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Antimicrobial Polymers Containing Melamine Derivatives. II. Biocidal Polymers Derived from 2-Vinyl-4,6-diamino-1,3,5-triazine

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ABSTRACT: Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) and a series of poly(styrene-*co*-2-vinyl-4,6-diamino-1,3,5-triazine) (PS-*co*-VDAT) copolymers were synthesized via conventional free-radical polymerizations. The polymer structures were confirmed by Fourier transform infrared, NMR, and elemental analysis. The molecular weights were determined by gel permeation chromatography studies, and the thermal properties were characterized by differential scanning calorimetry and thermogravimetric analysis. After treatment with chlorine bleach, PVDAT and PS-*co*-VDAT provided potent antimicrobial functions against multidrug-resistant Gram-negative and Gram-positive bacteria. The antimicrobial functions were durable for longer than 3 months and rechargeable for more than 50 times. The structure–property relationship of the polymers was further discussed. © 2005 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 43: 4089–4098, 2005

Keywords: antimicrobial polymers; chlorination; copolymerization; functionalization of polymers; melamine derivatives; polymerization; polystyrene; polystyrene copolymer

INTRODUCTION

Most recently, the wide spreading of emerging infectious diseases, especially those caused by multidrug-resistant pathogens, has become a growing global concern.[1] Polymeric materials play an important role in the transmission of infections because microorganisms often have strong abilities to survive on ordinary polymeric materials, turning the polymers into potential sources of cross-contaminations.[2–4] In response to these challenges, the development of biocidal polymers, polymers that can inactivate infectious pathogens upon contact, has attracted considerable research interest.[4–22] One of the most effective approaches to the preparation of biocidal polymers is to incorporate biocidal agents into polymer structures.[5–15] To date, a number of these agents, including quaternary ammonium salts,[5–8] phosphonium salts,[7] protoporphyrin-based light-activated singlet oxygen generators,[9,10] poly(ether ketone)s,[11] sulfonium salts,[12] antibiotics,[13] heavy-metal ions,[13,14] chlorophenyl derivatives,[15] biomimetic polyamides,[16,17] and *N*-halamines,[18–21] have been introduced into ordinary polymers. The biocidal performances of these materials differ significantly. It has been well established that ideal biocidal polymers do not exist;[13,14,18,19] different materials are suitable for different applications. Therefore, the availability of a wide range of biocidal polymers offers considerable flexibilities in the design and optimization of desirable performances for various uses.

The purpose of this study is to develop chloromelamine-based antimicrobial polymers that can effectively inactivate multidrug-resistant bacte-

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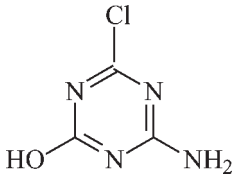

Figure 1. Chemical structure of ACHT.

ria. Monomeric chloromelamine derivatives, such as trichloromelamine and hexachloromelamine, have long been used as water and food disinfectants because of their potent antimicrobial activities, excellent stabilities, and low toxicities.[18] However, little work has been done in the development of polymeric chloromelamines for antimicrobial applications. In our previous studies,[22] we synthesized a reactive melamine derivative, 2-amino-4-chloro-6-hydroxy-*s*-triazine (ACHT), as shown in Figure 1. We found that ACHT could be readily covalently bound onto cellulosic materials through nucleophilic attacks of the hydroxyl groups of cellulosics to the C—Cl groups of the triazine rings. Upon chlorine bleach treatment, the covalently bound ACHT moieties were transformed into chloromelamine derivatives, providing potent, durable, and rechargeable antimicrobial functions against both Gram-negative and Gram-positive bacteria.[22] These findings suggest that polymeric chloromelamines can be a class of effective antimicrobial materials.

One of the drawbacks of ACHT is that it can only treat polymeric materials containing reactive sites such as hydroxyl groups or amino groups. To solve this problem, in this study, we used a polymerizable melamine derivative, 2-vinyl-4,6-diamino-1,3,5-triazine (VDAT), as shown in Figure 2, to synthesize a series of polymeric chloromelamines. We found that VDAT could readily form homopolymers and copolymers with styrene. After chlorine bleach treatment, the resultant polymers demonstrated potent, durable, and refreshable antimicrobial activities against multidrug-resistant Gram-negative and Gram-positive bacteria.

EXPERIMENTAL

Materials

VDAT was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan), 2,2′-azobisisobutyronitrile (AIBN) was provided by Sigma–Aldrich (St. Louis, MO), and styrene was purchased from Fisher Scientific (Hampton, NH). Before being used, VDAT and AIBN were recrystallized from hot water and methanol, respectively. Styrene was purified by distillation under reduced pressure. Other chemicals (Sigma–Aldrich) were used as received.

