Biocidal Polymers: Synthesis, Antimicrobial Activity, and Possible Toxicity of Poly(hydroxystyrene-*co*-methylmethacrylate) Derivatives

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Received 7 March 2010; accepted 2 July 2010
DOI 10.1002/app.33046
Published online 10 January 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Functionalized polymers have gained much interest in the last decades. This is due to their functional group and their polymer nature that give them unique properties and more advantages than the corresponding small molecules. In this trend, polyhydroxystyrene-*co*-MMA was modified to introduce amino group in the side chain of the polymer. The amine modified polymer was reacted with two classes of active compounds. The first class is aldehydes such as vanilline, *p*-hydroxybenzaldehyde, *p*-chlorobenzaldehyde, and anisaldehyde. The second class is phenolic esters such as *p*-hydroxymethylbenzoate, 2,4-dihydroxymethyl benzoate, and methyl salicylate. The antimicrobial activities of the polymer and modified polymer with these two classes were explored with Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Bacillus subtilus*), fugus like yeast (*Candida albicans SC5314*), and pathogenic molds (*Aspergillus flavus*, *Trycophyton rubrum*, and *F. oxysporium*). *In vitro* studies indicated that the start polymer did not affect on the test microorganisms, in contrary to its derivatives. The diameter of inhibition zone varied according to the active group in the modified polymer, polymer microstructure, and the test microorganism. Derivatives I, II, and III were selected among the most effective antimicrobial compounds. Their inhibitory effects on the ratio of surviving cell number (M/C) increased by increasing derivatives concentrations. Derivatives I and II were inhibitorier to *C. albicans* and molds than to bacteria while derivative III was only antibacterial. These derivatives seemed toxic to Brine shrimp by increasing their concentrations above 10 ppm, with derivative III being the less toxic, compared to others. To clarify this toxic effect and to decrease the toxicity of these derivatives, more detailed studies are necessary, and this will be focused in the nearest future. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 120: 2734–2742, 2011

Key words: antimicrobial polymers; poly(hydroxystyrene); poly(methylmethacrylate); bioactive polymers; self-sterilized materials; Brine shrimp

INTRODUCTION

Because of the increase of contamination and microbial infection by microorganisms in the last few years, there has been a great concern in various areas for producing new antimicrobial agents.[1] Bacterial contamination of biomedical devices is a major problem in those medical disciplines using biomaterials.

Antimicrobials agents gain interest from research and industry due to their potential to provide quality and safety benefits to various materials and environments depending on the type of applications.[2] However, these materials have the problems of residual toxicity of the agents, even when suitable amounts of the agents are used. Also, their protection is short-lived because of the difficulty in controlling the rate of diffusion in variety of areas, such as medical devices, healthcare products, water purification systems, hospitals, dental office equipment, food packaging, food storage, and household sanitation.[3,4]

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 selectivity, and prolonging the lifetime of the antimicrobial agents. Also, polymeric antimicrobial agents have the advantage that they are nonvolatile and chemically stable and do not permeate through skin. Therefore, they can reduce losses associated with volatilization, photolytic decomposition, and transportation.[1] Therefore, there has been increasing effort during the last two decades to synthesize antibacterial polymers through the chemical bonding or

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physical binding of low-molecular weight biocides to polymers.[5]

Polymers with antimicrobial activity are often required for healthcare products, food packaging, household sanitation, or medical applications, medical devices, water purification systems, filters, hospitals, and dental office equipment. They can also be used as a coating material for common objects such as doorknobs, children's toys, and computer keyboards to prevent transmission of microbial infections.[6] In this context, antimicrobial activity of some linear copolymers containing quaternary ammonium and phosphonium salts has been reported by Kenawy et al.[7,8] Rzaev and coworkers[9] reported using poly(*N*-vinyl-2-pyrrolidone-*alt*-maleic anhydride)/poly(ethylene amine) macro complexes as antimicrobial polymeric system. They found that with an increasing number of COOH groups in the macrocomplex of A–B, the antimicrobial activity of the macrocomplex of A–B was found to increase on a variety of Gram-positive bacteria. Both Gram-negative bacteria (*S. enteritidis* and *Escherichia coli*) were not affected by all the studied polymer systems. Patel et al.[10] found that homo- and copolymers of *N*-vinylpyrrolidone (VP) and 2,4-dichlorophenyl methacrylate (2,4-DMA) were effective in inhibiting selective microorganisms. It has been reported that polymers prepared using 2,4-DMA showed strong inhibitory effect toward *S. aureus*, *S. citreus*, and *E. coli*, molds, and yeasts, whereas poly(VP) has been shown to have relatively lower antimicrobial activity. Recently, a relatively new class of biocidal polymers known as cyclic *N*-halamines showed superior properties including biocidal efficacy, long-term stability, and recharge ability once the efficacy has been consumed during use. Several of such materials have been prepared and tested for antimicrobial properties.[11–15]

In this study, copolymer of hydroxystyrene and methylmethacrylate was modified with ethylenediamine (EDA), and the aminated copolymer was reacted with various bioactive agents. The antimicrobial activities of the modified copolymers against Gram-negative and Gram-positive test bacteria, fungus like yeast *Candida Albicans*, and pathogenic molds, in addition to their toxicity to *Artemia salina*, were explored.

