Synthesis, Characterization, and Antibacterial Activities of Novel *N*-Halamine Polymer Beads Prepared by Suspension Copolymerization

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Introduction

Free halogen, ozone, and chlorine dioxide are currently used water and wastewater disinfectants. However, all of these compounds have limitations such as short-term stability in aqueous solution and reactivity with organic impurities in water to form undesired (toxic) byproducts. As a result, there is a continuous effort to develop new insoluble polymeric disinfectants. For example, polymeric quaternary ammonium compounds,[1] iodine exchange resins,[1] and N-chlorinated sulfonamide derivatives[2] have been studied in order to be used in water treatment, but their own problems of low efficacies, low stability, and high cost limited their applications.

Recently, polymeric *N*-halamines have received considerable attention as novel water disinfectants.[3] It has been demonstrated that *N*-halamine structures could provide rapid kill against a wide spectrum of microorganisms without causing environmental concerns. Their antimicrobial activities are both durable and regenerable, proving them as ideal materials for water purification systems and filters. One of the most successful polymeric *N*-halamines that has been developed is poly[1,3-dichloro-5-methyl-5-(4′-vinylphenyl)hydantoin] (Poly I), as shown in Figure 1A. Poly I was synthesized first by a Friedel−Crafts acylation of polystyrene to produce poly(4-vinylacetophenone), followed by a Bucherer−Bergs synthesis of poly[5-methyl-5-(4′-vinylphenyl)hydantoin], and then a chlorination reaction in the presence of chlorine gas.[3]

The above preparation of Poly I from chemical modifications on polystyrene, though practical, brings in some difficulties in purification of the final product, especially when polystyrene beads are used as starting materials. There is a definite need in continued exploration on halamine polymers, particularly in searching for easy synthesis and easy recharging as well as improved durability in water treatment. In recent years, the interest in developing antimicrobial materials[4] in this research laboratory led to the design of several vinyl hydantoin monomers, such as 3-allyl-5,5-dimethylhydantoin (ADMH) and 3-(4′-vinylbenzyl)-5,5-dimethylhydantoin (VBDMH), as shown in Figure 1, parts B and C, respectively. Both monomers were employed in graft polymerizations on textile materials and copolymerizations with vinyl acetate, methyl methacrylate, and acrylonitrile and successfully introduced biocidal functions to the materials. On the basis of the requirements in water treatments, it is very interesting to study the possibility of the direct preparations of polystyrene hydantoin derivatives by suspension copolymerizations of these monomers with styrene. Additionally, this may allow us to systematically study the transformation from polystyrene to polymeric *N*-halamines by simply changing the vinyl hydantoin monomer contents in the polymerization.

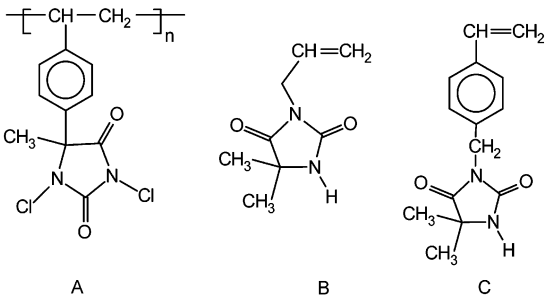

Figure 1. Chemical structures of (A) Poly I, (B) ADMH, and (C) VBDMH.

In this paper, we report the syntheses of antimicrobial polymer beads by suspension copolymerizations of styrene with VBDMH and ADMH, respectively. These polymers were characterized with elemental analysis, FT-IR, and SEM. The antibacterial activities of their *N*-halamine derivatives against gram-negative and gram-positive bacteria were further studied.

Experimental Section

Materials. All reagents were purchased from either Aldrich or Fisher Scientific. Styrene (ST) and divinylbenzene (DVS) were extracted with 10% aqueous sodium hydroxide and water, dried over anhydrous magnesium sulfate, and distilled under vacuum. 2,2′-Azobis(isobutyronitrile) (AIBN) and benzoyl peroxide (BPO) were recrystallized from EtOH and MeOH/chloroform, respectively.

