piperdine, which will be referred to as HAM-Sil and HAM-Sil1', respectively. In project three these new amine siloxane monomers were employed in the treatment of cellulose. The HAM-Sil and HAM-Sil1'-treated fabrics, once chlorinated, demonstrated sufficient bactericidal activities against both Gram-positive and Gram-negative bacteria, as well as increased stability during simulated laundering tests.

Project four is the chemical modification of silica gel with both HAM-Sil and HAM-Sil1' to create an inexpensive antimicrobial filter media which could have various industrial applications

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Introduction

Ancient 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. A recurring theme in history was the belief that epidemic diseases were spread by something in the air. A prime example of this idea is the record that Hippocrates attempted to drive the plague from Athens by lighting fires of aromatic wood in the streets.

Numerous disinfectants have been in use for 150-200 years and have stood the test of time. Chemicals such as pitch, wine, copper, and silver were the earliest disinfectants. Mercury had been known since the fourth century. Mercuric chloride was used by Arab physicians as an antiseptic and a wood preservative. Tar, coal, and wood tar were some of the first compounds to be used as disinfectants in the distilling industries and were used to preserve ship timber in the early 1700s. Chlorine and formaldehyde were discovered in 1744 and 1859, respectively. Chlorine was introduced for water treatment in 1843 and is still used for this purpose today. Copper sulfate, zinc chloride, sodium permanganate, acids, alkalis, sulfurs, and alcohols were disinfectants introduced in the mid-1800s.

In the 19th century, Louis Pasteur was credited with dispelling the belief in spontaneous generation of bacteria. John Tyndall, in the late 1800s, showed that spores were resistant to boiling and, if given time, could germinate. Their vegetative offspring could be killed by repetitive heating. This technique became known as Tyndallisation

Nicholas Appert (1810) demonstrated methods for preserving food during long sea voyages by sealing vegetables and fruit in glass jars and heating them.[1] William Henry published studies in the 1830s on the disinfection power of increased temperatures with a view for the suggestion of a substitute for quarantine.[2] Based on the first published results regarding the use of sterilizers, Pasteur, Koch, and Wolffhugel developed scientifically based procedures for both dry heat and steam sterilization in the late 1800s.

In the early ages it was proposed that filth led to disease which, in turn, led to loss of income and consequent poverty. Following this lead, Snow showed that cholera was transmitted by a contaminated water supply in 1855.[3] Budd (1856) demonstrated that typhoid fever was not caused by bad odors, but rather by an agent carried through sewage to the drinking water and milk.[3]

Pasteur (1857), who developed the process known as "pasteurization" to control microorganisms, showed that souring of milk was due to microorganisms.[1] In 1886 he helped solve spoilage problems in the wine industry. Semmelweiss proved that puerperal fever could be prevented if physicians washed their hands after performing autopsies and prior to assisting in childbirth.[2]

During this century, scientists have utilized better techniques for studying, growing and isolating microorganisms. This has furthered the development of a larger arsenal of more effective antimicrobials to solve specific problems.[4-6] Considerable effort today is spent studying efficacy and human and environmental toxicity in order to develop even better biocides.

When designing the ideal commercial biocide, many factors should be considered. A useful industrial microbial should posses the following characters: 1. the production cost

must be minimal, 2. it must have a low toxicity level, 3. it must not have an overpowering presence of odor or alter the appearance of the substrate upon which it is being used, 4. it should also be very effective to a wide array of microorganisms as well as environments, and 5. it needs to have an acceptable life span for its intended use.

In addition to identifying new biocides, scientists such as Worley, Sun, and others continue to improve their knowledge of the application of these materials.[7-13] Only minute quantities of biocidal active species are required for effective control. Today, the best biocide for a specific application comes from a partnership between the biocide manufacturer and user, identifying specific problems and then researching, developing, and testing to find a precise answer.

Present Need of Biocides

We can expect to live considerablely longer today than our grandparents did 90 years ago. A number of factors have contributed to our longer life expectancy, among them being medical innovation, nutritional improvements, and sociocultural changes. The effects of increased sanitation and the role of disinfection have also been very important. Infection control in the modern hospital environment depends heavily upon the use of a variety of specialized antimicrobial formulations. In addition to the cleaning and disinfectant solutions used in general housekeeping, there are various formulations used for especially demanding tasks such as operating room protection and sterilization or high-level disinfection of delicate and heat-sensitive medical devices. The appropriate and careful use of these products is a major factor in infection control.

Since bacteria, viruses, and fungi cannot be seen with the naked eye, extensive testing requirements and government review assure the validity of product claims. Depending on

their use, antimicrobial products are regulated by one or more federal agencies. The regulatory process also provides the added benefit of uniformly documented use instructions and well-characterized toxicology.

The increased use of new modern non-invasive surgery made possible by flexible endoscopy and other instruments with sophisticated optics and computers has placed increased demands on high-level disinfectants or sterilants.

As the average lifespan increases, and baby boomers age, there will be a dramatic increase in the number of older citizens and nursing home occupancy. Communal living, coupled with decreased resistance and general failing health, puts tremendous demands on sanitation and disinfection practices. Not only may the individuals have compromised immunity, sanitation challenges frequently occur with the problem of incontinence. Disinfection of surfaces is essential. To be effective, a disinfectant must remain in contact with a surface to be disinfected for the prescribed amount of time. A disinfectant cannot kill organisms that it does not contact.[1]

While the situation is not as serious as that in a hospital setting, nursing home caregivers may not have the same degree of infection control support that is available in the modern hospital. Health-care providers in these institutions must be trained in the use of appropriate disinfection of inanimate surfaces and use of antimicrobial soaps and/or other commercially available disinfectants against transmission of disease organisms through contact with unwashed hands and other contaminated surfaces.

Medical practitioners have at their disposal an arsenal of highly effective, proven biocides. Modern hospital sanitation depends heavily on specialized disinfectants and sterilants, which, because of performance demands upon them, are among the strongest

and most carefully applied of all antimicrobials used in society, and yet the number of nosocomial infections are still on the rise. In a survey conducted by the Center for Disease Control (CDC), Intensive Care Units (ICU), and Non-Intensive Units (Non-ICU) in health care establishments around the United States showed that cases related to drug resistance had risen quite significantly by approximately 20% from 1989 to 1998.[14] Clinics, physicians' and dentists' offices and nursing homes also require a high level of disinfection and sanitation which could possibly be augmented through *N*-halamine technology.[7, 9, 13]

The largest single use of antimicrobial agents is in the purification of drinking water. The disinfection of drinking water was introduced in the United States in the early 1900s to eliminate disease-producing contamination and unpleasant taste and odor, and it has substantially reduced the deaths and disease due to cholera, hepatitis, and amoebic dysentery. In third world countries and areas where water is not adequately disinfected, outbreaks of these diseases still occur. Worley and co-workers have developed water purification techniques based on *N*-halamine technology that deactivates a vast array of microbes and bacteria with only a minimal release of free halogen into the water.[15-17]

The most common methods now used to disinfect drinking water involve the use of free chlorine and chloramines (a mixture of chlorine and ammonia). Roughly 90 percent of the drinking water in the United States is disinfected with these compounds, while about one percent is disinfected through the use of ozone.

Chlorine and chloramines are the antimicrobial agents used to provide more than 200 million people with disinfected drinking water. Disinfecting drinking water at the treatment plant is a complicated process that can be done in a number of ways. A few

general steps apply for most treatment processes. In systems that use chlorine gas, raw water is dosed with chlorine as it enters the plant. Chemicals are added to form larger and heavier particles that settle out. Fine particles are removed by filtration. A chlorine dose is added to provide additional protection as the water leaves the plant and flows into the distribution system.[18]

The amount of chlorine and its contact time with the water are controlled by government regulation. Enough chlorine has to be added to disinfect the water inside the plant and to protect it from disease causing organisms it encounters as it flows through the distribution system. UV light or ozone may do a good job of disinfecting water in the plant, but may not provide the residual needed to keep the water disinfected until it is used. When UV light or ozone are used, a residual disinfectant, typically chlorine or chloramines, is usually needed to ensure the quality of the water.

Continuing incidents of food poisoning demonstrate the uncertainty of some food sanitation practices and the importance of antimicrobials in the maintenance of the safety of our food supply. Deaths and illness due to food-borne organisms are preventable with appropriate precautions including hand washing with an efficacious product and disinfection of surfaces that come in contact with contaminated food.

The cause of food-borne illness is widely recognized to be infection by pathogenic microorganisms. Organisms, typically responsible for food poisoning, are spread by the fecal-oral route. Contamination may occur via a person's hands or from a surface to non-contaminated food or prepared/cooked food which is then consumed.

Bacterial food poisoning may be caused in two ways: either by the direct presence of bacteria in consumed food, or by toxins produced by bacteria that remain in the food.

Bacterial organisms responsible for direct infection include *Salmonella spp., shigella spp., Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Vibrio spp.* and *Yersinia enterocolitica.* The organisms that produce toxins are: *Staphylococcus aureus*, *Bacillus cereus, Clostridium botulinum, Clostridium perfringens* and *Vibrio cholera*. The food supply system of the United States and disinfectants used to protect it are regulated by a network of federal, state, and local agencies. At the federal level the U.S. Food and Drug Administration regulates a variety of aspects, including the use of direct and indirect food additives. The U.S. Department of Agriculture (USDA) regulates meat and poultry processing plants including authorizations for hand cleaners and sanitizing products. State and local health departments use their Food Codes as the basis for their rules for food preparation and food service operations.

Dining out, whether at a fast food outlet or a five-star restaurant, is a part of the American way of life. Disinfecting, sanitizing, and antimicrobial cleaning products play an important role in ensuring healthy restaurants. Products such as kitchen cleaners, table wiping solutions, toilet and bathroom cleaners, antimicrobial soap, and floor cleansers help one enjoy a pleasant dining experience.

While the overwhelming majority of restaurants are clean, there are estimates that as much as 70 percent of all food poisonings occur in public eating establishments.[2] Restaurant sanitation and safety are highly regulated by state and local agencies that regularly conduct extensive inspections of eating establishments to protect the public. Inspections focus on areas where food is stored, served, and consumed. Equipment and facilities such as stoves, refrigerators, plumbing, and restrooms are inspected closely.

While the most common cause of food borne illness from restaurants is the failure to maintain the proper food holding and storage temperatures, contamination from food handlers and from bacterial material on food surfaces also cause problems. Restaurant owners and managers use a variety of antimicrobial products to keep their facilities clean and germ free. A variety of products are available for use on kitchen equipment and surfaces, dining area tables, and food serving surfaces (such as buffet tables), and restroom facilities.

Bacteria, fungi and viruses can live and breed where we live, endangering our health and quality of life.

There is evidence that many communicable diseases, such as hepatitis, and gastrointestinal problems, including diarrhea and upper respiratory diseases, are spread through contact with contaminated environmental surfaces and inanimate objects. Although the risk of infection in the home is lower than in a hospital, nursing home, or day care center, problems such as food poisoning and illnesses such as the common cold can result from bacteria and viruses found in households.

Studies of the bacteria found in the home environment suggest four major sites of potential household contamination: wet areas (baths, sinks, toilets, drains, and others), dry areas (floors, furniture, linen, clothing, and others), food and people, similar to the locations of concern for hotels, motels, and restaurants. In many homes bacteria can also be found on animals (pets and farm animals) and on lawns and gardens near the home. High bacteria counts are found mainly in wet areas associated with sinks, baths, toilets and diaper pails. High bacteria counts are also frequently recovered from washcloths,

dishcloths, and cleaning towels, thus proving an immense need for antimicrobial textiles which could possibly be achieved through the use of current *N*-halamine technology.

While the battle against communicable diseases is constant, society does have help. Along with medical breakthroughs in drugs, vaccinations and surgical procedures, today's society is equipped with modern-day disinfectants. Various researchers have acknowledged that disinfectants, applied to contaminated surfaces and objects, can play a key role in reducing the spread of colds and other potentially infectious diseases.[19]

Disinfection is an important way to protect all family members from illness. Those with vulnerable immune systems such as newborns and children, the elderly, and people with a serious illness, or recovering from hospitalization, are particularly vulnerable. However household antimicrobial products can also protect healthy family members from problems such as food poisoning, the common cold, or other transmittable diseases.

Hotels and motels use antimicrobials in routine cleaning of guest rooms and on-premise laundering to ensure clean and sanitary bed and bath linens. The same procedures apply to health clubs. Disinfectants are used in public restrooms in hotels, restaurants, shopping malls, and other facilities frequented by the public to reduce or prevent the spread of infections.

The routes of transfer of disease-causing microorganisms include sinks, faucet handles, bathrooms, counters, tables and desktops. Regular hand washing, the use of disinfectant cleaners on surfaces and objects, and general cleanliness are important practices.

Hotels and motels are also often equipped with indoor and/or outdoor swimming pools and whirlpools. These pools create an additional need for antimicrobials. Microbial growth, while encouraged by sunlight, still can flourish in the indoor environment. The

use of antimicrobials for biological control is therefore necessary in the treatment of swimming pool and spa waters; otherwise, biological contamination would result, indicated by the water's discoloration and bad odor. Hotels and motels rely on antimicrobials, namely chlorine, to lower the risk of disease and increase aesthetics for their guests' recreational enjoyment.

Proper treatment of cooling towers, which are an integral part of temperature control systems in hotels and motels, also relies on the use of antimicrobials. Cooling towers, like air scrubbers, remove chemicals and biological contamination from the air and trap it in the cooling liquid. Without antimicrobials, such systems can rapidly become contaminated with a variety of harmful microorganisms. Hotels and motels use antimicrobials in these systems to prevent the spread of disease through ventilation equipment.

Hand washing while at work is most often discussed in connection with the food handling and food preparation industry where the consequences of poor personal hygiene could cause outbreaks of disease. However, in addition to the obvious exposures inherent in food handling, there are other activities encountered at work that lead to exposure to potentially harmful microorganisms. These include handshaking, exposure to sick colleagues in meetings, contact with the public, sharing objects such as public toilets, telephones, exercise equipment, and money, as well as other obvious situations such as those encountered by animal handlers or sanitation workers.

Viruses can live for a few hours on surfaces such as a doorknob, pencil, or some other object touched by someone who has been sneezing, handling tissues, or wiping his

or her face.[2] If a healthy person comes in contact with the same surface and also touches his or her own face, he can transfer the virus to portals of entry.

It is reasonable to believe that the presence of infectious viruses in our environment, deposited on a surface or inanimate object, constitutes a threat to the human welfare. If one can reduce the amount of virus infectivity on inanimate objects by the use of virucidal disinfectants, it is axiomatic that the opportunity for this virus to be transmitted to humans will be significantly reduced. Therefore a coating, which could be applied to these various surfaces, which could potentially not only kill the previously present bacteria and other harmful organisms, but prevent the further growth of such, could be of considerable value to society as a whole with numerous practical applications. Such technologies have been developed by Worley and co-workers through the use of various types of *N*-halamine technologies.[7, 9, 13, 20-23]

The research discussed herein focused on the synthesis and application of novel *N*-halamine biocides. The first appearance of *N*-halamine compounds in the literature was by Kovacic and co-workers in 1968, which was followed by several more publications the following years.[24-30] In 1976 Kaminski and co-workers were the first to synthesize and demonstrate the antimicrobial behavior of halogenated oxazolidinone compounds I (3-chloro-4,4-dimethyl-2-oxazolidinone) and IB (3-bromoo-4,4-dimethyl-2-oxazolidinone) Figure1**.**[30]

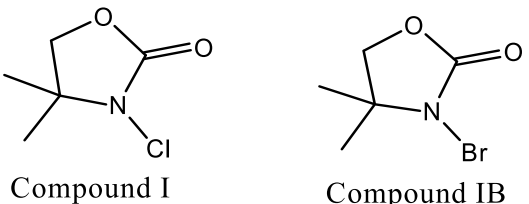


Figure 1. Structures of compound I and IB

However, it was not until 1987 that Worley and co-workers demonstrated that the oxazolidinones compounds were not adequate for use as a general-purpose disinfectant. However, a few years later they demonstrated that other types of *N*-halamines could be employed as effective biocides.[7-13, 15-17, 20-23]

Bacteria Review

" Biocide" is a general term used for describing a chemical substance which inactivates microorganisms, usually implying broad spectrum activity, meaning that it is effective in deactivating both Gram positive as well as Gram negative bacteria.[19] Since biocides range in their biocidal efficacies, many other terms can be used to describe more specific activity of the compound or procedure including "-cidal" when referring to compounds which kill the target microorganisms (e.g. bactericidal, sporicidal, and virucidal) as well as, "-static" implying compounds that can only inhibit the growth (e.g. bacteriostatic, sporistatic, and fungistatic) of such microorganisms.[19]

In order to discuss the efficacy of such various antimicrobial moieties, it becomes necessary to understand the mechanisms for their biocidal actions. Since the microorganisms of interest in this work are generally bacteria, a brief understanding of these bacterial cells is essential for further discussion.

Clinical bacteria are grouped into five categories based on Gram stain and appearance under the light microscope. Two groups have a general rounded shape, the cocci, and stain either red, indicating the stain was washed off implying Gram-negative, or blue indicating the stain was absorbed, implying Gram-positive. Two groups have a general rod-like shape and stain either red or blue, the bacilli. By far the largest group

numerically is the Gram-negative bacilli group. The bacteria of main concern for our discussion are *Staphylococcus aureus,* commonly notated as *S. aureus* a rounded shaped Gram-positive bacteria, and *Escherichia coli* commonly notated as *E. coli,* a rod shaped Gram- negative bacteria. *E. coli*, possesses an outer membrane layer which *S. aureus* does not have, and is attributed to not only the difference in stain indication, but the survival from some antimicrobials such as quaternary ammonium salts or quats. Gram-negative organisms have thicker cell walls, and the cell wall composition is different from that of Gram-positive organisms. This difference also accounts for some general distinctions in susceptibility to antibiotic drugs. Thus the Gram stain is considered by many as the single most important characteristic of clinical bacteria.

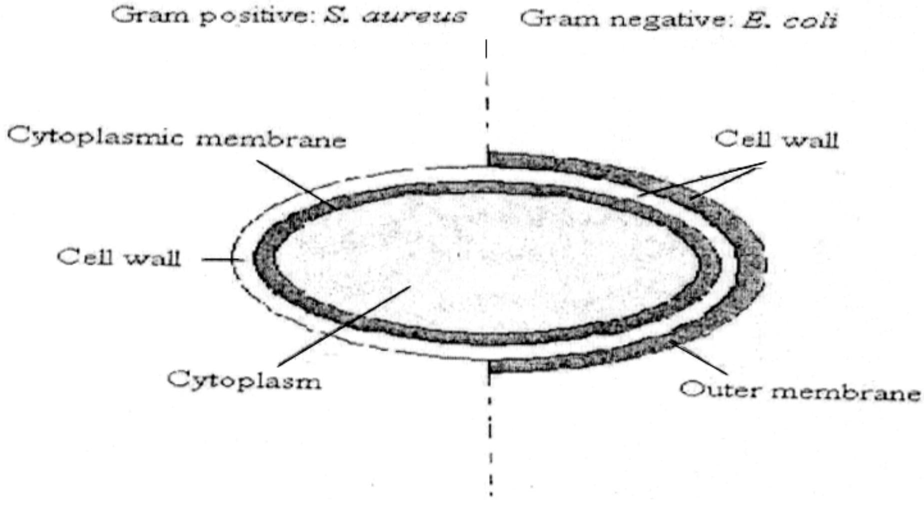


Figure 2. Structurial differences between *S. aureus* and *E. coli*[1]

Staphylococcus aureus causes a variety of suppurative pus-forming infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes, and

furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired nosocomial infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream.

Human staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defenses. The portal may be a hair follicle, but usually it is a break in the skin, which may be a minute needle-stick or a surgical wound. Foreign bodies, including sutures, are readily colonized by *Staphylococci*, which may make infections difficult to control. Another portal of entry is the respiratory tract. Staphylococcal pneumonia is a frequent complication of influenza. The localized host response to staphylococcal infection is inflammation, characterized by an elevated temperature at the site, swelling, the accumulation of pus, and necrosis of tissue. Around the inflamed area, a fibrin clot may form, walling off the bacteria and leukocytes as a characteristic pus-filled boil or abscess. More serious infections of the skin may occur, such as furuncles or impetigo. Localized infection of the bone is called osteomyelitis. Serious consequences of staphylococcal infections occur when the bacteria invade the blood stream. A resulting septicemia may be rapidly fatal; a bacteremia may result in seeding other internal abscesses, other skin lesions, or infections in the lung, kidney, heart, skeletal muscle, or meninges.

E. coli is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are faculatively anaerobic Gram-negative rods that live in the intestinal

tracts of animals in health and disease. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans.[2]

Mode of Activity and Spectrum of Biocides

For the last 30 years Dr. Worley's research labs have been concerned with the development of effective and regenerable biocides, namely *N*-halamines.[7, 8, 10-12, 14-17] This was in response due in part to the growing concerns over the wide spread of various antibiotic resistant bacteria and the increase of infectious diseases.[31-46] Nosocomial infections, are infections which were acquired or originated in a hospital or hospital-like settings such as nursing homes were reported to have occurred in one third of the patients who died while in the hospital, but there is no clear relationship between nosocomial infections and mortality.[47]

N-halamines in the broadest sence of the term simply refers to any organic compound containing any nitrogen halogen bond with the exception of fluorine as the halogen. However, later on we shall see that certain types of nitrogen halogen bonds are greatly preferred over others.

Numerous approaches have been employed such as the development of antimicrobial polymers, polymers which could potentially inactivate and/or inhibit the growth of microorganisms. This idea has attracted significant research interest from various researchers.[6-13, 15-17, 23, 30] One common thread among these groups is the effective approach of incorporating a wide variety of antimicrobial functional groups into existing

previously useful polymer structures. There has been a vast array of biocidal moieties which have been incorporated into various polymers such as quaternary ammonium salts,[4, 10, 15, 48-50] biguanides,[51-55] metal ions,[56-60] molecularly engineered peptides,[61] *N*-halamines,[7-9, 11, 13, 20-23] etc. which have been demonstrated to have shown some degree of usefulness in the development of new novel antimicrobial polymeric materials. It is of notable interest that the biocidal efficacies of these polymers demonstrate a significant difference from one another. Also, it should be pointed out that there are considerable differences in price between these various antimicrobials which may limit the actual commercial usefulness of the biocidal moiety in the variety of polymers mentioned. A brief discussion of the mode of action for these various biocidal moieties will help provide a more in depth understanding of why so many antimicobial agents are in current use today. For our purposes a review will only be discussed for those biocidal agents in wide spread use commercially.

Quaternary Ammonium Compounds

Surfactants, surface-active agents, have two primary regions in their molecular structures, one a water-attracting hydrophilic or polar group, and the other a hydrocarbon, water-repellent group. Depending on the presence of charge or lack of ionization of the hydrophilic group, surfactants can be classified into nonionic, anionic, and ampholytic, sometimes referred to as amphoteric compounds. Of these, the cationic agents that are of main concern for our purposes are exemplified by quaternary ammonium compounds (QACs), which are the most widely used in antiseptics and disinfectants. They are widely used as cationic detergents. Also, QACs have been used for a variety of clinical purposes, as well as hard surface cleaning and deodorization.

It has been well established for many years that QACs are membrane-active agents, with a predominately occurring target site of the cytoplasmic inner membrane of bacteria or the plasma membrane in yeasts.[62] In earlier works proposed by Salton, it was hypothesized that the following sequence of events for microorganisms exposed to cationic agents occurs: 1. adsorption and penetration of the agent into the cell wall; 2. a reaction with the cytoplasmic membrane, either lipid or protein, followed by membrane disorganization; 3. leakage of low molecular weight intracellular material; 4.degradation of proteins and nucleic acids; and 5. finally the wall lysis caused by autolytic enzymes. Therefore, a loss of structural integrity of the cytoplasmic membrane in bacteria accompanied by other damaging effects to the bacterial cell occurs.[63, 64]

QACs cause lysis of spheroplasts and protoplasts suspended in sucrose.[65, 66] The cationic reagents react with phospholipid components in the cytoplasmic membrane, thereby producing membrane distortion and protoplast lysis uder osmotic stress.[67] Isolated membranes do not undergo disaggregation on exposure to QACs, because the membrane distortion is not sufficiently drastic.

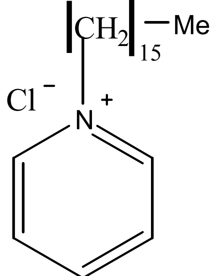
The proposed mechanism for the death of *S. aureus* by the use of QACs will now be evaluated. The cytoplasmic membrane provides the mechanism whereby metabolism is linked to solute transport, the generation of ATP, and flagellar movement. Protons are extruded to the exterior of the bacterial cell during metabolism. The combined potential of concentration or osmotic effect of the proton and its electropositivity is the PMF, which drives the ancillary activities. The QAC cetrimide or Alkyltrimethylammonium bromide is an atiseptic agent with detergent properties which can be seen in figure 3 and was found to have an effect on the PMF in *S. aureus*.[64] At its bacteriostatic concentration,

cetrimide caused the discharge of the pH component of the PMF and was also found to have the maximum amount of absorbing material. However, it is worth noting that some strains of Gram-positive bacteria such as *Staphylococci, Lactobacilli,* and Gram-negative *Pseudomonas* have become resistant to QACs.[68-70]

CH_3
Br^- +
R—N—CH_3
CH_3

Cetrimide is a generic name for Alkyltrimethylammonium bromide with the generic structure above. R= $C_{12}H_{25}$, or $C_{14}H_{29}$, or $C_{16}H_{23}$
Figure 3.

QACs, such as cetylpyridium chloride (CPC), as seen in figure 4., are also believed to have some effectiveness against Gram-negative bacteria. It is a common belief that QACs damage the outer membrane of gram-negative bacteria, thusly promoting their own uptake.



Cetylpyridium Chloride
Figure 4.

The QAC, cetylpyridium chloride, induces the leakage of K^+ and pentose material from yeast such as *S. cerevisiae* and induces protoplast lysis as well as interacting with crude cell sap.[71] In contrast it has been evidenced that QACs are ineffective in killing some Gram-negative bacteria such as strains of *P. stuartii*, which have been isolated from

urinary tract infections in paraplegic patients.[72] QACs are also ineffective against Gram-negatives such *as P. aeruginosa, Buvkholderia cepacia ,*and *Proteus spp.*[73, 74]

Biguanides

Chlorhexidine, as seen in figure 5, may be the most widely used biocide in antiseptic products, such as oral products and hand washing, as well as a disinfectant and preservative.[75] Its interaction and uptake by bacteria were initially studied by Hugo et al., who found the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and was dependant upon the chlorhexidine concentration and pH. In recent studies using labeled ^{14}C chlorhexidine gluctonate, the uptake by bacteria and yeast were shown to be very rapid with the maximum effect occurring within 20 s.[76, 77] While damage to the outer cell layer occurs, it is insufficient to cause lysiss or cell death.[78] The agent then crosses the cell wall or outer membrane, possibly by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. The yeast death is caused by partitioning into the cell wall, plasma membrane, and cytoplasm which causes leakage of intracellular constituents.[71] The leakage may not be the reason for cellular inactivation, but rather a consequence of cell death.

Figure 5 Chlorhexidine.

Chlorhexidine is not sporicidal even at high concentrations, and there is no affect of the viability of *Bacillus* spores at ambient temperatures,[79, 80] although a notable sporicidal effect is noticed at elevated temperatures. It is thought that sufficient changes occur to the spore structure to allow an increased uptake of the biguanide, but this is yet to be demonstrated experimentally. Very little is thoroughly understood about the uptake of chlohexidine by bacterial spores; however, it has been evidenced that coatless forms takeup more of the compound than do normal spores.[79] Biguanides such as chlorhexidine do have some draw backs, as reports of consumer irritability have been described in many cases where chlorhexidine is the active ingredient.[81] Also, the biocidal efficacy is not only pH dependant, but is greatly reduced by the presence of organic matter.[82]

Polymeric biguanides

Vantocil is a heterodisperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of around 3,000, as seen in figure 6. Polymeric biguanides have been employed as general disinfectants for use in the food industry and, very successfully, for the disinfection of swimming pools. Vantocil is active against Gram-positive and Gram-negative bacteria.

$$\left[\text{———NH—}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{—NH—}\overset{\text{NH}}{\overset{\|}{\text{C}}}\text{—NH—(CH}_2)_6\text{———} \right]_n \cdot x\ \text{HCl}$$

Figure 6 Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl) hydrochloride.

PHMB is a membrane active agent that is also capable of impairment of the integrity of the outer membrane of Gram-negative bacteria; however, the membrane could function as a permeability barrier.[54, 83] Activity has been seen to increase proportionally on a weight basis with increased levels of polymerization, which has been linked to enhanced inner membrane perturbation.[52, 84] Vantocil is not sporicidal and shows little antimicrobial efficacy toward *P. aeruginosa* and *Proteus vulgaris*.

Metal Ions

To narrow the scope of this discussion, the only metal of interest will be silver; however, the general idea and overall character of other metal biocides are similar. In one of its various forms silver and its derivatives have long been used as antimicrobial agents.[85, 86] The most widely used silver compound in current use is silver sulfadiazine (AgSD), as seen in figure 7., although there are numerous other silver containing antimicrobial agents such as silver metal and silver acetate.