EXPERIMENTAL

Materials

Polyhydroxystyrene-*co*-MMA was purchased from Aldrich, Milwaukee, WI, and used as received without further purification. EDA was purchased from Aldrich and distilled before use, and *p*-hydroxybenzoic acid was purchased from Aldrich and used as received without further purification. Methyl salicylate was purchased from El-Naser Pharmaceuticals Company, Cairo, Egypt. 4-Hydroxy-3-methoxybenzaldehyde (vanillin) was purchased from El-Gomhouria Chemicals Company, Tanta, Egypt, and used without further purification.

p-Hydroxybenzaldehyde was supplied by Aldrich and used as received without further purification. Diethyl ether was used as received from El-Naser Pharmaceutical Chemical Company, Cairo, Egypt. *p*-Chlorobenzaldehyde was purchased from Aldrich and was used as received without further purification. Glacial acetic acid was purchased from El-Gomhouria Chemical Company, Tanta, Egypt and used as received. Absolute ethanol was purchased from Merck-Schuchardt, Hohenbrunn, Germany, and distilled and dried before use. 1,4-Dioxane was purchased from Aldrich and distilled before using.

P-Hydroxymethylbenzoate was prepared in the laboratory as follows: *p*-hydroxybenzoic acid (50 g, 362 mmol) was dissolved in 250-mL dry methanol. Dry H_2SO_4 (16 mL) was added slowly to the colorless solution with stirring. The system was fitted to reflux at 80°C for 24 h in an oil bath with continuous stirring under anhydrous conditions. The system was cooled, and the excess alcohol was removed by rotary evaporator. A white precipitate was filtered off and washed with distilled water until no more acid appears in the TLC. The ester was collected as white powder and dried under vacuum at 40°C for 24 h. The yield was 47.5 g (95%), m.p (120°C). It was also characterized by ^{1}H NMR.

2,4-Dihydroxymethylbenzoate was prepared in the laboratory as follows: to the colorless solution of 50 g (324.42 mmol) of 2,4-dihydroxybenzoic acids in 250-mL dry methanol was added 16 mL of dry H_2SO_4. The mixture was refluxed with stirring at 80°C for 24 h in an oil bath under anhydrous conditions. The system was cooled and rotary evaporator removed the excess alcohol. Distilled water (300 mL) was added to the mixture and a white precipitate was formed. The precipitate was filtered off, washed with distilled water to ensure that no more unreacted acid still present in the precipitate. The ester was collected as white powder and was dried under vacuum at 40°C for 24 h. The yield was 45 g (95%) m.p. (110°C). It was also characterized by ^{1}H NMR.

Test microorganisms

The Gram-negative bacteria (*E. coli),* Gram-positive bacteria (*Bacillus subtilus*) the yeast *C. albicans* and the molds *Aspergillus flavus*, *Fusarium oxysporum*, and *Trycophyton rubrum* were obtained from the culture collection of Bacteriology Unit, Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

Media used

Nutrient and Sabouraud's broths, Nutrient, and Sabouraud's agar were used for growing and maintaining the test bacteria, yeast and molds, respectively.

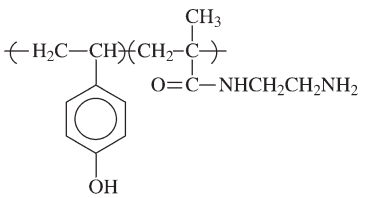
Instruments

Infrared spectra were recorded from KBr pellets on a PerkinElmer 1430 ratio recording infrared spectrophotometer, Wellesley, MA, USA. Elemental analyses were recorded on PERKIN-ERLMER 2400. Nuclear magnetic resonance spectrum (^{1}H NMR were recorded on Varian 300*M*, Mercury-oxford and on a Jeol JNM-PM ×90 SI NMR spectroscopy. Rotary Evaporator was supplied from Buchi, Switzerland. Vacuum Oven was supplied by LAB-LINE INSTRUMENTS, USA.

Polymer modification

Modification of poly(hydroxystyrene-*co*-methylmethacrylate) with EDA

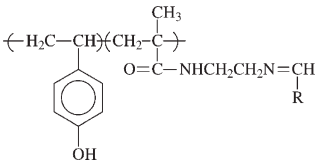
In 500-mL round-bottomed flask (20 g, 90.9 mmol) of P (HS-*co*-MMA) were added portion wise to ethylenediamine (EDA; 60.67 mL, 54.54 g, and 909 mmol). After the addition was completed, the system was fitted to reflux at 80°C for 5 days with continues stirring. The system was cooled, and the excess EDA was removed using rotary evaporator. Two hundred milliliters of dry 1,4-dioxane were added to the mixture, and dark yellow precipitate was formed. The precipitate was filtered off and washed five times with dry 1,4-dioxane to remove the unreacted ethylenediamine. The yield was 97.77% (22 g). The product was characterized by elemental microanalysis as in Table I and IR spectroscopy as in Table II. It was also characterized by ^{1}H NMR.

