Synthesis and Antibacterial Activities of Water-Soluble Methacrylate Polymers Containing Quaternary Ammonium Compounds

BEKIR DIZMAN,[1] MOHAMED O. ELASRI,[2] LON J. MATHIAS[1]

[1]Department of Polymer Science, University of Southern Mississippi, Hattiesburg, Mississippi 39406-0076

[2]Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406-0076

Received 24 May 2006; accepted 7 July 2006
DOI: 10.1002/pola.21678
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: New water-soluble methacrylate polymers with pendant quaternary ammonium (QA) groups were synthesized and used as antibacterial materials. The polymers with pendant QA groups were obtained by the reaction of the alkyl halide groups of a previously synthesized functional methacrylate homopolymer with various tertiary alkyl amines containing 12-, 14-, or 16-carbon alkyl chains. The structures of the functional polymer and the polymers with QA groups were confirmed with Fourier transform infrared and ^{1}H and ^{13}C NMR. The degree of conversion of alkyl halides to QA sites in each polymer was determined by ^{1}H NMR to be over 90% in all cases. The number-average molecular weight and polydispersity of the functional polymer were determined by size exclusion chromatography to be 32,500 g/mol and 2.25, respectively. All polymers were thermally stable up to 180 °C according to thermogravimetric analysis. The antibacterial activities of the polymers with pendant QA groups against *Staphylococcus aureus* and *Escherichia coli* were determined with broth-dilution and spread-plate methods. All the polymers showed excellent antibacterial activities in the range of 32–256 μg/mL. The antibacterial activity against *S. aureus* increased with an increase in the alkyl chain length for the ammonium groups, whereas the antibacterial activity against *E. coli* decreased with increasing alkyl chain length. © 2006 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 44: 5965–5973, 2006

Keywords: functionalization of polymers; NMR; radical polymerization; synthesis; water-soluble polymers

INTRODUCTION

Increasing effort has been made during the last two decades to synthesize antibacterial polymers through the chemical bonding or physical binding of low-molecular-weight biocides to polymers.[1–20] In comparison with low-molecular-weight biocides, antibacterial polymers show enhanced antibacterial activity, reduced residual toxicity, increased efficiency and selectivity, and prolonged lifetime.[1,3,12–15] Antibacterial polymers have been used as coatings in many areas, including food processing,[21] biomedical devices,[22] and filters.[23] They have also been used in the textile industry to form antibacterial fibers[24] and as disinfectants and preservatives in pharmaceuticals.[25] These polymers can be used in paints on hospital-room walls and everyday objects such as doorknobs, children's toys, computer keyboards, and telephones.[9]

Correspondence to: L. J. Mathias (E-mail: lon.mathias@usm.edu)

Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 44, 5965–5973 (2006)

Quaternary ammonium compounds (QACs) have long been the most widely used low-molecular-weight biocides.[13–21] They have several 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.[26] Common characteristics among QACs are that they possess both a positive charge and a hydrophobic segment.[27–29] QACs with a long alkyl chain substituent of at least eight carbon atoms can kill microorganisms such as bacteria and fungi by interacting with their cell membranes.[12,23,30] The lethal action of QACs is generally accepted to proceed as follows: 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 cell constituents, which results in the death of the microorganisms.[12,21] The antibacterial activity of QACs is strongly dependent on the overall molecular structure and chain length of the alkyl chain. An increase in the alkyl chain length of an amphiphilic compound, that is, to a 14-carbon chain, increases the antibacterial activity of the compound against both Gram-negative and Gram-positive bacteria.[5,21,31]

In general, two methods are employed for the immobilization of QACs on polymers.[12] The first method involves the incorporation of QACs into monomers with subsequent polymerization and use. This method has the advantage that the monomers can be polymerized with a variety of comonomers with various compositions. The second method involves linking the biocides directly onto preformed functional polymers.[32] The advantage of this method is that the functional polymers can be modified with a variety of different biocides and the degree of modification can be well controlled. The latter also allows more accurate structure–property evaluations by using a common base polymer structure and molecular weight to generate a family of derivatives of comparable structure.

Recently, our group has focused on the synthesis of antibacterial polymers containing pendant quaternary ammonium (QA) groups.[7] Previous efforts involved the synthesis of antibacterial polymers through the polymerization of methacrylate monomers containing QA groups (the first method mentioned previously). In this report, the synthesis of a preformed functional polymer with pendant alkyl halide moieties is described; it is then reacted with various tertiary alkyl amines containing 12-, 14-, or 16-carbon alkyl chains. This reaction of the functional polymer with the tertiary alkyl amines gives three water-soluble methacrylate polymers with pendant QA groups; these have been characterized and tested for antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* with broth-dilution[33] and spread-plate methods.[34] The shaking-flask method[35] has been used to investigate the antibacterial activity of the water-insoluble, neutral functional polymer.

