THE USE OF REACTIVE SILANE CHEMISTRIES TO PROVIDE DURABLE, NON-LEACHING ANTIMICROBIAL SURFACES

ABSTRACT

The application of a Silane quaternary amine (Si-quat) based antimicrobial has been proven effective as a finishing agent on textiles and construction products for almost 40 years. Antimicrobial agents of this type have been used on a wide variety of porous and non-porous systems with outstanding results. Successful applications can be achieved using almost any type of wet process, such as a pad or spray and but may also be extruded or molded into various synthetic materials. Once the material is cured onto or into the substrate it can then provide the antimicrobial protection necessary to safeguard the product from microbial contamination and subsequent breakdown.

This paper and presentation will cover not only the ease of use of the Si-Quat antimicrobials but will provide a review of the key data and test techniques relating to the demonstration of efficacy, durability and utility in dealing with microbial problems on non-porous surfaces under real-world in-use conditions. Durability and real-life performance are critical factors when choosing the proper antimicrobial treatment. This eco-friendly product falls in line with the current emphasis on sustainability and environmental impact that is dominating the world markets.

INTRODUCTION

Almost all materials have one thing in common; they face a common enemy. Bacteria, fungi, algae, and other organisms can consume and degrade surfaces during shipment, storage, and use, causing loss of product as well as exposing the manufacturer to potential liability. Contamination and colonization of microorganisms on surfaces can result in problems as small as an offensive odor to serious human infections and death. Imparting an antimicrobial agent into synthetic material can create microbial resistant, non-porous surfaces that can alleviate many of these problems. However, selecting the right antimicrobial is essential to provide the appropriate protection to the product as well as to protect our environment. The list of available agents becomes limited when the criteria selection includes durability, regulatory approvals (EU BPD, US EPA), spectrum of activity, and toxicity to both the manufacturer and the end-user.

The use of a silane quaternary amine based antimicrobial can provide durable antimicrobial protection against a wide variety of microorganisms without the worry of leaching heavy metals, phenolic compounds or other toxic compounds that continue to contaminate our environment and present situations that promote microbial resistance.

protection to the product as well as to protect our environment. The list of available agents becomes limited when the criteria selection includes durability, regulatory approvals (EU BPD, US EPA), spectrum of activity, and toxicity to both the manufacturer and the end-user.

The use of a silane quaternary amine based antimicrobial can provide durable antimicrobial protection against a wide variety of microorganisms without the worry of leaching heavy metals, phenolic compounds or other toxic compounds that continue to contaminate our environment and present situations that promote microbial resistance.

Altering surfaces with durable non-leaching antimicrobial agents such that they provide an active killing "field" for killing one celled organisms on contact is a reasonable and attainable goal. The use of quaternized nitrogen silanes has been demonstrated to provide such treatments on a wide variety of surfaces and end-use conditions. There are many ways to modify surfaces so that they are less receptive to the settling, attachment, and colonization of microorganisms. These modifications can create surfaces so that microorganisms that come into direct contact with the treated surface are inhibited or killed or more easily cleaned away.

Chemical and physical bonding mechanisms using covalent bonding mechanisms, using covalent or ionic associations done by simple condensation reactions, energy induced as in plasma deposition, or catalyzed reactions of reactive materials have been demonstrated. The success of these surface modifications at controlling the deposition, attachment and propagation of good (useful) or bad (destructive, interfering, or annoying) microorganisms has often been limited by many factors. These factors include the lack of durability of the coating and the practical and cost effective application of these agents during product manufacturing. Such is the challenge to find technologies that can be evaluated and utilized in a safe, long lasting, and cost effective manner. Silane quat monomeric agents can both self crosslink and can link with available surface sites to create fully cured polymer that binds directly to the surface providing an antimicrobial coating that becomes part of the substrate itself.   The non-leaching behavior of such a reactive surface allows for the control of surface microbial contamination without the continuous release of toxic components into the environment which can promote the formation of resistant organisms.