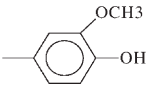
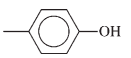
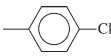
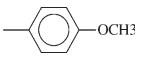


(I)

General procedure for modification of the EDA modified P (HS-*co*-MMA) with aromatic aldehyde derivatives

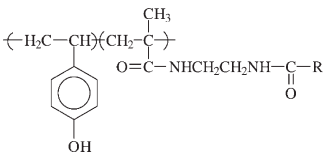
To a solution of aldehyde (8.06 mmol) in 20 mL of absolute ethanol was added with stirring modified poly(hydroxystyrene-*co*-methylmethacrylate) [MP (HS-*co*-MMA); 1 g, 4.03 mmol] and 1-mL glacial acetic acid. The reaction was continuous stirring at room temperature for 48 h, and then the system was fitted to reflux at 80°C for 10 h. The product was precipitate by adding it drop wise to diethyl ether; the product was filtered off and washed with diethyl ether to remove the excess aldehyde and acetic acid. The product was collected and characterized by elemental microanalysis as in Table I and IR spectroscopy as in Table II.



Polymer	Aldehyde	R	Yield (%)
II	Vanillin (4-Hydroxy-3-methoxy benzaldehyde)		66.66
III	p-hydroxy-benzaldehyde		64.3
IV	p-chloro-benzaldehyde		67.1
V	Anisaldehyde (ρ-Methoxy benzaldehyde)		61.2

General procedure modification of the EDA modified P (HS-*co*-MMA) with hydroxyl aromatic ester derivatives

To a solution of the aromatic ester (8 mmol) in 20 mL of absolute ethanol was added modified poly (hydroxystyrene-*co*-methylmethacrylate) [MP (HS-*co*-MMA); 1 g, 4.03 mmol] portion wise and 1-mL glacial acetic acid. Under anhydrous conditions, the reaction was stirred at room temperature for 48 h. Then, the system was refluxed at 80°C for 10 h. The product was precipitated by dropwise addition to diethyl ether. The precipitate was filtered off and washed with diethyl ether to remove the unreacted species. The modified polymer was collected. The structure of structure of the product was confirmed by elemental analysis as in Table I and IR spectroscopy as in Table II.



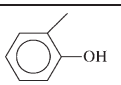
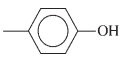
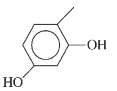
Polymer	Esters	R	Yield (%)
VI	Methylsalicylate		74.3
VII	p-hydroxy-methylbenzoatee		54
VIII	2,4-dihydroxy-methylbenzoate		54

TABLE I
Elemental Analyses of Aminated Copolymer and its Derivatives

	C%		H%		N%		Cl %	
Polymer	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
I	67.7	66.5	8.0	7.9	11.2	10.0	–	–
II	69.1	68.5	6.8	7.0	7.3	6.8	–	–
III	71.5	69.1	6.8	6.8	7.9	6.2	–	–
IV	68.0	67.5	6.2	6.5	7.5	6.9	9.5	8.71
V	72.1	69.7	7.1	7.0	7.6	6.9	–	–
VI	68.4	67.9	6.5	6.4	7.6	7.2	–	–
VII	68.4	67.0	6.6	6.7	7.1	6.9	–	–
VIII	65.6	65.8	6.3	6.5	7.3	6.8	–	–

Antimicrobial assessments

Antimicrobial activities

The antimicrobial spectrum of the prepared polymers bearing an active functional group were determined as powdered samples by the cut-plug method[16] on plates seeded with the tested bacteria: *E. coli* or *B. subtilus* on nutrient agar, which contained per liter: 3 g peptone; 5 g beef extract; 5 g NaCl; 20 g agar at pH 7. The test was performed also on plates seeded with *C. albicans*, *A. flavus*, *F. oxysporum*, or *T. rubrum* on Sabaraud's agar that contained per liter; 40 g glucose; 10 g peptone; 20 g agar at pH 6.0. After solidification, the wells were made, and each was filled with 20 mg of powdery polymer or its derivative. The plates were then incubated at 30°C for 24 h, after which the diameters of the inhibition zones were measured. The derivatives, which produced the highest inhibition zones, were selected and further assayed at different concentrations in aqueous suspensions to quantify its inhibitory effects.

Determination the ratio of surviving cell number (M/C)

The antimicrobial activity of the selected derivatives toward the sensitive representative microorganisms; *E. coli*, *B. subtilus*, *C. albicans*, and *A. flavus* were determined as the following: a loop full of either the test bacteria, fungus like yeast or molds was placed in 10 mL of nutrient broth or Sabaroud's broth, respectively, and incubated overnight at 30°C. One 10th of either nutrient or Sabaroud's broths were prepared, dispensed in test tubes containing 9.5 mL, and sterilized. The tubes were inoculated with 0.5 mL of the overnight growth from the corresponding organism. The selected effective polymers were added to the tubes containing the diluted broths and the most sensitive test microorganisms to give the final concentrations from 10 to 48 mg/mL. The cultures containing the test organisms and different concentrations of the selected antimicrobial derivatives were pre-equilibrated and shaken at 30°C as recommended by Nakashima et al.[17] and number of living cells as colony-forming unit/milliliter was determined after 24 h. The number of living cells obtained from derivative-supplemented cultures was divided by the number of living cells obtained from derivative-nonsupplemented control cultures to get the ratio (M/S).

