Novel Regenerable N-Halamine Polymeric Biocides. I. Synthesis, Characterization, and Antibacterial Activity of Hydantoin-Containing Polymers

YUYU SUN, GANG SUN

Division of Textiles and Clothing, University of California, Davis, California 95616

Received 14 April 2000; accepted 25 October 2000

ABSTRACT: Two novel cyclic-amine monomers, i.e., 3-allyl-5,5-dimethylhydantoin (ADMH) and 7,8-benzo-3 allyl-1,3-diazasprio[4.5]decane-2,4-dione (BADDD) were synthesized with good yields by reacting allyl bromide with 5,5-dimethylhydantoin (DMH) and 7,8-benzo-1,3-diazasprio[4.5]decane-2,4-dione (BDDD), respectively. The synthesized monomers were characterized by FTIR and ^{1}H-NMR spectra, and copolymerized with acrylonitrile (AN), vinyl acetate (VAC), and methyl methacrylate (MMA) in a small monomer ratio of ADMH and BDDD, respectively. The copolymers were characterized by FTIR, ^{1}H-NMR, and DSC studies. The N-halamine derivatives of the corresponding copolymers were found to exhibit high antibacterial activities against *Escherichia coli,* and the antibacterial properties were durable and regenerable. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2460–2467, 2001

Key words: antibacterial; regenerable; polymers; hydantoin-containing; synthesis

INTRODUCTION

Contamination of polymeric surfaces by microorganisms is of great concern in several areas, particularly in medical devices, health care products, water purification systems, hospital and dental office equipment, food packaging and food storage, etc. Consequently, biocidal polymers have received much attention in recent years.[1,2] Among the currently investigated biocidal materials, N-halamines have shown to provide almost instant and total kill of a wide range of microorganisms.[3] N-Halamine structures possess several useful features including good stability for long-term use and storage over a wide temperature range and ability to be regenerated in a chlorine solution repeatedly.[4–6] Most recently, N-halamine materials have been incorporated into cellulose-containing fabrics.[7–9] Results indicated that as little as 1% (wt) add-on of halamine structures provided powerful biocidal efficacy (6–7 log reduction) against most common pathogens, at a contact time of 2 min. The biocidal properties of the treated samples were durable and regenerable.

In this study, two novel cyclic amine monomers were synthesized and characterized. Copolymers of these monomers with several widely used vinyl monomers, i.e., acrylonitrile (AN), methyl methacrylate (MMA), and vinyl acetate (VAC), were prepared and characterized, and their antibacterial properties were investigated.

EXPERIMENTAL

Materials

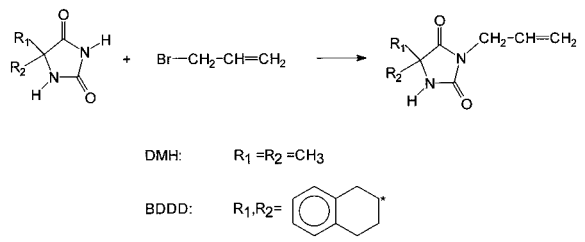
5,5-Dimethylhydantoin (DMH, Aldrich), 7,8-Benzo-1,3-diazaspiro[4.5]decane-2,4-dione (BDDD, Aldrich)

Correspondence to: Gang Sun (gysun@ucdavis.edu).

Contract grant sponsor: CAREER (National Science Foundation); Contract grant number: DMI 9733981.

Contract grant sponsor: HaloSource Corporation.

Journal of Applied Polymer Science, Vol. 80, 2460–2467 (2001)



Scheme 1

and allyl bromide (Acros) were used without further purification. AN, VAC, and MMA were distilled twice under reduced pressure. 2,2′-Azobisisobutyronitrile (AIBN) and potassium persulfate (PPS) was recrystallized from EtOH and distilled water, respectively.

Instruments

FTIR spectra were taken on a Nicolet Magana IR-560 spectrometer using either KBr pellets or polymer films. In both cases, the samples were made thin enough to ensure that the Beer-Lambert law was obeyed. ^{1}H-NMR spectra were recorded on a GE NMR QD-300 spectrometer. DSC measurements of the samples were performed by using a Shimadzu DSC-50 instrument at a heating rate of 20°C/min under a N_2 atmosphere.