EXPERIMENTAL

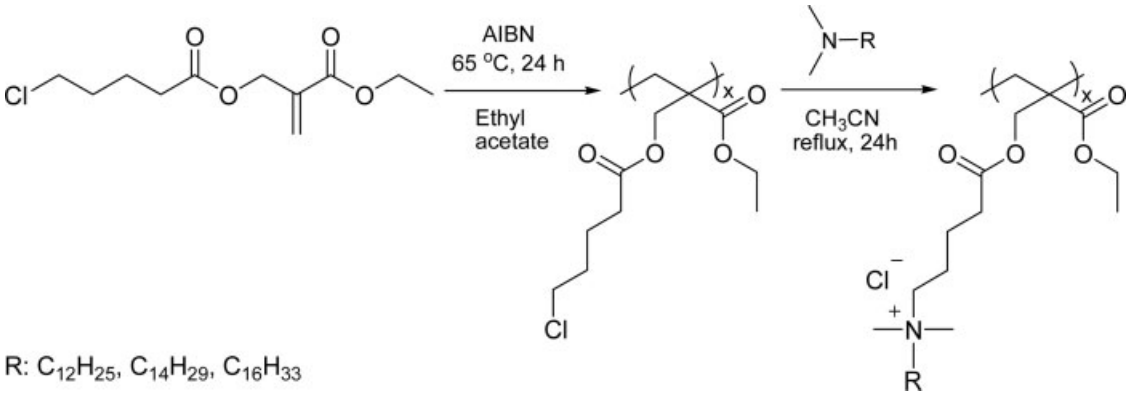
Materials and Bacterial Strains

The neutral functional polymer used in this work was previously synthesized by our group.[36] The number-average molecular weight, weight-average molecular weight, and molecular weight distribution of the polymer were 32,500 g/mol, 74,000 g/mol, and 2.25, respectively. *N,N*-Dimethyldodecylamine, *N,N*-dimethyltetradecylamine, and *N,N*-dimethylhexadecylamine were purchased from Aldrich. Sodium iodide (NaI) and all solvents were purchased from Acros, Fisher, or Aldrich. Azobisisobutyronitrile (AIBN) was purchased from Aldrich and recrystallized from methanol twice before use. All other chemicals were used without further purification.

Tryptic soy agar (TSA) and tryptic soy broth (TSB) were purchased from Difco Laboratories. TSA 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. TSB 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. The bacterial strains used for the antibacterial activity tests included *S. aureus* RN4220 and *E. coli* TOP10. The strains were kept at $-80\ ^{\circ}C$ in a freezer.

Measurements

^{13}C and ^{1}H NMR solution spectra were recorded on a Bruker AC-300 spectrometer at room temperature with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal reference. Fourier transform infrared (FTIR) spectra were obtained on a Mattson Galaxy series FTIR 5000 spectrometer with pressed KBr pellets. Thermal analyses



Scheme 1. Synthesis of the functional polymer and the polymers with QA groups.

were performed on a TA Instruments analyzer equipped with differential scanning calorimeter (2920 MDSC) and thermogravimetric analyzer (2960 SDT) cells with a heating rate of 10 °C/min under a nitrogen purge. The molecular weights and molecular weight distributions were measured with size exclusion chromatography on a system equipped with four styrene gel mixed-bed columns with tetrahydrofuran as the eluent and polystyrene standards for calibration.

Synthesis of the Functional Polymer (PCEMA)

Poly(α-chlorovalerylethylmethyl acrylate) (PCEMA) was synthesized by the free-radical solution polymerization of a methacrylate monomer – α-chlorovalerylethylmethyl acrylate (CEMA) – previously synthesized by our group.[36] Briefly, the monomer CEMA (16.0 g, 64 mmol) was homopolymerized with AIBN (0.11 g, 0.67 mmol, 1 mol %) as the initiator in ethyl acetate (60 mL) in a 250-mL, round-bottom flask. The flask was purged with N_2, and the temperature was adjusted to 65 °C. The polymerization was carried out for 24 h, and the final mixture was precipitated into hexane. The obtained homopolymer was dissolved in acetone and reprecipitated into hexane. The polymer was washed with hexane and dried in a vacuum oven at 50 °C for 12 h to remove any trapped solvents and give the homopolymer (13.0 g, 81% yield).

Typical Reaction of the Functional Polymer with Tertiary Alkyl Amines

The functional polymer (0.5 g, 0.015 mmol, 2.0 mmol alkyl halide), *N,N*-dimethyldodecylamine (3 g, 14.0 mmol), NaI (0.01 g), and CH_3CN (20 mL) were mixed in a 50-mL, round-bottom flask. A condenser was attached to the flask, the reaction temperature was adjusted to 80 °C, and the mixture was refluxed for 48 h. After the completion of the reaction, the solvent was evaporated with a rotary evaporator. The product left in the flask was first washed with diethyl ether and then dissolved in methanol and precipitated into diethyl ether twice. The diethyl ether was decanted, and the remaining solid product was washed extensively with diethyl ether. Finally, the product was dried in a vacuum oven at 50 °C for 12 h to remove any trapped solvents and give the polymer with pendant dimethyldodecyl ammonium groups (PCEMA-C12; 0.85 g, 92% yield).