Instruments

Fourier transform infrared (FTIR) spectra were recorded with a Thermo Nicolet 370 FTIR spectrometer. NMR measurements were carried out

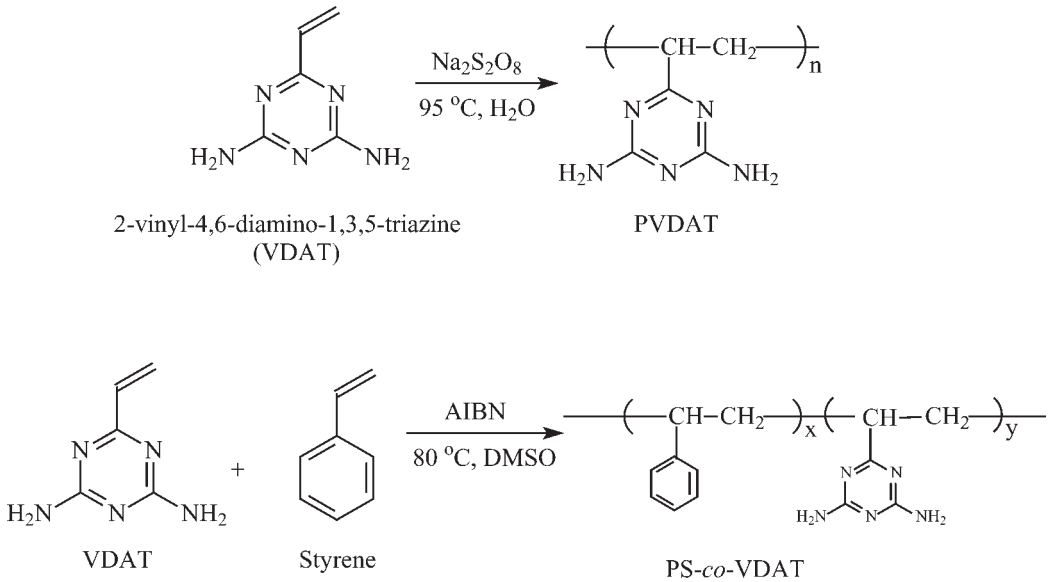

Figure 2. Preparation of PVDAT and PS-*co*-VDAT.

on a Varian Inova 500 spectrometer with $CDCl_3$ as the solvent. Gel permeation chromatography (GPC) studies were performed in tetrahydrofuran (THF) with the combination of an AMPolymer column and a Polymer Labs column equipped with a high-performance liquid chromatography pump (model 515, Waters, Milford, MA). The dual-detection system consisted of a refractometer (Optilab DSP, Wyatt Technology, Santa Barbara, CA) and a multi-angle laser light scattering detector (DAWN EOS, Wyatt Technology). The instrument was calibrated with polystyrene (PS) standards. Elemental analysis of the samples was conducted by Quantitative Technologies, Inc. (Whitehouse, NJ). The thermal properties of the samples were characterized with Shimadzu DSC-60 and Shimadzu TGA-50 instruments (Shimadzu, Kyoto, Japan) at a heating rate of 10 °C/min under an N_2 atmosphere.

Polymerization

Poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) was synthesized according to Overberger and Michelotti's method.[23] In a typical run, 0.5 g of VDAT was dissolved in 25 mL of distilled water at 95 °C under a nitrogen atmosphere. Polymerization was initiated by the addition of 0.01 g of sodium persulfate in 5 mL of distilled water. After a certain period of time, the precipitated polymers were filtered, washed with absolute ethanol, and dried at 50 °C for 24 h.

IR: 1650, 1539, 1458 (in-plane triazine ring vibrations), 835 cm^{-1} (out-of-plane triazine ring bending vibrations).[24–26]

In the synthesis of poly(styrene-*co*-2-vinyl-4,6-diamino-1,3,5-triazine) (PS-*co*-VDAT), 0.1 mol of styrene and VDAT mixtures (the VDAT concentration ranged from 1 to 20 mol % of the monomer mixtures) was added to a 250-mL, three-necked flask fitted with a condenser containing 100 mL of dimethyl sulfoxide (DMSO) under a nitrogen atmosphere. The reaction temperature was raised to 80 °C, 0.002 mol of AIBN was added, and the solution was vigorously stirred for 5 h. During this period of time, the colorless reaction mixtures gradually changed to viscous, yellowish solutions. The copolymers were obtained by precipitation in absolute ethanol. After filtration, the white powders were collected, washed successively with water and ethanol, and dried at 50 °C for 24 h.

IR: 1548, 1452 (triazine ring), 1605, 1491 cm^{-1} (benzene ring).[24–26]

Styrene homopolymers (PS) were prepared with the same method as the controls.

