Biologically Active Polymers. IV. Synthesis and Antimicrobial Activity of Polymers Containing 8-Hydroxyquinoline Moiety

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ABSTRACT: Polymers containing 8-hydroxyquinoline moiety were prepared. Modifications of the base polymer of glycidyl methacrylate were carried out in order to introduce chloromethyl groups, either by the hydrolysis of the poly(glycidyl methacrylate) and the chloroacetylation of the hydrolyzed polymer by the reaction with chloroacetyl chloride or by aminating the poly(glycidyl methacrylate) either with ethylenediamine or with hexamethylenediamine, followed by reacting the aminated polymers with chloroacetyl chloride. The polymers containing 8-hydroxyquinoline moiety were prepared by reacting the chloromethyl groups containing polymers with potassium salt of 8-hydroxy quinoline. The antimicrobial activity of the polymers obtained was examined against gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Bacillus subtilus*) as well as the fungus *Trichophyton rubrum*. Generally, all three polymers proved effective against the tested microorganisms, but growth inhibitory effects varied from one another. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 1364–1374, 2001

Key words: biomedical polymers; antimicrobial polymers; disinfectant polymers; biologically active polymers; polymeric biocides; 8-hydroxyquinoline

INTRODUCTION

Antimicrobial agents are those materials capable of killing pathogenic microorganisms.[1] In general, antimicrobial agents such as bactericides or disinfectants are low-molecular-weight compounds.[2] Phenols and cationic compounds are two main groups of compounds used almost exclusively for disinfectants. Application of aqueous hypochlorite and phenolic disinfectant compositions at kitchen and bathrooms sites produces a significant reduction in contamination level for only 3–6 h. Low-molecular-weight quaternary ammonium salts (QAS) possessing at least one alkyl group are able to kill microorganisms by interacting with the cell membrane.[3] However, most of these molecules are toxic to the environment, and their application is short-lived. Moreover, studies have hypothesized that more rapid contamination of sterilized surfaces is possible because of fresh contamination of surfaces.[4]

If these antimicrobial agents are attached or formed on a polymeric material, it would be an ideal solution to overcome problems associated with low-molecular-weight material. As compared with conventional antimicrobial agents of low molecular weight, advantages of polymeric antimicrobial agents are that they are nonvolatizable, chemically stable, and slow to permeate the skin of a human or animal[1] skin. Previous studies have shown that, although it is very difficult to rule out definitely the possibility that

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some leachable compounds take a small part in the observed biocidal activity, all results show that polymers containing permanently bound QAS have demonstrated an advantage over materials that function by controlled release of low-molecular-weight biocide, as they show much better durability with or without very low liberation of toxic products in the environment.[5] Therefore, they reduce losses associated with volatilization, photolytic decomposition, and transportation significantly. Moreover, increased efficiency, selectivity, and handling safety are additional benefits that may be realized.

Polymeric antimicrobial agents hold great promise for enhancing the efficiency of some existing antimicrobial agents, prolong their activity, reduce their toxicity, as well as reduce the environmental problems associated with others.[6] Therefore, investigation of polymeric antimicrobial agents represents a new and important direction that is developing in the field of antimicrobial agents.[5,7–10]

Previously, we have described antimicrobial polymeric systems based on the chemical modification of poly(glycidyl methacrylate) by the formation of quaternary ammonium and phosphonium salts on the modified polymers.[11] The polymeric phosphonium and ammonium salts of the modified glycidyl methacrylate polymers were inhibitory to the growth of gram-negative bacteria (*Escherichia coli, Pseudomones aeruginosa, Shigella* sp., and *Salmonella typhae*) and gram-positive bacteria (*Bacillus subtilis* and *B. cereus*), as well as the fungus (*Trichophyton rubrum*).

In the present work, the syntheses of polymers containing 8-hydroxyquinoline are described and their biological activities screened. The effect of a spacer group between the polymer main-chain and 8-hydroxyquinoline moiety on the antimicrobial activity is discussed.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA) was supplied by Aldrich and was de-inhibited before use as follows: 100 mL NaOH solution (10%) was added to 80 mL GMA and was then shaken for 5 min in a separating funnel. The last step was repeated three times. Calcium chloride was added to the GMA layer and left overnight in the refrigerator, followed by filtration of the calcium chloride; the GMA monomer was stored under refrigeration overnight before polymerization. Azobisisobutyronitrile (AIBN) (Merck) was recrystallized from a chloroform/petroleum ether mixture before use. Chloroacetyl chloride was used as supplied by Aldrich. Ethylenediamine was supplied by Aldrich and distilled before use (bp 118°C). Hexamethylenediamine and 8-hydroxyquinoline were supplied by Aldrich and were used as received. Pyridine was dried before use. All solvents were dried and distilled before use.

IR Spectroscopy

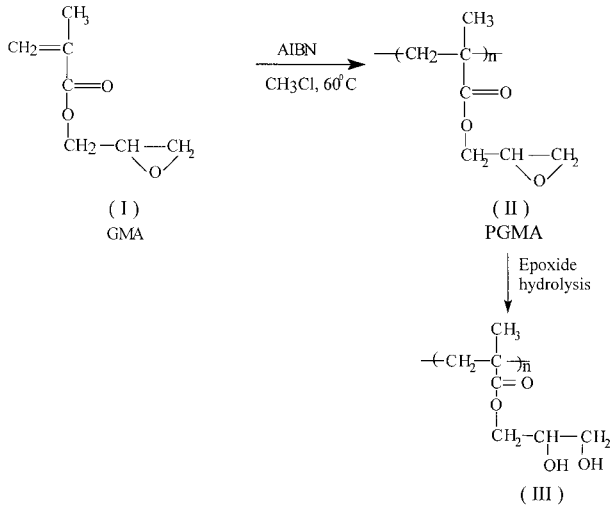
IR spectra were recorded on a Perkin–Elmer 1430 Ratio Recording Infrared Spectrophotometer from KBr pellets. Elemental microanalyses were determined on Heraeus instrument at Microanalysis Center, Cairo University (Giza, Egypt).

