Preparation of Iodine Containing Quaternary Amine Methacrylate Copolymers and Their Contact Killing Antimicrobial Properties

Supriya Punyani, Harpal Singh

Centre for Biomedical Engineering, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

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ABSTRACT: A novel quaternary amine methacrylate monomer (QAMA) was synthesized by amination of dimethacrylate with piperazine followed by its quaternization using an alkyl iodide. Copolymerization of QAMA with 2-hydroxyethyl methacrylate was carried out by free radical bulk polymerization technique at room temperature using ammonium persulfate and *N,N,N′,N′*-tetramethyl ethylenediamine as a redox initiator. The monomer as well as copolymers was characterized by FTIR and ^{1}H NMR spectral studies. Thermal and physical characteristics of copolymers of varying compositions of QAMA were evaluated by thermogravimetric analysis, differential calorimetry, contact angle and scanning electron microscopy. The antibacterial activity of the synthesized quaternary amine dimethacrylate copolymers against *Escherichia coli* and *Staphylococcus aureus* was studied by zone of inhibition and colony count method. QAMA copolymers showed broad-spectrum contact killing antibacterial properties without releasing any active agent as checked by iodide-selective ion meter. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 1038–1044, 2006

Key words: copolymerization; initiators; radical polymerization; NMR; monomers

INTRODUCTION

Disinfectants are chemical agents which kill microorganisms in short time, being usually used as solution.[1] Nowadays polymeric disinfectants have gathered considerable attentions because of their nontoxicity and nonirritant properties with prolonged antimicrobial activities compared with that of ordinary disinfectants like phenol, halogens, quaternary ammonium compounds (QACs) used for the same purpose.[2–9] Polymeric QACs have shown promising antimicrobial activities because of their amphiphilic structure and surfactant properties, which was first reported by Dogmak.[7] The antimicrobial action of the QACs is based on their damaging surfactant-like interaction with the membrane (cytoplasmic) of bacteria, resulting in loss of the membrane permeability. At convenient concentrations, they can cause cell leakage and death of the cell.[8] Quaternary structures are effective on both gram-positive and gram-negative bacteria but they have a stronger antibacterial effect on gram-positive ones, since gram negatives have an extra protective membrane. QACs are not affected from the protein concentration of the environment and do not lose their affectivity on bacteria over the course of time.[10] The effectiveness of these agents against microorganism is directly related to its area of contact with the microorganism's medium.

QACs containing polymers have been the subject of many investigations as water and air disinfectant and in antifouling coatings. Destais et al. studied biocidal properties of epoxy resins containing quaternary ammonium salts in which QACs containing oligomers were used as polyols to prepare polyurethane (PU) films by reaction with a triisocyanate and were found to have good bactericidal activities against *E. coli* and remain practically effective even after several months of immersion in water.[11] Tiller et al. designed contact killing antimicrobial surfaces by covalently attaching poly(4-vinyl-*N*-alkylpyridinuium) bromide to glass slides killing air borne bacteria on contact.[12] The antibacterial properties were assessed by spraying aqueous suspensions of bacterial cells on the surface followed by air drying and counting the number of cells remaining viable. It was found that modified glass surface was able to kill more than 90% of the deposited *S. aureus* cells in a dry state and a simple periodic washing could remove the dead deposited cells and rejuvenate such surface. Ikeda et al. synthesized polyesters/polyamides with well-defined spacer length, then quaternized it using alkyl bromide and checked its antimicrobial activity against *B. subtilis*, *P. aerugi-*

