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Biologically Active Polymers. V. Synthesis and Antimicrobial Activity of Modified Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) Derivatives with Quaternary Ammonium and Phosphonium Salts

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ABSTRACT: Antimicrobial copolymers bearing quaternary ammonium and phosphonium salts based on a copolymer of glycidyl methacrylate and 2-hydroxyethyl methacrylate were synthesized. Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) was modified for the introduction of chloromethyl groups by its reaction with chloroacetyl chloride. The chloroacetylated copolymer was modified for the production of quaternary ammonium or phosphonium salts. The antimicrobial activity of the obtained copolymers was studied against gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* sp., and *Salmonella typhae*), gram-positive bacteria (*Bacillus subtilus* and *B. cereus*), and the fungus *Trichophyton rubrum* by the cut-plug method. The results showed that the three copolymers had high antimicrobial activity. A control experiment was carried out on the main polymer without ammonium or phosphonium groups. The copolymer bearing quaternary salt made from tributyl phosphine was the most effective copolymer against both gram-negative and gram-positive bacteria and the fungus *T. rubrum*. The diameters of the inhibition zones ranged between 20 and 60 mm after 24 h. © 2002 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 40: 2384–2393, 2002

Keywords: antimicrobial polymers; bioactive polymers; biocides; biomedical polymers; disinfectant polymers; quaternary ammonium; quaternary phosphonium; copolymers; hydroxyethyl methacrylate; glycidyl methacrylate

INTRODUCTION

Antimicrobial agents of low molecular weight are used for the sterilization of water, as antimicrobial drugs, and for soil sterilization, but they have the problem of residual toxicity of the agents, even when suitable amounts of the agents are added.[1,2]

The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and

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selectivity, and prolonging the lifetime of the antimicrobial agents.[3,4]

Also, polymeric antimicrobial agents have the advantage that they are nonvolatile and chemically stable and do not permeate through the skin. Therefore, they can reduce losses associated with volatilization, photolytic decomposition, and transportation.[1]

In the field of biomedical polymers, infections associated with biomaterials represent a significant challenge to the more widespread application of medical implants.[5] Infection is the most common cause of biomaterial implant failure in modern medicine.[6,7]

Antimicrobial polymers play an important role; catheters made from a polymer that slowly releases an antibiotic could prevent thousands of hospital patients from dying from infections every year. Antimicrobial polymers could also thwart infections around more permanent implants, such as pacemakers.[8,9] In the field of textiles, work has been performed with the aim of developing new textile products with antimicrobial finishes for medical applications.[10] Because of the advantages of antimicrobial polymers, much attention has been paid to them.[11–19]

Copolymerization techniques have a number of advantages in controlling the degree of functional groups in the product, controlling its structure, and introducing the required properties into the polymer system.

It is known that quaternary ammonium compounds have been widely used as disinfectants. However, these suffer the disadvantages of low molecular weight compounds. In this study, quaternary ammonium and phosphonium copolymers were synthesized, and their antimicrobial properties were evaluated. The polymeric quaternary ammonium and phosphonium compounds in this investigation have antimicrobial properties and may be useful for many applications, such as biomedical devices and additives for antifouling paints.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA) was supplied by Aldrich and was deinhibited before use as described previously.[3] A NaOH solution (100 mL; 10%) was added to 80 mL of GMA and was shaken for 5 min in a separatory funnel. The second step was repeated three times, and then calcium chloride was added to the GMA layer and left overnight in a refrigerator. After filtration of the calcium chloride, the GMA monomer was stored under refrigeration overnight before polymerization. 2-Hydroxyethyl methacrylate (HEMA) was purchased from Aldrich, distilled under reduced pressure in the presence of hydroquinone, and stored at 4 °C until use. Triphenyl phosphine and tributyl phosphine were purchased from Aldrich and used without further purification. Triethyl amine was used as received from Merck–Schuchardt. Azobisisobutyronitrile (AIBN; Merck BDH, Ltd.) was recrystallized from a chloroform/petroleum ether mixture before use. Chloroacetyl chloride was used as supplied by Aldrich. Pyridine was dried before use via refluxing over calcium hydride and was distilled with the careful exclusion of moisture. All solvents were dried and distilled before use.

IR Spectroscopy and Elemental Microanalyses

IR spectra were recorded on a PerkinElmer 1430 ratio-recording IR spectrophotometer from KBr pellets.

