Biocidal Polystyrene Beads. III. Comparison of *N*-halamine and Quat Functional Groups

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ABSTRACT: A comparison has been made between the biocidal efficacies of *N*-chlorinated polymeric beads and two derivatives of polyquat beads. Biocidal effects were measured after brief contact exposures of aqueous suspensions of either *Staphylococcus aureus* or *Escherichia coli* to the water-insoluble beads. The polymeric backbone was held the same in all three types of beads, so they differed only in their biocidal derivative moieties. In all cases, functionalization of crosslinked chloromethylated polystyrene beads was performed to introduce the biocidal properties. Synthetic methods and test data will be presented. The most effective biocide, as measured by degree of inactivation in the shortest contact time of the two species of bacteria, was the *N*-chlorinated hydantoinyl derivative of methylated polystyrene. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 363–367, 2004

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INTRODUCTION

The most widely employed biocidal materials in use today in disinfecting household products are quaternary ammonium salt derivatives (quats) and their polymeric derivatives (polyquats). It is thought that the mechanism of action of quats and polyquats against pathogenic microorganisms involves first the adsorption of the positively charged site of the molecule onto the cell wall. This is then followed by diffusion through the cell wall to bind with and disrupt the cytoplasmic membrane, and then release of alkyl cations and constituents of the cytoplasmic membrane, causing death of the cell.[1] These materials have several advantages which have escalated their use and popularity in society. They are generally stable in aqueous solution for extended periods and have long shelf lives. If properly designed with structures containing satisfactory lipophilic alkyl groups, such as dodecyl, they can be effective against a broad spectrum of pathogenic microorganisms. Most important, many of them have received approval by the government-regulatory agencies for commercial sales. However, they also have limitations. Contact times for disinfection tend to be lengthy, and they cannot be regenerated once lost from a surface. The interested reader is referred to recent extensive reviews of quats and polyquats as biocidal materials[1,2] and an exhaustive patent literature.[3]

A relatively new type of biocide, which will soon be in use in the public sector, is the heterocyclic *N*-halamine. Molecules representative of this type of biocide contain either nitrogen–chlorine or nitrogen–bromine covalent chemical bonds. They may be prepared either as water-soluble monomers[4] or as water-insoluble polymers.[2] If derivatized with electron-donating alkyl groups adjacent to the nitrogen(s) on the heterocyclic rings, the molecules will be very stable toward release of free halogen into aqueous solution and function as biocides by the direct contact of the cells of microorganisms with the halogen atom. Following transfer of the oxidative halogen atom to the cell, inactivation most probably proceeds by an oxidation mechanism, as is presumed to be the case with free available halogen (e.g., as in hypochlorite bleach). Advantages inherent in the *N*-halamines as biocides are the capability of regeneration following loss of the halogen by simply exposing the molecules to additional free halogen, and, in general, more rapid inactivation of a broad spectrum of pathogens than is the case for quats and polyquats because oxidative chlorine and bromine are potent biocides. Their primary limitation is that being relatively new, some regulatory hurdles remain to be surmounted.

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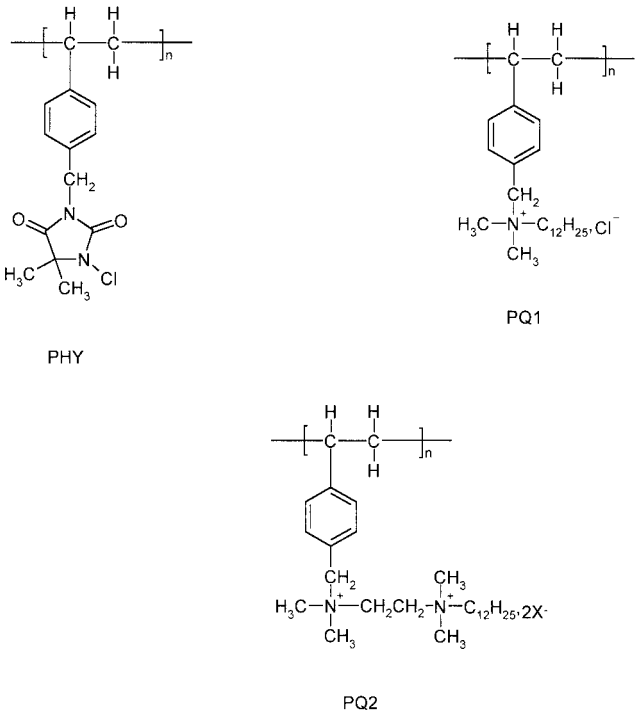

Figure 1 The structures of the biocidal polymeric beads used in this investigation.