Test Microorganisms

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

Synthesis of Linear Poly(Glycidyl Methacrylate) (II)

The procedure was reported previously by Kenawy et al.[11] as follows. To a solution of glycidyl methacrylate (I) (70.0 g, 492.96 mmol) in 300 mL chloroform was added 0.5 g AIBN. The reaction mixture was deoxygenated by bubbling nitrogen gas for 1 h. A reflux condenser was attached to the system and the reaction mixture was refluxed under nitrogen for 1 h on a water bath at 60°C. It was left overnight at room temperature; the polymer solution in chloroform was concentrated to one-fourth its volume. The resulting polymer (II) was precipitated from solution by dropwise addition of the product solution to a stirred diethyl ether. The precipitated polymer was filtered off and washed repeatedly with ether and kept under vacuum overnight at 30°C for 48 h. The polymer yield was 31.0 g (44.3% yield) (Scheme 1). The resulting polymer (II) was soluble in methylene chloride, chloroform, and dimethylformamide (DMF) and insoluble in water, toluene, benzene, ethanol, petroleum ether, *n*-hexane, and diethyl ether. The product (II) was characterized by IR and ^{1}H-NMR spectroscopy.



Scheme 1 Synthesis and acid hydrolysis of poly(glycidyl methacrylate).

Acid Hydrolysis of the Epoxide Group of Linear Poly(Glycidyl Methacrylate) (III)

Poly(glycidyl methacrylate) (II) (10.0 g, 70.42 mmol) was refluxed with 50 mL of 0.1*M* sulfuric acid at 60°C in a water bath with occasional stirring. The reaction was continued overnight. The hydrolyzed polymer (III) was filtered off and washed with water and ethanol and it was dried under vacuum at 30°C. The yield was 11.0 g (97.8% yield) (Scheme 1). The product (III) was characterized by IR spectroscopy to check the extent of hydrolysis.

Modification of Poly(Glycidyl Methacrylate (II) with Ethylenediamine (IV)

Poly(glycidyl methacrylate) was reacted with ethylenediamine and the reaction was performed as follows: To a solution of poly(glycidyl methacrylate) (II) (10 g, 71.0 mmol) in chloroform (20 mL) was added dropwise ethylenediamine (14.1 mL, 211 mmol). The reaction mixture was heated while stirring at 40°C for 7 h. The aminated polymer was filtered through a sintered glass funnel (G3) and washed with chloroform many times to remove the excess ethylenediamine. The product was dried under vacuum overnight at 25°C for 24 h. The resulting polymer was soluble in dimethylsulfoxide (DMSO), dimethylformamide, chloroform, and water. A yield of polymer (IV), 12 g (81%) was obtained (Scheme 2). The product was characterized by elemental analysis (Table I) and IR spectroscopy.

Modification of Poly(Glycidyl Methacrylate (II) With Hexamethylenediamine (V)

The amination of the poly(glycidyl methacrylate) with hexamethylenediamine was carried out similarly to the procedure described above for polymer (IV). The following quantities were used: 10 g of PGMA (71.0 mmol) and 24.5 g hexamethylenediamine (211.3 mmol). The yield of aminated polymer (V) was 14 g (77.8%) (Scheme 2). The product was characterized by IR spectroscopy and elemental analysis (Table I).

Modification of Polymers (III–V) Using Chloroacetyl Chloride

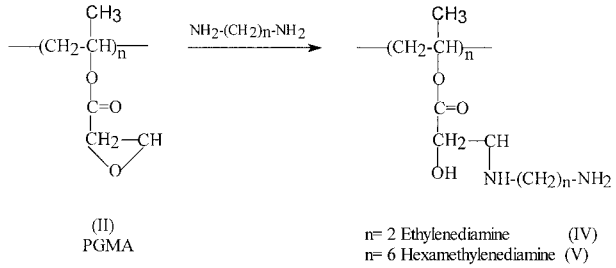
2.7.1. Chloroacetylation of Hydrolyzed Poly(Glycidyl Methacrylate) (VI)

To a suspension of hydrolyzed poly(glycidyl methacrylate) (III) (9.0 g, 56.25 mmol) in 90 mL dioxane was added 18.0 mL (55.9 mmol) pyridine. The mixture was cooled in an ice–salt bath and

Table I Elemental Microanalyses for Polymers (IV–XIII)

Polymer Code	% Cl		% N		% C		% H	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
IV	—	—	13.8	6.6	53.4	51.5	8.9	8.5
V	—	—	10.8	10.6	60.4	58	10.0	9.8
VI	22.6	20.0	—	—	42.2	42.8	4.5	5.1
VII	20	7.4	7.8	6.3	44.0	45.0	5.6	5.5
VIII	17.2	4.2	6.8	7.0	49.6	51.8	6.8	7.1
XI	—	—	5.2	2.7	65.6	62.7	4.9	4.2
XII	—	—	7.7	6.3	66.1	62.5	5.6	5.2
XIII	—	—	6.9	4.7	67.9	67.7	6.4	5.9

Calc, calculated value.