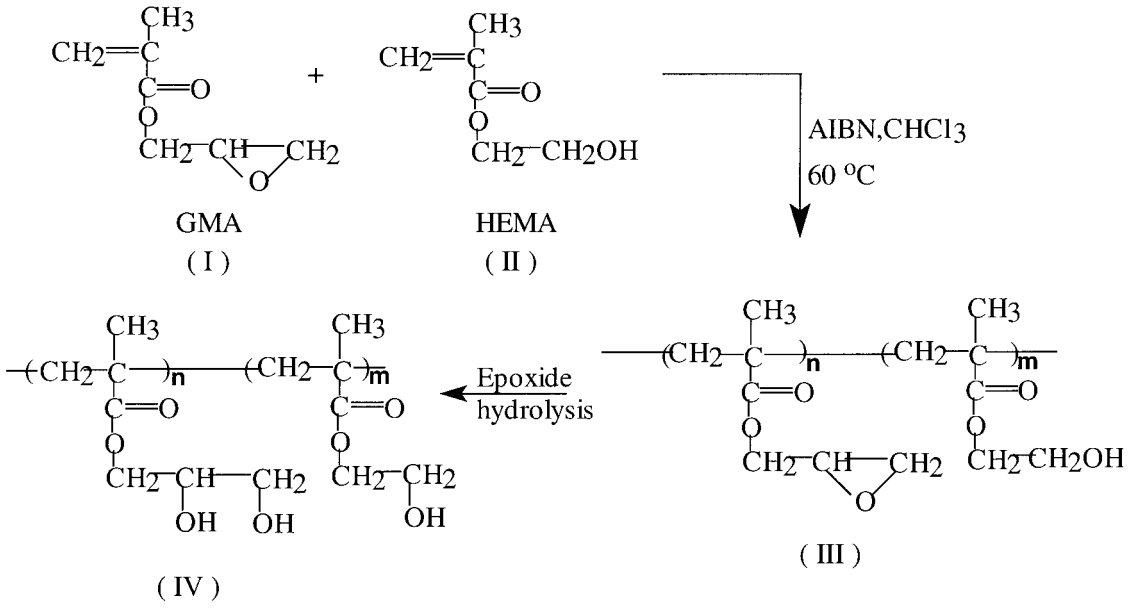
Elemental microanalyses were determined on a Heraeus instrument (Microanalysis Center, Cairo University, Giza, Egypt) and a Carlo Erba Strumentazione model 1106 elemental analyzer (Pisa University, Pisa, Italy).

Test Microorganisms

The microorganisms included the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* sp., and *Salmonella typhae* and the gram-positive bacteria *Bacillus subtilus* and *B. cereus*. The fungus *Trichophyton rubrum* represented dermatophyte fungi. Bacteria were maintained on nutrient agar, and the fungus was kept on Subouroud agar slopes.

Copolymerization of GMA and HEMA [Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (III)]

To a solution of GMA (**I**; 39.32 g, 276.9 mmol) and HEMA (**II**; 18.0 g, 138.46 mmol) in 100 mL of chloroform was added 0.2 g of AIBN. The solution was allowed to stand under a nitrogen atmosphere for 1 h for deoxygenation. A reflux condenser was attached to the system, and the reaction mixture was refluxed for 5 h under a nitrogen



Scheme 1. Synthesis and acid hydrolysis of poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate).

atmosphere in a water bath at 60 °C. The precipitated polymer (**III**) was filtered off, washed repeatedly with chloroform, and dried *in vacuo* overnight at 30 °C for 48 h to yield 57.0 g (99.5% yield; cf. Scheme 1). The product **III** was characterized by IR spectroscopy and elemental analysis (Table 1).

Acid Hydrolysis of the Epoxide Group of Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (IV)

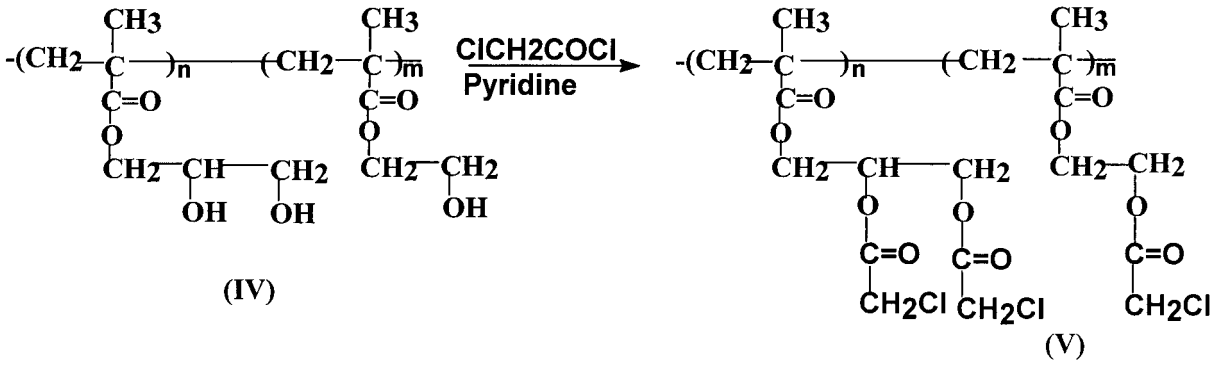
The hydrolysis of the epoxide groups of **III** to vicinal diol groups took place by catalysis with a mineral acid.[20] The procedure was as follows. To **III** (30.0 g, 72.46 mmol) was added 50 mL of 0.1 M sulfuric acid. A reflux condenser was attached to the system, the reaction mixture was stirred and heated in a water bath at 60 °C, and the stirring and heating were continued overnight. The hydrolyzed polymer was filtered off, washed with water and ethanol, and dried *in vacuo* at 30 °C to yield 30.5 g (93.6% yield; cf. Scheme 1). Hydrolyzed poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (**IV**) was characterized by IR spectroscopy to check the extent of hydrolysis.

