Synthesis and Characterization of Antibacterial and Temperature Responsive Methacrylamide Polymers

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ABSTRACT: A new methacrylamide monomer (MAMP) containing a pyridine moiety was synthesized by reacting methacrylic anhydride and 3-(aminomethyl) pyridine. The monomer was homopolymerized in 1,4-dioxane and copolymerized with *N*-isopropyl acrylamide in DMF at two different compositions using AIBN as an initiator. The pyridine groups of the homopolymer and copolymers were reacted with various bromoalkanes containing 12, 14, and 16 carbon alkyl chains to obtain the polymers with pendant pyridinium groups. The monomer and polymers were characterized by elemental analysis, NMR, FTIR, SEC, TGA, and DSC. The neutral and quaternized copolymers with low MAMP content were water-soluble and showed temperature-responsive behavior in aqueous solutions. The lower critical solution temperatures (LCSTs) of these polymers varied between the temperatures of 25 and 42 °C. The LCST of quaternized copolymers were higher than that of the neutral copolymer because they were more hydrophilic. The LCST of the quaternized copolymers decreased with an increase in the alkyl chain length on the pyridinium group because the copolymers became more hydrophobic this way. The antibacterial activities of water-soluble copolymers were investigated against *Staphylococcus aureus* and *Escherichia coli* using the broth dilution and spread plate methods, whereas the water-insoluble polymers were tested for the antibacterial activity against the same types of bacteria using the shaking flask method. The quaternized water-soluble copolymers showed excellent antibacterial activities against both types of bacteria, whereas the neutral polymers and quaternized water-insoluble homopolymers and copolymers were not active.

Introduction

In the last two decades, several antibacterial polymers have been synthesized by immobilization of low-molecular-weight antibacterial agents to polymers.[1−16] Compared to conventional low-molecular-weight biocides, antibacterial polymers have the advantages that they show enhanced antibacterial activity, reduced residual toxicity, increased efficiency and selectivity, and prolonged lifetime.[1,3,12,14,15] Antibacterial polymers have been used as coatings in many areas such as food processing,[17] filters,[18] and biomedical devices.[19] They have also been used in the textile industry to form antibacterial fibers[20] and as disinfectants and preservatives in pharmaceuticals.[21]

Some of the commonly used low-molecular-weight antibacterial agents are fluoroquinolones,[22−25] quaternary ammonium salts,[4−7] biguanide groups,[26] and phosphonium salts.[13,27,28] Among these antibacterial agents, quaternary ammonium compounds (QACs) have been the most widely used agents.[13−21] They have some advantages over other antibacterial agents, including excellent cell membrane penetration properties, low toxicity, good environmental stability, lack of skin irritation, low corrosivity, and extended residence time and biological activity.[29] Common characteristics among quaternary ammonium compounds are that they possess both a positive charge and a hydrophobic segment.[30−32] Quaternary ammonium compounds with a long alkyl chain have the ability to kill microorganisms such as bacteria, fungi, and molds by interacting with their cell membranes.[12,29,33] It is generally accepted that the positively charged QACs are adsorbed onto the negatively charged cell surface by electrostatic interaction, and then the long lipophilic chain promotes diffusion into and/or through the cell wall.[12] The long alkyl chains, especially as multiple groups acting in concert along the polymer chain, disrupt the cytoplasmic membrane and cause the loss of cytoplasmic constituents, which results in the death of the microorganisms.[12,17] The antibacterial activity of the QACs is strongly dependent on the overall molecular structure and chain length of the alkyl chain. It has been shown that an increase of the alkyl chain length of an amphiphilic compound, i.e., to 14 carbon alkyl chains, increases the antibacterial activity of the compound against both Gram-negative and Gram-positive bacteria.[5,17,34]

Two approaches are generally employed for the attachment of these antibacterial agents to polymers.[12] The first approach involves the introduction of the antibacterial agents to monomers, followed by their polymerization. This method has the advantage that the monomers can be polymerized with several other comonomers and the composition can be varied easily. The second approach, on the other hand, involves the linking of the antibacterial agents directly onto preformed functional polymers. The advantage of using this method is that the functional polymers can be modified with different antibacterial agents and the degree of modification can be controlled.

Recently, our group has focused on the synthesis of various antibacterial polymers containing pendant quaternary ammonium compounds.[7] In our previous effort, we have synthesized antibacterial polymers through the polymerization of methacrylate monomers containing quaternary ammonium groups. On the other hand, in this work, we synthesized preformed functional polymers with pendant pyridine moieties, which were then quaternized to obtain the antibacterial polymers with alkyl pyridinium pendant sites.

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A new methacrylamide monomer (MAMP) was synthesized and used in the polymerizations. The homopolymer of MAMP and its copolymers with *N*-isopropylacrylamide (NIPAAm) with two different compositions were synthesized by conventional free radical polymerization techniques. The quaternization of the homopolymer and copolymers were carried out using various bromoalkanes with 12, 14, and 16 carbon alkyl chains. The NIPAAm was used as a comonomer in the copolymers because its polymers show a lower critical solution temperature (LCST) behavior.[35−40] The homopolymer of NIPAAm, poly(*N*-isopropylacrylamide) (PNIPAAm), is one of the most extensively studied polymers with an LCST of 32 °C.[41−44] Several copolymers of NIPAAm have been synthesized in order to change the LCST. It has been found that the use of hydrophilic comonomers with NIPAAm increases the LCST of the copolymers, whereas the use of hydrophobic monomers decreases the LCST.[39,42] In our case, the copolymers with a high NIPAAm content and a low comonomer (MAMP) content showed temperature-responsive behavior in an aqueous environment. The quaternization of these copolymers resulted in the synthesis of both antibacterial and temperature-responsive copolymers. The obtained homopolymers and copolymers were tested for antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*. The minimum bactericidal concentration (MBC) values were determined for water-soluble copolymers using broth dilution[45] and spread plate methods.[46] The shaking flask method[47] was performed to investigate the antibacterial activity of water-insoluble polymers.

Experimental Section

Materials and Bacterial Strains. 3-Aminomethyl pyridine and methacrylic anhydride were purchased from Aldrich Chemical Co. All alkyl halides and solvents used in the synthesis were purchased from Acros Chemical Company, Fisher Scientific, or Aldrich Chemical Co. *N*-isopropylacrylamide was purchased from Acros and recrystallized from hexane twice. 2, 2′-Azobis(isobutyronitrile) (AIBN) was purchased from Aldrich and recrystallized from methanol twice before its use. All other chemicals were used as received.

Tryptic soy agar (TSA) was purchased from Difco Laboratories. It contained 15.0 g of pancreatic digest of casein, 5.0 g of enzymatic digest of soybean meal, 5.0 g of sodium chloride, and 15.0 g of agar. Tryptic soy broth (TSB) was also purchased from Difco Laboratories. It contained 17.0 g of pancreatic digest of casein, 3.0 g of enzymatic digest of soybean meal, 2.5 g of dextrose, 5.0 g of sodium chloride, and 2.5 g of dipotassium phosphate. Bacterial strains used for antibacterial activity tests included *S. aureus* RN4220 and *E. coli* TOP10 strain. The strains were kept at −80 °C in a freezer.