Synthesis of Monomers

The cyclic-amine monomers were synthesized according to Scheme 1. A solution of 6.4 g (0.05 mol) of DMH in 25 mL H_2O containing 2.8 g (0.05 mol) of KOH was combined with a solution of 4.4 mL (0.05 mol) allyl bromide in 10 mL of methanol. The solution was stirred at 60°C for 2 h, cooled, and dried under reduced pressure at room temperature. The resulted solid was recrystallized from petroleum ether, yielding, 7.7 g (92%); m.p., 74–75°C. ^{1}H-NMR (DMSO-d6, δ): 1.29 (6H, s, CH_3), 3.94 (2H, d, N—CH_2), 4.99–5.12 (1H, m, ═CH), 5.73–5.86 (2H, m, ═CH_2), 8.33 (1H, s, NH).

BADDD was prepared in a similar process and recrystallized from isopropyl alcohol, with a yield of 68% and m.p., 160–161°C. ^{1}H-NMR (DMSO-d6, δ): 1.83–2.08 (2H, m, CH_2), 2.77–2.96 (2H, m, CH_2), 3.08–3.34 (2H, m, CH_2), 3.98–3.99 (2H, d, N—CH_2), 5.04–5.14 (1H, m, ═CH), 5.76–5.89 (2H, m, ═CH_2), 7.14 (4H, m, benzene ring), 8.69 (1H, s, NH).

Synthesis of Copolymers

Synthesis conditions for the copolymers were summarized in Table I. About 5% wt of the two hydantoin-containing monomers was copolymerized with other monomers separately, to obtain desired antimicrobial properties without causing deterioration of the original properties of the polymers. AN, MMA, and VAC homopolymers were also synthesized using the same conditions as the corresponding copolymers.

Antibacterial Assessment

The antibacterial properties of VAC-*co*-ADMH and VAC-*co*-BADDD were explored according to the following procedure: polymer films were cast from acetone solution. The films (about 0.5 mm thick) were cut into small pieces (ca. 2 cm^2), and

Table I Synthesis Conditions and Some Properties of the Copolymers

	AN-*co*-ADMH	AN-*co*-BADDD	MMA-*co*-ADMH	MMA-*co*-BADDD	VAC-*co*-ADMH	VAC-*co*-BADDD
M1	ADMH	BADDD	ADMH	BADDD	ADMH	BADDD
M2	AN	AN	MMA	MMA	VAC	VAC
M1/M2 (wt)	1/19	1/19	1/19	1/19	1/19	1/19
Initiator (*M*)	PPS	AIBN	PPS	AIBN	AIBN	AIBN
Solvent	H_2O	DMF	H_2O	None	H_2O/CH_3OH	H_2O/CH_3OH
Temp (°C)	75	72	75	80	68	68
Time (h)	1.5	2.0	1.0	0.5	4.5	5.0
Conversion	38%	63%	22%	46%	54%	42%
[η] (dL/g)	1.344[a]	1.864[a]	0.446[b]	0.412[b]	0.776[b]	0.623[b]
W_{M1} (wt %)[c]	3.4	3.1	2.2	4.1	1.8	1.2

[a] Measured in DMF at 25°C, [b]measured in acetone at 25°C and [c]W_{M1} : ADMH or BADDD weight fraction in the copolymers (see Results and Discussion section for details).

then treated with 20 mL 1% regular chlorine bleach at 40°C for 1.5 h, then thoroughly washed with distilled water and dried under reduced pressure to remove free chlorine. Ten microliters of an aqueous suspension containing 10^5–10^6 colony-forming units (CFU)/mL of *Escherichia coli* were placed onto the surface of the film. The film was then "sandwiched" using an identical film. After different contact times, the entire "sandwich" was placed into 10 mL of 0.03% sodium thiosulfate aqueous solution. The resultant solution was then vigorously shaken for 5 min. An aliquot of the solution was then serially diluted, and 100 μL of each dilution was plated onto a nutrient agar plate. The same procedure was also applied to the unhalogenated samples as controls. Bacterial colonies on the agar plates were counted after incubation at 37°C for 48 h.

To study the durability of the antibacterial properties of the films, samples were immersed in 500 mL of distilled water at 45°C for 60 min, and air-dried at room temperature for 24 h. After each wash, the antibacterial properties of the treated samples were examined by using the same method.