Chloroacetylation of Hydrolyzed Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (V)

To a suspension of **IV** (20.0 g, 44.44 mmol) in 150 mL of chloroform was added 35.9 mL of pyridine (444.44 mmol). The mixture was cooled in an ice–salt bath, and chloroacetyl chloride (35.4 mL,

Table 1. Elemental Microanalysis for Copolymers **III–VIII**

Polymer Code	C (%)		H (%)		N (%)		CI (%)		P (%)	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
III	57.3	56.4	7.4	6.3	—	—	—	—	—	—
IV	53.7	50.9	7.6	6.4	—	—	—	—	—	—
V	43.9	48.2	4.8	4.6	—	—	20.4	15.6	—	—
VI	53.0	46.9	8.5	7.0	5.1	3.7	12.0	7.7	—	—
VII	67.1	57.0	5.4	5.2	—	—	8.2	8.30	7.0	4.0
VIII	58.0	52.0	9.0	8.4	—	—	9.0	5.2	8.2	5.7



Scheme 2. Chloroacetylation of modified poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate).

444.44 mmol) was added dropwise with cooling and stirring. The reaction mixture was stirred overnight at room temperature for an additional 4 days. The resin was filtered off and washed with chloroform and water. The washing with chloroform was continued until the washings were neutral. (cf. Scheme 2). Chloroacetylated poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (**V**) was characterized by elemental microanalysis (Table 1) and IR spectroscopy.

Immobilization of Phosphonium and Ammonium Salts onto Chloroacetylated Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (V)

Triethyl Ammonium Salt (VI)

The triethyl ammonium salt of chloroacetylated poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (**VI**) was synthesized as follows. To a stirred suspension of copolymer **V** (10.0 g, 12.01 mmol) in 100 mL of dry benzene was added 16.7 mL (12.13 g, 120.11 mmol) of triethyl amine. The reaction mixture was refluxed with stirring for 5 days. The product **VI** was filtered off and washed with benzene and dried *in vacuo* at 30 °C overnight to yield 10.5 g (65.3% yield; cf. Scheme 3). The product **VI** was characterized by elemental microanalysis (Table 1) and IR spectroscopy.

Triphenyl Phosphonium Salt (VII)

The title compound was prepared from copolymer **V** and triphenyl phosphine similarly to **VI** with the following quantities: 2.0 g (2.40 mmol) of copolymer **V** and 6.29 g (24.02 mmol) of triphenyl phosphine in 30 mL of dry benzene. The yield was 2.0 g (38.9% yield; cf. Scheme 3). The product **VII** was characterized by elemental microanalysis (Table 1) and IR spectroscopy.