Figure 7. Silver, [4-amino-N-(2-pyrimidinyl-κN1)benzenesulfonamidato-κO]

Silver sulfadiazine, AgSD, is essentially a combination of two antimicrobial agents, Ag^+ and sulfadiazine (SD). The question of whether the biocidal efficacy of AgSD occured predominately from only one of the compounds or through a synergestic pathway has been posed repeatedly. AgSD has a broad spectrum of activity and produces surface and membrane blebs in susceptible bacteria, but not in resistant bacteria.[87] AgSD

binds to cell components, including DNA.[88, 89] Fox et al. determined on the basis of chemical analysis that a polymeric structure of AgSD composed of six silver atoms bound to six SD ligands by silver-nitrogen bonds of the SD pyrimidine ring exists. Bacterial inhibition was hypothesized to be achieved when silver binds to sufficient base pairs in the DNA helix, thusly inhibiting transcription.[90] Comparatively, its antiphage properties have been attributed to the fact that AgSD binds to phage DNA.[91, 92] However, the exact mechanism of action of AgSD has yet to be accurately determined. One obviously glaring problem of AgSD as a common use biocide is its enormous price.

_N_-Halamines

Although *N*-halamines have a proven history of biocidal efficacy against both Gram-positive *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* there is little evidence in the literature supporting a definite mechanism for the mode of inactivation of such microorganisms.[20-23, 93] It is thought that the active moieties found in *N*-halamine compounds are both the free halogen cation species disassociated from the nitrogen, as well as the bound halogen in the N-X bond, which beares a partially positive charge. Both species are oxidizing agents and could possibly react with some chemical functionality of the microorganism's structure[93]. It is thought by Worley and others that the mode of inactivation of various microorganisms is similar to that of the class of compounds deemed chlorine-releasing agents or CRAs, only implying a pathway in which an oxidative halogen species is the active moiety, but emphasizing that free halogen is not the active species. Rather a direct transfer of the bound halogen from the *N*-halamine to the microorganism occurs.[93]

It has been hypothesized that the inactivation mechanism of *N*-halamines may involve such multiple pathways as: 1.alteration of key membrane integrity, 2. inactivation of key enzyme systems, 3. interference with nucleic acids, and 4. a combination of such with multiple points of attack. It has been established that chlorine oxidizes sulfhydryl-containing enzyme systems for normal cellular metabolism.[94-96] Chlorine has also been linked to the denaturization of the cell membrane resulting in the leakage of cellular components.[94, 97]

To adequately comprehend the mechanism proposed by Worley, an in depth examination of various structurally different *N*-halamines should be evaluated as well as some of the ideas previously put forth in the literature by Kaminiski. A listing of structurally different *N*-halamines can be seen in figure 9. This will help us understand the inverse relationship of stability of the halogen bond and the biocidal efficacy of the compound.[98] The cyclic organic *N*-halamines include the following: 1. 1,3-dichloro-5,5-dimetylhydantoin, 2. 1-bromo-3-chloro-5,5-dimethylhaydantoin, 3. 1-chloro-3-bromo-5,5-dimethylhydantoin, 4. N-chlorosuccinimide, 5. Tetrachloroglycoluril, 6. tetrachlorodimethylglycoluril, 7. 1-chloro-2,2,6,6-tetramethylpiperidine, 8. 1-chloro-2,2,6,6-tetramethylpiperidinol, 9. 1-chloro-2,2,6,6-tetramethylpiperidone, 10.1,3,5-tichloro-2,4-dioxohexahydrotriazine, 11. Trichlorisocyanurate, 12. 1,4-dichloro-2,2,5,5-tetramethyl-3,6-piperazinedione, 13. 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (TTDD), and 14.trichloromelamine.

The various stabilities of these numerous *N*-halamines can be accounted for by three factors:[25,117] 1. The presence or absence of hydrogen atoms adjacent to the N-X group is a structural key in a dehydrohalogenation reaction, which produces H-X. If hydrogen

atoms are present, then the reaction can proceed following the mechanism seen in figure 9. However, if alkyl groups are substituted in that position, it prevents this reaction from occurring, thusly stabilizing the N-X bond. 2. The presence of either electron donating or electron withdrawing groups adjacent to the N-X moiety also influences the overall stability of the N-X bond. In the case where there are electron donating groups such as methyl groups adjacent to the N-X moiety, electrons are released, thereby creating a destabilization effect of any negative charge that would start to build on N if X^+ would begin leaving, thusly preventing release of a cationic species such as X^+ through N-X bond dissociation. Conversely, if an electron-withdrawing group such as a carbonyl group were adjacent to the N-X bond of interest, the carbonyl would delocalize the nitrogen's lone pair by resonance and would act to stabilize the incurring negative charge developing on the N as X^+ began to disassociate, ultimately supporting the release of a cationic species such as X^+. 3.The presence of bulky hydrophobic alkyl groups may prohibit water molecules from hydrolyzing the N-X moiety through steric effects.

Figure 8 Dehydrohalogenation mechanism.

Since the transition state structure has a partial broken N-Cl bond just like the partially broken C-Cl bond in the S_N2 transition state, we expect that changing from one halide to another will produce the same change in rate that we see for the S_N2 mechanism: RI > RBr > RCl > RF, therefore implying that brominated *N*-halamines are far less stable than their chlorinated counter parts if no alkyl substitution is present adjacent to the N-X bond.

Figure 9. Structures of various *N*-halamines

The stability of the N-X bond is dependant upon which halogen X represents. For *N*-halamines, in general, X could represent either chlorine Cl, or bromine Br, but usally not iodine nor fluorine. In the case of fluorine it is simply too electronegative to participate in a nitrogen-fluorine bond. Typically the stability of the N-X bond is attributed to the extent in which bond overlap occurs between the halogen and nitrogen. The greater the bond overlap between the nitrogen and the halogen, the greater the stability of the *N*-halamine. For this reason iodine is usually excluded from possible candidates for the halogen in a N-halamine. Since chlorine is smaller than bromine it has a much greater degree of bond overlapping with nitrogen than does bromine. Thus, the order of stability as implied in terms of bond overlapping, is N-chloramines > N-bromamines > N-iodamines.

Predominately those microorganisms which are inactivated with free halogen follow the typical rule that the stability of the disinfectant is inversely related to its antimicrobial efficacy.[98] Kaminski and co-workers reinforce the concept of the inverse relationship between hydrolytic stability of *N*-halamines in aqueous solutions and the biocidal efficacy achieved, as well as the relationship these two phenomena have with the oveall structure of the *N*-halamine, especially those of the type classified as organic *N*-chloramines.[99-101] The stability of the nitrogen-chlorine bond with respect to hydrolysis and the corresponding biocidal efficacy of the *N*-chloramine was closely related to the ionization potential of the nitrogen lone pair in the *N*-chloramine. The N-chloramine, which possesses the most polar nitrogen-chlorine bond is most susceptible to hydrolysis, and therefore possesses the most unstable nitrogen-chlorine bond. Kaminski also determined a bacteristatic relationship between the bond polarity of the nitrogen-chlorine

bond which increased as the polarity of the nitrogen-chlorine bond increased. Thus, the most potent *N*-chloramine biocide is that which possesses the most polar nitrogen-chlorine bond, but it is also the most unstable. This general rule is also inversely applicable.

Review of *N*-halamine developments by the Worley group

Dr. Worley began his research of *N*-halamines in the 1980s with a goal to develop new halogen stabilizers for the purpose of disinfecting potable water. Earlier works from Bodor et.al convinced him to examine water soluble organic *N*-chloramines in the general class of oxazolidinones as potential commercially useful additives for water purification.[30, 99-101] This was due to their desirable chemical attributes such as most are solids which are soluble in water, bactericidal, and tend not to form any unwanted organic impurities i.e. trihalomethanes. Studies were carried out to determine the biocidal efficacy and halogen stability under various conditions of pH and temperature. After several studies interest shifted toward organic compounds of the general class imidazolidinones, compounds in this general family were found to be more stable in aqueous environments. However, once halogenated both classes of compounds possessed large UV absorption bands and were determined to be inadequate for most general purpose needs such as swimming pools and spas. Combined with the fact that novel water soluble antimicrobial materials typically require extensive and costly testing procedures before approval of such compounds could be given by various regulatory agencies. A fact which, is exacerbated for those materials with an intended use in disinfecting potable and recreational water supplies. Therefore, through the progression of innovation focus shifted once again this time to water insoluble compounds, which

could possibly be employed as water filters thereby reducing the effort needed for regulatory approval. This idea was achieved by chemical alterations of a uniformed, highmolecular weight, polystyrene beads.[11, 15, 17] These beads have been employed in various applications ranging from the removal of odor from cutting oils, selective oxidation of thiols, and the eradication of bacteria, microbes, and viruses from water. This leads us to our current projects of interest, the development of biocidal coatings and textiles. Numerous approaches have been investigated throughout the years, but the most successful has been the coatings of the *N*-halamine siloxane family.[7, 49, 99] Siloxanes have been proven to be extremely versatile with the ability to either chemically attach or physically adhere to a wide number of surfaces some of which include natural and synthetic fibers, as well as, stainless steal, silica gel, sand, formica, and ceramic.[99] This will be of great importance to the works discussed herein.

References

1. Mdigan MT, Martinko JM, parker J. Biology of Microorganisms Eighth ed. New Jersey: Pentice-Hall Inc., 1997.
2. Murray PR, Rosenthal KS, Kobbayashi GS, Pfaller MA. Medical Microbiology. Fourth ed. St. Louis: Mosby Inc., 2002.
3. Maloy SR, Cronan Jr JE, Freifelder D. Microbial Genetics. Boston: Jones and Bartlett Publishers, 1994
4. Majtan V, Majtanova L. Antibacterial efficacy of disinfectants against some gramnegative bacteria. Central European journal of public health 2002;10(3):104-106.
5. El-Masry AM, Moustafa HY, Ahmed AI, Shaaban AF. Halamine polymers: 1. Preparation and characterisation of new pyrimidinone biocidal polymers based on poly-4-vinylacetophenone. Pigment & Resin Technology 2004;33(2):75-84.
6. Chen Z, Sun Y. Antimicrobial polymers containing melamine derivatives. II. Biocidal polymers derived from 2-vinyl-4,6-diamino-1,3,5-triazine. Journal of Polymer Science, Part A: Polymer Chemistry 2005;43(18):4089-4098.
7. Liang J, Barnes K, Chen Y, Worley SD, Lee J, Broughton RM, et al. New rechargeable biocidal N-halamine siloxanes. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-048.
8. Liang J, Huang TS, Worley SD. Coating new N-halamine siloxanes on silica gel to prepare biocidal silica gels. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-049.

9. Barnes K, Liang J, Wu R, Worley SD, Lee J, Broughton RM, et al. Synthesis and antimicrobial applications of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]. Biomaterials 2006;27(27):4825-4830.
10. Xing X, Wang X. Quaternary ammonium salt grafted PE films. Gaofenzi Xuebao 2005(1):157-160.
11. Chen Y, Worley SD, Kim J, Wei CI, Chen T-Y, Suess J, et al. Biocidal Polystyrenehydantoin Beads. 2. Control of Chlorine Loading. Industrial & Engineering Chemistry Research 2003;42(23):5715-5720.
12. Nurdin N, Helary G, Sauvet G. Biocidal polymers active by contact. III. Aging of biocidal polyurethane coatings in water. Journal of Applied Polymer Science 1993;50(4):671-678.
13. Sun G, Worley SD. Halamine chemistry and its applications in biocidal textiles and polymers. Modified Fibers with Medical and Specialty Applications 2006:81-89.
14. Anonymous. Preventing Emerging Infectious Diseases: A Strategy for the 21st Century. Overview of the Updated CDC plan. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control 1998;47(RR-15):1-14.
15. Chen Y, Worley SD, Huang TS, Weese J, Kim J, Wei CI, et al. Biocidal polystyrene beads. III. Comparison of N-halamine and quat functional groups. Journal of Applied Polymer Science 2004;92(1):363-367.

16. Panangala VS, Liu L, Sun G, Worley SD, Mitra A. Inactivation of rotavirus by new polymeric water disinfectants. Journal of Virological Methods 1997;66(2):263-268.

17. Sun G, Chen TY, Habercom MS, Wheatley WB, Worley SD. Performance of a new polymeric water disinfectant. Water Resources Bulletin 1996;32(4):793-797.

18. White CG. Handbook of Chlorinationand Alternative Disinfectants. Fourth ed. Danvers: John Wiley and Sons, 1999.

19. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clinical Microbiology Reviews 1999;12(1):147-179.

20. Worley SD, Williams DE, Barnela SB, Elder ED, Swango LJ, Kong L. New halamine water disinfectants: Dep. Chem.,Auburn Univ.,Auburn,AL,USA.; 1987.

21. Worley SD, Williams DE, Barnela SB. The stabilities of new N-halamine water disinfectants. Water Research 1987;21(8):983-988.

22. Williams DE, Worley SD, Barnela SB, Swango LJ. Bactericidal activities of selected organic N-halamines. Applied and Environmental Microbiology 1987;53(9):2082-2089.

23. Barnela SB, Worley SD, Williams DE. Syntheses and antibacterial activity of new N-halamine compounds. Journal of Pharmaceutical Sciences 1987;76(3):245-247.

24. Kovacic P, Roskos PD. Chemistry of N-halamines. XIII. Amination of adamantanes and their precursors with trichloramine-aluminum chloride. Journal of the American Chemical Society 1969;91(23):6457-6460.

25. Kovacic P, Lowery MK, Roskos PD. Chemistry of N-halamines. XIV. Amination of exo-2-chloronorbornane and norbornane with trichloramine-aluminum chloride. Tetrahedron 1970;26(2):529-538.

26. Kovacic P, Lowery MK. Chemistry of N-halamines. XII. Amination of alkyl halides with trichloramine-aluminum chloride. Journal of Organic Chemistry 1969;34(4):911-917.

27. Kovacic P, Gormish JF, Hopper RJ, Knapczyk JW. Chemistry of N-halamines. XI. Side-chain amination of aryldialkylmethines with trichloramine-aluminum chloride-tert-butyl bromide. Journal of Organic Chemistry 1968;33(12):4515-4520.

28. Kovacic P, Field KW. Chemistry of N-halamines. XVI. Chlorination of alkenes with trichloramine. Journal of Organic Chemistry 1971;36(23):3566-3571.

29. Field KW, Kovacic P, Herskovitz T. Chemistry of N-halamines. XV. Amination of cycloalkanes with trichloramine-aluminum chloride. Journal of Organic Chemistry 1970;35(7):2146-2151.

30. Kaminski JL, Huycke MM, Selk SH, Bodor N, Higuchi T. N-Halo derivatives V. Comparative antimicrobial activity of soft N-chloramine systems. Journal of Pharmaceutical Sciences 1976;65(12):1737-1742.

31. Kenny JF, Isburg CD, Michaels RH. Meningitis due to Haemophilus influenzae type b resistant to both ampicillin and chloramphenicol. United States; 1980.

32. Weiss J, Beckerdite-Quagliata S, Elsbach P. Resistance of gram-negative bacteria to purified bactericidal leukocyte proteins: relation to binding and bacterial lipopolysaccharide structure. The Journal of clinical investigation 1980;65(3):619-628.
33. Voropaeva SD, Ankirskaia AS, Abramova ZI, Mironova TG. Antibacterial therapy and hospital infection. Antibiotiki 1980;25(8):619-622.
34. Stickler DJ, Thomas B. Antiseptic and antibiotic resistance in Gram-negative bacteria causing urinary tract infection. Journal of Clinical Pathology 1980;33(3):288-296.
35. Shaw WV, Brenner DG, Murray IA. Regulation of antibiotic resistance in bacteria: the chloramphenicol acetyltransferase system. Current topics in cellular regulation 1985;26:455-468.
36. Seifert J, Duswald KH, Lob G. What therapeutic possibilities exist in acute antibiotic-resistant and chronic infections. Fortschritte der Medizin 1982;100(13):592-598.
37. Schrinner E. Bacterial antibiotic resistance, development and distribution. Deutsche medizinische Wochenschrift (1946) 1981;106(9):276-280.
38. Saedler H. Antibiotic resistance factors and "jumping" genes (author's transl). Arzneimittel-Forschung 1980;30(3a):529-533.
39. Richmond M. Antibiotic resistance and the evolution of bacteria. Nature 1983;302(5910):657.
40. Marthi B, Fieland VP, Walter M, Seidler RJ. Survival of bacteria during aerosolization. Applied and environmental microbiology 1990;56(11):3463-3467.

41. Markowitz SM, Veazey JM, Jr., Macrina FL, Mayhall CG, Lamb VA. Sequential outbreaks of infection due to Klebsiella pneumoniae in a neonatal intensive care unit: implication of a conjugative R plasmid. The Journal of infectious diseases 1980;142(1):106-112.
42. Linton AH. Antibiotic resistance in bacteria associated with animals and their importance to man. Journal of Antimicrobial Chemotherapy 1985;15(4):385-386.
43. Kreger BE, Craven DE, Carling PC, McCabe WR. Gram-negative bacteremia. III. Reassessment of etiology, epidemiology and ecology in 612 patients. The American journal of medicine 1980;68(3):332-343.
44. Farrar WE, Jr. Plasmids and antibiotic resistance in gram-negative bacteria. Journal of the South Carolina Medical Association (1975) 1980;76(2):71-76.
45. Datta N. Bacterial resistance to antibiotics. Ciba Foundation symposium 1984;102:204-218.
46. Baaj AJ, Nejmi S, Mellouki W. Resistance phenotypes to 4 antibiotics of bacteria isolated in a hospital setting. Maroc medical 1981;3(1):501-514.
47. Gross PA, Van Antwerpen C. Nosocomial infections and hospital deaths. A case-control study. The American journal of medicine 1983;75(4):658-662.
48. Oosterhof Janine JH, Buijssen Kevin JDA, Busscher Henk J, van der Laan Bernard FAM, van der Mei Henny C. Effects of quaternary ammonium silane coatings on mixed fungal and bacterial biofilms on tracheoesophageal shunt prostheses. Applied and environmental microbiology 2006;72(5):3673-3677.

49. Liang J, Chen Y, Barnes K, Wu R, Worley SD, Huang TS. N-halamine/quat siloxane copolymers for use in biocidal coatings. Biomaterials 2006;27(11):2495-2501.

50. Gottenbos B, van der Mei HC, Klatter F, Nieuwenhuis P, Busscher HJ. In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials 2002;23(6):1417-1423.

51. MacGregor KM, inventor; (Ebiox Limited, UK). assignee. Antimicrobial skin composition comprising a biguanide or a quaternium compound. Application: WO Patent No. 2005-GB27552006013315, 2006 20050714.

52. Gilbert P, Pemberton D, Wilkinson DE. Synergism within polyhexamethylene biguanide biocide formulations. Journal of Applied Bacteriology 1990;69(4):593-598.

53. Broxton P, Woodcock PM, Heatley F, Gilbert P. Interaction of some polyhexamethylene biguanides and membrane phospholipids in Escherichia coli. Journal of Applied Bacteriology 1984;57(1):115-124.

54. Broxton P, Woodcock PM, Gilbert P. Binding of some polyhexamethylene biguanides to the cell envelope of Escherichia coli ATCC 8739. Microbios 1984;41(163):15-22.

55. Broxton P, Woodcock PM, Gilbert P. A study of the antibacterial activity of some polyhexamethylene biguanides towards Escherichia coli ATCC 8739. Journal of Applied Bacteriology 1983;54(3):345-353.

56. Wagener M, Vissing KD, Salz D, Steinruecke P, inventors; (Bio-Gate Bioinnovative Materials G.m.b.H., Germany; Fraunhofer-Gelellschaft zur Foerderung der Angewandten Forschung E. V.). assignee. Antimicrobial composite material. Application: WO Patent No. 2004-EP13030 2005048708, 2005 20041117.
57. Samad NA, Back DD, inventors; (Mainstream Engineering Corp., USA). assignee. Water disinfection method using metal-ligand complexes. Application: US Patent No. 95-417988 5632904, 1997 19950406.
58. Rioux ML, Ciccognani DT, Palys TJ, Turley PA, inventors; (Arch Chemicals, Inc., USA). assignee. Pyrithione biocides enhanced by zinc metal ions and organic amines. Application: US Patent No. 2002-325016 2004253194, 2004 20021220.
59. Garcia Rodriguez A, inventor; (Laboratorios Natysal, S.A., Spain). assignee. Biocidal glasses containing cations for treatment of water circuits. Application: ES Patent No. 2001-200101475 2190732, 2003 20010626.
60. Nagar R. Structural and microbial studies of some transition metal complexes. Journal of inorganic biochemistry 1989;37(3):193-200.
61. Jayaraman A, Yarmush ML, Roth CM. Molecular bioengineering. Industrial & Engineering Chemistry Research 2002;41(3):441-455.
62. Hugo WB, Frier M. Mode of action of the antibacterial compound dequalinium acetate. Applied microbiology 1969;17(1):118-127.
63. Salton MRJ. Lytic agents, cell permeability, and monolayer penetrability. Journal of General Physiology 1968;52(1)(Pt. 2):227S-252S.

64. Denyer SP, Hugo WB. The mode of action of tetradecyltrimethyl ammonium bromide (CTAB) on Staphylococcus aureus. Journal of Pharmacy and Pharmacology 1977;29(Suppl., Br. Pharm. Conf. 1977):66P.

65. Kanazawa A, Ikeda T, Endo T. A novel approach to mode of action of cationic biocides: morphological effect on antibacterial activity. Journal of Applied Bacteriology 1995;78(1):55-60.

66. Davies A, Field BS. Action of biguanides, phenols and detergents on Escherichia coli and its sphereoplasts. The Journal of applied bacteriology 1969;32(2):233-243.

67. Cabral JPS. Mode of antibacterial action of dodine (dodecylguanidine monoacetate) in Pseudomonas syringae. Canadian Journal of Microbiology 1992;38(2):115-123.

68. Sundheim G, Langsrud S, Heir E, Holck AL. Bacterial resistance to disinfectants containing quaternary ammonium compounds. International Biodeterioration & Biodegradation 1998;41(3-4):235-239.

69. Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. Society for Applied Microbiology Symposium Series 2002;31(Antibiotic and Biocide Resistance in Bacteria):121S-135S.

70. Holah JT, Taylor JH, Dawson DJ, Hall KE. Biocide use in the food industry and the disinfectant resistance of persistent strains of Listeria monocytogenes and Escherichia coli. Society for Applied Microbiology Symposium Series 2002;31(Antibiotic and Biocide Resistance in Bacteria):111S-120S.

71. Hiom SJ, Furr JR, Russell AD, Dickinson JR. Effects of chlorhexidine diacetate and cetylpyridinium chloride on whole cells and protoplasts of Saccharomyces cerevisiae. Microbios 1993;74(299):111-120.

72. Stickler DJ. Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: a perspective from the study of catheter biofilms. Society for Applied Microbiology Symposium Series 2002;31(Antibiotic and Biocide Resistance in Bacteria):163S-170S.

73. Russell AD, Russell NJ. Biocides: Activity, action and resistance. Symposium of the Society for General Microbiology 1995;53rd(Fifty Years of Antimicrobials: Past Perspectives and Future Trends):327-365.

74. Russell AD. Mechanisms of bacterial resistance to biocides. International Biodeterioration & Biodegradation 1995;36(3/4):247-265.

75. Denyer SP. Mechanisms of action of antibacterial biocides. International Biodeterioration & Biodegradation 1995;36(3/4):227-245.

76. Hiom SJ, Furr JR, Russell AD, Dickinson JR. Effects of chlorhexidine diacetate on Candida albicans, C. glabrata and Saccharomyces cerevisiae. The Journal of applied bacteriology 1992;72(4):335-340.

77. Fitzgerald KA, Davies A, Russell AD. Uptake of 14C-chlorhexidine diacetate to Escherichia coli and Pseudomonas aeruginosa and its release by azolectin. FEMS microbiology letters 1989;51(3):327-332.

78. el Moug T, Rogers DT, Furr JR, el-Falaha BM, Russell AD. Antiseptic-induced changes in the cell surface of a chlorhexidine-sensitive and a chlorhexidine-resistant strain of Providencia stuartii. The Journal of antimicrobial chemotherapy 1985;16(6):685-689.
79. Shaker LA, Furr JR, Russell AD. Mechanism of resistance of Bacillus subtilis spores to chlorhexidine. The Journal of applied bacteriology 1988;64(6):531-539.
80. Shaker LA, Dancer BN, Russell AD, Furr JR. Emergence and development of chlorhexidine resistance during sporulation of Bacillus subtilis 168. FEMS Microbiology Letters 1988;51(1):73-76.
81. Rosenberg A, Alatary SD, Peterson AF. Safety and efficacy of the antiseptic chlorhexidine gluconate. Surgery, gynecology & obstetrics 1976;143(5):789-792.
82. Russell AD, Day MJ. Antibacterial activity of chlorhexidine. The Journal of hospital infection 1993;25(4):229-238.
83. Gilbert P, Collier PJ, Brown MR. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrobial agents and chemotherapy 1990;34(10):1865-1868.
84. Gilbert P, Pemberton D, Wilkinson DE. Barrier properties of the Gram-negative cell envelope towards high molecular weight polyhexamethylene biguanides. Journal of Applied Bacteriology 1990;69(4):585-592.
85. Russell AD, Hugo WB. Antimicrobial activity and action of silver. Progress in Medicinal Chemistry 1994;31:351-370.
86. Brown MR, Anderson RA. The bactericidal effect of silver ions on Pseudomonas aeruginosa. The Journal of pharmacy and pharmacology 1968;20:Suppl:1S+.

87. Coward JE, Carr HS, Rosenkranz HS. Silver sulfadiazine: effect on the growth and ultrastructure of Staphylococci. Chemotherapy 1973;19(6):348-353.

88. Rosenkranz HS, Rosenkranz S. Silver sulfadiazine: interaction with isolated deoxyribonucleic acid. Antimicrobial agents and chemotherapy 1972;2(5):373-383.

89. Modak SM, Fox CL, Jr. Binding of silver sulfadiazine to the cellular components of Pseudomonas aeruginosa. Biochemical Pharmacology 1973;22(19):2391-2404.

90. Fox CL, Jr. Topical therapy and the development of silver sulfadiazine. United States; 1983.

91. Rahn RO, Setlow JK, Landry LC. Ultraviolet irradiation of nucleic acids complexed with heavy atoms. III. Influence of silver(+) ions and mercury(2+)ions on the sensitivity of phage and of transforming DNA to ultraviolet radiation. Photochemistry and Photobiology 1973;18(1):39-41.

92. Fox CL, Jr., Modak SM. Mechanism of silver sulfadiazine action on burn wound infections. Antimicrobial agents and chemotherapy 1974;5(6):582-588.

93. Williams DE, Elder ED, Worley SD. Is free halogen necessary for disinfection? Applied and Environmental Microbiology 1988;54(10):2583-2585.

94. Venkobachar C, Iyengar L, Rao AVSP. Mechanism of disinfection: effect of chlorine on cell membrane functions. Water Research 1977;11(8):727-729.

95. Knox WE, Stumpf PK, Green DE, Auerbach VH. The inhibition of sulfhydryl enzymes as the basis of the bactericidal action of chlorine. Journal of Bacteriology 1948;55:451-458.

96. Green DE, Stumpf PK. The mode of action of chlorine. Journal - American Water Works Association 1946;38:1301-1305.

97. Friberg L, Hammarstrom E. The action of free available chlorine on bacteria and bacterial viruses. Acta pathologica et microbiologica Scandinavica 1956;38(2):127-134.

98. Tsao TC, Williams DE, Worley CG, Worley SD. Novel N-halamine disinfectant compounds. Biotechnology Progress 1991;7(1):60-66.

99. Worley SD, Chen Y, Wang J-W, Wu R, Li Y, inventors; (Auburn University, USA; Vanson Halosource, Inc.). assignee. N-halamine siloxanes for use in biocidal coatings and materials. Application: WO Patent No. 2003-US18883 2003106466, 2003 20030612.

100. Kaminski JJ, Bodor N, Higuchi T. N-Halo derivatives IV. Synthesis of low chlorine potential soft N-chloramine systems. Journal of Pharmaceutical Sciences 1976;65(12):1733-1737.

101. Kaminski JJ, Bodor N, Higuchi T. N-halo derivatives. III: Stabilization of nitrogen-chlorine bond in N-chloroamino acid derivatives. Journal of Pharmaceutical Sciences 1976;65(4):553-557

BIOCIDAL HYDANTOINYLSILOXANE POLYMERS. V. SYNTHESIS AND APPLICATIONS OF 5,5'-ETHYLENEBIS[5-METHYL-3-(TRIETHOXY-SILYLPROPYL) HYDANTOIN] FOR DURABLE AND REGENERABLE ANTIMICROBIAL SURFACES

ABSTRACT

A novel, durable, long lasting, *N*-halamine siloxane monomer precursor, 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] has been prepared and characterized by ^{1}H-NMR and FTIR for the purpose of functionalizing the surfaces of various materials. The precursor *N*-halamine moiety was attached to the surfaces by siloxane covalent bonding or physical adhesion to the material surfaces. Simulated laundering tests indicated that the chlorinated *N*-halamine structure could survive many repeated home launderings. The materials were rendered biocidal after exposure to oxidative halogen solutions, ie. dilute household bleach. Once chlorinated, these materials were biocidal against *S .aureus* and *E. coli*. Upon loss of the halogen from either long-term use or consumption by the microbes on the surfaces, they could be simply re-charged by further exposure to dilute bleach to regain biocidal activity.