ADMH and VBDMH were synthesized in this lab as reported previously.[4a,4e] FT-IR spectra were taken on a Nicolet Magana IR-560 spectrometer using KBr pellets. The surface morphology of the beads was observed using a scanning electron microscope (SEM) Fei/Philip XL-30s FEG. Elemental analyses of the samples were conducted by Quantitative Technologies Inc. (Whitehouse, NJ). Active chlorine contents (wt %) of the chlorinated beads were determined by a titration method reported elsewhere.[4c]

Polystyrene-*co*-VBDMH (PS-*co*-VBDMH) Beads. An organic mixture containing 0.1 mol of ST and VBDMH mixtures (VBDMH was varied from 0−90 mol % of total monomers (mole %)), chlorobenzene (CB, 50−150 mol %), DVS (0−6 mol %) and BPO (1 mol %) was added to a 250 mL three-neck flask fitted with a mechanical stirrer and a condenser containing 60 mL of 0.6% poly(vinyl alcohol) (hydrolysis degree = 88% and polymerization degree = 2400) aqueous solution. The reaction temperature was then raised to 80 °C, and the stirring speed was kept at 150 rpm during the reaction period (6 h). The polymers obtained as beads were washed thoroughly with hot water, acetone, and methanol and were dried in a vacuum at 50 °C for 72 h to reach a constant weight.

Polystyrene-*co*-ADMH (PS-*co*-ADMH) Beads. To a 250 mL three-neck flask fitted with a mechanical stirrer and a condenser were added 55 mL of distilled water, 20 g of NaCl, and 1 g of $Na_3(PO_4)$. Under a high speed of stirring, 0.6 g of $CaCl_2$ in 5 mL of distilled water was added, followed by the addition of an organic phase containing 0.1 mol of ST and ADMH mixtures (ADMH was varied from 0−40 mol %), CB (0−100 mol %), DVS (0−6 mol %), and AIBN (1 mol %). The reaction temperature was then raised to 60−65 °C, and the stirring speed was kept at 400 rpm during the reaction period (6 h). The polymers obtained as beads were washed thoroughly

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with hot water, acetone, and methanol and were dried in a vacuum at 50 °C for 72 h to reach a constant weight.

Antibacterial Assessment. Five grams of the polymer beads was treated with 100 mL of a diluted chlorine bleach containing 3000 ppm of active chlorine at room temperature overnight, washed thoroughly with distilled water, and then dried under reduced pressure to remove any possible free chlorine. The antibacterial properties of the halogenated polymers were tested against *Escherichia coli*, a gram-negative bacterium, and *Staphylococcus aureus*, a gram-positive one. In the antibacterial study, 100 mL of an aqueous suspension containing 10^6-10^7 CFU/mL of bacteria passed through a column (diameter is about 0.5 cm, and the length is about 20 cm) packed with ca. 3 g of the corresponding sample beads, using a mini pump (Fisher Scientific) at various flow speeds. The effluent was collected, and the diluted solutions were plated onto nutrient agar plates. The highest flow speed for a total kill of the bacteria was reported for the characterization of the antibacterial efficacies of the samples. The same procedure was also applied to the un-halogenated samples as controls. Bacterial colonies on the agar plates were counted after incubation at 37 °C for 24 h.

Results and Discussion

Functionalized polymer beads have been the subjects of many investigations. However, most of these studies were aimed at the development of resins for applications in the areas of ion-exchange, chromatographic separation, and solid phase and combinatorial syntheses.[5−7] Our results showed that both VBDMH and ADMH could copolymerize with styrene, but their reactivities were quite different. For example, without cross-linkers, VBDMH could copolymerize with styrene when the molar content of VBDMH in the monomer mixtures was lower than 50%. If the molar content of VBDMH is higher than 50%, little high-molecular weight copolymers could be obtained, indicating the lower polymerization ability of VBDMH, possibly caused by its bulky hydantoin structure, which is in good agreement with our previous study.[4e]

