

Synthesis and Characterization of Quaternary Ammonium PEGDA Dendritic Copolymer Networks for Water Disinfection

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Received 16 July 2009; accepted 30 September 2009

DOI 10.1002/app.31510

Published online 5 January 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Quaternary ammonium compounds are some of the most widely used antimicrobial agents for various medical applications due to their low toxicity and broad spectrum antimicrobial activity. Various generations of poly(ethyleneglycol)diacrylate (PEGDA) based dendrimers were synthesized by Michael addition reaction of PEGDA with ethylene diamine and diethyl amine. The percentage yield of different generation of dendrimers were 70%, 66%, 60%, and 85% for G1.0 (=), G1.5 (NH₂), G2.0 (=), and G2.5 (=, NEt₂), respectively. Synthesized dendrimers were also copolymerized with ethyleneglycol dimethacrylate by free radical bulk polymerization at room temperature using ammonium persulphate/*N,N,N',N'*-tetramethyl ethylenediamine as a redox initiator system to form dendritic copolymer networks. These networks were quaternized with hydrochloric acid by continuously refluxing at 40°C for 6 h. Dendrimers and quaternized dendritic copolymer networks were character-

ized by ¹HNMR, FTIR, Differential scanning calorimetry, Thermogravimetric analysis, Scanning electron microscope, swelling, and leaching studies. Synthesized quaternary ammonium dendritic copolymer networks were found to be biostable and insoluble in water and capable of killing both Gram-positive and Gram-negative bacteria when contaminated water was treated with them. It was also observed that antimicrobial efficiency of dendritic copolymer networks increases with the increase in nitrogen atoms in the copolymer. The dendritic copolymer network with 16 quaternary ammonium groups (G2.5 (=, NEt₂): EGDMA QHCl) were highly efficient to disinfect 10 mL bacterial solution of 2000 cfu/mL within 2 min even at a very low concentration of 0.005 g/mL. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 116: 1640–1649, 2010

Key words: copolymerization; dendrimers; crosslinking; redox polymers; synthesis

INTRODUCTION

Microbial infection remains one of the most serious complications in several areas, particularly in medical devices, drugs, health care and hygienic applications, water purification systems, hospital and dental surgery equipments, textiles, food packaging, and food storage.^{1,2} Antimicrobials have gained interest from both academic research and industry due to their potential to provide quality and safety benefits to many materials. However, antimicrobial agents suffer from many disadvantages, such as high dose, toxicity to the environment and short-term antimicrobial ability. To overcome these problems antimicrobial functional groups can be introduced into polymer molecules. The use of antimicrobial poly-

mers offers promise for enhancing the efficacy and prolonging the life time of some existing antimicrobial agents and minimizing the toxicity and environmental problems associated with conventional antimicrobial agents. Research concerning the development of antimicrobial polymers represents a great challenge for both the academic world and industry.³

Quaternary ammonium compounds (QACs) are some of the most widely used antimicrobials. Although the exact mechanism of their antimicrobial action is still unclear but it is mostly due to cell membrane disruption by QACs, their ability to increase cell permeability, and their possible adverse effects on cell proteins. As biocidal action of QAC requires interaction with the cell membrane, it will be influenced by both the size of the molecule and the density of the quaternary ammonium functional groups. Larger molecules tend to have a lower permeation rate through the cell membranes and thus are less efficient. The antimicrobial action of QACs against Gram-positive and Gram-negative bacteria is also different as they have different cell structures.⁴ Some of the advantages of QAC over other

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Contract grant sponsors: Lockheed Martin Corporation (NJ, USA), UGC (New Delhi, India), CSIR (New Delhi, India).

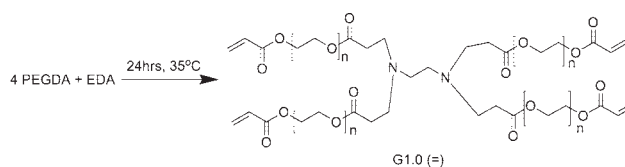
antimicrobial agents are that they are more stable, less corrosive, non irritating to the skin and have low mammalian toxicity, and broad spectrum antimicrobial activity.⁵