1. Introduction

Recently there has been a growing concern about how to reduce or eliminate completely infections, especially those caused by antibiotic-resistant, Gram-positive

bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), and Vancomycin-resistant *Enterococci* (VRE). These bacteria have been shown to have long survival times on commonly used hospital fabrics, such as hospital privacy drapes, scrub suits, and lab coats.[1,2] The survival and transfer of microorganisms between patients and health care workers have been documented.[3-6] The medical gowns and uniforms used currently have been proven to provide ineffective barriers for health care workers in numerous studies.[7-11] This demonstrates a great need for antimicrobial textiles and polymers that are able to protect against all major pathogens.[12-14] *N*-halamine compounds could provide such protection since they have shown excellent biocidal functions against a wide range of microorganisms such as fungi, bacteria, viruses, and yeasts.[15-17] In addition, *N*-halamines have demonstrated the capability of rapid and total inactivation of various microorganisms without causing the microorganisms to develop resistance to them.[18] The stability of *N*-halamines is directly related to their structures, which is evidenced by their dissociation constants in solution.[19] Furthermore, *N*-halamine structures are capable of killing microorganisms directly without the release of free chlorine into the system.[20] *N*-halamines can be composed of amine, amide, and imide halamine bonds, the dissociation constants for which are presented in Table 1.[19]

Table 1
Stability of N-halamine Structures[19]

Dissociation Reaction	Dissociation Constant For Example
Imide Structure: imide N–Cl $\overset{H_2O}{\rightleftharpoons}$ imide N–H + Cl^+	1.6×10^{-2} - 8.5×10^{-4} Trichlorocyanuric acid; 2.54×10^{-4} 1,3-dichloro-5,5-dimethylhydantoin
Amide Structure: amide N–Cl $\overset{H_2O}{\rightleftharpoons}$ amide N–H + Cl^+	2.6×10^{-8} 1,3-dichloro-2,2,5,5-tetramethylimidazolidin-4-one; 2.3×10^{-9} 3-chloro-4,4-dimethyl-2-oxazolidinone
Amine Structure: $R_2N–Cl \overset{H_2O}{\rightleftharpoons} R_2N–H + Cl^+$	$< 10^{-12}$ 1-Chloro-2,2,5,5-tetramethylimidazolidin-4-one

It is thought that those structures containing amide halamine bonds are of the most practical use since they exhibit a moderate rate of transfer of active chlorine from the *N*-halamine structures in aqueous solution to cells of organisms and provide reasonably rapid biocidal activity. The equilibrium of dissociation of a halamine in aqueous solution can best be understood from equations 1 and 2.

$$>N-Cl + H_2O \rightleftharpoons >NH + Cl^+ + OH^- \quad (1)$$

$$>N-Cl \underset{\text{Bleach}}{\overset{\text{Inactivate Pathogens}}{\rightleftharpoons}} >NH \quad (2)$$

The amine halamine bonds are the most stable, but offer a slower kill rate than do the amide halamines, and although the imide halamine offers a very rapid kill rate, it is the least stable structure and can rapidly lose active chlorine. Thus, the amide function seems to be a reasonable compromise between stability and biocidal efficacy. With this in mind, the precursor 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] was designed so that once chlorinated, it would provide an effective biocide which would be capable of having two or more siloxyl bonds to surfaces. This could enable a textile coated with it to withstand numerous home laundering cycles, providing a durable and effective broad spectrum biocidal textile. Examples of surfaces and materials which can be rendered biocidal with the *N*-halamine siloxanes include cellulose, synthetic fibers, ceramics, plastics, polyurethanes, and metals.[17] Upon loss of the halogen from either long-term use or consumption by the microbes on the surfaces, surfaces such as textiles can be simply recharged by further exposure to dilute bleach and thus regain their biocidal activity.[21-23] The limiting factor of the biocidal efficacy ultimately is how readily the *N*-halamine, and/or the precursor, can be washed off the surfaces of the materials. Prior work on a lower molecular-weight monomer 3-triethoxysilylpropyl-5,5-dimethylhydantoin has shown excellent biocidal efficacy on textiles, but a tendency to hydrolyze off the fabrics after extended machine washings.[22] Therefore, it seemed reasonable that increasing the molecular weight, hydrophobicity, and the number of chemical bonds to the surface would provide for an effective and durable antimicrobial coating for various materials.

2. Experimental

2.1 Materials.

All chemicals were purchased from the Aldrich Chemical Company, Milwaukee, WI and used as they were received without further purification unless otherwise noted. The fabric used was (Style 400 Bleached 100% Cotton print Cloth, Testfabics, Inc., West Pittston, PA). 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). The Trypticase soy agar employed was from (Difco Laboratories, Detroit, MI).

2.3 Instruments.

The NMR spectra were obtained using a Bruker 400 MHz spectrometer; the IR data were obtained with a Shimadzu IR Prestige-21 FTIR. Tensile strengths were measured with a model 1122 Instron Universal Materials Testing Machine. The reactor used was a Parr 4841 high pressure reactor.

2.4 Preparation of 5,5'-(1,2-ethanediyl)bis[5-methylhydantoin] Scheme (1).

Scheme 1

$H_3C-C(=O)-CH_2-CH_2-C(=O)-CH_3$ (Acetonylacetone) $\xrightarrow[\text{1:1 EtOH / } H_2O \text{ (V/V), 200mL; } 85^0C\text{, 72 h}]{\text{KCN (4 eq), } (NH_4)_2CO_3 \text{ (8 eq)}}$ 5,5'-(1,2-ethanediyl)bis[5-methylhydantoin]

5,5'-(1,2-ethanediyl)bis[5-methylhydantoin] was prepared, for example in one experiment, by adding 5.71g (0.05 mol) of 97% acetonylacetone to a 250 mL stainless steel vessel, along with 13.02 g (0.20 mol) of 97% potassium cyanide and 38.44 g (0.40 mol) of 98% ammonium carbonate in 200 mL of a 1:1 (V/V) mixture of ethanol and water as solvent. This mixture was then sealed and heated to 85^0C with constant stirring for 72 h. The reactor vessel was then allowed to cool to room temperature, and the reaction mixture was poured into a 500 mL beaker containing 300 mL of water. The precipitate formed was then vacuum filtered from the mother liquor which was then neutralized to a pH ≈7 by drop wise addition of a 6 N HCl acid solution over a period of 90 min. The precipitate was the desired product, which was dried in a vacuum oven overnight at 45^0C. A light tan solid was weighed, and (6.41g, 50.4% yield) was obtained. Spectroscopic data obtained for 5,5'-(1,2-ethanediyl)bis[5-methylhydantoin] were: ^{1}H NMR (d_6 DMSO) δ 1.27 (s, 6H), 1.58(m,4H), 7.97 (s, 1H), 7.99 (s, 1H) 10.60 (s, 2H); IR (KBr) 1715, 1735, 3207 cm^{-1}.

2.5 Preparation of 5,5'-ethylenebis[5-methyl-3-(3triethoxysilylpropyl)hydantoin] Scheme (2).

Scheme 2

1. NaOH (2 eq), EtOH, 25 mL, Reflux
2. DMF, 50mL, 115^0C, 16h, 3-Chloropropyltriethoxysilane (2eq)

5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] was prepared, for example in one experiment, by first preparing the sodium salt of 5,5'-(1,2-ethanediyl)bis[5-methylhydantoin]. This was done by mixing 1.00 g ($3.90x10^{-3}$ mol) of 5,5'-(1,2-ethanediyl)bis[5-methylhydantoin] in a 100 mL round bottom flask with 0.159 g ($3.90x10^{-3}$ mol) of 98% sodium hydroxide and 25 mL of absolute ethanol as solvent. This solution was then refluxed for 30 min with constant stirring. The sodium salt was isolated by removing the ethanol under reduced pressure and was then placed in a vacuum oven overnight at 45^0C to remove any water produced as a byproduct. Once 1.17 g of sodium salt were obtained, 50 mL of anhydrous DMF were added as solvent. When the salt had been dissolved, the reaction solution was heated to 45^0C, and 1.94 g ($7.80x10^{-3}$ mol) of 97% 3-chloropropyltriethoxy silane were added drop wise over a period of 25 min. Once all of the 3-chloro propyltriethoxysilane had been added, the temperature was then raised to 115^0C, and the reaction mixture was stirred constantly overnight. After cooling to ambient temperature, the KCl produced in the reaction and the DMF solvent were removed by filtration and evaporation, respectively. The KCl was then dried in an oven and weighed as one method to determine the percent yield of the reaction. Any DMF residual was removed with a hexane extraction, and the dark gold oil left behind was the desired product with a mass of 2.54 g which corresponded to a 97.7% yield. The mass of the KCl was 0.273 g which corresponded to a 94% yield. Some spectroscopic data for 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] were: ^{1}H NMR (d_6 DMSO) δ 1.16 (4 H), 1.19 (18 H), 1.20 (6 H), 1.36 (4 H), 1.37 (2 H), 3.7(4H), 3.9 (12 H), 7.96(1 H); IR (KBr) 773, 862, 1080, 1167, 1713, 1769, 2887, 2974, 3289, 3520 cm^{-1}.

2.6 Coating Procedure

The 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer was coated onto the surfaces of cotton swatches by soaking the swatches in baths containing 5% by weight of the crude product of the aforementioned monomer dissolved in a 1:1 (w/w) mixture of ethanol and water. After the soaking procedure, the coated swatches were cured at 95^0C for 1 h. Then, the swatches were soaked in 0.5% detergent solution for 15 min, followed by several water rinses to remove any weakly bonded coating.

2.7 Chlorination Procedure

The coated cotton swatches were chlorinated by soaking them in a 10% aqueous solution of NaOCl household bleach buffered to pH 7 at ambient temperature for 30 min. The Chlorinated swatches were washed with water and dried at 45^0C for 1 h to remove any occluded free chlorine. The loading of bound chlorine on the swatches was determined as described in the analytical titration procedure.

2.8 Analytical Titration Procedure

For the determination of oxidative chlorine (Cl^+) content, a standard iodometric/thiosulfate titration procedure was employed. For example, about 0.3 g of coated and chlorinated cotton swatch material was suspended in 50 mL of 0.1 N acetic acid solution. After addition of 0.3 g KI, and starch as an indicator, the solution was titrated with 0.0375 N sodium thiosulfate until the blue color disappeared at the end

point. The weight percent Cl^+ on the cotton swatches could then be determined from the equation below:

$$\% Cl^+ = [N \times V \times 35.45 / (2 \times W)] \times 100\% \quad (3)$$

where, N and V are the normality (eqv/L) and volume (L), respectively, of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in g of the cotton swatch sample.

2.9 Biocidal Efficacy Testing

One inch square cotton swatches, some uncoated to serve as controls, others coated with 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer, but unchlorinated, to serve as a second type of control, and others coated with chlorinated 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer were prepared. Dried swatches were then challenged with either *Staphylococcus aureus* ATCC 6538 or *Escherichia coli* O157:H7 ATCC 43895 using a "sandwich test". In this test, 25 μL of bacterial suspension were placed in the center of a swatch, and a second identical swatch was laid upon it which was held in place by a sterile weight to insure good contact of the swatches with the inoculum. The bacterial suspensions employed for the tests contained from 10^6 to 10^7 colony forming units (CFU), the actual number determined by counting after spread-plating on Trypticase soy agar plates. After contact times of 5.0, 10.0, and 30.0 min, the various swatches were placed in sterile conical centrifuge tubes, each containing 5.0 mL of sterile 0.01 N sodium thiosulfate to quench any oxidative free chlorine which might have been present, and vortexed for 150 s to remove bacteria. Then the swatches were removed, and serial dilutions of the quenched solutions were plated on

Trypticase soy agar. The plates were incubated at 37^0C for 24 h and then counted for viable CFU of bacteria.

2.10 Washing and Durability of Coatings

Laundering tests were performed on swatches of cotton coated with the 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer prepared as detailed above. Then one half of the swatches were chlorinated following the aforementioned procedure. All types of coated swatches were subjected to laundry washing cycles using AATCC Test Method 61 (Test 2A Procedure). Samples were evaluated after 5, 10, 25, and 50 washing cycles for retention of the coatings. Those samples not chlorinated before washing were chlorinated by the procedure described above in order to assess how much chlorine could be loaded after variable numbers of washing cycles. Those chlorinated before washing were divided into two groups, with half being assessed for chlorine loading without rechlorination, the other half being rechlorinated and then assessed for chlorine loading.

3 Results

For cotton swatches treated with 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer as described in the experimental section and chlorinated, it was found that the average oxidative chlorine loading on the swatches was 0.66% by weight. The treated cotton swatches were challenged with *S. aureus* and *Escherichia coli* O157: H7 at a concentration between 10^7 and 10^8 CFU/ mL in pH 7 phosphate buffer solution using a modified version of AATCC method 100. It was found

that all *S. aureus* colonies (>6.0 logs) were inactivated by the swatches treated with chlorinated 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer in the contact interval of 10 to 30 min; whereas, the swatches treated with the unchlorinated monomer experienced only a 0.2 log reduction at 30 min (Table 2). The control sample (untreated cotton swatches) gave only a 0.2 log reduction as well for the 30 min interval. It was also found that all *E. coli* (7.5 logs) were inactivated by the swatches treated with the chlorinated 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer in the contact interval of 10-30 min; whereas, the unchlorinated monomer and the untreated swatches experienced only a 0.15 log reduction in the same contact time interval (Table 3). Thus, it can be determined that the cotton cloth treated with 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer, once chlorinated, became capable of rapid and total inactivation of both Gram positive and Gram negative bacteria. In comparison, the siloxane monomer described previously[22] was found to have deactivated > 5.7 logs of *S. aureus* in a contact interval of 10-30 min., while a contact interval of 60-120 min. was necessary to inactivate >5.9 logs of *E.* coli.

Table 2

The biocidal efficacies of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]-treated cotton swatches against *S. aureus.*

Sample coating	Log reduction in a contact time of (min)		
	1.0	10.0	30.0
Exp. 1[a]			
Cotton Control	0.26	0.30	0.29
Unchlorinated Monomer	0.04	0.10	0.21
Chlorinated Monomer	2.04	3.52	6.90
Exp. 2[b]			
Cotton Control	0.09	0.18	0.20
Unchlorinated Monomer	0.02	0.07	0.06
Chlorinated Monomer	2.82	3.89	6.62

[a]Inoculum was 8.00 x 10^6 CFU; Cl^+ loading was 0.634%.

[b]Inoculum was 4.17 x 10^6 CFU; Cl^+ loading was 0.691%.

Table 3

The efficacies of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]- treated cotton swatches against *E. coli* O157:H7.

Sample Coating	Log reduction in a contact time of (min)		
	1.0	10.0	30.0
Exp. 3[a]			
Cotton Control	0.02	0.03	0.15
Unchlorinated Monomer	0.07	0.11	0.15
Chlorinated Monomer	0.39	4.18	7.56
Exp. 4[b]			
Cotton Control	0.04	0.13	0.22
Unchlorinated Monomer	0.17	0.21	0.29
Chlorinated Monomer	4.86	6.68	6.68

[a]Inoculum was 3.63 x 10^7 CFU; Cl^+ loading was 0.634%.

[b]Inoculum was 4.83 x 10^6 CFU; Cl^+ loading was 0.691%.

Finally, the results of the wash test performed on swatches of cotton containing the monomer coating, some chlorinated as outlined in the chlorination procedure, and others unchlorinated for comparison purposes, are presented in Table 4.

Table 4

Durability of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]- treated cotton swatches.

Number of Laundering Cycles	Cl^+ % When Chlorinated Before Laundering[a]	Cl^+ % When Chlorinated After Laundering[b]	Cl^+ % When Chlorinated After Recharge[c]
0	0.62	0.62	0.62
5	0.53	0.52	0.62
10	0.48	0.45	0.59
25	0.37	0.37	0.47
50	0.32	0.28	0.42

Number of Laundering Cycles	% Cl^+ Remaining On The Cotton Surfaces		
0	100	100	100
5	85	84	100
10	77	73	95
25	60	60	76
50	52	45	68

[a] The treated cotton swatches were chlorinated before they were laundered.

[b] The treated cotton swatches were chlorinated after they were laundered.

[c] The treated cotton swatches were chlorinated, laundered, and then re-chlorinated.

The average oxidative chlorine loading for the monomer coated sample was 0.62% by weight. Three observations are clearly evident from the data in Table 4. First, the monomeric coatings are partially washed off the surfaces upon successive laundering

cycles. Second, prechlorination reduces the rate of loss, perhaps due to the increased hydrophobicity of the chlorinated surfaces. Third, it is evident for all of the coating conditions that a sufficient biocidal efficacy could be regenerated upon rechlorination even after 50 laundering cycles. Furthermore, a low concentration of bleach added to the laundering cycles could possibly maintain biocidal activity of the cotton material for its lifetime. In comparison, the 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilyl propyl) hydantoin] monomer has a retention of 68 % on the cotton surfaces after 50 laundering cycles; whereas, the 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer only had 22 % retention after 50 laundering cycles.[22] As a result, the novel compound discussed herein is a much more effective and durable coating for textile fabrics.

4 Conclusion

The postulate proposed herein, ie. that increasing the potential covalent bonding to the surface by use of the 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl) hydantoin] instead of the lower molecular weight monomer 3-triethoxysilylpropyl-5,5-dimethylhydantoin, has been validated.

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References

1. Neely,A.N.; Maley, M.P. J Clin Microbiol 2000;38:724.
2. Slaughter, S.;Hayden, M.K.; Nathan,C.; Hu, T.C.; Rice,T.; Van Voorhis, J.; Matushek,M.; Franklin, C.; Weinstein, R.A. Ann Intern Med 1996;125: 448.
3. Lidwell, O. M.; Towers, A.G.; Ballard, J.; Gladstone, B. J Appl Bact 1974;37: 649.
4. Ransjo, U. J Hyg Camb 1979;82: 369.
5. Rubbo, S.D; Saunders, J. J Hyg Camb 1963;61:507.
6. Hambraeus, A. J Hyg Camb 1973;71:799.
7. Smith, J. W.; Nichols R. L. Arch Surg 1991;126:756.
8. Quebbeman, E. J.; Telford, G. L.; Hubbard, S.; Wadsworth, K.; Hardman B.; Goodman, H.; Cottlieb, M. S. Annal Surg 1991;214:614.
9. Lovitt, S.A.; Nichols, R. L.; Smith, J. W.; Muzik, A. C.; Pearce, P.E. Am J Infect Control 1992;20:185.
10. Beck W. C.; Collette, T. S. Am J Surg 1952;83:125.
11. Granzow, J. W.; Smith, J. W.; Nichols R.L.; Waterman, R. S.; Muzik, A. C. Am J Infect Control 1998;26:85.
12. Worley, S.D.; Sun, G. Trends Polym Sci 1996;11:364.
13. For example, see review articles: Worley SD, Williams DE. Crit Rev Environ Contrl 1988;18:133-175; Worley SD, Sun G. Trends Polym Sci 1996;4:364-370.
14. Liang, J.; Chen, Y.; Barnes, K.; Wu, R.; Worley, S. D.; Huang, T.-S. Biomaterials 2006;27:2495-2501.
15. Tsao, T. C.; Williams,D. C.; Worley, S. D.; Ind Eng Chem Res 1990;29:2161.

16. Sun, G.; Chen T. Y.; Habercom, M. S.; Wheatley, W. B.; Worley S. D. Water Resour Bull 1996;32:793.
17. Halamine siloxanes for use in biocidal coatings and materials. Worley, Shelby D.; Chen, Yongjun; Wang, Jia-Wang; Wu, Rong; Li, Yanjun. (Auburn University, USA; Vanson Halosource, Inc,) PCT Int. Appl. (2003).
18. Sun, G.; Xu, X.; Bickett, J. R.; Williams, J. F. Text Chem Colorist 1998;30:26-30
19. Qian, L.; Sun, G. J Appl Polym Sci 2003; 89: 2418-2425.
20. Williams, D. E.; Elder, E. D.; Worley, S. D. Appl. Environ. Microbiol. 1998;54;2583-2585.
21. Worley SD, Li F, Wu R, Kim J, Wei-C-I, Williams JF, et al. Surf Coat Int Part B: Coat Trans 2003;86:273-7.
22. Worley SD, Chen Y, Wang J-W, Wu R, Cho U, Broughton RM, et al. Surf Coat Int Part B: Coat Trans 2005;88:93-9.
23. Williams JF, Suess J, Santiago J, Chen Y, Wang J, Wu R, et al. Surf Coat Int Part B: Coat Trans2005;88:35-9

N-Halamine/Quat Siloxane Copolymers for Use in Biocidal Coatings

ABSTRACT

A series of copolymers incorporating N-halamine siloxane and quaternaryammonium salt siloxane units has been prepared. The primary function of the quat units was to render the siloxane copolymers soluble in water. The copolymers have been coated onto cotton swatches and evaluated for biocidal efficacy against *Staphylococcus aureus* and *Escherichia coli* O157:H7. It was determined that both N-halamine and quat functional groups were effective against *S. aureus*, but only the N-halamine units were effective against *E. coli* O157:H7. The copolymers should be useful for applications for which aqueous media is preferred over organic solvents to be used during coating procedures.

1. Introduction

Work in these laboratories for over two decades has proceeded concerning the development of novel heterocyclic biocidal N-halamine derivatives[1] which have long-term stabilities in contact with aqueous solution, as well as in dry storage. The compounds, deriving from the classes oxazolidinones, imidazolidinones, and hydantoins, function as contact biocides against a broad spectrum of pathogens including bacteria, viruses, fungi, and protozoa[1]. Although the original work done in these laboratories concerned water-soluble N-halamine monomers, more recently the direction of the studies has focused on functionalizing insoluble polymers with N-halamine moieties.[2-17]

The fields of biocidal and oxidative polymers have expanded rapidly in recent times, and outstanding work has been concentrated on several classes of polymers, eg. the halogenated poly(styrene-divinylbenzenesulfonamides)[18], polymeric phosphonium materials[19], and polymeric quaternary ammonium compounds[20]. The N-halamine polymers developed in our laboratories possess several attributes which are advantageous such as the ability to immobilize high concentrations of chlorine to enable rapid biocidal activities, the capability of regeneration upon exposure to aqueous free halogen after the initial charge is spent, and the liberation of very low amounts of corrosive free chlorine into water (less than 1 mg/L).

Probably the most important N-halamine polymers which have been developed in our laboratories are the N-halogenated poly(styrenehydantoins),[2-6,11-14] because of their potential for economical disinfection of potable water, thus improving world health. However, in recent work here[7-10,15-17] and elsewhere[21-24] the technology has been extended to the creation of N-halamine biocidal coatings for textiles and hard surfaces. A very promising biocidal coating developed in these laboratories applicable to a variety of surfaces is an N-chlorohydantoinyl siloxane which can be employed in a coating bath either in its monomeric, or in an oligomeric, form.[16,17] Both forms are soluble in organic solvents such as ethanol or ethanol/water mixtures. However, there are applications for which an aqueous coating bath may be desirable in order to avoid the presence of organic solvents. In the current work copolymers of the hydantoinyl siloxane and a quaternary ammonium salt siloxane, which are adequately soluble in water alone, have been prepared and employed to coat cotton swatches. After chlorination with household bleach, the coated swatches have been shown to exhibit excellent biocidal efficacy

against the Gram positive and Gram negative bacteria *Staphylococcus aureus* and *Escherichia coli* O157:H7, respectively. The structures of the copolymers are illustrated in Scheme 3.

2. Experimental Section

2.1 Preparation of Hydantoinyl/Quat Siloxane Polymers

The starting material for all of the homopolymers and copolymers discussed herein was poly(3-chloropropylsiloxane)(PCPS) prepared from the monomer 3-chloropropyltriethoxysilane (Aldrich Chemical Company, Milwaukee, WI) as discussed previously.[17] The homopolymer poly[3-(5,5-dimethylhydantoinylpropyl)siloxane](PHS) was synthesized by reacting PCPS with the potassium salt of 5,5-dimethylhydantoin (Aldrich Chemical Company, Milwaukee, WI); characterization data (1H NMR (Bruker 400 MHz), IR (Shimadzu IR Prestige-21 FTIR), and EA (Atlantic Microlabs)) have been reported.[17] Yields, based upon a repeating unit, exceeded 95 %. The quat homopolymer poly[3-dimethyldodecylammoniumsiloxane chloride](PQS) was prepared by reacting PCPS with dimethyldodecylamine (Aldrich Chemical Company, Milwaukee, WI) in a 1:1 molar ratio based upon a repeating unit of PCPS. For example, in one experiment 6.92 g (0.05 mol) of PCPS were dissolved in 50 mL of DMF. To this solution were added 11.0 g (0.05 mol) of 97 % dimethyldodecylamine. The reaction mixture was stirred at 100^0C for 12 h. After cooling to ambient temperature, the KCl produced in the reaction and the DMF solvent were removed by filtration and evaporation, respectively. Any DMF residual was removed with a hexane extraction, and the white solid product was dried under vaccum overnight at 50^0C before further use. The yield, as determined by a titration procedure to be described, was about 85 % based upon a repeating unit.

Some spectroscopic data for the PQS were: ^{1}H NMR (d_6 DMSO) δ 0.86, 1.25, 1.66, 1.79, 3.07, 3.33, 3.63; IR (KBr) 702, 914, 1129, 1468, 1484, 1628, 2854, 2925, 2956, 3000-3700 cm^{-1}.

The hydantoinyl/quat siloxane copolymers, poly[3-(5,5-dimethylhydantoinylpropyl)siloxane-co-3-dimethyldodecylammoniumpropylsiloxane chloride](PHQS) were prepared by two different procedures. In a two-step process (Scheme 3) the molar ratio of hydantoin salt and PCPS were controlled so as to produce approximately a desired value of n in the first step, and then this was repeated in a second step with the product of the first step and an approriate amount of dimethyldodecylamine to produce a desired value of m. For example, to produce a PHQS with values of n and m of 0.5, 6.92 g (0.05 mol) of PCPS was mixed with 4.15 g (0.025 mol) of the potassium salt of 5,5-dimethylhydantoin in 50 mL of DMF. After stirring the mixture for 5 h at 100^0C, and removing the KCl produced by filtration, 5.50 g (0.025 mol) of dimethyldodecylamine were added, and the reaction mixture was stirred at 100^0C for an additional 8 h. Removal of DMF and drying overnight under vacuum at 50^0C produced a 78.3 % yield of a white solid product (^{1}H NMR (d_6 DMSO) δ 0.56, 0.85, 1.26, 1.56, 1.66, 2.68, 3.04, 3.33, 3.60; IR (KBr) 699, 774, 912, 1122, 1279, 1421, 1450, 1469, 1707, 1768, 2855, 2927, 3000-3700 cm^{-1}.

Scheme 3.

In a one-step process (Scheme 4) PCPS, the potassium salt of 5,5-dimethylyhydantoin, and dimethyldodecylamine were simply mixed in a molar ratio of 0.5:0.25:0.25, respectively, in one pot. The reaction was run with stirring at 100^0C for 12 h and worked up as discussed above; the yield of white solid was 77.5 % in this case. The two methods gave very similar products as determined by ^{1}H NMR and IR.

Scheme 4

2.1. Coating Procedure

The various polymers and copolymers were coated onto the surfaces of cotton swatches (Style 400 Bleached 100 % Cotton print Cloth, Testfabrics, Inc., West Pittston, PA) by soaking the swatches in baths containing about 0.15 mol/L of each compound dissolved in distilled water for 15 min. Since PHS has very low solubility in water, a 1:1 w/w mixture of ethanol and water was used for this homopolymer; this procedure was also necessarily followed for the copolymer in Scheme 3 in which the values of n and m were 3 and 1, respectively. After the soaking procedure, the coated swatches were cured at 95^0C for 1 h and then further at 145^0C for 20 min. Then the swatches were washed in 0.5 % detergent solution for 15 min followed by several water rinses to remove any weakly bonded coating.

2.2. Chlorination Procedure

The coated cotton swatches were chlorinated by soaking them in a 10 % aqueous solution of NaOCl household bleach (Clorox, Inc., Oakland, CA) buffered to pH 7 at ambient temperature for 45 min. The chlorinated swatches were washed with water and dried at 45^0C for 1 h to remove any occluded free chlorine. The loading of bound chlorine on the swatches was determined as described below.

2.3. Analytical Titration Procedures

Two types of titration procedures were used, one to estimate oxidative chlorine loadings in PHS and in the copolymers PHQS, the other to estimate quat content in PQS and in the PHQS copolymers. For determination of oxidative chlorine (Cl^+) content, a modified iodometric/thiosulfate titration procedure was employed in which the usual solvent water was necessarily replaced by a mixture of ethanol and 0.1 N acetic acid (9:1 v/v). For example, about 0.3 g of coated and chlorinated cotton swatch material was suspended in a solution of 90 mL ethanol and 10 mL 0.1 N acetic acid. After addition of 0.2 g KI, the mixture was titrated with 0.0375 N sodium thiosulfate until the yellow color disappeared at the end point. The weight percent Cl^+ on the cotton swatch could then be determined from the equation below:

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

where N and V are the normality and volume, respectively, of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in g of the cotton swatch sample.