Chlorination of PVDAT and PS-*co*-VDAT

To transform the $—NH_2$ groups of PVDAT and PS-*co*-VDAT into chloromelamines, polymer samples were crushed into fine powders. A standard sieve was used to collect powders in the range of 60–80 mesh (0.42–0.31 mm). The powders were immersed in 0.6% NaOCl solutions containing 0.05% of a wetting agent (Triton X-100) at room temperature for 8 h under constant shaking. The pH value of the solutions was adjusted to 4.5 with pH buffers. Our previous studies showed that this weak acidic condition could provide a high chlorine content in the transformation of melamine derivatives into chloromelamines.[22] After chlorination, the powders were washed thoroughly with a large amount of distilled water (the washing water was tested with chlorine testing strips with an accuracy of 0.05 ppm chlorine to ensure that free chlorines were removed), filtered, and dried at the ambient temperature under reduced pressure. The active chlorine contents of the samples were determined by iodometric titration, as reported previously.[22]

Antimicrobial Properties

The antimicrobial properties of the chlorinated samples were challenged with multidrug-resistant bacteria. All the microorganisms were purchased from the American Type Culture Collection (ATCC; Manassas, VA). *Staphylococcus aureus* (ATCC 14154), which is resistant to tetracycline, penicillin, streptomycin, and erythromycin, was selected to represent Gram-positive bacteria, and *Escherichia coli* (ATCC 29214), which is resistant to sulfonamide, was used as a typical example of Gram-negative bacteria.

Both species are biosafety level-2 microorganisms, which are of moderate hazard to personnel and instruments.[27] A biosafety level-2 cabinet and appropriate personal protective equipment, including gloves, respirators, gowns, and foot covers, were used in the antimicrobial tests. All the microbial studies followed the guidelines provided by the U.S. Department of Health and Human Services.[27]

Table 1. PVDAT and PS-*co*-VDAT (Copol) Synthesized in This Study

Samples	VDAT Content in the Original Monomer Mixtures (mol %)	Yield (%)	VDAT Content in the Polymers (mol %)[a]	M_n ($\times 10^4$)[b]
PVDAT	100	100	100	1.00[c]
Copol-1	1	29.2	0.93	1.67
Copol-2	5	35.6	4.7	1.71
Copol-3	10	38.4	8.6	2.03
Copol-4	15	39.6	11.5	2.39
Copol-5	20	46.7	16.1	2.49

[a] Calculated on the basis of the elemental analysis data of the polymer samples.
[b] Determined by GPC studies.
[c] Average molecular weight (M_v) of PVDAT, calculated with $[\eta] = K_v M_v^a$, where $[\eta]$ is the intrinsic viscosity, K_v is 3.0×10^{-3}, and a is 0.51 at 30 °C in acetic acid.[23,28]

In the antimicrobial studies, 1 g of a chlorinated powder sample in the range of 60–80 mesh (0.42–0.31 mm) was packed into a column (i.d. = 6 mm), which was sealed with sterilized fiberglass on both sides. An aqueous suspension (1 mL of) containing 10^{7-8} CFU/mL bacteria was passed through the column. The flow rate of the liquid was controlled by compressed air. The effluent was collected and serially diluted, and each dilution was placed on nutrient agar plates (ATCC medium 3). The same procedure was also applied to the unchlorinated samples as controls. Bacterial colonies on the plates were counted after incubation at 37 °C for 24 h.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Polymers

The polymerization reactions are illustrated in Figure 2. Our studies showed that both PVDAT and PS-*co*-VDAT could be synthesized via conventional free-radical polymerizations. Under these reaction conditions, VDAT could be quantitatively polymerized into PVDAT (100% conversion rate, as shown in Table 1) in less than 5 min. The high polymerization activity of VDAT could be explained by its triazine structure: in the propagation process, because of the conjugated ring structures and the electron-withdrawing effect of the nitrogen atoms, the triazine moiety considerably stabilized the active sites of the polymer chains, resulting in a longer half-life time of the free radicals and thus higher monomer conversion rates. In the synthesis of the copolymers, however, the yield was relatively low. An extension of the reaction time did not significantly increase the conversion rate.

