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Synthesis and Antimicrobial Activity of Polysiloxanes Polyurethane Biocidal Polymers as Surface Modifiers

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ABSTRACT

Complex epidemiological situation, nosocomial infections, microbial contamination, and infection risks in hospital and dental equipment have led to an ever-growing need for prevention of microbial infection in these various areas. Macromolecular systems, due to their properties, allow one to efficiently use them in various fields, including the creation of polymers with the antimicrobial activity. N-halamine siloxane coatings can be rendered biocidal by exposure to dilute bleach. Once the bound chlorine is lost from the coatings, it can be regenerated by further exposure to dilute bleach. Polysiloxanes with 3-(alkyldimethylammonio) propyl pendant groups was synthesized by quaternization of n-octyldimethylamine with linear polysiloxanes containing 3-chloropropyl groups attached to silicon atoms. The polysiloxanes bearing quaternary ammonium salts (QAS) showed bactericidal activity when incorporated in a polysiloxane network. The activity was retained after 60 days of immersion in water. This paper briefly highlights mechanism of action, synthetic schemes and biocidal efficacy data as well as major applications of antimicrobial polymers.

Keywords: Biocidal coatings, Biocidal efficacy data, *N*-halamines, Antimicrobial activity, Functional polysiloxanes, Quaternary ammonium salts, Macromolecular biocide.

INTRODUCTION

The invasion of polymers by bacteria, fungi and other micro-organisms is manifested by loss of mechanical properties, surface degradation, discoloration, staining and other polymer deteriorations leading to loss of its appearance and properties. Addition of a biocide during compounding is the most usual way to prevent the colonization of polymers by micro-organisms. However, the biocide released from polymer may be a hazard to the environment and protection is limited in time. It is known that QAS exert their biocidal activity by interaction with the cell

wall of bacteria. In the search of nonleaching "environmentally friendly" biocides, the recent concept of QAS biocides fixed to a polymer in a permanent way by a stable chemical bond has been developed [1].

The development of novel biocidal water-soluble cyclic *N*-halamine derivatives have been employed are superior because of their long-term stabilities in aqueous solution and in dry storage. This exceptional stability is a result of their chemical structures; all have electron-donating alkyl groups substituted on the heterocyclic rings adjacent to the oxidative N–Cl or N–Br moieties which hinder the release of "free halogen" into aqueous solution. The combined *N*-halamines thus serve as the contact biocides. Although combined *N*-halamine monomers generally require longer contact times at a given halogen concentration than does "free halogen" to inactivate pathogens. It is possible to concentrate *N*-halamine moieties on insoluble polymers, thus producing a substantial reservoir of combined halogen for enhanced disinfection purposes. Furthermore, the functionalized *N*-halamine polymers are superior in overall performance such as taking into account biocidal efficacy, stability at varying pH and in the presence of organic receptors, rechargeability, lack of toxicity and cost to other biocidal polymers which have been developed over the years [2].

The QAS-substituted hydroxytelechelic polybutadiene have been prepared and incorporated in a polyurethane network [3,4]. The same was done with a polysiloxane bearing both QAS and alcohol functions [5]. In both cases, a very high biocidal activity and a fairly good permanency in water were observed and interpreted as an intrinsic surface property of the polymer films being able to kill bacteria by a direct contact between the solid film and the bacteria cells [6,7]. The purpose of this work is to explore a new route for the synthesis of biocidal QAS-containing polysiloxane involving the direct quaternization of a 3-halogenopropyl-substituted polysiloxane used as a precursor.

This paper concern the synthesis, mechanism of action of different antimicrobial polymers and non-leaching microbiocidal surfaces and factors influencing their activity and toxicity, the stabilities of the bound chlorine on the surfaces. Substrates employed include wood and paint. Potential uses for the technology are discussed as well as major applications of antimicrobial polymers.

Mechanism of Action

The main strategy for designing synthetic antimicrobial polymers has been determined by the common structural features of the outer envelope of different bacterial cells. The important characteristic of the outer envelope of the cells is a net negative charge (often stabilized by the presence of divalent cations such as Mg^{2+} and Ca^{2+}). It is provided by the teichoic (or lipoteichoic) acid molecules of Gram-positive bacteria cell wall (CW), the lipopolysaccharides and phospholipids of Gram-negative bacteria outer membrane (OM), and the cytoplasmic membrane (CM) itself, which is composed of a phospholipid bilayer with embedded essential functional proteins, such as enzymes.