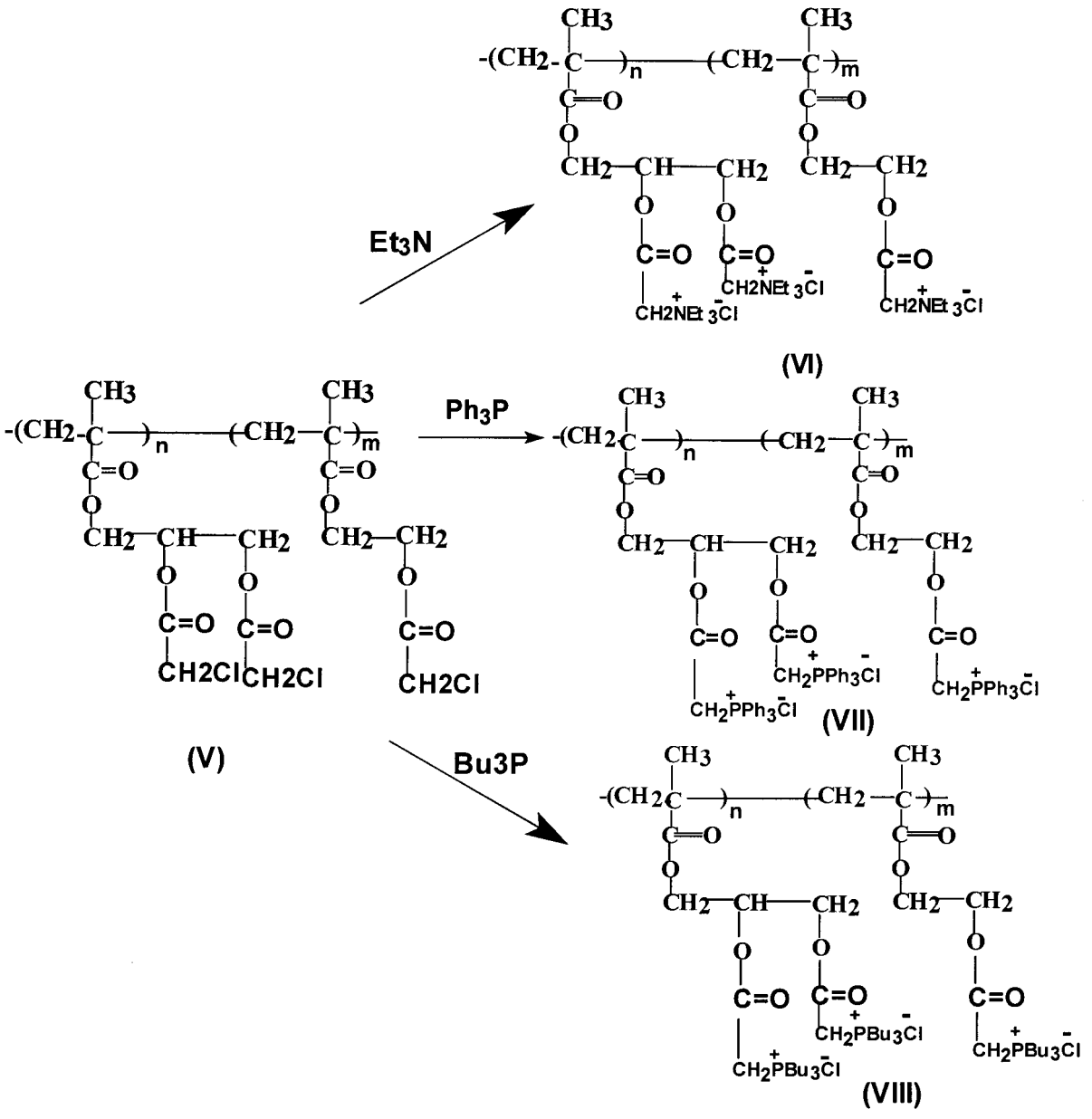
Tributyl Phosphonium Salt (VIII)

The title compound was prepared from copolymer **V** and tributyl phosphine similarly to (**VI**) with the following quantities: 2.0 g (2.40 mmol) of copolymer **V** and 6.0 mL (4.85 g, 24.02 mmol) of tributyl phosphine in 30 mL of dry benzene. The yield was 2.5 g (54.4% yield; cf. Scheme 3). The product **VIII** was characterized by elemental microanalysis (Table 1) and IR spectroscopy.

Evaluation of Antimicrobial Activity

The antimicrobial spectrum of the prepared polymers was determined against the aforementioned test bacteria on powdery samples by the cut-plug method[21] on nutrient agar that contained, per liter, 10 g of peptone, 5.0 g of NaCl, 5 g of beef extract, and 20.0 g of agar at pH 7. The assay plates were seeded with the test bacteria, and after solidification, the wells were made and filled with 20.0 mg of powdery polymer. The plates were incubated at 30 °C for 24 h, after which the diameters of the inhibition zones were measured, and the compounds, which produced the inhibition zones, were further assayed at different concentrations in aqueous suspensions for quantification of their inhibitory effects.

A loopful of each culture was placed in 10 mL of broth diluted 10 times, which was then incubated overnight at 30 °C. At this stage, the cultures of the test bacteria contained 11.65 and 12×10^4 cells/mL *B. subtilis* and *P. aeruginosa*, respectively, and 2.4×10^3 cells/mL *E. coli*, and the test



Scheme 3. Immobilization of ammonium and phosphonium salts onto modified poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate).

fungus contained 8.4×10^4 spores/mL, which were used for the antimicrobial test.