Dendrimers are novel highly branched three dimensional macromolecules of nanodimension and are usually synthesized by either convergent or divergent methods.^{6–10} Dendrimers can be tailored to generate uniform or discrete functionalities and possess tunable inner cavities, surface moieties, sizes, molecular weights, and solvent interactions.^{11–16} The novel architecture of dendrimer provides a very high number of functional groups in a compact space. These properties have attracted great research interest in exploring their potential biomedical applications, such as in drug delivery, gene transfection, and imaging.^{8,9,17–22} Cooper and coworkers^{4,23} have synthesized quaternary ammonium poly(propylene imine) dendrimer and showed that these novel biocides have excellent broad spectrum antimicrobial properties. However, these dendrimers have low mechanical properties, difficult to separate and may cause contamination of the medium. Few attempts have been made to improve the mechanical properties of dendrimers by crosslinking for coating and adhesive applications.^{24,25} Gitsov and coworkers^{26–30} have used different dendrimers as multifunctional building elements for amphiphilic hydrogels with poly(ethyleneglycol) as the linear hydrophilic segment for various biomedical applications. The purpose of this study was to synthesize various generations of poly(ethyleneglycol)diacrylate (PEGDA) based dendrimers, their free radical polymerization, and evaluation of biocidal activity of synthesized dendritic copolymer networks after quaternizing with hydrochloric acid (HCl).

EXPERIMENTAL SECTION

Materials

PEGDA (M_w = 575) was obtained from Sigma-Aldrich (USA). Ethylene diamine (EDA), diethyl amine (DEA), dichloro methane (CH_2Cl_2), HCl, and ammonium persulfate (APS) were purchased from CDH (India). Ethyleneglycol dimethacrylate (EGDMA), N,N,N',N' -tetramethylethylenediamine (TEMED) were procured from Merck (Mumbai, India). HPLC grade methanol (MeOH) was obtained from Loba Chemicals (Mumbai, India). Luria broth and nutrient agar were purchased from Hi-Media Laboratories, (Mumbai, India). Bacterial strains *Escherichia coli* (ATCC 25,922) and *Staphylococcus aureus* (ATCC 33,807) for antimicrobial assessment studies were obtained from Hi-Media Laboratories, (Mumbai, India).



Scheme 1 Synthesis of G1.0 (=).

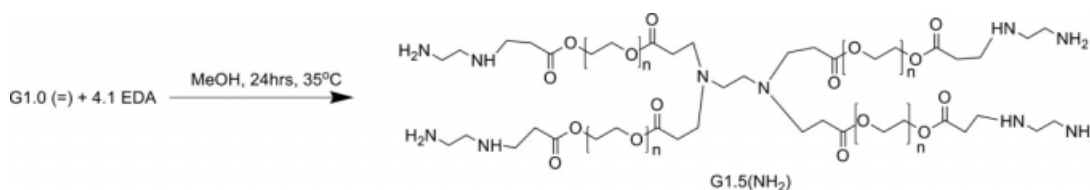
Synthesis of functionalized dendrimers of various generations

G1.0 (=): To a 250 mL two necked round bottomed flask equipped with a stirrer, 20 mM of PEGDA-575 and 5 mM of EDA were added. EDA was always added drop wise to diacrylate and the temperature of the solution was not allowed to increase above 5°C. After complete addition, the solution temperature was slowly allowed to increase to 35°C and stirred continuously for 24 h at 35°C (Scheme 1). The crude product obtained was purified by column chromatography using a solvent mixture (MeOH : CH_2Cl_2 = 0.5 : 9.5) as eluent to produce a colorless, transparent, viscous liquid (yield = 70%) and was identified by attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) and proton nuclear magnetic resonance spectroscopy (^1H NMR).

G1.5 (NH_2): G1.0 (=) prepared in scheme 1, was further reacted with EDA in 1 : 4.1 mole ratio using methanol (20% w/v) as a solvent for 24 h, so that all double bonds were converted into amino functional groups (Scheme 2). The reaction product was distilled under reduced pressure to remove excess of amine, methanol, and was purified by column chromatography using a solvent mixture (MeOH : CH_2Cl_2 = 0.5 : 9.5) as eluent to produce a light yellow viscous liquid (yield = 66%) and was characterized by ATR-FTIR and ^1H NMR.

G2.0 (=): G1.5 (NH_2) prepared in scheme 2 was again reacted with PEGDA-575 in 1 : 12 mole ratio using methanol (20% w/v) as a solvent for 24 h, so that all N—H bonds were converted into N—C bonds with terminal double bonds (Scheme 3). The crude product obtained was purified by column chromatography using a solvent mixture (MeOH : CH_2Cl_2 = 0.5 : 9.5) as eluent to produce a light yellow viscous liquid (yield = 60%) and was characterized by ATR-FTIR and ^1H NMR.