Powder samples were used to study their antibacterial properties when copolymers could not be made into films. One gram of the powder was treated with 20 mL regular chlorine bleach (sodium hypochlorite 5.25%) at 40°C for 1.5 h, washed thoroughly with distilled water, and then dried under reduced pressure to remove any free chlorine. The antibacterial properties of the halogenated copolymers were tested according to a modified method reported previously.[5] In the present study, 10 mL of an aqueous solution containing 10^5–10^6 CFU/mL of *Escherichia coli* passed through a column containing about 1-cm length of the corresponding sample powders, using gravity feed. The effluent was collected and the diluted solutions were placed onto nutrient agar plates. Same procedure was also applied to unhalogenated samples as controls. Bacterial colonies on the agar plates were counted after incubation at 37°C for 24 h.

RESULTS AND DISCUSSION

Characterization of the Monomers

Both ADMH and BADDD showed significant differences from their corresponding parent chemicals in FTIR and ^{1}H-NMR spectra. The FTIR spectra of DMH, ADMH, BDDD, and BADDD are shown in Figure 1. It can be seen clearly that unlike DMH bearing two absorption bands in the region of 3000–3500 cm^{-1}, ADMH has only one band in this region. The C═O stretching band shifted from 1727 cm^{-1} in DMH to 1710 cm^{-1} in ADMH. Similar changes from BDDD to BADDD can also be observed in their FTIR spectra, as shown in Figure 1. ^{1}H-NMR spectra of DMH, ADMH, BDDD, and BADDD (Fig. 2) further confirmed the structures of ADMH and BADDD. The characteristic peaks of ADMH can be found at 3.94 (2H, d, N—CH_2), 4.99–5.12 (1H, m, ═CH), and 5.73–5.86 (2H, m, ═CH_2), indicative of the allylic structure. Furthermore, DMH shows two peaks in the region of 7.8–11.0, corresponding to the N-1 and N-3 protons, respectively,[10] but in the ^{1}H–NMR spectrum of ADMH, only one band around 8.5 is observed. Similar changes are shown in the NMR spectra of BDDD and BADDD, suggesting that a typical N-3 substitution of hydantoins occurred in the reactions (Scheme 1).

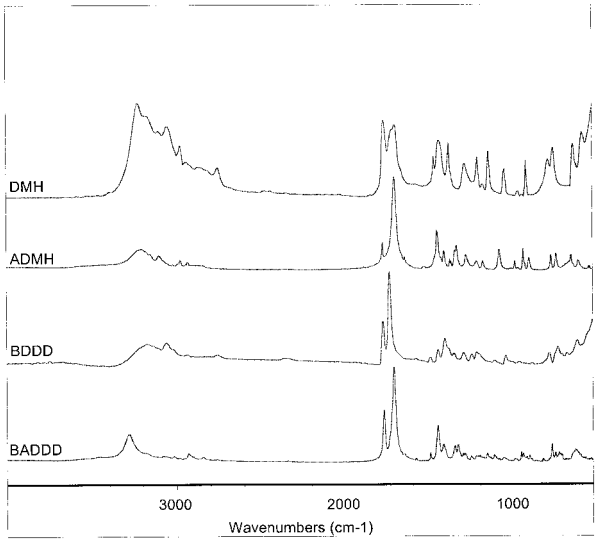


Figure 1 FTIR spectra of DMH, ADMH, BDDD, and BADDD.

Synthesis and Characterization of Polymers

Much difficulty was encountered in attempts to prepare ADMH and BADDD homopolymers due to the radical "autoinhibition" of the allylic structures of the two monomers. This antoinhibition effect occurs to allylic monomers in radical polymerization processes. Once allylic radicals are formed, the radicals are stabilized due to two equivalent allyl resonance structures. The degra-

