Biocidal Polystyrene Beads. IV. Functionalized Methylated Polystyrene

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Received 5 August 2003; accepted 8 November 2003

ABSTRACT: Crosslinked chloromethylated polystyrene beads were reacted with hydantoin and imidazolidinone derivatives to produce functionalized beads which could be rendered biocidal upon reaction with free chlorine or bromine. The biocidal efficacies of the *N*-chlorinated, and in one case, the *N*-brominated polymeric beads against *Staphylococcus aureus* and *Escherichia coli* O157:H7 in aqueous suspension have been determined. Synthetic methods and test data have been presented.© 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 368–372, 2004

Key words: biocidal polymers; *N*-halamine polymers; methylated polystyrene

INTRODUCTION

Although a variety of biocidal polymers (e.g., quaternary ammonium salts, phosphonium materials, halogenated sulfonamides, and biguanides) has been synthesized and tested for biocidal activity,[1] a relatively new class known as cyclic *N*-halamines has been shown to have superior properties including biocidal efficacy, long-term stability, and rechargeability once the efficacy has been consumed during use. Several such materials have been prepared and tested in these laboratories. Of particular value in water and air disinfection applications are the *N*-halamine polymers prepared in the form of highly crosslinked porous beads.

The first article of this series discussed the preparation and biocidal efficacy testing of the novel polymer poly-1,3-dichloro-5-methyl-5-(4′-vinylphenyl)hydantoin[2,3] (Poly1-Cl in Fig. 1) that result from functionalization of highly crosslinked, porous polystyrene beads.[4] Poly1-Cl beads, with their excellent efficacies against bacteria, fungi, protozoa, and virus particles, are being evaluated for their potential in potable water disinfection applications. The second article of the series concerned the control of chlorination of the precursor 5-methyl-5-(4′-vinylphenyl)hydantoin beads such that lesser amounts of chlorine could be bonded to the polymer. They could be used in applications other than for potable water disinfection, for which contact times could be extended and chlorine outgassing could be minimized.[5] The third article of the series compared the biocidal efficacies of beads produced by functionalization of methylated polystyrene with an *N*-chlorinated hydantoin and two quaternary ammonium salt derivatives; the *N*-halamine polymer beads required much shorter contact times than did the polyquat beads to achieve the desired disinfection performance.[6]

The current article will address the functionalization of methylated polystyrene by halogenated hydantoin and imidazolidinone derivatives to illustrate the broad variety of polymers that can be produced as biocidal beads. Sun and Sun[7] have prepared a similar polystyrene–*N*-halamine compound, although by a different procedure starting from the monomer 3-(4′-vinylbenzyl)-5,5-dimethylhydantoin and polymerizing it, rather than by functionalizing commercial chloromethylated polystyrene, as will be described in this work. The structures of the several types of functionalized polymeric beads prepared in this work are shown in Figure 1.

EXPERIMENTAL

Preparation of halogenated methylated polystyrene hydantoin beads

Complete details concerning the preparation of methylated polystyrene hydantoin beads have been pre-

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Contract grant sponsor: U.S. Air Force; contract grant number: FO8637-02-C-7020.
Contract grant sponsor: Vanson-HaloSource Corp.

Journal of Applied Polymer Science, Vol. 92, 368–372 (2004)