Because the polymers were not soluble in water or in different solvents, they were suspended in a sterile, 10-fold dilution of the aforementioned nutrient broth medium to yield a 0.05 g/mL concentration, and 0.5 mL was transferred to flasks containing a sterile, 10-fold dilution of the nutrient broth to give final concentrations of 10, 5, and 2.5 mg/mL. The exposure of bacterial cells to biocide polymers was started when 0.2 mL of a culture containing 11.65 and 12×10^4 cells/mL *B. subtilis* and *P. aeruginosa*, respectively, 2.4×10^3 cells/mL *E. coli*, or 8.4×10^4 fungal spores/mL *T. rubrum* was added to 10 mL of the aforementioned biocide suspension, which was pre-equilibrated and shaken at 30 °C as recommended by Nakashima et al.[22] At the same time, 0.2 mL of the same culture was added to 10 mL of a 10-fold dilution of the nutrient broth, decimal dilutions were prepared, and the starting number of cells was counted by the spread-plate method. After

24 h of contact, 1.0-mL portions were removed and mixed with 9.0 mL of the 10-fold dilution of the nutrient broth, and then decimal serial dilutions were prepared from these dilutions; the surviving bacteria or fungi were counted by the spread-plate method. After inoculation, the plates were inoculated at 30 °C, and the number of colonies was counted after 24 h for bacteria and after 48 h for fungi. The ratio was carried out in triplicate every time. The ratio of the colony numbers for the media containing the polymer (*M*) to those without these compounds (*C*) was taken as the surviving cell number, and with this value, the antimicrobial activity was evaluated. A control experiment was carried out with polymer **IV**.

RESULTS AND DISCUSSION

Copolymerization of GMA and HEMA (III)

The copolymerization of GMA (**I**) and HEMA (**II**) was carried out with a free-radical polymerization technique in chloroform at 60 °C with AIBN as an initiator (cf. Scheme 1). The IR spectrum of the product **III** showed peaks at 3400 (OH), 2856 (CH), 1728 (C═O), 1456 (CH_3), and 1269 cm^{-1} (epoxide).

Acid Hydrolysis of the Epoxide Group of Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (IV)

The epoxide groups of the copolymer were hydrolyzed when treated with dilute sulfuric acid at 60 °C for 15 h. The IR spectra showed that the epoxide groups underwent hydrolysis. The hydrolysis of the epoxide groups in the polymer resulted in substantially selective hydroxylation of epoxide group into α,β-diol only when the reaction mixture was heated at 60 °C and catalyzed by 0.1 M sulfuric acid (cf. Scheme 1). When the reaction was carried out at room temperature, the hydrolysis was not complete. The IR spectrum of the product **IV** showed peaks at 2856 (CH), 1455, 2923 (CH3), 3425 (OH), 1118 (sec. OH), and 1730 cm^{-1} (C═O). The results of the elemental analysis are shown in Table 1.

Chloroacetylation of Hydrolyzed Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (V)

The introduction of chloroacetyl groups was readily achieved by the treatment of the hydrolyzed copolymer **IV** with chloroacetyl chloride in the presence of pyridine in dry chloroform under dehydrating conditions. An analysis of the polymer by IR spectroscopy confirmed that the acetylation reaction was complete by the disappearance of the hydroxyl groups. The product **V** was characterized by elemental microanalysis, which showed 15.6% Cl (found) and, therefore, indicated a high percentage of conversion of the diol polymer to the chloroacetylated derivative (cf. Scheme 2). The same conclusion was confirmed from the IR studies. The IR spectrum of the product **V** showed peaks at 782 and 1270 (—CH_2Cl), 1728 (—C═O), 2856 (CH, CH_2), and 1453 and 2926 cm^{-1} (CH_3).

Immobilization of Phosphonium and Ammonium Salts onto Chloroacetylated Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) (VI–VIII)

Compounds **VI–VIII** were synthesized to study the influence of the nature of the active group on the antimicrobial activity of the copolymer. Quaternization of the chloroacetylated polymers was carried out in benzene by the reaction of **V** with triethyl amine, triphenyl phosphine, and tributyl phosphine, respectively, at room temperature, followed by heating at 80 °C for 2 days (cf. Scheme 3).

Generally, triethyl amine, triphenyl phosphine, and tributyl phosphine were quaternized with the chloromethylated copolymer at room temperature. However to confirm quaternization of the polymers, we heated the reaction mixtures at 80 °C (under benzene refluxing). This method favored the formation of phosphonium and ammonium salts. The amount of triphenyl phosphine, tributyl phosphine, and triethyl amine was in excess (10 times) with respect to the chloromethyl group content because it was reported earlier that under stoichiometric conditions, the yield of quaternization was limited.[23] The phosphorus and nitrogen contents of the biocidal polymers **VI–VIII** were determined by elemental microanalysis (Table 1), and the data were in good agreement with the calculated values. The degrees of modifications for polymers **VI–VIII** were 72.54, 57.14, and 69.51%, respectively. Also, the IR spectra of polymers **VI–VIII** indicated the presence of peaks at 1580–1475 (P—CH_2) and 1475–1397 cm^{-1} (P—Phenyl), which confirmed the formation of phosphonium salts.

