Synthesis, Characterization, and Antimicrobial Properties of Novel Quaternary Amine Methacrylate Copolymers

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Received 18 January 2007; accepted 22 August 2007
DOI 10.1002/app.27340
Published online 19 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A novel amine methacrylate monomer trimethylolpropane trimethacrylate–piperazine–ethyleneglycol dimethacrylate (TMPTMA-PPZ-EGDMA) was synthesized by amination of trimethylolpropane trimethacrylate (TMPTMA) with excess of piperazine (PPZ) followed by reaction with ethyleneglycol dimethacrylate (EGDMA). Copolymerization of TMPTMA-PPZ-EGDMA with 2-hydroxyethyl methacrylate (HEMA) was carried out by free radical polymerization using ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED) as a redox initiator. The copolymers obtained were then quaternized with 1-iodooctane. The monomers were characterized by FTIR and ^{1}H NMR spectral studies. The molecular weights and polydispersity values of the monomers were determined with gel permeation chromatography. Quaternized copolymers containing more than 20% amine methacrylate monomer showed microporosity in the range of 9.9–10.4 μm. The antibacterial activity of the quaternized copolymers against *Escherichia coli* and *Staphylococcus aureus* was studied using UV–vis spectrophotometer and scanning electron microscopy. Quaternized copolymers showed broad-spectrum contact-killing antibacterial properties without releasing any active agent as checked by iodide selective ion meter. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2861–2870, 2008

Key words: radical polymerization; copolymerization; antimicrobial activity; NMR; TGA

INTRODUCTION

Quaternary ammonium compounds (QACs) of low molar mass are widely used as cationic disinfectants. Common characteristics among QACs are that they possess both a positive charge and a hydrophobic segment.[1–3] Classification and biological activity of QACs depend upon the nature of the organic groups attached to nitrogen, the number of nitrogen atoms present, and the counterion.[1] QACs usually contain four organic groups linked to nitrogen, which may be similar or different in chemistry and structure. The organic substituents are either alkyl, aryl, or heterocyclic.[4] At least one of the organic substituents should be a long alkyl chain to provide a hydrophobic segment compatible with the bilayer of the outer cell wall of the microorganism.[5–7] It has been reported that the antimicrobial activity increases with increasing alkyl chain length of an amphiphilic compound upto 14 carbon alkyl chains.[7,8] Any anion may be attached to the nitrogen cation of QACs to form a salt, although the chloride and bromide salts are most commonly used.[2] QACs are effective against both gram-positive and gram-negative bacteria at medium concentrations and also have moderate effectiveness against viruses, fungi, and algae.[7,9,10] Some of the advantages of QACs over other antimicrobial agents are that they are more stable, less corrosive, nonirritating to the skin, and have low mammalian toxicity.[2] Continuous efforts have been made during the last two decades to synthesize polymers with QAC substituents.[1,3–5,11–13] The literature suggests that the polymers containing QACs, either in the backbone or as pendant groups, not only enhanced efficacy over corresponding small QAC molecule, but also shows reduced residual toxicity, increased efficiency and selectivity, and prolonged lifetime.[1,5,14–16] Polymeric antimicrobial agents also have the advantage that they are nonvolatile, chemically stable, and do not permeate through the skin. As a result, they significantly reduce losses associated with volatilization, photolytic decomposition, and migration.[17] QACs containing antimicrobial polymers have been used as coatings in many areas such as food processing,[18,19] biomedical devices,[20] filters,[21] and additives for antifouling paints.[22] The use of cationic antimicrobial polymers can eliminate bacterial infection of implanted devices such as catheters.[23] These polymers can be used in paints on hospital room walls and everyday objects such as doorknobs, children's toys, computer keyboards, and telephones. This renders them antiseptic and thus prevent the transmission of bacterial infections.[24] They are used in the textile industry to form antimicrobial

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Contract grant sponsor: Council of Scientific and Industrial Research (CSIR).

fibers[25,26] and as disinfectants and preservatives in pharmaceuticals.[27] Other likely uses of these polymers are as cleaning solutions for contact lenses[28] as well as coatings and chemically bound components of biomaterials.[29] In polymeric biocides, there are many factors, which can be expected to affect their antibacterial activity such as molecular weight and its distribution, configuration and conformation, hydrophilic–lipophilic balance (HLB), and spacer length between active groups in the polymer molecule that will result in a change in both conformation and charge density of the polymer, thus affecting the mode of interaction with cytoplasmic membranes and leading finally to different antimicrobial activities.[30]

Iodine-based antimicrobial polymers have gathered considerable attention because of their nontoxicity and nonirritant properties with prolonged antimicrobial activities.[31] Many researchers have tried to immobilize or entrap iodine into various polymeric matrices to develop antimicrobial polymers with sustained release of active iodine for disinfection of water. Physiological effects due to long-term ingestion of iodine, even though at ppm levels, could be a serious issue because of its toxicity. Many researchers have also reported kidney failure[32] and hyperthyroidism due to continuous and uncontrolled release of iodine.[33] Therefore, various attempts are being made to make polymeric disinfectants with inbuilt antimicrobial properties without releasing of any bioactive agent.