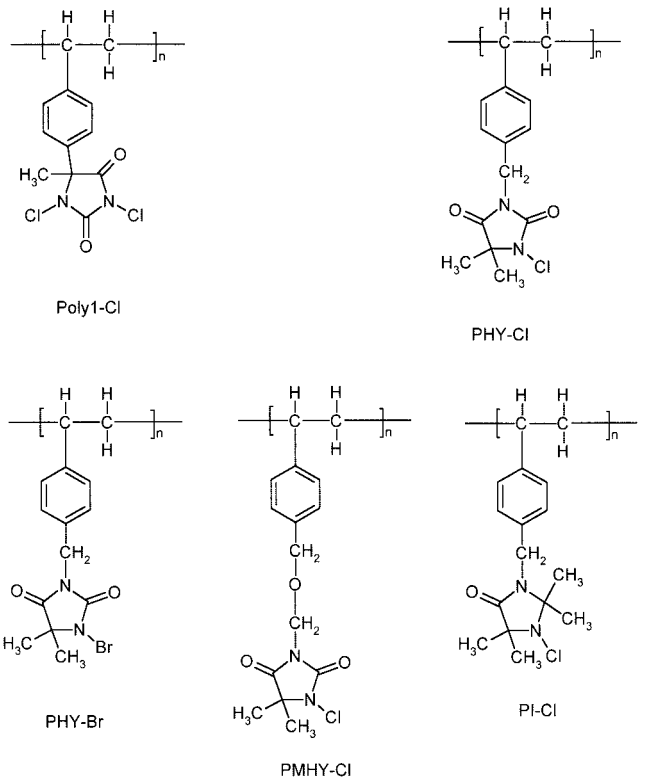

Figure 1 The structures of the biocidal polymeric beads used in this investigation.

sented.[6] Briefly, the potassium salt of 5,5-dimethylhydantoin was prepared by reacting equimolar amounts of 5,5-dimethylhydantoin with potassium hydroxide in boiling ethanol. Following removal of the ethanol and product water under vacuum, the white salt was dissolved in anhydrous dimethyl formamide (DMF) at 95°C. Porous beads of 5.6% crosslinked chloromethylated polystyrene (containing 20.85% by weight chlorine), obtained from Suqing Group (Jiangyin, Jiangsu, China), were first cleaned by soaking in acetone, before being added to the DMF solution of hydantoin salt. These beads were in the size range 180–425 μm in diameter, but had internal pores of undetermined sizes. The mixture was heated with stirring at about 100°C for 12 h, and following removal of the solvent, potassium chloride, and unreacted hydantoin salt, the functionalized beads were washed with water, and then ethanol, and dried under vacuum at 85°C until constant weight (35.5% by weight add-on). An infrared spectrum of a small sample of crushed beads in a KBr pellet exhibited prominent bands at 1715 and 1776 cm^{-1}, demonstrating the presence of the hydantoin functional group.

N-chlorination was accomplished by suspending 10.0 g, for example, of the porous beads functionalized as described above, in a flask containing 50 mL of 5.25% sodium hypochlorite and 50 mL of water, and the pH was adjusted to 7.5 by the addition of 2*N* acetic acid. The mixture was stirred for 45 min at 25°C, filtered, and washed with three 100-mL portions of water at 25°C. The thus chlorinated beads were dried under vacuum at 50°C until their weight became constant. A sodium thiosulfate/iodometric titration indicated that the chlorine loading of the dried beads was 6.2% by weight. An infrared spectrum of a small sample of crushed beads in a KBr pellet exhibited prominent bands at 1726 and 1790 cm^{-1} as expected for a monochlorinated hydantoin functional group. The structure of this functionalized *N*-chloramine polymer is labeled PHY-Cl in Figure 1.

Methylated polystyrene hydantoin beads (5.0 g) prepared as described above were also suspended in a solution containing 40 mL of 10% sodium hypobromite and 40 mL of water. The pH was adjusted to 7.0 by using 2*N* acetic acid. The mixture was stirred for 1 h at 25°C. The brominated beads were removed by filtration, washed with three 100-mL portions of water, and dried under vacuum until constant weight was obtained. The bromine content determined by sodium thiosulfate/iodometric titration was 8.2% by weight. An infrared spectrum of a small sample of crushed beads in a KBr pellet exhibited prominent bands at 1714 and 1776 cm^{-1}, consistent with the presence of a monobrominated hydantoin functional group (see structure PHY-Br in Fig. 1).