Measurements. ^{13}C and ^{1}H NMR spectra were collected on a Varian 300 MHz NMR in $CDCl_3$ and DMSO-d_6 with tetramethylsilane (TMS) as the internal reference. FTIR spectra were recorded on a Mattson Galaxy series FTIR 5000 spectrometer using pressed KBr pellets. Thermal analyses were performed on a TA Instruments analyzer equipped with a 2920 differential scanning calorimeter and a 2960 thermal gravimetric analyzer cells using heating rates of 10 °C/min under nitrogen purge. Elemental analysis results were obtained from Quantitative Technologies, Inc. Antibacterial assays were incubated in a Gyromax 737 shaker at 30 °C with a gyration speed of 220 rpm. Absolute molecular weights and molecular weight distributions were obtained by the Viscotek SEC with low-angle light scattering.

Synthesis of 3-(Methacrylamidomethyl)-pyridine (MAMP). 3-(aminomethyl) pyridine (31.35 g, 0.29 mol) and triethylamine (44.67 g, 0.29 mol) were mixed in 80 mL of methylene chloride in a 250 mL round-bottom flask. The flask was kept in an ice bath to adjust the temperature to 0 °C. Methacrylic anhydride (44.67 g, 0.29 mol) was added to the mixture dropwise in 30 min. The final mixture was allowed to react for 30 min at 0 °C and 11 h at ambient temperature. After completion of the reaction, the reaction mixture was extracted with water (100 mL), and the organic layer was separated and extracted two more times with water. The organic layer was passed through a phase separation filter paper in order to remove residual water remaining. Finally, the methylene chloride was evaporated using a rotary evaporator at ambient temperature to give a yellowish solid product in 91% yield (46.48 g, 0.26 mol). Elem. Anal. Calcd for MAMP: C, 68.16; H, 6.86; N, 15.90; O, 9.08. Found: C, 67.84; H, 7.03; N, 15.70; O, 9.43.

Homopolymer Synthesis. MAMP (8.81 g, 0.05 mol) and AIBN (0.08 g, 0.5 mmol) were dissolved in 1,4-dioxane (40 mL) in a 100 mL round-bottom flask. The flask was closed with a rubber septum, and N_2 was passed through the flask using syringe needles. The temperature was adjusted to 70 °C, and the mixture was stirred for 24 h. 1,4-Dioxane was evaporated using a rotary evaporator. The solid product was dissolved in *N*,*N*-dimethylformamide and precipitated into diethyl ether. It was then reprecipitated from methanol into petroleum ether twice. The final white solid product was dried in a vacuum oven at ambient temperature to give the homopolymer (5.01 g) in 57% yield. Elem. Anal. Calcd for homopolymer: C, 68.16; H, 6.86; N, 15.90; O, 9.08. Found: C, 64.94; H, 7.30; N, 14.35; O, 13.41.

Copolymer Synthesis (50/50 Composition). MAMP (5.29 g, 0.03 mol), NIPAAm (3.40 g, 0.03 mol), and AIBN (0.10 g, 0.6 mmol) were dissolved in DMF (20 mL) in a 50 mL round-bottom flask. The flask was closed with a rubber septum, and N_2 was passed through the flask using syringe needles. The temperature was adjusted to 70 °C, and the mixture was stirred for 24 h. The final mixture was precipitated into diethyl ether. The product was dissolved in methanol and precipitated into petroleum ether. The final white solid product was dried in a vacuum oven at ambient temperature to give the 50/50 copolymer (5.39 g) in 62% yield. Elem. Anal. Calcd for 50/50 copolymer: C, 65.92; H, 8.33; N, 14.14; O, 11.61. Found: C, 61.82; H, 8.36; N, 13.65; O, 16.17.

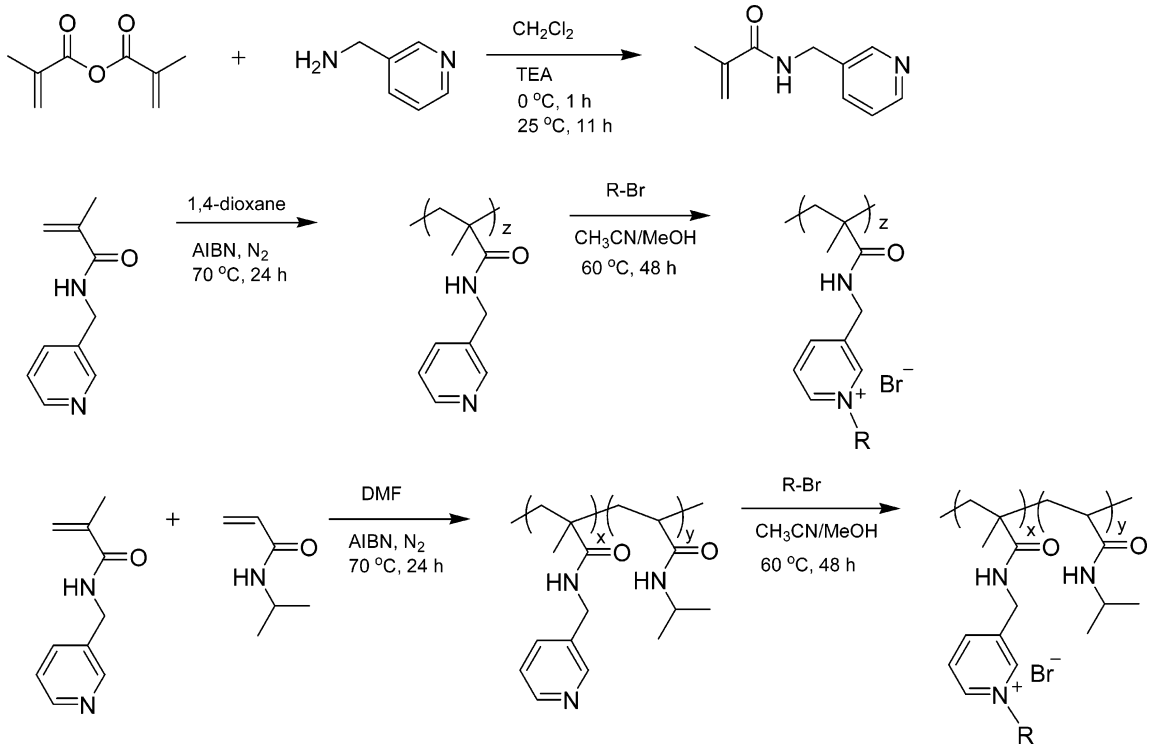
Copolymer Synthesis (90/10 Composition). MAMP (0.88 g, 5 mmol), NIPAAm (5.09 g, 45 mmol), and AIBN (0.16 g, 1 mmol) were dissolved in DMF (20 mL) in a 50 mL round-bottom flask. The flask was closed with a rubber septum, and N_2 was passed through the flask using syringe needles. The temperature was adjusted to 70 °C, and the mixture was stirred for 24 h. The final mixture was precipitated into diethyl ether. The white solid product was dissolved in methanol and reprecipitated into diethyl ether. The final white solid product was washed with diethyl ether extensively and dried in a vacuum oven at 40 °C for 12 h to give the 90/10 copolymer (3.73 g) in 63% yield. Elem. Anal. Calcd for 90/10 copolymer: C, 64.13; H, 9.51; N, 12.73; O, 13.63. Found: C, 59.80; H, 9.68; N, 11.98; O, 18.54.