In copolymerization of ADMH/styrene systems, a much more significant inhibition phenomena was observed. At molar contents of ADMH higher than 30% in the monomers, no polymers could be obtained. It should be noted that, unlike VBDMH, the much lower polymerization activity of ADMH was most likely caused by its allylic structure. The allylic radical, once formed by initiation, is very stable. Because of this stability, degradative chain transfer competes exceptionally well with normal propagation, and the polymer chains are terminated after the addition of only a few monomer units.[4a−c,8]

Our previous studies showed that the polymerization activities of the hydantoin monomers could be remarkably enhanced by the addition of a small amount of multifunctional monomers.[4] Similar results were also obtained in the copolymerization of VBDMH with styrene. In the presence of 1% DVS, copolymer beads could be obtained at a satisfactory yield when molar contents of VBDMH in the monomer mixtures were up to 70%. The cross-linked polymer beads were insoluble in any solvents. The presence of DVS promoted the copolymerization of ADMH with styrene too, but to a different extent. In these cases, with the molar contents higher than 30% in the monomer mixtures, ADMH could not form copolymers with styrene despite the fact that the DVS concentration was raised to 6%. These results could also be explained by the allylic structure of ADMH and the so-called autoinhibition process in its polymerization reactions.[4a−c,8]

Table 1. PS-*co*-VBDMH and PS-*co*-ADMH Beads Produced[a]

sample no.	comonomer	comonomer content (%)[b]	DVS (%)[b]	CB (%)[b]	polymer composition (%)[c]	yield (%)
1	no	N/A	1	50	N/A	95
2	VBDMH	70	1	50	71	92
3	ADMH	30	2	50	16	77

[a] Samples 1 and 2 were made by using 1% BPO (mole %) as an initiator and 0.6% (wt %) PVA as stabilizer at 80 °C, while sample 3 was prepared by using 1% AIBN as initiator and $Ca_3(PO4)_2$ formed in situ by the reaction of $Na_3(PO_4)$ with $CaCl_2$, as stabilizer at 60−65 °C. [b] Mole % in total monomers. [c] VBDMH or ADMH molar contents in the polymers, which were calculated according to the element analysis.

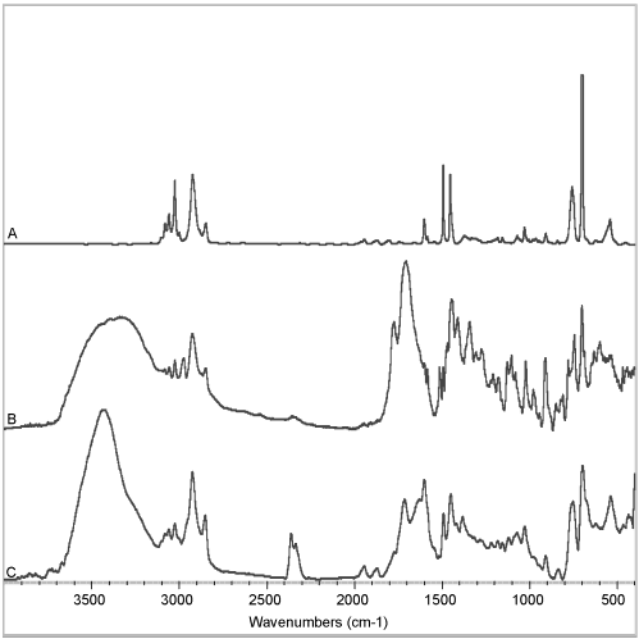


Figure 2. FT-IR spectra of (A) polystyrene, (B) PS-*co*-VBDMH, and (C) PS-*co*-ADMH.

Because of the intention to demonstrate the hypothesis that antimicrobial polymer beads can be synthesized by suspension copolymerization of styrene with VBDMH or ADMH in this paper, only the samples with the highest VBDMH and ADMH contents were further studied, and their preparation conditions are summarized in Table 1.