Extensive work in these laboratories over the past decade[2] has established that *N*-halamine groups, such as *N*-halo hydantoins, oxazolidinones, and imidazolidinones, can be covalently attached to a variety of polymers used in water-disinfection applications,[5–7] surface coatings,[8–10] and elastomers.[11] Sun and coworkers, in pioneering work on textile fabrics, have extended the technology to its use in rendering fabrics containing cellulose (cotton and cotton blends) biocidal.[12–15] More recently, it has been demonstrated that nylon and PET can also be rendered biocidal with similar technology.[16–18]

In this work, a direct comparison will be made of the antibacterial efficacious of polymeric beads functionalized with an *N*-halamine or two different quat derivatives. To provide the best comparison, the polymeric backbone will be held the same. In all cases, functionalization of representative samples of the same batch of crosslinked chloromethylated polystyrene beads will be performed. Similar polyquat derivatives have been proposed by Kourai and Yabuhara.[3] Sun and Sun[19] 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 done in this work. The structures of the three types of functionalized polymeric beads are shown in Figure 1.

EXPERIMENTAL

Preparation of chlorinated methylated polystyrene hydantoin beads

The potassium salt of 5,5-dimethylhydantoin was prepared by reacting 25.6 g (0.2 mol) of 5,5-dimethylhydantoin with 11.2 g (0.2 mol) of potassium hydroxide in 100 mL of boiling ethanol with stirring. The ethanol and product water were removed under vacuum to obtain the white salt. The salt was added to 200 mL of anhydrous dimethyl formamide (DMF) (Sigma-Aldrich, Milwaukee, WI) and heated to 95°C until all of the salt dissolved.

Porous beads of 5.6% crosslinked chloromethylated polystyrene (containing 20.85% by weight chlorine) obtained from Suqing Group (Jiangyin, Jiangsu, China) having particle sizes in the range of 180 to 425 μm, but undetermined pore sizes, were cleaned by soaking them in acetone (400 mg/mL) for 30 min at 25°C and then by passing three portions of acetone (0.5 mL/g) through them in a filter funnel. Then, 8.12 g (about 0.048 mol) of the cleaned, chloromethylated polystyrene beads were added to the DMF solution of hydantoin salt, and the mixture was heated with stirring at about 100°C for 12 h. Following removal of the solvent and unreacted hydantoin salt, the functionalized beads were washed and dried under vacuum at 85°C until constant weight [11.0 g (35.5% by weight add-on)]. An infrared spectrum of a small sample of the beads (crushed to a powder) in a KBr pellet exhibited prominent bands at 1715 and 1776 cm^{-1}, the two expected carbonyl stretching bands, which demonstrated the presence of the hydantoin functional group.

Then, 10.0 g of the porous beads functionalized as described above were suspended in a flask containing 50 mL 5.25% sodium hypochlorite and 50 mL 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 the beads (crushed to a powder) 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 in Figure 1.

Preparation of polyquat beads

Two types of polyquat beads were prepared from 5.6% crosslinked chloromethylated polystyrene by a procedure analogous to that reported by Kourai and Yabuhara.[3] Briefly, following thorough cleaning of the

beads as described above, 15.3 g (about 0.09 mol of active chlorine) of the beads were suspended in 150 mL ethanol in a 500-mL flask fitted with a condenser. To this suspension was added 19.2 g (0.09 mol) of dimethyldodecylamine (Aldrich Chemical Co., Milwaukee, WI), and the mixture was refluxed with stirring for 12 h. Then, the functionalized beads were removed by filtration, extracted in 100 mL of boiling ethanol for 15 min, washed with three portions of near boiling ethanol in a filter funnel (0.5 mL/g), and dried to constant weight under vacuum at 50°C. The yield was 32.0 g (87.1% based upon add-on weight) of the polyquat labeled PQ1 in Figure 1.

The polyquat-labeled PQ2 in Figure 1 was prepared by first reacting 10.0 g of cleaned 5.6% crosslinked chloromethylated polystyrene beads (about 0.059 mol of active chlorine) in 100 mL of ethanol with 8.7 g (0.075 mol) of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (Aldrich Chemical Co.) under reflux and stirring for 12 h. The product was purified in the same manner as described above for PQ1. The yield was 15.66 g (83% based upon the add-on weight of 5.66 g). Then, 15.10 g of these functionalized beads were suspended in 150 mL ethanol and reacted with 4.5 g (0.018 mol) dodecyl bromide (Aldrich Chemical Co.) under reflux and stirring for 18 h. By using the same purification procedure as described above, the yield of PQ2 was only 15.86 g (7.3% based upon the amount of tertiary amine attached to the beads in the previous reaction step).

Biocidal efficacy

Two types of biocidal tests were conducted for aqueous solutions of the Gram-positive bacterium *Staphylococcus aureus* (ATCC 5368)—a column filter test and a shaking test. The Gram-negative bacterium *Escherichia coli* O157:H7 (ATCC 43895) was also subjected to the column filter test.