The cytoplasmic membrane has selective permeability properties (it is semi-permeable) and regulates the transfer of solutes and metabolites in and out of the cell cytoplasm. Based on the features of the CW/OM and CM of a cell, the major part of antimicrobial polymers was designed

as cationic hydrophilic–hydrophobic macromolecular systems, a target site for which the cytoplasmic membrane was considered (so-called membrane active agents). There are polymers with links containing a hydrophilic polar functional block bearing cationic charge and a hydrocarbon non-polar hydrophobic block (or hydrophobic structure of a whole link), or random copolymers formed by a hydrophobic monomer and a hydrophilic co monomer with a functional group. Such polymer/copolymer structures provide surface-activity properties and adsorption/absorption ability (so-called surfactants) and high binding affinity for bacterial cells enhanced by high lipophilicity in order to cause effective damage of the structural organization and integrity of cell membranes, followed by CM disruption (in the major part of cases), leakage of cytoplasmic contents and cell lysis [8,9].

Mode of Action

N-halamine polymeric compounds: There is a large class of biocidal polymers and copolymers, cyclic N-halamine polymeric compounds, whose mechanism of action is distinct from the aforesaid of membrane active antimicrobial polymers. N-halamines and later polymers with N-halamine functional groups have been developed and stabilize the antimicrobial properties of free halogens (chlorine or bromine). In N-halamines, one or more halogen atoms are covalently bonded to the nitrogen atoms of the compounds which provide stability and slowly release free active halogen species into the environment. The main biocidal impact of the N-halamines relates to a specific action of oxidative halogen (Cl^+ or Br^+) targeted at a biological receptor (thiol groups or amino groups in proteins) upon direct contact with a cell, leading to cell inhibition or cell inactivation, rather than polymer action itself, for instance polymeric quaternary ammonium salt with a long alkyl radical [10,11].

METHODS

i) Synthesis of siloxane polymers: Polymers of the trialkoxysilylpropylhydantoin derivatives were prepared according to following procedure. First, the appropriate 5, 5-dialkylhydantoin was prepared by reaction of ammonium carbonate, potassium salt and the necessary dialkyl ketone in a 2.0:1.0:0.67 molar ratios in a water/ethanol (1:1 by volume) solvent mixture at 50–60°C for 4–10 h. The crude products were isolated by exposure to dilute HCl and filtration; purification was effected by recrystallization from water/ethanol. The synthesized compounds were characterized by melting points and their structures have been confirmed by suitable spectroscopic techniques such as ^{1}H NMR; yields ranged from 90 to 98% by weight. Then the potassium salts of the dialkylhydantoins were prepared by mixing the dialkylhydantoins with equimolar quantities of KOH in ethanol and heating at refiux for about 10 min. The salts were isolated by removal of the solvent under vacuum. After drying overnight at 60°C, the salts were dissolved in dimethyl formamide at 65°C. An equimolar solution of 3-chloropropyltriethoxysilane in dimethyl formamide was then added drop wise at 110°C and the resulting mixture was held at 110°C for 3–7 hours. The KCl produced in the reaction was removed by filtration and the dimethyl formamide was removed at reduced pressure. Purification was effected by dissolving the resulting oil or solid in ethyl acetate, shaking with water and removing the ethyl acetate by evaporation. The structures of the 3-triethoxysilylpropyl-5-alkyl-5-methylhydantoin derivatives were confirmed by ^{1}H NMR; yields ranged from 80 to 90% by weight [12]. The polymers could be chlorinated before or after a coating procedure using a 15% solution of sodium hypochlorite bleach buffered to pH 7.

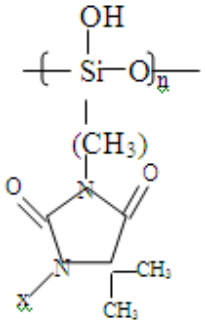

Fig. Structures of polymer

ii) Synthesis of poly [(3-chloropropyl) methylsiloxane-co-dimethylsiloxane]: The mixture of (3-chloromethyl) methyldichlorosilane hydrolysate 22 g, octamethylcyclotetrasiloxane 36 g, hexamethyldisiloxane 0.51 g and 0.3 ml of CF_3SO_3H was kept in room temperature for 24 hours. Then the polymer formed was washed four times with water and precipitated from its methylene chloride solution in methanol. The precipitation procedure was repeated five times. After drying 24 hours over $CaCl_2$, the polymer was heated 7 hour in 90°C/1023 mmHg. 28 grams, yield 50% of copolymer Poly [(3-chloropropyl) methylsiloxane-co-dimethylsiloxane] was obtained. The synthesized compound was characterized by Size-Exclusion Chromatography shown in Table 1 and their structures have been confirmed by suitable spectroscopic techniques such as ^{1}H-, ^{13}C-, and ^{29}Si-NMR.