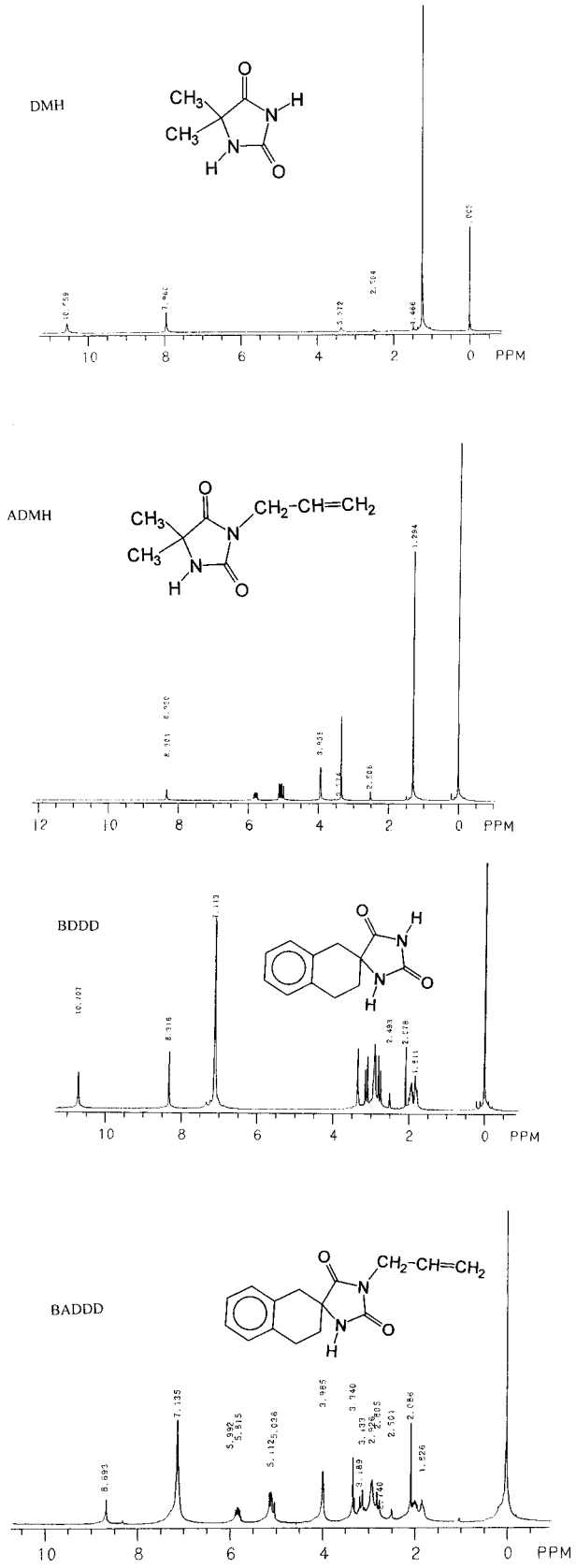


Figure 2 ^{1}H-NMR spectra OF DMH, ADMH, BDDD, AND BADDD.

dative chain transfer of allylic radicals competes exceptionally well with normal propagation reactions of the polymer chains; thus, the polymer chains are terminated after the addition of only a few monomer units.[11,12] This character of allyl monomers is not a desired property for preparing high molecular weight polymers; as a result, little allyl monomer was found applications in polymerization. But it could be an advantageous feature for grafting reactions, where short chains of polymers are needed. In addition, the poor homopolymerization ability ensures the excellent yields of ADMH and BADDD in the syntheses and prolonged storage stability at ambient temperatures.

Although ADMH and BADDD could not easily polymerize by themselves in radical reactions, they can still form copolymers with many vinyl monomers in satisfactory yields. ADMH and BADDD have been copolymerized with several acrylic, substituted-acrylic, and vinyl monomers smoothly under regular radical polymerization conditions, as shown in Table I. Despite the fact that only small amounts (ca. 5% wt) of ADMH and BADDD were employed in most of the examples listed in the table, copolymers could be formed in systems containing up to 50% (mol ratio) of these two allyl monomers. It is another feature different from regular allyl monomers. The ability of copolymerization of ADMH and BADDD is attributable to the imide structures of the two monomers. The main difference between allyl copolymerization and ordinary vinyl copolymerization is that chain transfer between an active growing chain and an allylic monomer can lead not only to termination of the growing polymer chain, but also termination of the kinetic chain.[12,13] But in this case, the strong electron-withdrawing ability of the imide groups lowers the electron density of the allylic carbon, and thus the termination steps involving the abstraction of allylic hydrogen in allyl copolymerizations, is retarded. As a result, degradative chain transfer decreases, and satisfactory copolymerization takes place. Similar behavior has been reported on copolymerizations of electron abstracting group-substituted allylic monomers with a vinyl monomer.[13–15]

Figure 3 shows the FTIR spectra of polyacrylonitrile (PAN) and AN-*co*-ADMH. PAN shows absorbance bands at 2243, 1454, 1248, and 1073 cm^{-1}, which are attributed to the ν(CN), $\delta(CH_2)$, γ_w(CH), and $\nu_+(0)$, respectively, in agreement with the literature data.[16] In the spectrum of AN-*co*-ADMH, besides the characteristic bands of PAN, two bands centered at 1770 at 1710 cm^{-1}, assigned to the amide and imide bonds of the

