Polymerization of a Hydantoinylsiloxane on Particles of Silicon Dioxide To Produce a Biocidal Sand

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Received 17 September 2004; accepted 17 December 2004
DOI 10.1002/app.21814
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The monomer 3-triethoxysilylpropyl-5,5-dimethylhydantoin has been polymerized on the surfaces of sand particles to produce an adhered film that, upon chlorination with dilute sodium hypochlorite bleach, becomes biocidal. The biocidal efficacy of this coated sand has been demonstrated in a cartridge filter experiment against the bacterial pathogens *Staphylococcus aureus* and *Escherichia coli*. Complete inactivation was observed within 1 min of contact for the former bacterium and in the interval of 1–5 min for the latter. Upon a loss of biocidal activity due to the depletion of bound chlorine, the coated sand particles can be recharged by further exposure to dilute bleach. Synthetic methods and test data are presented. Potential uses of biocidal sand include disinfection and odor control in water treatment facilities and recirculating baths. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 1161–1166, 2005

Key words: polysiloxanes; biological applications of polymers; water-soluble polymers

INTRODUCTION

Work in these laboratories for the past 2 decades has focused on the preparation and testing of novel monomeric[1] and polymeric[2] *N*-halamine biocidal compounds and materials. Molecules representative of this type of biocide contain either nitrogen–chlorine or nitrogen–bromine covalent chemical bonds. The biocidal *N*-halamines that have been developed in these laboratories are very stable toward the release of free halogen into aqueous solutions, and they function as biocides through the direct contact of the cells of microorganisms with the oxidative halogen atom. Advantages inherent in *N*-halamines as biocides are their preparation *in situ* by the exposure of the precursor molecules to free chlorine or free bromine, their regeneration following the loss of the halogen by simple re-exposure of the molecules to additional free halogen, and the rapid inactivation of a broad spectrum of pathogens because oxidative chlorine and bromine are potent biocides.

N-Halamine groups such as *N*-halohydantoins, oxazolidinones, and imidazolidinones can be covalently attached to a variety of polymers used in water disinfection applications,[3–9] surface coatings,[10–12] and elastomers.[13] Sun and coworkers,[14–17] in pioneering work on textile fabrics, extended the technology to its use in rendering fabrics containing cellulose (cotton and cotton blends) biocidal. Moreover, nylon and poly(ethylene terephthalate) can be made biocidal with similar technology.[18–20]

In this work, we demonstrate that a novel hydantoinylsiloxane can be polymerized on the surfaces of sand particles so that it adheres to the sand. The subsequent exposure of the treated sand to sodium hypochlorite bleach causes covalent bonding of the oxidative chlorine to the amide nitrogen of the hydantoin heterocyclic ring moiety, and this renders the surface of the sand biocidal. The reaction scheme to be used is shown in Figure 1. Some efficacy data for N-halogenated hydantoinyl siloxane monomers and polymers have been presented recently.[21,22] Prior work elsewhere has shown that quaternary ammonium salt derivatives of siloxanes can be used to create biocidal surfaces,[23,24] although the polyquats are weakly biocidal in comparison with analogous *N*-halamines.[8] Also, it has recently been reported that with similar technology, an imidazole moiety can be attached through a siloxy tether to polysaccharides to create a weakly biocidal surface that helps to prevent corrosion on aluminum substrates.[25,26]

EXPERIMENTAL

Preparation of the 3-triethoxysilylpropyl-5,5-dimethylhydantoin monomer (I)

The triethoxysilylpropylhydantoin derivative was prepared according to a procedure similar to that out-

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Contract grant sponsor: U.S. Air Force; contract grant number: F08637-02-C-7020.
Contract grant sponsor: Vanson-HaloSource Corp.

