Biologically Active Polymers. IV. Synthesis and Antimicrobial Activity of Tartaric Acid Polyamides

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ABSTRACT: New bactericidal polyamides with quaternary ammonium or phosphonium salts were prepared, and their antimicrobial activities were explored. The polyamides were synthesized by the polycondensation of diethyl-*l*-tartrate or chloromethylated diethyl-*l*-tartrate with ethylenediamine in dry absolute ethanol. The polyamides were modified to yield polymers with either quaternary ammonium or phosphonium salts. The polymers were characterized with elemental microanalysis and ^{1}H-NMR and IR spectra. The antimicrobial activity of the polymers bearing onium salts was studied against Gram-negative bacteria (*Escherichia coli*, *Pseudomones aeruginosa*, *Shigella* sp., and *Salmonella typhae*), Gram-positive bacteria (*Bacillus subtilis* and *Bacillus cereus*), and a fungus (*Trichophytum rubrum*) by the cut-plug and viable-cell-count methods. Although all the polymers showed high antibacterial activity, some had no antifungal activity. The tributyl phosphonium salt of the polyamide was more effective against both Gram-negative and Gram-positive bacteria than the triethyl ammonium and triphenyl phosphonium salts of the polyamide. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 4780–4790, 2006

Key words: biological applications of polymers; polyamides; Polycondensation; antimicrobial

INTRODUCTION

Antimicrobial agents are used for many applications, such as water sterilization, drugs, soil sterilization, biomedical-device sterilization, and prevention of the microbial contamination of shipboard, water-compensated, and hydration fuel tanks.[1–4] They are also in common use in the areas of health care and hygienic applications such as sterile bandages and clothing (e.g., antimicrobial surgical gowns and antifungal athletic socks).[5] Furthermore, antimicrobial agents are commonly used in coating of surfaces such as ship hulls, shower walls, and many kinds of tubing to minimize the problems of biofouling.[6]

However, these materials have the problems of residual toxicity of the agents, even when suitable amounts of the agents are used.[4,7] Also, their protection is short-lived because of the difficulty in controlling the rate of diffusion.[4,8] Methyl bromide is commonly used in agricultural fields for soil sterilization in primary fumigants to control nematodes, weeds, and fungi. However, there is a lot of environmental concern because it destroys the stratospheric ozone layer: methyl bromide releases bromine, which is believed to be 40 times more efficient in breaking down stratospheric ozone on a per atom basis.[9] To prevent the disadvantages that accompany antimicrobial agents, such as residual toxicity and environmental damage, recently such reagents have been bonded to polymeric materials. Polymeric antimicrobial agents have become a subject of intense research because of their promising advantages over monomeric forms.[10,11]

In this study, we selected polyamides based on tartaric acid, which is widely distributed in nature, is classified as a fruit acid, and occurs in many fruits.[12] Also, polymers based on tartaric acid are biodegradable, and this is a desirable property for many applications.[13,14] We report the synthesis of polyamides based on diethyl-*l*-tartrate (**I**) or chloromethylated **I** and the modification of the polyamides to produce polyamides with either quaternary ammonium or phosphonium salts. The antimicrobial activities of these modified polyamides were also screened.

EXPERIMENTAL

Materials and instruments

I, chloroacetyl chloride, tributylphosphine, and triphenylphosphine were purchased from Aldrich (Aldrich, Milwaukee, WI) and were used without further

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purification. Ethylenediamine was purchased from Aldrich and was distilled before use (bp = 118°C). Triethylamine was the purest grade available from Merck–Schuchardt (Merck-Schuchardt, Hhenbrun, Germany) and was used without further purification. All solvents were dried and distilled before use.

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). IR spectra were recorded on a PerkinElmer 1430 ratio recording IR spectrophotometer (Perkin Elmer, Wellesley, MA) with KBr pellets. ^{1}H-NMR spectra were recorded on a JEOL JNM-PM X90 Si-NMR spectroscopy instrument (Microanalysis Center). Thin-layer chromatography (TLC) was carried out with silica-gel-precoated plastic sheets containing a Multib fluorescent indicator (UV-254/366 NM) and supplied by SAN Gabriel (SAN Gabriel, CA).

Test microorganisms

These included Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* sp., and *Salmonella typhae*) and Gram-positive bacteria (*Bacillus subtilis* and *Bacillus cereus*). The fungus *Trichophytum rubrum* represented dermatophyte fungi. Bacteria were maintained on the nutrient agar, and the fungus was maintained on Subouroud agar slopes.

Chloroacetylation of I

To **I** (15.46 g, 75.05 mmol), chloroacetyl chloride (29.9 mL, 42.4 g, 375.25 mmol) was added dropwise with a dropping funnel. The reaction mixture was heated under dry conditions at 140°C for about 24 h. The viscous product was dissolved in benzene (100 mL) and washed with distilled water (3 × 100 mL) to remove excess chloroacetyl chloride. The benzene solution was dried with anhydrous magnesium sulfate, and charcoal was used for further purification. The product, chloroacetylated diethyl-*l*-tartrate (**II**), was dried *in vacuo* at room temperature to give 25.0 g (92.8% yield) as a viscous oil (Scheme 1). **II** was characterized with elemental microanalysis and ^{1}H-NMR and IR spectroscopy.

Polymer synthesis and modification

Polycondensation of II with ethylenediamine [poly(chloroacetylated diethyl-*l*-tartrate-*co*-ethylenediamine) (III)]

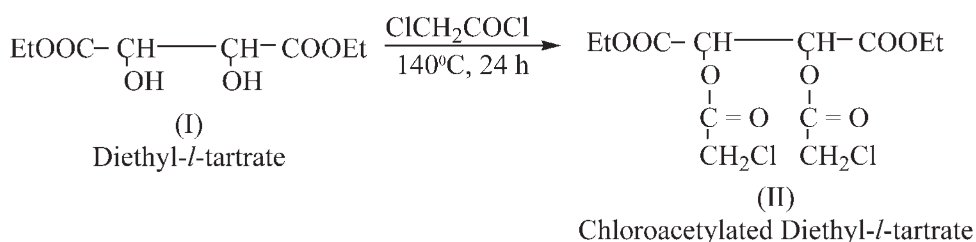
The procedure was performed as described previously.[15] To **II** (10.40 g, 28.97 mmol) in a 100-mL, round-bottom flask, ethylenediamine (1.74 g, 28.97 mmol) was added. The reaction mixture was stirred at room temperature for 3 days and was heated at 60°C for 4 h. The product (**III**) was filtered off and washed with ethanol. **III** was dried *in vacuo* at 30°C overnight to give 2.5 g (26.4% yield; Scheme 2). **III** was characterized with elemental analysis and IR spectroscopy.