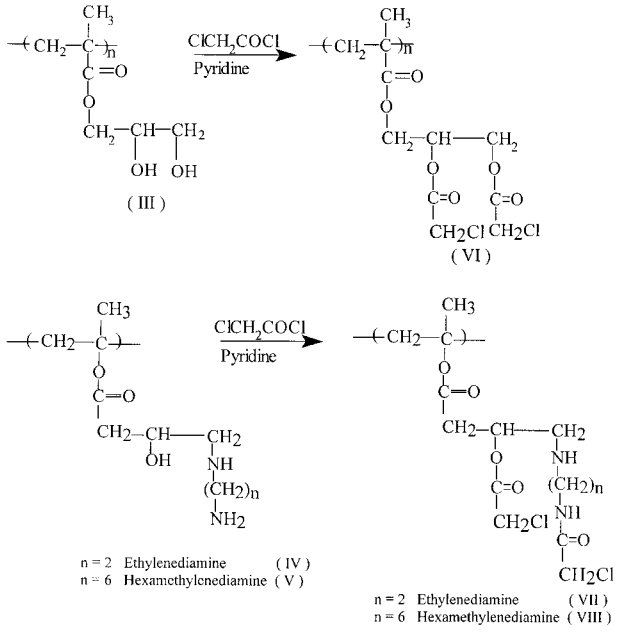


Scheme 2 Chemical modification of poly(glycidyl methacrylate) using various diamines.

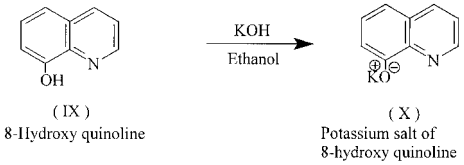
17.8 mL (55.9 mmol) chloroacetyl chloride was added dropwise with cooling and stirring. The reaction mixture was stirred overnight at room temperature for 3 days. The resin was filtered and washed with water and dioxane (Scheme 3). Washing with dioxane continued until no acid was indicated using an acidity test. The product (VI) was characterized by elemental microanalysis and IR spectroscopy.

Chloroacetylation of Polymer (IV)

To a suspension of polymer (IV) (9.0 g, 56.25 mmol) in 90 mL chloroform was added 18.0 mL (55.9 mmol) pyridine. The mixture was cooled in an ice–salt bath and 17.8 mL (55.9 mmol) chloroacetyl chloride was added dropwise with cooling



Scheme 3 Chloroacetylation of polymers (III–V) using chloroacetyl chloride.



Scheme 4 Formation of potassium salt of 8-hydroxyquinoline.

and stirring. The reaction mixture was stirred overnight at room temperature for 3 days. The resin that formed was filtered off and washed with water and chloroform (Scheme 3). Washing with chloroform was continued until no acid was indicated using the acidity test. The product (VII) was characterized by elemental microanalysis and IR spectroscopy.

Chloroacetylation of Polymer (V)

To a suspension of polymer (V) (9.0 g, 56.25 mmol) in 90 mL chloroform was added 18.0 mL (55.9 mmol) pyridine. The mixture was cooled in an ice–salt bath and 17.8 mL (55.9 mmol) chloroacetyl chloride was added dropwise with cooling and stirring. The reaction mixture was stirred overnight at room temperature for 3 days. The resin that formed was filtered off and washed with water and chloroform (Scheme 3). Washing with chloroform was continued until no acid was indicated using the acidity test. The product (VIII) was characterized by elemental microanalysis and IR spectroscopy.

Formation of Potassium 8-Quinolinolate (X)

The procedure was as described previously.[12] 8-Hydroxyquinoline (IX) (10 g, 68.97 mmol) was dissolved in 150 mL ethanol; ethanolic potassium hydroxide (4.25 g, 75.89 mmol) was then added. The reaction mixture was refluxed with stirring for 2 h; stirring continued at room temperature overnight. Ethanol was evaporated on a rotary evaporator. The product was washed with diethyl ether, filtered and dried under vacuum at 30°C for 24 h. The yield was 11.5 g (91.2%) (Scheme 4). The product (X) was characterized by elemental microanalysis and IR spectroscopy.

Immobilization of Potassium 8-Quinolinolate (X) onto the Chloroacetylated Polymers

Immobilization of Potassium 8-Quinolinolate (X) onto the Chloroacetylated Poly(Glycidyl Methacrylate) (VI)

Chloroacetylated poly(glycidyl methacrylate) (VI) (2 g, 6.39 mmol) was suspended in 200 mL DMF.