Toxicity test

The cytotoxicity [lethal dose $(LD)_{50}$] of the selected derivatives I, II, and III was determined to the larvae of *Artemia salina* using Brine shrimp lethality bioassay.[18] Different concentrations of each selected polymer (10, 100, and 1000 ppm) were suspended in 5-mL vials containing saline solution and 10 shrimps

TABLE II
IR Analysis of Aminated Copolymer (I) and its Derivatives (I–VIII)

		—CONH						
Polymer	(CH) alph.	C=O	NH	—OH	C—Cl	C=N	OCH_3	Ar—H
I	2940	1718	1552	3365	–	–	–	831
II	2942	1716	1552	3383	–	1649	2613	831
III	2940	1713	1554	3350	–	1601	–	833
IV	2939	1715	1552	3263	770	1649	–	834
V	2939	1715	1554	3263	–	1649	2608	834
VI	2940	1715	1554	3409	–	–	–	831
VII	2941	1716	1554	3395	–	–	–	831
VIII	2942	1714	1552	3402	–	–	–	832

in each. Three replicates were used for each concentration, and dead larvae were counted after 72 h.

RESULTS AND DISCUSSION

Synthesis of modified polyhydroxystyrene-*co*-methylmethacrylate polymers

The modification of P (HS-*co*-MMA) aimed to introduce active amino group to the copolymer by reacting it with EDA. The aminated P (HS-*co*-MMA) expected to have highly activity toward aldehyde and esters.

Modification of P (HS-*co*-MMA) with ethylene diamine

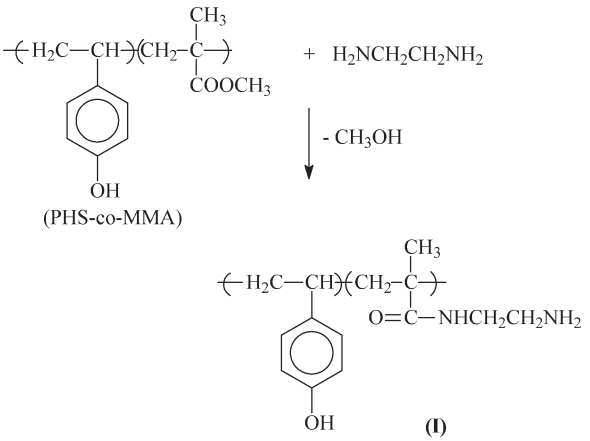
The copolymer poly(hydroxystyrene-*co*-methylmethacrylate) P (HS-*co*-MMA) was modified with EDA to give aminated MP (HS-*co*-MMA) (I); the amination reaction takes place in methanol as a solvent (Scheme 1). Many attempts were carried to determine the molar ratio between the polymer and diamine. When the molar ratio was 1 : 10, the reaction gives the highest degree of amination. The reaction yield was 97.77%, and the polymer was collected as yellow fine powder. At lower ratios of the copolymer, diamines, crosslinked polymer, were produced. It was also noticed that the reaction occurs at higher temperature better than lower temperature in presence of high-molar ratio of the diamine Also, addition of solvent promotes the reaction to yield unacross-linked polymer. The product was washed many times with dry 1,4 dioxane and dry ethanol to remove unreacted diamine. The resulting polymer (I) was for further modification reactions due to the presence of different functional groups.

The product was characterized by elemental analysis, and it was in a good agreement with the calculated values as shown in Table I and the IR spectra of (**I**) as in Table II. The IR shows peaks at 3365 cm^{-1} for (NH_2), strong absorption band at 1718 cm^{-1} for (C=O), 2940 cm^{-1} for (CH aliphatic), and band at 1661 cm^{-1} for (C—N) and showed peaks at 1551–1554 cm^{-1} for —NH secondary amine.

^{1}H NMR spectrum of (I) in (d_6-DMSO) is characterized by the appearance of singlet signal at 0.9 ppm due to CH_3 proton, whereas NH_2 protons resonated at 3.1 ppm (signal), 1.4 ppm (CH_2 duplet), 2.2 ppm (CH_2 triplet), 3.3 ppm (CH_2 triplet), 2.5 ppm (CH_2 duplet) and 2.4 ppm (CH triplet) 6.4–7 ppm (m,H, ArH), 4 ppm (signal for H in OH), and 8 ppm (NH signal).