MICROORGANISMS

Mold, mildew, fungus, yeast, bacteria, and virus (microorganisms), are part of our everyday lives. There are both good and bad types of microorganisms. The thousands of species of microorganisms that exist are found everywhere in the environment, on our garments, on our bodies and on virtually every surface around us. Microorganisms, their body parts, metabolic products, and reproductive parts, cause multiple problems to synthetic materials. They are human irritants, sensitizers, toxic -response agents, causers of disease, and simple discomforting agents. Clearly, microorganisms are the most potent pollutants in our environment, on our clothes, and on our furnishings.

Microorganisms need moisture, appropriate temperatures, nutrients, and most of them need to be associated with a surface. Textiles, apparel, bathrooms, carpets, draperies, wall coverings, furniture, bedding and ceiling tiles create ideal habitats for microorganisms due to the high levels of humidity seen in these environments during common use. Nutrients utilized by microorganisms can be organic material, inorganic material, and/or living tissue. For example, bacteria play an important role as part of the body's microflora, and along with the skin, are shed continuously. Given acceptable growth conditions, they can multiply from one organism to more than one billion in just 18 hours. Over time, microorganisms can form highly complicated and durable microbial colonies that attach themselves to surfaces. These microbial biofilms are a prime concern in the medical industry and must be controlled before they form on the surface themselves.

Microorganisms cause problems with raw materials and processing chemicals, wet processes in mills, roll or bulk goods in storage, finished goods in storage and transport, and goods as they are used by the consumer. They are also an annoyance and aesthetic problem to architects,

builders, and home owners. The economic impact of microbial contamination is significant and the consumer interests and demands for protection are at an all-time high.

ANTIMICROBIALS The term antimicrobial refers to a broad range of technologies that provide varying degrees of protection for both organic and synthetic products against microorganisms. Antimicrobials are very different in their chemical nature, mode of action, impact on people and the environment, in plant-handling characteristics, durability on various substrates, costs, and how they interact with good and bad microorganisms. Antimicrobials are used in and on a variety of substrates to control bacteria, fungi, and algae. This control reduces or eliminates the problems of deterioration, staining, odors, and health concerns that they cause. Additionally, antimicrobial agents may prevent the loss of product during transport and can potentially reduce legal liability when microbial contamination occurs. In the broad array of microorganisms there are certainly both good and bad types. Antimicrobial strategies for bad organisms must include ensuring that non-target organisms are not affected or that adaptation of microorganisms is not encouraged. For instance, antimicrobial agents applied to textiles must control all microorganisms on the textile without leaching into the environment and affecting the natural biological skin flora. In addition, as sublethal doses of antimicrobial agents may lead to adaptation. The antimicrobial agent should not lose effectiveness over time and cannot diminish in effective concentration. Antimicrobial agents can be classified in two main types; leaching and non-leaching. Leaching antimicrobial agents are defined as agents that must come off the treated substrate in order to exert the antimicrobial properties. Any antimicrobial agent that must enter the cell to work is considered a leaching agent. Non-leaching agents are fixed to the treated surface (usually by covalent bonds) and subsequently do not need to leave this surface to provide antimicrobial action. As these agents are physically attached, there is generally no means for removal and therefore no means to diminish the overall strength. The need for new and safer antimicrobial technologies is obvious. These new agents must be safer to the end-use, the applicator, and also to the earth. Antimicrobial agents that do not leach from the original treatment site can provide for this protection. But even non-leaching is not enough. Antimicrobial agents in general must have broad spectrum antimicrobial activity (equally effective against bacteria, fungi, and algae), have little to risk to the product or to the people applying the product, must easily fit current production systems, must be environmentally friendly, and must be compliant with all global biocidal regulations (U.S. EPA, EU BPD, REACH). SILANE QUATERNARY AMMONIUM COMPOUNDS In the mid-1960's, researchers discovered that antimicrobial organofunctional silanes could be chemically bound to receptive substrates by what were believed to be Si-O linkages. The method was described as orienting the organofunctional silane in such a way that hydrolysable groups on the silicon atom were hydrolyzed to silanols and the silanols formed chemical bonds with each other and the substrate. The resultant surface modification, when an antimicrobial moiety such as quaternary nitrogen was included, provided for the antimicrobial to be oriented away from the surface 1. The attachment of this chemical to surfaces appears to involve two processes. First and most important is a very rapid process that coats the substrate with the cationic species one molecule deep. This is an ion exchange process by which the cation of the silane quaternary ammonium compound replaces protons from water on the surface. It has long been known that most surfaces in contact with water generate negative electrical charges at the interface between water and the surface. This mechanism is further supported by data generated with a radioactive silane quaternary ammonium compound. During the treatment, depletion of the radioactivity from solution was almost immediate by an amount corresponding to that sufficient to cover the surface