Figure 2 shows the FT-IR spectra of Polystyrene (PS, Figure 2A), PS-*co*-VBDMH (Figure 2B), and PS-*co*-ADMH (Figure 2C). In the spectra of the two copolymers, besides the characteristic bands of PS, two new bands centered at 1710 and 1768 cm^{-1} could be also detected. These bands are attributed to the imide and amide groups in the copolymers, respectively,[4] indicating that the two monomers can form copolymers with styrene by suspension copolymerizations.

The surface morphologies of the polymer beads were studied by SEM observations, and the results are shown in Figure 3. Most of the beads are in the range of 10−20 mesh, indicating that the current suspension copolymerization is a promising approach to prepare polymer beads with spherical particle shape and narrow particle size distribution. Polystyrene beads (Figure 3A) show regular and smooth surfaces. However, the presence of VBDMH or ADMH in the copolymers leads to porous, irregular surfaces (Figure 3, parts B and C). Similar phenomena were observed in the suspension

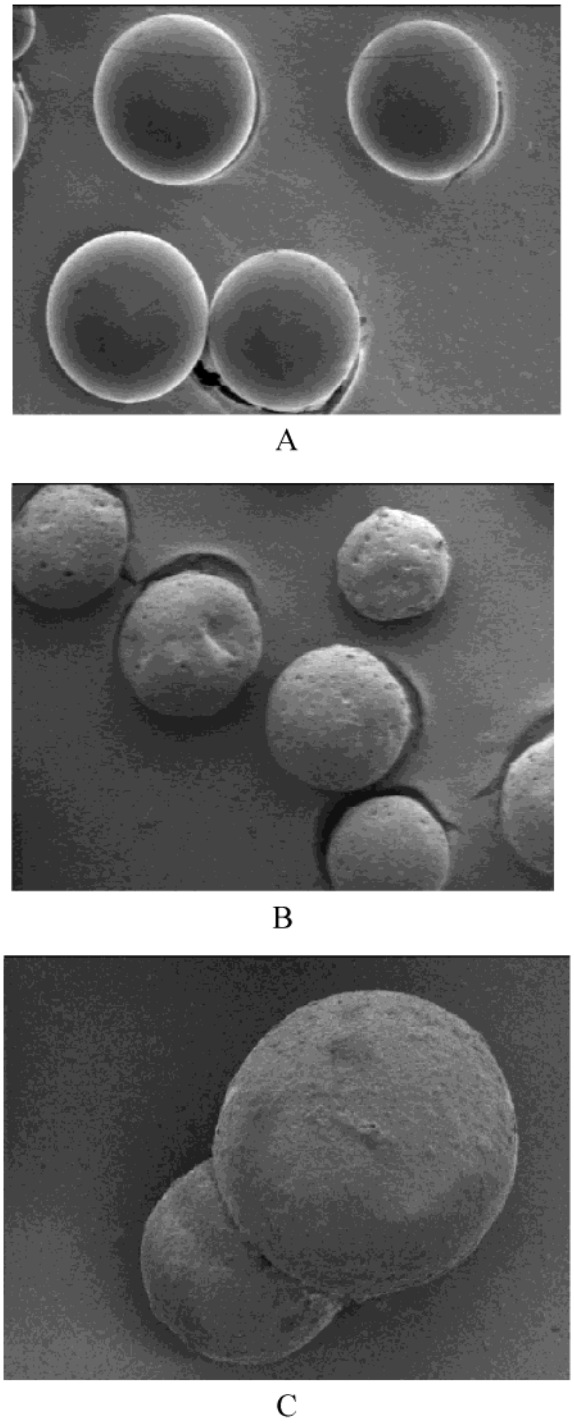


Figure 3. Scanning electron micrographs of (A) polystyrene, (B) PS-*co*-VBDMH, and (C) PS-*co*-ADMH.

polymerization of other functional styrene monomers, which could be attributed to decreased mechanical stabilities of the new beads.[6] It should be pointed out that such a surface morphology change after copolymerization is very desirable in the present study. The rough/porous structures will certainly increase the surface areas of the *N*-halamine beads. And our previous studies[4] have established that polymeric *N*-halamines will have the maximum antimicrobial efficiencies only when their active sites can make full contact with the microorganisms.