Quaternization of Polymers. Homopolymer (0.5 g) and excess 1-bromododecane, 1-bromotetradecane, or 1-bromohexadecane (5.0 g) were mixed in 8 mL of a 5/3 volume mixture of methanol and acetonitrile solvent system in a test tube. The test tube was closed with a rubber septum and kept in an oil bath at 60 °C. The mixture was stirred at this temperature for 48 h. At the end of the reactions, each mixture was precipitated into diethyl ether. Each solid product obtained was dissolved in methanol and reprecipitated into diethyl ether three times. Finally, the polymers were dried in a vacuum oven at 40 °C for 8 h and at ambient temperature overnight. The quaternized homopolymers obtained using 1-bromododecane, 1-bromotetradecane, and 1-bromohexadecane, respectively, were 0.83 g (yield: 69%), 0.87 g (yield: 67%), 0.72 g (yield: 53%).

The same procedure and quantities were used for the quaternization of the 50/50 copolymer. The amounts of the quaternized copolymers obtained with 1-bromododecane, 1-bromotetradecane, or 1-bromohexadecane were 0.70 g (yield: 75%), 0.63 g (yield: 64%), 0.55 g (yield: 54%), respectively.

A slightly different procedure was used for the quaternization of the 90/10 copolymer. Although the same quantities were used, a trace amount of NaI was added into the reaction mixture in order to optimize the degree of quaternization. The amounts of the quaternized copolymers obtained with 1-bromododecane, 1-bro-

Scheme 1. Synthesis of the Monomer MAMP and Its Polymers



motetradecane, or 1-bromohexadecane were 0.45 g (yield: 90%), 0.43 g (yield: 86%), 0.41 g (yield: 82%), respectively.

Antibacterial Assessment. *S. aureus* and *E. coli* were streaked out on TSA plates and incubated at 37 °C for 24 h. A representative colony was lifted off with a wire loop and placed in 5 mL of TSB, which was then incubated with shaking at 37 °C for 24 h. At this stage, the cultures of *S. aureus* and *E. coli* contained approximately 10^9 colony-forming units (CFU) per mL. Cultures of *S. aureus* and *E. coli* containing 10^7 CFU/mL were prepared by dilution with TSB, which were used for antibacterial tests.

The antibacterial activities of the water-soluble copolymers were determined by testing different concentrations of the copolymers against *S. aureus* and *E. coli* using broth dilution and spread plate methods. A range of concentrations from 20480 to 40 μg/mL of each copolymer was prepared using sterile double-deionized water (autoclaved) in 96-well microtiter plates. The test organisms (2×10^5 CFU, 20 μL TSB) were added into each well. In the end, each well contained 180 μL of water, 20 μL TSB, and the test organism. The microtiter plates were incubated at 30 °C for 24 h in a shaker. At the end of this period, a small amount of the mixture from each well was pulled out and spread on agar plates using a swab, and the plates were incubated at 30 °C for 48 h. The growth of bacterial cells was observed on agar plates. The lowest concentration of antibacterial copolymer at which no growth was observed was determined as the minimum bactericidal concentration (MBC) value. The test was repeated at least four times for each antibacterial copolymer. The water/TSB mixture (180/20 μL) and water/TSB mixture (180/20 μL) inoculated with test bacterium were used as negative and positive controls, respectively.

The neutral and water-insoluble polymers were tested against *S. aureus* and *E. coli* using the shaking flask method, where 5, 10, 20, and 40 mg of each polymer were mixed with bacteria solution (2×10^6 CFU) in a 2.5 mL liquid medium (2 mL of water and 0.5 mL of TSB) in culture tubes. The tubes were incubated in an oven at 30 °C for 24 h, and the growth of bacteria was observed visibly.

Results and Discussion

Synthesis and Characterization of the Monomer and Polymers. The general route for the synthesis of the new monomer and its polymers is shown in Scheme 1.

Figure 1 shows ^{13}C NMR results of the monomer MAMP, its homopolymer, and the 50/50 copolymer with NIPAAm. The double-bond peaks of the monomer were observed at 120 and 140 ppm. These peaks disappeared upon polymerization. The appearance of the backbone peaks (b, c, k, l) of the homopolymer and copolymer at around 46−54 ppm also confirms the formation of the polymers. The appearance of three backbone peaks was a result of the tacticity present in the polymers. These peaks were more noticeable in the homopolymer than they were in the copolymer. The tacticity effects were also observed for the polymers at around 17−18 ppm, where the methyl peak of the MAMP monomer is present. The methylene peak e and pyridine peaks were observed at around 41 and 124−150 ppm, respectively, in the monomer, hopolymer, and copolymer spectra. In the copolymer spectrum, all of the additional peaks of the NIPAAm unit were observed. The isopropyl peaks (o and n) and the carbonyl peak were observed at 23, 35, and 175 ppm, respectively.

In Figure 2, ^{1}H NMR spectra of the monomer, homopolymer, and 50/50 copolymer are shown. Upon polymerization, the double-bond peaks of the MAMP monomer (b and c) observed at 5.3 and 5.7 ppm disappeared, whereas the backbone peaks of the homopolymer and copolymers (bc″, j, and k) appeared at around 1.2−2.1 ppm. The pyridine peaks (g, h, i, j) were observed at around 7.2−8.5 ppm in all three spectra. The amide peak shifted from 6.8 ppm in the monomer to 7.8 ppm in the polymers. The methylene peak next to amide group shifted upfield and broadened in the polymers. The methyl peak of the monomer shifted from 1.9 to 0.7−0.8 ppm in the polymers. The other peaks of the NIPAAm unit (n, m, and l) were all observed in the copolymer spectrum at 1.0, 3.8, and 7.0 ppm.