Samuel et al. patented a composition, which when applied to a substrate, forms an adherent, transparent, water-insoluble polymeric film on the substrate surface that provided sustained antimicrobial disinfecting action for prolonged periods, without necessity for reapplication.[34] The coating claimed to provide surface-disinfecting action by a contact-killing mechanism and does not release its components into contacting solutions at levels that would result in solution disinfection. In another patented work, a linear polymer (polyionenes) was immobilized on a chemically inert carrier material and a matrix having an antimicrobial was developed and used in the form of microporous membranes as filters for water purification or in form of beads as surface disinfectant.[35]

A new concept of contact type of disinfection by iodine-based resin formed by adsorption of iodine on a strong alkaline anion-exchange resin was reported by Liwang and Bisang.[36] He claimed that iodine resin prepared could remove and kill more than 99.9% of *E. coli*, *S. aureus*, and *C. albicans* in water at 25°C and pH 6.5 after contact for 1.07, 1.71, and 6.37 s, respectively. Another quaternary ammonium exchange resin binding tri-iodide or penta-iodide was reported by Sanden et al. for their ability to disinfect water containing *L. pneumophile*.[37] Results indicated that the iodinated resins are stable demand release disinfectants. There was no residual iodine detected by amperometric titration. When an aqueous suspension containing 2.7×10^9 cfu of *L. pneumophile* per milliliter was passed through tri-iodinated resins, less than 0.004% were recovered. No viable cells were detected by direct plating from a suspension of 2.3×10^9 cfu/mL eluted through penta-iodinated resins (>99.999% viability reduced). Nakagawa et al. prepared poly(*N*-dodecyl-4-vinylpyridine-*co*-divinylbenzene) by copolymerizing 4-vinyl pyridine with divinylbenzene using suspension polymerization, followed by quaternization of the 4-vinylpyridine groups with 1-iodododecyl. When *E. coli* cell suspension was passed through a column packed with beads, the number of cells in the effluent decreased.[38]

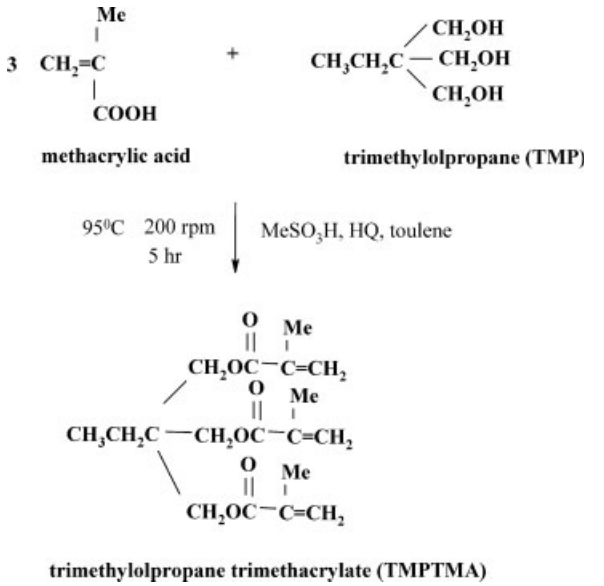
Kanazawa et al. synthesized various novel monomers with phosphonium salts from 3- (and 4-) chloromethylstyrene and trialkylphosphine and polymerized these monomers to obtain antibacterial polymers.[39] Antibacterial activities of the polymers were examined against *S. aureus* and *E. coli* by viable cell counting method in sterilized water. Compounds with the longest alkyl chain (octyl) were found to exhibit particularly high activity, and this fact may be ascribable to the contribution of the increased hydrophobicity of the compound to the microbiocidal activity.

The present study involves synthesis and characterization of trimethylolpropane trimethacrylate (TMPTMA)-based novel class of amine methacrylate monomer, its copolymerization with hydrophilic monomers, followed by quaternization with alkyl iodide and evaluation of their physical and antimicrobial properties.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA), trimethylol propane (TMP), and 1-iodooctane (octyl iodide) were obtained from Sigma, USA. Ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED), ethyleneglycol dimethacrylate (EGDMA), methacrylic acid, methanesulfonic acid, and hydroquinone were procured from E. Merck, Germany. Piperazine (PPZ), toluene, and methanol (HPLC grade) were obtained from Loba Chemicals (Mumbai, India) and were used as received. Luria agar and peptone (bacteriological grade) for microbiological assay were obtained from Hi-Media Laboratories, Mumbai (India) and were used for antimicrobial studies. *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) for antimicrobial assessment