The same procedure and amounts were used in reactions with *N,N*-dimethyltetradecylamine and *N,N*-dimethylhexadecylamine. The molar ratios of the amines and alkyl halides were adjusted in such a way that the amines were at least in 5 times excess of the alkyl halides. The amounts and yields of the polymers containing pendant dimethyltetradecyl and dimethylhexadecyl ammonium groups (PCEMA-C14 and PCEMA-C16) were 0.88 (90% yield) and 0.60 g (58% yield), respectively. The degrees of quaternization for the homopolymer with these alkyl amines were determined by ^{1}H NMR to be 94, 93, and 91% for PCEMA-C12, PCEMA-C14, and PCEMA-C16, 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 CFU/mL. Cultures of *S. aureus* and *E. coli* containing 10^7 CFU/mL were prepared by dilution with TSB and were used for antibacterial tests.

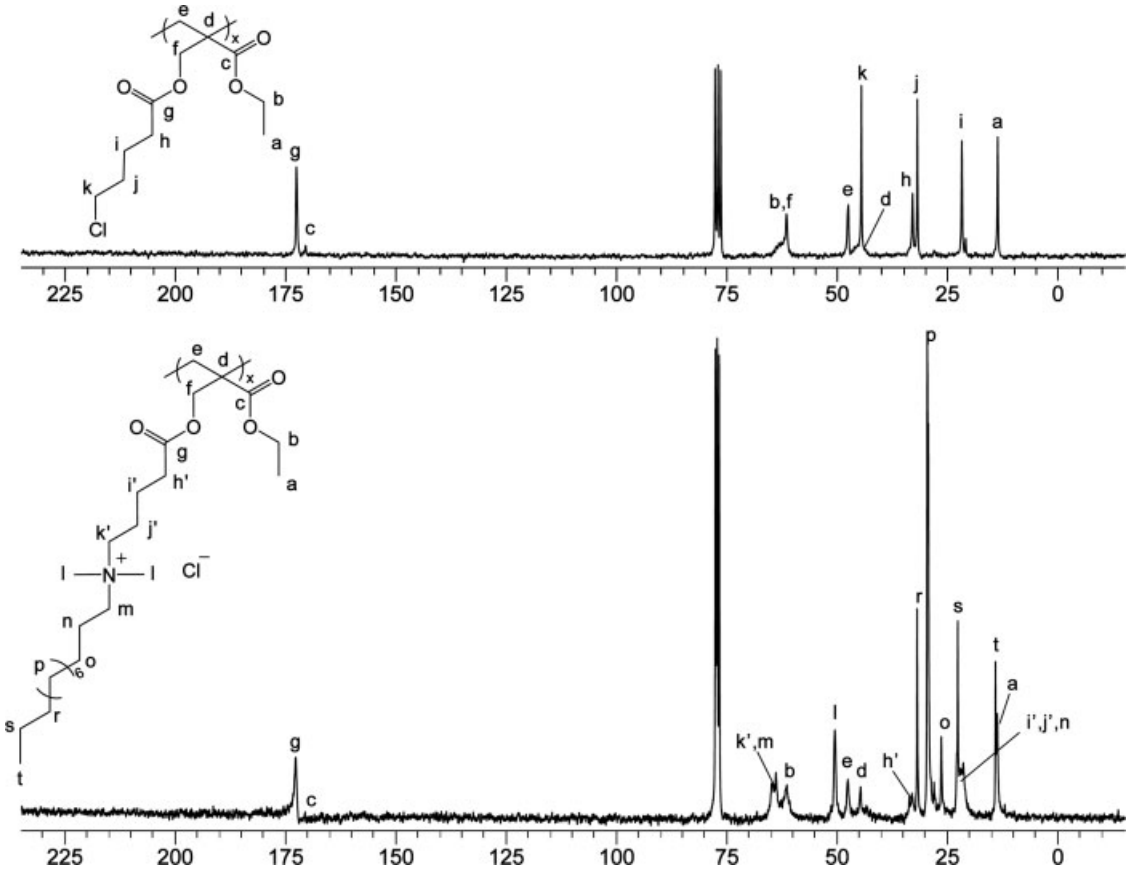


Figure 1. ^{13}C NMR spectra of the functional polymer (top) and PCEMA-C12 (bottom).

The antibacterial activities of the water-soluble polymers with pendant QA groups were determined through the testing of different concentrations of the polymers against *S. aureus* and *E. coli* with broth-dilution and spread-plate methods. A range of concentrations (from 4096 to 4 μg/mL) for each polymer was prepared with sterile, double-deionized water (autoclaved) in 96-well microtiter plates. The test organisms (5×10^5 CFU, 50 μL of TSB) were added to each well. In the end, each well contained 350 μL of water, 50 μL of TSB, and the test organism. The microtiter plates were incubated at 37 °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 with a swap, and the plates were incubated at 37 °C for 48 h. The growth of bacterial cells was observed on agar plates. The lowest concentration of the 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 polymer. The water/TSB mixture (350 μL/50 μL) and water/TSB mixture (350 μL/50 μL) inoculated with each test bacterium in the microtiter plates were used as negative and positive controls, respectively. There was no growth in the wells with the negative controls; whereas the growth of the test bacterium was observed in all wells with positive controls.