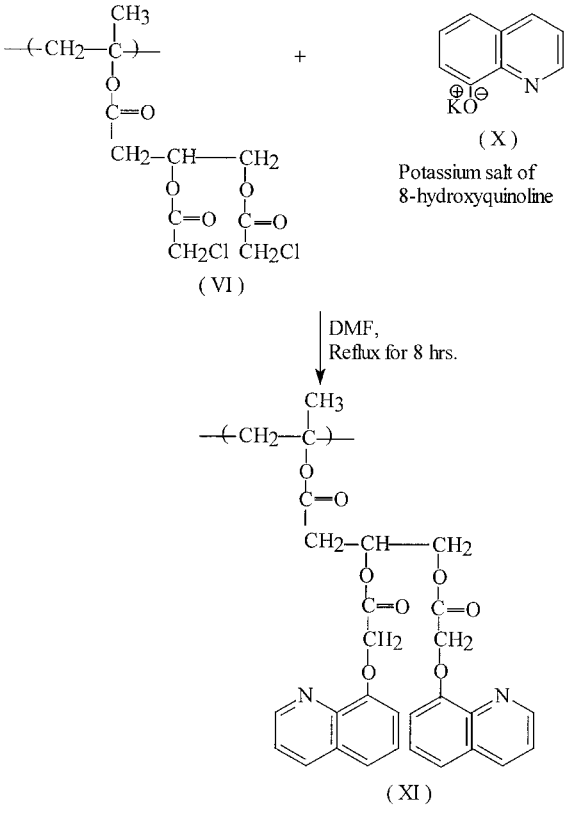
Potassium 8-quinolinolate (X) (2.34 g, 12.78 mmol) was dissolved in DMF and added dropwise to a boiling solution of chloroacetylated poly(glycidyl methacrylate) (VI). The reaction mixture was refluxed for 8 h. The product was filtered and dried under vacuum at 30°C. The yield was 1.6 g (47.2%) (Scheme 5). The product (XI) was characterized by elemental microanalysis and IR spectroscopy.

Immobilization of Potassium 8-Quinolinolate (X) onto the Chloroacetylated Polymer (VII)

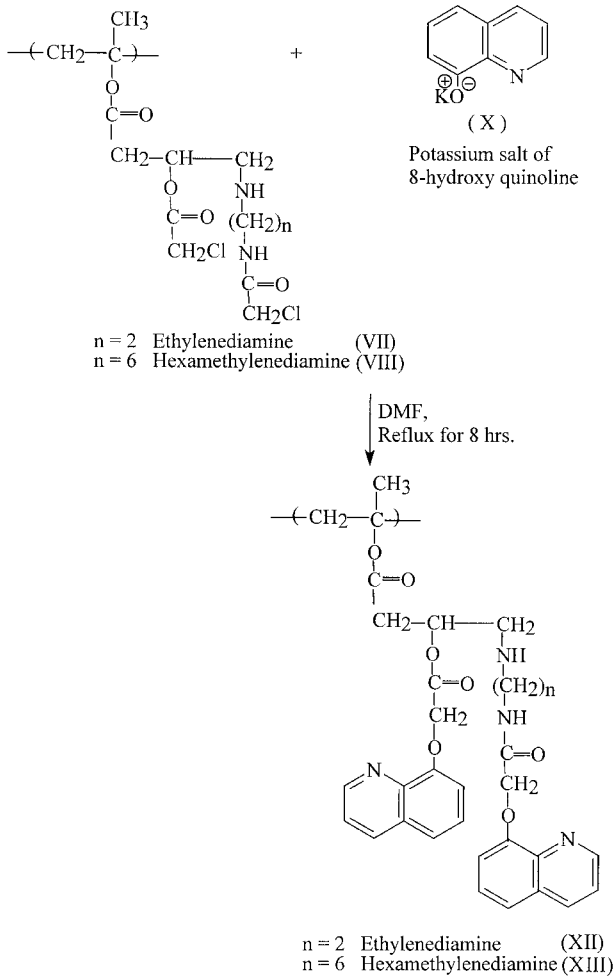
Chloroacetylated polymer (VII) (2 g, 6.39 mmol) was suspended in 200 mL DMF. Potassium 8-quinolinolate (X) (2.34 g, 12.78 mmol) was dissolved in DMF and added dropwise to the boiling solution of chloroacetylated polymer (VII). The reaction mixture was refluxed for 8 h. The product was filtered and dried under vacuum at 30°C. The yield was 3.4 g (72.2%) (Scheme 6). The product (XII) was characterized by elemental microanalysis and IR spectroscopy.

Immobilization of Potassium 8-Quinolinolate (X) onto the Chloroacetylated Polymer (VIII)

Chloroacetylated polymer (VIII) (2 g, 6.39 mmol) was suspended in 200 mL DMF. Potassium



Scheme 5 Immobilization of potassium salt of 8-hydroxyquinoline onto polymer (VI).



Scheme 6 Immobilization of potassium salt of 8-hydroxyquinoline onto polymers (VII, VIII).

8-quinolinolate (X) (2.34 g, 12.78 mmol) was dissolved in DMF and added dropwise to the boiling solution of chloroacetylated polymer (VIII). The reaction mixture was refluxed for 8 h. The product (XI) was filtered and dried under vacuum at 30°C. The yield was 2.2 g (59.0%) (Scheme 6). The product (XIII) was characterized by elemental microanalysis and IR spectroscopy.

Evaluation of Antimicrobial Activity

The antimicrobial spectrum of the prepared polymers was determined against the above test bacteria on powdery samples by the cut plug method[13] on nutrient agar, which contained per liter 10 g peptone, 5 g NaCl; 5 g beef extract; and 20 g agar; pH 7. The assay plates were seeded with the test bacteria. After solidification, the wells were made and filled with 20 mg powdery polymer. The plates were in-

cubated at 30°C for 24 h, after which the diameters of inhibition zones were measured; the compounds, which produced inhibition zones, were further assayed at different concentrations in aqueous suspension to quantify their inhibitory effects.

A loopful of each culture was placed in 10 mL of broth or 10× dilute broth, which was then incubated overnight at 30°C. At this stage, the cultures of the test bacteria contained 1.6×10^5 cells/mL, *B. subtilis,* 1×10^4 cells/ml, *E. coli.* The test fungus contained 5.4×10^4 fungal spores/mL, which was used for the antimicrobial test.