After treated with a bleach solution containing 3000 ppm of active chlorine, the hydantoin groups of the polymers could be transformed into *N*-halamine structures following the reaction (eq 1). To confirm the

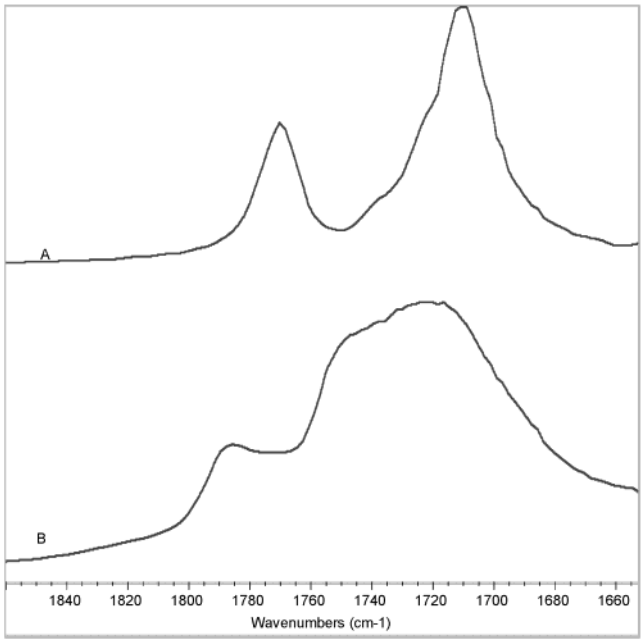
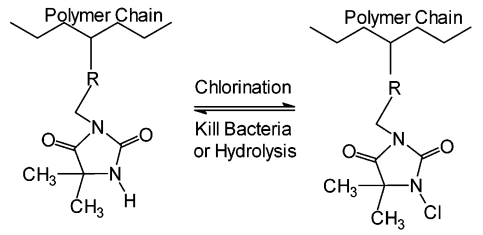


Figure 4. FT-IR spectra in the region of 1600−1850 cm^{-1} of PS-*co*-VBDMH (A) before chlorination and (B) after chlorination.



(1)

transformation, as an example, Figure 4 shows the FT-IR spectra in the region of 1600−1850 cm^{-1} of PS-*co*-VBDMH before and after the bleach treatment, respectively. Before the treatment, the sample displays a band around 1768 cm^{-1}, attributing to the amide carbonyl structure of the hydantoin ring (Figure 4, A). After the treatment, the 1768 cm^{-1} band disappears; and instead, a new band at 1786 cm^{-1} appears. These 18 cm^{-1} differences of vibrations between the treated and untreated samples strongly indicate that the hydantoins are transformed into *N*-halamine structures.[4,9]

The *N*-halamine structures in all of the copolymers were very stable. After being immersed in distilled water (water-to-sample ratio > 100:1 by weight) for 1 month, the FT-IR spectra were recorded, and no difference was observed between the water treated and untreated *N*-halamines. After treated with a 0.1 M sodium thiosulfate aqueous solution, a common reducing agent, at 60 °C for 2 h, the *N*-halamine structures were finally returned into the precursor hydantoins, and the 1786 cm^{-1} band changed back to 1768 cm^{-1}. However, after another bleach treatment, the 1768 cm^{-1} band could be converted to 1786 cm^{-1} again. The same "1786−1768−1786" cycle was repeated 10 times, and each time the same result was observed. As the antibacterial activities are provided by the *N*-halamine structures,[3,4] the durability and regenerability of these structures could provide the copolymers with corresponding durable and regenerable antibacterial properties.