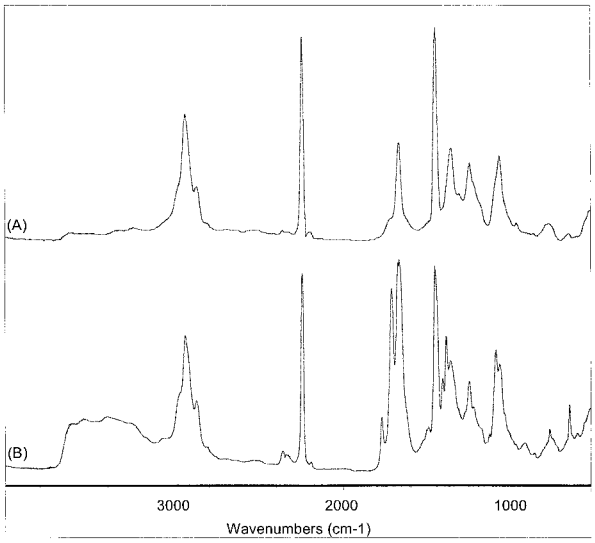


Figure 3 FTIR spectra of (A), PAN, and (B) AN-*co*-ADMH.

hydantoin structure,[10] respectively, can be observed. The same characteristic bands of the hydantoin structures can be found from other copolymers.

Structural ADMH and BADDD contents of the copolymers can be estimated from their FTIR spectra.[17,18] Figure 4 shows the FTIR spectra of PAN and ADMH physical mixture films cast from DMF solution, in the range of 1725 to 2330 cm^{-1}. With the increase in ADMH content, the intensity of the 1770 cm^{-1} band increases, while that of the

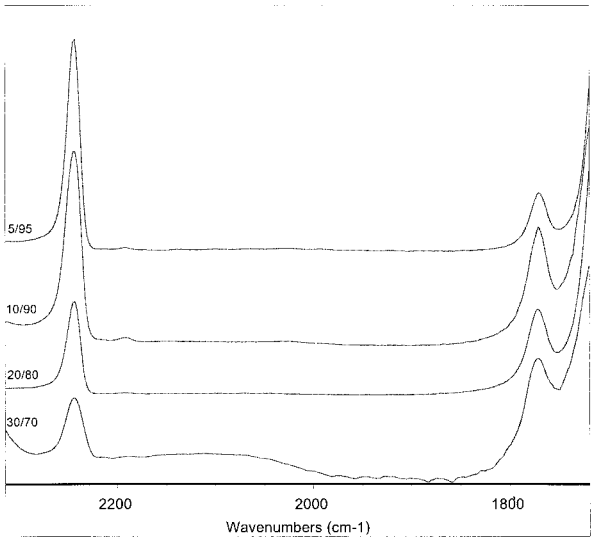


Figure 4 FTIR spectra of PAN and ADMH physical mixtures of different ADMH/PAN weight ratio in the range of 1725 to 2325 cm^{-1}.

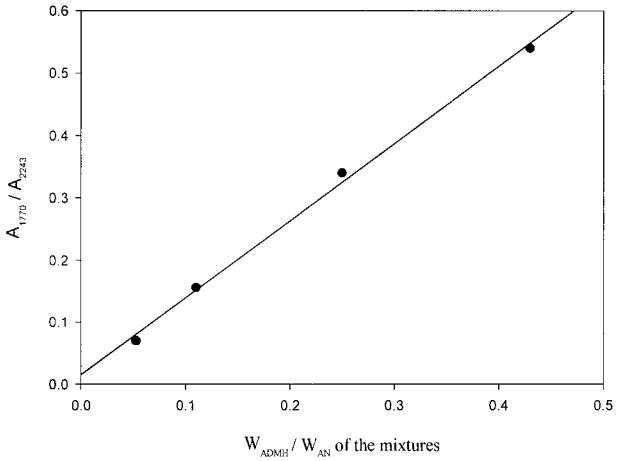


Figure 5 Plot of 1770 and 2243 cm^{-1} peak area ratio vs. ADMH and PAN weight ratio.

2243 cm^{-1} band decreases. Figure 5 shows the plot of A_{1770}/A_{2243} vs. W_{ADMH}/W_{AN}, where A_{1770} and A_{2243} represents the area of the 1770, 2243 cm^{-1} band and W_{ADMH}, W_{AN}, the weight of ADMH and AN in the physical mixtures, respectively. A linear relationship is obtained, and the slope is determined to be 0.74. Thus, the weight content of ADMH units in the AN-*co*-ADMH was found to be 3.4%. Similarly, the hydantoin contents of other copolymers were also obtained, as shown in Table I. In the copolymers, the hydantoin structure contents were all below 5% wt (the original monomer ratio), and the BADDD contents are usually lower than ADMH, except in the case of MMA-*co*-BADDD, where bulk copolymerization process was employed.