Because the polymers were not completely soluble in either water or different solvents, they were suspended in sterile 10 times dilution of the above nutrient broth medium to make 0.05 g/mL, and 0.5 mL were transferred to flasks containing sterile 10× dilute nutrient broth to give the final concentrations 10, 5, and 2.5 mg/mL. Exposure of the bacterial cells to biocides started when 0.2 mL of the culture containing 6×10^4 bacterial cells/ml or 8×10^2 fungal spores/mL was added to 10 mL of the above biocide suspension, which was preequilibrated and shaken at 30°C as recommended by Nokashima et al.[14] At the same time, 0.2 mL of the same culture was added to 10 mL of 10× dilute nutrient broth, decimal dilutions were prepared, and the starting number of cells was counted by the spread-plate method. After 24-h contact, 1.0-mL portions were removed and mixed with 9.0 mL of 10× dilute nutrient broth. Decimal serial dilutions were then prepared from these dilutions, and 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 48 h for fungi. The ratio was carried out in triplicate each time. The ratio of the colony numbers for the media containing the polymer (M) and those without these compounds (C) was taken as surviving cell number; this value was used to evaluate antimicrobial activity. Each experiment was repeated three times and the average was taken, with an error of approximately 3%.

RESULTS AND DISCUSSION

Synthesis of Linear Poly(Glycidyl Methacrylate) (II)

Polymerization of glycidyl methacrylate was carried out in chloroform at 60°C, with AIBN as the initiator (Scheme 1). The polymer was precipitated into a large excess of diethyl ether. The ratio of chloroform to ether should be at least 1 : 5 to afford good precipitation of the product.[11] Trying to polymerize the glycidyl methacrylate by bulk polymerization technique, the product was a crosslinked homopolymer insoluble in ordinary solvents. This is in good agreement with the results obtained earlier.[14]

The ^{1}H-NMR spectrum showed peaks at δ 5.62 ppm (—CH_2Cl), 1.96 ppm (—CH_3), 4.2 ppm (—O—CH_2—CH), 3.26 ppm (CH-epoxide), 2.75 ppm (CH_2-epoxide). IR spectrum of the product (II) showed peaks at 2,800 cm^{-1} (CH), 1730 cm^{-1} (C═O), 1,450 cm^{-1} (CH_3), 1,250 cm^{-1} (epoxide).

Acid Hydrolysis of the Epoxide Group of Linear Poly(Glycidyl Methacrylate) (III)

The epoxide groups of the polymer were hydrolyzed when treated with dilute sulfuric acid. The IR spectra showed that all the epoxide groups were completely hydrolyzed. 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 0.1M sulfuric acid. When the reaction was carried out at room temperature, hydrolysis was not completed (Scheme 1).

The IR spectrum of the product (III) showed peaks at 2,800 cm^{-1} (CH), 1,450 cm^{-1} and 3,000 cm^{-1} (CH_3), 3,500 cm^{-1} (OH), and 1,100 cm^{-1} (sec OH).

Amination of Poly(Glycidyl Methacrylate (II) With Ethylenediamine (IV) and Hexamethylenediamine (V)

Epoxide reacts with amines and ammonia.[16] PGMA was reacted with ethylenediamine and hexamethylenediamine at 40°C in chloroform for 7 h. Their reactions are described in scheme 3. The ratio of epoxide containing polymer to the diamine should be 1 : 3. The diamine should be at least threefold of the polymer. It was noted that if the ratio of the diamine is less than three times that of the polymer, some crosslinking occurs. Therefore, it is recommended to use excessive diamine. It is also noted that the reaction of the epoxide containing polymer should take place by the addition of the polymer to the excess diamines, and not the reverse, to avoid the problem of crosslinking.

The aminated polymers were characterized by elemental analysis, indicating that the extent of

amination of poly(glycidyl methacrylate) with hexamethylenediamine was higher than in the case of amination with ethylenediamine (Table I). This may be attributed to the chain length of the hexamethylenediamine, which is higher than that of ethylenediamine.

The IR spectrum of the product (IV) showed peak at 3,400 cm^{-1} (NH, NH_2) and the disappearance of peak at 1,250 cm^{-1} (epoxide group). The IR spectrum of product (V) showed peak at 3,413 cm^{-1} (NH, NH_2) and the disappearance of epoxide peak at 1,250 cm^{-1}.

Modification of Polymers (III–V) Using Chloroacetyl Chloride

Chloroacetylation of Hydrolyzed Poly(Glycidyl Methacrylate) (VI)

Introduction of chloroacetyl groups was readily achieved by treatment of the diol polymer with chloroacetyl chloride in the presence of pyridine in dry dioxane under dehydrating conditions (Scheme 3).

The analysis of the polymer by infrared spectroscopy confirmed that the acetylation reaction was completed. The product was characterized by elemental microanalysis, which showed Cl% (found) 20.0% (Table I). This indicated a high percentage of conversion of the diol polymer to the chloroacetylated derivative. The same conclusion was confirmed by the IR studies. The IR spectrum of the product (VI) showed peaks at 750 cm^{-1} and 1,260 cm^{-1} (—CH_2Cl), 1,750 cm^{-1} (—C═O), 2,800 cm^{-1} (CH), (CH_2), and 1,450 cm^{-1} and 3,060 cm^{-1} (CH_3).

Chloroacetylation of Aminated Polymers

The aminated polymers (IV, V) were chloroacetylated aiming at the formation of chloromethyl group at the side-chain of the polymers. The chloromethylation of the amino group was carried out through acetylation by treatment with chloroacetyl chloride in the presence of pyridine as the base.