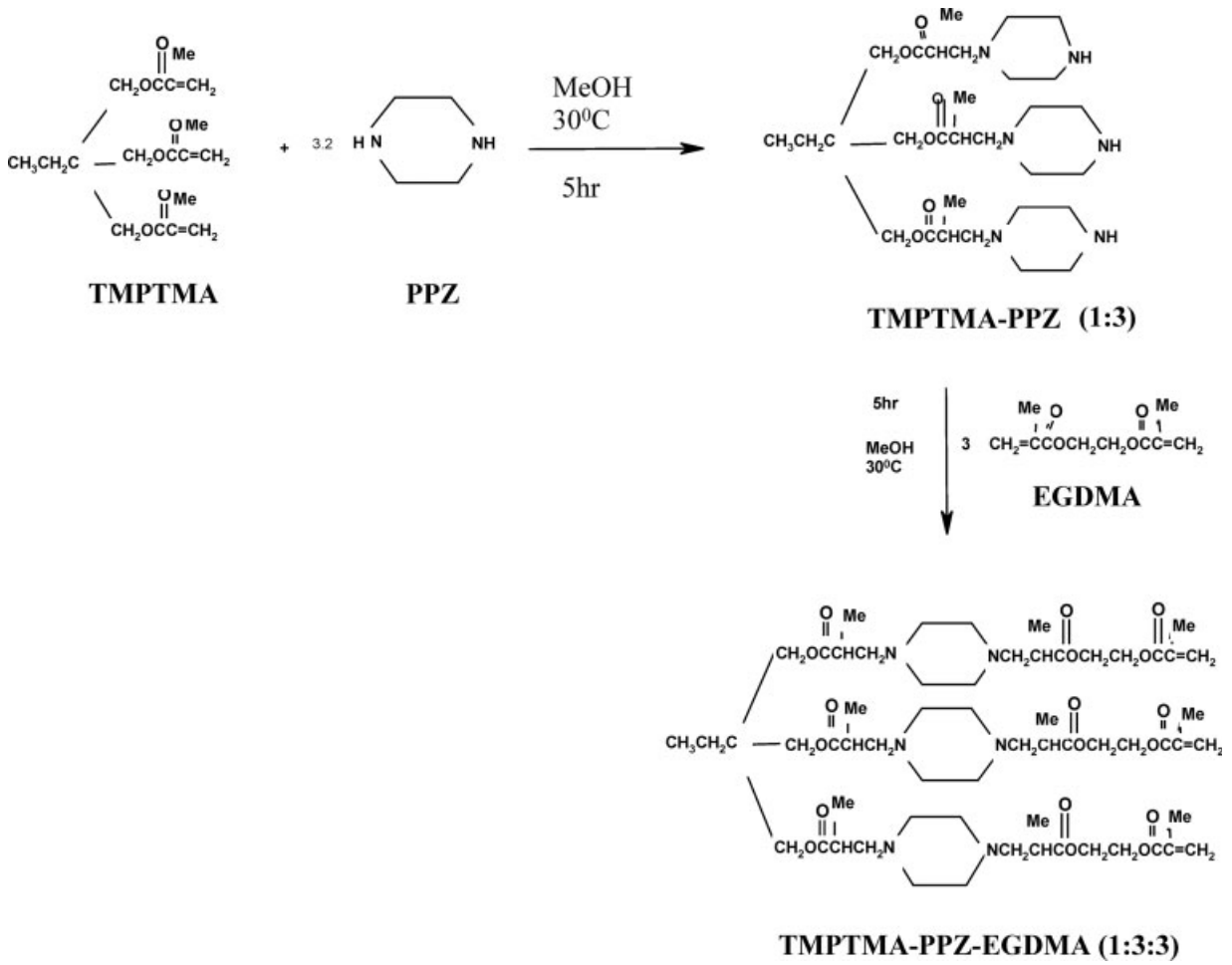


Scheme 1 Synthesis of trimethylopropane trimethacrylate (TMPTMA).

studies were obtained from Department of Biochemical Engineering and Biotechnology, IIT Delhi, India.

Monomer synthesis

Trimethylolpropane trimethacrylate–piperazine–ethyleneglycol dimethacrylate (TMPTMA-PPZ-EGDMA) was synthesized via three-step reaction (Schemes 1 and 2). Firstly, TMPTMA was synthesized by esterification of 1 mole of trimethylol propane with excess of methacrylic acid (3.5 moles). Methane sulfonic acid (1.4%) was used as catalyst, hydroquinone (0.15%) was used as inhibitor, and 20% toluene was used as azeotropic solvent. The amounts of catalyst, inhibitor, and solvent were taken as w/w percentage of total monomer (reaction in Scheme 1). The reaction was carried out in a three-necked round bottom flask fitted with a mechanical stirrer, a reflux condenser, and a dean stark apparatus in heavy paraffin oil bath at 95°C at 200 rpm for 5 h. The esterification



Scheme 2 Synthesis of TMPTMA-piperazine-EGDMA (TMPTMA-PPZ-EGDMA) (1 : 3 : 3).

reaction was stopped when calculated amount of water was collected in the arm of dean stark apparatus. The acid value of the obtained product was determined by titrating with alcoholic KOH, which was standardized using oxalic acid and cresol red as an indicator. The product was first washed with chilled 50-mL distilled water and then depending on the acid value, the product was washed several times by slowly adding 5% $NaHCO_3$ solution with continuous stirring to remove the unreacted acid and inhibitor. The two layers thus obtained were separated using separating funnel; the top layer was of product and the bottom layer consisted of unreacted acid and sodium salt of hydroquinone, which was responsible for the pink color in aqueous solution appearing at the bottom. The process of washing the upper layer (containing synthesized monomer) was repeated till the acid value reached below 1.0. Finally, the product obtained was washed with 50 mL of distilled water and vacuum-distilled at 70–75°C with 110 ppm of inhibitor and stored at 4°C.

In the second step, amination of TMPTMA with PPZ was carried out in a 250-mL two-neck flask equipped with oil bath, temperature controller, reflux condenser, and mechanical stirrer. PPZ (27.2 g; 3.2 moles), TMPTMA (39 g; 1 mole), and methanol (30% of total monomer) as solvent was taken (reaction in Scheme 2). The mixture was stirred continuously for 6 h at a controlled temperature of 35°C. TMPTMA-PPZ (1 : 3) obtained was then kept in vacuum oven for 3 h to remove the solvent. Finally, 1 mole of the product obtained was reacted with 3 moles of EGDMA under the same reaction condition to obtain TMPTMA-PPZ-EGDMA (1 : 3 : 3). The monomer synthesized was stored at 4°C and was identified by ATR-FTIR and ^{1}H NMR ($CDCl_3$, 300 MHz). For gel permeation chromatography experiment, a waters model 150 CV, GPC system with ultrasyragel columns was used; a refractive index detector was used to monitor the molecular weight and its distribution. Polystyrene and dimethyl sulfoxide (DMSO) were used as the standards and the eluent solvent, respectively.