Modification of the MP (HS-*co*-MMA) with aromatic aldehyde derivatives

The Schiff base formation between modified poly(hydroxystyrene-*co*-methylmethacrylate) MP (HS-*co*-MMA) and different aldehydes was carried out in absolute ethanol. Series of various derivatives of aminated P (HS-*co*-MMA) was prepared by condensation of it with vanilline, or *p*-hydroxy benzaldehyde, or, *p*-chlorobenzaldehyde and *p*-methoxybenzaldehyde in the presence of glacial acetic acid as catalyst in an oil bath at 80–90°C with stirring. In all condensation reactions, excess amounts of aldehyde (1 : 2), respectively, were used to ensure that complete condensation occurred. The Schiff base was formed at room temperature, but heating was used to ensure the condensation and to increase the reaction yield. The product was precipitated in diethyl ether, filtered off, and washed with diethyl ether to remove excess of aldehyde and acetic acid. The reaction scheme is as outlined in Scheme 2. The resulting polymers (**II–V**) were used for further studies.

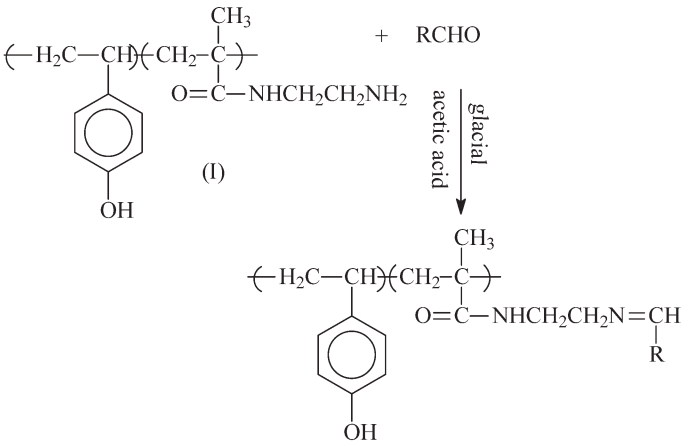


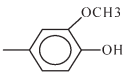
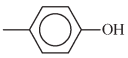
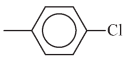
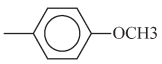
Scheme 1

The products were characterized by elemental analysis, and it was in a good agreement with the calculated values as shown in Table I. The IR spectra of the polymer (**II–V**) showed strong band at 3263–3383 cm^{-1} due to OH of secondary anime, band at 1713–1718 due to (C=O) at 2841–2942 cm^{-1} due to (CH aliphatic), and the appearance of band at 1649 cm^{-1} due to (C=N). The IR spectra of polymer (**II**) and (**V**) showed an absorption bands at 2618 and 2608 cm^{-1}, respectively, region due to the methoxy group (OCH_3) and polymer (**IV**) showed strong band at 770 cm^{-1} region due to the C—Cl group, peaks at 1552–1554 cm^{-1} for (—NH—) and 3365 cm^{-1} for (NH_2) in polymer (**I**) as in Table II.

Modification of the amine modified P (HS-*co*-MMA) (**I**) with various hydroxy aromatic esters

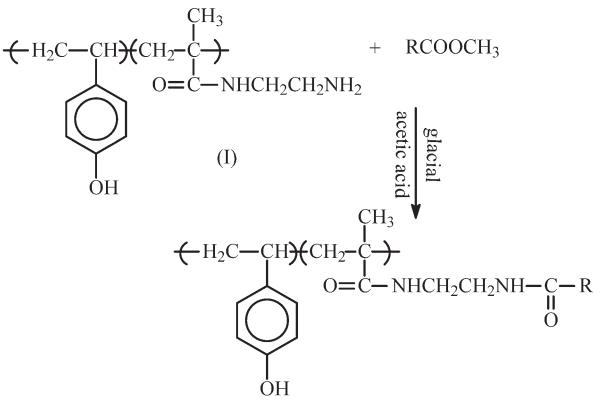
Various phenolic esters were prepared by reacting corresponding acid with absolute methyl alcohol in the presence of sulfuric acid. The esterification was carried out in high-conversion yield (90–95%). The ^{1}H NMR spectrum of 4 hydroxymethyl-benzoate in DMSO showed peaks at 10.3 ppm (1H, singlet, OH in position 4), 7.8 ppm (2H, doublet, CH in position

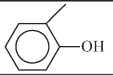
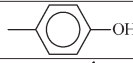
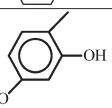


Compound	Aldehyde	R	Yield (%)
II	Vanilline (4-Hydroxy-3-methoxy benzaldehyde)		66.66
III	p-hydroxy-benzaldehyde		64.3
IV	p-chloro-benzaldehyde		67.1
V	Anisaldehyde (p- Methoxy benzaldehyde)		61.2