The quat contents of PQS and the PHQS copolymers could not be determined for the coated cotton swatches. However, a modified ion association titration method developed for quaternary ammonium salts[25] was used to estimate the percentage of quat

functional groups present in the polymers. In this method 0.020 to 0.050 g of PQS or PHQS was dissolved in 50 mL of 0.05 N acetic acid. To the solutions were added 3 drops of 0.5 % bromophenol blue/ethanol as an indicator. The titrant was 0.0100 N sodium tetraphenylborate, and the end point was determined by a color change from blue to light yellow. The weight percent quat was calculated according to the following equation:

(4) % Quat = [N X V X M / W] X 100 %

where N and V are the normality and volume consumed, respectively, of sodium tetraphenylborate solution, M is the molecular weight of a quat repeating unit, and W is the weight in g of the PQS or PHQS sample.

2.4. Biocidal Efficacy Testing

One inch square cotton swatches, some uncoated to serve as controls, others coated with PHS, but unchlorinated, to serve as a second type of control, and others coated with chlorinated PHS, chlorinated PHQS, or PQS, were rinsed thoroughly with water. All samples containing quat functional groups were vortexed for 30 s in 10 mL of distilled, deionized water to remove any occluded quaternary ammonium salt. These swatches were removed from the vortex tube, and the water was tested for the presence of eluted quat by adding 2 drops of 0.5 % bromophenol blue indicator. A blue color indicated the presence of some eluted quat in the water. Such samples were subjected to further washing in 0.5 % detergent solution and subsequent rinsing until the eluted water from the vortex tube, after exposure to bromophenyl blue, remained yellow.

Dried swatches were then challenged with either *Staphylococcus aureus* ATCC 6538 or *Escherichia coli* O157:H7 ATCC 43895 (American Type Culture Collection,

Rockville, MD) using a “sandwich test”. In this test 25 μL of bacterial suspension was placed in the center of a swatch, and a second identical swatch was laid upon it, held in place by a sterile weight to insure good contact of the swatches with the inoculum. The bacterial suspensions employed for the tests contained from 10^6 to 10^7 colony forming units (CFU), the actual number determined by counting after spread-plating on Trypticase soy agar (Difco Laboratories, Detroit, MI) plates. After contact times of 0.5, 5.0, 10.0, and 30.0 min, the various swatches were placed in sterile conical centrifuge tubes, each containing 5.0 mL of sterile distilled, deionized water, and vortexed for 15 s to remove bacteria. Then the swatches were removed, 50 μL of sterile 0.01 M sodium thiosulfate were added to quench any oxidative free chlorine which might have been present, and serial dilutions of the quenched solutions were plated on Trypticase soy agar. The plates were incubated at 37^0C for 24 h and then counted for viable CFU of bacteria.

3. Results and Discussion

3.1 Preparation of Hydantoinyl/Quat Siloxane Polymers

Characterization of the various polymers and copolymers synthesized in this work is problematic. For the hydantoinyl siloxane homopolymer PHS, the 95 % yield based upon a repeating unit, the ^{1}H NMR, IR, and EA data,[17] and oxidative chlorine titration results all indicated that almost all of the propyl groups on PCPS were substituted with hydantoinyl functional groups. However, the molecular weight of PHS homopolymer does vary depending upon the preparation procedure (eg. about 11,000 D using the method cited herein, but about 4000 D if a hydantoinylpropylsilane is polymerized in acidic solution)[17]; nevertheless, the biocidal properties of PHS do not seem to vary with molecular weight.[17] In the case of the quat siloxane homopolymer

PQS, the modified titration procedure described in Section 2.4 suggested that the yield was only about 85 %. Probably the reason for less conversion for PQS than for PHS can be attributed to steric hindrance caused by the large dodecyl group.

For the copolymers PHQS characterization becomes more difficult. It can be assumed that the copolymers are random ones with varying substitution patterns on the backbone with hydantoin and quat functional groups. That both types of functionalities are present can be seen from the IR spectra in Figure 1 for a copolymer PHQS which was designed to be about 1:1 in the two types of functionalities. The two carbonyl stretching vibrational modes for any hydantoin compound give rise to two IR bands in the 1700-1810 cm^{-1} range which are clearly present for PHS and the 1:1 PHQS polymers. Furthermore, the presence of the C-H stretching modes with corresponding IR bands at 2854-2855 and 2925-2927 cm^{-1} for PQS and PHQS indicate the presence of quat functional groups in the PHQS copolymer. The data in Table 5 illustrate further the relative difficulty of functionalization of the siloxane polymers with quats versus hydantoins. Clearly as the value of m in Scheme 3 increases relative to n, the reactivity of the siloxane with the dimethyldodecylamine declines.

Table 5
Comparison of the weight percent quat functional group in the siloxane polymers as predicted theoretically with those determined by ion association titration

Designed Polymer or Copolymer	**% Quat Predicted Theoretically**	**% Quat Determined by Titration**
PQS	100	85.23 $\pm$ 0.48
PHQS (1H:1Q)	60.44	51.24 $\pm$ 0.58
PHQS (3H:1Q)	33.75	23.25 $\pm$ 0.68

3.2. Coating Procedures

The primary goal of this work was to enhance solubility of PHS in water, so as to avoid as much as possible organic solvents. Although exact solubilities were not measured, it was found that greater than the 5 % solubility necessary in an aqueous coating bath was obtained for PQS and PHQS for n values (Scheme 3) less than or equal to 0.5, ie. a hydantoin:quat ratio of 1:1. For a hydantoin:quat ratio of about 3:1, the solubility decreased to about 3 %. The PHS homopolymer could only be dissolved in ethanol/water mixtures.

The cotton swatches coated in this work, and subsequently chlorinated, retained their white color and did not undergo significant deterioration.

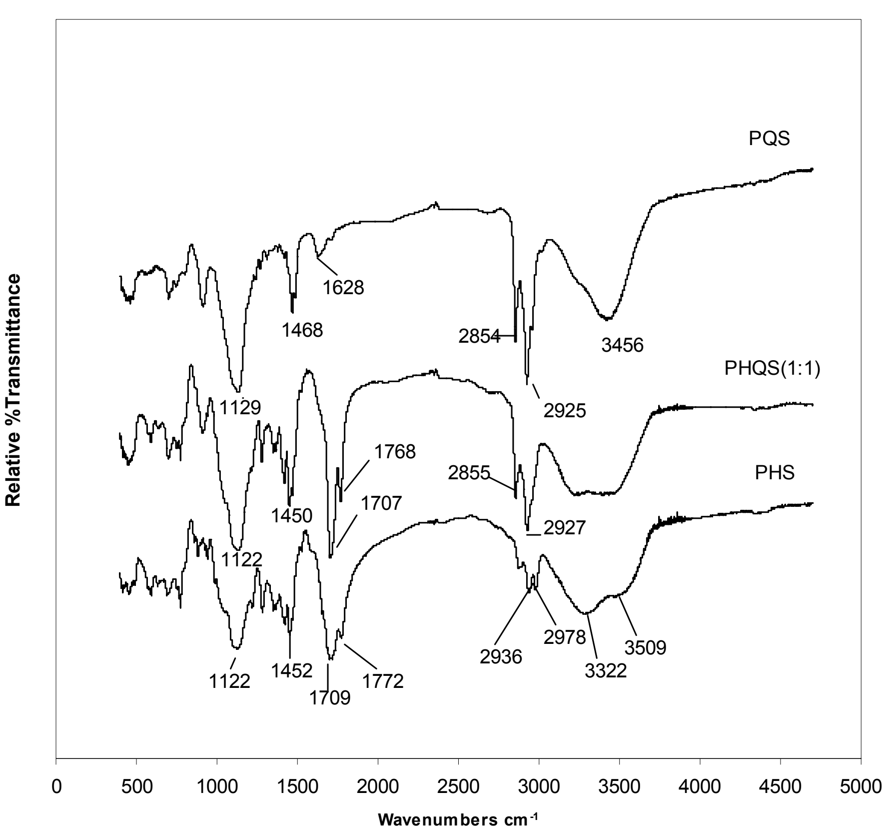

Figure 10. The FTIR spectra of homoploymers PHS and PQS and of copolymer PHQS (1H:1Q)

3.2 **Determination of Chlorine Loadings**

For the cotton swatches coated with all of the polymers, it was found that the traditional iodometric/thiosulfate titration procedure employing dilute acetic acid, KI, and starch was best modified by use of a solution containing a 9:1 (v/v) ratio of ethanol and 0.1 N acetic acid without the usual starch indicator. The problems were accentuated as the quat content of the coatings increased. As can be seen in Table 6, the modification greatly accelerated the titration procedure by producing an easily observed end point (yellow to clear).

Table 6

Comparison of oxidative chlorine titration methods for coated cotton swatches containing chlorinated siloxane polymers and copolymers.

Designated Polymer or Copolymer	Weight % Cl^+ Using Traditional Titration[a]	Time Required to End Point (min)[a]	Weight % Cl^+ Using Modified Titration[b]	Time Required to End Point (min)[b]
PHS	0.92 ± 0.01	20	0.89 ± 0.01	1 - 2
PHQS (3H:1Q)	0.74 ± 0.01	30	0.78 ± 0.01	2
PHQS (1H:1Q)	0.40 ± 0.01	>180	0.50 ± 0.01	2 - 3
PHQS (1H:3Q)	0	>180	0.19 ± 0.01	3 - 4
PQS	0		0	

[a] Sample suspended in 0.1 N acetic acid solution for iodometric/thiosulfate titration (see text).

[b] Sample suspended in ethanol/0.1 N acetic acid (9:1 v/v) solution for iodometric/thiosulfate titration (see text).

The presence of starch in the titration causes a deep blue color on the swatches themselves which does not disappear during the titration leading to difficulty in determining end points. In addition, for those samples containing PQS and PHQS copolymers, the I_3^- formed during the iodometric titration procedure can associate strongly with the positively charged nitrogen of the quat functionality in the coating leading to large errors in the oxidative Cl^+ determination in a normal titration procedure. The presence of ethanol circumvents this problem as I_3^- is very soluble in this solvent leading to rapid and accurate titration with the sodium thiosulfate. From the data in Table 6 one can see that PHQS swatches containing a hydantoin:quat ratio of about 3:1 did not indicate the presence of any oxidative chlorine using the traditional iodometric/thiosulfate

titration procedure. We strongly recommend the use of the modified titration procedure discussed herein for oxidative chlorine determinations for N-halamine polymeric coatings.

Although the typical chlorine loadings reported in Table 6 may seem low, it will be demonstrated in the next section that the biocidal efficacies of the coated swatches are quite good.

3.3 Biocidal Efficacy Testing

The biocidal efficacy data for PHS, PHQS (1:1), and PQS for Gram positive *S. aureus* and Gram negative *E. coli* O157:H7 are presented in Tables 7 and 8, respectively. The tests were conducted in duplicate in separate experiments. In the first experiment the swatches coated with PHS contained 0.89 % Cl^+; those coated with PHQS (1:1) contained 0.26 % Cl^+. In the second experiment the corresponding loadings were 0.86 % and 0.26 %, respectively. The reason why the the PHQS (1:1) swatches contained considerably less Cl^+ than indicated in Table 6 was that several detergent/rinsing cycles were necessary to prevent the elution of quat as determined in the bromophenol blue test (see Experimental Section 2.5). Thus some hydantoinyl/quat siloxane coating washed away from the surface leading to a lower titrated loading of Cl^+ for the PHQS (1:1) samples.

Several observations are apparent from Tables 7 and 8. All of the biocidal polymer coatings were effective against Gram positive *S. aureus* including the PQS homo polymer even at the short 30 s contact time. The 4.4 log reduction effected by PHQS (1:1) in the 30 s contact time in experiment 1 was representative of only 2 CFU's counted, and since a complete inactivation was noted for experiment 2 at this contact

time, it can be assumed that this was just an anomalous result. However, it is evident that the coatings containing hydantoinyl siloxane functional groups (PHS and PHQS (1:1)) were much more effective against Gram negative *E. coli* O157:H7 than was PQS. This has been observed here for PHQS (1:3) as well, so it is evident that copolymers containing at least some chlorinated hydantoinyl functional group are preferred for inactivation of the *E. coli* O157:H7. This result can be rationalized by consideration of the compositions of the bacterial cells. It is well known that the primary mechanism of action for quat biocides is disruption of the cell membrane, followed by leakage of critical cell components, leading to cell inactivation.[26] On the other hand, the mechanism of action of N-halamine biocides involves direct transfer of oxidative halogen to the cell where cell inactivation occurs by an oxidation mechanism. Gram negative bacteria such as *E. coli* O157:H7 have very complex cell walls which resist penetration by quats, but evidentally much less so by oxidative chlorine. Gram positive bacteria such as *S. aureus* have much less complex cell walls which are easily penetrated by either quats or oxidative chlorine.

Finally, it should be noted that a quat functional group of trimethyl or triethyl ammonium chloride in PHQS renders the copolymer soluble in water at the 1H:1Q level as well. Such copolymers would be less expensive to prepare, but one of the alkyl groups necessarily must be C_{12} to C_{18} (dodecyl to octadecyl) in order for the quat functionality to provide any biocidal activity for the copolymer once oxidative chlorine is expended from the hydantoinyl functional group. Although much of the biocidal activity of the hydantoinyl functionality can be regenerated by simple exposure to dilute bleach,

retaining some activity due to the quat functionality would be advantageous, at least for Gram positive bacteria.

Table 7

The efficacies of various copolymer-treated cotton swatches against S. aureus

Sample Coating	**Log Reduction in a contact time of (min).**				
	0.5	**1.0**	**5.0**	**10.0**	**30.0**
Exp. 1[a]					
Cotton Control	0.37	0.44	0.44	0.37	0.52
PHS Control[b]	0.14	0.15	0.25	0.22	0.26
PHS[c]	6.52	6.52	6.52	6.52	6.52
PHQS (1H:1Q)[c]	4.40	6.52	6.52	6.52	6.52
PQS	6.52	6.52	6.52	6.52	6.52
Exp. 2[d]					
Cotton Control	0.51	0.56	0.57	0.60	0.68
PHS Control[b]	0.35	0.40	0.37	0.62	0.70
PHS[c]	6.68	6.68	6.68	6.68	6.68
PHQS (1H:1Q)[c]	6.68	6.68	6.68	6.68	6.68
PQS	6.68	6.68	6.68	6.68	6.68

[a] Inoculum was 3.33×10^6 CFU; see text for Cl^+ loadings.

[b] Unchlorinated. [c] Chlorinated; see text for loading. [d] Inoculum was 4.76×10^6 CFU; see text for Cl^+ loadings.

Table 8

The efficacies of various copolymer-treated cotton swatches against E. coli *O157:H7*

Sample Coating	**Log Reduction in a contact time of (min).**				
	0.5	**1.0**	**5.0**	**10.0**	**30.0**
Exp. 1[a]					
Cotton Control	0.18	0.20	0.33	0.30	0.42
PHS Control[b]	0.06	0.24	0.22	0.26	0.45
PHS[c]	6.62	6.62	6.62	6.62	6.62
PHQS (1H:1Q)[c]	2.07	6.62	6.62	6.62	6.62
PQS	0.49	0.54	0.75	2.02	2.60
Exp. 2 [d]					
Cotton Control	0.06	0.10	0.24	0.22	0.13
PHS Control[b]	0.10	0.10	0.13	0.17	0.22
PHS[c]	3.80	7.00	7.00	7.00	7.00
PHQS (1H:1Q)[c]	0.97	3.17	4.48	7.00	7.00
PQS	1.03	1.50	2.24	2.34	2.38

[a] Inoculum was 4.18×10^6 CFU; see text for Cl^+ loadings.

[b] Unchlorinated. [c] Chlorinated; see text for loading. [d] Inoculum was 1.00×10^7 CFU; see text for Cl^+ loadings.

4 **Conclusions**

It has been demonstrated that hydantoinyl/quat siloxane copolymers can be prepared which are adequately soluble in water to be used for coating cotton swatches. The swatches possessed biocidal efficacy against Gram positive *S. aureus* as a result of the quat functional group alone, as well as with the chlorinated hydantoinyl functional group present (greater than 6 logs within 30 s contact). However, for Gram negative *E. coli* O157:H7 the presence of the chlorinated hydantoinyl functional group was necessary to achieve 6 to 7 log inactivation within 1 to 10 min contact. Also, it was found that a modified iodometric/thiosulfate titration procedure was needed to analytically determine oxidative chlorine loadings on the cotton swatches. Although this study focused exclusively on coated cotton, the technology could be extended to any surface to which siloxanes will bind.

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References

1. Worley SD, Williams DE. Halamine water disinfectants. Crit Rev Environ Contrl 1988;18:133-175.
2. Worley SD, Sun G. Biocidal polymers. Trends Polym Sci 1996;4:364-370.
3. Sun G, Wheatley WB, Worley SD. A new cyclic N-halamine biocidal polymer. Ind Eng Chem Res 1994;33:168-170.
4. Sun G, Allen LC, Luckie EP, Wheatley WB, Worley SD. Disinfection of water by N-halamine biocidal polymers. Ind Eng Chem Res 1995;34:4106-4109.
5. Sun G, Chen TY, Wheatley WB, Worley SD. Preparation of novel biocidal N-halamine polymers. J Bioact Compat Polym 1995;10:135-144.
6. Sun G, Chen TY, Habercom MS, Wheatley WB, Worley SD. Performance of a new polymeric water disinfectant. J Amer Wat Res Assoc 1996;32:793-797.
7. Panangala VS, Liu L, Sun G, Worley SD, Mitra A. Inactivation of rotavirus by new polymeric water disinfectants. J Virol Meth 1997;66: 263-268.
8. Lin J, Winkelmann C, Worley SD, Broughton RM, Williams JF. Antimicrobial treatment of nylon. J Appl Polym Sci 2001;81:943-947.
9. Lin J, Winkelmann C, Worley SD, Kim J, Wei CI, Cho U, Broughton RM, Santiago JI, Williams JF. Biocidal polyester. J Appl Polym Sci 2002;85:177-182.
10. Elrod DB, Figlar JG, Worley SD, Broughton RM, Bickert JR, Santiago JI, Williams JF. A novel biocidal elastomer. Rub Chem Tech 2001;74:331-337.

11. Eknoian MW, Worley SD, Bickert J, Williams JF. Novel antimicrobial N-halamine polymer coatings generated by emulsion polymerization. Polym 1999;40:1367-1371.
12. Chen Y, Worley SD, Kim J, Wei C-I, Chen TY, Santiago JI, Williams JF, Sun G. Biocidal polystyrenehydantoin beads for disinfection of water. Ind Eng Chem Res 2003;42:280-284.
13. Chen Y, Worley SD, Kim J, Wei C-I, Chen TY, Suess J, Kawai H, Williams J F. Biocidal polystyrene beads. II. Control of chlorine loading. Ind Eng Chem Res 2003;42:5715-5720.
14. Chen Y, Worley SD, Huang TS, Weese J, Kim J, Wei C-I, Williams JF. Biocidal polystyrene beads. III. Comparison of N-halamine and quat functional groups. J Appl Polym Sci 2004;92:363-367.
15. Chen Y, Worley SD, Huang TS, Weese J, Kim J, Wei C-I, Williams JF. Biocidal polystyrene beads. IV. Functionalized methylated polystyrene. J Appl Polym Sci 2004;92:368-372.
16. Worley SD, Li F, Wu R, Kim J, Wei C-I, Williams JF, Owens JR, Wander JD, Bargmeyer AM, Shirtliff ME. A novel N-halamine monomer for preparing biocidal polyurethane coatings. Surf Coat Intern Part B: Coat Trans 2003;86:273-277.
17. Williams JF, Suess J, Santiago J, Chen Y, Wang J, Wu R, Worley SD. Antimicrobial properties of novel N-halamine siloxane coatings. Surf Coat Intern Part B: Coat Trans 2005;88:35-39.

18. Worley SD, Chen Y, Wang J-W, Wu R, Cho U, Broughton RM, Kim J, Wei C-I, Williams JF, Chen J, Li Y. Novel N-halamine siloxane monomers and polymers for preparing biocidal coatings. Surf Coat Intern Part B: Coat Trans 2005;88:93-99.

19. Emerson DW, Shea DT, Sorensen EM. Functionally modified poly(styrenedivinylbenzene). Preparation, characterization, and bactericidal action. Ind Eng Chem Prod Res Dev 1978;17:269-274.

20. Emerson DW. Polymer-bound active chlorine: disinfection of water in a flow system. Polymer-supported reagents. 5. Ind Eng Chem Res 1990;29:448-450.

21. Emerson DW. Slow release of active chlorine and bromine from styrene-divinylbenzene copolymers bearing N,N-dichlorosulfonamide, N-chloro-N-alkylsulfonamide and N-bromo-N-alkylsulfonamide functional groups. Polymer-supported reagents. 6. Ind Eng Chem Res 1991;30:2426-2430.

22. Zhang Y, Emerson DW, Steinberg SM. Destruction of cyanide in water using N-chlorinated secondary sulfonamide-substituted macroporous poly(styrene-co-divinylbenzene). Ind Eng Chem Res 2003; 42:5959-5963.

23. Bogoczek R, Balawejder EK. N-monohalogeno- and N,N-dihalogenopoly(styrene-co-divinylbenzene)sulfonamides. Polym Commun 1986;27: 286-288.

24. Bogoczek R, Balawejder EK. Studies on a macromolecular dichloroamine – the N,N-dichloro poly(styrene-co-divinylbenzene) sulfonamides. Angew Makromolec Chem 1989;169:119-135.

25. Kanazawa A, Ikeda T, Endo T. Polymeric phosphonium salts as a novel class of cationic biocides. III. Immobilization of phosphonium salts by surface photografting and antibacterial activity of the surface-treated polymer films. J Polym Sci: Part A: Polym Chem 1993;31:1467-1472.

26. Lambert JL, Fina GT, Fina LR. Preparation and properties of triiodide-, pentaiodide-, and heptaiodide-quaternary ammonium strong base anion-exchange resin disinfectants. Ind Eng Chem Prod Res Dev 1980;19:256-258.

27. Hazziza-Laskar J, Nurdin N, Helary G, Sauvet G. Biocidal polymers active by contact. I. Synthesis of polybutadiene with pendant quaternary ammonium groups. J Appl Polym Sci 1993;50:651-662.

28. Sauvet G, Fortuniak W, Kazmierski K, Chojnowski J. Amphiphilic block and statistical siloxane copolymers with antimicrobial activity. J Polym Sci Part A: Polym Chem 2003;41:2939-2948.

29. Isquith AJ, Abbott EA, Walters PA. Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride. Appl Microbiol 1972;24:859-863.

30. Sun G, Xu X. Durable and regenerable antibacterial finishing of fabrics: biocidal properties. Text Chem Colorist 1998;30:26-30.

31. Sun Y, Sun G. Novel regenerable N-halamine polymeric biocides. III. Grafting hydantoin-containing monomers onto synthetic fabrics. J Appl Polym Sci 2001;81:1517-1525.

32. Sun Y, Chen TY, Worley SD, Sun G. Novel refreshable N-halamine polymeric biocides containing imidazolidin-4-one derivatives. J Polym Sci Part A: Polym Chem 2001;39:3073-3084.

33. Braun M, Sun Y. Antimicrobial polymers containing melamine derivatives. I. Preparation and characterization of chloromelamine-based cellulose. J Polym Sci Part A: Polym Chem 2004;42:3818-3827.

34. Sakai T. Stepwise determination of quaternary ammonium salts and aromatic amines in pharmaceuticals by an ion association titration. Anal Sci 2001;17:1379-1382.

35. Gottenbos B, van der Mei HC, Klatter F, Nieuwenhuis P, Busscher HJ. In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials 2002;23:1417-1423.

New More Durable Amine *N*-halamine Siloxane Treated Textiles

Abstract

A new series of *N*-halamine precursors 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine, and 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine, which will be referred to as HAM-Sil and HAM-Sil1', respectively, for the rest of this discussion, were prepared in a two step process with the overall yield in the range of 84% by mass of theoretical, and characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT, mass, and FTIR. HAM-Sil1' and/or HAM-Sil-treated fabrics, once chlorinated, could provide exceptional durable biocidal properties, with a superior kill rate compared to other amine *N*-halamine structures discussed in the literature to date.[1, 2] This is due to the ample amount of coating attached to the surface of the cotton leading to a sizeable increase of oxidative chlorine and an overall increase in biocidal efficacies for both Gram-positive, as well as Gram-negative, bacteria. HAM-Sil1' in particular should be the more stable coating on the newly attached surface, since it has a sterically favorable methyl group bonded to the siloxane to prevent hydrolysis and the ultimate loss of the coating from the surface. As one might think, the amine *N*-halamine structures derived are much more stable, thereby providing an enhanced durability, compared to other types of *N*-halamine structures containing different types of nitrogens, i.e. amide and/or imide functional groups. The results discussed herein demonstrate that this type of amine *N*-halamine structures could withstand repeated home launderings and would also

require less frequent chlorine recharging to sufficiently provide the desirable antimicrobial properties.

1. Introduction

The primary objective of this research was to develop more durable antimicrobial textiles by chemically attaching a series of novel amine, halamine siloxane precursors, HAM-Sil1', and HAM-Sil, to the surface of cotton in order to promote greater durability of the chlorinated treated fabric, with improved biocidal vitality, and durability of the coating, which the previously employed amide/imide halamine compounds could not offer. To incorporate the amine halamine precursors onto the surface of cellulose, two new N-halamine precursors, HAM-Sil and HAM-Sil1', have been synthesized in a commercially feasible two-step process, and were then attached to the fabrics' surfaces, by employing a condensation reaction between the hydrolyzed siloxane monomers and the chemically active OH groups of the cellulose.

N-halamine compounds have proven to demonstrate exceptional biocidal efficacies toward a large number of various microorganisms including both Gram-positive and Gram-negative bacteria, fungi, viruses, and yeast.[3-6] Contaminated environmental surfaces, and equipment, have been linked to outbreaks of infection or colonization because of vancomycin-resistant *Enterococci* (VRE) and *Pseudomonas Aeruginosa* (PSAE). Bacteria commonly encountered in hospitals are capable of prolonged survival and may promote cross transmission even after these surfaces were cleaned utilizing manufacturers' recommendations of natural, commercial, or hospital-approved products and methods,[7-11] One contribution to the transmission of bacteria, diseases, and nosocomial infections acquired in hospitals may be due to the long survival rates of these

pathogens on various surfaces within the hospitals themselves.[7] As a means to subdue the risk of bacterial infections in healthcare settings may require the implementation of biocidal textiles to limit the amount of cross-contamination, which occurs during the daily activity of healthcare workers. For example, it has been proven that durable and antimicrobial fabrics have been developed by Worley and co-workers by chemically incorporating hydantoin rings to the cotton surfaces using siloxanes as the chemical means for attachment.[12-14] Compounds of this type, once chlorinated, incorporate an amide halamine structure, which possesses moderate attributes of stability and biocidal efficacy.[12,15] However, hydantoin rings have also been incorporated onto cotton fabrics by grafting dimethylol-5,5-dimethylhydantoin (DMDMH) onto the surfaces of cellulose.[16-18] Once chlorinated, these hydantoin rings introduce dominantly imide halamine structures, which readily loose active chlorine and therefore require persistent chlorine recharging either after laundering or after a given time in dry storage.[5]

Typically the stability of the N-X bond is attributed to the extent in which bond overlap occurs between the halogen and nitrogen. The overall stability of *N*-halamines is usually attributed to their structures, which may include imide, amide, and amine halamine bonds.[6, 19] The dissociation rate of a *N*-halamine in aqueous environments can be used to indirectly infer the transfer rate of the oxidative chlorine directly to the bacteria, since, it was determined by Worley et. al[20] that the mode of biocidal action occurs from direct transfer of the halogen from the *N*-halamine to the bacteria by a direct transfer process. The dissociation rates for a series of typical *N*-halamines can be seen in table 1 from chapter two.[12] The dissociation of chlorine from halamine structures is in the following order: Imide halamine > amide halamine > amine halamine. Therefore, we can

reasonably predict that amine halamines once attatched to cotton surfaces could provide a more stable biocide with overall lasting ability.

2. Experimental

2.1 Materials

All chemicals were purchased from the Aldrich Chemical Company, Milwaukee, WI and used as they were received without further purification unless otherwise noted. The fabric used was (Style 400 Bleached 100% Cotton print Cloth, Testfabics, Inc., West Pittston, PA). 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). The Trypticase soy agar employed was from (Difco Laboratories, Detroit, MI).

2.2 Instruments

The NMR spectra were obtained using a Bruker 400 MHz spectrometer; the IR data were obtained with a Shimadzu IR Prestige-21 FTIR. Toluene was purified by the use of a M Braun SPS 5 solvent system. The mass spectra data were obtained by the employment of a Micro Mass Trio 2000. Tensile strengths were measured with a model 1122 Instron Universal Materials Testing Machine.