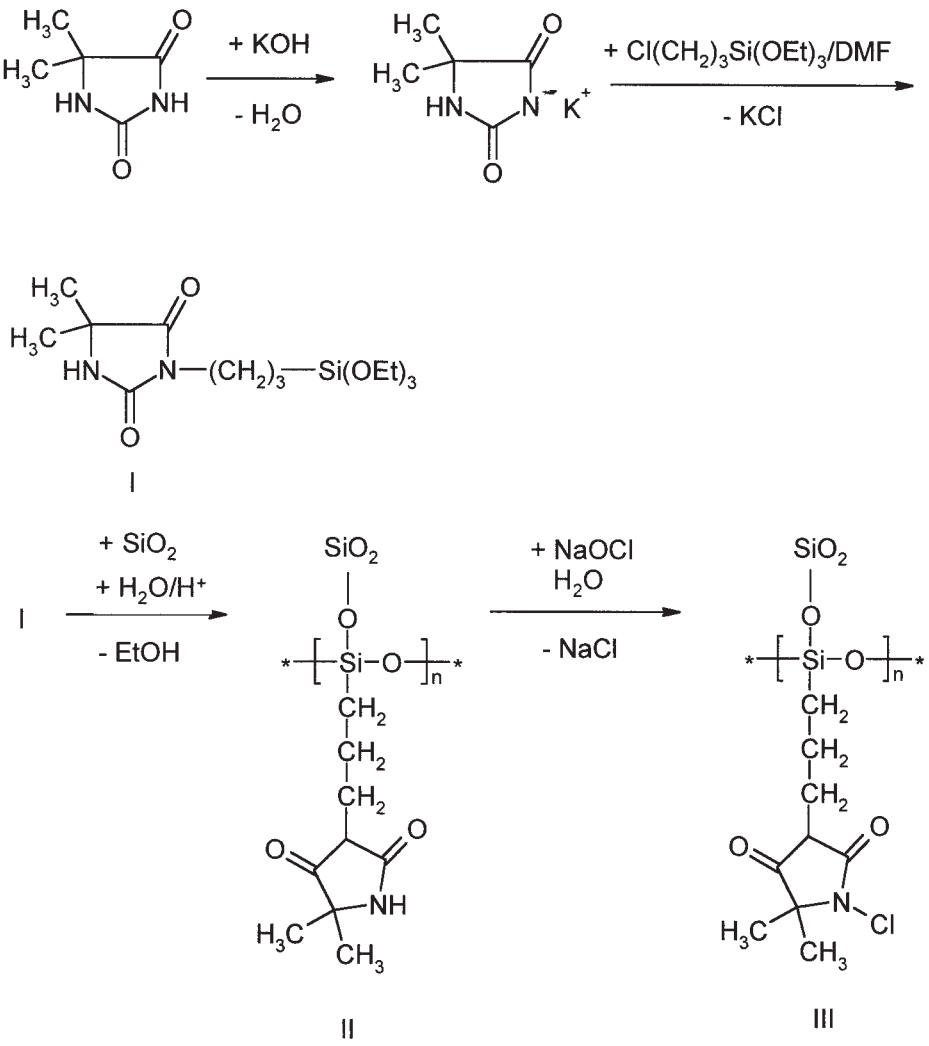

Figure 1 Polymerization scheme for producing biocidal sand.

lined in U.S. Patent 4,412,078.[27] A mixture of 500 mL of ethanol, 64.0 g (0.5 mol) of 5,5-dimethylhydantoin (Aldrich Chemical Co., Milwaukee, WI), and 28.0 g (0.5 mol) of potassium hydroxide was refluxed until the solution became clear. Then, the solid potassium salt of 5,5-dimethylhydantoin was isolated by the evaporation of the ethanol solvent and water produced in the reaction under reduced pressure. This salt was dried *in vacuo* at 60°C for 4 days to form the anhydrous potassium salt. The dry salt was then mixed with 500 mL of anhydrous *N,N*-dimethylformamide (DMF), and the mixture was heated at 60°C until a clear solution formed. Then, 120.4 g (0.5 mol) of 3-chloropropyltriethoxysilane (Aldrich Chemical) was added dropwise over a 1-h period with stirring at the ambient temperature. The mixture was heated at 95°C for 4 h and cooled, and the potassium chloride produced in the reaction was removed by filtration. The DMF solvent was removed by distillation to produce 150.0 g of a brown, viscous oil, which was identified as the desired precursor monomer **I** (see Fig. 1), the yield being 90.3% of the theoretical amount. The product was further purified by distillation under reduced pressure (16 mmHg; the fraction was collected at 235–238°C) for elemental and spectroscopic characterization.