Polymer synthesis

2-Hydroxyethyl methacrylate (HEMA) was copolymerized and crosslinked with TMPTMA-PPZ-EGDMA using redox (APS 0.6%, TEMED 0.6% by weight of total monomer concentration) free radical bulk polymerization technique. The formulation consisted of variable percentage of TMPTMA-PPZ-EGDMA (5–100%) with HEMA and distilled water (8% of total monomer). The reaction mixture was then poured into a polypropylene mold with an inner diameter of 5 mm. Within a time span of 5–20 min, a solid polymeric tube was obtained and was washed thoroughly with hot distilled water (80°C). Polymer tube was wiped dry using Whatman filter paper and then dried to constant weight at room temperature. They were then quaternized using excess of 1-iodooctane at 85°C for 3–4 h. Quaternized copolymers were again washed thoroughly with hot distilled water (80°C) to remove the residual monomer, dried and used for characterization and other studies.

Polymer characterization

ATR-FTIR spectroscopy

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrum of TMPTMA-PPZ-EGDMA and various poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymers before and after the quaternization were recorded on a Perkin Elmer spectrum one spectrometer.

Nuclear Magnetic Resonance (^{1}H NMR)

A Bruker AC 300 spectrophotometer at a frequency of 300 MHz was used for recording NMR of various samples in $CDCl_3$ solvent with monomer concentration of 4 mg/mL. Tetramethylsilane (TMS) was used as an internal standard.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) of all vacuum-dried samples was carried out on Perkin Elmer TGA-7 system. The thermograms were obtained under nitrogen atmosphere at a uniform heating rate at 10°C/min in the temperature range of 50–600°C. Relative thermal stability of the samples was evaluated in terms of initial decomposition temperature (IDT) and final decomposition temperature (FDT).

Scanning electron microscopy

The surface characteristics and porosity of synthesized copolymers of various compositions were studied using STEREOSCAN 360 (Cambridge Scientific Industries, U.K.), scanning electron microscope (SEM), after coating them with silver to provide conduction.

Leaching studies

UV–visible scan of TMPTMA-PPZ-EGDMA and HEMA monomer was done from 200 to 400 nm wavelength to know the maximum absorption peak of the monomers. The leaching of the monomers from synthesized copolymers before and after washing them with 250 mL of hot distilled water (80°C)

TABLE I
Average Molecular Weights Data by GPC for Synthesized Monomers

Monomer	M_n	M_w	Polydispersity (M_w/M_n)
TMPTMA-PPZ (1 : 3)	583	591	1.01
TMPTMA-PPZ-EGDMA (1 : 3 : 3)	1051	1190	1.1

was carried out at 210 and 275 nm. Simultaneously, leaching of alkyl iodide before and after washing was also checked by iodide selective ion meter, which was standardized using 0.1*M* iodide solution.

Evaluation of antimicrobial properties of quaternized copolymers

The synthesized copolymers of different compositions quaternized with alkyl iodide were tested against *E. coli* and *S. aureus*. Bacterial strains were grown in nutrient broth. Five percent (v/v) inoculum of bacterial culture was used to inoculate solution of nutrient broth (control) and test media (100-mL solution of nutrient broth + 50 mg polymer sample) and incubated on rotary shaker (200 rpm) at 37°C. Half milliliter of liquid was withdrawn at specified time intervals (24–48 h) from the test media. After suitable dilution with DNS reagent and distilled water, optical density was measured at 660 nm and calculated as optical density per milliliter (i.e., growth). The inhibition percentage (I) was obtained as follows:

$$I = \frac{100(X - Y)}{X}$$

where X is the optical density of the bacterial suspension in the control set and Y is the optical density of the bacterial suspension in the polymer containing test set.[40] This method is based on the principle that as the growth proceeds, cell number increases, which leads to increase in the optical density of the medium.

Evaluation of antimicrobial properties using SEM

The antimicrobial activities of quaternized amine methacrylate copolymers were also evaluated using STEREOSCAN 360 (Cambridge Scientific Industries, UK) SEM. Control unquaternized amine methacrylate copolymer and quaternized poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) (40/60) with 1-iodooctane were incubated separately in 50-mL erlenmeyer flask with luria broth inoculated with 10^6 CFU/mL of *E. coli*. The flasks were agitated at 37°C at a rate of 80 times/min for 24 h. After the incubation period, the copolymers were taken out and placed in sterile glass test tubes in an inclined position. Enough PBS (phosphate-buffer saline) was added to immerse the material and gently agitated by tapping the test tube with the index finger. The PBS was removed and fresh PBS added, and the process was repeated three times. This facilitated removal of loosely adhered bacteria and media components, which might interfere in the further processing. The retained bacteria were fixed with 5% glutaraldehyde for 1 h, and were then kept immersed in distilled water, and freeze-dried.[41] The surfaces of the copolymer samples were examined using SEM, after sputter-coating them with silver.