The copolymers were characterized by ^{1}H-NMR studies. Unfortunately, due to the low contents of the hydantoin monomer units, their characteristic peaks are barely detectable. Figure 6 shows an NMR spectrum of another AN-*co*-ADMH sample at the copolymerization ratio of $1/6(W_{ADMH}/W_{AN})$. The characteristic peaks of ADMH at 1.29 (CH_3) and 8.5 (NH, weak) can be detected. Furthermore, there are no peaks in the region of 4.99–5.12 and 5.73–5.86, indicating the sample studied here is a polymer, not a mixture of monomers.

The thermal behavior of the copolymers was also studied. As an example, Figure 7 shows the DSC results of PAN, AN-*co*-ADMH, and AN-*co*-BADDD. The first two copolymers did not exhibit melting points or thermal degradations under 300°C. But the DCS spectrum of AN-*co*-BADDD copolymer displays an exothermic peak at 280°C, indicating the decomposition temperature of the

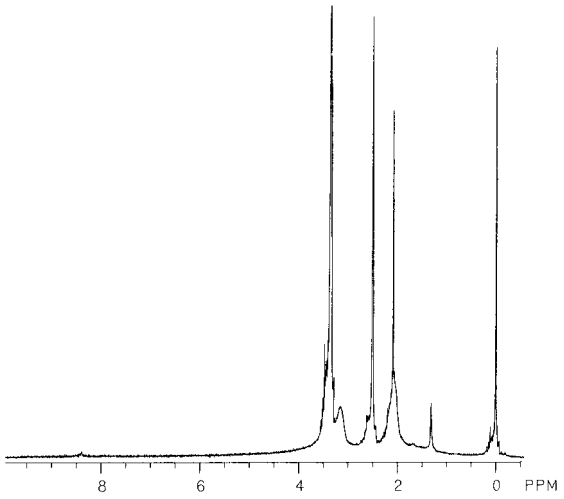


Figure 6 ^{1}H-NMR spectrum of a AN/ADMH copolymer.

polymer. It has been widely accepted[19–21] that PAN has a "semicrystalline" structure, particularly the PAN copolymers containing a small amount of other monomers. PAN polymers also possess strong dipole–dipole interactions between polar CN groups of the polymer chains. As a result, the polymers do not melt under regular conditions, but will decompose under elevated temperatures. The existence of a Td in the AN-*co*-BADDD is very likely, due to the large side groups of BADDD, i.e., not all of the AN units can pack favorably. Consequently, "physical crosslinking" due to dipole–dipole interactions between CN groups is actually diluted, resulting in a detectable Td under 300°C. In case of the AN-*co*-ADMH copolymer, although the ADMH

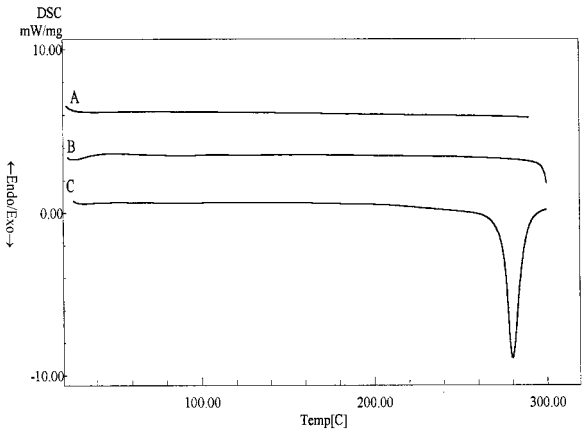


Figure 7 DSC cures of (A) PAN, (B) AN-*co*-ADMH and (C) AN-*co*-BADDD.