Polycondensation of I with ethylenediamine [poly(diethyl-*l*-tartrate-*co*-ethylenediamine) (IV)]

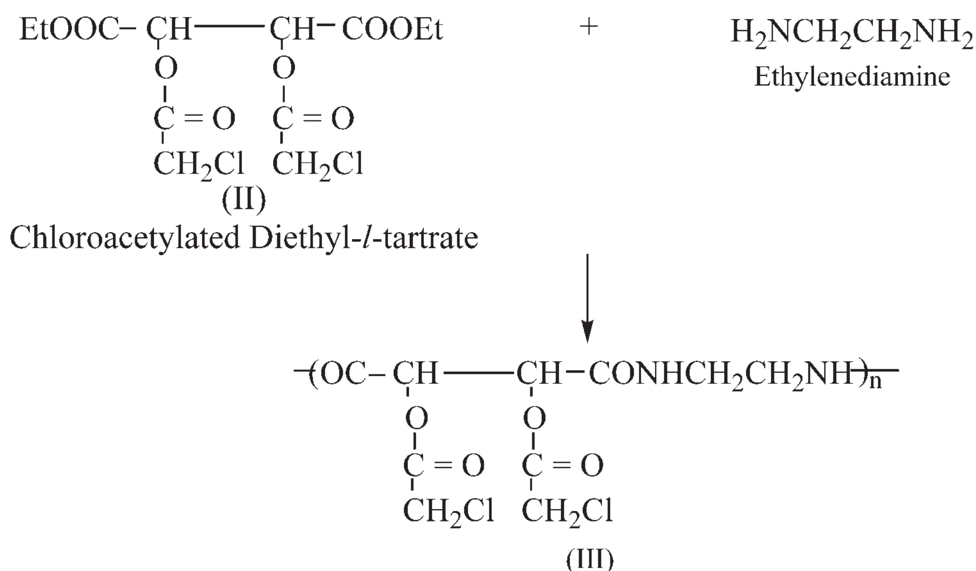
To **I** (20.00 g, 97.08 mmol) in a 100-mL, round-bottom flask, ethylenediamine (5.82 g, 97.08 mmol) was added. The mixture was stirred at room temperature for 4 h. The product (**IV**) was filtered off and washed with ethanol. **IV** was dried *in vacuo* at 30°C for 2 days to give 16.89 g (100.0% yield), as indicated in Scheme 3. **IV** was characterized with elemental analysis and ^{1}H-NMR and IR spectroscopy.

Chloroacetylation of polytartaramide (V)

To a suspension of polyamide **IV** (6.00 g, 34.48 mmol) in dry chloroform, 16.7 mL of pyridine (16.34 g, 206.89 mmol) was added. The reaction mixture was cooled in an ice–salt bath, and 16.3 mL of chloroacetyl chloride (23.17 g, 206.89 mmol) was added to the cooled, stirred reaction mixture. The reaction mixture was stirred at room temperature for 4 days. The resin that formed ($\mathbf{V_a}$) was filtered off and washed with chloroform and was dried *in vacuo* to give 2.5 g of a product that contained 12.3% Cl. Chloroform was evaporated on a rotary evaporator from the filtrate containing the highly chloroacetylated product, and the residue was added to stirred distilled water. The precipitated polymer was filtered off and washed several times with distilled water. The product [modified poly(diethyl-*l*-tartrate-*co*-ethylenediamine) ($\mathbf{V_b}$)] was dried *in vacuo* at 30°C for 2 days to give 11.0 g (97.6% yield; Scheme 4). $\mathbf{V_b}$ was characterized with elemental



Scheme 1 Chloroacetylation of **I**.



Scheme 2 Polycondensation of **II** with ethylenediamine.

microanalysis and IR spectroscopy and was used for further work.

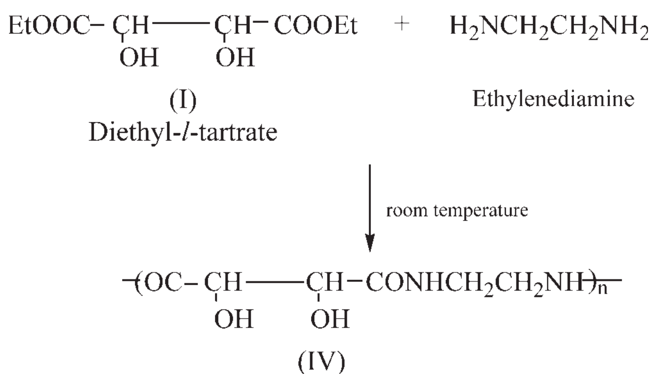
Immobilization of phosphonium and ammonium salts onto chloroacetylated polyamides III and V_b

Triethyl ammonium salt of poly(chloroacetylated diethyl-*l*-tartrate-*co*-ethylenediamine) (VI)

To a stirred suspension of polymer **III** (2.00 g, 6.12 mmol) in 20 mL of dry benzene, triethylamine (3.4 mL, 2.47 g, 24.46 mmol) was added. The reaction mixture was refluxed with stirring for 5 days. The product (**VI**) was filtered off, washed with benzene, and dried *in vacuo* at 30°C overnight to yield 2.5 g (77.3% yield; Scheme 5). **VI** was characterized with elemental analysis and ^{1}H-NMR and IR spectroscopy.

Triphenyl phosphonium salt of poly(chloroacetylated diethyl-*l*-tartrate-*co*-ethylenediamine) (VII)

The title compound was prepared from polymer **III** and triphenylphosphine similarly to the procedure described for **VI** with the following quantities: 2.00 g (6.12 mmol) of polymer **III** and 6.41 g (24.46 mmol) of triphenylphosphine in 20 mL of dry benzene. The yield was 3.7 g (71.2% yield), as indicated in Scheme 5. The product (**VII**) was characterized with elemental analysis and ^{1}H-NMR and IR spectroscopy.



Scheme 3 Polycondensation of **I** with ethylenediamine.