Correspondence to: H. Singh (harpal@cbme.iitd.ernet.in).
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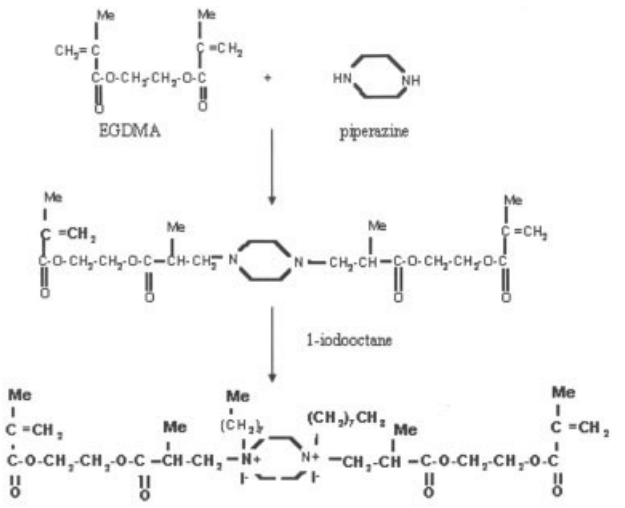
nosa etc.[13] Li et al. synthesized copolymer of 4-vinylpyridine with styrene and then quaternized it using benzyl bromide to introduce microbiocidal properties into the copolymer.[14] The studies revealed their broad prospects for development and application in the field of bioengineering, bioinstrumentation, and environmental protection. Another research group, Lee et al., introduced antimicrobial properties onto the glass surface by direct polymerization of tertiary amine 2-(dimethylamino) ethyl methacrylate via atom transfer radical polymerization and quaternized using an alkyl halide and then its antimicrobiocidal properties were tested against *E. coli* and *B. subtilis* and it was observed that 6.25 cm^2 modified glass surface was sufficient to kill 10^9 bacteria in few minutes.[15] Similarly, Augusta et al. attempted immobilization of quaternary ammonium salts on hydrophilic gels based on sucrose methacrylate and tested their antibacterial properties and it was found that they can be applied without contamination of the substrate and can be removed easily and used repeatedly.[5] Hazziza-Laskar et al. synthesized water-insoluble PU quaternary ammonium salts and found that these films exhibit biocidal activity against gram-positive and negative bacteria and yeast and molds. It was found that the bioactivity was controlled by many parameters such as the contact time of film with bacteria, the NCO/OH ratio used to prepare the cure PU, and the length of alkyl chain linked to the quaternary nitrogen atom.[16] Water insoluble macroreticular macromolecules based on spherical copolymers were prepared by suspension polymerization of glycidylmethacrylate with 1,4 divinylbenzene. The glycidyl groups were allowed to open by the reaction with hydrogen chloride, followed by the reaction with alkyl amines. Antibacterial activity against *E. coli* and *S. aureus* was evaluated and it was found that antibacterial activity of macromolecule increases with increasing amount of quaternary ammonium groups in the macromolecule.[17]

In all the earlier-mentioned cases, nitrogen bearing polymers have been quaternized to impart antimicrobial properties; but this method of quaternization results in less extent and surface quaternization which may lead to loss of antimicrobial properties with time due to surface erosion. Not much attempts have been made to check the polymerization behavior of quaternary ammonium monomers and physical and antimicrobial properties of their polymeric products. Hong et al. copolymerized reactive diluent containing quaternary ammonium salts with urethane acrylate using UV light for antistatic coatings.[18] Similarily, Gong et al. prepared copolymers based on 2-ethyl hexyl methacrylate and *N,N*-dimethyl amino ethylmethacrylate for humidity sensors.[19] The present study involves synthesis and characterization of a novel class of quaternary amine methacrylate monomer (QAMA) monomer, its copolymerization with hydrophilic monomers, and evaluation of their physical and antimicrobial properties. This particular approach for synthesis has been adopted to obtain chemically reactive component, which is active enough to be coated onto devices to impart antimicrobial property to them.



Scheme 1 Synthesis of quaternary amine methacrylate (QAMA).

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA), ammonium persulfate (APS) from CDH, India, *N,N,N′,N′*-tetramethyl ethylenediamine (TEMED), ethylene glycol dimethacrylate (EGDMA) (E. Merck, Germany), 1-iodooctane (Sigma, USA), piperazine and methanol (HPLC grade; GS Chemicals, India) 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. *E. coli* (gram negative) and *S. aureus* (gram positive) for antimicrobial assessment of copolymers containing quaternized monomer (QAMA) was obtained from Department of Chemistry, IIT Delhi, India.