RESULTS AND DISCUSSION

Monomer synthesis

M_n and M_w of TMPTMA-PPZ (1 : 3) and TMPTMA-PPZ-EGDMA (1 : 3 : 3) are summarized in Table I. The polydispersity of TMPTMA-PPZ (1 : 3) and TMPTMA-PPZ-EGDMA (1 : 3 : 3) are 1.05 and 1.1, respectively. The polydispersity index shows that synthesized monomers are ideal monomers as the value lies between 1.0 and 1.5, further strengthening the idea that they are almost monodispersed system.

Polymer synthesis

Poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymer upto 60% TMPTMA-PPZ-EGDMA was successfully synthesized using redox free radical initiator, but on increasing TMPTMA-PPZ-EGDMA concentration beyond 60%, incomplete polymerization was observed. Increasing TMPTMA-PPZ-EGDMA content in the monomer mixture increases the hydrophobic content during the copolymerization, which is not supported by the redox initiator (APS/TEMED) that is hydrophilic in nature.

Polymer characterization

ATR-FTIR spectroscopy

The ATR-FTIR spectra of TMPTMA, TMPTMA-PPZ (1 : 3), and TMPTMA-PPZ-EGDMA (1 : 3 : 3) are presented in Figure 1. TMPTMA showed characteristic peaks of C=O at 1719 cm^{-1}, C=C at 1637 cm^{-1}, C—O of carbonyl at 1295 cm^{-1} [Fig. 1(a)]. TMPTMA-PPZ (1 : 3) [Fig. 1(b)] showed band of N—H at 3000–3200 cm^{-1}, and no peak of C=C at 1637 cm^{-1} was observed, which confirmed that the Michael addition reaction has taken place between

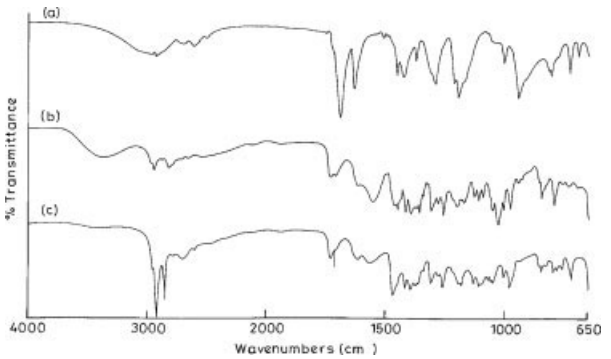


Figure 1 ATR-FTIR spectra of (a) TMPTMA, (b) TMPTMA-PPZ (1 : 3), and (c) TMPTMA-PPZ-EGDMA (1 : 3 : 3).

TMPTMA and PPZ. TMPTMA-PPZ-EGDMA (1 : 3 : 3) showed peak of C=C at 1637 cm^{-1} and of C—N at 1445 cm^{-1} confirming the reinsertion of double bond in the final product. ATR-FTIR spectra of poly (HEMA-*co*-TMPTMA-PPZ-EGDMA) before and after the quaternization are presented in Figure 2. Poly (HEMA-*co*-TMPTMA-PPZ-EGDMA) showed characteristic peaks of C=O at 1719 cm^{-1}, C—O of carbonyl at 1295 cm^{-1}, and band at 3386 cm^{-1} due to OH group of HEMA, while additional peaks were observed at 748 and 945 cm^{-1} due to iodide in the IR spectra of copolymer after quaternization [Fig. 2(b)]. In addition to this, there was absence of peak at 1638 cm^{-1} of C=C in IR spectra of both copolymer samples (Fig. 2(a,b)], which confirmed the polymerization.

Nuclear magnetic resonance (^{1}H NMR)

Proton peaks at 6.13 and 5.59 ppm of —C=CH_2, 4.28 ppm of —OCH_2 of ester group, 1.9 ppm of —CH_2, and 0.93 ppm of —CH_3 group were observed in TMPTMA. In case of TMPTMA-PPZ, new peaks at 2.46 ppm of —CH_2—N— and at 2.9 ppm of —NH were observed. There was absence of peaks at 6.13 and 5.59 ppm of —C=CH_2 confirming PPZ-terminated TMPTMA product is formed. While in case of TMPTMA-PPZ-EGDMA, reappearance of proton peaks at 6.13 and 5.59 ppm confirms the presence of vinyl group in the final product due to insertion of EGDMA (Fig. 3).

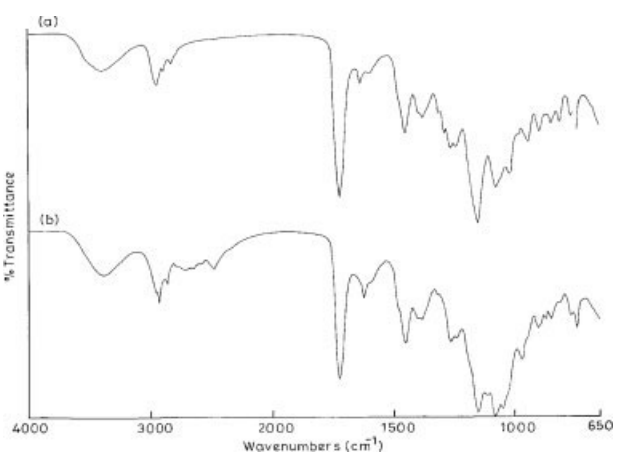


Figure 2 ATR-FTIR spectra of (a) poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) and (b) poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternize with 1-iodooctane.