The neutral functional polymer was tested against *S. aureus* and *E. coli* with the shaking-flask method, in which 5, 10, 20, or 40 mg of the polymer was mixed with a bacterial solution (5×10^6 CFU) in 2.5 mL of a liquid medium (2 mL of water and 0.5 mL of TSB) in culture tubes. The tubes were incubated in an oven at 37 °C for 24 h, and the growth of bacteria in the culture tubes was observed visibly. The test was repeated at least four times.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Polymers

The synthesis of the functional polymer and the polymers with QA groups is shown in Scheme 1.

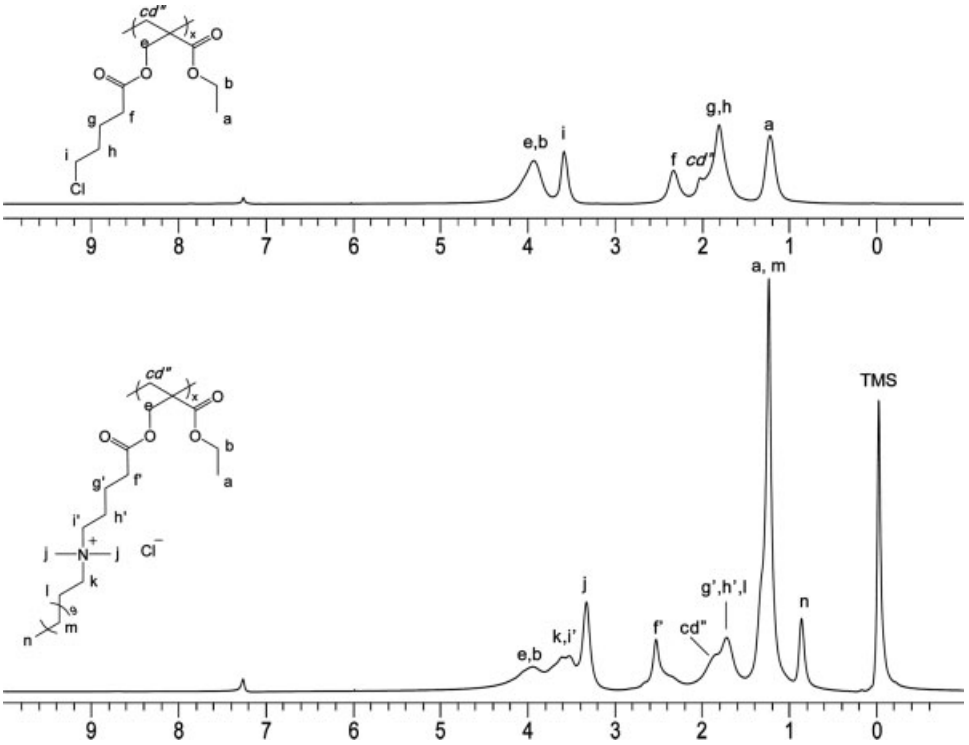


Figure 2. ^{1}H NMR spectra of the functional polymer (top) and PCEMA-C12 (bottom).

The synthesis and characterization of the CEMA monomer and the functional polymer were discussed in detail previously.[31] In this report, the synthesis, characterization, and antibacterial activities of the polymers containing pendant QA groups derived from this functional polymer are discussed.

The reaction of the functional polymer with tertiary alkyl amines was followed by ^{1}H NMR, ^{13}C NMR, and FTIR. In Figure 1, the ^{13}C NMR spectra and peak assignments of both the functional polymer and PCEMA-C12 are shown. The ^{13}C NMR spectrum of the functional polymer shows all the peaks from the CEMA monomer, except the vinylic carbons, which disappeared upon polymerization. The polymer backbone peaks resonate at 46 and 48 ppm, and peak c of —C=O shifts downfield upon polymerization. The intensity of the peaks on the polymer backbone and close to it (peaks c, e, d, b, h, and f) are diminished in the functional polymer compared with that of the CEMA monomer. In the ^{13}C NMR spectrum of PCEMA-C12, the chemical-shift changes of the carbons from the alkyl halide as well as new methyl and methylene carbon peaks of the dimethyldodecyl amine group can be observed. Upon the reaction with *N,N*-dimethyldodecyl amine (DDA), the methylene peaks α and β to —Cl (peaks k and j at 45 and 32 ppm, respectively) disappeared. These peaks, now α and β to the ammonium group, resonated at 64 and 22 ppm, respectively, in the PCEMA-C12 spectrum. The methylene peak of the DDA group α to the QA group also resonated at 64 ppm. Although the chemical shifts for peaks i and h do not change significantly, the peak intensities are reduced upon reaction. The methylene peaks of the dimethyldodecyl amine group (peaks o, p, r, and s) can be observed at 23–31 ppm. The methyl peaks both on the QA group (peak l) and at the end of the alkyl chain (peak t) can be observed at 51 and 14 ppm, respectively.

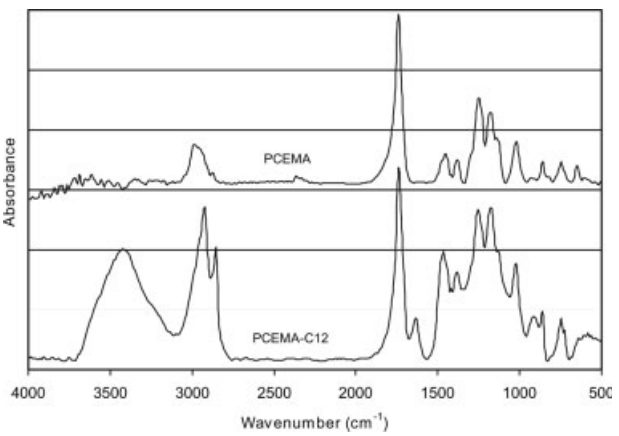


Figure 3. FTIR spectra of the functional polymer (PCEMA) and PCEMA-C12.