The halogen analysis in the produced polymeric products (VII, VIII) showed the extent of conversion as indicated in (Table I). IR spectra of the chloroacetylated polymer (VII) showed peaks at 1,255.5 cm^{-1} (—CH_2Cl), 3,402 cm^{-1} (NH), 1,722 cm^{-1} (C═O), 1,633 cm^{-1} (amide group NHCO). The IR spectrum of product (VIII) showed peaks at 3,402 cm^{-1} (NH), 2,902 cm^{-1} (CH, CH_2), 1,633 cm^{-1} (amide group NHCO), 1,733 cm^{-1} (C═O), and at 1,260 cm^{-1} (—CH_2Cl).

Immobilization of Potassium 8-Quinolinolate (X) onto the Chloroacetylated Polymers (VI–VIII)

Functionalization of the chloroacetylated polymers (VI–VIII) with potassium 8-quinolinoxyl groups was carried out by refluxing the potassium 8-quinolinolate with the chloroacetylated polymers (VI–VIII) for 8 h. The level of functionalization was confirmed by the elemental analysis and IR spectroscopy. From the nitrogen content found by elemental analysis, the conversion percentage was calculated to be 52% for polymer (XI), 82% for polymer (XII), and 68.2% for polymer (XIII).

The IR spectrum of product (XI) showed peaks at 1,600 cm^{-1} (strong peak), 1,455 cm^{-1} (weak peak) (hydroxyquinoline moiety), 1391 cm^{-1}, 1461 cm^{-1} (C—CH_3), 3,000 cm^{-1} (CH aromatic), and 2913 cm^{-1} (CH, CH_2 aliphatic).

The IR spectrum for product (XII) showed peaks at 1,566 cm^{-1}, 1,470 cm^{-1} (hydroxyquinoline moiety), 1722 cm^{-1} (C═O), 3,000 cm^{-1} (CH aromatic), 3,400 cm^{-1} (NH), 1,461 cm^{-1} (C—CH_3). The IR spectrum for compound (XIII) showed peaks at 1,453 cm^{-1}, 1566 cm^{-1} (hydroxyquinoline moiety), 1,726 cm^{-1} (C═O), 3,428 cm^{-1} (NH), 2,927 cm^{-1} (CH aromatic).

Antimicrobial Assessment of Polymers (XI–XIII)

The antimicrobial activities of polymers containing 8-hydroxyquinoline moiety (XI–XIII) against *E. coli, Shigella* sp., *B. subtilis, B. cereus,* and *T. rubrum* were explored by the cut plug method and viable cell counting methods, as described under Materials and Methods.

The capability of the prepared polymers to inhibit the growth of the microorganisms tested on solid media is shown in (Table II). It was found that the diameter of the inhibition zone varied according to the space between the bioactive group and the polymer backbone and according to the test microorganism. The growth-inhibiting effect was quantitatively determined by ratio (M/C) of the surviving cell number. The antimicrobial activity of polymers (XI–XIII) is shown in figures 1–3. The antimicrobial activities of the three polymer samples against the selected bacteria were high. They killed 80–95% at a concentration of 2.5 mg/mL. However, a concentration of 5 mg/mL of polymers XII and XIII killed 100% of the tested bacteria. The antimicrobial activity of the polymers against *E. coli* varied from polymer to polymer. For example, using a concentration of 2.5 g/mL of polymer XIII killed 98% of the *E. coli* whereas using the same concentration of polymer

Table II Average Diameters of Inhibition Zones* (mm) Produced by 10.0-mg Powdery Polymers of Modified Poly(Glycidyl Methacrylate) Containing 8-Quinolinyl Against Different Test Bacteria After 24 h by the Cut Plug Method on Nutrient Agar at 30°C

	Polymer		
Organisms	XI	XII	XIII
Escherichia coli	20	40	30
Shigella sp.	10	30	40
Salmonella typhae	10	30	45
Bacillus subtilis	15	20	25
Bacillus cereus	10	35	30

* Each experiment was repeated three times; the average was taken, with an error of 3%.

XII killed 80% of *E. coli.* Polymer XI was less effective against *E. coli* at this concentration compared with other polymers.

Increasing the concentrations from 2.5 mg/mL to 5 mg/mL of polymer XII and XIII killed 100% of the *E. coli,* whereas polymer XI killed 20% of *E. coli.* However, increasing the concentration of polymer XI to 10 mg/mL increased the percentage killed to 55. For the gram-positive bacteria *B. subtilis,* a concentration of 2.5 mg/mL of polymer XII was sufficient to kill 97%, whereas the same concentration of polymer XI and XIII killed 80% and 90% of *B. subtilis,* respectively.

For the fungus *T. rubrum,* polymer XI was found to be the most effective material among these polymers. A concentration of 2.5 mg/mL of polymer XI killed 80% of the *T. rubrum,* whereas polymers XII and XIII killed 68% and 35% of *T. rubrum,* respectively. However, increasing the concentration of polymer XII to 5 mg/mL killed all *T. rubrum* in the test, whereas increasing the concentration of polymers XI and XIII raised the percentage killed to 90% and 65%, respectively.