Tributyl phosphonium salt of poly(chloroacetylated diethyl-*l*-tartrate-*co*-ethylenediamine) (VIII)

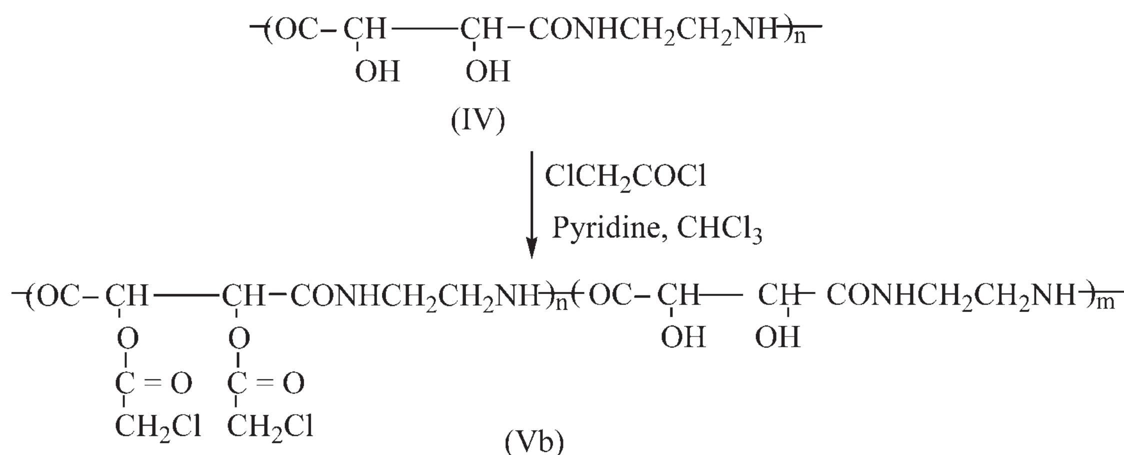
The title compound was prepared from polymer **III** and tributylphosphine similarly to product **VI** with the following quantities: 2.0 g (6.12 mmol) of **III** and 6.1 mL (4.94 g, 24.46 mmol) of tributylphosphine in 20 mL of dry benzene. The yield was 4.0 g (89.5% yield), as indicated in Scheme 5. The product (**VIII**) was characterized with elemental analysis and ^{1}H-NMR and IR spectroscopy.

Triethyl ammonium salt of modified poly(diethyl-*l*-tartrate-*co*-ethylenediamine) (IX)

The title compound was prepared from polymer $\mathbf{V_b}$ and triethylamine similarly to product **VI** with the following quantities: 2.0 g (6.12 mmol) of chloroacetylated polyamide $\mathbf{V_b}$ and 5.1 mL (3.7 g, 36.69 mmol) of triethylamine in 20 mL of dry benzene. The yield was 2.7 g (83.3% yield), as indicated in Scheme 6. The product (**IX**) was characterized with elemental analysis and IR spectroscopy.

Triphenyl phosphonium salt of modified poly(diethyl-*l*-tartrate-*co*-ethylenediamine) (X)

The title compound was prepared from chloroacetylated polyamide $\mathbf{V_b}$ and triphenylphosphine similarly to **VI** with the following quantities: 2.0 g (6.12 mmol)



Scheme 4 Chloroacetylation of **IV**.

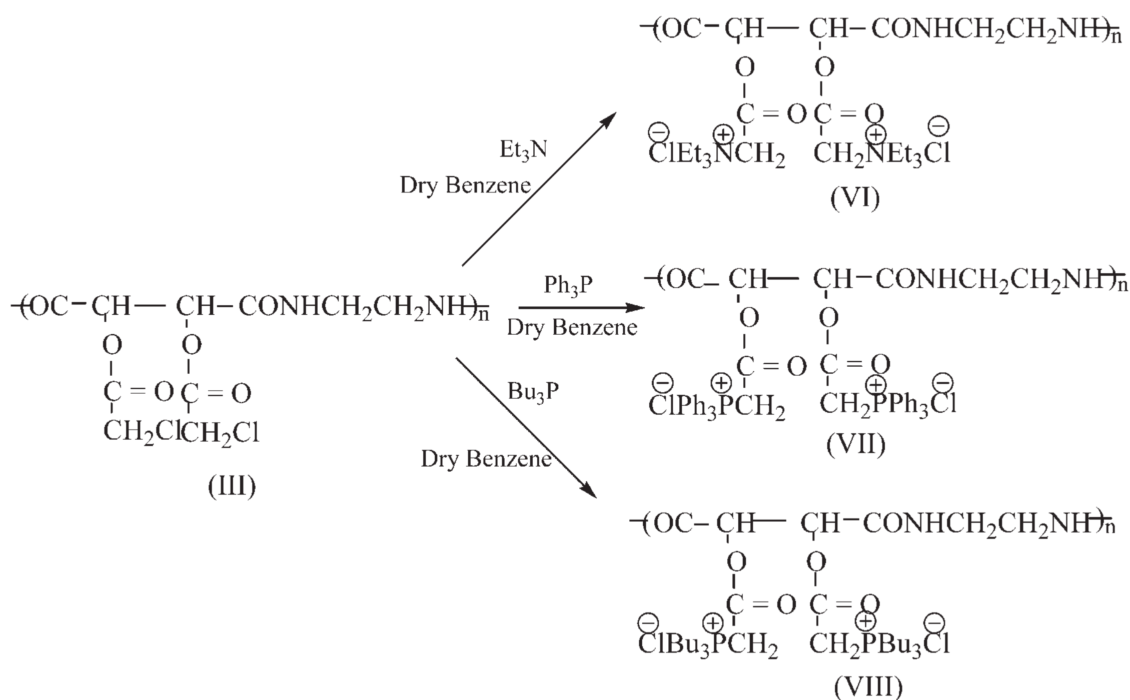
of $\mathbf{V_b}$ and 9.61 g (36.69 mmol) of triphenylphosphine in 20 mL of dry benzene. The yield was 3.2 g (61.5% yield; Scheme 6). The product (**X**) was characterized with elemental analysis and IR spectroscopy.