The compositions and molecular weights of the samples are summarized in Table 1. In the synthesis of the copolymers, the VDAT contents varied from 1 to 20 mol % of the original monomer mixtures to establish the lowest VDAT composition in the copolymers to achieve antimicrobial functions. The compositions of the copolymers were calculated on the basis of the elemental analysis data (N percentage of the samples). The general trend is that the molar contents of the VDAT moieties in the copolymers are only slightly lower than those in the original monomer mixtures, and this indicates that copolymerization can be an effective approach to introduce VDAT moieties into the chemical structures of the copolymers. The number-average molecular weights (M_n's) of the copolymers were determined by GPC studies in THF. With the increase in the VDAT contents in the monomer mixtures, the M_n value of the copolymers slightly increases. On the other hand, PVDAT is only soluble in acetic acid and concentrated mineral acid aqueous solutions. Therefore, the molecular weight of PVDAT was determined with a viscous method in acetic acid at 30 °C, as reported previously.[23,28] The low molecular weight of PVDAT could be caused by its low solubility: unlike VDAT, PVDAT is not soluble in hot water. In the polymerization process, once the molecular weights reached a certain limit, the polymers precipitated, and this led to the termination of chain propagations. A similar phenomenon was observed by other investigators in the synthesis of VDAT-containing polymers.[23,28]

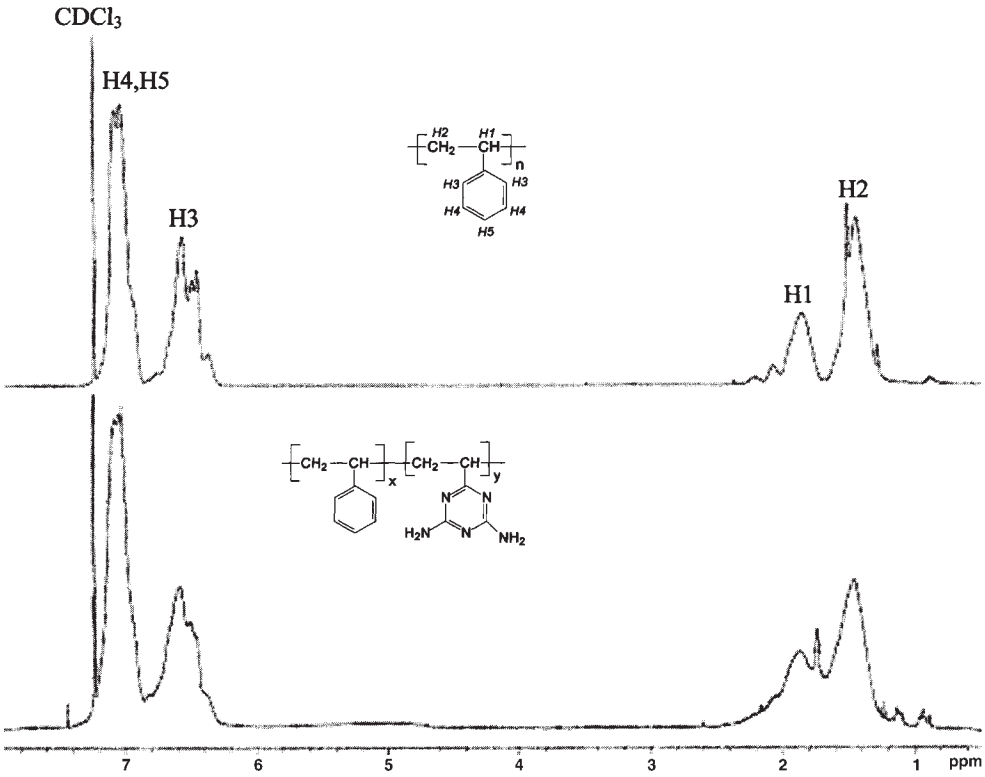

Figure 3. ^{1}H NMR spectra of PS and Copol-4.

The chemical structures of the samples were characterized. Shown in Figure 3 are the ^{1}H NMR spectra of PS and Copol-4 in $CDCl_3$. In the spectrum of PS, the peaks in the range of 1.2–1.9 ppm are related to the protons of the polymer backbones, and the bands in the region of 6.2–7.2 ppm are correspondent to phenyl protons.[29] Because of the relatively low VDAT content in the sample, the copolymer shows a similar ^{1}H NMR spectrum. However, the presence of VDAT moieties in the copolymer was confirmed by ^{13}C NMR analysis. As shown in Figure 4, the carbon atoms in PS backbones display signals around 40 ppm, and the phenyl carbons show peaks in the range of 124–146 ppm, in good agreement with the literature data.[29] In the ^{13}C NMR spectrum of the copolymer, in addition to these characteristic PS signals, a new peak centered at 167 ppm can be detected, which is assigned to the carbon atoms of the triazine rings.[29]

The thermal properties of the samples were characterized by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). As shown in Figure 5, PS shows a glass-transition temperature (T_g) at 92.4 °C. On the other hand, no melting point (T_m) can be observed, and this indicates that the crystallinity of the sample was rather low. In the case of PVDAT, because of the strong intermolecular interactions, neither T_g nor T_m can be observed. In the copolymers, with the increase in the VDAT content, T_g gradually increases. For instance, T_g of Copol-1 (0.93 mol % VDAT; see Table 1 for the compositions of the copolymers) is 99.5 °C, that of Copol-3 (8.6 mol % VDAT) is 119.1 °C, and T_g of Copol-5 (16.1 mol % VDAT) is as high as 141.3 °C. It is believed that this trend can be attributed to the presence of stronger intermolecular forces. In other words, increasing the VDAT content introduces more hydrogen bonds and dipole–dipole interactions, which decrease the mobility of the polymer chains, and this results in higher T_g's.