Figure 3 shows the ^{1}H NMR spectra of the 50/50 and 90/10 copolymers. The peaks e and m were integrated, and the ratio was used to calculate the copolymer compositions. The compositions were essentially the same as the feed ratios of the monomers. As the content of the MAMP unit was increased from 10 to 50%, the intensities of the pyridine peaks (g, h, i,

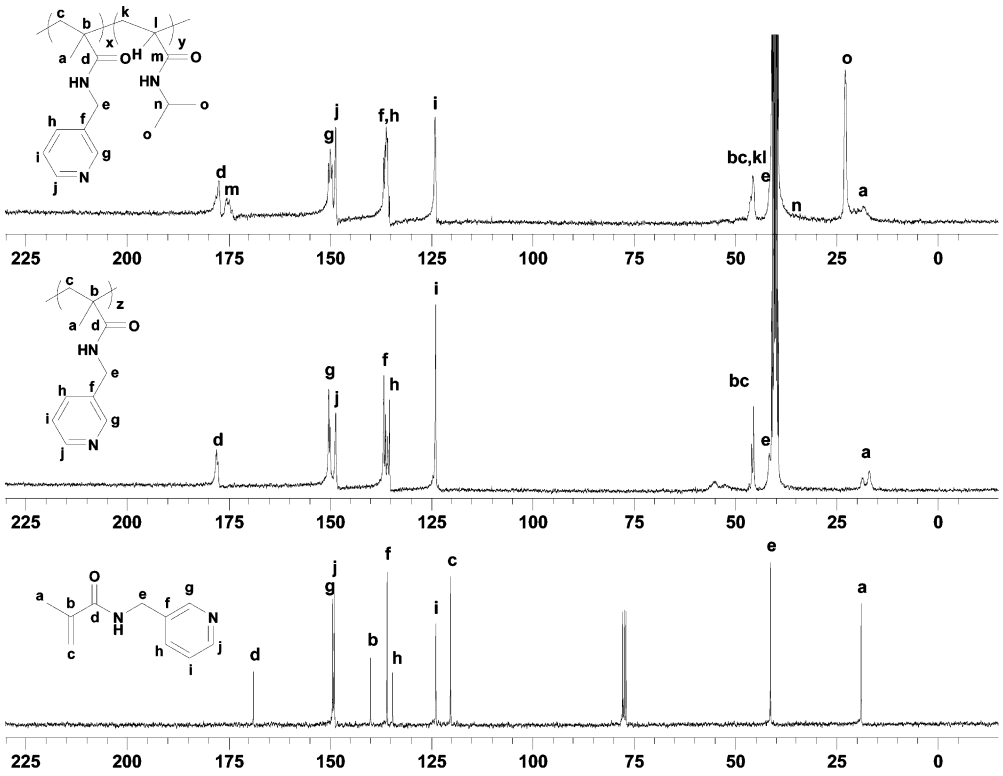


Figure 1. ^{13}C NMR spectra of the monomer (MAMP), homopolymer, and 50/50 copolymer (bottom to top).

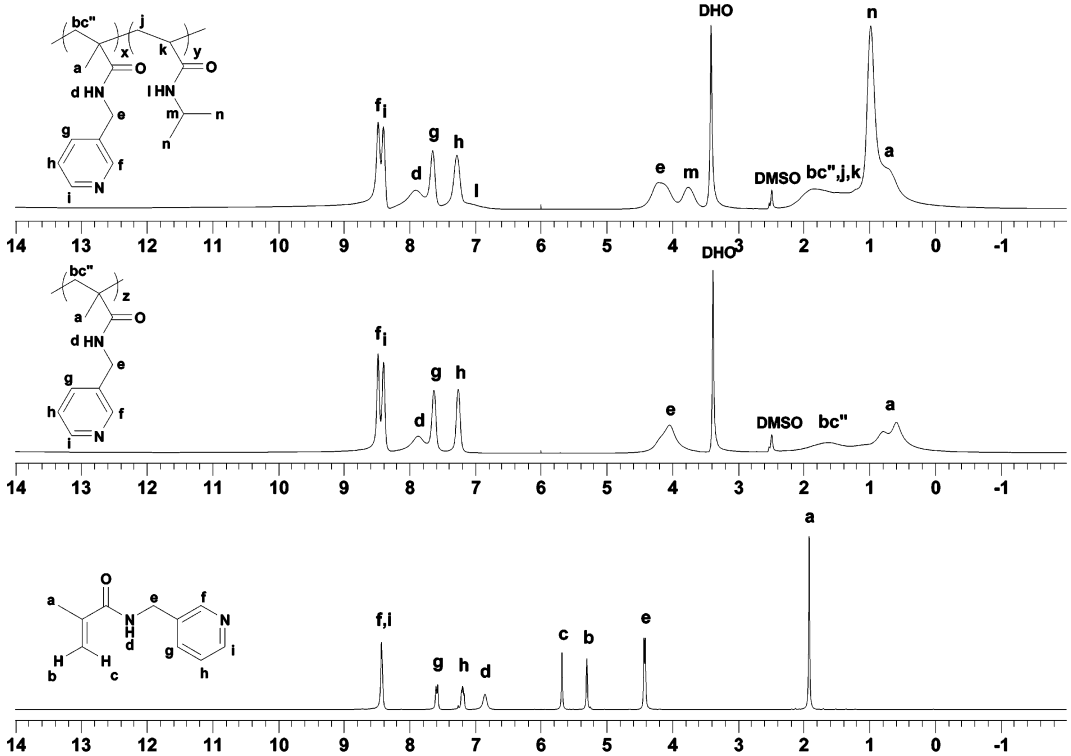


Figure 2. ^{1}H NMR spectra of the monomer, homopolymer, and 50/50 copolymer (bottom to top).

f), amide peak (d), methyl peak of MAMP unit (a), and methylene peak next to amide in the MAMP (e) increased.

Figure 4 shows the ^{1}H NMR spectra of the 90/10 copolymer, 90/10 copolymer-C12, 90/10 copolymer-C14, and 90/10 copolymer-C16. As the neat copolymer was quaternized with bromoalkanes, the methyl and methylene peaks of the alkyl units were observed in the ^{1}H NMR spectra of all quaternized copolymers at around 0.8 and 1.2 ppm, respectively. All pyridine peaks shifted downfield after the quaternization of the pyridine. The degree of quaternization (DQ) for each copolymer was calculated by integrating methyl and methylene peaks of the alkyl units against methyl groups of NIPAAm unit. The DQs were found to be more than 90% for all polymers synthesized here.

Table 1 shows the molecular weights and molecular weight distributions of the homopolymer and copolymers. The molecular weight of the homopolymer was quite high, perhaps due to the strong hydrogen bonding capability of the amide groups