Scheme 2

3 and 5), 6.8 ppm (2H, doublet, CH at positions 2 and 6), and 3.8 ppm (3H, singlet, CH_3). The ^1H NMR spectrum of 2,4-dihydroxy-methylbenzoate in DMSO showed peak at 10.7 ppm (1H, singlet, OH in position 2), 10.42 ppm (1H, singlet, OH in position 4), 7.62 ppm (1H, singlet, CH in position 3), 6.25 ppm (1H, doublet, CH in position 5), 6.35 ppm (1H, doublet, CH in position 6), and at 3.82 ppm (3H, singlet, OCH3). These compounds were prepared to improve the antimicrobial activities of the polymers by introducing phenol in the side chain of the polymer. Reaction of amine containing copolymer I with the esters was carried out to introduce the phenol groups to the side chain of the polymer. Copolymers (**VI**, **VII**, and **VIII**) were prepared in absolute ethanol, and excess amount (1 : 2), respectively, of the ester was used to ensure that the reaction was completed. The product was precipitated in diethylether. Removal of the unreacted ester was carried out by washing with diethyl ether. The modified polymers were then collected, dried, and characterized. Generally, the reaction occurred easily and without any problems as expected with higher yields (cf. Scheme 3). The products were characteristic by elemental analysis, and it was in a good agreement with the



Compound	Ester	R	Yield (%)
VI	Methylsalicylate		74.3
VII	p-hydroxy-methylbenzoate		54.0
VIII	2,4-dihydroxy-mehtylbenzoate		54.0

Scheme 3

TABLE III
Diameter of Inhibition Zone (mm) Produced by the Powdery Form of P (HS-*co*-MMA) and its Derivatives Against Test Microorganisms

	Inhibition zone diameter (mm)					
	Bacteria		Yeast		Molds	
Polymers	*Escherichia coli*	*Bacillus subtilus*	*Candida albicans*	*Aspergillus flavus*	*Tricophyton rubrun*	*Fusarium oxysporium*
P(HS-*co*-MMA)	0.0	0.0	0.0	0.0	0.0	0.0
I	38.0	22.5	38.0	30.0	40.0	30.0
II	10.0	22.5	10.0	22.5	22.5	30.0
III	20.0	22.5	10.0	0.0	0.0	0.0
IV	20.0	18.2	0.0	0.0	0.0	0.0
V	0.00	10.0	0.0	0.0	0.0	0.0
VI	0.00	0.0	10.0	0.0	0.0	0.0
VII	10.0	10.0	0.0	0.0	0.0	0.0
VIII	12.0	10.0	0.0	0.0	0.0	0.0

calculated values as shown in Table I. The characteristic bands of IR spectra of the polymers (VI–VIII) were listed in Table II. Absorption bands at 3395–3490 cm^{-1} region are due to the stretching vibrations of the OH group, and a strong band at 1714–1716 cm^{-1} region is due to the C=O group. The absorption band appeared at 2940–2942 cm^{-1} region is due to the CH aliphatic group and showed peaks at 1551–1554 cm^{-1} for (NH).

Antimicrobial activity of P (HS-*co*-MMA) and its derivatives

The capability of the start polymer and its modified polymers (derivatives) in its powdery form to inhibit the growth of the selected test microorganisms on solid media is shown in Table III. The prepared antimicrobial polymers as insoluble materials have contact killing antimicrobial properties. This occurs by dispersion of the polymer in the diluted liquid media that allows easier dispersion, through pre-equilibration and shaking at 30°C as recommended by Nakashima et al.[17] Shaking helps microorganism to contact with the dispersed polymer. When the polymer formulation contacts the microorganism, the biocidal moiety could be transferred to or contact the microorganism in amounts sufficient to kill it.

Although P (HS-*co*-MMA) has no inhibitory effect on the test microorganisms (0.0 inhibition zone), modification helped detectable antimicrobial effect with variable clear zones (diameters of inhibition zones ranged between 10 and 40 mm after 24 h of incubation), according to the derivative substitutions or additive group(s) and the test microorganism. The derivatives **I** and **II** were inhibitory to tested yeast, molds, and bacterial species. Derivative III has antibacterial and anticandidal activities. Other derivatives (**IV**, **V**, **VII**, and **VIII**) have no antifungal activity but have antibacterial activities, on the other hand, derivative (**VI**) has no antibacterial activity, but has low activity only against *C. albicans*. Derivatives II and III have similar polymer microstructure except II has extra OCH_3 group, this group made polymer II much more active than III, which contains only the hydroxyl groups. This means that OCH_3 group promoted the interaction between the cells and the polymer leading to more activity. However, it also seems that this may be due to the presence of both hydroxyl group and methoxy group as in case of polymer (V). Although the latter has methoxy group, it has no antifungal activity but low-antibacterial effect. The results of the above screening indicated that derivatives **I**, **II**, and **III** were the most active ones compared to other derivatives. Thus, the antimicrobial activity of different concentrations of these derivatives against representative Gram-positive bacteria (*B. subtilus*), Gram-negative bacteria (*E. coli*), and the fungus like *C. albicans* and the mold *A. flavus* were assessed in more details in the subsequent experiments.

Antimicrobial assessment of polyhydroxy styrene-*co*-MMA derivative (**I**)

The antimicrobial activities of modified polymer (**I**) against the test microorganisms were estimated by the ratio (M/C) of the surviving cell number. From the results illustrated in Figure 1, the potency of the inhibition increased by increasing the derivative concentration and varied according to the test microorganism. The modified polymer was more inhibitory to yeast than the test fungus and bacteria as in the following order; *C. albicans* > *A. flavus* > *B. subtilus* > *E. coli*.