Generally, all three polymers were found to be active against the test microorganism. Polymer XI was found to be more active against the fungus *T. rubrum,* polymer XII was found more active against *B. subtilis,* and polymer XIII was found to be more active against *E. coli* even at low concentration (2.5 mg/mL). Comparing these results with the previous published results obtained under the same conditions and concentrations, we conclude in general, that the polymers of this work are more effective than the materials based on modification of poly(glycidyl methacrylate).[11]

Generally, the potency of inhibition varied according to the polymer and the test strain. Antimicrobial activity of the materials tested was selective toward certain microorganisms, obviously resulting from the structural affinity between the wall of the microorganism and the tested materi-

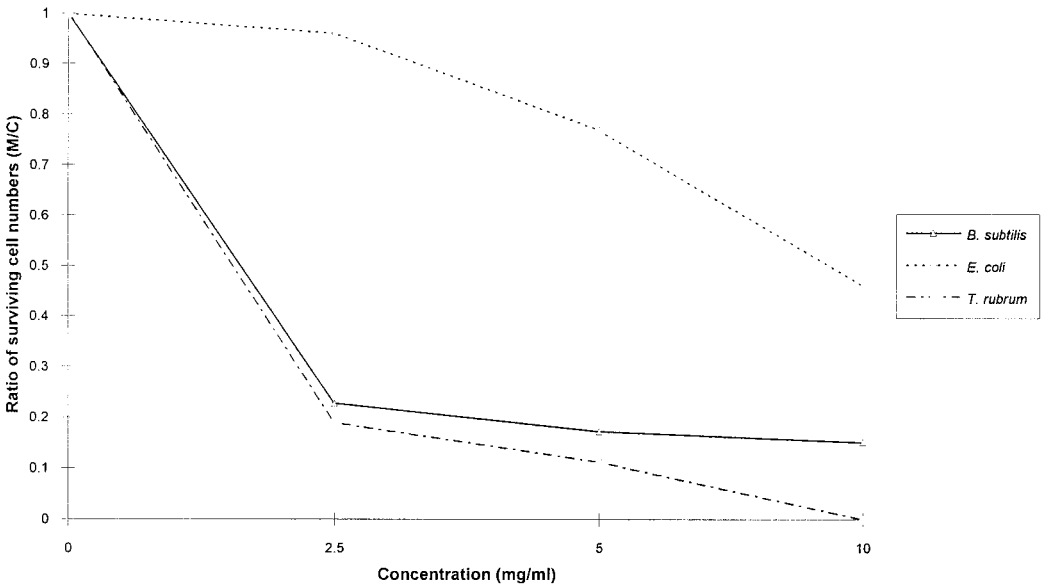


Figure 1 Growth inhibition of different concentration of polymer XI. Inoculation: 1.6 $\times$ 10^5 cells/ml, *B. subtilis;* 1 $\times$ 10^4 cells/ml *E. coli,* and 5.4 $\times$ 10^4 fungal spores/ml *T. rubrum.*

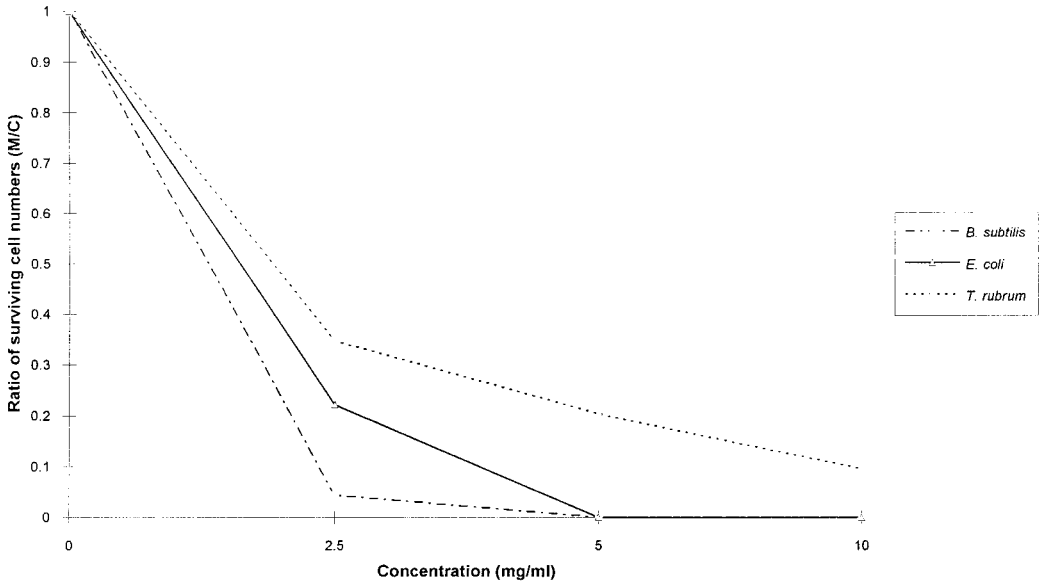


Figure 2 Growth inhibition of different concentration of polymer XII. Inoculation: 1.6 $\times$ 10^5 cells/ml, *B. subtilis;* 1 $\times$ 10^4 cells/ml *E. coli,* and 5.4 $\times$ 10^4 fungal spores/ml *T. rubrum.*

als. Similar results were reported previously using chitosan or derivatives as the materials.[8]

At the base of all antimicrobial chemotherapy lies the concept of selective toxicity. The necessary selectivity can be achieved in several ways: vulnerable targets within the microbe may be absent from the cells of the host; alternatively, the analogous targets within the host cells may be sufficiently different, or at least sufficiently inaccessible, for selective attack to be possible. The minute size and capacity for very rapid multiplication of bacteria ensure that they are structurally and metabolically very different from mammalian cells; theoretically, there are numerous