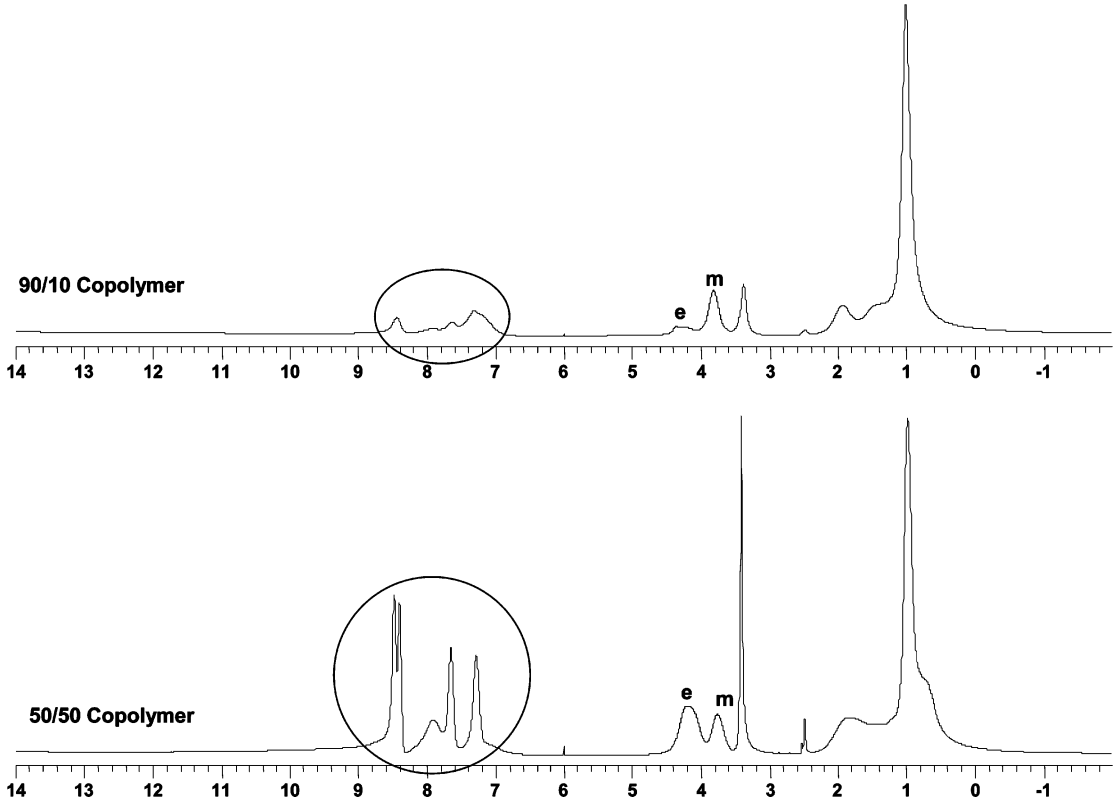


Figure 3. ^{1}H NMR spectra of 50/50 copolymer and 90/10 copolymer.

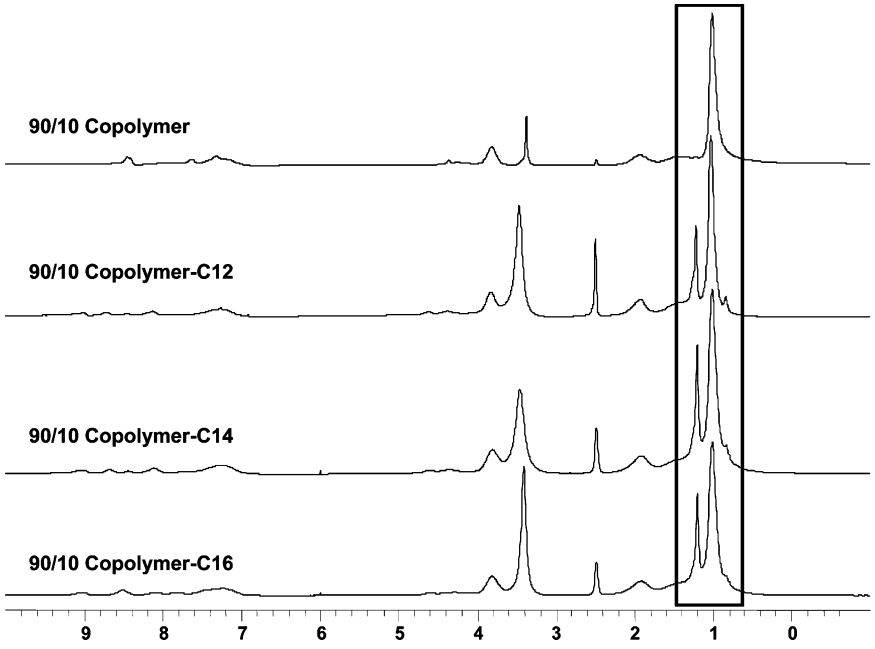


Figure 4. ^{1}H NMR spectra of 90/10 copolymer, 90/10 copolymer-C12, 90/10 copolymer-C14, 90/10 copolymer-C16.

Table 1. Molecular Weights and Molecular Weight Distributions of Homopolymer and Copolymers

polymer	M_n	M_w	PDI
homopolymer	426 000	658 000	1.54
50/50 copolymer	76 000	107 000	1.41
90/10 copolymer	49 000	76 000	1.55

of the MAMP monomer. Intermediate molecular weights were obtained for the copolymers. As the NIPAAm content was increased from 50 to 90% in the copolymers, the molecular weight decreased. PDI values of all polymers were relatively low.

Fourier transform infrared spectroscopy (FT-IR) was utilized to follow the polymerizations and quaternization reactions of the homopolymer and copolymers (Figure 5). The peak assignments for the monomer were made as follows: (1) C−H out-of-plane deformation in pyridine (805 cm^{-1}), (2) C═C stretching in CH_2═R_1R_2 (945 cm^{-1}), (3) symmetric C−N stretching vibration (1430 cm^{-1}), (4) CH_2 scissors vibration and CH_3 antisymmetric deformation (1465−1485 cm^{-1}), (5) N−H deformation bend (amide II band) (1530 cm^{-1}), (6) C═N stretching in pyridine (1590 cm^{-1}), (7) C═C stretching in pyridine and methacrylamide (1600−1635 cm^{-1}), (8) C═O

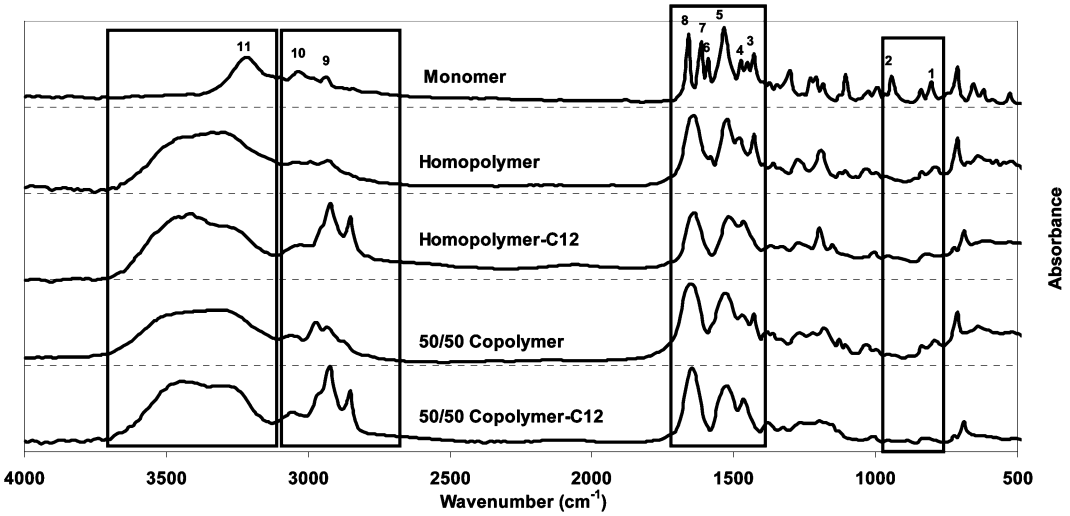


Figure 5. FTIR spectra of MAMP, homopolymer, homopolymer-C12, 50/50 copolymer, and 50/50 copolymer-C12.