ANAL. Calcd. for $C_{14}H_{28}SiN_2O_5$: C, 50.6%; H, 8.4%; N, 8.4%. Found: C, 50.3%; H, 8.4%; N, 9.0%. ^{1}H-NMR ($CDCl_3$, δ): 0.61 (2H), 1.22 (9H), 1.43 (6H), 1.73 (2H), 3.48 (2H), 3.82 (6H), 7.17 (1H). IR (KBr) 740, 813, 1081, 1104, 1713, 1774, 2879, 2989, 3279, 3485 cm^{-1}. Mass spectrometry (CI/CH_4): m + 1, 333.

Preparation of the 3-triethoxysilylpropyl-5,5-dimethylhydantoin polymer bound to sand

A quantity of commercial sand (20–30-mesh Ottawa standard, Fisher Scientific, Pittsburgh, PA) was placed in an open beaker containing distilled, deionized water, which was generally acidified with HCl, and a

quantity of **I** was added. The mixture was allowed to stand at the ambient temperature without stirring for a measured time. Then, the treated sand was removed by filtration and rinsed three times with distilled, deionized water. The tested variables were the concentration of **I** added, the concentration of HCl, and the time of polymerization. Typically, 5.0 g of the sand was placed in a 100-mL beaker with 10 mL of aqueous HCl (0.01–1.0*M*) and 0.1–0.5 g of **I**; the reaction times varied from 1 to 40 h. Also, some experiments were performed with prepolymerized **I**, prepared as described previously,[21] which was allowed to come into contact with the sand in the same manner.

Preparation of the biocidal 3-triethoxysilylpropyl-5,5-dimethylhydantoin polymer bound to sand

The polymer-bound sand (e.g., 26.0 g) was rendered biocidal by exposure to a 10% aqueous solution (e.g., 300 mL) of commercial sodium hypochlorite bleach (Clorox Co., Oakland, CA) buffered to pH 7.0 for 1 h at the ambient temperature. The chlorinated polymer-bound sand was removed by filtration, rinsed with three 30-mL portions of distilled, deionized water, and dried at 45°C for 1 h to remove any occluded free chlorine. The amounts of chlorine (wt %) on the polymer-bound sand samples were determined by standard iodometric/thiosulfate titrations. Some experiments were performed to determine the stability of the chlorine on the sand over time under flow conditions, as well as its regeneration capabilities.

Biocidal efficacy testing

Polymer-bound sand (26.0 g) containing about 0.28 wt % chlorine (expressed as Cl^+) was packed into a glass column with an inside diameter of 1.0 cm and a length of 25.0 cm, the sand column being 18.0 cm long; the empty-bed volume was measured to be 6.42 mL. Two identical control columns were prepared containing unchlorinated polymer-bound sand and untreated sand, respectively. Then, 50-mL portions of aqueous solutions (buffered to pH 7.0) containing pathogenic bacteria were circulated through the three columns with a peristaltic pump (Gelman Sciences, Ann Arbor, MI) at a flow rate of about 1.5 mL/s. By the repeated recirculation of these 50-mL portions, the contact times could be varied. Measured portions of the effluent (25 μL) were collected at specific time intervals in sterile tubes; they were immediately quenched with 0.02*N* sodium thiosulfate to prevent any subsequent inactivation of the bacteria by any free chlorine that might have leached out of the biocidal sand. Then, serial dilutions of the quenched effluents were plated onto trypticase agar, and colony counts were performed after incubation at 37°C for 24 and 48 h. The two bacterial pathogens employed were Gram-positive *Staphylococcus aureus* (ATCC 6538) and Gram-negative *Escherichia coli* O157:H7 (ATCC 43859). The cell density of *S. aureus* was about 6.13×10^4 CFU/mL (where CFU is colony forming unit); that of *E. coli* was about 5.60×10^5 CFU/mL.