The inoculates were 8.2×10^5 cells/mL for *E. coli*, 7.3×10^4 cells/mL for *C. albicans*, 2.9×10^4 cells/mL for *B. subtilus*, and 5.9×10^5 cells/mL for *A. flavus.*

The polymer I was tested for 6 months and remained active when was in contact with the microorganism, with no growth of the microorganisms at concentration of 40 mg/mL.

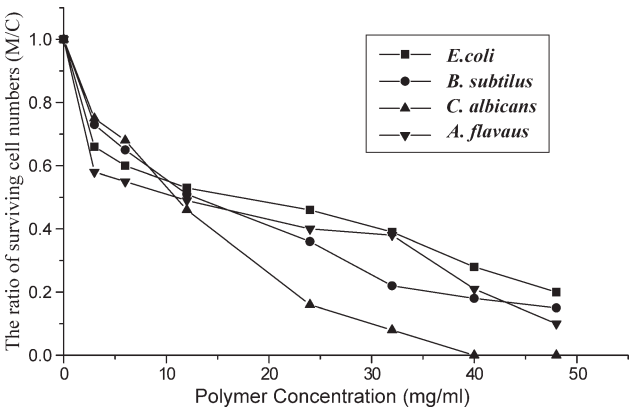


Figure 1 Growth inhibition of different concentrations of P (HS-*co*-MMA) derivative (I).

Antimicrobial assessment of polyhydroxy styrene-*co*-MMA derivative (**II**)

The antimicrobial activities of derivative (**II**) were quantitatively determined by the ratio (M/C) of the surviving cell number of *C. albicans*, *A. flavus*, *B. subtilus*, and *E. coli*, and the results were shown in Figure 2. Generally, the potency of inhibition increased by increasing the concentration of the polymer, and the inhibitory effect was more clearer on molds, yeast than on bacteria as in the following order *A. flavus* > *C. albicans* > *E. coli* > *B. subtilus*. The polymer II was tested for 6 months and remained active when was in contact with the microorganism, with no growth of the microorganisms at concentration of 40 mg/mL. The inoculates were 8.2×10^5 cells/mL for *E. coli*, 7.3×10^4 cells/mL for *C. albicans*, 2.9×10^4 cells/mL for *B. subtilus*, and 5.9×10^5 cells/mL for *A. flavaus*.

Antimicrobial assessment of polyhydroxy styrene-*co*-MMA derivative (**III**)

According to the results in Table III, the antimicrobial activities of derivative (**III**) were assessed only

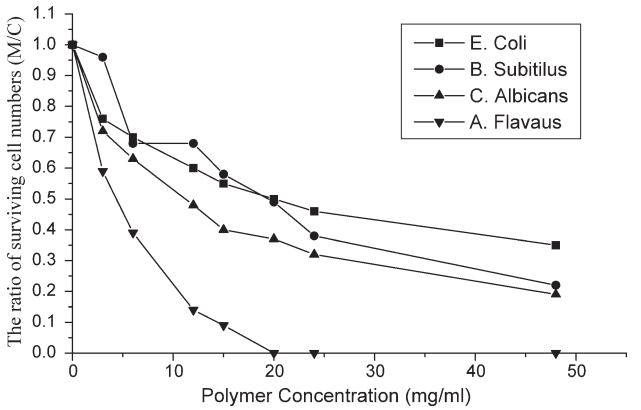


Figure 2 Growth inhibition of different concentrations of P (HS-*co*-MMA) derivative (II).

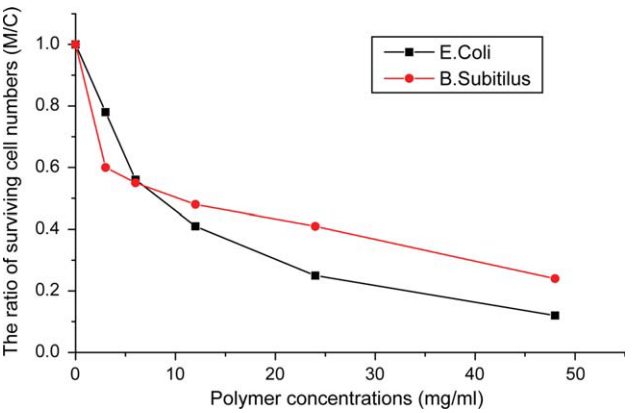


Figure 3 Growth inhibition of different concentrations of P (HS-*co*-MMA) derivative (III). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

against *B. subtilus* and *E. coli* by quantitative determination of the ratio (M/C) to the surviving cell number. The results were illustrated in Figure 3, and the potency of the inhibition was increased by increasing the concentration of the derivative. Derivative (**III**) was more inhibitory to *E. coli* than *B. subtilus*.