stretching (amide I band) (1660 cm^{-1}), (9) aliphatic CH, CH_2, CH_3 symmetric and antisymmetric stretching (2850−3000 cm^{-1}), (10) aromatic ═CH stretching in pyridine (3050 cm^{-1}), and (11) N−H stretching (3230 cm^{-1}). The C═C stretching peaks 2 and 7 of the monomer disappeared upon polymerization. The aliphatic CH stretching peak (peak 9) compared to the aromatic CH peaks (peak 10) of the 50/50 copolymer were more intense than that of the homopolymer because the aliphatic CH content in the 50/50 copolymer is higher. As the quaternization of the pyridine was carried out, the intensity of the aliphatic CH peaks (peaks 9 and 4) increased both in the quaternized homopolymer and copolymer. The NH stretching peak of the monomer appeared at around 3230 cm^{-1}. On the other hand, this peak was seen at around 3150−3390 cm^{-1} in the polymers and was split into two peaks. The C−N stretching peak 3 of the monomer was observed in the neat homopolymer and 50/50 copolymer, whereas this peak disappeared as the polymers were quaternized. Additionally, the CH out-of-plane deformation peak 1 was also present in the neat polymers, whereas this peak disappeared in the quaternized polymers. The amide C−N and C═O stretchings were broader in the polymers compared to the monomer. The same observations applied to the 50/50-C14 copolymer and the 50/50-C16 copolymer. The FT-IR spectra of the 90/10 copolymer and its quaternized forms showed similar behaviors. The increase in the intensities of the aliphatic CH stretching peaks was less because a smaller amount of pyridine was present in this copolymer compared to 50/50 copolymer.

TGA and DSC were used for thermal analysis of the polymers. Figure 6 shows both the TGA thermograms and decomposition and glass transition temperatures (T_d and T_g) of neat and quaternized polymers. The T_d values for the neat polymers were around 280−290 °C. The T_d values for the quaternized polymers were lower than that of the neat polymers. The quaternized copolymers had similar T_d values around 260−270 °C, whereas the quaternized homopolymers had lower T_d values around 210−230 °C. The T_g values for neat and quaternized copolymers were very close to each other. The hydrogen bonding between the (meth)acrylamide groups of the polymers was more dominant than the van der Waals interactions between the aliphatic pendant side chains in determining the T_g values of the copolymers. The T_g value for the homopolymer PMAMP was 169 °C (Figure 7). No T_gs were observed for the quaternized homopolymers, which might either be due to the plasticization effect of the aliphatic side chains and/or adventitious water that was absorbed by the samples.

Table 2. Minimum Bactericidal Concentrations of the Quaternized Water-Soluble 90/10 Copolymers against *S. aureus* and *E. coli*

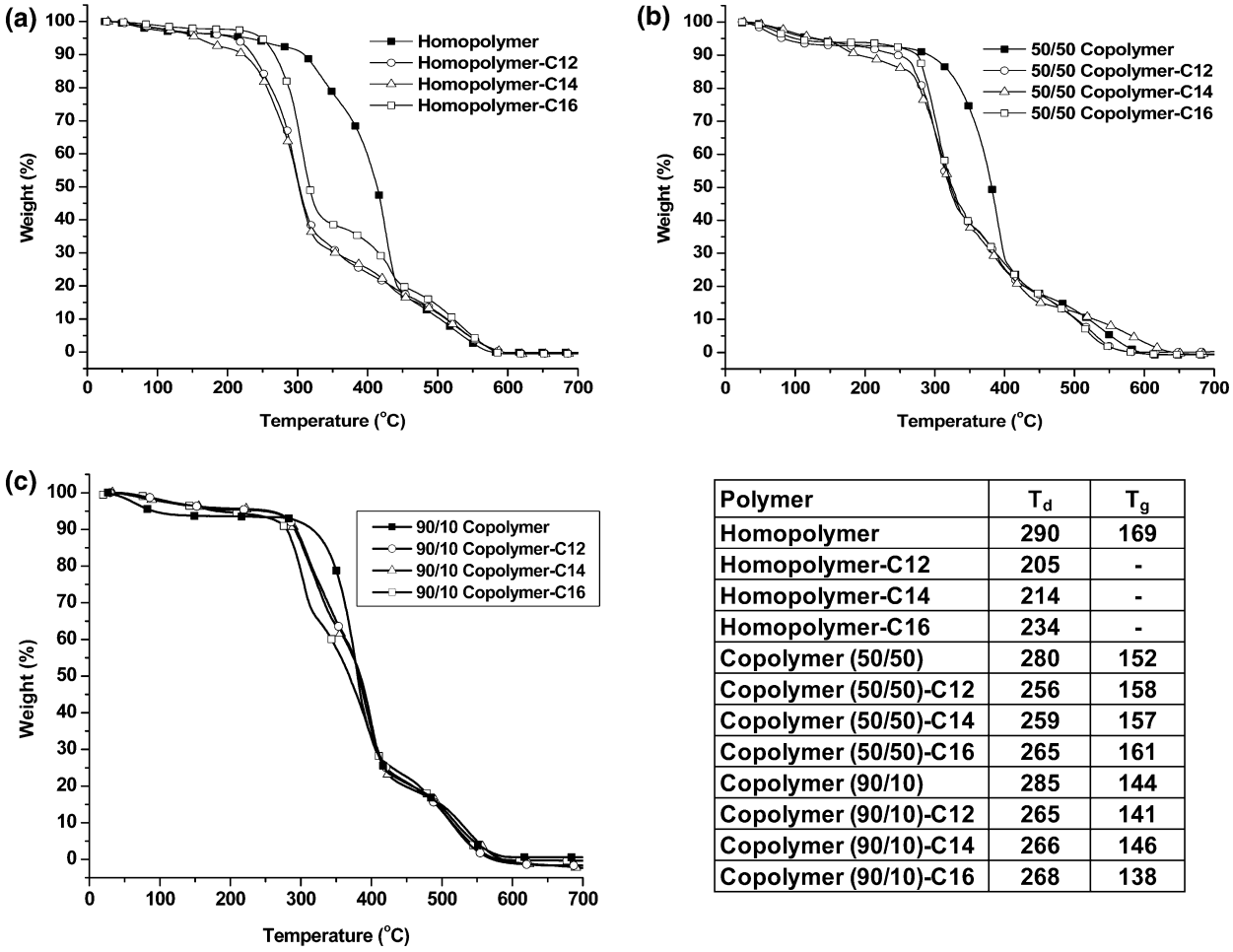
	MBC (μg/mL)	
polymer	S. aureus	E. coli
90/10 copolymer-C12	640	320
90/10 copolymer-C14	320	160
90/10 copolymer-C16	640	320

The 90/10 copolymer showed a transition at temperatures between 25 and 30 °C. The transition was observed by both DSC (Figure 8) and melting point apparatus (data not shown). The copolymer was water-soluble below 25 °C and water-insoluble above this temperature. As the temperature was raised above 25 °C, a white dispersion was formed.