Tributyl phosphonium salt of modified poly(diethyl-*l*-tartrate-*co*-ethylenediamine) (**XI**)

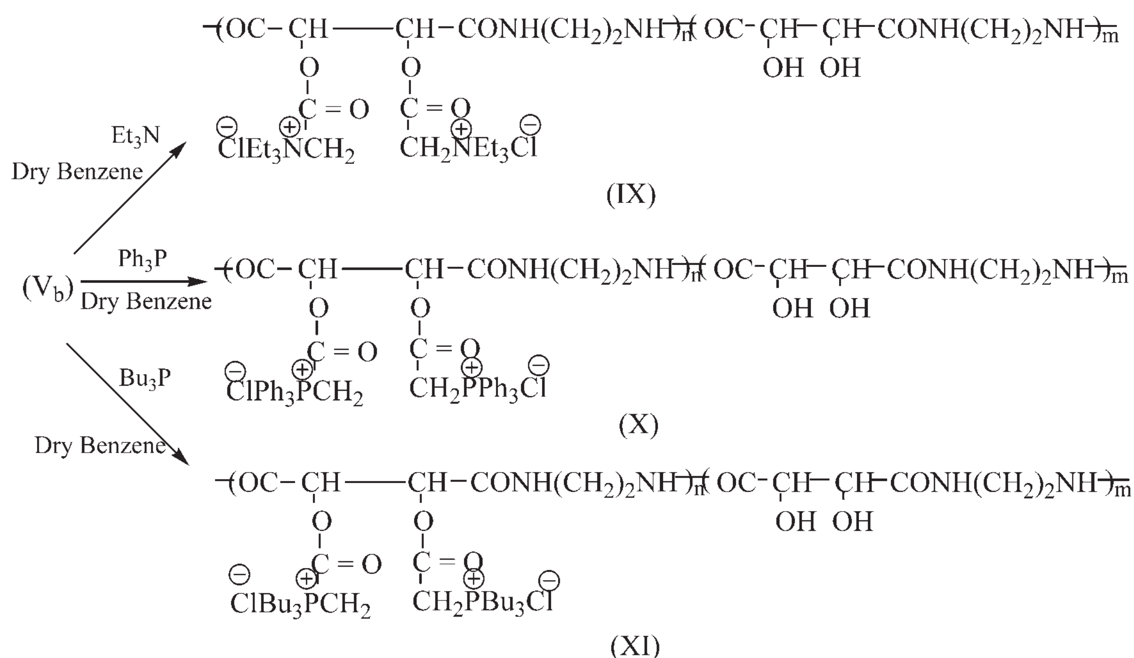
The title compound was prepared from chloroacetylated polyamide $\mathbf{V_b}$ and tributylphosphine similarly to product **VI** with the following quantities: 2.00 g (6.12 mmol) of $\mathbf{V_b}$ and 7.41 g (36.69 mmol) of tributylphosphine in 20 mL of dry benzene. The yield was 1.1 g (24.6% yield; Scheme 6). The product (**XI**) was characterized with elemental analysis and IR spectroscopy.

Evaluation of the antimicrobial activity

The antimicrobial spectrum of the prepared polymers was determined against the aforementioned test bacteria on powdery samples by the cut-plug method[16] on nutrient agar for bacteria that contained per liter 10 g of peptone, 5 g of NaCl, 5 g of beef extract, and 20 g of agar (pH 7); Subouroud agar was used for the fungus. The assay plates were seeded with the test bacteria; after solidification, the wells were made and filled with 20 mg of the 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 that produced the inhibition zones were further assayed at



Scheme 5 Immobilization of ammonium and phosphonium salts onto **III**.



Scheme 6 Immobilization of ammonium and phosphonium salts onto $\mathbf{V_b}$.

different concentrations in aqueous suspensions to quantify their inhibitory effects.

A loopful of each culture was placed in 10 mL of a 10-fold diluted broth, which was then incubated overnight at 30°C. At this stage, the cultures of the test bacteria contained 8.4, 6.96, and 7.2 × 104 cells/mL *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and the culture of the test fungus contained 4.3 × 105 fungal spores/mL, which were used for the antimicrobial tests for polymers **VI–VIII**. On the other hand, the cultures of the test bacteria used for the antimicrobial tests for polymers **IX–XI** contained 9, 1.76, and 3.37 × 104 cells/mL *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and the culture of the test fungus contained 1.29 × 105 spores/mL.

Because polymers **VI–VIII** were completely soluble in water, they were dissolved in the aforementioned nutrient broth medium (sterile and full-strength) to make the concentration of 0.05 g/mL, and 0.5 mL was transferred to flasks containing the sterile, full-strength nutrient broth to give the final concentrations of 10, 5, and 2.5 mg/mL. Polymers **IX–XI** were suspended in sterile, 10-fold dilutions of the aforementioned nutrient broth medium to make a concentration of 0.05 g/mL, and 0.5 mL was transferred to flasks containing the sterile, 10-fold diluted nutrient broth to give the final concentrations of 10, 5, and 2.5 mg/mL.

For polymers **VI–VIII**, the exposure of bacterial cells to biocides was started when 0.2 mL of the cultures containing 8.4, 6.96, and 7.2 × 104 cells/mL *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and 4.3 × 105 fungal spores/mL *T. rubrum* was added to 10 mL of the aforementioned biocide solution, which was pre-equilibrated and shaken at 30°C, as recommended by Nakashima et al.[17]

For polymers **IX–XI**, the exposure of bacterial cells to biocides was started when 0.2 mL of the culture containing 9, 1.76, and 3.37 × 104 cells/mL *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and 1.29 × 105 fungal spores/mL *T. rubrum* was added to 10 mL of the full-strength 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 full-strength nutrient broth; then, decimal serial dilutions were prepared from these dilutions, and the surviving bacteria or fungi were counted by the spread-plate method. After inoculation, the plates were incubated 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 and those without these compounds (M/C) was taken as the surviving cell number, and with this value, the antimicrobial activity was evaluated.

RESULTS AND DISCUSSION

Tartrate-based polyamides were selected on account of their biodegradability and the safety of using these polymer because they are natural and present in many fruits. This is a very important requirement, especially when these polymers are intended for use as antimicrobial drugs. Also, chloromethylated tartrate was selected as a monomer instead of the tar-

trate itself to increase the quaternary salt polymer content. This was due to the high conversion percentage of tartrate to chloromethyl tartrate by the chloromethylation process.

Chloroacetylation of I

The chloroacetylation of **I** was carried out by the reaction of **I** with excess chloroacetyl chloride at 140°C. The formation of the chloroacetylated monomer was in a relatively high yield and was followed by TLC with a mixture of methylene chloride and chloroform in a ratio of 3:1, respectively as the eluent. The product (**II**) was dissolved in benzene and washed several times with water to remove excess chloroacetyl chloride, and charcoal was also used for further purification (Scheme 1).

The elemental analysis of **II** showed a calculated Cl value of 19.74% (found: 18%), as listed in Table I, thus indicating a high conversion percentage of the hydroxyl groups of the monomer to the chloroacetylated derivative. This conclusion was also confirmed by the ^{1}H-NMR studies. The ^{1}H-NMR spectrum of **II** ($CDCl_3$) showed the following δ values: 7.43 (—CH—, singlet), 5.9 (—CH_2Cl, singlet), 4.3 (—CH_2—, quartet), and 1.3 ppm (—CH_3, triplet). Also, the IR spectrum confirmed the same conclusion, showing peaks at 791 and 1277 cm^{-1} (assigned to —CH_2Cl) and at 1756 cm^{-1} (assigned to —C=O).