2.3 Preparation of 4-(Allyloxy)-2,2,6,6-tetramethylpiperidine Scheme 5.

$$\text{2,2,6,6-tetramethyl-4-piperidinol (1 eq.)} + \text{NaH (1 eq.)} + \text{allyl bromide (1 eq.)} \xrightarrow[\text{1. R.T, 30 min; 2. } 65^{0}\text{C, 4 h.}]{\text{THF, Dry}} \text{4-(allyloxy)-2,2,6,6-tetramethylpiperidine (87\% yield)}$$

Scheme 5. Preparation of 4-(Allyloxy)-2,2,6,6-tetramethylpiperidine

The preparation of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine was carried out, for example, in one experiment by modifying a 3-necked round bottom flask, which was fitted with a Krytox® greased ground glass stopper so that it could be removed as necessary for the addition of chemicals. The middle neck was fitted with a nitrogen bubbler so as to keep the system purged of any atmospheric oxygen or moisture. The last neck was fitted with a rubber stopper. To this flask 2.00 g (0.05 mol) of sodium hydride (60% dispersion in mineral oil) were added, and then washed with three 20 mL aliquots of hexanes, which were siphoned from the flask using a cannulae that entered through the rubber stopper. Separately, in a single neck 250 mL round bottom flask 100 mL of THF was freshly dried by distillation over sodium metal into the nitrogen purged flask. To this flask 7.942 g (0.05mol) of 2,2,6,6-tetramethyl-4-piperidinol (99%) were added, and the flask was sealed with a Krytox® greased ground glass stopper. The solution was then allowed to mix at room temperature until it became clear. This solution was then quickly added to the 3-necked round bottom flask, which held the previously prepared sodium hydide. The flask was then sealed as the rubber stopper was removed and replaced by a

Krytox® greased ground glass stopper. This solution was allowed to mix for 30 min at which time 6.110 g (0.05 mol) of ally bromide (99%) were quickly added bulk-wise by the removal of one of the glass stoppers while the nitrogen flow was increased so as to maintain a purged system. After this addition, the solution was allowed to mix at room temperature for an additional 30 min. The flask was then fitted with a water condenser and the solution was then heated to 65° C and allowed to mix for another 4 h. Once the reaction was completed, the sodium bromide by-product was then removed along with the THF by vacuum filtration and distillation, respectively. The distillation process also served for the removal of any unreacted allyl bromide. The resultant oil was then cooled to room temperature ambiently at which time any unreacted starting material or its salt had precipitated out of solution. The desired product was then simply decanted and 8.628 g of pure 4-(allyloxy)-2,2,6,6-tetramethylpiperidine were obtained, which corresponded to a 87% yield by mass of theoretical. Spectroscopic data obtained for 4-(allyloxy)-2,2,6,6-tetramethylpiperidine were: ^{1}H-NMR (250 MHz, d_6 DMSO) 1H, m, 5.87; 1H, d, 5.26; 1H, d, 5.12; 2H, d, 3.99; 1H, m, 3.73; 2H, dd, 1.84; 6h, s, 1.10; 6H, s, 1.02; 2H, td, 0.96; ^{13}C NMR (75MHz, d_6 DMSO) 29.73, 35.27, 44.69, 51.19, 68.25, 72.09, 115.88, 136.50; mass calculated for $C_{12}H_{23}NO$ m/z 197, found m/z 197; FTIR 1130, 1423, 1458, 1645, 1821, 2927, 2956, 3319 cm^{-1}.

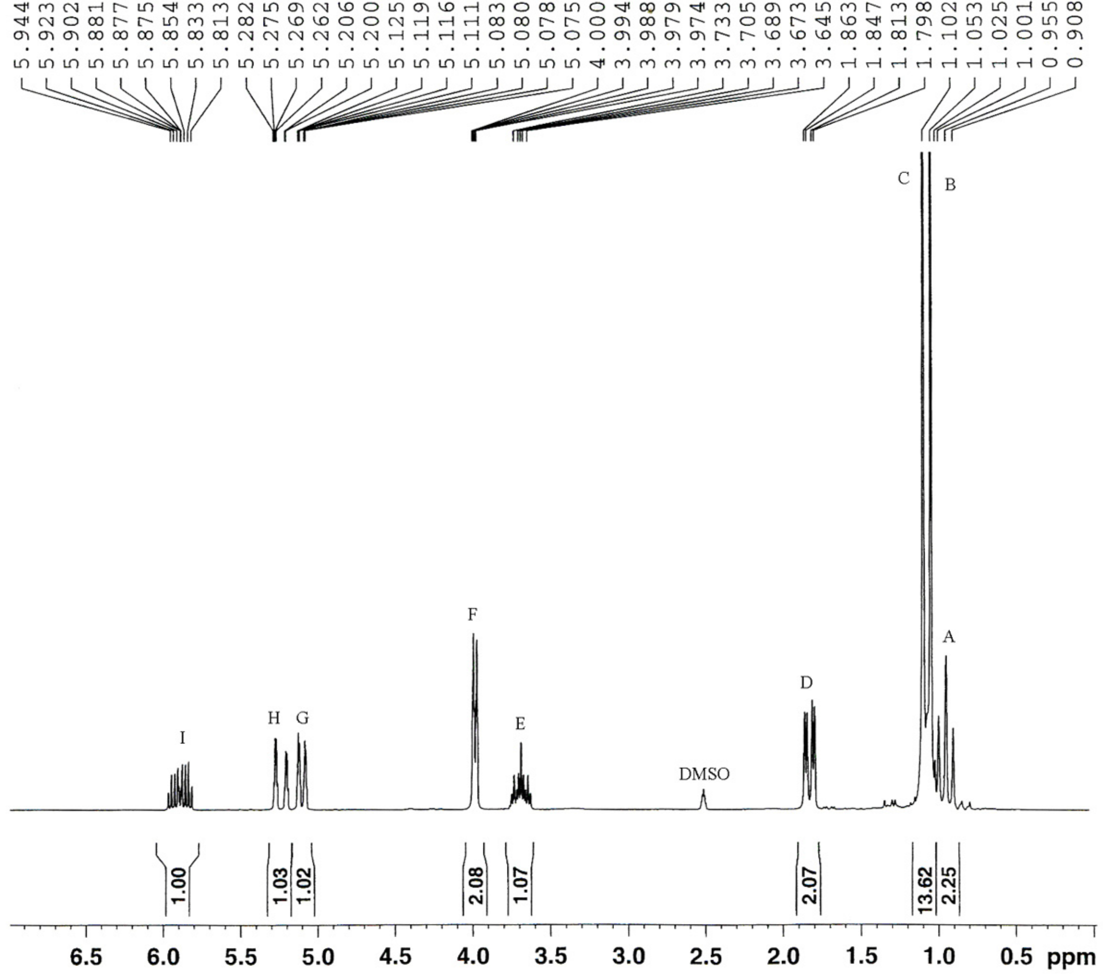

Figure 11a ^{1}H NMR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

Figure 11b proton map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

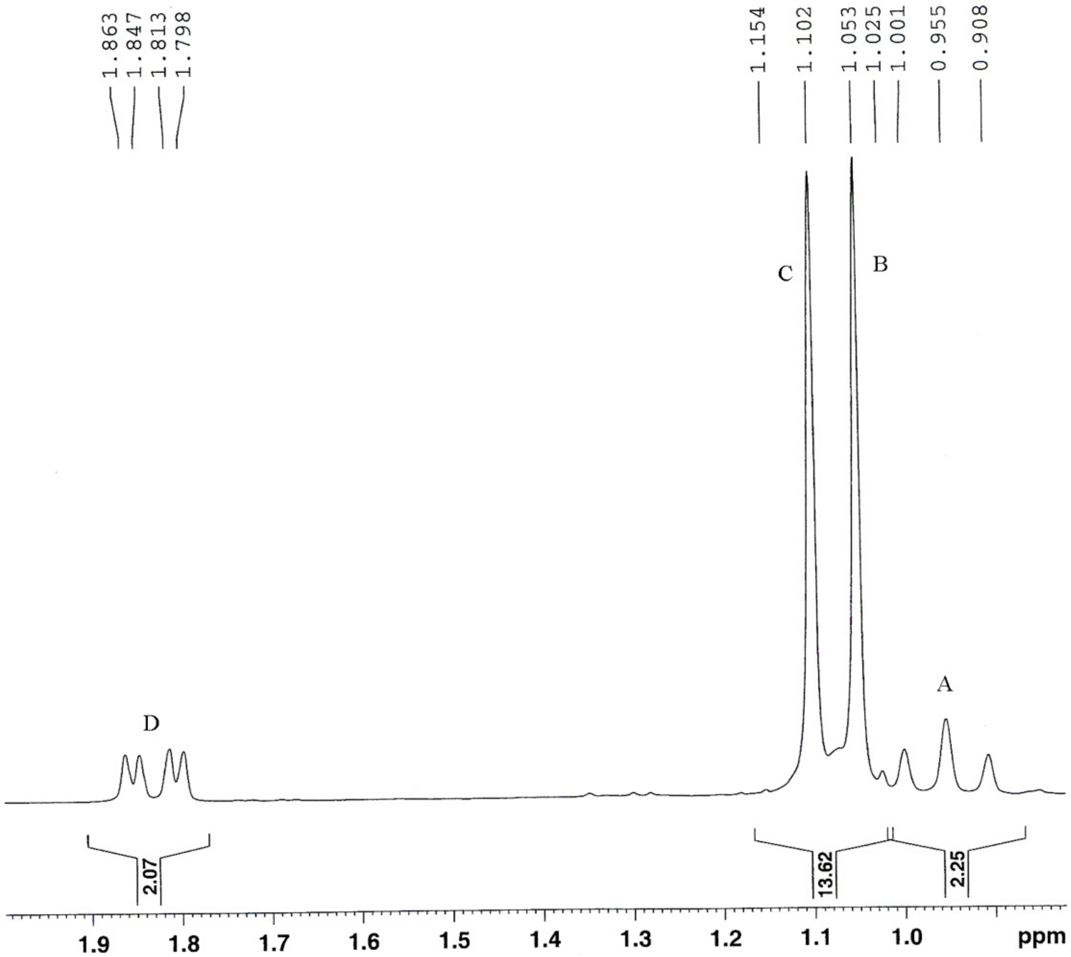

Figure 12 ^{1}H NMR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine expanded.

Figure 11b proton map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

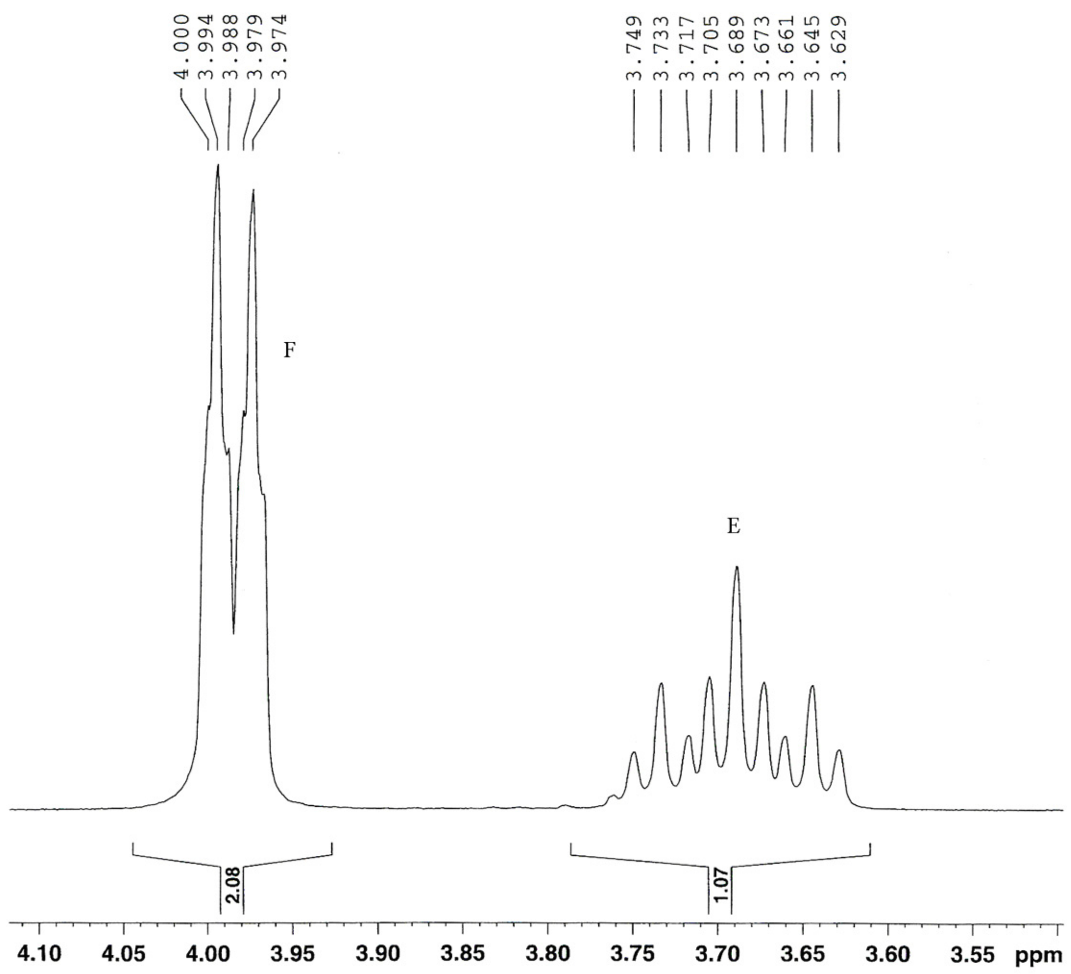

Figure 13 ^{1}H NMR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine expanded.

Figure 11b proton map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

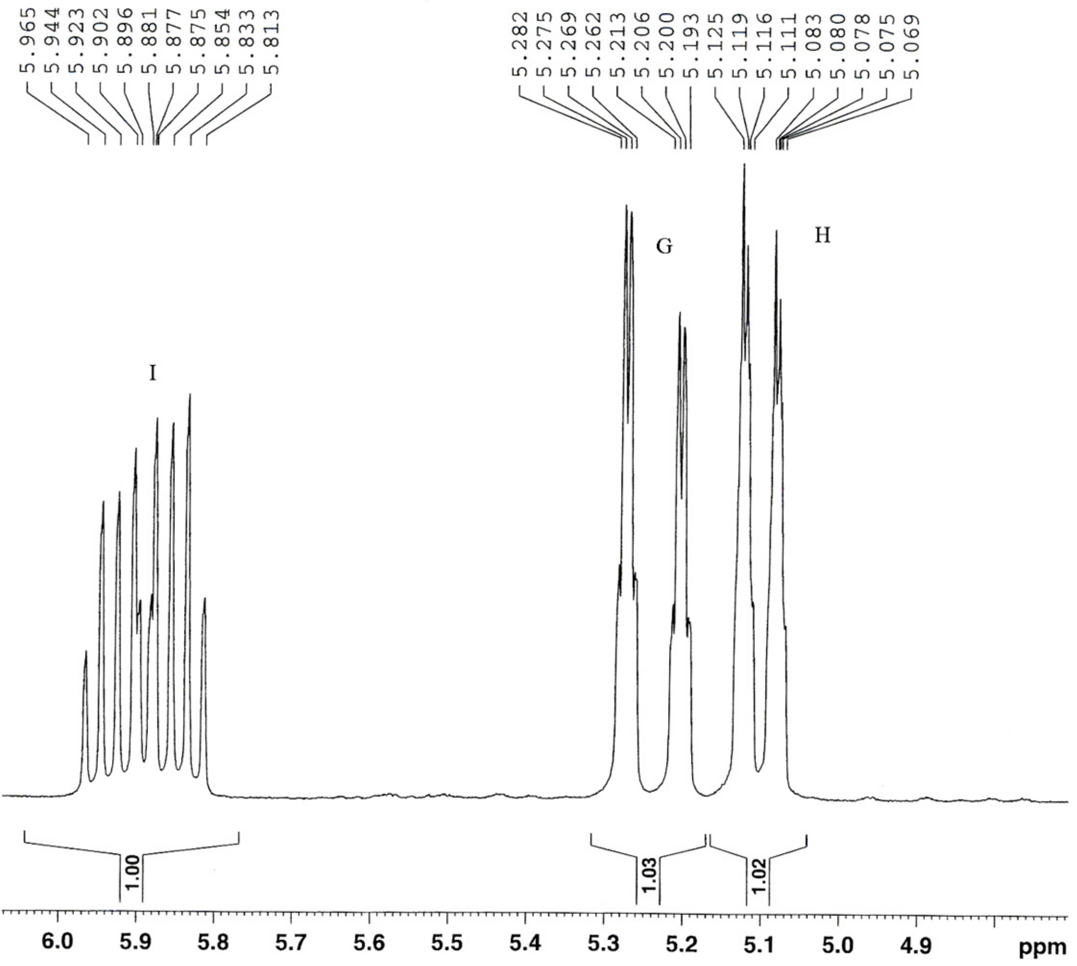

Figure 14 ^{1}H NMR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine expanded.

Figure 11b proton map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

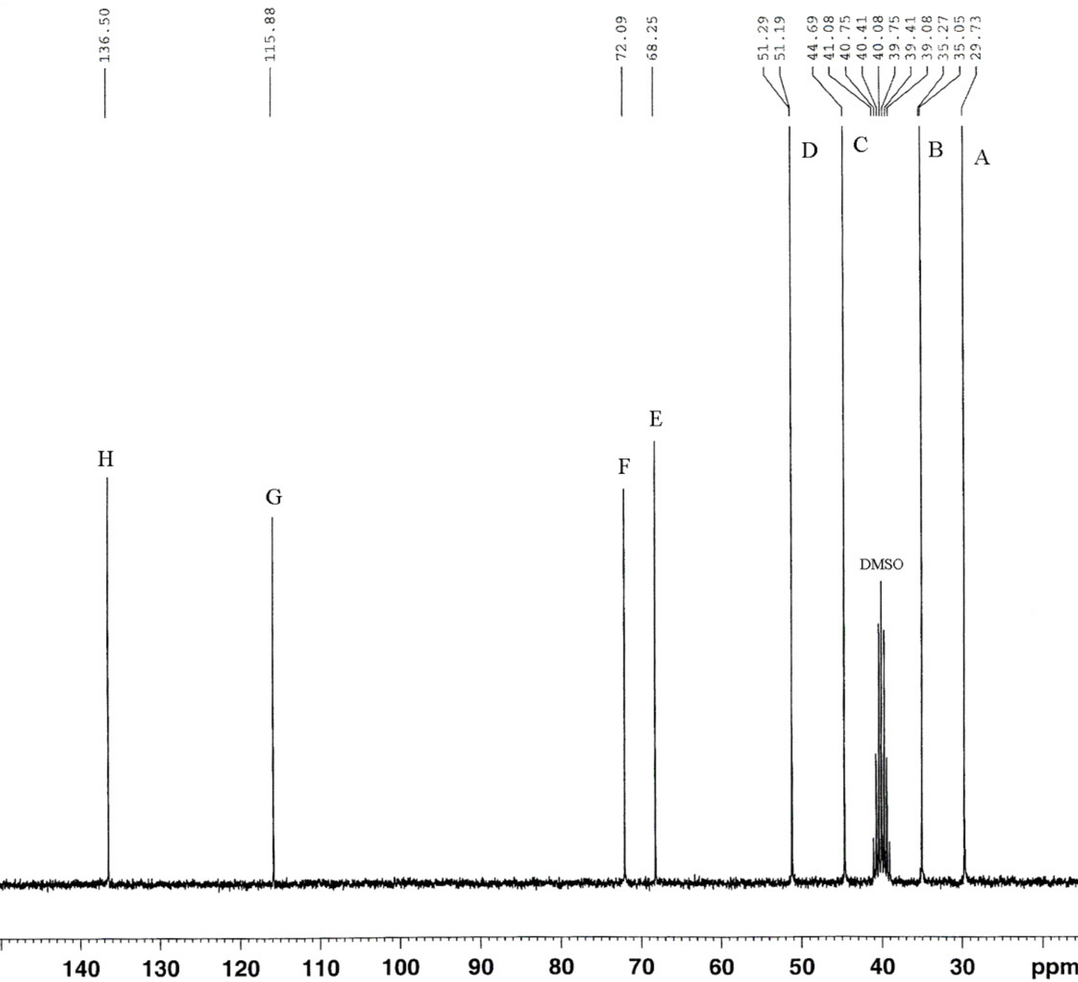

Figure 15 ^{13}C NMR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

Figure 15b ^{13}C map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

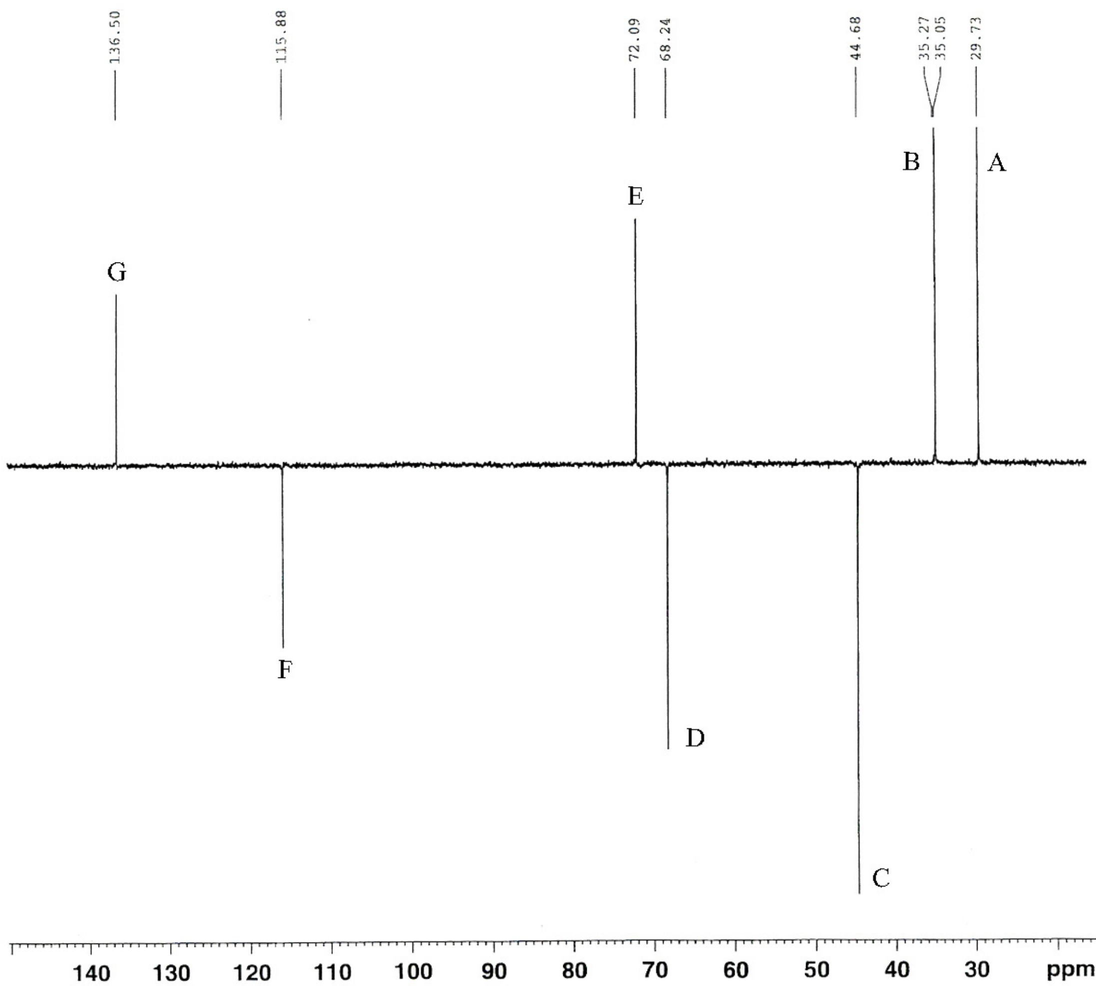

Figure 16 ^{13}C DEPT-135 spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

Figure 16b ^{13}C map of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

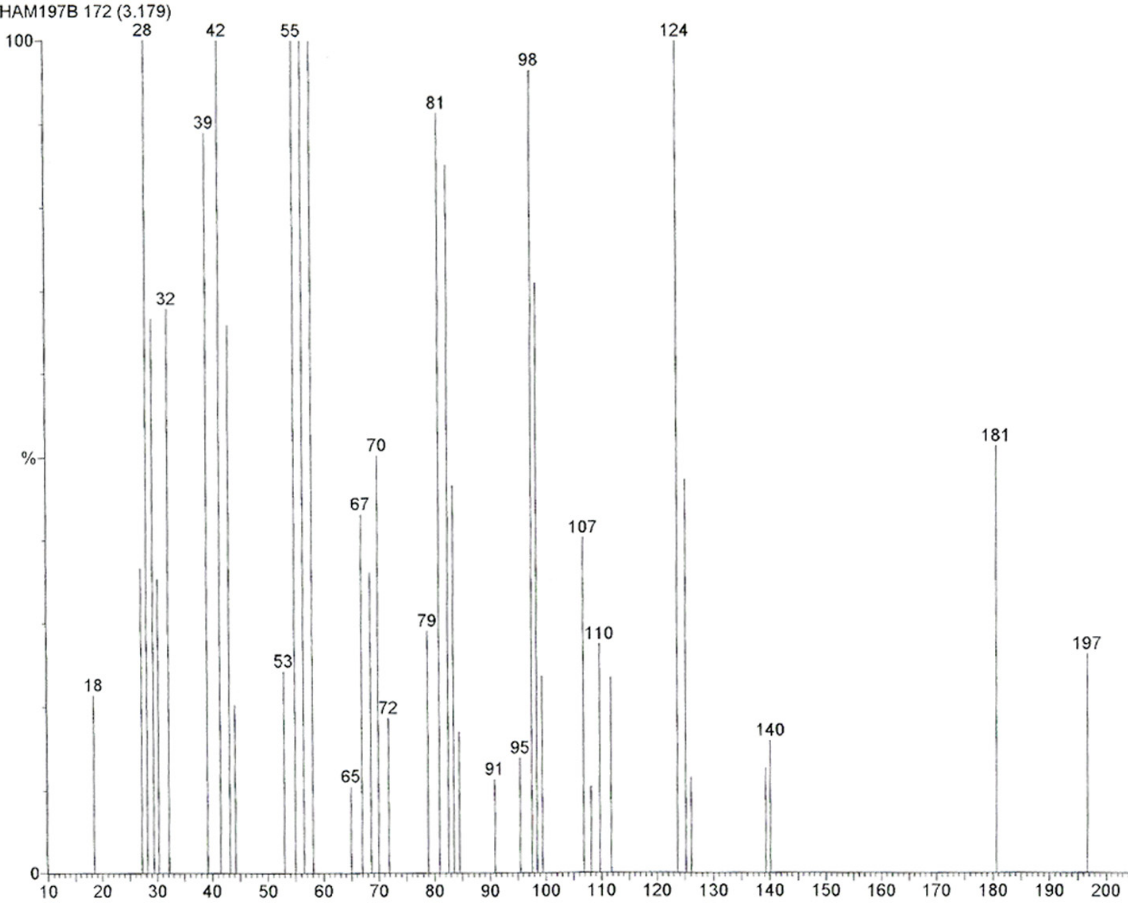

Figure 17 Electron Impact mass spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

$C_{12}H_{23}ON$

Figure 17b structure of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

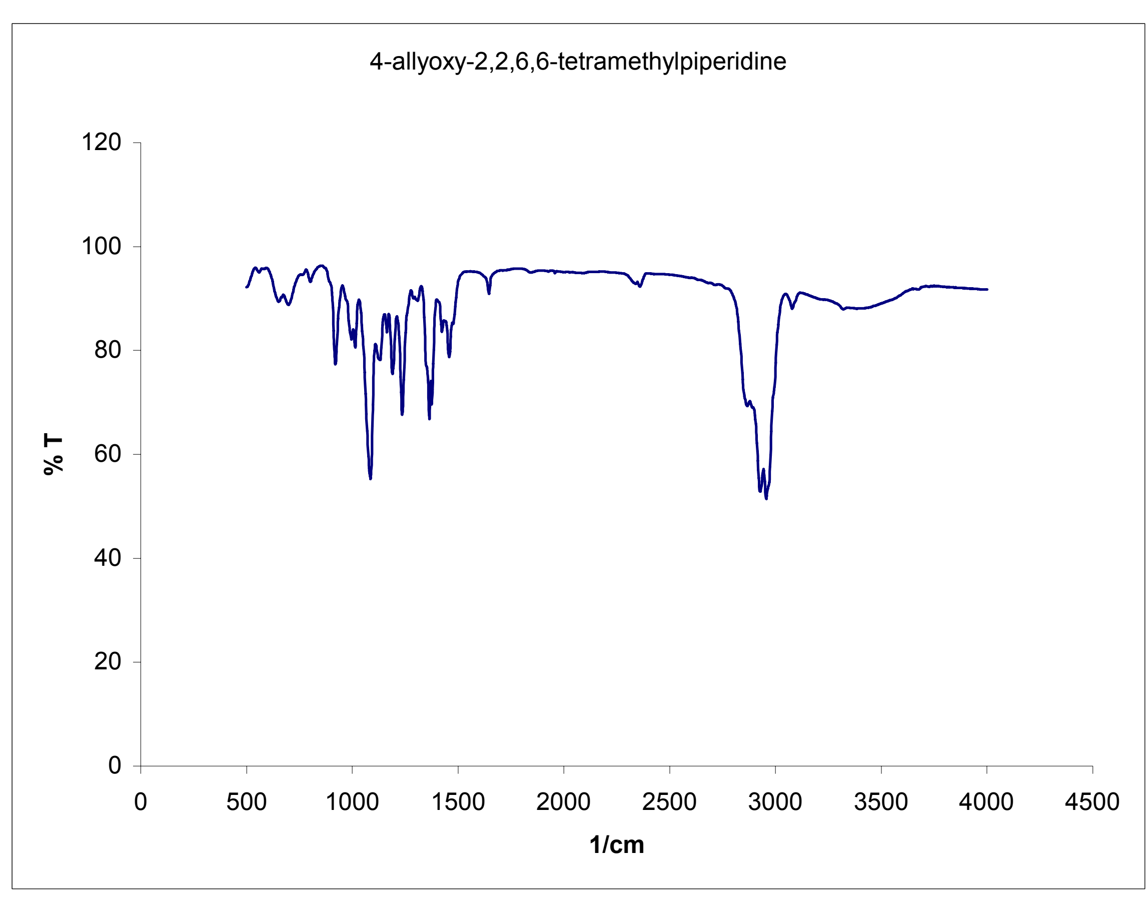

Figure18 FTIR spectrum of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine.