one layer deep, even on surfaces which contain no functionality. Similar results are published for many organic quaternary ammonium compounds. The second process is unique to materials such as silane quaternary ammonium compounds that have silicon functionality enabling them to polymerize, after they have coated the surface, to become almost irremovable even on surfaces with which they cannot react covalently. Covalent bonding to the surface can also occur and through a series of heating and cooling steps, it is also possible to have intermolecular polymerization creating interpenetrating network in which the reactive silane forms anchors for additional polymer formation. Once hydrolyzed, the silanol groups become functionalized and are able to react with itself and available sites on the surface to form a dense polysiloxane network with an extremely high cationic charge density capable of destroying microbes.

ANTIMICROBIAL ACTIVITY OF SILANE QUAT ANTIMICROBIAL This section summarizes the broad spectrum antimicrobial activity of the Si-Quat antimicrobial agent applied onto a variety of both porous and non-porous surfaces. The data represent over 35 years of experience and microbiological and chemical testing measuring the effectiveness of the Si-Quat antimicrobial agent after being applied onto surfaces such as furniture, carpets, wood and vinyl flooring, non-woven textiles (air filters), aquariums, etc. Surfaces treated with the SiQuat technology have been shown to be resistant to the formation of biofilm. This resistance is due to two specific mechanisms which will be described below.

Since inception in the mid-1960's, the antimicrobial activity of the [3-(trimethoxysilyl) propyldimethyloctadecyl] ammonium chloride (Si-Quat) has been studied extensively on a variety of treated surfaces. The antimicrobial activity of solid surfaces treated with the Si-Quat agent was first described by Isquith et al1 and later elaborated on by others, most notably, by Speier and Malek2. In their study, dose dependent antibacterial activity was demonstrated against both the Gram – Escherichia coli and the Gram + Staphylococcus aureus after treating a solid surface of clearly defined dimensions. The rate of kill and surface kinetics of these treated surfaces were further defined and demonstrated by Isquith and McCollum3. This work was followed by a companion study which measured the broad spectrum antimicrobial activity against a mixed fungal spore suspension (Aspergillus niger, Aspergillus flavus, Aspergillus versicolor, Penicillium funiculosum, Chaetomium globosum). With the use of radioactive tracers, Isquith and McCollum demonstrated that "biological activity of the Si-Quat bonded to surfaces may offer a method of surface protection without addition of the chemical to the environment". Algicidal (Chlorophyta, Cyanophyta and Chrysophyta) activity of the Si-Quat applied to glass was demonstrated by Walters et al4.

Further work demonstrates the ability to apply this material to a variety of substrates. This work includes surfaces from glass and aquariums to entire hospitals (Walters et al4., Lewbart et al5., and Kemper et al6). Kemper studied the microbial colonization of environmental surfaces in hospitals and the effectiveness of the Si-Quat to control these organisms. This 30 month study measured persistent antimicrobial activity on surfaces treated with the Si-Quat agent. Isquith demonstrated antimicrobial activity on a variety of porous and non-porous surfaces. The Si-Quat antimicrobial agent was applied to surfaces as diverse as stone and ceramic, cotton and wool, vinyl and viscose, aluminum, stainless steel, wood, rubber, plastic, and Formica (Isquith et al1). These authors state that these surfaces "were found to exhibit durable antimicrobial activity when treated with Si-Quat, against a spectrum of microorganisms of medical and economic importance". Further independent testing confirms antimicrobial activity on air filters and fabrics treated and used directly in the hospitals settings.

The property of the Dow Corning 5772 Antimicrobial that provides for the physical contact and rupturing of the cell membranes of single celled organisms revolves around the chemical structure of the monomer and subsequent final polymer. Contact with the oleophilic moieties of the long carbon chain and high cationic charge density exerted by the quaternized nitrogen of the polymer by the cell membranes of single celled organisms causes the physical rupture and inactivation of the membrane and the inhibition and death of the microbe.