The phase transition temperatures were also visibly observed for the quaternized 90/10 copolymers (Figure 9). Upon quaternization of 90/10 copolymer, the phase transition temperature increased. This is due to the fact that the quaternized polymers are more hydrophilic than the neat copolymer. As the length of alkyl chain attached to the pyridine group increased from 12 to 14 to 16, the phase transition temperature decreased as a result of the increased hydrophobicity of the copolymer.

Antibacterial Assessment. The antibacterial activities of the water-soluble copolymers were determined by testing different concentrations of the copolymers against *S. aureus* and *E. coli* using broth dilution and spread plate methods. A range of concentrations of each copolymer was prepared according to the experimental procedure mentioned earlier. The addition of TSB into a 96-well microtiter plate wells containing different concentrations of the copolymers in water resulted in precipitation of the copolymers. As a result of the precipitation of the copolymers, the antibacterial activities might be limited because there is less interaction between the copolymers and bacteria when the copolymers are solid compared to the case when they are in solution. The MBC values of the quaternized water-soluble copolymers obtained from broth dilution and spread plate tests are summarized in Table 2.

The best antibacterial activity was obtained for the 90/10 copolymer-C14, which had MBC values of 320 and 160 μg/mL against *S. aureus* and *E. coli*, respectively. The other quaternized water-soluble copolymers had MBC values of 640 μg/mL against both types of bacteria. Considering that only around 10% of the composition of the copolymers carries pyridinium groups, these copolymers show very good antibacterial activities. The agar plates showing the MBC results of



Polymer	T_d	T_g
Homopolymer	290	169
Homopolymer-C12	205	-
Homopolymer-C14	214	-
Homopolymer-C16	234	-
Copolymer (50/50)	280	152
Copolymer (50/50)-C12	256	158
Copolymer (50/50)-C14	259	157
Copolymer (50/50)-C16	265	161
Copolymer (90/10)	285	144
Copolymer (90/10)-C12	265	141
Copolymer (90/10)-C14	266	146
Copolymer (90/10)-C16	268	138

Figure 6. TGA thermograms of neat and quaternized homopolymer (a), 50/50 copolymer (b), and 90/10 copolymer (c) (Inserted table provides decomposition temperature (T_d) and glass transition temperature (T_g) of each polymer (data obtained from DSC)).

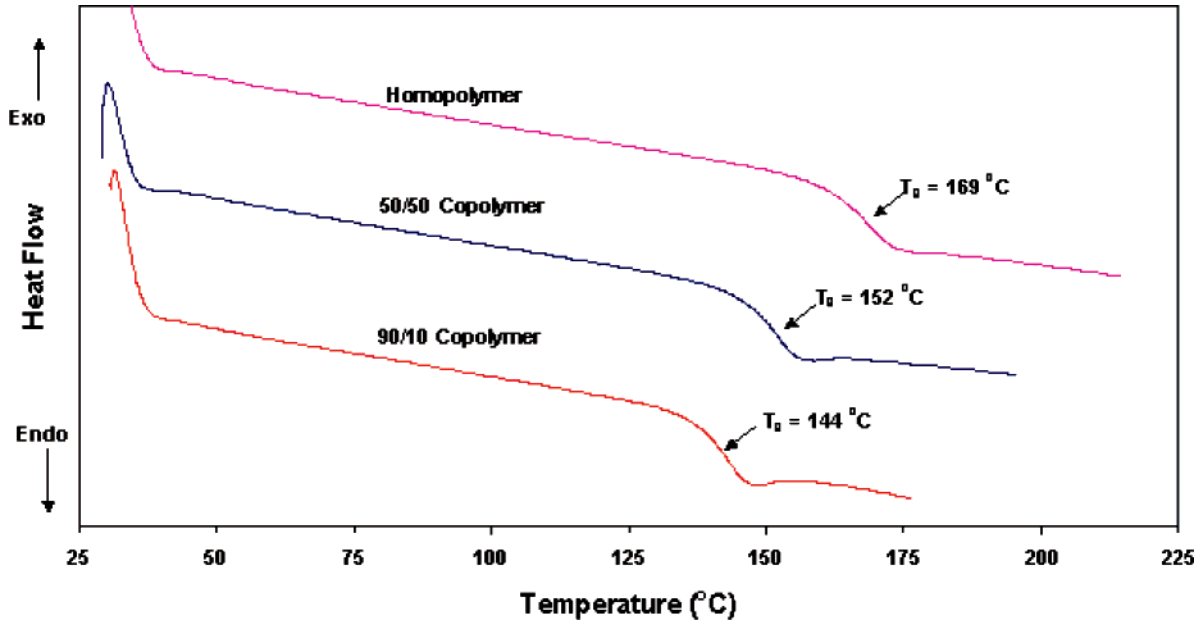


Figure 7. DSC thermograms of homopolymer, 50/50 copolymer, and 90/10 copolymer.

neutral and quaternized water-soluble 90/10 copolymers against *S. aureus* are shown in Figure 10. The neutral copolymer did not show any antibacterial activity as expected. No growth was observed for the negative control, which is just the water/TSB (180/20 μg/mL) mixture, whereas the bacterial growth was observed for the positive control, which is the water/TSB (180/20 μg/mL) mixture inoculated with *S. aureus*.

The neutral and water-insoluble polymers were tested against *S. aureus* and *E. coli* using the shaking flask method, where 5, 10, 20, and 40 mg of each polymer was mixed with bacteria

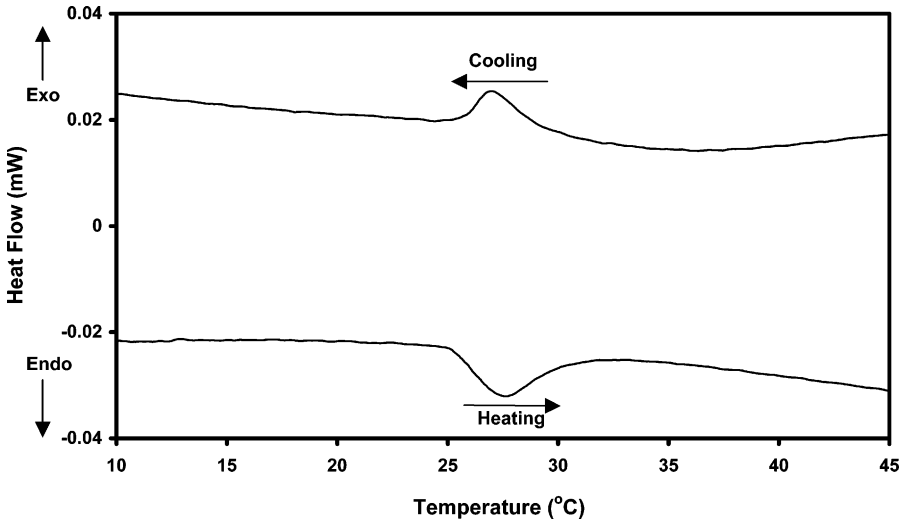


Figure 8. DSC thermograms of the 90/10 copolymer obtained upon heating and cooling between 10 and 45 °C at a rate of 1 °C/min.