2.4 Preparation of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil) Scheme 6.

1eq. + 1 eq.

Toluene, Dry

Pt catalyst

1. 75°C, 4h
2. 100°C, 4h

86% yield

Scheme 6. Preparation of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperdine

The preparation of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil) was carried out, for example in one experiment, by modifying a 3-necked round bottom flask so as the middle neck was fitted with a water condenser, which was fitted with a nitrogen bubbler so as to keep the system purged of any atmospheric oxygen or moisture. The other two additional necks were fitted with ground glass stoppers. All of the joints were greased with Krytox® to ensure good seals. To this flask, 9.862 g (0.05 mol) of 4-(allyloxy)-2,2,6,6-tetramethylpiperidine were added along with 200 mL of dry toluene, which were directly added to the flask from a solvent purification system. This solution was allowed to mix at room temperature until the solution became clear. Then 8.646 g (0.05 mol) of trietoxysilane 95% were added to the solution and mixed until clear. Then 20 μL of a 2 % by weight solution of chloroplatinic acid dissolved in isopropanol were

added, and the reaction solution was then heated to 75^0 C with constant stirring for 4 h, and then at 100^0 C for an additional 4 h. At the end of this time spectroscopic evidence showed that the starting material 4-(allyloxy)-2,2,6,6-tetramethylpiperidine had completely reacted with the disappearance of the band at 1645 cm^{-1}. The reaction mixture of an oily appearance was then purified by vacuum distillation with the portion being collected at 95° C (0.1 torr) being the desired product with 13.538 g obtained corresponding to a 86 % yield by weight of theoretical for the silation reaction, and an overall yield of 75 % by weight of theoretical. Spectroscopic data obtained for 4-[3-triethoxysilylpropyl]-2,2,6,6-tetramethylpiperdine were: ^{1}H-NMR (250 MHz, $CDCl_3$) 2H, td, 0.61; 2H, td, 0.98; 9H, t, 1.12; 6H, s, 1.15; 2H, m, 1.62; 2H, dd, 1.87; 2H, td, 3.60; 1H, m, 3.72; 6H, q, 3.77; ^{13}C NMR (75 MHz, $CDCl_3$) 6.50; 18.23; 23.45; 28.96; 34.93; 44.91; 51.31; 58.25; 70.02; 72.25; mass calculated for $C_{18}H_{39}NO_4Si$ m/z 361, found m/z 361; FTIR 952, 1081, 1186, 1237, 2924, 2972, 3317 cm^{-1}.

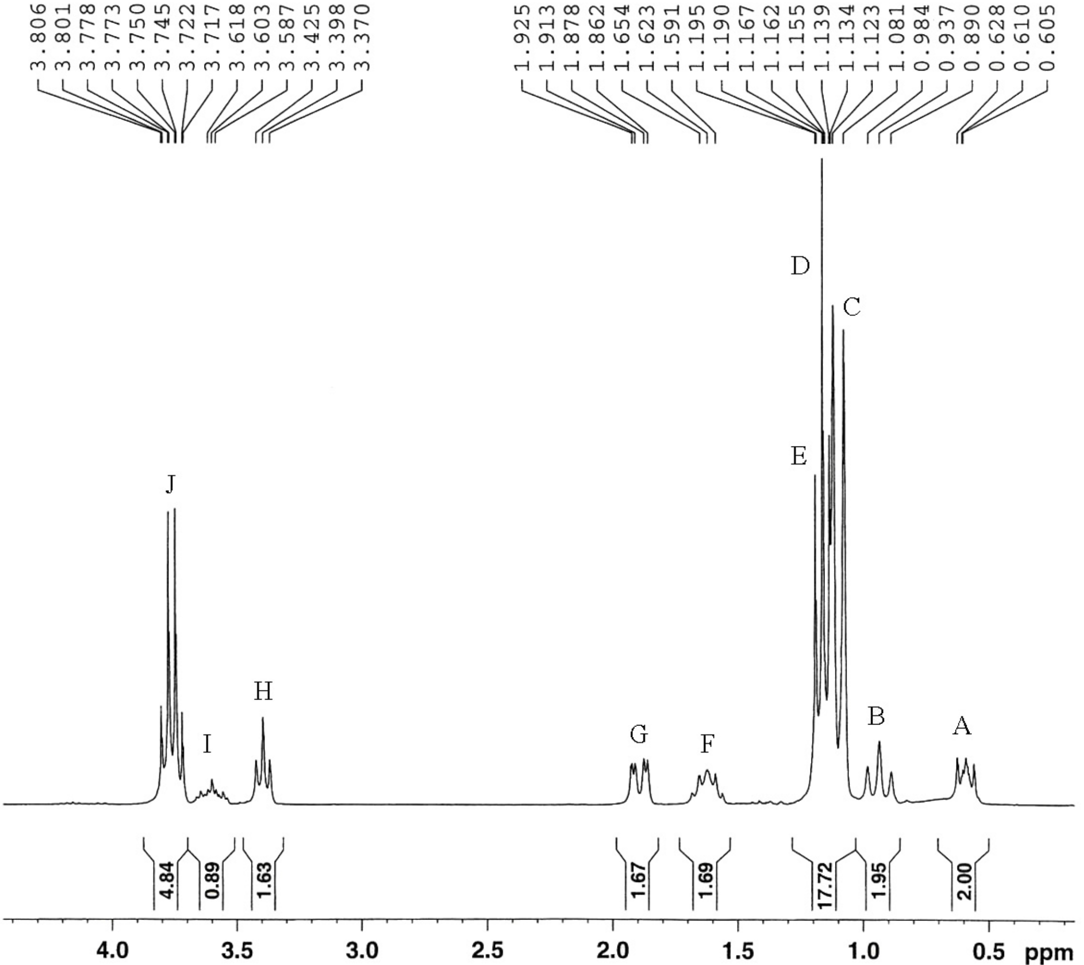

Figure 19 ^{1}H NMR spectrum of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil).

Figure 19b proton map of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil).

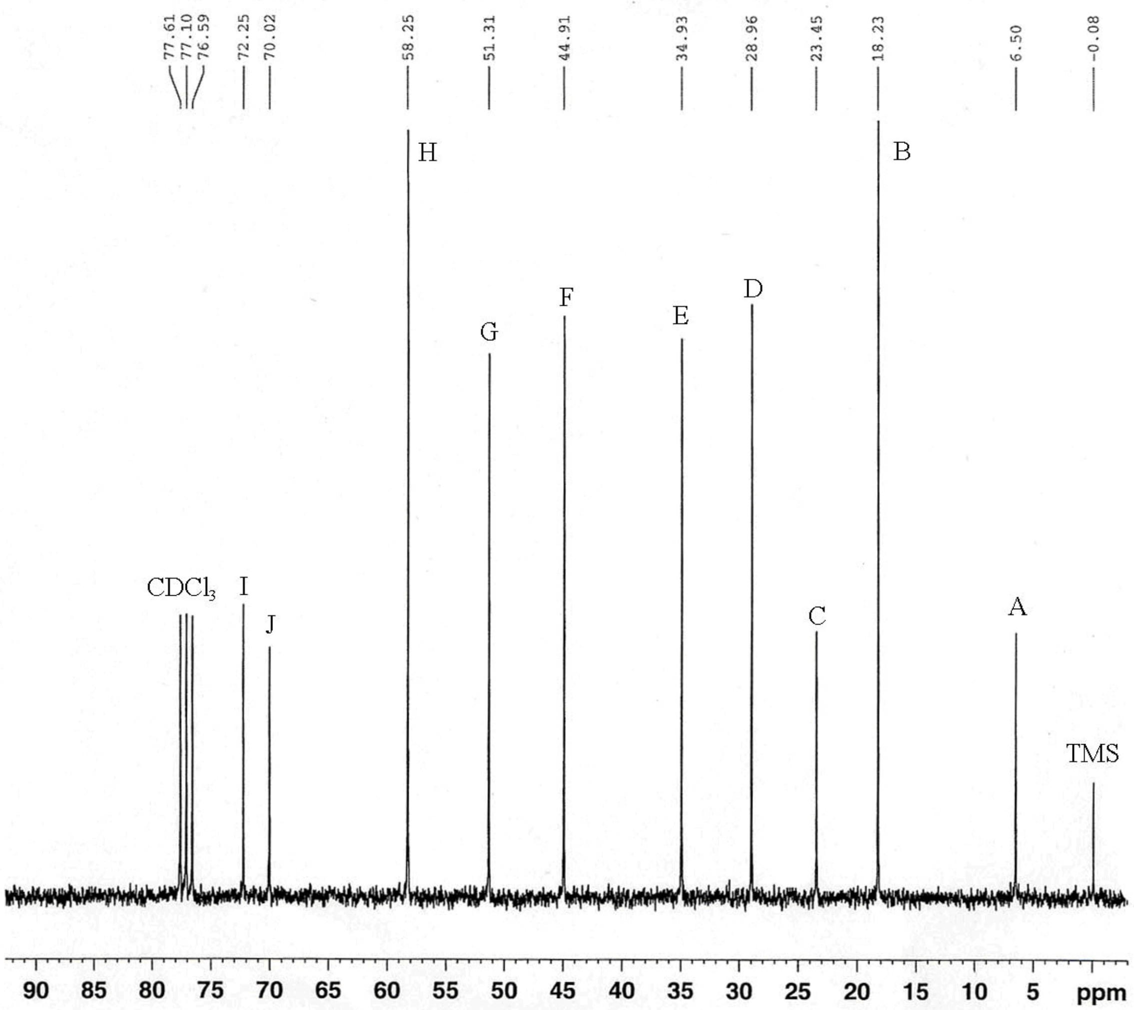

Figure 20 ^{13}C NMR spectrum of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

C_D C_E C_G C_F HN C_I—O—C_J—C_H—C_A—Si (O—C_C—C_B)$_3$ C_D C_G C_F C_E

Figure 20b ^{13}C map of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

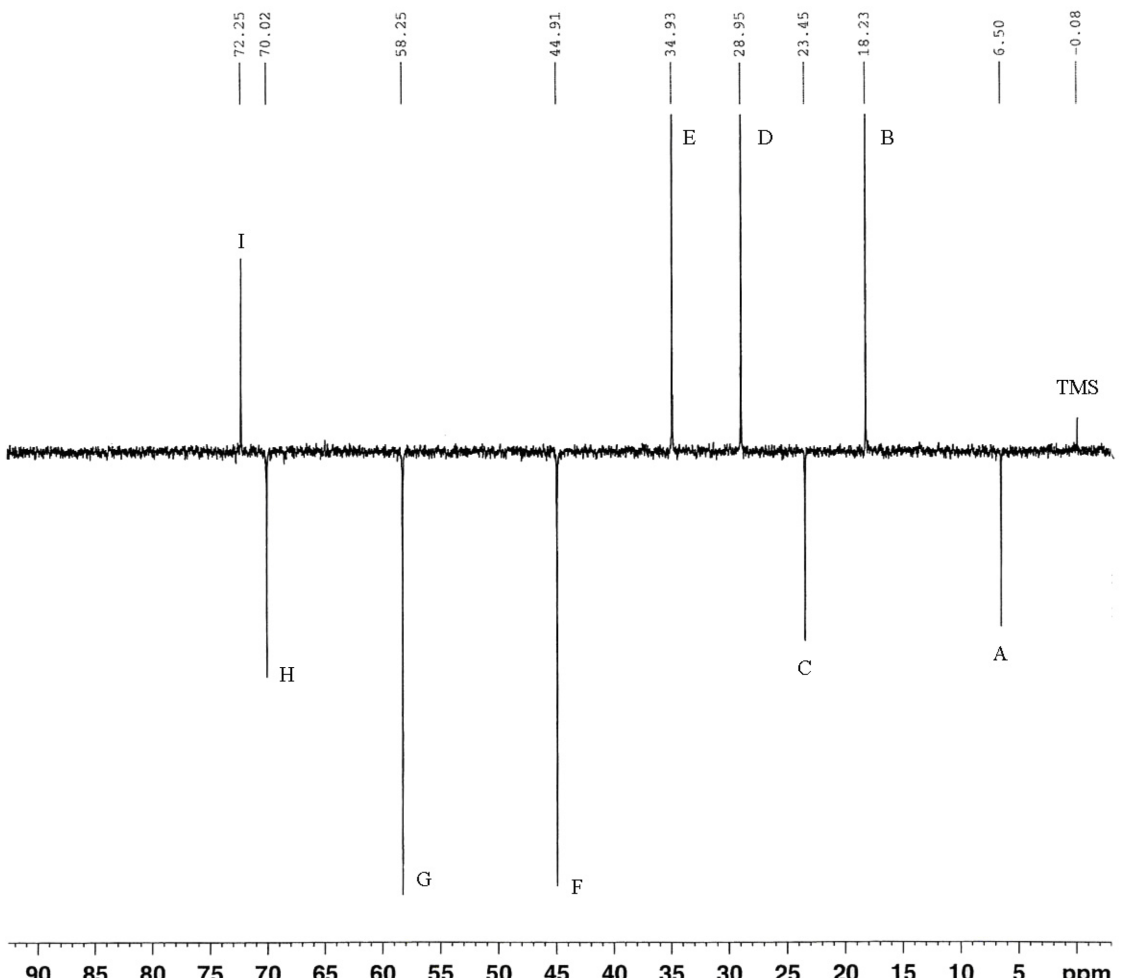

Figure 21 ^{13}C DEPT-135 spectrum of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

Figure 21b ^{13}C map of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

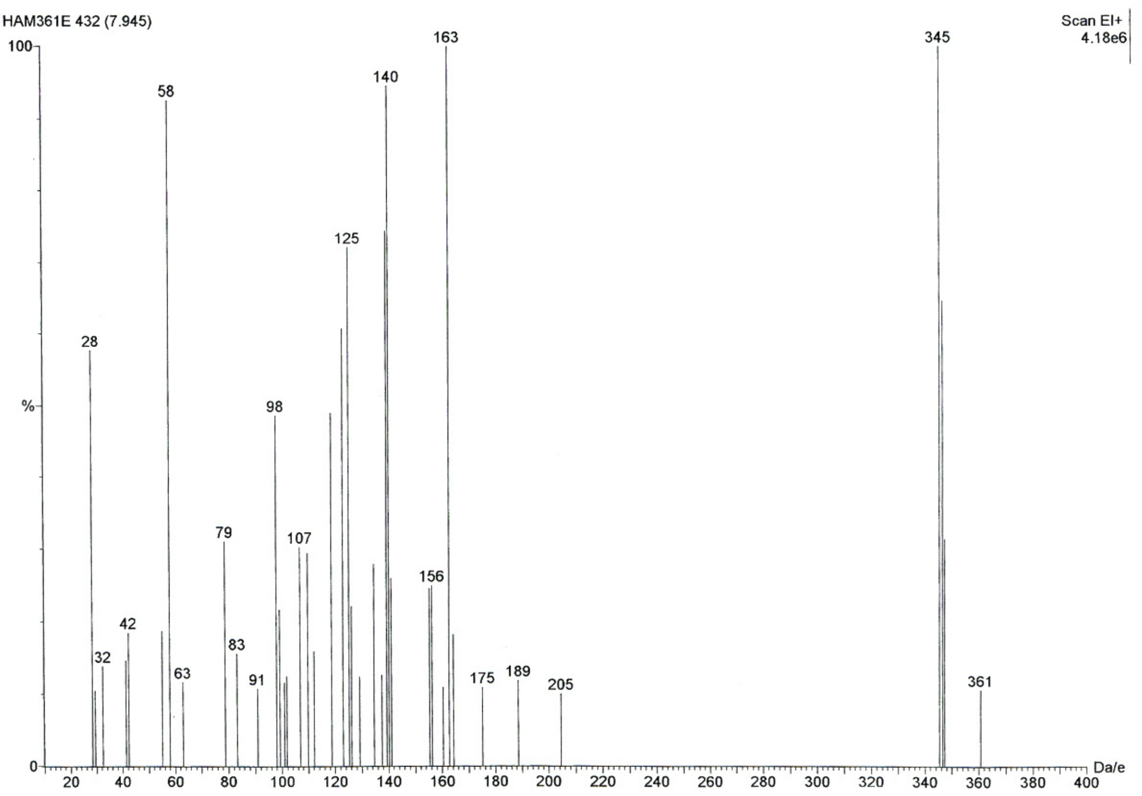

Figure 22 Electron Impact mass spectrum of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

$C_{18}H_{39}O_4NSi$

Figure 22b Structure of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

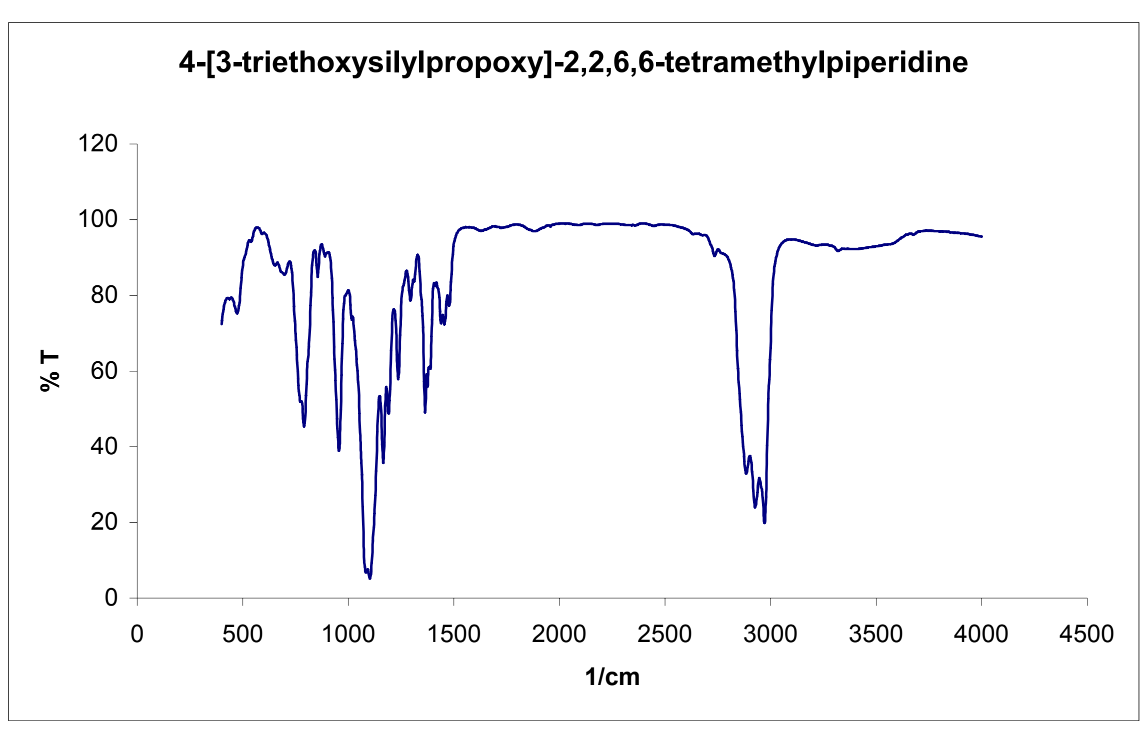

Figure 23 FTIR spectrum of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

2.5 Preparation of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

The preparation of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1') was carried in a similar manner as 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine with the exception of the use of diethoxymethylsilane was substituted for triethoxysilane. Spectroscopic data obtained for of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine were: ^{1}H-NMR (250 MHz, $CDCl_3$) 3H, s, 0.001; 2H, td, 0.61; 2H, td, 0.98; 6H, t, 1.12; 6H, s, 1.15; 2H, m, 1.62; 2H, dd, 1.87; 2H, td, 3.60; 1H, m, 3.72; 4H, q, 3.77; ^{13}C NMR (75 MHz, $CDCl_3$) – 4.96; 9.97; 18.38; 23.59; 28.95; 34.97; 44.95; 51.39; 58.06; 70.30; 72.34; mass calculated for $C_{17}H_{37}NO_3Si$ m/z 331, found m/z 331; FTIR 950, 1083, 1188, 1238, 1255, 1408, 2926, 2970, 3311 cm^{-1}.

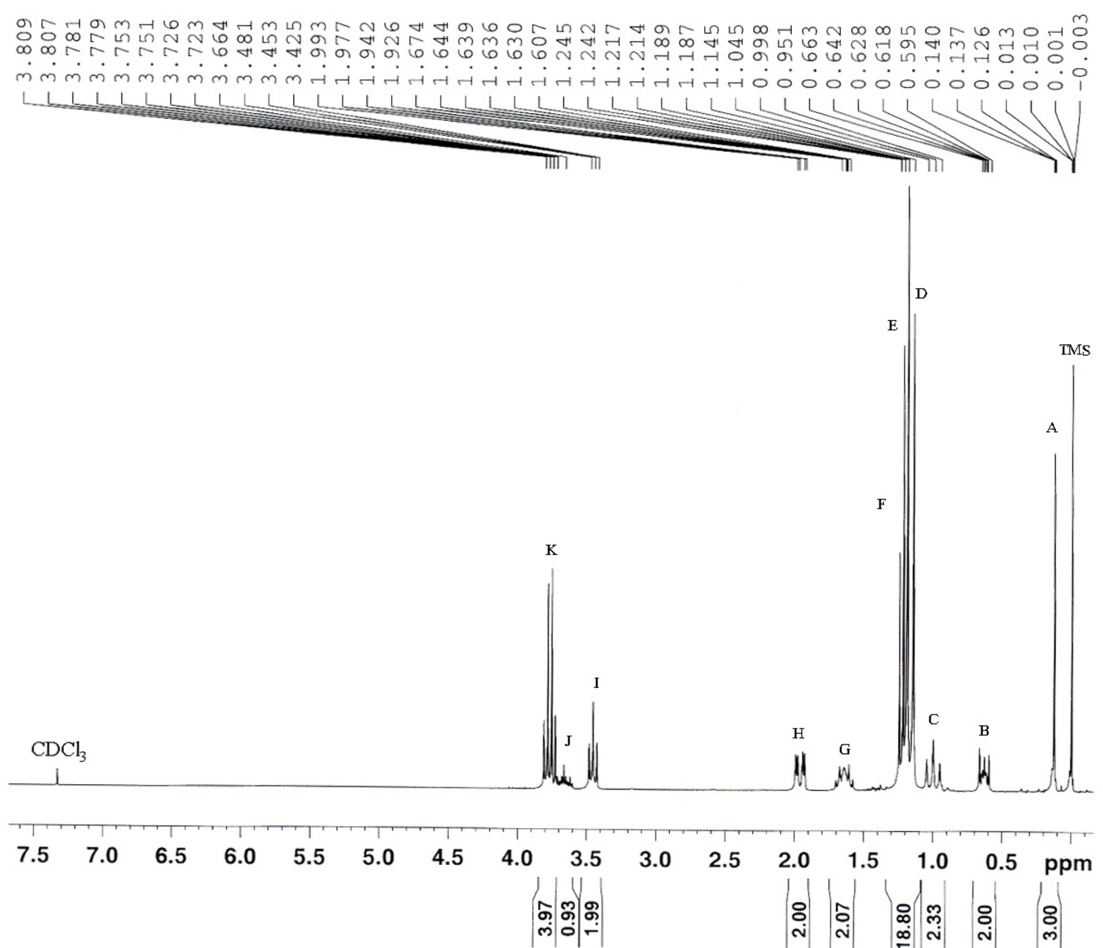

Figure 24 ^{1}H NMR spectrum of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

Figure 24b proton map of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

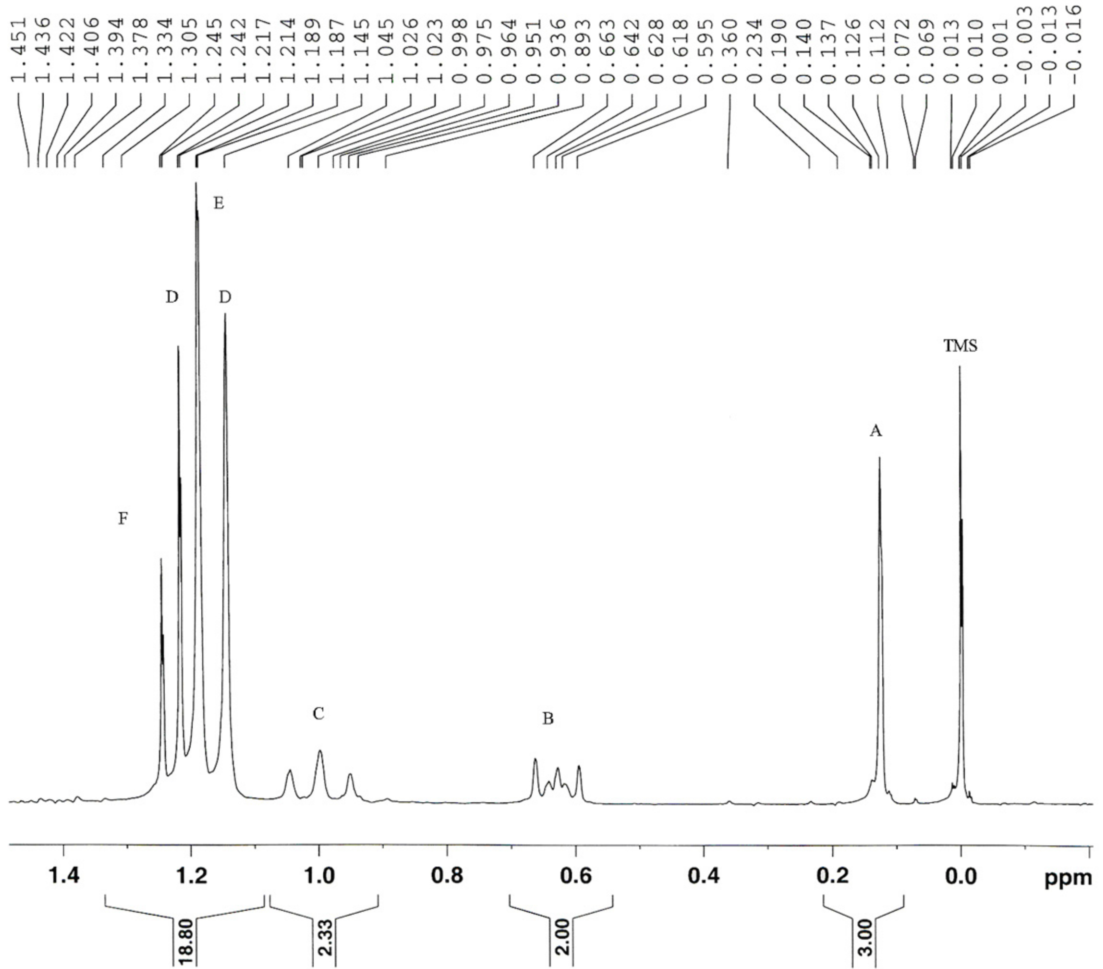

Figure 25 ^{1}H NMR spectrum expanded of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