Bactericidal Properties of Siloxane Polymers

a. Coating surfaces: The 5,5-dimethylhydantoinylsiloxane derivative (polymeric forms) was simply mixed into a commercial floor enamel at weight percent ranging from 3.0 to 5.0%. The polymer was dissolved in ethanol/water (1:1 by volume) solution which was miscible with the paint; the paint was then brushed onto wood and was allowed to dry in air at ambient temperature for 24 hours before chlorination.

b. Chlorinating treated surfaces: The treated surfaces were rendered biocidal by either spraying them with or soaking them in 7–12% aqueous solutions of household bleach (sodium hypochlorite). After rinsing with distilled, deionized water and drying in air at 30–50°C, samples of the materials were analyzed for oxidative chlorine coverage using an iodometric/thiosulfate titration procedure. In some cases this was repeated over an extended time period to evaluate the stability of the chlorinated surfaces.

c. Biocidal effcacy testing: Treated surfaces were challenged with *Staphylococcus aureus* (ATCC 6538) and/or *Escherichia coli* (ATCC 43895) bacterial suspensions in pH 7 phosphate buffer solution. The surfaces were quenched with 0.02 N sodium thiosulfate solutions at various contact times. Serial dilutions of the solutions contacting the surfaces were plated on tryptic soy agar, incubated for 48 hours at 40°C, and colony counts were made to determine the presence or absence of viable bacteria.

Bactericidal properties of poly [(3-chloropropyl) methylsiloxane-co-dimethylsiloxane]

a. Test by contact: Copolymer poly [(3-chloropropyl) methylsiloxane-co-dimethylsiloxane] (120 g) fully quaternized with dimethyldodecylamine was added to 171 g of a mastic made of a

mixture of polydimethylsiloxanes and a curing system. The mastic was spread in thin films (100 mm thickness) on a plate of glass or molded in the shape of small disks (thickness 5 mm, diameter 27 mm). The samples were immersed into a suspension of bacteria at room temperature and left in contact for 2 hours. The initial number of bacteria and the number of survivors after the contact with the mastic were counted [13]. Data for three species of bacteria are presented in Table 2.

b. Test of aging: The film of the mastic was immersed for 60 days in water and continuously stirred by a mechanical stirrer. Results are presented in Table 3.

c. Test in solution: A solution containing the quaternized polysiloxane at a concentration of 5.3 x 10^{-4} mol^{-1} was inoculated with a known number of *Escherichia coli*. After to 2 hours, the survivors were counted using the usual procedure.

RESULTS AND DISCUSSION

Siloxane polymers: For the experiments involving the addition of the monomer and polymer of 3-triethoxysilylpropyl-5, 5-dimethylhydantoin to commercial floor enamel, the stabilities of the bound chlorine over a 60 day period are shown in Table 4. One can see from Table 4 that the chlorine loading does decline over a 60 day period for the monomer and polymer additives, the polymer stabilizing the chlorine somewhat better. It should be noted that prior work in these laboratories has demonstrated that a Cl^+ loading of 1.10 x 10^{16} atom/cm^2 on a surface utilizing an *N*-chloramine is sufficient to provide biocidal activity[11]. The chlorine loading could be partially restored upon rechlorination after 60 days, e.g., for the 6.0% monomer sample a rechlorination yielded 1.10 x 10^{17} atom /cm^2. However, we have observed a decline in chlorination potential with time if the hydantoinylsiloxane compounds are allowed to remain in the original wet paint. It is recommended that the compounds be added immediately before use of the paint.