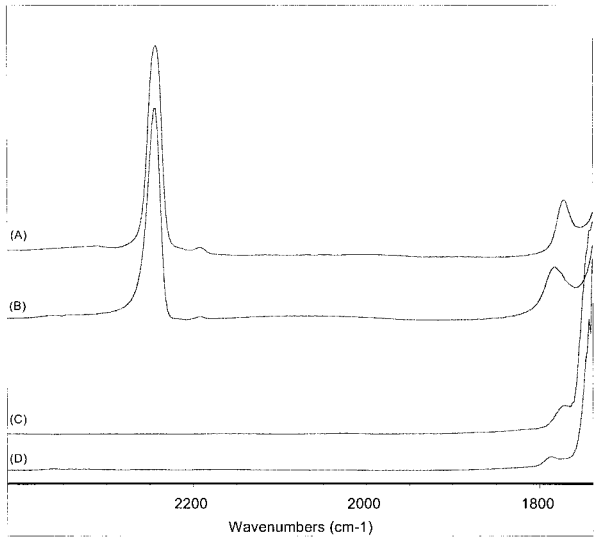


Figure 8 FTIR spectra in the region of 1750 and 2440 cm^{-1} of (A), AN-*co*-ADMH, before bleaching, (B), (A) after bleach treatment, (C), VAC-*co*-ADMH before bleaching, and (D), (C) after bleaching.

content is higher than that of BADDD in AN-*co*-BADDD, the side groups of ADMH are much smaller, the structural impact to polymer properties is limited. Little difference can be observed between the DSC curves of AN-*co*-ADMH and PAN polymers.

Antibacterial Properties of Halogenated Copolymers

After treatment with regular chlorine bleaching, the amide groups of the copolymers are readily transformed into N-halamine structures. Such a transformation can be observed with an FTIR spectrometer. The FTIR spectra of AN-*co*-ADMH and VAC-*co*-ADMH copolymers before and after bleach treatment, respectively, are shown in Figure 8. Before the treatment, both of the two samples show a band at 1770 cm^{-1}, attributed to the amide structures of the hydantoin rings. After the treatment, the 1770 cm^{-1} band disappears, and a new band centered at 1784 cm^{-1} appears. The 14-cm^{-1} difference between the treated and untreated samples strongly suggests that the hydantoin structures are transformed into N-halamine structures.[10] The N-halamine structures in all of the copolymers are very stable. After being immersed in distilled water for a period of 2 months, the FTIR spectra were recorded again, and no difference was observed between the water-treated and untreated N-halamines.

Table II Antibacterial Properties of the Copolymers Against *E. coli*

	AN-*co*-ADMH	AN-*co*-BADDD	MMA-*co*-ADMH	MMA-*co*-BADDD	VAC-*co*-ADMH	VAC-*co*-BADDD
Form of samples	Powder	Powder	Powder	Powder	Film	Film
E. coli concentration (CFU/mL)	10^6	10^6	10^5	10^6	10^6	10^5
Flow speed for total kill (mL/min)[a]	0.4	0.6	0.5	0.2	NA[c]	NA
Contact time for total kill (min)[b]	NA	NA	NA	NA	15	25

[a] For powdered samples and [b]for film samples.
[a] and [b]: flow rate or contact time shown here are minimum conditions necessary for total kill of *E. coli.* See text for details.
[c] NA: not applicable.

After treatment with 0.1 mol/L sodium thiosulfate aqueous solution at 60°C for 2 h, the N-halamine structures are converted to hydantoins, and the 1784 cm^{-1} bands changed back to 1770 cm^{-1}. However, after another bleach treatment, the 1770 cm^{-1} band can be changed to 1784 cm^{-1} again. The same "1784 → 1770 → 1784" cycle has been repeated 10 times, and each time the same result was observed. As the antibacterial properties are provided by the N-halamine structures,[3–9] the durability and regenerability of these structures certainly will provide corresponding antibacterial properties.

The halogenated polymers were tested for biocidal efficacy by using the bacterium *Escherichia coli* in forms of films or powders. The results are shown in Table II. It can be seen that the powdered halogenated copolymers demonstrate a total kill against *Escherichia coli* at a flow rate of 0.2–0.6 mL/min, while the polymer films need a contact time of less than 30 min to execute the total kill. No significant differences can be noticed in the antibacterial properties from the copolymers, indicating the similar functions possessed by N-halamine structures.