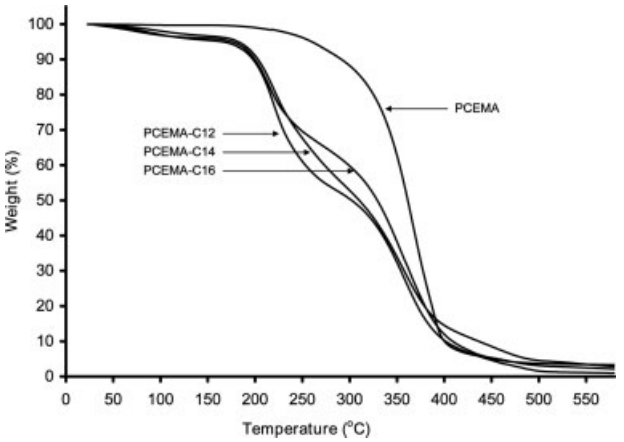


Figure 4. TGA thermograms of the functional polymer (PCEMA) and the polymers with pendant QA groups (PCEMA-C12, PCEMA-C14, and PCEMA-C16).

The chemical shifts observed in the ^{13}C NMR spectra of PCEMA-C14 and PCEMA-C16 are essentially the same as those of PCEMA-C12, varying only slightly in intensity for peaks associated with the longer alkyl chain.

In Figure 2, the ^{1}H NMR spectra and peak assignments of PCEMA and PCEMA-C12 are shown. The vinylic protons of the monomer CEMA can be seen at 5.8 and 6.3 ppm. The disappearance of the double-bond peaks of the monomer and the formation of the backbone peaks confirm that the functional polymer was obtained. Methylene peak e also shifts upfield as a result of polymerization. Other monomer peaks can be observed at the same chemical shifts found in the functional polymer spectrum. In the ^{1}H NMR spectrum of PCEMA-C12, new peaks from the dimethyldodecyl ammonium group can be observed. The methyl and methylene peaks α to the QA group (peak j and peaks k and i′) can be observed at 3.3–3.5 and 3.4–3.7 ppm, respectively. The methylene peaks β to the QA group (peaks h′ and l) can be observed at 1.7 ppm. The methyl peak and other methylene peaks of the dimethyldodecyl ammonium group (peaks n and m) appear at 0.9 and 1.2–1.4 ppm, respectively. There were no significant changes in the chemical shifts of the other peaks of the functional polymer (peaks a, b, cd″,e, f, and g). The chemical shifts observed in the ^{1}H NMR spectra of PCEMA-C14 and PCEMA-C16 were the same as those of PCEMA-C12.

FTIR spectroscopy was used to follow the reactions between PCEMA and tertiary alkyl amines. In Figure 3, the FTIR spectra of PCEMA and PCEMA-C12 are shown. The peak assignments for the functional polymer (PCEMA) were made as follows: (1) C—Cl stretching (650 cm^{-1}); (2) CH_2 rocking (750 cm^{-1}); (3) C—O stretching of O—CH_2 (1025 cm^{-1}), C—O—C antisymmetric stretching of O=C—O—C (1175 cm^{-1}, 1300 cm^{-1}), and C—O stretching of O—C=O (1265 cm^{-1}); (4) CH_2 and CH_3 bending and stretching (1370 cm^{-1}, 1450 cm^{-1}); (5) C=O stretching (1740 cm^{-1}); and (6) aliphatic CH, CH_2, and CH_3 symmetric and asymmetric stretching (2850–3000 cm^{-1}). All the functional polymer peaks except the C—Cl stretching peak can be observed in the PCEMA-C12 spectrum. The intensities of the aliphatic CH, CH_2, and CH_3 symmetric and asymmetric stretching around 2850–3000 cm^{-1} and CH_2 and CH_3 bending and stretching peaks at 1370 and 1450 cm^{-1} increased in PCEMA-C12 because there were additional methylene and methyl groups from the dimethyldodecyl ammonium group in this polymer. Two new peaks can be observed around 3100–3600 and 1600–1675 cm^{-1} in the PCEMA-C12 spectrum because of the presence of H_2O. The same peaks and similar peak intensities can be observed in the FTIR spectra of PCEMA-C14 and PCEMA-C16. All three polymers with pendant QACs were amphiphilic. Because the QA groups were very hygroscopic, the polymers absorbed water up to 5% [as measured by thermogravimetric analysis (TGA)].

TGA and differential scanning calorimetry (DSC) were used for the thermal analysis of the polymers. The TGA thermograms of all the polymers are shown in Figure 4. The decomposition temperature of the functional polymer was around 200 °C, whereas the decomposition temperatures of the polymers with pendant QA groups were around 180 °C. No glass-transition or melting temperatures were observed by DSC for the polymers within the temperature range between the ambient temperature and 180 °C.