Thermogravimetric analysis

TGA thermograms of poly(HEMA) and poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternize with 1-iodooctane with different percentage of TMPTMA-PPZ-EGDMA are presented in Figure 4. Thermogram of the poly(HEMA) and quaternized poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymers showed clean single-step degradation. The initial decomposition

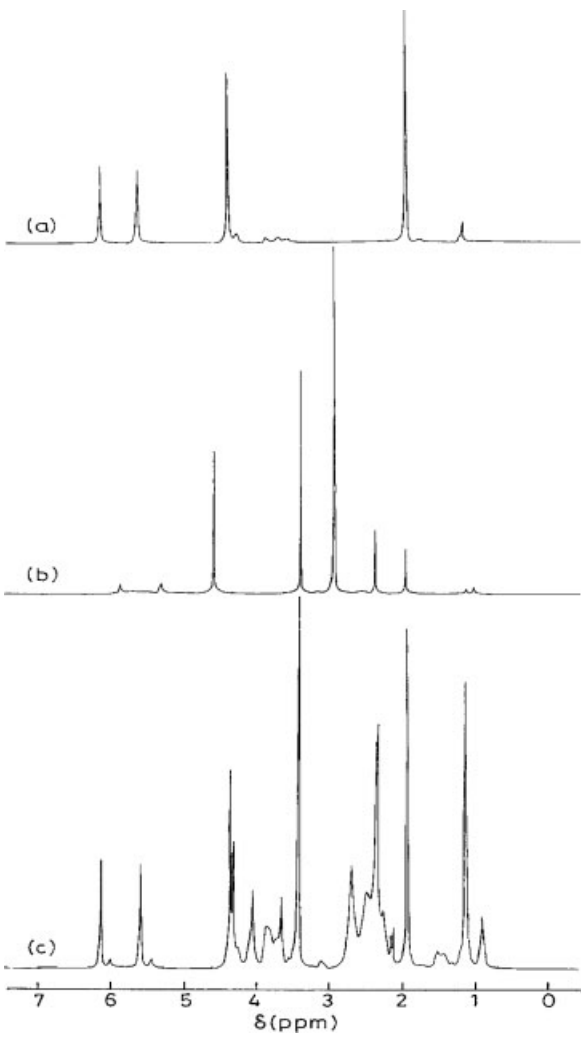


Figure 3 ^{1}H NMR spectra of (a) TMPTMA monomer, (b) TMPTMA-PPZ (1 : 3), and (c) TMPTMA-PPZ-EGDMA (1 : 3 : 3).

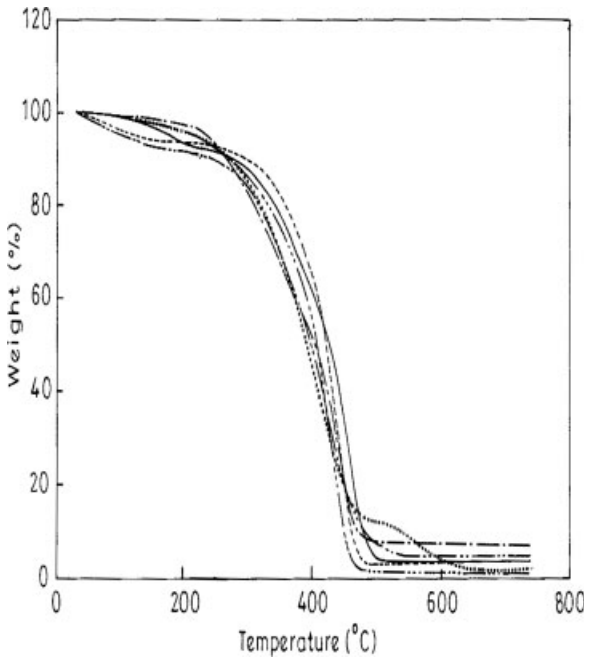


Figure 4 Representative TGA curves of (···) pure HEMA and poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternize with 1-iodooctane with different % of TMPTMA-PPZ-EGDMA: (—), 20%; (- - -), 30%; (-···-), 40%; (-···-), 50%; (····), 60%.

temperature of the poly(HEMA) is 394°C, which decreased to 304°C on insertion of 60% TMPTMA-PPZ-EGDMA in the copolymer. Thermal stability of the quaternized copolymer decreased with the increase of TMPTMA-PPZ-EGDMA content due to increase of iodine content with the increasing percentage of TMPTMA-PPZ-EGDMA in the copolymer used for quaternization.

Scanning electron microscopy

The SEM photographs of poly(HEMA) and copolymers containing 5–60% TMPTMA-PPZ-EGDMA monomer in poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) system quaternized with 1-iodooctane are presented in Figure 5. The poly(HEMA) and quaternized copolymer upto 10% TMPTMA-PPZ-EGDMA content showed zero porosity, whereas the copolymer containing 20% or higher TMPTMA-PPZ-EGDMA showed pore size in range of 9.9–10.1 μm. It is the presence of three vinylic groups present in TMPTMA-PPZ-EGDMA, which are responsible for the porosity. Ingavle et al. synthesized a series of macroreticular, beaded, glycidyl methacrylate (GMA) and divinylbenzene (DVB) polymers with variance in pore size distribution by suspension polymerization.[42] They showed dependence of surface functional group, surface area and porosity on copolymer composition, porogen type, and volume. He concluded that there were many factors that contribute to pore size and pore size distribution including porogen type, the volume of porogen relative to the volume of the monomers, the mole ratio between GMA, the reactive monomer, and the crosslinker, DVB, termed as crosslink density.