Preparation of chlorinated methylated polystyrene hydroxymethylhydantoin beads

Porous, crosslinked chloromethylated polystyrene beads [10.57 g (about 0.062 mol of potentially reactive chlorine for nucleophilic substitution)] were suspended in 150 mL of anhydrous DMF. Then, 10.7 g of anhydrous potassium carbonate (0.078 mol) and 12.3 g (0.078 mol) of 3-hydroxymethyl-5,5-dimethylhydantoin (TCI America, Portland, OR) were added, and the mixture was stirred for 48 h at 100°C. After cooling the mixture to 25°C, suction filtration was used to isolate the functionalized beads. The beads were then washed with three 100-mL portions of water, soaked in 250 mL of boiling water for 15 min, and subsequently washed with two 100-mL portions of water. Then, the beads were dried under vacuum at 85°C to constant weight (13.98 g or 32.3% add-on weight). An infrared spectrum of a small sample of the crushed beads in a KBr pellet exhibited prominent bands at 1715 and 1777 cm^{-1}, which demonstrated the presence of the hydantoin functional group (the two expected carbonyl stretching bands).

Then, 5.0 g of the porous beads functionalized as described above were suspended in a flask containing 40 mL of 5.25% sodium hypochlorite and 40 mL of water, and the pH was adjusted to 7.5 by the addition of 2*N* acetic acid. The mixture was stirred for 1 h at 25°C, filtered, and washed with three 100-mL portions

of water at 25°C. The thus chlorinated beads were dried under vacuum at 50°C until their weight became constant. A sodium thiosulfate/iodometric titration indicated that the chlorine loading of the dried beads was 6.83% by weight. An infrared spectrum of a small sample of the beads (crushed to a powder) in a KBr pellet exhibited prominent bands at 1728 and 1792 cm^{-1} as expected for a monochlorinated hydroxymethylhydantoin functional group. The structure of the polymer labeled PMHY-Cl is shown in Figure 1.

Preparation of chlorinated methylated polystyrene imidazolidinone beads

A 2.84 g (0.02 mol) portion of 2,2,5,5-tetramethylimidazolidin-4-one (TMIO) prepared as described previously[8] and 0.49 g (0.02 mol) of sodium hydride were added to 100 mL of anhydrous DMF. After stirring the mixture for 2 h at 25°C, 6.0 g (0.035 mol of active chlorine) of chloromethylated polystyrene beads were added. The mixture was stirred at 95°C for 48 h, cooled, and filtered, and the functionalized beads were washed with two 100-mL portions of water and then held in boiling water for 15 min. After filtration, the beads were again washed with two 100-mL portions of water and then dried under vacuum at 75°C until constant weight (6.65 g) was obtained. The percentage by weight add-on was 10.8%. This add-on percentage was lower than for the other functionalized beads described above. An infrared spectrum of a small sample of crushed beads in a KBr pellet exhibited prominent bands at 1613 and 1696 cm^{-1}, which demonstrated the presence of the imidazolidinone functional group, most probably bonded to the polymer beads at the amide nitrogen of the heterocyclic moiety.

Then, 3.4 g of the functionalized beads were soaked in 20 mL of 5.25% sodium hypochlorite and 20 mL water at a pH of 7.5 (adjusted by addition of 4*N* acetic acid) at 25°C for 1 h. After filtration and washing with three 100-mL portions of water, the beads were dried to constant weight under vacuum at 50°C. A sodium thiosulfate/iodometric titration indicated that the chlorine loading of the dried beads was 2.85% by weight. An infrared spectrum of a small sample of crushed beads in a KBr pellet exhibited prominent bands at 1609 and 1717 cm^{-1}, indicative of a rather low chlorine loading.

Biocidal efficacy

Column filter biocidal efficacy tests were conducted for aqueous suspensions of the Gram-positive bacterium *Staphylococcus aureus* (ATCC 5368) and the Gram-negative bacterium *Escherichia coli* O157:H7 (ATCC 43895) for the four types of beads in this study. The column filter test has been used extensively in these laboratories for testing polymeric *N*-halamines against pathogens in flowing water. The protocols have been extensively described,[2,3,9] so only a brief protocol will be repeated here. Seven glass columns (1.11 cm ID; 1.27 cm OD) were packed with beads of PHY-Cl, PHY-Br, PMHY-Cl, PI-Cl, and three controls to a length of about 7.6 cm. The controls were the unhalogenated precursor polymers of the four halogenated polymers. The weight of the packed beads and empty-bed volume for each of the column filters is given in Table I. Before each new run, the control columns were sterilized in an autoclave; the halogenated polymer columns were sterilized by passing through a solution of sodium hypochlorite, or sodium hypobromite in the case of PHY-Br, as in their original preparations. All of the columns were extensively washed with autoclaved distilled, deionized water before subjecting them to suspensions of bacteria. Washing was performed until the concentration of free halogen in the effluent was unmeasurable (<0.2 mg/L for Cl^+ and <0.5 mg/L for Br^+).