Polymer synthesis and modification

Polycondensation of II with ethylenediamine (III)

The polycondensation of **II** with ethylenediamine was carried out in absolute ethanol at room temperature without the use of any catalyst, as shown in Scheme 2. The elemental analysis of product **III**, as listed in Table I, showed the following calculated values: 21.68% Cl (found: 16.8%) and 8.6% N (found: 8.6%). The elemental analysis proved that the reaction of the diamine not only occurred with the ethyl ester of the chloroacetylated diethyl tartrate but took place to a small extent on the chloromethyl ester. This was calculated to be 22.5%. This meant that the reaction of the ethylenediamine with the diethyl ester occurred to an extent of 77.5%. This was clear from the chlorine analysis, which showed a decrease in the found chlorine. However, in previous work, when this ester was the bulky group, it was proved by the synthesis of model compounds that the reaction occurred only with the diethyl ester.[15] The IR spectrum of **III** showed peaks at 1637 cm^{-1} (assigned to —CONH—) and at 784 and 1232 cm^{-1} (assigned to —CH_2Cl).

Polycondensation of I with ethylenediamine (IV)

The polycondensation of **I** with ethylenediamine was carried out in a high yield at 30°C without the use of any catalyst, as shown in Scheme 3. The produced functionalized polymer may be useful in many applications because of the presence of different functional groups (e.g., OH and NH). The elemental microanalysis was in good agreement with the calculated values, as listed in Table I.

The IR spectrum of product **III** showed the disappearance of the ethyl ester group and the appearance of peaks at 1658 cm^{-1} (assigned to —CONH—) and at 3353 cm^{-1} (assigned to *sec*-OH). The ^{1}H-NMR spectrum [dimethyl sulfoxide (DMSO)] of **III** showed peaks at δ values of 1.15 (CH_3—CH_2—, triplet), 3.2 (—CH_2—NH—, singlet), 3.7–4.5 (CH_3—CH_2—, —OH), and 7.7 ppm (—CH_2—NH_2, singlet).

Chloroacetylation of polyamide IV

The chloroacetylation of **IV** was carried out by the treatment of **IV** with chloroacetyl chloride in the presence of pyridine under dehydrating conditions, as shown in Scheme 4. The chloroacetylation resulted in two products. One had a low degree of acetylation (insoluble product), as indicated by the elemen-

TABLE I
Elemental Microanalyses for Monomer II and Polymers III–XI

Code	N (%)		P (%)		Cl (%)		C (%)		H (%)	
	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
II	—	—	—	—	19.74	18.0	40.1	39.4	4.49	5.0
III	8.6	9.6	—	—	21.68	16.8	36.7	33.4	3.7	5.0
IV	16.0	14.6	—	—	—	—	41.3	38.9	5.7	5.6
Vb	8.6	9.0	—	—	21.6	20.4	36.7	37.0	3.7	4.0
VI	10.6	11.4	—	—	13.4	13.5	49.0	44.0	7.99	8.0
VII	3.3	4.0	7.2	7.0	8.4	9.4	64.8	64.4	5.0	5.8
VIII	3.9	4.0	8.5	8.5	9.7	10.0	55.8	57.0	9.1	10.0
IX	10.6	8.0	—	—	13.4	13.0	49.0	48.9	7.9	7.3
X	3.3	4.0	7.2	2.2	8.4	9.0	64.8	57.5	5.0	4.7
XI	3.9	4.0	8.5	1.3	9.7	14.0	55.8	46.0	9.1	6.3

tal analysis, and this might have been the high-molecular-weight fraction of **IV**. This product represented 2.5% of the total polymer yield and was not further used in this work because of the low halogen content. The second product was highly chloroacetylated, as indicated by the elemental microanalysis; this fraction might be have been the lower molecular weight part. The last product represented 97.5% of the yield ($\mathbf{V_b}$). This indicated the high conversion of **IV** to chloroacetylated product $\mathbf{V_b}$, as listed in Table I.

The IR spectrum of product $\mathbf{V_b}$ showed peaks at 683 and 1237 cm^{-1} (assigned to —CH_2Cl), at 1728 cm^{-1} (assigned to —C=O), at 2859 cm^{-1} (assigned to —CH— and —CH_2—), and at 1436 and 2929 cm^{-1} (assigned to CH_3).

Immobilization of phosphonium and ammonium salts onto polymer III

Compounds **VI–VIII** were synthesized to study the influence of the nature of the active group on the antimicrobial activity of the polymer. The quaternization of the chloroacetylated polymers was carried out in benzene by the reaction of **III** with triethylamine, triphenylphosphine, and tributylphosphine at 80°C for 5 days, as shown in Scheme 5.

Generally, triethylamine, triphenylphosphine, and tributylphosphine were quaternized with the chloromethylated polymer at room temperature. However, to confirm the quaternization of the polymers, the reaction mixtures were heated at 80°C under refluxing benzene. This method favored the formation of phosphonium and ammonium salts. Triethylamine, triphenylphosphine, and tributylphosphine were present in a slight excess over the chloromethyl group because it was reported previously that under stoichiometric conditions, the yield of quaternization was limited.[4,18] The phosphorus and nitrogen contents of biocidal polymers **VI–VIII** were determined by elemental microanalysis, as listed in Table I, and the data were in good agreement with the calculated values.

Also, the IR spectra of polymers **VI–VIII** indicated the presence of peaks at 1540–1554 cm^{-1} (assigned to P—CH_2) and at 1434–1455 cm^{-1} (assigned to P—Ph), which confirmed the formation of ammonium and phosphonium salts.

The ^{1}H-NMR spectrum (DMSO) for polymer **VI** showed peaks at δ values of 1.25 (CH_3—CH_2—, triplet), 3.25 (—CH_2—NH—), 4 (CH_3—CH_2—) 8.3 (—CH_2—NH_2, quartet), and 9 ppm (—CH—, —CO—, singlet). Also, the ^{1}H-NMR spectrum (DMSO) for polymer **VII** showed peaks at δ values of 3.2 (—CH_2—NH—, singlet), 7.5 (C_6H_5—), and 8.9 ppm (—CH—, CO—, singlet). The ^{1}H-NMR spectrum (DMSO) for polymer **VIII** showed peaks at δ values of 0.85 (CH_3—CH_2—, triplet), 1.35 (CH_3—CH_2—CH_2—), 2.2 (CH_3—CH_2—CH_2—P), 3.2 (—CH_2—NH_2), and 9.1 ppm (—CH—, CO—, singlet).