Table 1: Structures of the halogenated polysiloxanes

% Halogenated units (NMR)	*Mn* (g. mol^{-1}) SEC (PS Equiv.)	*Mw*/*Mn*
22 (Cl)	26621	1.65

Table 2: Bactericidal activity of silicone mastic containing the QAS-containing polysiloxane
Time of contact: 2 hours at 20°C

Strain	Initial number of bacteria (N_o)	Number of survivors (N)	Logarithmic reduction ratio log (N_o/N)
Escherichia coli	$1.25x10^6$	$2.8x10^4$	1.65
Aeromonas h.	$3.67x10^6$	$1.1x10^6$	0.52
Pseudomonas a.	8.10^7	$>1.1x10^7$	<0.87

Table 3: Effect of aging in water of silicone mastic containing the QAS-containing polysiloxane Q17, Strain: *Escherichia coli*, Time of contact: 2 hours at 20°C

Sample	Initial number of bacteria (N_o)	Number of survivors (N)	Logarithmic reduction ratio log (N_o/N)
Thin film aged 60 days	$1.15x10^6$	$1.95x10^4$	1.76
Thick sample aged 60 days	$1.68x10^6$	$1.25x10^3$	3.13

Poly [(3-chloropropyl) methylsiloxane-*co*-dimethylsiloxane]: by co equilibration of (3-chloromethyl) methyldichlorosilane hydrolysate with octamethylcyclotetrasiloxane and hexamethyldisiloxane. The characteristics of this copolymer are presented in Table 1. The composition of the copolymer was determined by ^{1}H, ^{13}C and ^{29}Si-NMR spectrum. It was used to determine the distribution of the functional siloxane units by a sequential analysis made on the triad level. The experimental values are compared in Table 5 with those calculated for a random placement. The distribution was close to statistical.

Table 4: Stability of bound chlorine in a commercial paint

S. No.	Sample type and loading	Cl^+ content immediately after chlorination of dried surface in atom/cm^2	Cl^+ content 60 days after chlorination of dried surface in atom/cm^2
01	3.0% polymer on painted wood	0.75×10^{17}	0.50×10^{17}
02	5.0% polymer on painted wood	1.99×10^{17}	1.50×10^{17}

The error range for these values as determined by iodometric/thiosulphate titration was 5-10%

Table 5: Sequential analysis of poly [(3-chloropropyl) methylsiloxane-co-dimethylsiloxane]

Triad	Triad ^{29}Si NMR (ppm/TMS)	Experimental	Calculated *
DD^XD	-25.5	10.3	13.5
DD^XD^X	-25.3	9.4	8.7
$D^XD^XD^X$	-25.9	4.6	2.1
DDD	-23.9	48.6	47.9
DDD^X	-23.7	25.5	26.9
D^XDD^X	-23.5	5.6	4.9

**Assuming random placement of units D and D^X.*

CONCLUSIONS AND FUTURE DEVELOPMENTS

It can be concluded that *N*-halamine siloxane polymers can be very useful in constructing biocidal surface coatings. This has been demonstrated herein for wood. Numerous potential applications can be envisualized.

Polysiloxanes containing (3-halogenopropyl) - methylsiloxane units are easy to prepare and are suitable for the introduction of QAS groups. The resulting QAS-modified polysiloxanes used as additive during the compounding of a silicone mastic confer to it excellent bactericidal properties that are retained after a 2-month contact with water. These results confirm those semi-interpenetrated network consisting of a macromolecular biocide in a polymer network are an interesting solution when long-term protection against micro-organism growth is needed with a minimum release of toxic compounds in the surrounding medium. These kinds of compounds avoid the drawbacks of the protection by small molecules (active by diffusion) and those due to the covalently bound biocidal polymers (active by contact).

A rather large class of polymers was referred to as membrane active agents. During the last decade, many data were received, which support the membrane-disrupting mechanism as a final result of antimicrobial action of the quaternary biocidal polymers as well as some non-quaternary polymers with protonated amine groups, whereas the proper mechanism of the interactions between membrane active macromolecules and bacterial or mammalian cells or model

liposomes, is not yet clear to the end. It may be expected that, due to progress in modern biophysical techniques, the studies of antimicrobial activity of polymers, either in solution or immobilized on surface, will provide interesting data and possibly new insight into the mechanisms of their antimicrobial action.

Research activity will be focused also on the studies of cytotoxicity to human cells of synthetic antimicrobial polymers to attain high selectivity and biocompatibility. These factors as well as more clear understanding of the mechanism (s) of antimicrobial action of polymers are necessary to design and synthesize innovative effective antimicrobial systems, which are environmentally safe and have no negative impact on human health.

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