Monomer synthesis

QAMA was synthesized via two step reaction (Scheme 1). To a 250-mL two-neck flask equipped with oil bath, temperature controller, refluxing condenser, and mechanical stirrer, 8.6 g (1 mol) of piperazine and 59 g (3 mol) of EGDMA and methanol (30% of total monomer) as solvent was added. The mixture was stirred continuously for 6 h at a controlled temperature of 35°C. The reaction product obtained was then kept in vacuum oven for 3 h to remove the solvent and the product was identified by attenuated total reflec-

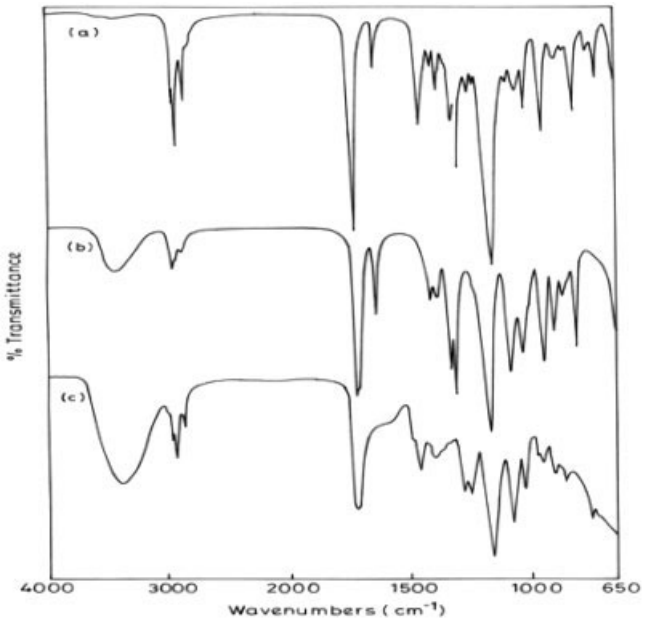


Figure 1 ATR-FTIR spectra of (a) QAMA, (b) HEMA, and (c) poly(HEMA-*co*-QAMA).

tance-Fourier transform infrared spectroscopy (ATR-FTIR) and nuclear magnetic resonance (^{1}H NMR) ($CDCl_3$, 300 MHz). The synthesized amine acrylate monomer was then quaternized using 1.1 eq/amine of 1-iodooctane by refluxing for 2 h continuously to give pale yellow liquid and then cooled and stored at 4°C.

Polymer synthesis

HEMA was copolymerized with QAMA by a redox initiator (APS 0.6%, TEMED 0.6% by weight of total monomer concentration) in an aqueous solution. The formulation consisted of variable percentage of QAMA (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 this was washed thoroughly with boiling distilled water. Polymer tube was wiped dry using laboratory tissue paper and weighed and then dried to constant weight at room temperature for characterization and other studies.

Polymer characterization

Attenuated total reflectance (ATR-FTIR spectroscopy)

ATR-FTIR spectrum (attenuated total reflectance-Fourier transform infrared spectroscopy) of QAMA and various poly(HEMA-*co*-QAMA) copolymers were recorded on a Perkin–Elmer spectrum one spectrometer.

Nuclear magnetic resonance

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. Tetramethyl silane was used as an internal standard.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies on various polymeric samples were carried out using Perkin–Elmer DSC-7 system. Vacuum-dried samples were loaded into the DSC system and the thermogram was run in the temperature range of 0–200°C under the nitrogen atmosphere at the heating rate of 10°C/min.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) studies of all vacuum-dried samples were 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 and final decomposition temperature.