Immobilization of phosphonium and ammonium salts onto chloroacetylated polyamide $\mathbf{V_b}$

Compounds **IX–XI** were synthesized to study the influence of the nature of the active group on the antimicrobial activity of the polymer. The quaternization of the chloroacetylated polymers was carried out in dry benzene by the reaction of $\mathbf{V_b}$ with triethylamine, triphenylphosphine, or tributylphosphine, as described previously for the quaternization of **III** (Scheme 6).

The phosphorus and nitrogen contents of biocidal polymers **IX–XI** were determined by elemental microanalysis, as shown in Table I, and the data were in good agreement with the calculated values.

Also, the IR spectra of polymers **IX–XI** indicated the presence of peaks at 1537–1443 cm^{-1} (P—CH_2) and at 1443–1401 cm^{-1} (assigned to P—Ph), which confirmed the formation of ammonium and phosphonium salts.

Antimicrobial assessment of polymers VI–VIII

Polymeric biocides are powerful candidates for polymeric drugs with a high activity, which can be ascribed to their characteristic nature of carrying the high local density of the active groups in the vicinity of the polymer chains.[19]

Many studies have been performed on the antibacterial activity of low-molecular-weight and polymeric quaternary ammonium salts.[19–21] The target size of the cationic biocides is the cell envelope of bacteria; thus, an increase in the molecular size due to polymerization, which may result in reduced permeability, is not regarded as a factor seriously affecting their activity. In fact, polycationic biocides have been shown to possess a higher activity against bacteria.[19,20,22]

In addition, polymeric biocides are particularly important because they possess promising advantages over monomeric forms.[10,11,23,24] Polycations with main-chain or pendant quaternary ammonium salts show outstandingly high antibacterial activity against Gram-positive and Gram-negative strains and exhibit a wide spectrum of antimicrobial activity.[2,23,25,26]

Also, it has been demonstrated that polycationic biocides with pendant phosphonium salts exhibit much higher antibacterial activity than the monomeric analogues, and the phosphonium polymers show a higher activity by about 2 orders of magnitude than a polymeric quaternary ammonium salt with the same structure except for the cationic part.[23,27]

To the best of our knowledge, the prepared compounds in these studies have not been reported in the literature, and it is safe to mention here that it is the first report to evaluate their antimicrobial activities.

TABLE II
Diameter of the Inhibition Zone (mm) Produced by 20.0 mg of Polymers VI–VIII

Organism	Polymer		
	VI	VII	VIII
E. coli	30	72	64
P. aeruginosa	44	80	90
Shigella sp.	44	30	30
S. typhae	10	72	92
B. subtilis	20	60	68
B. cereus	20	64	80

The antimicrobial activities of the salts of **III** compounds (**VI–VIII**) against *E. coli*, *P. aeruginosa*, *Shigella* sp., *B. subtilis*, *B. cereus*, and *T. rubrum* were explored by the cut-plug method and viable-cell-count methods, as described in the Experimental section.

The capability of the prepared polymers to inhibit the growth of the tested microorganisms on solid media is shown in Table II. The diameter of the inhibition zone varied according to the active group in the polymer and the test bacteria. The compounds inhibited the growth of the test bacteria on a solid agar medium, with **VIII** being the most effective on both Gram-negative and Gram-positive bacteria (the diameters of the inhibition zones ranged between 30 and 92 mm) after 24 h. This meant that **VIII** was better than other effective groups as an antimicrobial agent in comparison with salts from triethylamine and triphenylphosphine. The growth-inhibiting effect was quantitatively determined by M/C.

As shown in Figure 1, the growth-inhibitory effects of polymer **VI** differed among the bacterial and fungal strains. The inhibition became stronger in the order of *T. rubrum* < *E. coli* < *B. subtilis* < *P. aeruginosa*. The results showed also that the inhibitory effect increased with an increasing concentration of the polymer, except for the fungus *T. rubrum*, for which no effect was recorded. For example, when the concentration of polymer **VI** was increased from 2.5 to 10 mg/mL for *P. aeruginosa*, M/C decreased from 0.4 to 0.1; that is, a 10 mg/mL concentration killed up to 90% of *P. aeruginosa*. Figure 2 shows the inhibitory effect of polymer **VII**. The

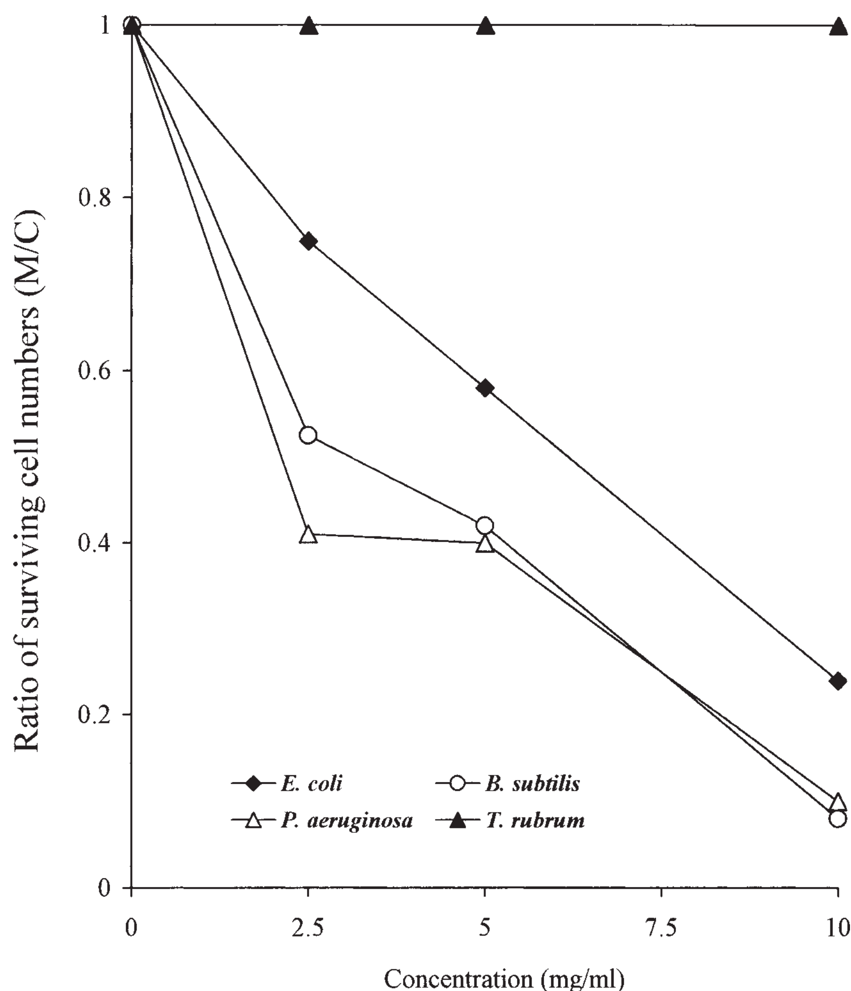


Figure 1 Growth inhibition of different concentrations of polymer **VI** (inoculation: 8.4, 6.96, and 7.2 × 10^4 cells/mL of *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and 4.3 × 10^5 fungal spores/mL of *T. rubrum*).