Antibacterial Assessment

The antibacterial activities of the water-soluble polymers with pendant QA groups were deter-

Table I. MBCs of the Water-Soluble Polymers with Pendant QA Groups against *S. aureus* and *E. coli*

	MBC (μg/mL)	
Polymer	*S. aureus*	*E. coli*
PCEMA-C12	128	64
PCEMA-C14	64	256
PCEMA-C16	32	256

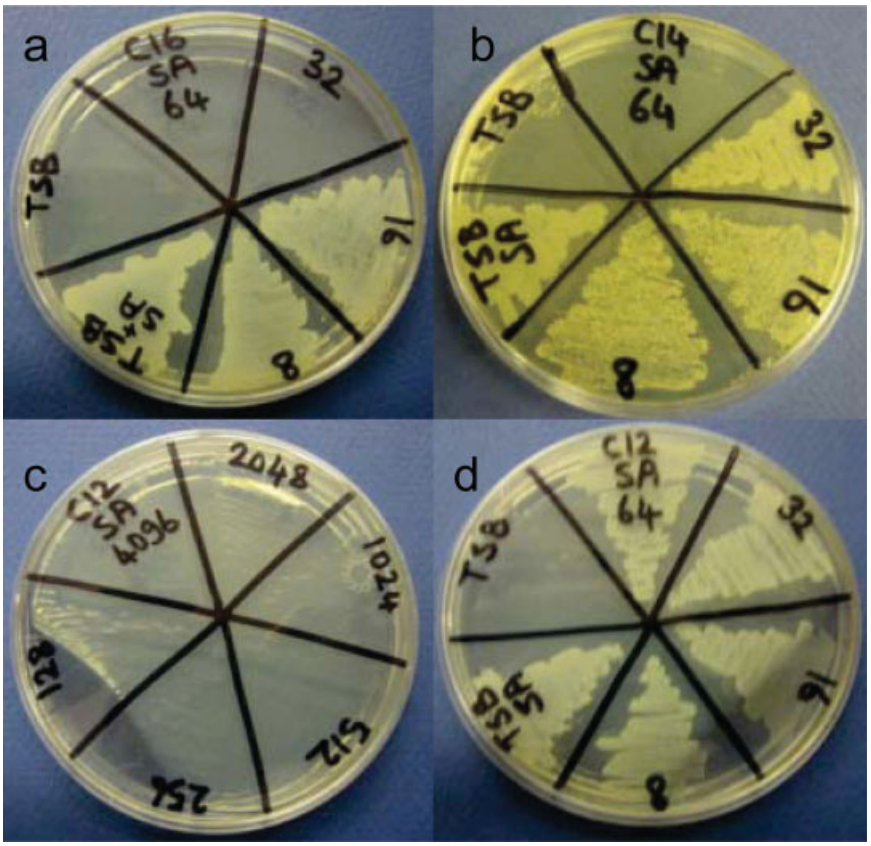


Figure 5. MBC results for the polymers with pendant QA groups against *S. aureus*: (a) PCEMA-C16, (b) PCEMA-C14, and (c,d) PCEMA-C12 (TSB and TSB/*S. aureus* are the negative and positive controls, respectively; the concentrations are given as micrograms per milliliter). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mined through the testing of different concentrations of the polymers against *S. aureus* and *E. coli* with broth-dilution and spread-plate methods. A range of concentrations for each polymer was prepared according to the experimental procedure mentioned earlier. The addition of TSB to 96-well microtiter plate wells containing different concentrations of the polymers in water resulted in the precipitation of the polymers. As a result of the precipitation, the antibacterial activities of the polymers may have been limited because there was less interaction between the polymers and bacteria when the polymers were solid versus when they were in solution. Although precipitation of the polymers occurred, the polymers showed excellent antibacterial activities. The MBC values of the water-soluble polymers with pendant QA groups obtained from broth-dilution and spread-plate tests are summarized in Table 1.

The MBC values of the polymers with pendant QA groups against *S. aureus* are shown in Figure 5 and show increased activity with an increase in the length of the alkyl chain on QA. This was probably due to the longer alkyl chains providing better compatibility with the bilayer structure of the bacterial cell wall, allowing the polymer to more easily be diffused through the bacterial cell wall and leading to the rupture of the cytoplasmic membrane and death of the bacteria.

The MBC values of the polymers with pendant QA groups against *E. coli* are shown in Figure 6 and show decreased activity with an increase in the length of the alkyl chain on QA. This behavior against *E. coli* was in the opposite direction of the activity of the polymers against *S. aureus*. This might be due to the fact that the activity against *E. coli* was more dependent on the solubility of the polymer in the water/TSB mixture than their activity against *S. aureus*. An increase in the alkyl chain length resulted in an increase in the hydrophobic character of the polymer and a decrease in water solubility.