Contact angle measurements

Contact angle was determined by angle formed by a drop of water on the surface of films using a Rame-

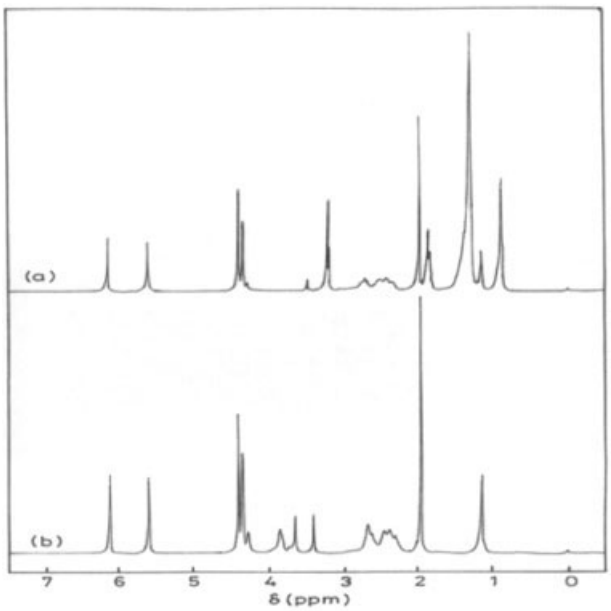


Figure 2 ^{1}H NMR spectra of (a) QAMA monomer and (b) EGDMA–piperazine amine acrylate monomer.

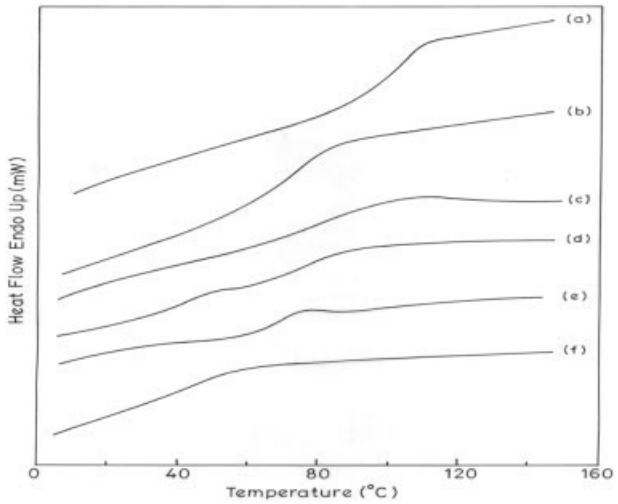


Figure 3 DSC thermograms of poly(HEMA-*co*-QAMA) with different percentages of QAMA (a) 0%, (b) 5%, (c) 10%, (d) 20%, (e) 30%, and (f) 40%.

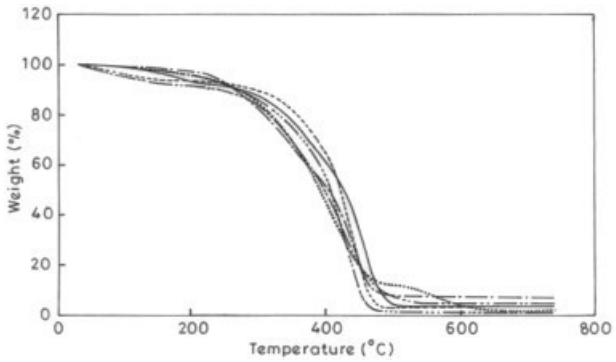


Figure 4 Representative TGA curves of (–˙–) pure HEMA and poly(HEMA-*co*-QAMA) with different percentages of QAMA (—) 5%, (– – –) 10%, (–¨–) 20%, (– ¨–) 30%, and (¨¨) 40%.

Hart Goniometer (model 100–00-230, USA). Polymer was sliced into thin sections and was kept on constant angle platform and a drop of distilled water was applied over the surface using a microsyringe. The angle formed between water and solid surface called contact angle was measured using the telescope.

Scanning electron microscopy

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

Leaching studies

UV–visible scan of QAMA 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 boiling distilled water was carried out at 210 and 275 nm. Simultaneously, leaching of iodooctane before and after washing was checked by iodide-selective ion meter which was standardized using 0.1*M* iodide solution.