Additionally, the presence of VDAT moieties in the copolymers improved the thermal stabilities of the samples. As shown in Figure 6, PS begins to decompose around 300 °C. However, no weight loss can be observed in the TGA curve of PVDAT below 350 °C. In the copolymers, increasing the

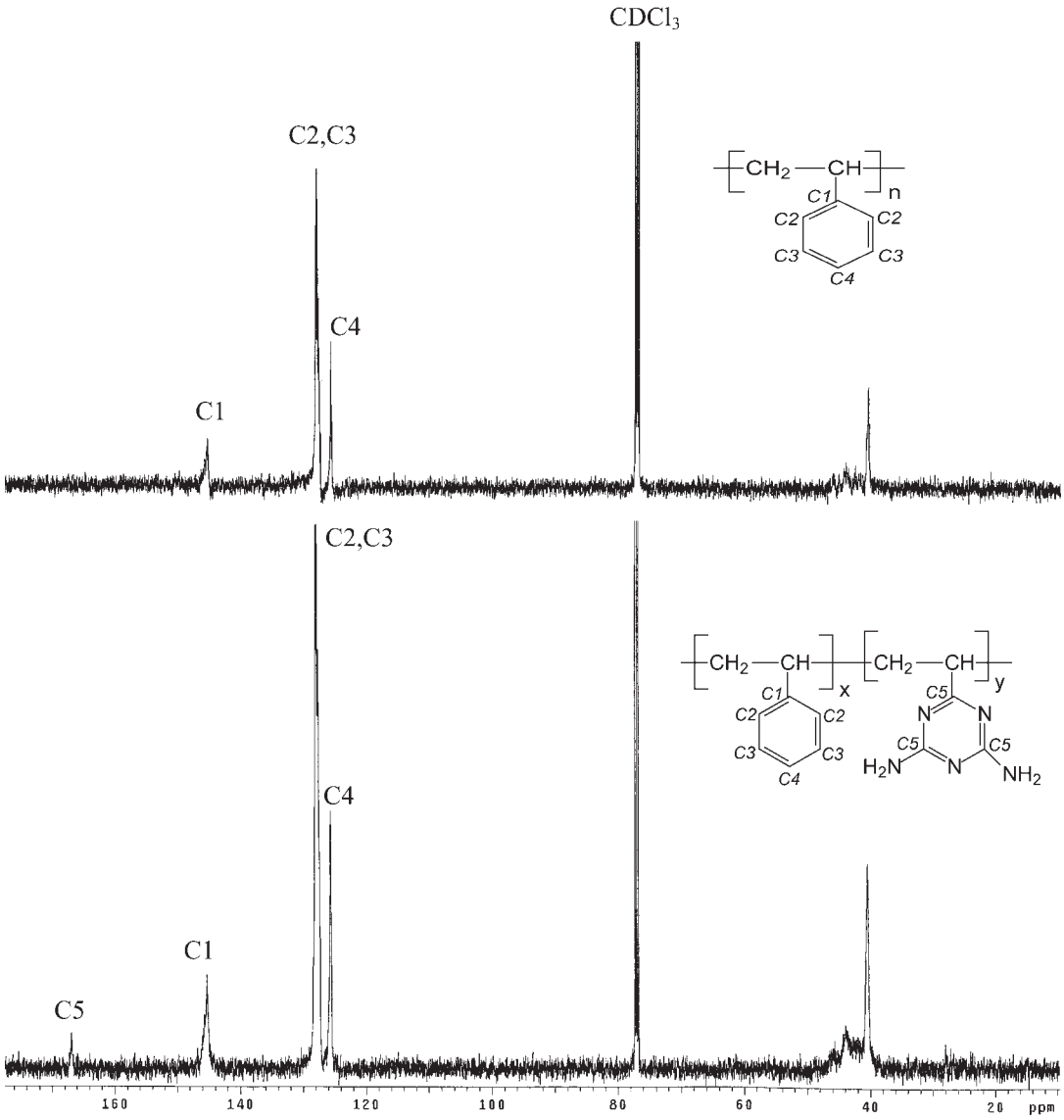

Figure 4. ^{13}C NMR spectra of PS and Copol-4.

VDAT content considerably increased the decomposition temperatures of the samples (see Fig. 6 for details). It has been well established that in the thermal degradation of PS, free radicals play an important role; they cause chain scissions and depolymerizations, resulting in weight loss.[30(a)] Upon copolymerizations, VDAT moieties were introduced, which could serve as free-radical scavengers at high temperatures.[30(b)] Therefore, at higher VDAT contents, better thermal stabilities were observed.