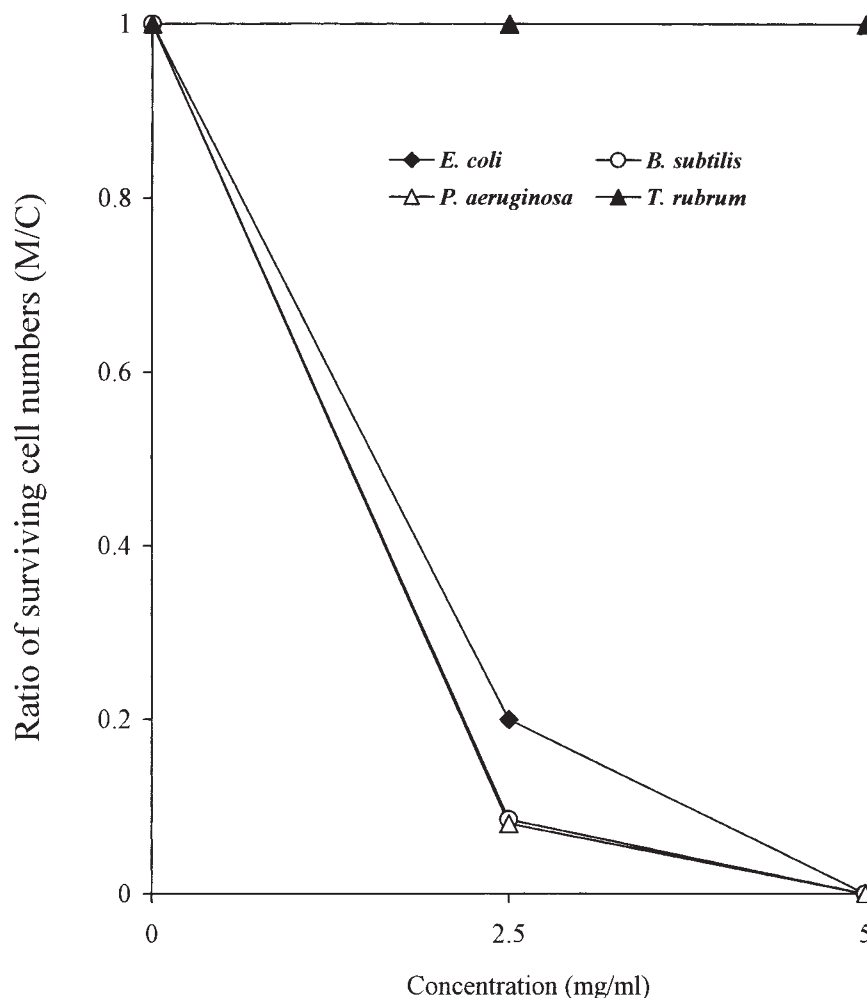


Figure 2 Growth inhibition of different concentrations of polymer **VII** (inoculation: 8.4, 6.96, and 7.2 $\times$ 10^4 cells/mL of *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and 4.3 $\times$ 10^5 fungal spores/mL of *T. rubrum*).

results showed that the inhibition became stronger in the order of *T. rubrum* < *B. subtilis* < *E. coli* < *P. aeruginosa*, and the inhibition was increased with an increasing concentration of the polymer, except for the fungus *T. rubrum*, for which no effect was recorded. Increasing the concentration of **VII** from 2.5 to 5.0 mg/mL caused 100% killing for *E. coli*, *P. aeruginosa*, and *B. subtilis*. However, no effect was observed for *T. rubrum* with increasing concentration. Figure 3 shows the inhibitory effect of polymer **VIII**. The results showed that the inhibition became stronger in the order of *T. rubrum* < *E. coli* < *B. subtilis* < *P. aeruginosa*, and the inhibition was increased with an increasing concentration of the polymer, except for the fungus *T. rubrum*, for which no effect was recorded. A concentration of 5.0 mg/mL for polymer **VIII** showed 100% killing for *P. aeruginosa*; 100% killing of *E. coli* and *B. subtilis* required a 10 mg/mL concentration of **VIII**. Generally, the potency of inhibition varied according to the polymer and the test strain, and these groups of compounds had no effect on the fungus *T. rubrum*. This may indicate that these compounds have different modes of action than others. This may lead to the suggestion that the mode of action of these compounds depends on the cell wall of the test organism.

Antibacterial assessment of polymers IX–XI

The antimicrobial activities of **IX–XI** against *E. coli*, *P. aeruginosa*, *Shigella* sp., *B. subtilis*, *B. cereus*, and *T. rubrum* were explored by the cut-plug method and viable-cell-count methods, as described in the Experimental section.

The capability of the prepared polymers to inhibit the growth of the tested microorganisms on solid media is shown in Table III. The compounds inhibited the growth of the test bacteria on a solid agar medium, with **IX** being the most effective against both Gram-negative and Gram-positive bacteria (the diameters of the inhibition zones ranged between 10 and 80 mm after 24 h. This meant that **IX** was better than other effective groups as an antimicrobial agent in comparison with triphenylphosphine or tributyl-