This active ingredient monomer, when applied to surfaces and polymerized, provides a mode of antimicrobial activity that physically ruptures the cell membranes of microorganisms by ionic association (cell membranes carry a negative charge) and lipophilic attraction (the C18 associating with the lipoprotein of the membrane) causing disruption and lyses of the microbial cell. Speier and Malek7 showed this lysis on treated nonwoven fabric surfaces through electron microscopic observations. The distortion of the overall cellular structure could be seen on both Gram + and Gram – bacteria on treated and untreated surfaces. The depletion of the cellular electrochemical potential across the membrane and release of cytoplasmic materials provides complete destruction of the microbe.

CONTROLLING BIOFILM DEVELOPMENT

Microbial contamination and subsequent biofilm formation is a major cause of infection, contamination, and product deterioration. Controlling or even removing the biofilm after its development is difficult. A useful strategy is to control biofilm formation before it starts. For the prevention of biofilm formation, control of both adherence and colonization of the microorganisms on the substrate surface is critical. One of the strategies to prevent biofilm formation is to modify the physiochemical properties of a surface in order to minimize or reduce the attraction of the surface to the microorganism thereby controlling adherence. Reducing the attraction simplistically can be done either by manipulating the ionic charge of the surface altering the electrostatic interface or changing the hydrophobic/hydrophilic properties through surface energy manipulations (or both) (Gottenbos et al8).

Controlling or minimizing the adhesion of microorganisms to the surface can be done using several techniques. Strategies used in the modification of surface characteristics range from altering the physical properties of the surface via mechanical abrasion to covalently attaching functional components to the surface (Marshell9, MacKintosh10, Bouloussa11). However, controlling the physical surface properties through water repellency does not appear to be enough to prevent biofilm formation. Bacteria can still adhere to highly hydrophobic surfaces.

Creating an active antimicrobial surface will destroy the adhering microorganisms, single celled organisms, thereby preventing further proliferation. Several groups have recently studied the ability to permanently create antimicrobial surfaces by covalently binding cationic polymers directly to surfaces (Kenawy12, Huang13, Lin14, Kurt15).

The idea of creating active antimicrobial surfaces via the treatment with non-leaching quaternary amine compounds is certainly not new as presented above and using very similar approaches to the Si-Quat technology, these groups have created highly active antimicrobial surfaces. Using elaborate application techniques, long poly quaternary chains could be produced that create varied chain length polymers on surfaces with varying thickness. This work is summarized well in the review by Kenawy et at12. These groups demonstrated that a high cationic charge density and specific chain length polymerization were critical in the formation of permanent, non-leaching biocidal surfaces. In theory these long chain quaternary polymers are permanently fixed to the surface via covalent linkages but act directly on the cell membrane. This interaction is either through a physical association with the membrane via the long polymeric carbon chains and/or through direct ion exchange reactions with specific membrane components. The ion exchange theories in particular are interesting with the evidence that high surface charge density is directly related to killing efficiency. The killing efficiency and required charge density is dependent on organism, cellular components, surface charge of particular organisms and growth rate. (Murata16, Kugler17, Neu18).

It is critical, of course, that to use an antimicrobial agent in the prevention of biofilm formation, the agent must be broad spectrum and active against the particular biofilm causing organisms. Demonstration of the broad spectrum antimicrobial activity of surfaces treated with the Si-Quat antimicrobial agent can be found in the peer reviewed literature on a monthly basis. The Si-Quat technology, as reference above, is specific against all tested organisms typically responsible for biofilm formation.