Table 2. Diameters of the Inhibition Zones (mm) Produced by 20.0 mg of Powdery Copolymers of Modified Poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) against Different Test Bacteria after 24 h by the Cut-Plug Method on Nutrient Agar at 30°C

		Copolymer		
Organisms	**IV**	**VI**	**VII**	**VIII**
E. coli	0	26	40	30
P. aeruginosa	0	40	50	60
Shigella sp.	0	20	30	30
Salmonella typhae	0	20	44	42
B. subtilis	0	20	30	40
B. cereus	0	20	12	20

Antibacterial Assessment of Copolymers VI–VIII

The antimicrobial activities of modified copolymers **VI–VIII** against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *T. rubrum* were explored by the cut-plug method and viable cell counting methods as described previously. A control experiment was carried out with polymer **IV** to check the effect of the introduction of the ammonium or phosphonium groups to the polymer. From the data shown in Table 2, it was concluded that polymer **IV** showed no inhibition zone. The ability of the prepared copolymers to inhibit the growth of the tested microorganisms on solid media is shown in Table 2. The diameter of the inhibition zone varied according to the active group in the polymer and test microorganism. The compounds inhibited the growth of the test bacteria on a solid agar medium, with the tributyl phosphonium salt **VIII** of the modified copolymer **VIII** being the most effective on both gram-negative and gram-positive bacteria (the diameters of the inhibition zones ranged between 20 and 60 mm) after 24 h. This means that the tributyl phosphonium salt is better than the other groups studied here as an antimicrobial agent. The growth inhibitory effect was quantitatively determined by the ratio (*M*/*C*) of the surviving cell number. As shown in Figure 1, the growth inhibitory effect of polymer **VI** differed among the bacterial and fungal strains. Polymer **VI** at a concentration of 2.5 mg/mL killed only 70% of *T. rubrum*, 25% of *E. coli*, 17% of *P. aeruginosa*, and 15% of *B. subtilis*. However, increasing the polymer **VI** concentration to 10 mg/mL killed 100% of *T. rubrum*, 80% of *E. coli*, 70% of *P. aeruginosa*, and 40% of *B. subtilis*. The inhibition becomes stronger in the order *B. subtilis* < *E. coli* < *P. aeruginosa* < *T. rubrum*. The results show also that the inhibitory effect increased with the concentration of the polymer increasing. Figure 2 shows the inhibitory effect of polymer **VII**. The results showed that the inhibition becomes stronger in the order *B. subtilis* < *P. aeruginosa* < *E. coli* < *T. rubrum*, and the inhibition was increased with the concentration of the polymer increasing. Polymer **VII** was effective against *T. rubrum*; it killed 99% of *T. rubrum* at a

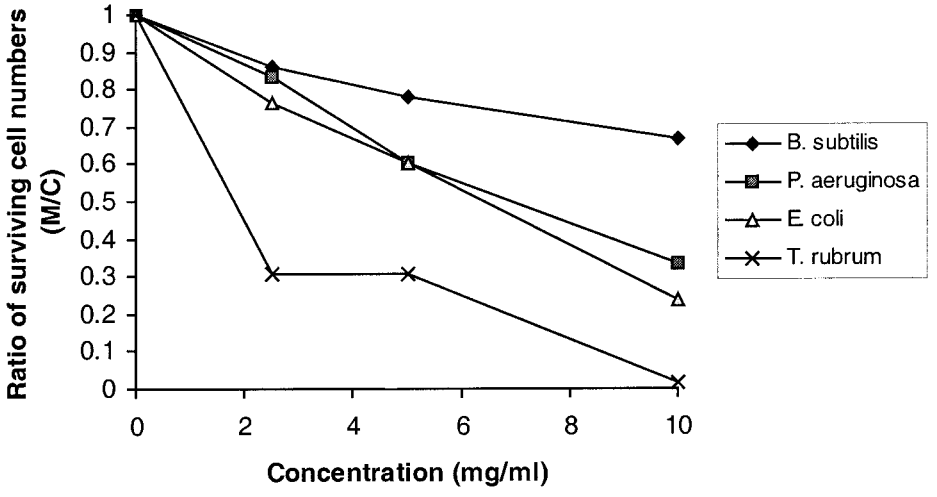


Figure 1. Growth inhibition of different concentrations of polymer **VI**. The inoculations were 11.65 and 12 × 10^4 cells/mL *B. subtilis* and *P. aeruginosa*, respectively; 2.4 × 10^3 cells/mL *E. coli*; and 8.4 × 10^4 fungal spores/mL *T. rubrum*.