TABLE I
Thermal Decomposition Temperature (TGA), Glass Transition Temperature (T_g), and Contact Angle Values of Copolymers with Different Compositions of QAMA

	% QAMA in poly (HEMA-*co*-QAMA)					
	0	5	10	20	30	40
T_g (°C) from DSC	102	99	95	88	76	70
T_i (°C) from TGA	416	394	386	363	330	304
T_f (°C) from TGA	489	483	476	468	416	407
Contact angle (°) ±2°	48	52	58	76	82	88

Evaluation of antimicrobial properties of QAMA copolymers

Nutrient agar plates were prepared by dissolving 35 g of readymade Luria agar in 1 L of water, and pH was adjusted to 7.0 ± 0.2. The contents were then sterilized by autoclaving at 15 lbs pressure (121°C) for 30 min. A lawn of bacteria (*E. coli* and *S. aureus*) was laid over the plates using sterile cotton swab. A weighed amount of QAMA copolymer was placed on the plate using sterile forceps. A nonquaternized copolymer was also placed as a control. Plates were incubated at 37°C at 24 h and the clear zone of inhibition around the sample was measured the next day as a measure of antimicrobial activity of quaternary amine acrylate HEMA copolymers. The antibacterial activity of copolymers with variable percentage QAMA against *E. coli* was also evaluated by colony count method.[20] Copolymer (20 g) was kept in 200 mL of bacterial solution with initial count of 2×10^5 cells/mL and aliquots were withdrawn at different time interval and viable cell count was seen.

RESULTS AND DISCUSSION

Polymer synthesis

Poly(HEMA-*co*-QAMA) copolymer up to 40% QAMA were successfully synthesized using redox free radical initiator but on increasing QAMA percentage beyond 40%, incomplete polymerization was observed. Increasing QAMA content in the monomer mixture increases iodine, which acts as inhibitor for copolymerization after a certain extent.