TABLE I
Column Filter Characteristics

Polymer bead sample[a]	Weight of beads (g)	Empty-bed volume (mL)
PHY-Cl	3.26	4.41
PHY-Br	3.26	3.77
PHY control	3.26	5.47
PMHY-Cl	3.40	2.88
PMHY control	3.40	5.35
Pl-Cl	3.28	4.00
Pl control	3.20	4.00

[a] See text and Figure 1.

The suspensions of bacteria (in 50 mL portions) were pumped through the columns by using a peristaltic pump (Gelman Sciences, Ann Arbor, MI) with the flow rates carefully controlled such that the contact times for each pass through a column (empty-bed volume/flow rate) were about 1.0 s. Thus, recycling the solution for at least six times provided a total contact time with the polymer beads of about 6 s. An aliquot was withdrawn after each pass for bacterial viability enumeration; a 0.02*N* solution of sodium thiosulfate was used to quench any active free halogen which might have passed into the effluent from the halogenated polymer bead columns. It has been shown in these laboratories that this quenching treatment does not affect the viability of the bacteria. The initial concentrations of bacteria added to sterilized pH 7.0 phosphate buffer and flowed through the columns ranged from $3.6–5.5 \times 10^6$ colony forming units (CFU)/mL of the Gram-positive bacterium *S. aureus* (ATCC 6538), or $4.9–6.8 \times 10^6$ CFU/mL of the Gram-negative bacterium *E. coli* O157:H7 (ATCC 43895). Dilutions of the effluents were deposited onto Trypti-

TABLE II
Efficacies of the Four Polymeric Bead Columns against *S. aureus*

Polymer bead sample[a]	Contact time (s)	Remaining CFU/mL
PHY-Cl	0	6.7×10^6
PHY-Cl	1	TNTC[b]
PHY-Cl	2	0
PHY-Cl	3	0
PHY-Cl	5	0
PHY-Cl	6	0
PHY-Br	0	5.4×10^6
PHY-Br	1	0
PHY-Br	2	0
PHY-Br	3	0
PHY-Br	4	0
PHY-Br	5	0
PHY-Br	6	0
PMHY-Cl	0	4.4×10^6
PMHY-Cl	1	0
PMHY-Cl	2	0
PMHY-Cl	3	0
PMHY-Cl	4	0
PMHY-Cl	5	0
PMHY-Cl	6	0
Pl-Cl	0	3.6×10^6
Pl-Cl	1	TNTC[b]
Pl-Cl	2	6.3×10^3
Pl-Cl	3	0
Pl-Cl	4	0
Pl-Cl	5	0
Pl-Cl	6	0

[a] See text and Figure 1; the control columns showed no significant reductions at contact times of 60 s.
[b] Too numerous to count accurately.

case soy agar (TSA) plates which were incubated at 37°C with enumeration at 24 and 48 h.

RESULTS AND DISCUSSION

The results showing the efficacies of the seven bead columns for inactivation of *S. aureus* and *E. coli* 0157:H7 are presented in Tables II and III. The PHY-Cl polymer beads were able to cause complete inactivation (>6.6 log) of both species of bacteria in the contact time interval of 1 to 2 s. In contrast, the polymer labeled Poly1-Cl in Figure 1 has been shown to accomplish the same task in less than 1 s, which is not surprising given that it contains generally about 20% by weight chlorine.[9] The PMHY-Cl polymer (see Fig. 1) also completely inactivated both species of bacteria in a contact time of less than or equal to 1 s. The probable reason for its slightly superior performance when compared to the PHY-Cl polymer is that it contained 6.8% by weight oxidative chlorine, as compared to 6.2% for PHY-Cl; the difference is accentuated when the slightly higher molecular weight of PMHY-Cl than that of PHY-Cl is taken into account. It is also conceivable that under the conditions of the experiment (100°C for 48 h) formaldehyde was emitted from 3-hydroxymethyl-5,5-dimethylhydantoin such that the PMHY-Cl contained PHY-Cl as a coproduct.[10]