TABLE I
Polymerization/Adhesion Efficiency as a Function of the Weight of Monomer I

I (g)[a]	Titrated C^+ (wt %)
0.10	0.00
0.20	0.04
0.30	0.09
0.40	0.15
0.50	0.27

[a] The weight of the sand in each case was 5.0 g; the polymerization was performed in 10 mL of 0.1*M* HCl for 16 h at 25°C.

RESULTS AND DISCUSSION

Preparation of the biocidal sand

Compound **I** is only sparingly soluble in water under neutral or acidic conditions, and it has greater density than water. Thus, upon the addition of **I** to a beaker containing the sand and water, it effectively precipitates and coats the sand particles. The acid-promoted polymerization of **I** to form the polymer-bound sand (**II** in Fig. 1) through a hydantoinyltrihydroxysiloxane intermediate occurs on the surfaces of the sand particles. The chlorination of **II** then occurs via direct contact of the oxidative chlorine in aqueous sodium hypochlorite with the amide nitrogen on the hydantoin ring to form the biocidal sand particles (**III** in Fig. 1). The efficiencies of the polymerization and adhesion processes can be assessed by an analytical determination of the weight percentage of bound oxidative chlorine.

Table I shows the effect of varying the concentration of **I** in a typical experimental run: the amount of biocidal sand was held constant at 5.0 g in 10 mL of 0.1*M* HCl, and the time of polymerization was fixed at 16 h at the ambient temperature. The weight percentage of titratable chlorine (Cl^+) increased dramatically as the concentration of **I** increased, as might be expected. Of course, economics will dictate the loading concentration of **I** to achieve the desired biocidal performance. A 5 wt % starting concentration of **I** with respect to the weight of the sand may be the maximum feasible.

Table II illustrates the effect of varying the acidity of the aqueous solution supporting the polymerization of **I** on the sand particles in a typical experimental run. In this case, 5.0 g of sand was mixed with 0.5 g of **I** in 10 mL of 0, 0.01, 0.1, and 1.0*M* aqueous solutions of HCl,

TABLE II
Polymerization/Adhesion Efficiency as a Function of the Concentration of HCl

Molar concentration of HCl[a]	Titrated Cl^+ (wt %)
0.00	0.00
0.01	0.00
0.10	0.27
1.00	0.18

[a] The weight of the sand in each case was 5.0 g; the polymerization was performed with 0.5 g of monomer **I** in 10 mL of HCl for 16 h at 25°C.

and the polymerization was allowed to proceed for 16 h at the ambient temperature in each case. The observation that the solution containing no HCl seemed to support slightly more efficient polymerization and adhesion than the one containing 0.01*M* HCl is without doubt an experimental aberration within the error of the titration method. At a polymerization time of 2 h, the titrated result was 0.0013 wt % Cl^+ for the 0.01*M* HCl solution; this illustrates the variability in the analytical determination, in that the loading should not actually decline at 16 h (it was 0.0292% at 40 h). The surprising and significant result is that the 0.1*M* HCl solution supported a more efficient polymerization/adhesion than the 1.0*M* HCl solution. Evidently, an optimum concentration of acid is needed to hydrolyze the ethoxy groups on **I** to produce the trihydroxy intermediate, which then proceeds to polymerize and attach to the sand particles. Higher concentrations of acid may lead to detachment from the sand and/or decomposition of the hydantoin ring over extended time periods.