Toxicity of polyhydroxy styrene-*co*-MMA derivatives

The cytotoxicity of the P (HS-*co*-MMA) derivatives **I**, **II**, and **III** was determined by using larvae of Brine shrimp (*Artemia salina*).[19–22] In Table IV, the LD_{50} of the antimicrobial derivatives on Brine shrimp differed according to the compound and its concentration. The lethality% to the larvae increased by increasing the concentration of each polymer. Derivatives II and III being with more lethal effect, than derivative **I**, above 10 ppm. Derivative **III** being with less lethality, than derivatives I and II, at 100 ppm. The lethal effect of derivatives I and II may be due to that compounds are with anticandidal and antifungal effect than antibacterial, such as most known antifungal drugs. To clarify the mode of action of these compounds on yeasts and molds need more study in the future and also to search for more modifications to decrease the toxicity of such compounds.

TABLE IV
Toxicity of Poly (HS-*co*-MMA) Derivatives to *Artemia salina* in Cultures Supplemented with Different Concentrations

Derivatives	0	10 ppm	100 ppm	1000 ppm
Lethality % of *Artemia salina*				
I	0.0	0.0	100	100
II	0.0	40	100	100
III	0.0	30	90	100

CONCLUSIONS

The results of this study indicated successful modification of copolymer of hydroxystyrene and methylmethacrylate with EDA, and the aminated copolymer was reacted with various bioactive agents. The antimicrobial activity of the synthesized derivatives is mostly anticandidal and antifungal than antibacterial polymers. Polymers I and II showed high-antimicrobial activity against all the tested microorganisms. However, polymer I showed the highest activity against *T. rubrum*. At the same time, polymer II showed the highest activities against *F. oxysporium*. Generally, polymers III and IV showed higher activity against bacterial species more than the other species. The synthesized derivatives seemed to be toxic to the larvae of Brine shrimp in high concentrations. This needs more detailed study to explain the toxicity and more research for less toxic antimicrobial derivatives among these groups.

References

1. Kenawy, E.-R.; Worley, D.; Broughton R. Biomacromolecules 2007, 8, 1359.
2. Radheshkumar, C.; Münstedt, H. React Funct Polym 2006, 66, 780.
3. Kenawy, E.-R.; Abdel-Hay, I.; Shahada, L.; El-Shanshoury Abd El-Raheem, R.; El-Newehy, M. H. J Appl Polym Sci 2006, 102, 4780.
4. Jones, D. S.; Djokic, J.; Gorman, S. P. Biomaterials 2005, 26, 2013.
5. Dizman, B.; Elasri, M. O.; Mathias, L. J. J Polym Sci Part A: Polym Chem 2006, 44, 5965.
6. Hea-Sun, Y.; Eun-Soo, P. Macromol Mater Eng 2006, 291, 621.
7. Kenawy, E.-R.; Mahmud, Y. A. G. Macromol Biosci 2003, 3, 107.
8. Kenawy, E.-R.; Abdel-Hay, F. I.; El-Shanshoury Abd El-Raheem, R.; El-Newehy, M. H. J Polym Sci Part A: Polym Chem 2002, 40, 2384.
9. Temiz, A.; Togay, S. O.; Sener, A.; Guven, G.; Rzaev, Z. M. O.; Piskin, E. J Appl Polym Sci 2006, 102, 5841.
10. Patel, M. B.; Patel, D. A.; Ray, A.; Patel, R. M. Polym Int 2003, 52, 367.
11. Chen, Y.; Worley, S. D.; Huang, T. S.; Weese, J.; Kim, J.; Wei, C.-I.; Williams J. F. J Appl Polym Sci 2004, 92, 368.
12. Qian, L; Sun, G. J Appl Polym Sci 2004, 91, 2588.
13. Sun, Y; Sun, G. J Appl Polym Sci 2001, 81, 617.
14. Sun, Y.; Sun, G. J Appl Polym Sci 2003, 88, 1032.
15. Sun, Y.; Chen, T.-Y.; Worley, S.D.; Sun, G. J Polym Sci Part A: Polym Chem 2001, 39, 3073.
16. Pridham, T. G.; Lindenfelser, L. A.; Shotwell, O. L.; Sodola, F.; Benedict, R. G.; Foleg, C.; Jacks, P. W.; Zaumeyer, W. J.; Perston, W. H.; Mitchell, J. W. Phytopathology 1956, 46, 568.
17. Nakashima, T.; Enoki, A.; Fuse, G. Bokin Bobai 1987, 15, 325.
18. Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; Mclaughlin, J. L. Plant Med 1982, 45, 31.
19. McLaughlin, J. L.; Chang, C. J.; Smith, D. L. In Studies in Natural Products Chemistry; Rahman, A. U., Ed.; Elsevier: Amsterdam, 1991.
20. De Siqueira, J. M.; Ziminianini, M. G. Quím Nova 2001, 24, 185.
21. Lima, N. M. F.; Dos Santos, A. F.; Porfirio, Z.; Goulart, M. O. F.; Sant'Ana, A. E. G. Acta Trop 2002, 83, 43.
22. Luna, J. S.; Dos Santos, A. F.; Lima, M. R. F.; Omena, M. C.; Mendonça, F. A. C.; Sant'Ana, A. E. G.; Moreau, N. J. J Ethnopharmacol 2005, 97, 199.