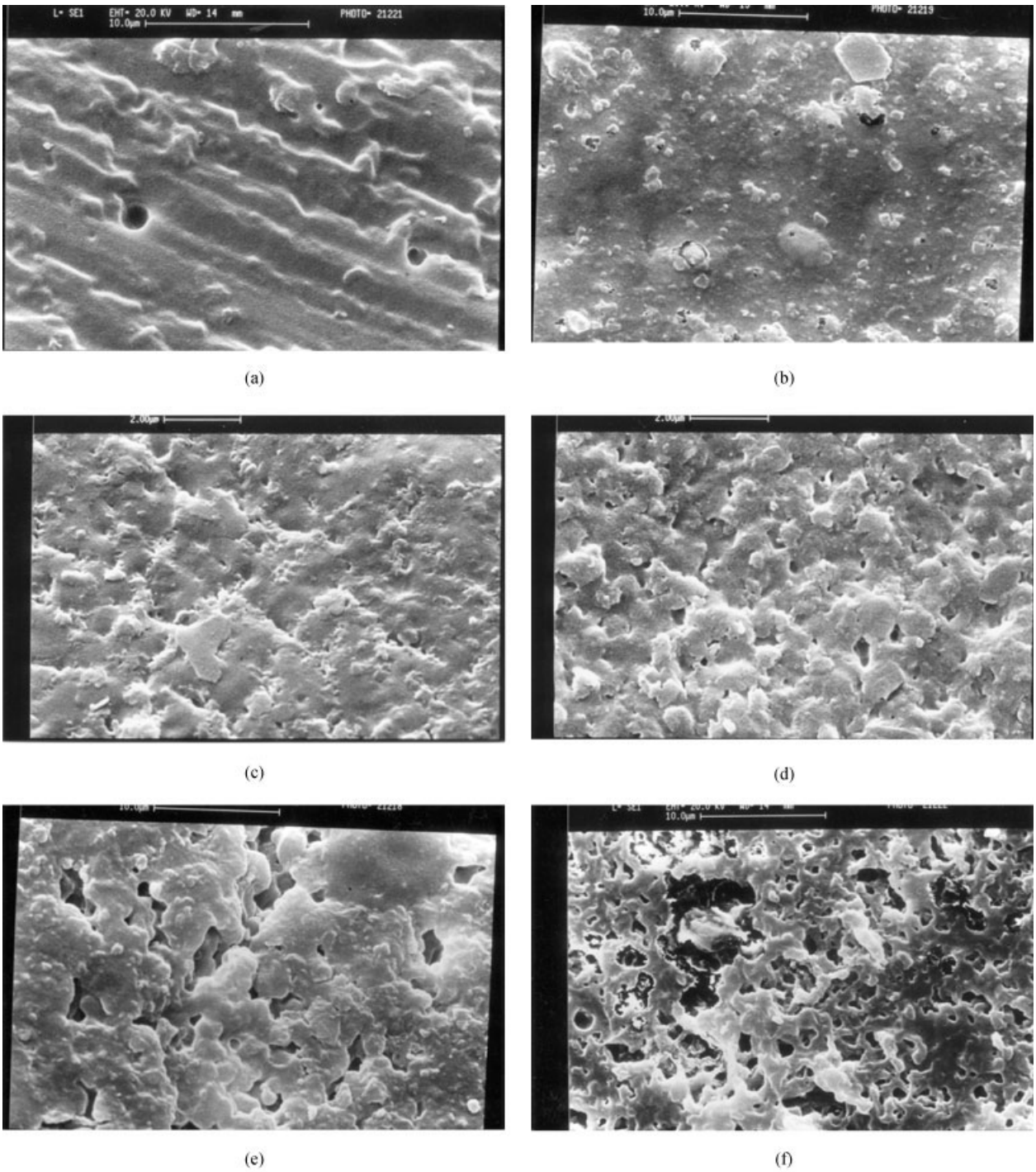


Figure 5 SEM photographs of poly(HEMA-*co*-QAMA) with different percentages of QAMA (a) 0%, (b) 5%, (c) 10%, (d) 20%, (e) 30%, and (f) 40%.

Polymer characterization

Attenuated total reflectance (ATR-FTIR) spectroscopy

The ATR-FTIR of HEMA, QAMA monomer, and poly-(HEMA-*co*-QAMA) are presented in Figure 1. QAMA showed characteristic peaks of C═O at 1719 cm^{-1}, C═C at 1637.13 cm^{-1}, C—O of carbonyl at 1295 cm^{-1}, C—N at 1445 cm^{-1}, and of iodide group at 747–900 cm^{-1}. HEMA showed characteristic peaks of OH at 3399 cm^{-1}, C═O at 1714 cm^{-1}, C—O of carbonyl at 1200 cm^{-1}, C—O—C stretching at 1022 cm^{-1}, and C═C at 1637.13 cm^{-1}. Whereas in the spectrum of poly(HEMA-*co*-QAMA) copolymer, the stretching vibration of C═C group of the monomer at 1638 cm^{-1} disappeared and peak at 3359 cm^{-1} due to OH group of HEMA, peaks at 747 and 900 cm^{-1} due to iodide

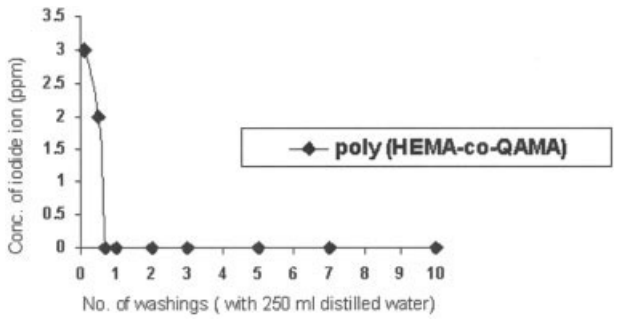


Figure 6 Leaching studies of alkyl iodide using iodide-selective ion meter.

group of QAMA were observed. This confirms the insertion of QAMA group into the copolymer during copolymerization with HEMA.