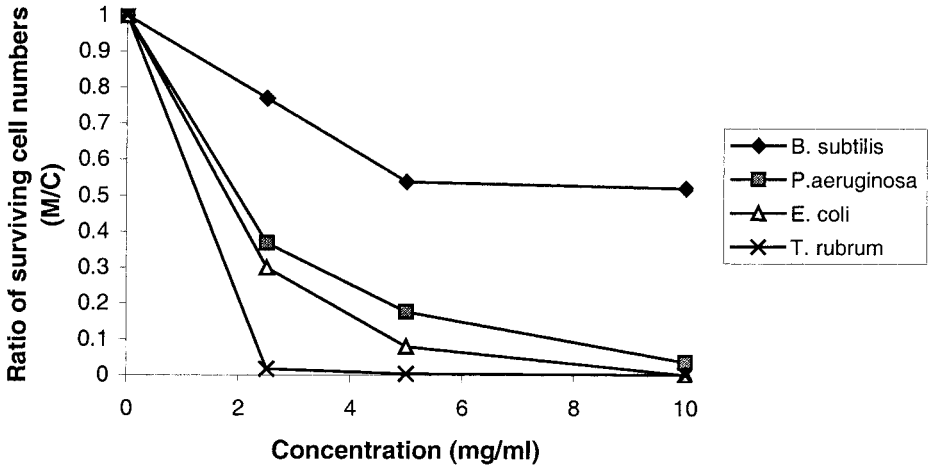


Figure 2. Growth inhibition of different concentrations of polymer **VII**. The inoculations were 11.65 and 12 $\times$ 10^4 cells/mL *B. subtilis* and *P. aeruginosa*, respectively; 2.4 $\times$ 10^3 cells/mL *E. coli*; and 8.4 $\times$ 10^4 fungal spores/mL *T. rubrum*.

concentration of 2.5 mg/mL. Increasing the concentration of polymer **VII** to 5 mg/mL killed 100% of *T. rubrum*. The same concentration (2.5 mg/mL) of polymer **VII** killed 70% of *E. coli*, 64% of *P. aeruginosa*, and 24% of *B. subtilis*. However, increasing the concentration of polymer **VII** to 10 mg/mL killed 100% of *E. coli*, 97% of *P. aeruginosa*, and only 50% of *B. subtilis*. The inhibitory effect of polymer **VIII** is shown Figure 3. The results showed that the inhibition becomes stronger in the order *B. subtilis* < *P. aeruginosa* < *T. rubrum* < *E. coli*, and the inhibition was increased with the concentration of the polymer increasing. Polymer **VIII** was also very effective against *T. rubrum*; a concentration of 2.5 mg/mL killed 100% of *T. rubrum*. Polymer **VIII** was also active against *E. coli*; at a concentration of 5 mg/mL, it killed 100%. A polymer **VIII** concentration of 10 mg/mL killed 98% of *B. subtilis* and 91.7% of *P. aeruginosa*.

For the fungus *T. rubrum*, polymers **VII** and **VIII**, which had phosphonium groups in the side chain, were very effective even at the lower concentration of 2.5 mg/mL. These two polymers are more effective against *T. rubrum* than previously reported.[3,4] Generally, the potency of inhibition varied according to the polymer and the test strain. The polymers were found to be more active