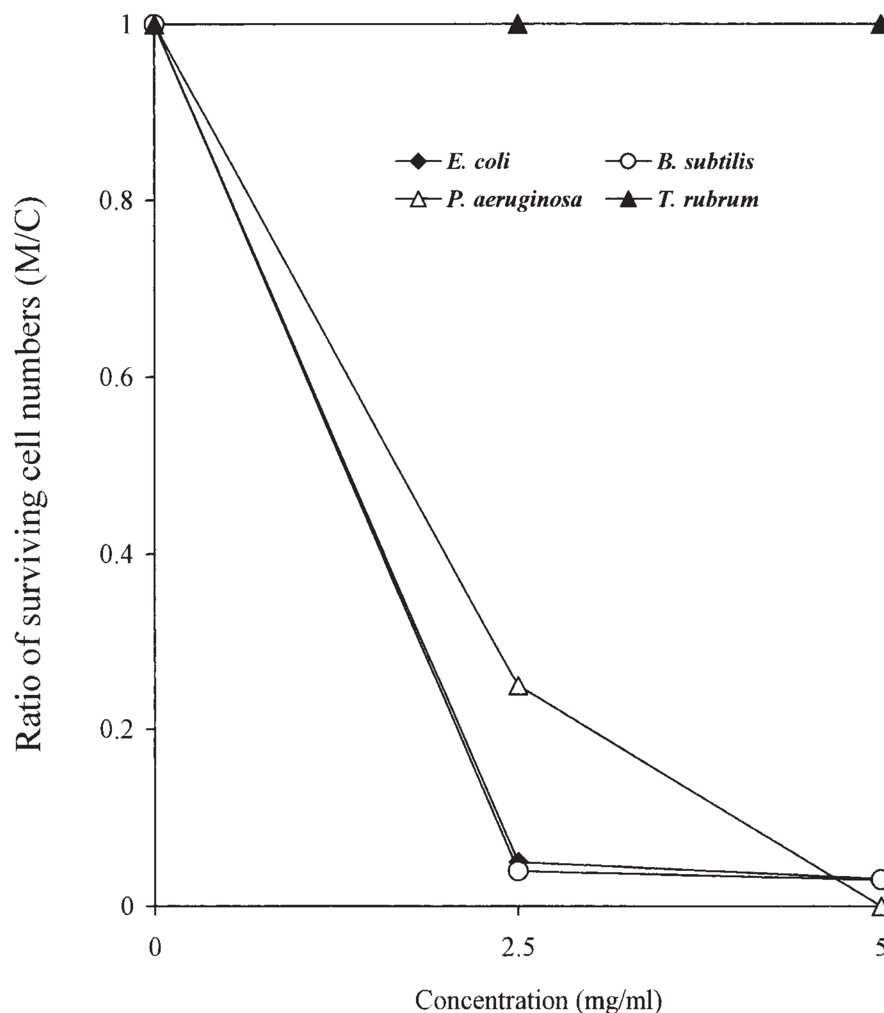


Figure 3 Growth inhibition of different concentrations of polymer **VIII** (inoculation: 8.4, 6.96, and 7.2 × 10^4 cells/mL of *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, and 4.3 × 10^5 fungal spores/mL of *T. rubrum*).

phosphine. The growth-inhibiting effect was quantitatively determined by *M/C*. The growth-inhibitory effect of polymer **IX** differed among the bacterial and fungal strains. The inhibition became stronger in the order of *B. subtilis* < *E. coli* < *P. aeruginosa* < *T. rubrum*.

Polymer **IX** showed 100% killing of *T. rubrum* at a concentration of 2.5 mg/mL and 98% killing of both *E. coli* and *B. subtilis* at 2.5 mg/mL. However, when the concentration of polymer **IX** was increased to 5.0 mg/mL, it showed 100% killing of both *E. coli* and *B. subtilis*. The same phenomenon was observed for *P. aeruginosa*: 2.5 mg/mL killed 90%, whereas 5.0 mg/mL killed 100%. Polymer **X** showed 100% killing of *B. subtilis*, *E. coli*, and *T. rubrum* at 2.5% mg/mL, whereas it showed 99% killing of *P. aeruginosa* at 2.5 mg/mL, which was increased to 100% when the concentration was increased to 5.0 mg/mL. Polymer **XI** showed 100% killing of *E. coli* and *P. aeruginosa* at a concentration of 2.5 mg/mL; however, it showed 95 and 98% killing of *T. rubrum* and *B. subtilis*, respectively, at 2.5 mg/mL. Again, when the concentration of polymer **XI** increased to 5.0 mg/mL, the killing percentage of *T. rubrum* and *B. subtilis* increased to 100%.

TABLE III
Diameter of the Inhibition Zone (mm) Produced by 20.0 mg of Polymers IX–XI

	Polymer		
Organism	IX	X	XI
E. coli	12	32	60
P. aeruginosa	80	40	70
Shigella sp.	12	26	20
S. typhae	28	18	26
B. subtilis	12	32	22
B. cereus	10	20	32

Mode of action

It is now generally accepted that the mode of action of polycationic biocides based on quaternary ammonium salts can be interpreted on the basis of adsorp-

tion onto the bacterial cell surface, binding to the cytoplasmic membrane, release of cytoplasmic constituents such as K^+ ions, DNA, and RNA, and the death of the cell, which have been observed for polycationic biocides.[19,20,22,27–30] It is well known that bacterial cell surfaces are negatively charged. Then, adsorption onto the negatively charged cell surface is expected to be enhanced with increasing charge density of the cationic biocides. Therefore, it is reasonable to assume that the adsorption onto the bacterial cell surface is much more enhanced for polymers, compared with that for model compounds (monomers).[19,26] A similar situation can also be expected via binding to the cytoplasmic membrane because there are many negatively charged species present in the cytoplasmic membrane, such as acidic phospholipids and some membrane proteins.[19,31,32] The disruption of the membrane is a result of the interaction of the bound polymers with the membrane; thus, it is expected to be facilitated with increasing amounts of the bound polymers. It is known that there is much difference in the structures of cell walls of Gram-positive and Gram-negative bacteria. In Gram-positive bacteria, there are plenty of pores that allow foreign molecules to come into the cell without difficulty.[19,33] On the other hand, in Gram-negative bacteria, the outer membrane is a potential barrier against foreign molecules with a high molecular weight.[19,33]

Consequently, the overall activity is determined by two factors: one is favored for polymers (adsorption onto the bacterial cell surface, binding to the cytoplasmic membrane, and disruption of the cytoplasmic membrane), and the other is not favored for polymers (diffusion through the cell wall).[19]

CONCLUSIONS

The synthesis of polyamides based on tartaric acid was investigated. Synthesized polytartaramides deserve special attention because they are easily accessible and display desirable properties. This may be because tartaric acid is widely distributed in nature in many fruits. The polyamides were synthesized from **I** and ethylenediamine; then, the hydroxyl group of the polytartaramides was further modified with chloroacetyl chloride to introduce the chloromethyl functionality, which was used as a support for the quaternary ammonium and phosphonium groups. For comparison, **I** was chloroacetylated and then was polymerized with ethylenediamine. However, the last polymerization process occurred partially at the chloromethyl group and mainly at the diethyl ester. All the synthesized polymers were quaternized at the chloromethyl groups with triethylamine, triphenylphosphine, and tributylphosphine. The quaternized polymers showed high activity against various microorganisms.

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