The durability of the antibacterial properties of the copolymers was studied under different testing conditions. After the halogenated polymers were stored at 25°C and 65% RH for 3 months, antibacterial properties of the samples were essentially unchanged, indicating the polymers possessing proper stability in dry state. VAC-*co*-ADMH polymer films were also washed using distilled water, and after each wash, the antibacterial properties were retested with results shown in Table III. After different wash periods, the contact time necessary for a total kill of *E. coli* becomes longer and longer, meaning the decrease of antibacterial properties. Such a decrease of the antimicrobial properties can be viewed as a result of reduced active halamine structures on the polymers films. However, the FTIR spectra of the washed samples still show bands centered at 1784 cm^{-1}, not at 1770 cm^{-1}, suggesting that the overall N-halamine structure remains almost unchanged after washing. It is highly possible that only the N-halamines structures on the surface of the films returned to their hydantoin precursors, but those inside the bulk film are unchanged due to less contact between internal halamine bonds and water. Because only the surface of the films provides the antibacterial functions, the antibacterial properties will decrease as the halamine structures are reduced after washing. However, after rebleaching, the sample can provide a total kill at a contact time of 15 min again. And after 20 cycles of this "bleach → wash 10 times → rebleach," the antibacterial properties of the sample are essentially restored by the last bleaching, indicating the regenerable functions.

CONCLUSION

Novel cyclic-amine-containing vinyl monomers could be synthesized in good yields by reacting

Table III Relationship between Wash Times (*N*) and Contact Time (*T*) Necessary for a Total Kill of *E. coli* by VAC-*co*-ADMH Film (*E. coli* Concentration: 10^5–10^6 CFU/mL)

N	0	3	5	10	15
T (min)	15	15	20	45	60

allyl halides (Br, Cl, etc.) with corresponding 5,5-disubstitued hydantoins in alkaline solutions. Due to a common "autoinhibition" effect of the allylic structures, these monomers could not form high molecular weight homopolymers under ordinary conditions. However, they could be readily copolymerized with most acrylic, substituted acrylic and vinyl monomers, with satisfactory yields. The hydantoin units in the copolymers could be easily transformed into N-halamine structures upon exposure to a chlorine bleach solution. N-Halamine derivatives of the corresponding copolymers exhibited powerful, durable, regenerable antibacterial properties against *Escherichia coli.* Those polymers could find applications in medical devices, hygienic materials, and the food-processing industry.

The research is support by a CAREER award from National Science Foundation (DMI 9733981) and financially sponsored by HaloSource Corporation, Seattle, WA.

REFERENCES

1. Vigo, T. L. Manmade Fibers: Their Origin and Development; Seymour, R. B.; Porter, R. S., Eds.; Elsevier Appl Sci, New York, 1992, p. 214.
2. Vigo, T. L. Biotechnology and Bioactive Polymers; Gebelein, C.; Carraher, C., Eds.; Plenum Press, New York, 1994, p. 225.
3. Worley, S. D.; Sun, G. Trends Polym Sci 1996, 11, 364.
4. Sun, G.; Chen, T. Y.; Worley, S. D. Polymer 1996, 37, 3753.
5. Worley, S. D.; Sun, G. Polym Mater Encyclopedia 1996, 1, p. 550, A-B.
6. Sun, G.; Chen, T. Y.; Habercom, M. S.; Wheatley, W. B.; Worley, S. D. Water Res Bull 1996, 32, 793.
7. Bickert, J. R.; Xu, X.; Sun, G.; Williams, J. F. International Conference on Safety & Protective Fabric '98, 1998, p. 1.
8. Sun, G.; Xu, X. Textile Chem Colorist 1998, 6, 26.
9. Sun, G.; Xu, X. Textile Chem Colorist 1999, 31, 21.
10. Ware, E. Chem Rev 1950, 46, 403.
11. Bartlett, P. D.; Tate, F. A. J Am Chem Soc 1953, 75, 91.
12. Schildknecht, C. E. Allyl Compounds and Their Polymers; John Wiley & Sons: New York, 1973, p. 30.
13. Shigetomi, Y.; Kojima, T.; Ono, N. J Polym Sci Part A Polym Chem 1990, 28, 3317.
14. Shigetomi, Y.; Ono, N.; Kato, H.; Oki, M. Polym J 1992, 24, 87.
15. Shigetomi, Y.; Ono, N.; Kato, H.; Oki, M. Polym J 1992, 24, 99.
16. Liang, C. Y.; Krimm, S. J Polym Sci 1958, 31, 513.
17. Nyquist, R. A. Appl Spectrosc 1987, 41, 797.
18. Liu, M. X.; Dwyer, J. L. Appl Spectrosc 1996, 50, 349.
19. Allen, R. A.; Ward, I. M.; Bashir, Z. Polymer 1994, 35, 2063.
20. Bashir, Z. J Polym Sci Part B Polym Phys 1994, 32, 1115.
21. Liu, X. D. Macromolecules 1993, 26, 3030.