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,[5–7] so only a brief protocol will be repeated here. Five glass columns (1.11 cm ID; 1.27 cm OD) were packed with beads of PHY, PQ1, PQ2, and two controls. The control for PHY was its unchlorinated precursor; the control for the two polyquats was the original chloromethylated polystyrene beads. The weights of the packed beads and empty-bed volumes for each of the column filters are given in Table I. Before each new run, the polyquat columns and the two control columns were sterilized in an autoclave: the PHY column was sterilized by passing through a solution of sodium hypochlorite as in its original preparation. All of the columns were extensively washed with autoclaved distilled, deionized water before subjecting them to solutions of bacteria. In the case of the PHY column, washing was continued until the concentration of free chlorine in the effluent was unmeasurable (<0.2 mg/L). 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 *n* times provided a total contact time with the polymer beads of *n* 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 chlorine which might have passed into the effluent from the PHY column. 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 5.2×10^4 to 3.7×10^5 colony forming units (CFU)/mL. Dilutions of the effluents were deposited onto Trypticase soy agar (TSA) plates, which were incubated at 37°C with enumeration at 24 and 48 h.

TABLE I
Column Filter Characteristics

Polymer bead sample[a]	Weight of beads (g)	Empty-bed volume (mL)
PHY	3.30	3.75
PHYcontrol	3.38	4.65
PQ1	3.30	4.97
PQ2	3.30	5.48
PQ control	3.30	3.05

[a] See text and Figure 1.

For the shaking tests, 1.0×10^7 CFU of *S. aureus* added to 50 mL of sterile pH 7.0 phosphate buffer were mixed with 150 mg of each of the types of polymeric beads (PHY, PQ1, PQ2, and the two controls) and agitated in sterile tubes with a shaker (2568S/N, ThermoForma, Marietta, OH) at 250 rpm. At contact times of 5, 15, and 45 min, aliquots of 1 mL were removed; the PHY aliquots were quenched with 0.02*N* sodium thiosulfate, and the viable bacteria were enumerated after incubation at 37°C for 24 and 48 h.

Stabilities of PHY beads

A 5.0-g quantity of PHY beads was dried under vacuum at 50°C until constant weight was obtained. The beads were stored in a capped brown bottle at ambient. Periodically over 96 days, samples were removed for analytical determination of remaining total oxidative chlorine by using an iodometric/thiosulfate titration procedure.

RESULTS AND DISCUSSION

The results showing the efficacies of the five bead columns for inactivation of *S. aureus* and *E. coli* are

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

Polymer bead sample[a]	Contact time (s)	Remaining CFU/mL
PHY	0	1.1×10^5
PHY	1	4.7×10^4
PHY	2	0
PHY	3	0
PHY	5	0
PHY control	0	1.1×10^5
PHY control	1	1.1×10^5
PHY control	2	7.4×10^4
PHY control	3	6.9×10^4
PHY control	5	5.7×10^4
PQ1	0	5.2×10^4
PQ1	1	1.8×10^4
PQ1	2	1.4×10^4
PQ1	3	1.2×10^4
PQ1	5	6.3×10^3
PQ2	0	1.1×10^5
PQ2	1	2.7×10^5
PQ2	2	6.5×10^4
PQ2	3	8.1×10^4
PQ2	5	6.3×10^4
PQ control	0	1.1×10^5
PQ control	1	6.9×10^4
PQ control	2	8.5×10^4
PQ control	3	6.1×10^4
PQ control	5	4.5×10^4

[a] See text and Figure 1.

TABLE III
Efficacies of the Five Polymeric Bead Columns against *E. coli*

Polymer bead sample[a]	Contact time (s)	Remaining CFU/mL
PHY	0	3.7×10^5
PHY	1	0
PHY	2	0
PHY	3	0
PHY	5	0
PHY control	0	3.7×10^5
PHY control	1	2.2×10^5
PHY control	2	1.9×10^5
PHY control	3	1.8×10^5
PHY control	5	1.8×10^5
PQ1	0	3.7×10^5
PQ1	1	2.1×10^5
PQ1	2	1.8×10^5
PQ1	3	1.8×10^5
PQ1	5	1.8×10^5
PQ2	0	3.7×10^5
PQ2	1	2.0×10^5
PQ2	2	1.8×10^5
PQ2	3	2.0×10^5
PQ2	5	1.6×10^5
PQ control	0	3.7×10^5
PQ control	1	2.1×10^5
PQ control	2	2.1×10^5
PQ control	3	2.0×10^5
PQ control	5	1.8×10^5

[a] See text and Figure 1.