Somewhat stimulated by the renewed understanding of the role of Si-Quat modified surfaces in the prevention of biofilm formation, several investigators renewed the investigation of the relationship between surfaces treated with the Dow Corning chemistry and the formation of microbial biofilm. The application of the Dow Corning onto surfaces structurally changes the surface. To further understand the relationship between water repellency and adsorption on surfaces treated with the Si-Quat, researchers from North Carolina State University, College of Textiles applied the Si-Quat technology directly onto polyester textiles and measured the water absorptive properties. This group demonstrated that the siloxane polymer that forms upon final hydrolysis and condensation of the silane monomer is directly related to time, temperature, and pH of treatment solution. Both hydrophilic and hydrophobic surfaces could be created depending on application procedure (Abo El Ola et al.19) while antibacterial activity of the surface remained intact. Saito et al20., from Hiroshima University, used treated silica particles to measure the relationship between the adherence of Oral Streptococci and surface hydrophobicity and Zeta potential. Gottenbos et al8 from the University of Groningen demonstrated both in vitro and in vivo activity of Si-Quat treated silicone rubber used in the biological implants. As an expansion of this work from the same laboratory, Oosterhof, measured the inhibitory effects of positively charged coatings on the viability of yeasts and bacteria in mixed biofilm. Significant reduction in both adherence and colonization of organisms associated with tracheoesophageal shunt prosthetic biofilm (Oosterhof et al.21).

The Si-Quat technology when applied to surfaces affects both the adhesion properties of microorganisms due to increased hydrophobic properties of the long carbon chain fully polymerized and also directly destroys one celled organisms on contact through mechanisms described above. Nikawa et al22 from Hiroshima University studied both the adhesion and colonization of mixed biofilm suspensions as a means to control biofilm formation on medical devices. This group demonstrated that commercially pure wrought titanium treated with the SiQuat technology significantly reduced the adherence and colonization of both Candida albicans and Streptococcus mutans, even when the surface was coated with a proteinaceous layer like saliva or serum. Clearly this biofilm control mechanism was directly related to both the decreased adhesion due to the hydrophobicity created by the octadecyl alkyl chain and also due to the killing of the quaternary ammonium which killed initial adherent cells and also retarded or inhibited subsequent microbial growth. Furthermore, cell culture and cytotoxicity studies were performed in order to demonstrated the non-leaching behavior of the antimicrobial coating. No significant cytotoxicity of Si-Quat was observed in cell viability tests or inflammatory assays.

SUMMARY AND CONCLUSIONS

The use of reactive silanes functionalized with antimicrobial agents has been demonstrated to provide surfaces which are resistant to microbial growth and subsequent biofilm formation. These surfaces become resistant due to both the biostatic repulsions of microorganisms to the surface and due to the highly charged cationic density and physical attraction of the resulting polymer network. These non-leaching antimicrobial surfaces can be applied to a variety of substrates due to the highly reactive silanol groups associated with the antimicrobial agent. These reactive groups bind both to the surface and itself forming highly cross-linked networks that form durable protective coatings on virtually any surface.

With the increase in awareness of multiple antibiotic resistant bacteria, the recognition of increased sensitivity of our environment that bioaccumulates toxic chemicals and formation of strict regulatory agencies, it is paramount that new uses for older, safer, antimicrobial agents are investigated. These antimicrobial agents must be safe for the environment and end-user but still protect our products from the detrimental effects caused my rampant microbial contamination. The use of reactive silane chemistry to provide durable, non-leaching antimicrobials on synthetic

material has been demonstrated to be a way of controlling microbial contamination in a safe and effective manner