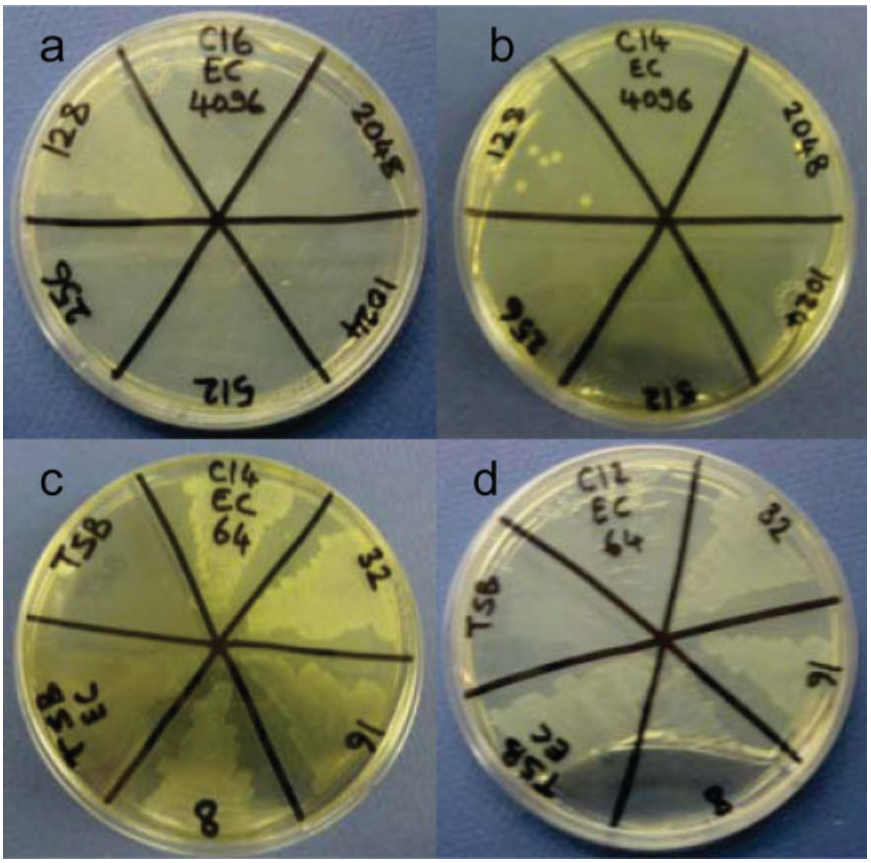


Figure 6. MBC results for the polymers with pendant QA groups against *E. coli*: (a) PCEMA-C16, (b,c) PCEMA-C14, and (d) PCEMA-C12 (TSB and TSB/*E. coli* are the negative and positive controls, respectively; the concentrations are given as micrograms per milliliter). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

This effect is more pronounced with *E. coli* because it has a much more complicated cell wall structure than *S. aureus*. There is an outer membrane in the cell wall of *E. coli*, which protects it from attack by antibacterial agents, and with a less water-soluble polymer in the water/TSB mixture, it is more difficult to kill *E. coli*.

The antibacterial activity tests for the neutral functional polymer showed no activity against either *S. aureus* or *E. coli*. The antibacterial activity results for the negative and positive controls were also observed as expected. There was no growth of bacteria in the negative controls; whereas both bacteria grew well in the positive controls.

The antibacterial action of polymers containing QA groups 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.[12,14,21] The same type of action is expected for the newly synthesized water-soluble polymers with pendant QA groups. Although the water-soluble polymers precipitate upon the addition of TSB, there might still be sufficient soluble polymer present in the mixtures to cause the death of bacteria. Alternatively, strong electrostatic interactions between the bacteria and the precipitated polymer may result in sufficient penetration of the surface-bound active groups to result in membrane disruption. Whatever the mechanism of action is, it is clear that these materials offer excellent opportunities for real-world applications.

CONCLUSIONS

New water-soluble polymers containing pendant QA groups with various long alkyl chains were

synthesized and tested for their antibacterial activity against *S. aureus* and *E. coli*. The polymers with pendant QA groups showed excellent antibacterial activities. The antibacterial activity of the polymers against *S. aureus* increased with an increase in the alkyl chain length on QA as a result of the increased compatibility with the bacterial cell wall. On the other hand, the antibacterial activity of the polymers against *E. coli* decreased as the alkyl chain length increased. This behavior is due to the fact that the solubility of the polymers, which becomes less with increasing alkyl chain length, plays a more important role in the antibacterial activity of the polymers against *E. coli*. These polymers can be used as potent antibacterial agents in coatings and pharmaceutical applications.

This material is based on work supported by the National Science Foundation under Materials Research Science and Engineering Center (grant DMR 0213883). The authors also thank the National Science Foundation Major Research Instrumentation program (grant DMR-0079450) for funding to upgrade and expand the NMR capability at the University of Southern Mississippi.