presented in Tables II and III, respectively. The *N*-chloramine polymer beads (PHY) provided a complete 5 log inactivation of *S. aureus* in a contact time of between 1 and 2 s and in less than or equal to 1 s for *E. coli*. The necessary contact time for *S. aureus* inactivation was slightly longer than that (less than 1 s), demonstrated earlier for poly[1,3-dichloro-5-methyl-5-(4′-vinylphenyl)hydantoin] beads, which have been shown to be useful in cartridge filters for potable water disinfection.[20] The PHY control beads showed a slight loss (about 0.3 log) of bacteria over the 5-s contact, which, if significant, could indicate a very small amount of filtration, rather than inactivation. PQ1 caused about a 1.2 log inactivation of *S. aureus* over a 5-s contact time, but no more inactivation of *E. coli* than did the PQ control beads in that time. PQ2 did not function any better than did the PQ control for either bacterium in the column test for the 5-s contact. Thus, it is clear that, for brief contact times in a cartridge filter application, the *N*-chloramine beads provide considerably better inactivation of the two bacteria studied than do the polyquat beads.

The results showing the efficacies of the five types of beads in the shaking test experiments are given in Table IV. As expected from the results obtained from the column tests, the *N*-chloramine polymer beads provided a complete 4.7 log inactivation of *S. aureus*

TABLE IV
Efficacies of the Five Polymeric Beads against *S. aureus* in a Shaking Test

Polymer bead sample[a]	Contact time (min)	Remaining CFU/mL
PHY	0	5.2×10^4
PHY	5	0
PHY	15	0
PHY	45	0
PHY control	0	5.2×10^4
PHY control	5	5.2×10^4
PHY control	15	4.8×10^4
PHY control	45	3.2×10^4
PQ1	0	5.2×10^4
PQ1	5	0
PQ1	15	0
PQ1	45	0
PQ2	0	5.2×10^4
PQ2	5	5.1×10^4
PQ2	15	4.8×10^4
PQ2	45	3.3×10^4
PQ control	0	5.2×10^4
PQ control	5	5.3×10^4
PQ control	15	4.7×10^4
PQ control	45	4.0×10^4

[a] See text and Figure 1.

TABLE V
Oxidative Chlorine Stability of the PHY Beads[a]

Time (days)	Weight percent CI	% Decrease in CI
0	6.30	—
14	6.13	2.7
28	5.90	6.3
60	5.68	9.8
96	5.49	12.9

[a] See text and Figure 1.

within 5 min of agitated contact, with no decline for the PHY control beads in that timeframe. After 5 min of exposure, even undetectable free chlorine concentrations (<0.2 ppm) would be expected to have a deleterious effect on *S. aureus*, so it cannot be concluded that all the decline in viable bacteria in this experiment was due to contact with the beads. Perhaps surprisingly, however, the polyquat PQ1 beads also gave a complete 4.7 log inactivation within 5 min, but the PQ2 beads were able to cause only a 0.1 log reduction in 45 min, a number which is probably not significant. The most plausible explanation for the poor performance of the PQ2 beads is that the preparation procedure resulted in only a small fraction of dicationic sites (i.e., the yield was only 7.3% in the last step of the process). Thus, most of the sites on PQ2 refer to the monocation, as in PQ1, except that the fourth substituent on the quaternary nitrogen is now predominantly the ethylene dimethyl amino group rather than the dodecyl group. It is well known that for quats to be effective biocides, a long alkyl group such as dodecyl is required to provide the necessary lipophilicity for effective cell-wall penetration.[1] Reaction of dodecyl bromide with a tertiary nitrogen, as required to produce PQ2, is a difficult process due to steric constraints. If PQ2 could be produced in high yield as a dication, it would undoubtedly function more effectively as a biocide than would PQ1.

The data in Table V show that the *N*-chlorinated beads are quite stable to the loss of oxidative chlorine, losing only 12.9% of their chlorine under storage at ambient temperature over a period of 96 days. The reason for the excellent stability is that the oxidative chlorine atom is covalently bound to the amide nitrogen of the hydantoin ring (Fig. 1) adjacent to two electron-donating alkyl groups to counteract any N—Cl bond weakening brought about by the adjacent electron-withdrawing carbonyl functional group. The imide nitrogen is bonded to the polymer backbone and thus contains no chlorine. It has been demonstrated previously that chlorine can be recharged onto such beads once the chlorine has been exhausted because of loss processes by simply exposing them once again to aqueous free chlorine.[20]

CONCLUSION

It has been shown that *N*-chlorinated polymeric beads are more efficacious in an aqueous disinfection application against the bacteria *S. aureus* and *E. coli* than are structurally similar polyquat beads. Upon loss of the antimicrobial chlorine atom, activity can be restored by exposure to a source of free chlorine such as hypochlorite bleach. In most disinfection applications, *N*-chlorinated polymers may be superior to, or at least competitive with, similar polyquats.

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