LITERATURE CITED

1 Isquith, A.J. et al, Applied Microbiology (Dec., 1972), p. 859-863. 2 Malek, J.R. and Speier, J.L., Development of an Organosilicone Antimicrobial Agent for the Treatment of Surfaces, J. for Coasted Fabrics, Vol. 12 (July, 1982), P. 38-46. 3 Isquith and McCollum. Surface Kinetic Test Method for Determining Rate of Kill by an Antimicrobial Solid. Applied and Environmental Microbiology, p. 700-704. November 1978. 4 Walters, P.A. et al, , Applied Microbiology, Vol. 25, No. 2, (Feb., 1973), p. 256. 5 Lewbart et al. Safety and Efficacy of the Environmental Products Group Masterflow Aquarium Management System with AEGIS Microbe Shield. Aquacultural Engineering 19 (1999) p. 9398. 6 Kemper et al. Improved Control of Microbial Exposure Hazards in Hospitals: A 30-month Field study. National convention for Association of Practitioner for Infection Control (APIC) .1992. Gottenbos et al, , Antimicrobial Effects of Positively Charged Surfaces on Adhering Gram-positive and Gram-negative Bacteria, Journal of Antimicrobial Chemotherapy (2001) 48, pp. 7-13. 7 Speier, J.L. and Malek, J.R., Destruction of Microorganisms by Contact with Solid Surfaces, J. of Colloid and Interface Science, Bol. 89, No. 1 (Sept. 1982), p. 68-76. 8 Gottenbos et al. In vitro and in vivo Antimicrobial Activity of Covalently Coupled Quaternary Ammonium Silane Coatings on Silicone Rubber. Biomaterials 2002; 23: 1417-1423. 9 Marshall et al. , J. Gen. Microbiology. 68 (1971), p. 337. 10 MacKintosh et al., Effects of Biomaterial Surface Chemistry on the Adhesion and Biofilm Formation of Staphylococcus Epidermidis in Vitro, Wiley Inter-Science (2006), pp. 836-842. 11 Bouloussa et al., A New, Simple Approach to Confer Permanent Antimicrobial Properties to Hydroxylated Surfaces by Surface Functionalization, Chem. Commun., 2008, pp. 951-953. 12 Kenawy et al, The Chemistry and Applications of Antimicrobial Polymers: A State-of-theRoth, C., Canadian Pat. No. 2,010,782 (May 24, 1977). 13 Huang et al, Nonleaching Antibacterial Glass Surfaces via "Grafting Onto": The Effect of the Number of Quaternary

Ammonium Groups on Biocidal Activity, Langmuir 2008, 24, pp.67856795. 14 Lin et al, Insights into Bactericidal Action of Surface-attached Poly (vinyl-N-hexylpyridinium) Chains, Biotechnology Letters 24:2002, pp. 801-805. 15 Kurt et al, Highly Effective Contact Antimicrobial Surfaces via Polymer Surface Modifiers, Langmuir 2007, 23, pp. 4719-4723. 16 Murata et al. L, Permanent, Non-leaching Antibacterial Surfaces-2: How High Density Cationic Surfaces Kill Bacterial Cells, Biomaterials 28 (2007) pp. 4870-4879. 17 Kugler et al. Evidence of a charge-density threshold for Optimum Efficiency of Biocidal Cationic Surfaces, Microbiology (2005) 151, pp. 1341-1348. 18 Neu, T.R. Significance of Bacterial Surface-Active Compounds in Interaction of Bacteria with Interfaces, Microbiological Reviews, Mar. 1996, pp. 151-166. 19 Abo El Ola et al., Unusual Polymerization of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride on PET Substrates, Polymer 45 (2004) pp. 3215-3225. 20 Saito et al. Adherence of Oral Streptococci to an Immobilized Antimicrobial Agent, Archs Oral Biol. Vol. 42, No. 8, 1997 pp. 539-545. 21 Oosterhof et al., Effects of Quaternary Ammonium Silane Coatings on Mixed Fungal and Bacterial Biofilms on Tracheoesophageal Shunt Prostheses, Applied and Environmental Microbiology, May 2006, pp. 3673-3677. 22 Nikawa et al. Immobilization of Octadecyl Ammonium Chloride on the Surface of Titanium and Its Effect on Microbial Colonization In Vitro. Dental Materials Journal 24(4), 2005, pp.570-582.

ADDITIONAL REFERENCE LITERATURE

23 Gettings, R.L. and Triplett, B.L., A New Durable Antimicrobial Finish for Textiles, Book of Papers, AATCC National Conference (1978). 24 Hayes, S.F. and White, W.C., How Antimicrobial Treatment can Improve Nonwovens, American Dyestuff Reporter (June, 1984). 25 McGee, J.B. et al., New Antimicrobial Treatment for Carpet Applications, American Dyestuff Reporter, (June, 1983). 26 Lawrence, C.A., Germicidal Properties of Cationic Surfactants, Chap. 14, Cationic Surfactants 27 Baier, R.E., Substrate Influences on Adhesion of Microorganisms and Their Resultant New Surface Properties, Adsorption of Microorganisms to Surfaces (John Wiley and Sons, 1980). 28 Andresen et al., Nonleaching Antimicrobial Films Prepared from Surface-Modified Microfibrillated Cellulose, Biomacromolecules 2007, 8, pp. 2149-2155.