REFERENCES AND NOTES

1. Li, G.; Shen, J.; Zhu, Y. J Appl Polym Sci 2000, 78, 668.
2. Talaro, K.; Talaro, A.Foundations in Microbiology; WCB: 1993; p 286.
3. Goodson, B. A.; Ehrhardt, A.; Ng, S.; Nuss, J.; Johnson, K.; Giedlin, M.; Yamamoto, R.; Moos, W. H.; Krebber, A.; Ladner, M.; Giacona, M. B.; Vitt, C.; Winter, J. Antimicrob Agents Chemother 1999, 43, 1429.
4. Sauvet, G.; Dupont, S.; Kazmierski, K.; Chojnowski, J. J Appl Polym Sci 2000, 75, 1005.
5. Abel, T.; Cohen, J. I.; Engel, R.; Filshtinskaya, M.; Melkonian, A.; Melkonian, K. Carbohydr Res 2002, 337, 2495.
6. Borman, S. Sci Tech 2001, 79, 13.
7. Dizman, B.; Elasri, M. O.; Mathias, L. J. J Appl Polym Sci 2004, 94, 635.
8. White, D. G.; Acar, J.; Anthony, F.; Franklin, A.; Gupta, R.; Nicholls, T.; Tamura, Y.; Thompson, S.; Threlfall, E. J.; Vose, D.; Vuuren, M. V.; Wegener, H. C.; Costarrica, M. L. Rev Sci Tech Off Int Epiz 2001, 20, 849.
9. Tiller, J. C.; Liao, C. J.; Lewis, K.; Klibanov, A. M. Proc Nat Acad Sci 2001, 98, 5981.
10. Robertson, J. R. U.S. Patent 5,358,688, 1994.
11. Kawabata, N.; Nishiguchi, M. Appl Environ Microbiol 1988, 54, 2532.
12. Tashiro, T. Macromol Mater Eng 2001, 286, 63.
13. Kenawy, E.; Abdel-Hay, F. I.; El-Raheem, A.; El-Shanshoury, R.; El-Newehy, M. H. J Polym Sci Part A: Polym Chem 2002, 40, 2384.
14. Ikeda, T.; Hirayama, H.; Yamaguchi, H.; Tazuke, S.; Watanabe, M. Antimicrob Agents Chemother 1986, 30, 132.
15. Nonako, T.; Noda, E.; Kurihara, S. J Appl Polym Sci 2000, 77, 1077.
16. Seong, H.; Whang, H. S.; Ko, S. J Appl Polym Sci 2000, 76, 2009.
17. Beyth, N.; Farber, I. Y.; Bahir, R.; Domb, A. J.; Weiss, E. I. Biomaterials 2006, 27, 3995.
18. Cen, L.; Neoh, K. G.; Ying, L.; Kang, E. T. Surf Interface Anal 2004, 36, 716.
19. Park, E. S.; Kim, H. S.; Kim, M. N.; Yoon, J. S. Eur Polym J 2004, 40, 2819.
20. Arnt, L.; Nusslein, K.; Tew, G. N. J Polym Sci Part A: Polym Chem 2004, 42, 3860.
21. Schroeder, J. D.; Scales, J. C. U.S. Patent 20,020,051,754, 2002.
22. Rosinskaya, C.; Weinberg, A. U.S. Patent 20,040,106,912, 2004.
23. Kawabata, N.; Fujita, I.; Inoue, T. J Appl Polym Sci 1996, 60, 911.
24. Broughton, R. M.; Worley, S. D.; Slaten, B. L.; Mills, G.; Sunderman, C.; Sun, G.; Michielsen, S. Natl Text Center Annu Rep 1998, 347.
25. Kyba, E. P.; Park, J. U.S. Patent 6,051,611, 2000.
26. Gabrielska, J.; Sarapuk, J.; Przestalski, S.; Wroclaw, P. Tenside Surf Detergents 1994, 31, 296.
27. Ikeda, T.; Tazuke, S.; Suzuki, Y. Makromol Chem 1984, 185, 869.
28. Mcdonnell, G.; Russell, A. D. Clin Microbiol Rev 1999, 12, 147.
29. Ranucci, E.; Ferruti, P.; Neri, M. G. J Biomater Sci Polym Ed 1991, 2, 255.
30. Przestalski, S.; Sarapuk, J.; Kleszczynska, H.; Gabrielska, J.; Hladyszowski, J.; Trela, Z.; Kuczera, J. Acta Biochim Pol 2000, 47, 627.
31. Birnie, C. R.; Malamud, D.; Schanaare, R. L. Antimicrob Agents Chemother 2000, 44, 2514.
32. Kenawy, E.; Abdel-Hay, F. I.; El-Magd, A. A.; Mahmoud, Y. React Funct Polym 2006, 66, 419.
33. White, D. G.; Acar, J.; Anthony, F.; Franklin, A.; Gupta, R.; Nicholls, T.; Tamura, Y.; Thompson, S.; Threlfall, E. J.; Vose, D.; Vuuren, M. V.; Wegener, H. C.; Costarrica, M. L. Rev Sci Tech Off Int Epiz 2001, 20, 849.
34. Goodson, B. A.; Ehrhardt, A.; Ng, S.; Nuss, J.; Johnson, K.; Giedlin, M.; Yamamoto, R.; Moos, W. H.; Krebber, A.; Ladner, M.; Giacona, M. B.; Vitt, C.; Winter, J. Antimicrob Agents Chemother 1999, 43, 1429.
35. Moon, W.; Kim, J. C.; Chung, K.; Park, E.; Kim, M.; Yoom, J. J Appl Polym Sci 2003, 90, 1797.
36. Dizman, B.; Mathias, L. J. J Polym Sci Part A: Polym Chem 2005, 43, 5844.