Chlorination of the Polymers

Our studies showed that upon chlorine bleach treatment, some of the $—NH_2$ groups of PVDAT and PS-*co*-VDAT copolymers could be transformed into chloromelamine structures, in good agreement with the chlorination studies of monomeric melamine derivatives.[18] The simplified chlorination mechanism is illustrated in Figure 7. The chlorine contents of the chlorinated samples were determined by iodometric titrations with a method reported previously;[22] the data are shown in Table 2. In VDAT-containing polymers, either one or both hydrogen atoms of the $—NH_2$ groups could be chlorinated. For example, in PVDAT, if $—NH_2$ were transformed into $—NCl_2$, the theoretical active chlorine content would be 51.6%; if only one hydrogen atom were substituted, $—NH_2$ would be transformed into —NHCl, and the theoretical chlorine con-

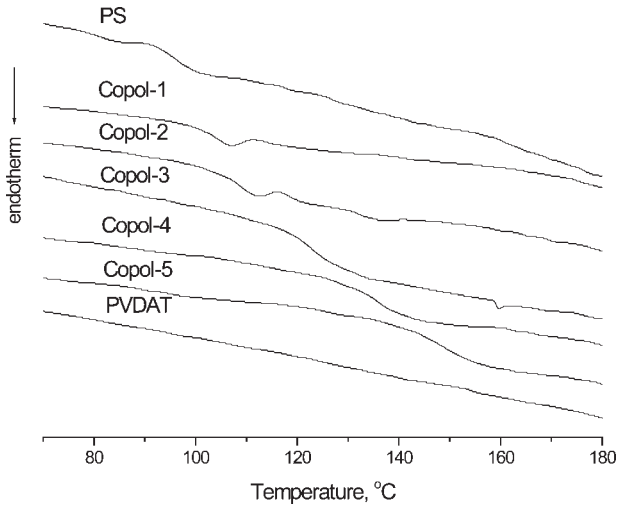

Figure 5. DSC curves of PS, PVDAT, and PS-*co*-VDAT.

tent would be 34.5%. Our iodometric titration result showed that chlorinated PVDAT had 30.9% active chlorine. Therefore, it is most likely that the chlorinated PVDAT samples had a structure of —NHCl.

This can be a reasonable hypothesis because under our chlorination conditions (pH 4.5), chlorine cations (Cl^+) that formed according to the following equation could act as the chlorinating agents:

$$HOC1 \rightleftharpoons C1^+ + OH^- \quad (1)$$

That is, the chlorinating reactions could proceed via direct substitutions of the hydrogen atoms by Cl^+ at the nitrogen atom.[31] After one hydrogen atom was substituted, the $—NH_2$ group would be transformed into —NHCl. Because of the electron-withdrawing effect of the chlorine atom, the nitrogen atom in —NHCl would have lower electron density than the nitrogen atom in $—NH_2$. Consequently, it would be much more difficult for the second hydrogen atom in —NHCl to be substituted by Cl^+. Similar results have been reported for the chlorination of other monomeric[18] and polymeric chloromelamine derivatives.[32]

If —NHCl were the dominant structure, the percentage of chlorinated nitrogen atoms in the corresponding samples could be calculated on the basis of the VDAT contents (see Table 1) and the chlorine contents (Table 2). The data are listed in Table 2. In all the samples, more than 85% of $—NH_2$ was transformed into —NHCl, and this suggests that VDAT-containing polymers are effective chloromelamine precursors.

The active chlorines in the samples were both durable and rechargeable. After 3 months of storage at 25 °C and 80% relative humidity, the available active chlorine contents of the samples were essentially unchanged. To establish the rechargeability of the active chlorines, freshly bleached samples were first treated with a 0.03% sodium thiosulfate solution at room temperature for 30 min to quench the chlorine and then rebleached. After each rebleaching, the active chlorine contents were tested. As typical examples, shown in Table 3 are the active chlorine contents of PVDAT and Copol-1 after 50 cycles of bleaching–quenching–rebleaching treatments. Little change can be detected after recharging, and this indicates that the active chlorines were fully refreshable; this is in good agreement with our previous studies of ACHT-treated cellulosic materials.[22] Very similar results were also obtained for other chlorinated PS-*co*-VDAT copolymer samples.