Leaching studies

The copolymers synthesized were washed several times with hot distilled water (80°C) to remove any residual monomer and the unattached alkyl iodide in the quaternized copolymers. The residual leaching of monomer from the copolymer showed distinct absorption peak at 210 and 275 nm with zero washing, but no peak at 210 and 275 nm was observed in the filterate after washing the polymer several times with hot distilled water (80°C). Presence of free 1-iodooctane from the copolymer was also checked using iodide selective ion meter. With zero washing, the copolymer showed the presence of 2 ppm of free 1-iodooctane, which was brought to zero with two to three washings with hot distilled water (80°C) as checked by iodide selective ion meter (Fig. 6).

Evaluation of antimicrobial properties of quaternized copolymers

Antimicrobial activity of poly(HEMA) and poly (HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternized with 1-iodooctane with varying percentage of TMPTMA-PPZ-EGDMA from 5 to 60% in the copolymer against bacteria is shown in Figure 7. The poly (HEMA) showed maximum growth (97%) for the bacteria, while the quaternized poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymers showed an appreciable reduction in the bacterial growth. It was observed that as the TMPTMA-PPZ-EGDMA content in the copolymer increases, the site of quaternization increases; hence, the bacterial growth percentage decreases. Kenawy[15] synthesized quaternary ammonium groups containing poly(glycidyl methacrylate) and found that the growth inhibitory effect varied according to the structure of the polymer and the composition of the active group and increased with increasing the concentration of the polymer.

Evaluation of antimicrobial properties using SEM

Bacterial retention onto copolymer surfaces of control poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) and poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternized with 1-iododoctane were examined by SEM. The copolymer surfaces were washed with PBS to remove the loosely adhered bacteria. Bacterial attachment leading to the formation of a coherent biofilm is a two-step process: the initial step being reversible wherein the physical forces come into play and the

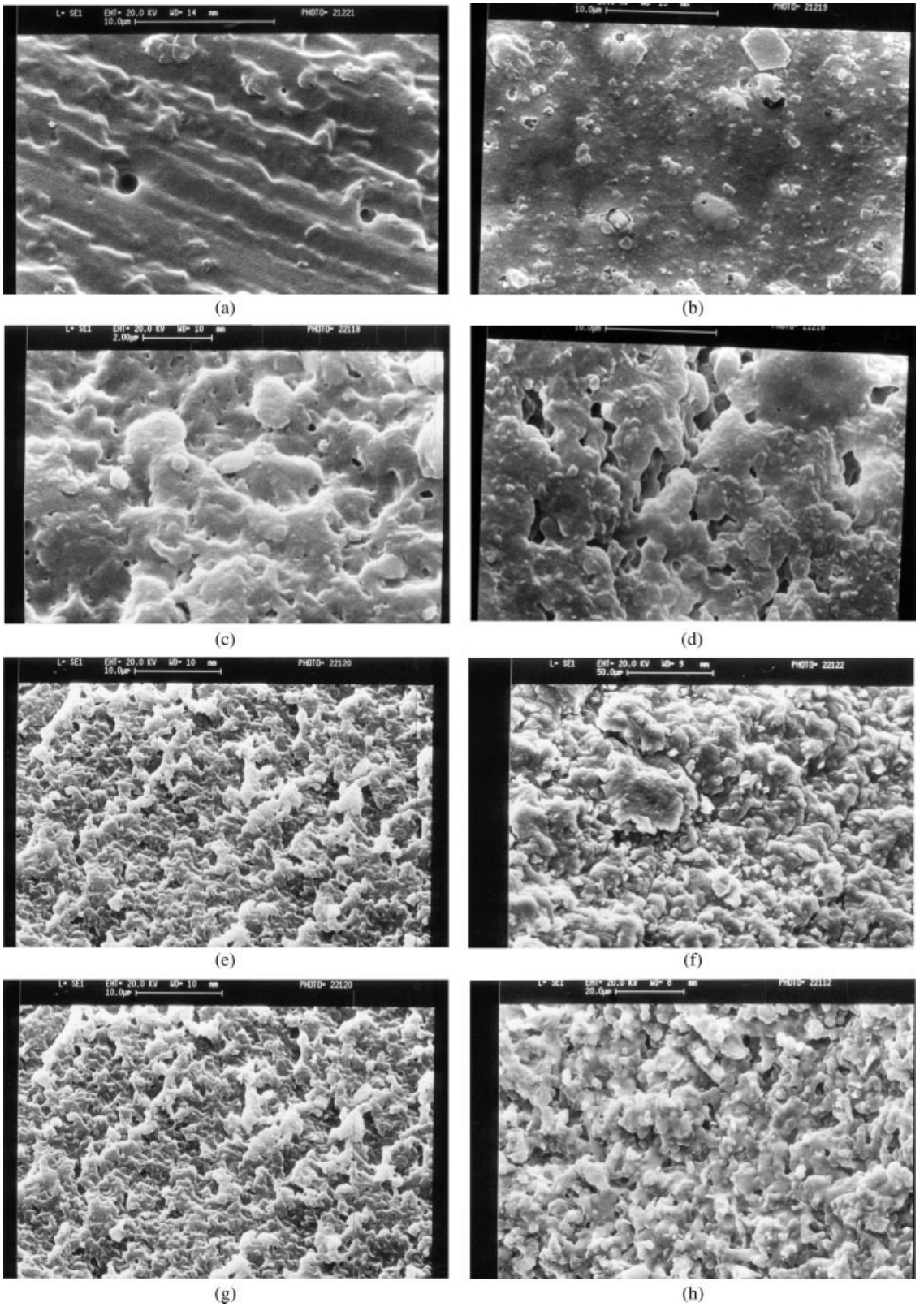


Figure 5 SEM photographs of poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternize with 1-iodooctane with different % of TMPTMA-PPZ-EGDMA: (a) 0%, (b) 5%, (c) 10%, (d) 20%, (e) 30%, (f) 40%, (g) 50%, (h) 60%.