Table III shows the effect of varying the time of polymerization for a typical experimental run. In this experiment, 5.0 g of sand was mixed with 0.5 g of **I** in 10 mL of 0.1*M* aqueous solutions of HCl, and the polymerization was allowed to proceed for 1–24 h at the ambient temperature. The weight percentage of Cl^+ increased dramatically with time until it became essentially constant at about 0.27% after 16 h of polymerization.

A simple experiment designed to evaluate the stability of **III** and its potential for recharging was also performed. In this experiment, 50.4 L of distilled, deionized water flowed through a column of **III**, which was identical to the column discussed previously, continually for a period of 168 h with a gravity feed. The initial titrated loading of Cl^+ on the sand was 0.274 wt %. After 168 h, the loading only declined to 0.251 wt %. At that time, further exposure to dilute bleach yielded a loading of 0.271 wt %. Thus, at the flow rate employed (ca. 5 mL/min), the loss of chlorine over 1 week was low, and the loss of bound hydantoinylsiloxane, as indicated by the recharge experiment, was very small. The concentration of free chlorine in the effluent (measured as Cl^+) was only about 0.2 mg/L.

The effect of the prepolymerization of **I**[21] followed by contact with sand was studied also. In this case, 30 mL of a 2.5 wt % solution of poly(3-triethoxysilylpropyl-5,5-dimethylhydantoin) in ethanol was mixed with 5.0 g of sand. The mixture was refluxed for 2 h. The treated sand was then filtered from the solution and cured at 120°C for 30 min. Chlorination was performed in 100 mL of 10% sodium hypochlorite bleach buffered to pH 7.0 for 1 h at the ambient temperature. After rinsing and drying at 45°C for 1 h, an iodometric/thiosulfate titration revealed an oxidative chlorine loading of only 0.030 wt % Cl^+. Even under the drastic treatment conditions (refluxing and curing before chlorination), this loading was significantly lower than that obtained for **I** directly polymerized on the sand in the presence of 0.1*M* HCl for 1 h. Thus, direct polymerization upon the sand particles would appear to the recommended method of producing the biocidal sand.

The efficiency of the polymerization/adhesion process for compound **I** binding to sand particles to produce **II** can probably be increased in at least three ways. First, all the data collected herein for monomer **I** polymerization on sand were for the polymerization reaction at the ambient temperature. Raising the temperature should cause an increase in the rate of the polymerization reaction. Second, the treated sand was not cured before chlorination. Curing at higher temperatures should cause better adhesion of the polyhydantoinylsiloxane to the sand particles. Third, the 0.1*M* concentration of HCl used during the polymerization may not be optimum. These three optimization experiments will be performed in due course.

Biocidal efficacy studies

The biocidal test results for the inactivation of *S. aureus* and *E. coli* O157:H7 by column filters containing **III**

TABLE III
Polymerization/Adhesion Efficiency as a Function of the Time of Polymerization

Time of polymerization (h)[a]	Titrated Cl^+ (wt %)
1	0.00
2	0.00
4	0.03
6	0.12
8	0.18
12	0.23
16	0.27
24	0.26

[a] The weight of the sand in each case was 5.0 g; the polymerization was performed with 0.5 g of monomer **I** in 10 mL of 0.1*M* HCl at 25°C.

are presented in Tables IV and V, respectively. For *S. aureus*, the sand control column caused a 1.9 log loss of CFUs in 1 min of contact and a 2.9 log loss in 15 min of contact. The column treated with unchlorinated polyhydantoinylsiloxane (**II**) produced a 0.8 log loss of CFUs in 1 min of contact and a 2.4 log loss in 15 min of contact. The losses for both control columns must be due to filtration or selective adsorption because neither can be biocidal. Viable bacteria were isolated from the two control columns after the experiment. On the other hand, the column containing chlorinated **III** at a loading of about 0.28 wt % Cl^+ produced a complete 6.5 log inactivation in 1 min or less and at all subsequent contact times. Contact times of less than 1 min are not readily achievable with our equipment. However, these data show that **III** definitely possesses significant biocidal efficacy. There were no viable bacteria isolated from the column containing **III** after the experiment.