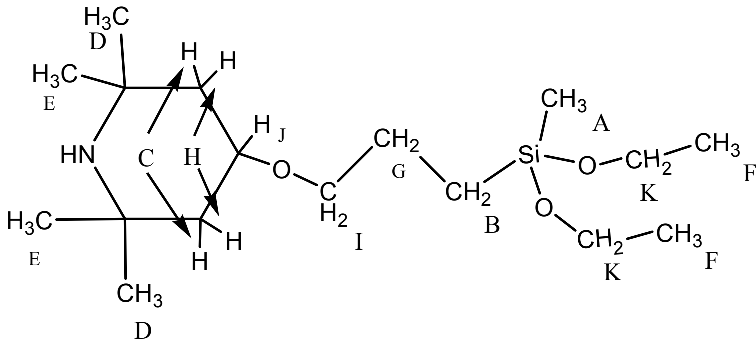

Figure 24b proton map of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

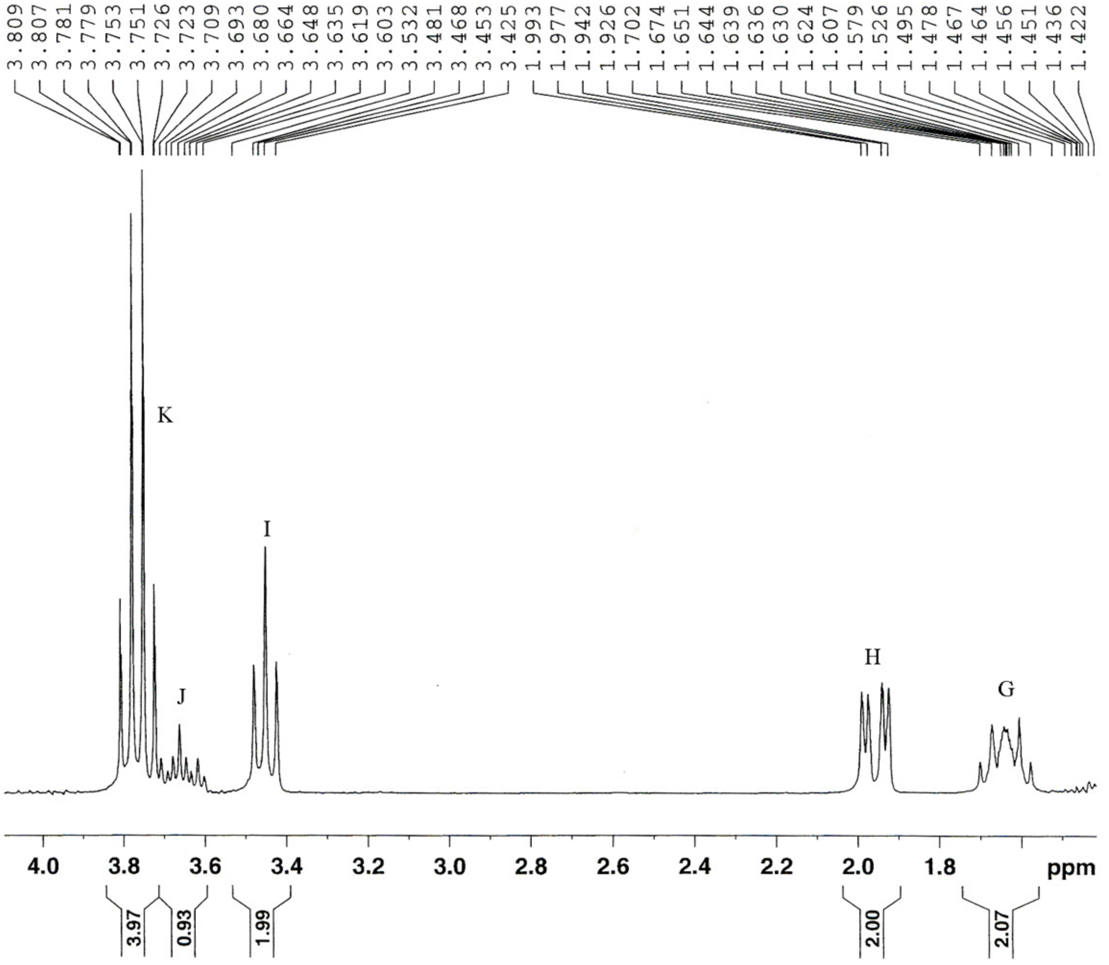

Figure 26 ^{1}H NMR spectrum expanded of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

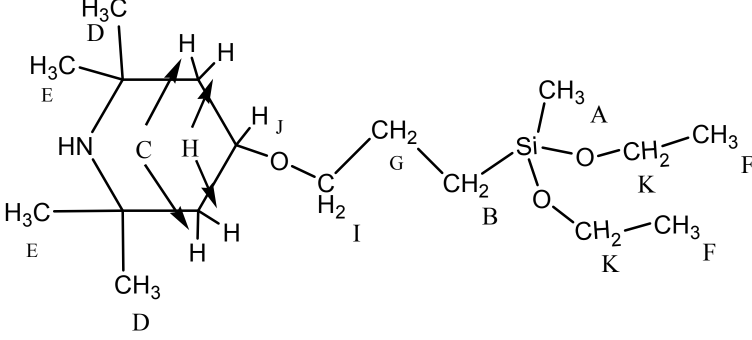

Figure 24b proton map of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine.

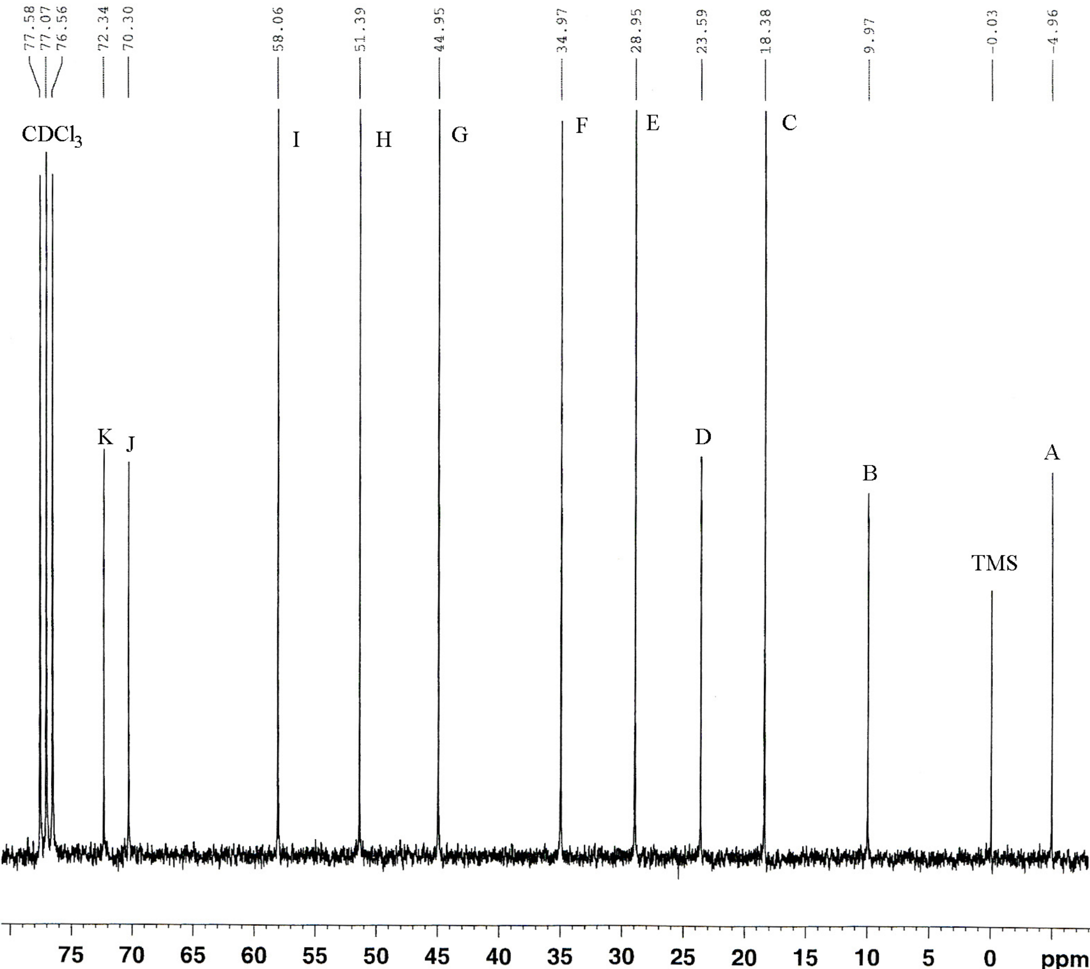

Figure 27 ^{13}C NMR spectrum of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

Figure 27b ^{13}C map of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

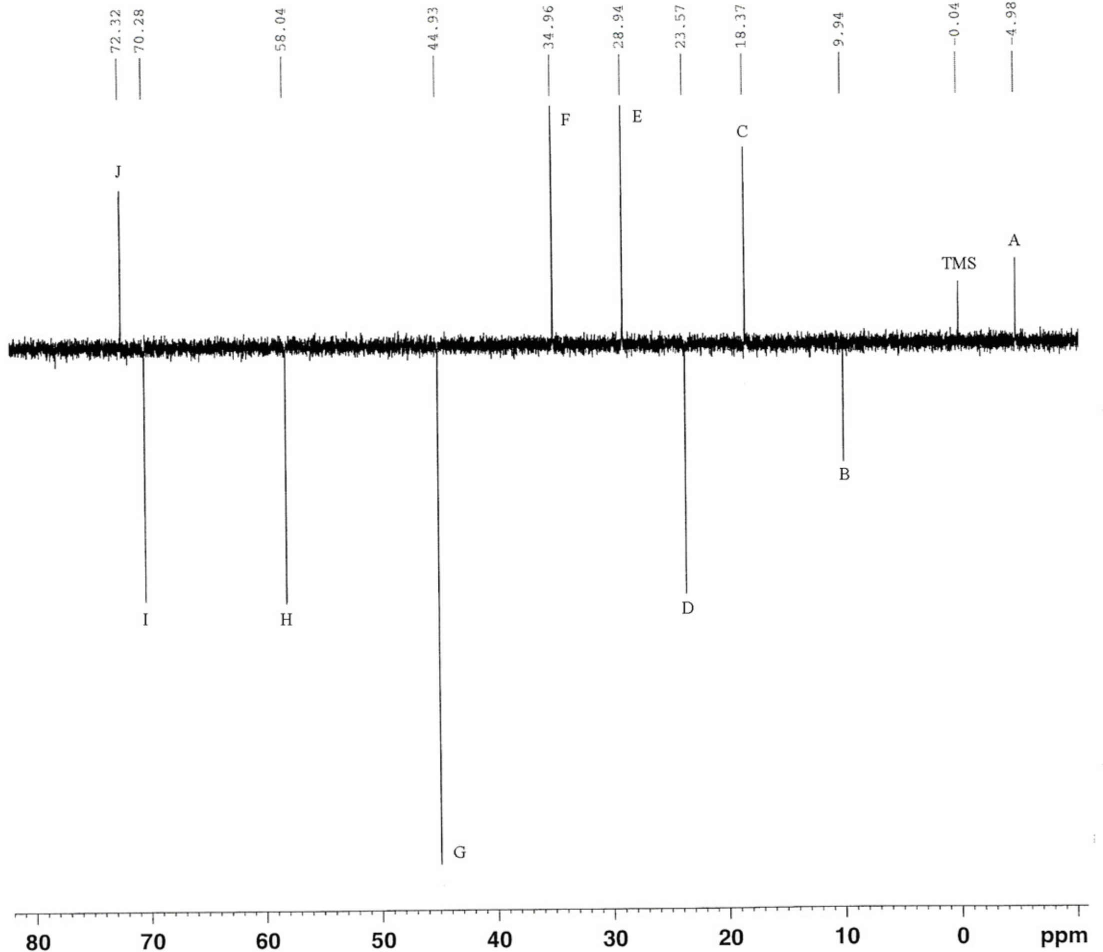

Figure 28 ^{13}C DEPT-135 spectrum of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

Figure 28b ^{13}C map of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

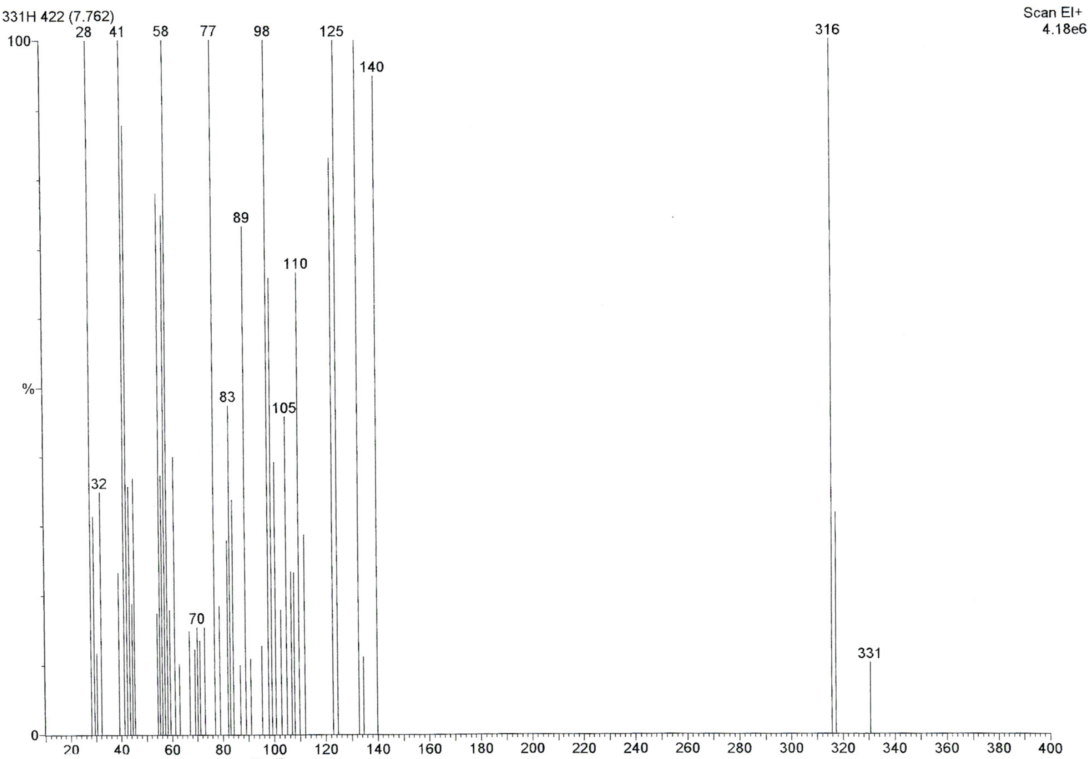

Figure 29 Electron Impact mass spectrum of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine (HAM-Sil1').

$C_{17}H_{37}O_3NSi$

Figure 29b Structure of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

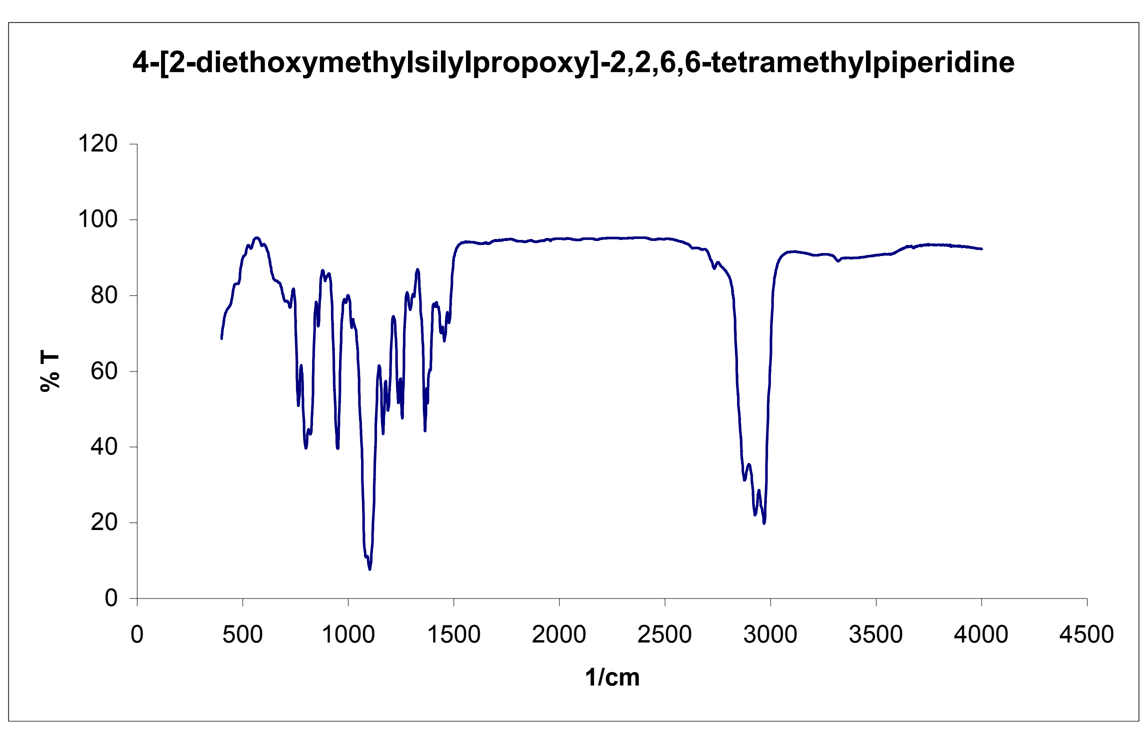

Figure 30 FTIR spectrum of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperidine.

2.6 Coating Procedure

The HAM-Sil and HAM-Sil1' monomers were coated onto the surfaces of cotton swatches by soaking the swatches in baths containing 5% by weight of the aforementioned monomers dissolved in a 1:1 (w/w) mixture of ethanol and water. After the soaking procedure, the coated swatches were cured at 95^{0}C for 1 h. Then, the swatches were soaked in 0.5% detergent solution for 15 min, followed by several water rinses to remove any weakly bonded coating. The typical percent add-on weight observed for both the HAM-Sil and HAM-Sil1' monomers were 4.6 % and 4.4 %, respectively. No significant loss of strength was observed for either of the treated fabrics compared to that of an untreated control swatch.

2.7 Chlorination Procedure

The coated cotton swatches were chlorinated by soaking them in a 10% aqueous solution of NaOCl household bleach buffered to pH 7 by the drop-wise addition of 6 M HCl at ambient temperature for 30 min. The chlorinated swatches were then washed with deionized water and dried at 45^0C for 1 h to remove any occluded free chlorine. The loading of bound chlorine on the swatches was determined as described in the analytical titration procedure.

2.8 Analytical titration procedure

For the determination of oxidative chlorine (Cl^+) content, a modified iodometric/thiosulfate titration procedure was employed which has been discussed previously in the literature.[21] For example, about 0.3 g of coated and chlorinated cotton swatch material was suspended in 90 mL of ethanol and 10 mL of a 0.1 N acetic acid solution. After the addition of 0.3 g KI, the solution was then titrated with 0.0375 N sodium thiosulfate until the yellow color disappeared at the end point. The weight percent Cl^+ on the cotton swatches could then be determined from the equation below:

$$\% \ Cl^+ = [N \times V \times 35.45 / (2 \times W)] \times 100\% \quad (3)$$

where N and V are the normality (eqv/L) and volume (L), respectively, of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in g of the cotton swatch sample.

2.9 Biocidal efficacy testing

One inch square cotton swatches, some uncoated to serve as controls, others coated with HAM-Sil monomer, but unchlorinated, to serve as a second type of control, and others

coated with chlorinated HAM-Sil monomer were prepared. It was deemed unnecessary to challenge the HAM-Sil1' swatches due to the costly microbial analysis, since it is reasonable to infer that a similar oxidative loading of chlorine would deactivate the bacteria in a similar manner. Dried swatches were then challenged with either *Staphylococcus aureus* ATCC 6538 or *Escherichia coli* O157:H7 ATCC 43895 using a "sandwich test". In this test, 25 μL of bacterial suspension were placed in the center of a swatch, and a second identical swatch was laid upon it, which was held in place by a sterile weight to insure good contact of the swatches with the inoculum. The bacterial suspensions employed for the tests contained 10^6 colony forming units (CFU), the actual number determined by counting after spread-plating on Trypticase soy agar plates. After, contact times of 5.0, 10.0, and 30.0 min, the various swatches were placed in sterile conical centrifuge tubes, each containing 5.0 mL of sterile 0.01 M sodium thiosulfate to quench any oxidative free chlorine which might have been present, and vortexed for 150 s to remove bacteria. Then the swatches were removed, and serial dilutions of the quenched solutions were plated on Trypticase soy agar. The plates were incubated at 37^0C for 24 h and then counted for viable CFU of bacteria.

3.0 Washing and durability

Laundering tests were performed on swatches of cotton coated with the HAM-Sil and HAM-Sil1' monomers prepared as detailed above. Then one half of the swatches were chlorinated following the aforementioned procedure. All types of coated swatches were subjected to laundry washing cycles using AATCC Test Method 61 (Test 2A Procedure). Samples were evaluated after 5, 10, 25, and 50 washing cycles for retention of the

coatings. Those samples not chlorinated before washing were chlorinated by the procedure described above in order to assess how much chlorine could be loaded after variable numbers of washing cycles. Those chlorinated before washing were divided into two groups, with half being assessed for chlorine loading without re-chlorination, the other half being re-chlorinated and then assessed for chlorine loading.

3.1 Results

For cotton swatches treated with HAM-Sil monomers as described in the experimental section and chlorinated, it was found that the average oxidative chlorine loading on the swatches was 0.33% by weight. The treated cotton swatches were challenged with *S. aureus* and *E. coli* O157: H7 at a concentration of 10^6 CFU/ mL in pH 7 phosphate buffer solution using a modified version of AATCC method 100. It was found that all *S. aureus* colonies (>6.0 logs) were inactivated by the swatches treated with chlorinated HAM-Sil monomer in the contact interval of 0 to 5 min; whereas, the swatches treated with the unchlorinated monomer experienced only a 0.48 log reduction at 5 min (Table 9). The control sample (untreated cotton swatches) gave only a 0.03 log reduction as well for the 5 min interval. It was also found that all *E. coli* (> 6 logs) were inactivated by the swatches treated with the chlorinated HAM-Sil monomer in the contact interval of 0 to 5 min; whereas, the unchlorinated monomer and the untreated swatches experienced only a 0.71 and 0.04 log reduction, respectively, in the same contact time interval (Table 9). It is thought that the HAM-Sil1'-treated and chlorinated fabrics would have had a similar result. Thus, it can be determined that the cotton cloth treated with HAM-Sil monomer, once chlorinated, became capable of rapid and total inactivation of both

Gram positive and Gram negative bacteria. In comparison, the 3-triethoxysilylpropyl-5,5-dimethylhydantoin siloxane monomer described previously[22] with amide functional groups was found to have deactivated > 5.7 logs of *S. aureus* in a contact interval of 10-30 min., while a contact interval of 60-120 min. was necessary to inactivate >5.9 logs of *E. coli.* For other reported amine halamines in the literature such as, 3-methylol-2,2,5,5-tetramethyl-imidazolidin-4-one (MTMIO), a contact time of 6 h was necessary for a 6 log reduction of bacteria[2] due to the insufficient oxidative chlorine loading provided by those coatings.

Table 9

The efficacies of Ham-Sil-treated cotton swatches against *S. aureus*

Sample coating	Log reduction in a contact time of (min)		
	5.0	10.0	30.0
Exp. 1[a]			
Cotton Control	0.37	0.54	0.71
Unchlorinated Monomer	0.88	1.60	2.08
Chlorinated Monomer	6.99	6.99	6.99
Exp. 2[b]			
Cotton Control	0.03	0.04	0.20
Unchlorinated Monomer	0.48	0.49	1.22
Chlorinated Monomer	6.89	6.89	6.89

[a]Inoculum was 9.67 x 10 x 10^6 CFU; Cl^+ loading was 0.331% by weight.

[b]Inoculum was 7.67 x 10^6 CFU; Cl^+ loading was 0.344 % by weight.

Table 10

The efficacies of Ham-Sil-treated cotton swatches against *E. coli* O157:H7

Sample Coating	Log reduction in a contact time of (min)		
	5.0	10.0	30.0
Exp. 3[a]			
Cotton Control	N/A	0.09	0.19
Unchlorinated Monomer	N/A	0.11	0.28
Chlorinated Monomer	6.94	6.94	6.94
Exp. 4[b]			
Cotton Control	0.04	0.24	0.24
Unchlorinated Monomer	0.71	0.93	1.41
Chlorinated Monomer	6.76	6.76	6.76

[a]Inoculum was 8.67 x 10^7 CFU; Cl^+ loading was 0.334 % by weight.

[b]Inoculum was 5.67 x 10^6 CFU; Cl^+ loading was 0.331 % by weight.

Finally, the results of the wash test performed on swatches of cotton containing the two monomer coatings, some chlorinated as outlined in the chlorination procedure, and others unchlorinated for comparison purposes, are presented in Table 11. The average oxidative chlorine loading for the HAM-Sil and HAM-Sil1' monomer-coated samples were 0.49% and 0.44% by weight, respectively. Five observations are clearly evident from the data in Tables 11 and 12. First, the monomeric coatings are partially washed off

the surfaces upon successive laundering cycles. Second, HAM-Sil1' did not demonstrate the expected increase of durability due to the methyl attached to the siloxane inhibiting the hydrolysis of the monomer. Perhaps, the hydrophobic effects of the chlorinated monomers supercede what benefits may be gained form that of the methyl present on the HAM-Sil1' monomer coatings. Third, prechlorination reduces the rate of loss, perhaps due to the increased hydrophobicity of the chlorinated surfaces. Fourth, from the small difference observed from the prechlorinated swatches and the rechlorinated swatches it seems as though the loss of oxidative chlorine is mainly due to the loss of the monomer coatings from the surface. Fifth, it is evident for all of the coating conditions that a sufficient biocidal efficacy could be regenerated upon rechlorination even after 50 laundering cycles. Furthermore, a low concentration of bleach added to the laundering cycles could possibly maintain biocidal activity of the cotton material for its lifetime. In comparison, the HAM-Sil and HAM-Sil1'monomers had a retention of 50 % and 48 %, respectively, on the cotton surfaces after 50 laundering cycles; whereas, the 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer only had 22 % retention after 50 laundering cycles.[22] As a result, the novel compound discussed herein is a much more effective and durable coating for textile fabrics than any other discussed in the literature to date. There are also three possible factors which influence this marked improvement. First, due to lack of neighboring hydrogens next to the chlorinated amine functionality, it is impossible for the hindered amine compound to undergo a dehydohalogenation process to lose any oxidative chlorine. Second, due to the steric hinderance surrounding the amine chlorine bond, it becomes less likely that the amine halamine can lose oxidative

chlorine through a hydrolysis type mechanism. Third, it is well understood that the amine halamine bond is the strongest of all halamine-type bonds.

Table 11

Durability of the HAM-Sil coating on the surface of cotton

Number of Laundering Cycles	Cl^+ % When Chlorinated Before Laundering[a]	Cl^+ % When Chlorinated After Laundering[b]	Cl^+ % When Chlorinated After Recharge[c]
0	0.490	0.490	0.490
5	0.451	0.431	0.461
10	0.417	0.397	0.431
25	0.343	0.309	0.368
50	0.216	0.176	0.245

Number of Laundering Cycles	% Cl^+ Remaining On The Cotton Surfaces		
0	100	100	100
5	92	88	94
10	85	81	88
25	70	63	75
50	44	36	50

[a] The treated cotton swatches were chlorinated before they were laundered.

[b] The treated cotton swatches were chlorinated after they were laundered.

[c] The treated cotton swatches were chlorinated, laundered, and then re-chlorinated.

Table 12

Durability of the HAM-Sil1' coating on the surface of cotton

Number of Laundering Cycles	Cl^+ % When Chlorinated Before Laundering[a]	Cl^+ % When Chlorinated After Laundering[b]	Cl^+ % When Chlorinated After Recharge[c]
0	0.436	0.436	0.436
5	0.397	0.374	0.405
10	0.357	0.344	0.371
25	0.296	0.265	0.318
50	0.183	0.148	0.209

Number of Laundering Cycles	% Cl^+ Remaining On The Cotton Surfaces		
0	100	100	100
5	91	86	93
10	82	79	85
25	68	61	73
50	42	34	48

[a] The treated cotton swatches were chlorinated before they were laundered.

[b] The treated cotton swatches were chlorinated after they were laundered.

[c] The treated cotton swatches were chlorinated, laundered, and then re-chlorinated.

4. Conclusion

The novel hindered amine siloxane compounds discussed herein are a logical possibility for commercialization due to their relatively inexpensive cost of synthesis and their outstanding performance in both durability during laundering and biocidal efficacy.

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References

1. Qian L, Sun G. Durable and regenerable antimicrobial textiles: Improving efficacy and durability of biocidal functions. Journal of Applied Polymer Science 2004;91(4):2588-2593.
2. Qian L, Sun G. Durable and regenerable antimicrobial textiles: Synthesis and applications of 3-methylol-2,2,5,5-tetramethyl-imidazolidin-4-one (MTMIO). Journal of Applied Polymer Science 2003;89(9):2418-2425.
3. Worley SD, Sun G. Biocidal polymers. Trends in Polymer Science (Cambridge, United Kingdom) 1996;4(11):364-370.
4. Sun G, Chen TY, Habercom MS, Wheatley WB, Worley SD. Performance of a new polymeric water disinfectant. Water Resources Bulletin 1996;32(4):793-797.
5. Worley SD, Williams DE. Halamine water disinfectants. Critical Reviews in Environmental Control 1988;18(2):133-175.
6. Tsao TC, Williams DE, Worley SD. A new disinfectant compound. Industrial & Engineering Chemistry Research 1990;29(10):2161-2163.
7. Lankford Mary G, Collins S, Youngberg L, Rooney Denise M, Warren John R, Noskin Gary A. Assessment of materials commonly utilized in health care: implications for bacterial survival and transmission. American journal of infection control 2006;34(5):258-263.
8. Neely AN. A survey of gram-negative bacteria survival on hospital fabrics and plastics. The Journal of burn care & rehabilitation 2000;21(6):523-527.
9. Belkin NL. Survival of gram-positive bacteria on hospital fabrics. Journal of clinical microbiology 2000;38(10):3912.