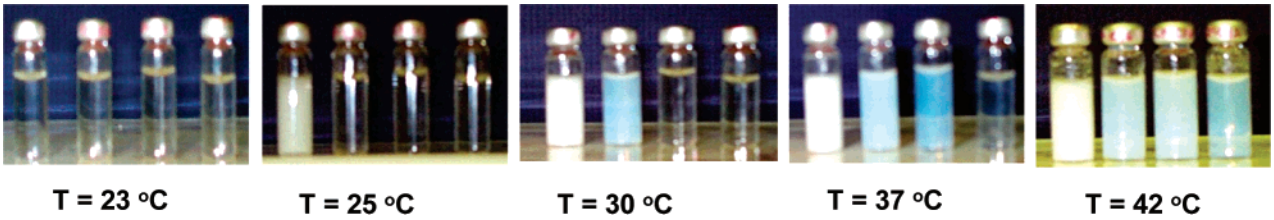


Figure 9. Phase transition temperatures for 90/10 copolymers (left to right in each photo: neutral copolymer, copolymer-C16, copolymer-C14, copolymer-C12).

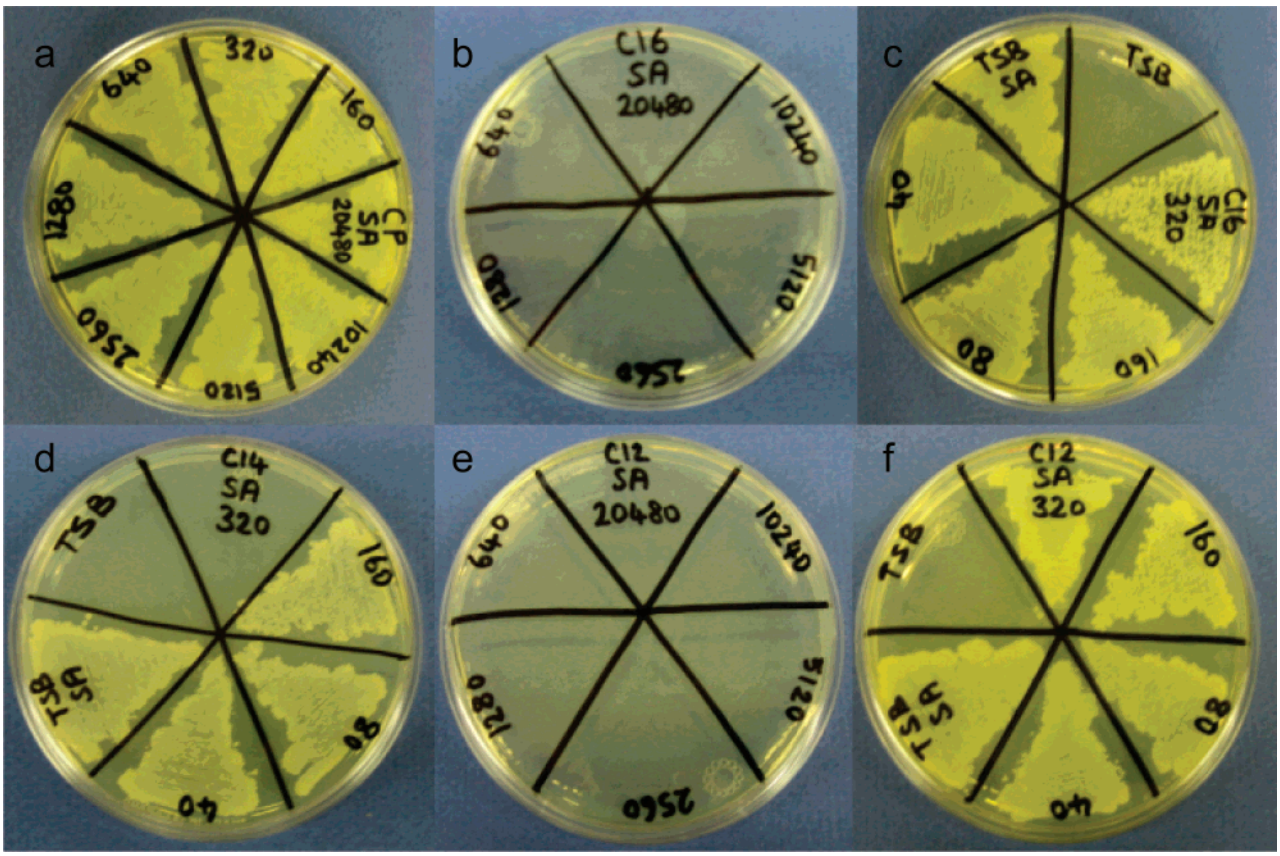


Figure 10. The MBC results of the water soluble copolymers: (a) neutral 90/10 copolymer, (b, c) 90/10 copolymer-C16, (d) 90/10 copolymer-C14, (e,f) 90/10 copolymer-C12.

solution in H_2O/TSB mixture. None of the polymers showed antibacterial activity against both types of bacteria at these concentrations.

The antibacterial action of polymers containing QACs is mechanistically complex. The target sites of these polymers are the cytoplasmic membranes of bacterial cells. The polymers

adsorb onto the bacterial cell surface, diffuse through the cell wall, bind to the cytoplasmic membrane, and disrupt the cytoplasmic membrane. This causes the release of the cytoplasmic constituents and death of the bacteria.[12,14,17] The same type of action is expected for the newly synthesized temperature-responsive polymers. The water-soluble polymers interact better with the test bacteria, therefore, they show better antibacterial activities compared to the water-insoluble polymers. Although the water-soluble copolymers precipitate upon addition of TSB, there might be still some soluble polymer present in the mixtures that causes the death of bacteria.

Conclusions

A new methacrylamide monomer (MAMP) with two functional sites was synthesized, homopolymerized, and copolymerized with NIPAAm at two different compositions. The quaternization of the homopolymer and copolymers were carried out using various bromoalkanes with 12, 14, and 16 carbon alkyl chains. The quaternized copolymers with low MAMP and high NIPAAm content were water-soluble and showed temperature-responsive behavior, which was controlled by changing the alkyl chain length in the pyridinium group. The quaternized water-soluble copolymers showed very good antibacterial activities against both *S. aureus* and *E. coli*. The neutral and water-insoluble polymers did not show any antibacterial activities under the conditions used here.

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