Antimicrobial Properties of the Polymeric Chloromelamines

The antimicrobial properties of the chlorinated samples were challenged with 10^7–10^8 CFU/mL multidrug-resistant *S. aureus* (Gram-positive and resistant to tetracycline, penicillin, streptomycin, and erythromycin) and *E. coli* (Gram-negative and resistant to sulfonamide). As described in the Experimental section, in the antimicrobial tests, chlorinated powders in the

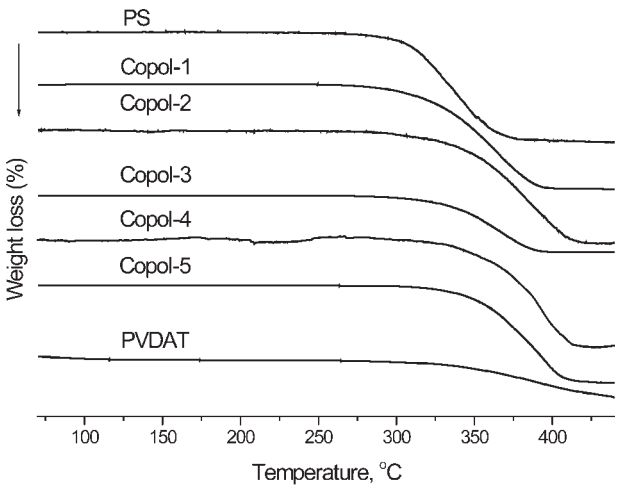

Figure 6. TGA curves of PS, PVDAT, and PS-*co*-VDAT.

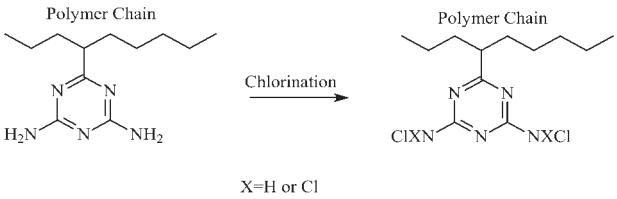

Figure 7. Simplified mechanism of transforming VDAT-containing polymers into polymeric chloromelamines.

range of 60–80 mesh were packed into columns, and bacterial suspensions were passed through the columns. The flow rate was controlled by compressed air. In this study, a rate of 1 min/mL was the fastest flow that could be achieved. At even higher rates, the flow became unstable.

Among the polymeric chloromelamines prepared in this study, chlorinated PVDAT could provide the highest active chlorine content (30.9%), and chlorinated Copol-1 had the lowest active chlorine content (0.74%), as shown in Table 2. In the antimicrobial tests, we found that both of them could provide 99.99999% kill (7-log reduction) of 10^7–10^8 CFU/mL multidrug-resistant *S. aureus* and *E. coli* (see Table 4, nos. 1 and 11). These findings suggest that not only VDAT homopolymer but also copolymers containing as little as 0.93 mol % VDAT moieties (see Table 1 for the composition of Copol-1) are potent antimicrobial materials, indicating that copolymerization with VDAT can be an effective approach to turn ordinary polymers into antimicrobial materials.

The antimicrobial functions were durable upon storage. For all the chlorinated samples, after storage at 25 °C and 80% relative humidity for 3 months, the antimicrobial functions were essentially unchanged (see Table 4, nos. 2–4 and 12–14) when challenged with 1 mL of 10^7–10^8 CFU/mL bacteria. On the other hand, higher bacterial loads resulted in decreased antimicrobial activities. For instance, after exposure to 50 mL of 10^7–10^8 CFU/mL bacteria, although PVDAT showed similar killing power, Copol-1 could only inactivate 99.99% of the cells. At even higher bacterial loads, the antimicrobial efficacy of PVDAT also decreased (see the decreasing trend in Table 4, no. 5 to no.6 and no. 15 to no. 16). These phenomena can be explained by the antimicrobial mechanisms of *N*-chloromelamines. As pointed out by Bodor et al.,[33] the bactericidal action of chloramines is believed to be a manifestation of a chemical reaction involving the direct transfer of positive chlorines from the chloramines to appropriate receptors in the bacteria cells. This reaction could effectively destroy or inhibit the enzymatic or metabolic cell processes, resulting in the expiration of the organisms. This process consumes chlorines. Therefore, with other conditions kept constant, at a higher chlorine content, the sample could inactivate more cells.

Rechargeability is another important feature of polymeric *N*-chloromelamines. After exposure to 100 mL of 10^7–10^8 CFU/mL bacteria, the samples were rebleached, and this regenerated the original antimicrobial functions of PVDAT and Copol-1 (see Table 4, nos. 7 and 17). To provide more information about rechargeability, the chlorinated powders were also treated with a 0.03% sodium thiosulfate solution at room tem-

Table 2. Chlorine Contents of the Chlorinated Polymeric Samples

Samples	Calculated for NCl_2 (%)[a]	Calculated for NHCl (%)[b]	Found (%)[c]	Chlorinated Nitrogen Atoms in the Samples Based on the Structure of NHCl (%)
Chlorinated PVDAT	51.6	34.5	30.9	89.6
Chlorinated Copol-1	1.61	0.77	0.74	97.4
Chlorinated Copol-2	5.95	3.06	2.97	97.1
Chlorinated Copol-3	10.2	5.38	5.22	97.0
Chlorinated Copol-4	13.2	7.05	6.71	95.2
Chlorinated Copol-5	17.4	9.51	8.33	87.6

[a] Chlorine contents of the samples if both hydrogen atoms of the $-NH_2$ groups were chlorinated to form $-NCl_2$ groups. The data were calculated on the basis of the elemental analysis data of the polymer samples.