10. Vanhems P, Lepape A, Savey A, Jambou P, Fabry J. Nosocomial pulmonary infection by antimicrobial-resistant bacteria of patients hospitalized in intensive care units: risk factors and survival. The Journal of hospital infection 2000;45(2):98-106.
11. Neely AN, Maley MP. Survival of enterococci and staphylococci on hospital fabrics and plastic. Journal of clinical microbiology 2000;38(2):724-726.
12. Barnes K, Liang J, Wu R, Worley SD, Lee J, Broughton RM, et al. Synthesis and antimicrobial applications of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]. Biomaterials 2006;27(27):4825-4830.
13. Liang J, Huang TS, Worley SD. Coating new N-halamine siloxanes on silica gel to prepare biocidal silica gels. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-049.
14. Liang J, Barnes K, Chen Y, Worley SD, Lee J, Broughton RM, et al. New rechargeable biocidal N-halamine siloxanes. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-048.
15. Worley SD, Chen Y, Wang J-W, Wu R, Li Y, inventors; (Auburn University, USA; Vanson Halosource, Inc.). assignee. N-halamine siloxanes for use in biocidal coatings and materials. Application: WO Patent No. 2003-US18883 2003106466, 2003 20030612.
16. Sun G, Worley SD. Halamine chemistry and its applications in biocidal textiles and polymers. Modified Fibers with Medical and Specialty Applications 2006:81-89.

17. Sun G, Worley SD. Chemistry of durable and regenerable biocidal textiles. Journal of Chemical Education 2005;82(1):60-64.

18. Xu X, Sun G. Multifunctional antibacterial cellulose. Polymer Preprints (American Chemical Society, Division of Polymer Chemistry) 2001;42(1):532-533.

19. Tsao TC, Williams DE, Worley CG, Worley SD. Novel N-halamine disinfectant compounds. Biotechnology Progress 1991;7(1):60-66.

20. Williams DE, Elder ED, Worley SD. Is free halogen necessary for disinfection? Applied and Environmental Microbiology 1988;54(10):2583-2585.

21. Liang J, Chen Y, Barnes K, Wu R, Worley SD, Huang TS. N-halamine/quat siloxane copolymers for use in biocidal coatings. Biomaterials 2006;27(11):2495-2501.

22. Worley SD, Chen Y, Wang JW, Wu R, Cho U, Broughton RM, et al. Novel N-halamine siloxane monomers and polymers for preparing biocidal coatings. Surface Coatings International, Part B: Coatings Transactions 2005;88(B2):93-99.

Biocidal N-Halamines VI. Hindered Amine *N*- Halamine Siloxane-Functionalized Silica Gel

Abstract

A new *N*-halamine precursor, 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine, which will be referred to as HAM-Sil has been employed to functionalize the surfaces of silica gel particles so as to produce a more stable film, that once chlorinated by a dilute solution of household bleach, can be rendered biocidal. The biocidal efficacies of the newly functionalized silica gel particles have been demonstrated using a cartridge filter experiment by challenging both Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* 0157:H7. Complete 6 log inactivations of both bacteria were observed within a contact time as rapid as 30 s. However, some longer contact times were needed dependant upon the oxidative chlorine loading of the silica gels. It has also been demonstrated that once the bound chlorine has been depleted, that it can be simply recharged by the further introduction of dilute household bleach. Some possible implementations of this product include filter media for various uses such as for swimming pools, spas, and cutting oils to serve as disinfectants and for odor control.

1. Introduction

Recent work in our laboratories has been concentrated on the development of new regenerable filter medias. A variety of *N*-halamine siloxane monomers and polymers have been incorporated into various medias such as sand and particles of silica gel.[1-3] These *N*-halamine siloxane monomers or polymers have employed amide halamines to provide their biocidal function.[2, 4-8] Whereas, the work described herein employs a new amine halamine to provide biocidal functionality. The hindered amine siloxane monomer, 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperdine, employed in this study had two significant advantages over its amide counterparts. First, the methyl groups surrounding the amine chlorine bond served to protect it against hydrolyses; that is, the release of free halogen into an aqueous environment. Second, it is well known that the amine halamine bond is the strongest of all the halamine-type bonds, ie. the dissociation constant for an amine halamine in aqueous solution is three to four orders of magnitude lower than that of the amide halamines as can be seen in Table 1 from chapter 2.[4]

Worley et al.[9] has previously proven that pathogenic organisms are deactivated by direct contact to the N-halamine functionality; therefore, it seems reasonable that the newly modified amine halamine particles of silica gel may be more stable toward hydrolyses and could provide an enhanced biocidal activity for a longer duration of time without being recharged with dilute bleach. However, it is also reasonable to infer that the inactivation time may be longer for a more stable amine halamine than that of an amide halamine with a lesser bond strength due to the ease of direct transfer of the halogen to the bacteria. It could be possible by the implementation of these *N*-halamine filter medias that a lower concentration of free halogen than that of current use could be

employed while maintaining the safety of our drinking waters.[2, 6-8, 10-12] The novel hindered amine siloxane compound developed in our laboratories can be used to chemically modify particles of silica gel, which demonstrate sufficient biocidal efficacies once chlorinated with dilute household bleach. Since particles of silica gel possess a voluminous amount of surface area and are relatively inexpensive, they prove to be an effective precursor filter media. The modification of silica gels has gained some attention in the literature recently.[13-15] Thus, the chemical modifications of silica gel particles could be of importance.

2. Experimental

2.1 Materials

All chemicals were purchased from the Aldrich Chemical Company, Milwaukee, WI and used as they were received without further purification unless otherwise noted. The 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). The Trypticase soy agar employed was from (Difco Laboratories, Detroit, MI). The peristaltic pump employed was from (Gelman Sciences, Ann Arbor, MI).

2.2 Preparation of 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperdine (HAM-Sil).

The HAM-Sil monomer employed for this work was synthesized according to the method previously discussed in chapter 4, which also contained the characterization data. Its structure, designated as **I**, can be seen in figure 31.

HAM-Sil, **I** + Silica gel + EtOH/H_2O (1:1 w/w), Reflux → **II** + NaOCl, H_2O → **II-Cl**

Figure 31 The prepartion scheme for producing biocidal silica gel with monomer **I**.

2.3 Preparation of 4-[3-diethoxymethylsilylpropoxy]-2,2,6,6-tetramethylpiperdine (HAM-Sil1').

The HAM-Sil1' monomer used for this work was also synthesized according to the method previously discussed in chapter 4. The HAM-Sil1' structure, designated as **III,** is shown in figure 32.

HAM-Sil1', **III** + Silica gel + EtOH/H_2O (1:1 w/w), Reflux → **IV** + NaOCl, H_2O → **IV-Cl**

Figure 32 The prepartion scheme for producing biocidal silica gel with monomer **III**.

2.4 Coating Procedures

In the case of the HAM-Sil monomer **I**, commonly 5.0 g of 30-60 mesh silica gel were added to a 100 mL round bottom flask along with 20.0 g of an ethanol/water (1:1 w/w)

mixture, as well as the HAM-Sil monomer in the range of 0.25 to 1.25 g. The mixture was then refluxed for 0.5 to 7 h. The newly modified silica gel (**II**, fig. 31) was isolated by vacuum filtration and washed with three 100 g aliquots of an ethanol/water (1:1 w/w) mixture, and was then dried under open ambient conditions. The results of these various conditions are represented in figure 33 and table 13. In the case of the HAM-Sil1'-modified silica gel, the same process was performed, and the results can also be seen in figure 33, as well as in table 13.

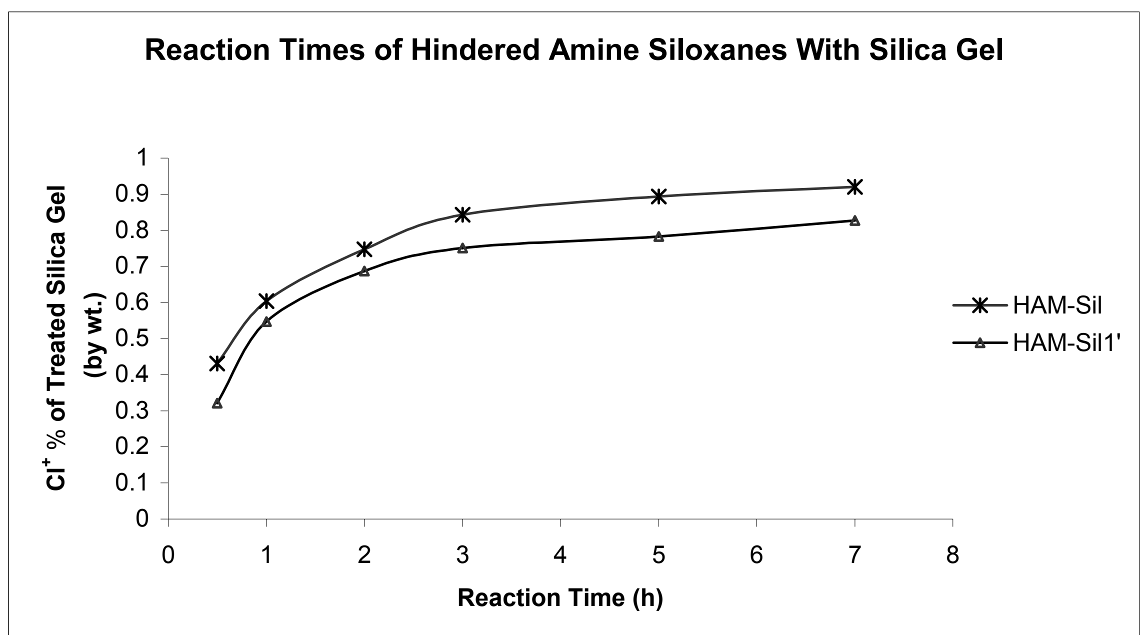

Figure 33 Coating efficiencies of hindered amine siloxane monomers as a function of time.

Table 13

Coating efficiency of monomers **I** and **III** on silica gel as a function of reaction time.

Reaction time (h)[a]	Titrated Cl^+ (wt %)[b]	Titrated Cl^+ (wt %)[c]
0.5	0.431	0.321
1.0	0.604	0.547
2.0	0.747	0.687
3.0	0.843	0.751
5.0	0.894	0.783
7.0	0.920	0.827

[a]The weights of silica gel, **I** or **III**, and ethanol/water (1:1 w/w) in each case were respectively, 2.5 g, 0.75 g, and 10.0 g; the reaction was ran under reflux conditions.

[b] Silica gel treated with the HAM-Sil monomer, **I.**

[c] Silica gel treated with the HAM-Sil1' monomer, **III.**

2.5 Chlorination Procedure

The coated modified particles of silica gel were chlorinated by soaking them at ambient temperature for 30 min in a 10% aqueous solution of Clorox® brand NaOCl, a household bleach, which was buffered to pH ≈7 by the drop-wise addition of 6 M HCl. The chlorinated particles of silica gel were then washed with 3 aliquots (50 mL per 2.5 g of silica gel) of deionized water and dried at 45^0C for 2 h to remove any occluded free chlorine. The loading of bound chlorine on the silica gel was determined as described in the analytical titration procedure.

2.6 Analytical titration procedure.

For the determination of oxidative chlorine (Cl^+) content, a modified iodometric/thiosulfate titration procedure was employed which has been discussed previously in the literature.[16] For example, approximately 0.2 g of treated and chlorinated silica gel was ground into a powder using a mortal and pestle. The freshly ground silica gel was then suspended in 90 mL of ethanol and 10 mL of a 0.1 N acetic acid solution. After the addition of 0.3 g of KI, the solution was then titrated using with 0.0375 N sodium thiosulfate until the yellow color disappeared at the end point. The weight percent of Cl^+ on the chlorinated, modified particles of silica gel could then be determined from the equation below:

% Cl^+ = [(X V X 35.45)/(2 X W)] X 100% **3**

where N and V are the normality (eqv/L) and volume (L), respectively, of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in g of the modified silica gel.

2.7 Biocidal efficacy testing

In these experiments glass columns (25.0 cm length, 1.0 cm inside diameter) were packed to a height of 18.0 cm with the varying forms of silica gel comprising of treated-chlorinated and two controls treated-unchlorinated and untreated silica gel. The empty bed volumes of the packed columns were measured to be approximately 4.0 mL, so that a controlled flow rate of 4.0 mL/s would indicate a contact time of 1 s for the bacteria in the columns. A peristaltic pump was employed to control the flow rate of 50 mL aliquots of a pH 7 buffered inoculum solution through the columns. The contact times were adjusted by the consecutive recirculation of the inoculum through the columns. Once the adjusted contact times had elapsed, 200 μL aliquots were collected in sterile centrifuge

tubes and immediately quenched by the addition of 0.02 N sodium thiosulfate to ensure that any oxidative chlorine that may have leached from the silica gel was inactivated and unable to further the reduction of viable bacteria. Then, serial dilutions of the quenched solutions were plated on Trypticase soy agar. The plates were incubated at 37°C for 24 h and then counted for viable CFU of bacteria. The bacteria employed to challenge the columns were a Gram-positive *Staphylococcus aureus* ATCC 6538 or a Gram-negative *Escherichia coli* O157:H7 ATCC 43895. The results can be seen in tables 14 and 15.

Table 14

The efficacies of Ham-Sil-treated silica gel against *S. aureus*

Sample coating	Log reduction in a contact time (sec)		
	10.0	30.0	60.0
Exp. 1[a]			
Silica gel Control	0.13	0.09	0.14
Unchlorinated Monomer	0.75	0.81	1.01
Chlorinated Monomer	3.19	3.49	6.31
Exp. 2[b]			
Silica gel Control	0.13	0.09	0.14
Unchlorinated Monomer	1.44	2.06	2.18
Chlorinated Monomer	3.46	6.28	6.28

[a]Inoculum was 2.07 x 10 x 10^6 CFU; Cl^+ loading was 0.854 % by weight.

[b]Inoculum was 1.93 x 10^6 CFU; Cl^+ loading was 1.01 % by weight.

Table 15

The efficacies of HAM-Sil-treated silica gel against *E. coli* O157:H7

Sample Coating	Log reduction in a contact time of (min)			
	10.0	30.0	60.0	120.0
Exp. 3[a]				
Silica gel Control	N/A	0.09	0.19	0.39
Unchlorinated Monomer	0.50	0.57	0.76	0.89
Chlorinated Monomer	1.544	2.08	2.68	6.20
Exp. 4[b]				
Silica gel Control	0.02	0.07	0.05	0.05
Unchlorinated Monomer	0.07	0.13	0.41	0.72
Chlorinated Monomer	1.63	6.13	6.13	6.13

[a]Inoculum was 1.67×10^6 CFU; Cl^+ loading was 0.854 % by weight.

[b]Inoculum was 1.33×10^6 CFU; Cl^+ loading was 1.01 % by weight.

2.8 Stability and biocidal efficacy of chlorinated coatings.

The stability of the HAM-Sil coating on particles of silica gel has been examined for overall stability as well as the chlorine regeneration capacity. The HAM-Sil1' monomer was not tested under these conditions due to the lesser oxidative loading of chlorine would probably imply a less likelihood of commercial utilization. It was thought that the HAM-Sil monomer and/or the chlorine could be disassociated from the surface or

monomer, respectively, either by a hydrolysis type reaction, or could be simply washed off through physical abrasion of the flowing water. Therefore, a cartridge filter-type experiment was employed to test the overall durability of treated chlorinated particles of silica gel. The results of this test will provide both the rate of dissociation of the chlorine in a flowing aqueous environment in which an equilibrium can not be established due to the fact that the water is not recirculated through the cartridge providing a constant volume of water and the bonding of the coating to the surface of the silica gel.

3. Results and Discussion

The biocidal efficacies of the newly modified silica gel with the chlorinated HAM-Sil monomer can be directly compared to the hydantoin siloxane monomer with the data available in the literature.[1-3] The oxidative chlorine loadings, as well as the biocidal efficacies, were reported for surfaces such as sand or silica gel modified with the 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer; however, it is important to note that the polmerized derivatives have been reported as well, and that data will not be used for comparative purposes of stability and biocidal efficacies due to the difficulty in attributing the dominating factors. It is well known that polymeric versions of compounds can demonstrate notable differences of performance. Experiments performed previously in our laboratories by Liang et al.[3] have employed the use of amide halamine monomers to treat the surface of sand. These experiments produced a modified surface with the capability to hold 0.28 % Cl^+ by weight. This newly modified sand was demonstrated to totally inactivate a 6.50 log concentration of *S. aureus* in a contact interval of 0-1 min, and 7.40 log concentration of *E. coli* in a contact interval of 1-5 min. However, the HAM-Sil- modified silica gel discussed herein was capable of providing a 1.01 % Cl^+ by

weight loading, and inactivating a 6.0 log concentration of either *E. coli* or *Staph* in a contact interval of 0-30 sec. Although there is a notable difference in the cost of the starting filter media material, a significant increase of oxidative loading is achieved. Thus, a much shorter contact time was needed to provide similar reductions of bacterial colonies with the HAM-Sil-modified silica gel. These differences are mainly due to the starting filter media itself, and not the monomer used to modify the surface. Therefore, a direct comparison can be made between the literature data for the 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer-modified silica gel with that of the HAM-Sil-modified silica gel discussed herein. The 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer modified silica gel is capable of supporting a 1.03 % Cl^+ by weight oxidative chlorine loading and a total 7 log reduction of either *Staph* or *E.coli* in a contact interval of 0-30 s.[1] The HAM-Sil modified silica gel was capable of demonstrating similar biocidal efficiency with a 6 log reduction of either bacteria in the same contact interval. The relative stabilities under flowing water conditions can also be compared to literature results as seen in table 16.[1]

TABLE 16

Stability of Coated Silica Gel Particles under Flowing Water Conditions

Coating material[a]	Time of flow (h)	Titrated Cl^+ (wt %)[b]	Titrated Cl^+ (wt %)[c]
Hydantoin Siloxane Modified Silica gel	0	1.05	
	168	1.02	1.04
Hindered Amine Siloxane silica gel	0	0.89	
	168	0.85	0.86

[a]The material was packed into a glass column (see text).

[b]The chlorine loadings were measured before and after flowing distilled, deionized water through the column at a rate of about 5 mL/min in the hydantoin siloxane case whereas a flow rate of about 8 mL/min in the HAM-Sil case.

[c]The chlorine loadings were measured after recharging the material in the column with household bleach (see text).

The 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer modified silica gel only lost 4 % of its oxidative chlorine loading after 168 h of continous flow at a rate of 5 mL/min; whereas, the HAM-Sil modified silica gel only lost 4.5 % of its oxidative chlorine loading after the 168 h of continous flowing water at an increased rate of about 8mL/min. The recharge data shows that the hydantoin siloxane-modified silica gel could be recharged almost completely. However, in the case o the HAM-Sil-modified silica gel the recharge data reveals that only 97 % of its original oxidative chlorine loading cold be reclaimed. From this data it is possible to infer that the true reason for loss of the 4.5 % of the original oxidative chlorine present on the HAM-Sil modified silica gel can be attributed to the loss of HAM-Sil monomer from the surface of the silica gel, and not the dissociation of the chlorine into the water. This loss of monomer can be attributed to the increased flow rate from the literature experiment to the one discussed herein. Also, it is

important to note that almost no difference is seen between the oxidative chlorine loading after the flow experiments and that of the recharge data determined for the Ham-Sil-modified silica gel.

4 Conclusions

From the data taking into consideration the difference of flow rates observed for the stability test and the difference of bacterial concentrations employed while determining the biocidal efficacies of these *N*-halamine-modified types of silica gel, two observations are apparent. First, N-halamines, no matter the type of nitrogen chlorine bond, can serve as effective biocides. Second, the N-Cl bond of the amine halamine may be more stable toward hydrolyses than that of the siloxane bond to the silica gel. The modified silica gel discussed herein, once chlorinated, demonstrated suitable performance levels of both stability, as well as endurance, and therefore could have potential commercial use in such practical applications as cutting oil filtration and spas, applications in which a low, but constant demand for biocidal efficacy exist.

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References

1. Liang J, Owens JR, Huang TS, Worley SD. Biocidal hydantoinylsiloxane polymers. IV. N-halamine siloxane-functionalized silica gel. Journal of Applied Polymer Science 2006;101(5):3448-3454.
2. Liang J, Huang TS, Worley SD. Coating new N-halamine siloxanes on silica gel to prepare biocidal silica gels. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-049.
3. Liang J, Wu R, Huang TS, Worley SD. Polymerization of a hydantoinylsiloxane on particles of silicon dioxide to produce a biocidal sand. Journal of Applied Polymer Science 2005;97(3):1161-1166.
4. Barnes K, Liang J, Wu R, Worley SD, Lee J, Broughton RM, et al. Synthesis and antimicrobial applications of 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]. Biomaterials 2006;27(27):4825-4830.
5. Liang J, Barnes K, Chen Y, Worley SD, Lee J, Broughton RM, et al. New rechargeable biocidal N-halamine siloxanes. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 2006:IEC-048.
6. Chen Y, Worley SD, Huang TS, Weese J, Kim J, Wei CI, et al. Biocidal polystyrene beads. IV. Functionalized methylated polystyrene. Journal of Applied Polymer Science 2004;92(1):368-372.
7. Chen Y, Worley SD, Huang TS, Weese J, Kim J, Wei CI, et al. Biocidal polystyrene beads. III. Comparison of N-halamine and quat functional groups. Journal of Applied Polymer Science 2004;92(1):363-367.

8. Chen Y, Worley SD, Kim J, Wei CI, Chen T-Y, Suess J, et al. Biocidal Polystyrenehydantoin Beads. 2. Control of Chlorine Loading. Industrial & Engineering Chemistry Research 2003;42(23):5715-5720.
9. Williams DE, Elder ED, Worley SD. Is free halogen necessary for disinfection? Applied and Environmental Microbiology 1988;54(10):2583-2585.
10. Sun G, Chen TY, Habercom MS, Wheatley WB, Worley SD. Performance of a new polymeric water disinfectant. Water Resources Bulletin 1996;32(4):793-797.
11. Williams DE, Worley SD, Barnela SB, Swango LJ. Bactericidal activities of selected organic N-halamines. Applied and Environmental Microbiology 1987;53(9):2082-2089.
12. Worley SD, Williams DE, Barnela SB. The stabilities of new N-halamine water disinfectants. Water Research 1987;21(8):983-988.
13. Lukasiewicz A, Chmielewska D, Walis L, Rowinska L. New silica materials with biocidal active surface. Polish Journal of Chemical Technology 2003;5(4):20-22.
14. Pirogov AV, Kuzavlev AP, Shpigun OA. New stationary phases for normal-phase HPLC based on silica gel modified with magnesium, calcium, strontium, and barium salts. Journal of Analytical Chemistry 2005;60(8):747-751.
15. Otsuka M, Tokumitsu K, Matsuda Y. Solid dosage form preparations from oily medicines and their drug release. Effect of degree of surface-modification of silica gel on the drug release from phytonadione-loaded silica gels. Journal of Controlled Release 2000;67(2-3):369-384.

16. Liang J, Chen Y, Barnes K, Wu R, Worley SD, Huang TS. N-halamine/quat siloxane copolymers for use in biocidal coatings. Biomaterials 2006;27(11):2495-2501.

Conclusions And Recommendations For Future Work

In chapter one the postulate proposed was that increasing the number of potential covalent bonds to the surface would provide a more durable coating on the substrate of interest, namely fabrics employed in the use of making hospital garments. Therefore, the 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer was designed to double the number of potential bonds to the surface relative to the previously developed 3-triethoxysilylpropyl-5,5-dimethylhydantoin while maintaining other factors such as dissociation constants of the oxidative chlorine from the newly modified chlorinated surface. Thus, a direct comparision could be inferred from the difference in performance during practical applications such as simulated household launderings. It was of interest to determine the relative durability of the siloxane bonds to the cotton. It was found that increasing the number of potential bonds to the surface did indeed increase the amount of bound coating to the surface, which was determined directly by the increase in percentage of add-on weight as well as the increase of oxidative chlorine which could be loaded on the 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer-treated fabrics. The results of these tests also help to indicate the stability of the hydantoin ring itself in withstanding the stringent conditions of a washing environment. The results showed that the ring was not decomposing in the elevated pH and temperature environment. Rather, the reason for the loss of the ability of treated hydantoinsiloxane monomers was in fact due to the loss of

the monomer itself from the surface and not to the decomposition of the hydantoin ring as once thought. The 5,5'-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin] monomer-treated fabrics were capable of higher oxidative chlorine loadings and increased durability during repeated launderings. They out performed the previously developed 3-triethoxysilylpropyl-5,5-dimethylhydantoin-treated fabrics in both durability and overall biocidal efficacy due to the increase in bound oxidative chlorine which they possessed.

In chapter two a series of questions were proposed for the co-polymerization of hydantoin siloxane monomers with that of quaternary ammonium salt siloxane monomers: 1. could *N*-halamines perform more efficiently in deactivating bacteria than that of the widely commercialized quaternary ammonium salt compounds, 2. could a synergistic effect be seen with the combination of both QAC's and *N*-halamine moieties, and 3. is it possible to make an industrially friendly water soluble monomer by varying the quat functionality with respect to that of the hydantoin pendant group? It was determined that both N-halamine and quat functional groups were effective in inactivating *S. aureus*; however, only the N-halamine units were responsible for the rapid inactivation of *E.coli.* There seemed to be no synergistic effects in the inactivation of the bacteria tested in these studies. After evaluating several ratios of quat to *N*-halamine functionalities, it was found that the least amount of quat necessary was a 1:3 ratio of quat to *N*-halamine, respectively. This ratio allowed for a 5 % by weight solution in water to be employed during the treatment of fabrics. Therefore, the practices of green chemistry could be successfully employed during the treatment process making this type of co-polymer coatings potentially more industrially advantageous to any previously

developed N-halamine siloxane monomer coatings. Also, the quat functionality could provide a longer lasting biocidal moiety even after the loss of oxidative chlorine after extended use. This could find applications for which recharging the surface may be impractical, such as military use or internal medical devices. However, the N-halamine functional groups provided superior biocidal efficacy against *E. coli* as compared to that of the QAC functional groups and are thought to be unsurpassed in overall performance.

The amine halamine siloxane monomers discussed in chapter 3 demonstrated exceptional performances once bonded to fabrics and chlorinated in both biocidal activity and endurance during repeated simulated washing tests. The newly developed 4-[3-triethoxysilylpropoxy]-2,2,6,6-tetramethylpiperidine monomer was designed to provide increase stability toward hydrolysis of the N-Cl bond during the washing environment. The results of simulated washing tests indicated that not only did it out perform the previously developed hydantoin siloxane monomers in the loss of oxidative chlorine from the surface of the treated swatches, but it also was a more durable coating implying that a lower percentage wash washed off the surface during the laundering process. It is thought that the inceased hydrophobicity of the amine N-halamine siloxane coatings is the dominant factor for its stability. Since the amine chlorine is less likely to be hydrolyzed than that of an amide halamine, it provides a more hydrophobic coating therefore protecting the siloxane bonds from hydrolyses. The increased biocidal efficacy should be further explored.

The amine halmine siloxane monomers were employed to treat the surface of silica gel as explained in chapter 4. It was determined that the amine N-halmine type bonds were stable during flowing water tests and could provide reasonable inactivation of bacteria.

Once again the increase in bond strenth of the amine halamine plays an important role in not allowing the loss of oxidative chlorine from the filter media surface. Also, the experiments in chapter 4 demonstrated the versatility of the siloxane type of monmers to bind to various substrates.

The works herein were focused on developing more stable and durable N-halamine siloxane coatings that could potentially have industrial usefulness. It has been demonstrated numerous times in a number of experiments that *N*-halamines are extremely versatile and potent biocides. Since these types of coatings discussed herein use an inexpensive bleach solution to render them biocidal, and harness the oxidative power of chlorine, for which there is no known tolerance by any bacteria, it seems reasonable that coatings of this general type could find many practical uses and potentially increase the quality of living for all of mankind.

Future work

Further works should be done to determine why various types of N-halamine precursors show an extreme difference for the potential to be chlorinated during normal laundering conditions in the presence of bleach. Also, additional microbiological investigations to determine if the types of *N*-halmine bonds, ie. amine, amide, imide, are influencing the kill rates of microorganisms. Lastly, work should be done to find new cost-effective ways to attach N-halamine precursors to various substates of interest. One potentially useful pathway may be to employ 1,2,3,4-butanetetracarboxylic acid as a cross linking agent for substrates with active OH groups such as cellulose with chemically modified halamine precursors, which also contain active OH groups.