A very significant decline in efficacy for both species of bacteria was observed for the PI-Cl polymer beads. This was due to two factors. First, the weight percentage of chlorine in PI-Cl was only 2.85% because of the difficulty in chlorinating the sterically hindered amine nitrogen, as well as to the less favorable reaction of the amide nitrogen of the imidazolidinone ring with the chloromethylated polystyrene. Second, for the hydantoin derivatives (PHY-Cl and PMHY-Cl), the oxidative chlorine moiety is bonded to an amide nitrogen; whereas, for the imidazolidinone derivative (PI-Cl), it is bonded to an amine nitrogen. The covalent N—Cl bond is stronger for an amine nitrogen than for an amide nitrogen. Thus, oxidative chlorine is released to bacterial cells upon direct contact with more difficulty for the amine N—Cl moiety. On the other hand, the oxidative chlorine bonded in PI-Cl should be more stable to loss processes such as reaction with receptor sites on organic impurities. However, it has been demonstrated that even the polymer PHY-Cl is quite stable to loss of chlorine, losing only12.9% of its chlorine

TABLE III
Efficacies of the Four Polymeric Bead Columns against *E. coli* O157:H7

Polymer bead sample[a]	Contact time (s)	Remaining CFU/mL
PHY-Cl	0	6.2×10^6
PHY-Cl	1	TNTC[b]
PHY-Cl	2	0
PHY-Cl	3	0
PHY-Cl	5	0
PHY-Cl	6	0
PHY-Br	0	6.7×10^6
PHY-Br	1	0
PHY-Br	2	0
PHY-Br	3	0
PHY-Br	4	0
PHY-Br	5	0
PHY-Br	6	0
PMHY-Cl	0	5.9×10^6
PMHY-Cl	1	0
PMHY-Cl	2	0
PMHY-Cl	3	0
PMHY-Cl	4	0
PMHY-Cl	5	0
PMHY-Cl	6	0
Pl-Cl	0	5.6×10^6
Pl-Cl	1	TNTC[b]
Pl-Cl	2	TNTC[b]
Pl-Cl	3	TNTC[b]
Pl-Cl	4	TNTC[b]
Pl-Cl	5	7.9×10^3
Pl-Cl	6	5.2×10^2

[a] See text and Figure 1; the control columns showed no significant reductions at contact times of 60 s.
[b] Too numerous to count accurately.

under storage at ambient temperature over a period of 96 days.[6]

The brominated polymer beads (PHY-Br in Fig. 1) also inactivated both species of bacteria in less than or equal to 1 s even though they contained only 8.2% by weight oxidative bromine (equivalent to 3.6% by weight oxidative chlorine). This was expected because the N—Br bond is weaker than the N—Cl bond, facilitating transfer of the halogen moiety to the bacterial cells. Of course, concomitantly the stability of the bromine on the polymer beads should be less than that for the chlorine on its chlorinated analog. It has been observed frequently in these laboratories that *N*-brominated heterocyclic monomers and polymers inactivate bacteria at lower contact times than do their *N*-chlorinated analogs, but the former are always less stable to loss of oxidative halogen than are the latter in aqueous solution or dry storage.[1,11]

Finally, it should be noted that the polymeric beads can be recharged repeatedly with free halogen following loss of the original charge. This is accomplished by simply exposing them to aqueous solutions of hypochlorite or hypobromite.

CONCLUSION

It has been shown that *N*-halogenated polymeric beads are efficacious in an aqueous disinfection application, requiring short contact times for inactivation of the bacteria *S. aureus* and *E. coli* 0157:H7. The functionalized polymers can be tailored to the application, depending upon whether rapid biocidal activity or long-term stability to loss of oxidative halogen is desired. The various polymeric beads will be particularly useful in the disinfection of water and moist air flowing through cartridge filters containing them.

This work was supported by U.S. Air Force Contract F08637-02-C-7020 and by the Vanson-HaloSource Corp.

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