[b] Chlorine contents of the samples if only one hydrogen atom of the $-NH_2$-groups was chlorinated to form $-NHCl$ groups. The data were calculated on the basis of the elemental analysis data of the polymer samples

[c] Determined by iodometric titration with a method reported previously.[22]

Table 3. Active Chlorine Contents in PVDAT and Copol-1 after Different Cycles of Bleaching–Quenching–Rebleaching Treatments

Sample	Active Chlorine Content (%)					
	As Prepared	10 times	20 times	30 times	40 times	50 times
Chlorinated PVDAT	30.9	30.8	32.6	28.4	31.5	30.2
Chlorinated Copol-1	0.74	0.68	0.72	0.71	0.65	0.73

perature for 30 min to quench the chlorine and then rebleached. After 50 cycles of this bleaching–quenching–rebleaching treatment, little change could be observed in the antimicrobial activities of the samples (see Table 4, nos. 8–10 and 18–20), and this indicated that the antimicrobial activities were fully refreshable.

CONCLUSIONS

Our results showed that VDAT could be smoothly homopolymerized and copolymerized with styrene through conventional free-radical polymerizations. The homopolymer and copolymers were characterized with FTIR, NMR, GPC, elemental

Table 4. Antimicrobial Activities of Selected Samples against 10^7–10^8 CFU/mL Multidrug-Resistant *S. aureus* and *E. coli*[a]

No.	Chlorinated Polymer Sample	Age (day)[b]	Regenerated[c]	Regeneration Times[d]	Kill of *S. aureus* (%)	Kill of *E. coli* (%)
1	PVDAT	3	N	N/A	99.99999	99.99999
2	PVDAT	30	N	N/A	99.99999	99.99999
3	PVDAT	60	N	N/A	99.99999	99.99999
4	PVDAT	90	N	N/A	99.9999	99.999
5[e]	PVDAT	5	N	N/A	99.9999	99.99999
6[f]	PVDAT	5	N	N/A	99.999	99.999
7[g]	PVDAT	6	Y	1	99.99999	99.99999
8	PVDAT	6	Y	10	99.99999	99.99999
9	PVDAT	10	Y	30	99.9999	99.9999
10	PVDAT	12	Y	50	99.9999	99.9999
11	Copol-1	3	N	N/A	99.99999	99.99999
12	Copol-1	30	N	N/A	99.9999	99.99999
13	Copol-1	60	N	N/A	99.9999	99.9999
14	Copol-1	90	N	N/A	99.9999	99.9999
15[e]	Copol-1	4	N	N/A	99.999	99.999
16[f]	Copol-1	4	N	N/A	99.99	99.9
17[g]	Copol-1	6	Y	1	99.99999	99.9999
18	Copol-1	8	Y	10	99.99999	99.99999
19	Copol-1	10	Y	30	99.9999	99.9999
20	Copol-1	14	Y	50	99.9999	99.9999

[a] All the samples were chlorinated powders in the range of 60–80 mesh. The flow rate was 1.0 mL/min.

[b] Time in days elapsing between the sample preparation and biocidal efficacy test with storage at 25 °C and 80% relative humidity.

[c] "Y" indicates that the samples were recharged, and "N" indicates that the samples were not recharged.

[d] Times that the regenerating processes were repeated if the chloromelamine powders were regenerated.

[e] These samples were challenged with 50 mL of 10^7–10^8 CFU/mL bacteria.

[f] These samples were challenged with 100 mL of 10^7–10^8 CFU/mL bacteria.

[g] After exposure to 100 mL of 10^7–10^8 CFU/mL bacteria, the samples were rebleached, and the antimicrobial functions were retested.

analysis, DSC, and TGA studies. Upon chlorine bleach treatment, chloromelamine structures could be formed in the VDAT-based polymeric materials. The chlorinated samples demonstrated powerful, durable, and rechargeable antimicrobial functions against multidrug-resistant Gram-negative and Gram-positive bacteria. These findings suggest that copolymerization with VDAT can be an effective approach to turn ordinary polymers into antimicrobial materials. More VDAT-containing antimicrobial polymers are being studied in this laboratory to provide further information concerning the structure–property relationships of polymeric chloromelamines.

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