Table 2. Antimicrobial Activities of Selected Samples Against *E. coli*

polymer[a]	W_{ct} (%)[b]	W_{ce} (%)[c]	W_{ce}/W_{ct} (%)	age (days)[d]	regenerated (Y/N)[e]	regeneration times[f]	$V_{E.\ coli}$ (mL/min)[g]	$V_{S.\ aureus}$ (mL/min)[g]
PS-*co*-VBDMH	10.9	6.4	58.7	3	N	N/A	3.4	4.7
PS-*co*-VBDMH	10.9	6.2	56.9	60	N	N/A	2.9	4.7
PS-*co*-VBDMH	10.9	5.9	54.1	3	Y	2	3.5	4.4
PS-*co*-VBDMH	10.9	6.0	56.9	3	Y	10	3.1	4.5
PS-*co*-ADMH	3.7	2.9	78.4	5	N	N/A	0.93	2.3
PS-*co*-ADMH	3.7	2.5	67.6	60	N	N/A	0.88	2.2
PS-*co*-ADMH	3.7	3.1	83.8	5	Y	2	0.92	1.9
PS-*co*-ADMH	3.7	2.7	73.0	3	Y	10	0.89	2.0

[a] The chlorinated beads are in the range of 10−20 mesh. [b] Theoretical chlorine content (wt %) of the beads. [c] Real chlorine contents (wt %) of the beads determined by titration. [d] Time in days elapsing between sample preparation and biocidal efficacy test with storage at 21 °C, 65% RH. [e] Whether or not the chlorinated beads were first reduced by using 0.1 M sodium thiosulfate aqueous solution at 60 °C for 2 h, and then rebleached. [f] The number of times the regenerating process repeated. [g] The Highest flow speeds for a total kill of 100 mL of aqueous suspensions containing 10^6−10^7 CFU/mL *E. coli* and 10^6−10^7 CFU/mL *S. aureus*, respectively.

The biocidal properties of the chlorinated copolymers were examined against *E. coli* and *S. aureus*, and the results are shown in Table 2. All the samples show much stronger antibacterial activity against *S. aureus*, a typical gram-positive species, than that of *E. coli*, a gram-negative one. For example, PS-*co*-VBDMH can provide a total kill (6 log reduction) of *S. aureus* at a flow speed range of 4.5−4.7 mL/min, but in the case of *E. coli*, the highest flow speed is 2.9−3.4 mL/min. Such an antibacterial activity is somewhat lower than that of Poly I, as reported previously.[3] A similar trend can be observed in the case of PS-*co*-ADMH (see Table 2 for details). These findings are most likely due to the different structures of gram-positive and gram-negative bacteria: The lipid bilayer cell membranes of the gram-positive bacteria are covered by a porous peptidoglycan layer, which does not exclude most antimicrobial agents. On the other hand, gram-negative bacteria are surrounded by two membranes. The outer membrane serves as an efficient permeability barrier because it contains lipopolysaccharides and porins. As a result, gram-negative bacteria are better protected than gram-positive ones against antimicrobial agents.[17] The stronger antibacterial efficiencies of PS-*co*-VBDMH than that of PS-*co*-ADMH could be caused by the much higher active chlorine content of former samples, as shown in Table 2.

The durability and regenerability are two important features of these polymeric biocides. For all of the samples, after the storage for two months at an environment of 21 °C and 65% RH, the chlorine contents and their antibacterial properties of the *N*-halamine polymers were essentially unchanged (see Table 2). The *N*-halamine beads were also first reduced to hydantoins by the treatment of 0.1 M sodium thiosulfate aqueous solution at 60 °C for 2 h, and then rebleached using the same condition as the preparation of the first generation *N*-halamine beads. After 10 cycles of this "bleaching → reducing → rebleaching" treatment, the chlorine contents and the antibacterial efficiencies of the samples were essentially unchanged (see Table 2), indicating that they were not only durable but also fully regenerable.

Conclusions

PS-*co*-VBDMH and PS-*co*-ADMH polymer beads were prepared by direct suspension copolymerizations of styrene with VBDMH and ADMH, respectively, with the addition of a cross-linker, DVB. After chlorination, the hydantoin structures in the copolymers could be transformed into *N*-halamines, providing the samples with powerful, durable, and regenerable antimicrobial activities against *E. coli* and *S. aureus*. The simple and environmental-friendly synthesis process and the flexibility in the control of *N*-halamine content and surface morphologies of the materials are the most important advantages of these biocidal polymer beads.

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