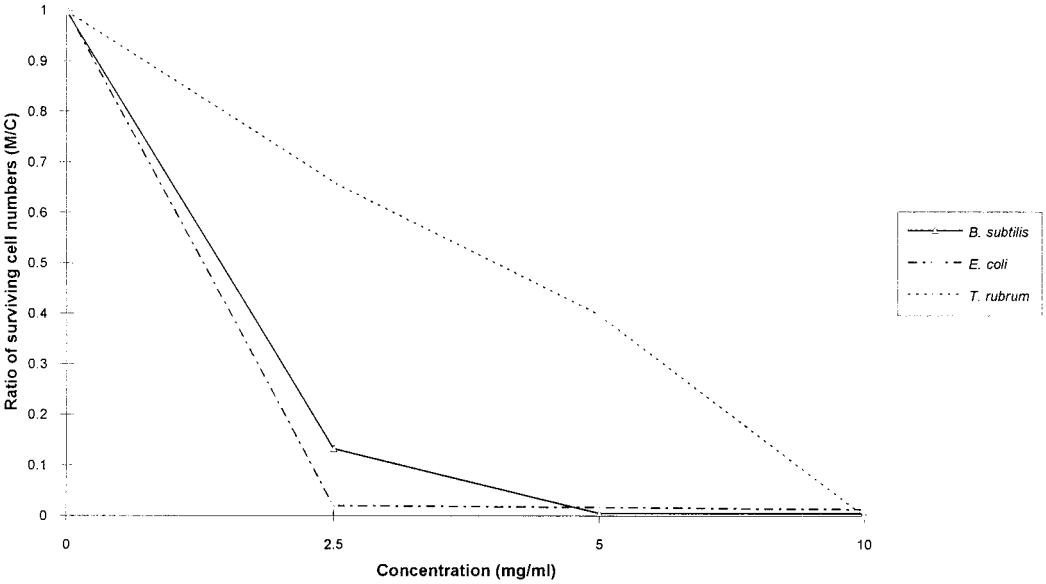


Figure 3 Growth inhibition of different concentration of polymer XIII. Inoculation: 1.6 $\times$ 10^5 cells/ml, *B. subtilis;* 1 $\times$ 10^4 cells/ml *E. coli,* and 5.4 $\times$ 10^4 fungal spores/ml *T. rubrum.*

ways in which bacteria can be selectively killed or disabled. In the event that, it turns out that only the bacterial cell wall is structurally unique, other subcellular structures, including the cytoplasmic membrane, ribosomes, and DNA, are built on the same pattern as those of mammalian cells. Fortunately, sufficient differences in construction and organization exist at these sites to make exploitation of the selective toxicity principle feasible.[17]

The mode of action is similar to that of cationic biocides, which is interpreted in terms of the following sequence of elementary processes[18–21]: (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. These compounds appear to interfere with construction of the bacterial cell wall, synthesis of protein, and replication and transcription of DNA. Access though the cytoplasmic membrane is usually achieved by simple diffusion or by active transport, for example, with aminoglycosides and tetracyclines.[17]

Generally, the mode of action of these can be interpreted on the basis of each elementary process described above; the same physiological events as in processes 1, 3, and 5 have been observed for the polycationic biocides.[22–25] Since the disruption process is believed to involve the interaction of the bound polymers with charged membrane,[23,26,27] it is expected that increasing the concentration of 8-hydroxyquinoline bound polymers will facilitate the binding process.

It is known that there is considerable difference in the structure of cell walls between gram-positive and gram-negative bacteria. In the case of gram-negative organisms, the antibiotic must also negotiate an outer membrane, consisting of a characteristic lipopolysaccharide–lipoprotein complex, which is responsible for preventing many antibiotics from reaching an otherwise sensitive intracellular target. This lipophilic outer membrane contains aqueous transmembrane channels which selectively allow passage of hydrophilic molecules depending on their molecular size and ionic charge.[22,28] Many antimicrobial agents use these channels to gain access to gram-negative organisms, although other pathways are used as well.[29] By contrast, gram-positive cells have a simple cell wall structure; the outside cytoplasmic membrane has only a rigid peptidoglycan layer. The peptidoglycan layer, although relatively thick, is composed of networks with numerous pores, which allow foreign molecules to come into the cell without difficulty.[23,28] Consequently, the overall activity would be determined by two factors: one is favored for polymers (processes 1, 3, and 4) and the other is not favored for the polymers (process 2).[23]

CONCLUSIONS

This work demonstrates that it is feasible to synthesize polymers containing 8-hydroxyquinoline. Three polymers were synthesized; their antimicrobial properties were evaluated using the cut plug method. These antimicrobial polymers showed high antimicrobial activity against *Escherichia coli* (gram-negative), *Bacillus subtilus* (gram-positive), and the fungus *Trichophyton.*

Generally, these polymers are very potent biocides against the selected organisms. More specifically, polymer (XII) killed about 95% (±3%) of *B. subtilis* at a concentration 2.5 mg/mL, whereas for the same organism, a concentration 2.5 mg/mL of a polymer XI, XIII killed about 80% and 85%, respectively. For *E. coli,* a concentration of 2.5 mg/mL of polymer XIII killed 98%, whereas the same concentration of polymer XI, XII killed about 80%. For the fungus *T. rubrum,* polymer XI was the most effective at a concentration of 2.5 mg/mL and killed about 80%, whereas polymers XII and XIII killed 65% and 35% at the same concentration respectively.

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