second step and the final step being the synthesis of extracellular polysaccharide cementing film.[43] The washing step causes detachment and removal of bacteria in the initial phase of adhesion.[44] This step becomes essential as bacteria have to withstand the sheer stress experienced *in vivo* to form a biofilm.

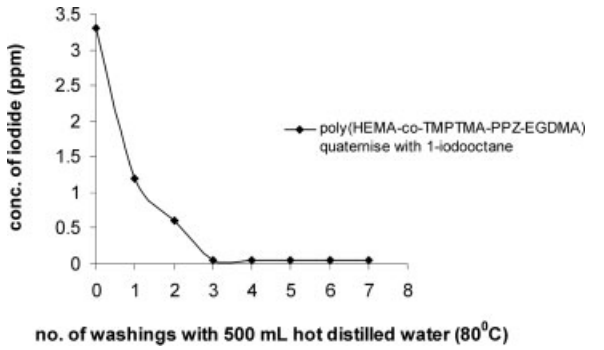


Figure 6 Leaching studies of alkyl iodide using iodide selective ion meter.

Thus, the bacteria that have adhered and retained leads to biofilm formation, and it is this population, which is analyzed by SEM. Figure 8(a) shows retention of *E. coli* onto the surface of control poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymer, whereas there is complete prevention of retention of *E. coli* onto the poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) (40/60) copolymer surface quaternized with 1-iododoctane [Fig. 8(b)], which is due to contact-killing antimicrobial properties of quaternized copolymer without releasing any bioactive agent. Leaching studies using iodide selective ion meter showed no release of iodine, which further confirms the permanent attachment of active iodine through alkyl chain. The contact-killing microbiocidal role of the copolymer can be attributed to the hydrophobic interaction of the alkyl chain with lipid bilayer of the cell wall[7] and the amphiphilic nature of the copolymer, which disrupts the cell membrane and leads to leakage of K^+ ions and cytoplasmic fluid and thus inhibits various cell processes.[45]

CONCLUSION

Trimethylolpropane–piperazine–ethyleneglycol dimethacrylate (TMPTMA-PPZ-EGDMA) monomer was successfully synthesized and copolymerized with

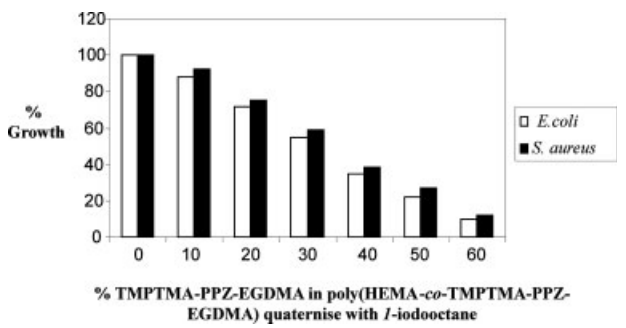


Figure 7 Effect of quaternized copolymers on percentage growth of bacteria.

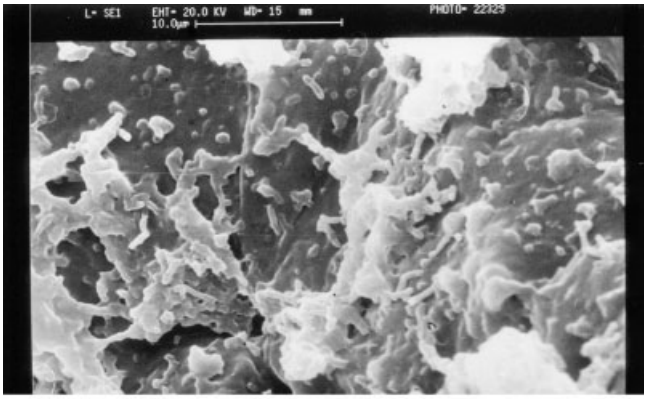


Figure 8 SEM photographs: (a) poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) with *E. coli* adhered on its surface; (b) poly (HEMA-*co*-TMPTMA-PPZ-EGDMA) quaternize with 1-iodooctane, no bacteria grown on its surface.

2-hyroxyethyl methacrylate by free radical redox polymerization technique. The copolymers of poly (HEMA-*co*-TMPTMA-PPZ-EGDMA) of different compositions were quaternized with 1-iodooctane. Leaching studies showed no release of iodide from copolymer as checked by iodide selection ion meter. Poly(HEMA-*co*-TMPTMA-PPZ-EGDMA) copolymer quaternized with 1-iodooctane showed broad spectrum contact-killing antimicrobial properties without release of any bioactive agent. Antimicrobial activities increased with increasing TMPTMA-PPZ-EGDMA concentration in the quaternized copolymers. These quaternized copolymers have strong potential to be used as contact-killing antimicrobial polymers for various biomedical applications.

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