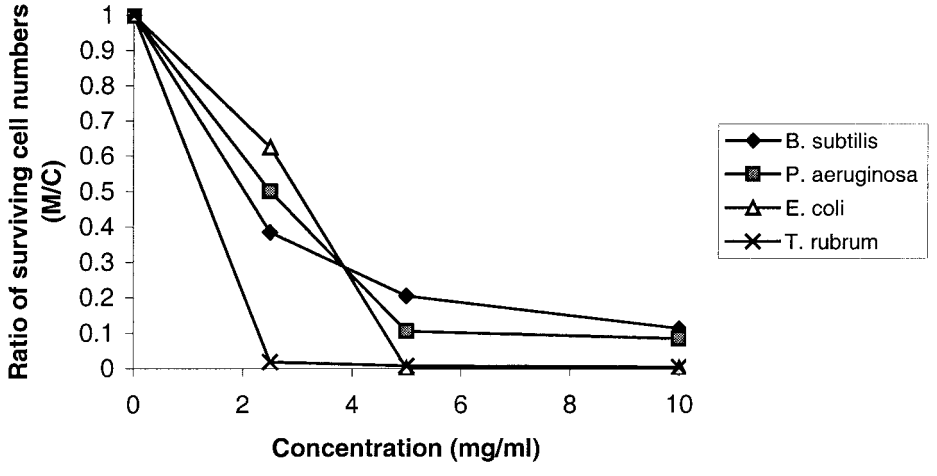


Figure 3. Growth inhibition of different concentrations of polymer **VIII**. The inoculations were 11.65 and 12 $\times$ 10^4 cells/mL *B. subtilis* and *P. aeruginosa*, respectively; 2.4 $\times$ 10^3 cells/mL *E. coli*; and 8.4 $\times$ 10^4 fungal spores/mL *T. rubrum*.

against the fungus *T. rubrum* and the gram-negative bacteria (*E. coli* and *P. aeruginosa*) than the gram-positive bacteria (*B. subtilis*). The mode of action of cationic biocides is interpreted in terms of the following sequence of elementary processes:[24–27] (1) adsorption onto the bacterial cell surface; (2) diffusion through the cell wall; (3) binding to the cytoplasmic membrane; (4) disruption of the cytoplasmic membrane; (5) release of cytoplasmic constituents such as K^+ ions, DNA, and RNA; and (6) death of the cell.

It is now generally accepted that the mode of action of the polycationic biocides based on quaternary ammonium and phosphonium salts can be interpreted on the basis of each elementary process previously described because the same physiological events found in processes 1, 3, and 5 have been observed for the polycationic biocides.[28–30] It is well known that bacterial cell surfaces are negatively charged. Therefore, adsorption onto the negatively charged cell surface (process 1) is expected to be enhanced with the increasing charge density of the cationic biocides. Therefore, it is reasonable to assume that process 1 is much more enhanced for polymers than for model compounds.[28,31] A similar situation can also be expected in process 3 because there are many negatively charged species present in the cytoplasmic membrane, such as acidic phospholipids and some membrane proteins.[28,32,33] The disruption of the membrane (process 4) is a result of the interaction of the bound polymers with the membrane disruption and, therefore, is expected to be facilitated with increasing amounts of the bound polymers. The hydrophobicity of the substituent affected the antibacterial activity of the phosphonium salts. It has been previously shown that with increasing hydrophobicity of the foreign compounds, they become more active, interacting with the cytoplasmic membranes (processes 3 and 4).[21] It is known that there is much difference in the structures of the cell walls between gram-positive and gram-negative bacteria. The former cells have a simple cell wall structure, in which outside the cytoplasmic membrane there is only a rigid peptidoglycan layer. The peptidoglycan layer, although relatively thick, is composed of networks with plenty of pores that allow foreign molecules to come into the cell without difficulty.[21,27] However, gram-negative bacteria have very complicated cell walls. There is another membrane outside the peptidoglycan layer, the outer membrane, and it has a structure similar to that of the cytoplasmic membrane. Because of the bilayer structure, the outer membrane is a potential barrier against foreign molecules of high molecular weight.[28,34] Consequently, the overall activity would be determined by two factors: one is favored for polymers (processes 1, 3, and 4), and the other is unfavorable for polymers (process 2).[27]

CONCLUSIONS

A copolymer from GMA and HEMA was easily prepared by a free-radical polymerization technique. The copolymer was functionalized by its reaction with chloroacetyl chloride for the introduction of chloromethyl groups, followed by quaternization with triethyl amine, triphenyl phosphine, and tributyl phosphine.

The antimicrobial properties of the quaternary ammonium and phosphonium copolymers based on the modified poly(glycidyl methacrylate-*co*-hydroxyethyl methacrylate) were tested *in vitro* against both gram-positive and gram-negative bacteria and the fungus *T. rubrum*.

The copolymers showed high antimicrobial activity against the fungus *T. rubrum* and gram-negative bacteria (*E. coli* and *P. aeruginosa*) but were not relatively active at low concentrations against gram-positive bacteria (*B. subtilis*).

Polymers **VII** and **VIII** showed excellent antimicrobial activity against *T. rubrum*; they killed 100% of *T. rubrum* at a concentration of 2.5 mg/mL. However, *T. rubrum* required a concentration of 10 mg/mL polymer **VI** to kill 100% of *T. rubrum*. To kill 100% of *E. coli*, it required a concentration of 10 mg/mL polymer **VII** or 5 mg/mL polymer **VIII**. Polymer **VII** proved to be able to kill 100% of *E. coli*, 97% of *P. aeruginosa*, and 48% of *B. subtilis* at a concentration of 10 mg/mL. In conclusion, it seems that these materials could be promising candidates for preventing biomaterial-related infections.

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