In the case of *E. coli* O157:H7, the sand control column caused a 0.2 log loss of CFUs in 1 min of contact and a 0.5 log loss in 15 min of contact. The **II** control column also produced a 0.2 log loss of CFUs in 1 min of contact and a 0.5 log loss in 15 of contact. The column containing **III** at a loading of about 0.28 wt % Cl^+ produced a 3.0 log inactivation in 1 min of contact and a complete 7.4 log inactivation in the interval of 1–5 min and at all subsequent contact times. Perhaps the larger cell density of this bacterium can explain why not all CFUs were inactivated in 1 min, as was the case for *S. aureus*.

TABLE IV
Biocidal Efficacy Against *S. aureus*

Sample in column filter	Contact time (min)	Total bacteria (CFU)	Log reduction
Sand control[a]	0	2.9×10^6	
	1	3.6×10^4	1.9
	5	7.3×10^3	2.6
	10	4.0×10^3	2.9
	15	3.3×10^3	2.9
II control[b]	0	3.0×10^6	
	1	4.5×10^5	0.8
	5	4.6×10^4	1.8
	10	2.2×10^4	2.1
	15	1.3×10^4	2.4
III[c]	0	3.1×10^6	
	1	nd[d]	6.5
	5	nd[d]	6.5
	10	nd[d]	6.5
	15	nd[d]	6.5

[a] Untreated sand control (see text).
[b] Sand treated with unchlorinated polymer **II** (see Fig. 1 and text).
[c] Sand treated with **II** and then chlorinated to form **III** (see Fig. 1 and text).
[d] nd = none detected. The detection limit was 40 CFU/mL.

TABLE V
Biocidal Efficacy Against *E. coli* O157:H7

Sample in column filter	Contact time (min)	Total bacteria (CFU)	Log reduction
Sand control[a]	0	2.1×10^7	
	1	1.5×10^7	0.2
	5	1.3×10^7	0.2
	10	1.3×10^7	0.2
	15	6.9×10^6	0.5
II control[b]	0	3.0×10^7	
	1	1.9×10^7	0.2
	5	1.9×10^7	0.2
	10	1.7×10^7	0.2
	15	9.6×10^6	0.5
III[c]	0	2.8×10^7	
	1	3.1×10^4	3.0
	5	nd[d]	7.4
	10	nd[d]	7.4
	15	nd[d]	7.4

[a] Untreated sand control (see text).
[b] Sand treated with unchlorinated polymer **II** (see Fig. 1 and text).
[c] Sand treated with **II** and then chlorinated to form **III** (see Fig. 1 and text).
[d] nd = none detected. The detection limit was 40 CFU/mL.

The performance of the biocidal sand in this study is not as efficacious as those of the various polystyrene-hydantoin porous bead derivatives reported earlier, which provide similar inactivation in 1–2-s contact times.[6–8] However, those beads contain loadings in the 9–20 wt % Cl^+ range. Also, their preparations are much more expensive than that of **III**. The potential fields of use will be quite different for the two types of biocidal agents. Furthermore, the experiments were conducted with distilled water rather than water containing a large chlorine demand. However, prior work in these laboratories has demonstrated that *N*-halamine-derivatized biocidal surfaces function extremely well even when EPA worst-case water is employed; under these conditions, more frequent recharging is necessary.[4]

CONCLUSIONS

It has been demonstrated that a hydantoinylsiloxane monomer can be polymerized upon the surfaces of sand particles so that a coating adheres that can be rendered biocidal by contact *in situ* with dilute sodium hypochlorite bleach. The biocidal sand inactivates *S. aureus* and *E. coli* O157:H7 in a column filter application in contact times of approximately 1 min. Once the biocidal activity is lost through the loss of oxidative chlorine from the surface, it can be at least partially regenerated by further exposure to free chlorine.

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