Nuclear magnetic resonance

Proton peaks at 6.13, 5.59 ppm of C═CH_2, 2.46 ppm of —CH_2—N—, 4.28 ppm of —OCH_2 of ester group, 1.12 ppm of α—CH_3 group were observed both in amine methacrylate and quaternized amine methacrylate monomer. In addition to these, peaks at 2–1.9 ppm of CH_2 protons of long alkyl chain and characteristic peak of —CH_3 proton at the chain end of alkyl chain at 0.9 ppm observed in quaternized amine methacrylate confirms quaternization of amine methacrylate (Fig. 2).

Differential scanning calorimetry

The DSC thermograms of poly(HEMA), poly(HEMA-*co*-QAMA) with different percentages of QAMA are presented in Figure 3. It was found that T_g of copolymer decreases with the increase in percentage of QAMA in poly(HEMA-*co*-QAMA) copolymer because of increase in hydrocarbon chain length of octyl iodide (Table I).

Thermogravimetric analysis

TGA thermograms of poly(HEMA), poly(HEMA-*co*-QAMA) with different percentages of QAMA are presented in Figure 4. Thermogram of the poly(HEMA) and poly(HEMA-*co*-QAMA) copolymers showed clean single-step degradation. The initial decomposition temperature of the poly(HEMA) is 394°C, which decreased to 304°C on insertion of 40% QAMA in the copolymer (Table I). Thermal stability of the copolymer decreased with the increase of QAMA content because of increase of iodine content with the increasing percentage of QAMA in the copolymer.

Contact angle measurements

As given in Table I, it is observed that HEMA is hydrophilic in nature and has contact angle of 48° but as QAMA content is increased in the poly(HEMA-*co*-QAMA) copolymer, the contact angle increases as hydrophobic nature of copolymer increases with the increase of QAMA due to introduction of long hydrocarbon chain of octyl iodide.

Scanning electron microscopy

The SEM photographs of poly(HEMA) and copolymers containing 5–40% QAMA monomer in poly(HEMA-*co*-QAMA) system are presented in Figure 5. The poly(HEMA) and copolymer up to 10% QAMA content showed zero porosity whereas the copolymer beyond 20% QAMA showed pore size in range of 9.74–9.91 μm.

Leaching studies

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

Evaluation of antimicrobial properties of QAMA copolymers

No growth of bacteria on the copolymers was observed the next day after the incubation. The contact killing antibacterial properties of copolymers contain-

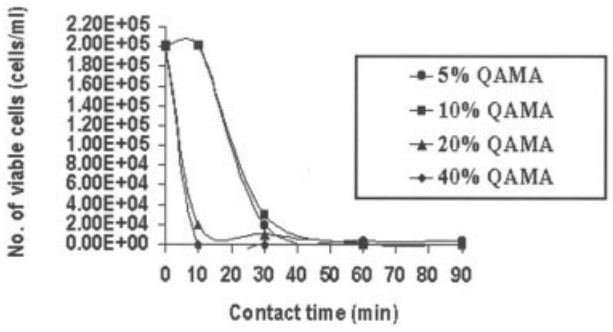


Figure 7 Decrease in contact time of killing of *E. coli* with the increase in QAMA content in the copolymer.

ing different percentages of QAMA against *E. coli* with viable cell count of 2×10^5 cells/mL in aqueous solution was also checked by colony count method to see the effect of increasing QAMA percentage on the contact killing antimicrobial properties of the copolymer, and it was observed that contact time of killing of *E. coli* reduces to a great extent with increasing QAMA monomer in the copolymer system (Fig. 7).

CONCLUSIONS

Amine methacrylate monomer was successfully synthesized and quaternized with alkyl iodide. Quaternary amine methacrylate was then copolymerized with 2-hyroxyethyl methacrylate by redox free radical polymerization technique. Leaching studies showed no release of iodide from copolymer as checked by iodide selective ion meter. QAMA copolymer showed broad spectrum contact killing antimicrobial properties without release of any bioactive agent. Antimicrobial activities increased with increasing QAMA concentration in the copolymers.

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