G2.5 (=, NET_2): G2.0 (=) prepared in scheme 3 was reacted with DEA in 1 : 6 mole ratio for 24 h using methanol (20% w/v) as a solvent, so that half of the double bonds were converted into tertiary amine groups to further increase the N-content of the dendrimer (Scheme 4). Similar procedure was followed as in G1.0 (=). The reaction product was then purified by column chromatography using a solvent mixture (MeOH : CH_2Cl_2 = 0.5 : 9.5) as



Scheme 2 Synthesis of G1.5 (NH₂).

eluent to produce a light yellow viscous liquid (yield = 85%). The product obtained was characterized by ATR-FTIR and ¹H NMR. G2.5 (=, NEt₂) was a mix generation, where half of the double bonds of G2.0 (=) were reacted with DEA to create tertiary amine functionalization and half double bonds remained intact for further crosslinking and polymerization using free radical redox polymerization.

Synthesis of dendritic copolymer networks

Copolymer networks of various generations of PEGDA dendrimers were synthesized using 30% EGDMA as comonomer by free radical redox polymerization technique using APS/TEMED initiator system. Aqueous solution of APS and TEMED (0.06% w/w of total monomer) was prepared separately. Dendrimer (of any generation) and EGDMA were mixed in the ratio of 70 : 30 (w/w) in a glass tube. Aqueous APS solution was added to it followed by TEMED solution to synthesis the dendritic copolymer network at room temperature. The dendritic copolymer networks were represented as G1.0 (=): EGDMA, G2.0 (=): EGDMA, and G2.5 (=, NEt₂): EGDMA for the copolymer networks of G1.0 (=), G2.0 (=), and G2.5 (=, NEt₂), respectively. Synthesized copolymer networks were kept at 70°C for 1 h to attain complete curing and were taken out after breaking the glass tube. All the dendritic copolymer networks were washed several times with hot

distilled water to remove any trace of unreacted monomer.

Quaternization of dendritic copolymer networks

Two grams of each synthesized dendritic copolymer networks of various generations, cut into very fine pieces, were taken in a 100 mL round bottomed flask and 30 mL of 6N HCl was added. The setup was kept under reflux at 40°C for 6 h. After that quaternized product was washed several times with hot distilled water to remove excess HCl and dried under vacuum. Quaternized dendritic copolymer networks were represented as G1.0 (=): EGDMA QHCl, G2.0 (=): EGDMA QHCl, and G2.5 (=, NEt₂): EGDMA QHCl.

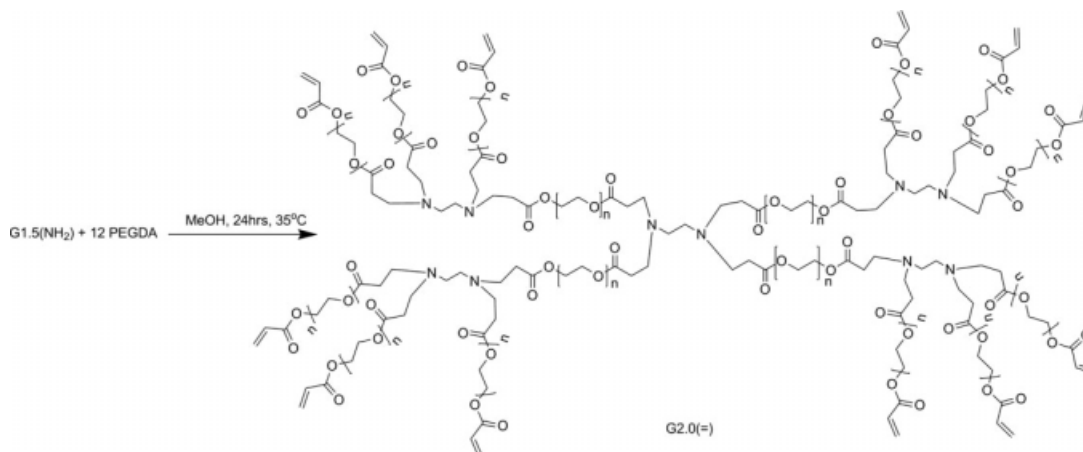
CHARACTERIZATION

Attenuated total reflectance spectroscopy

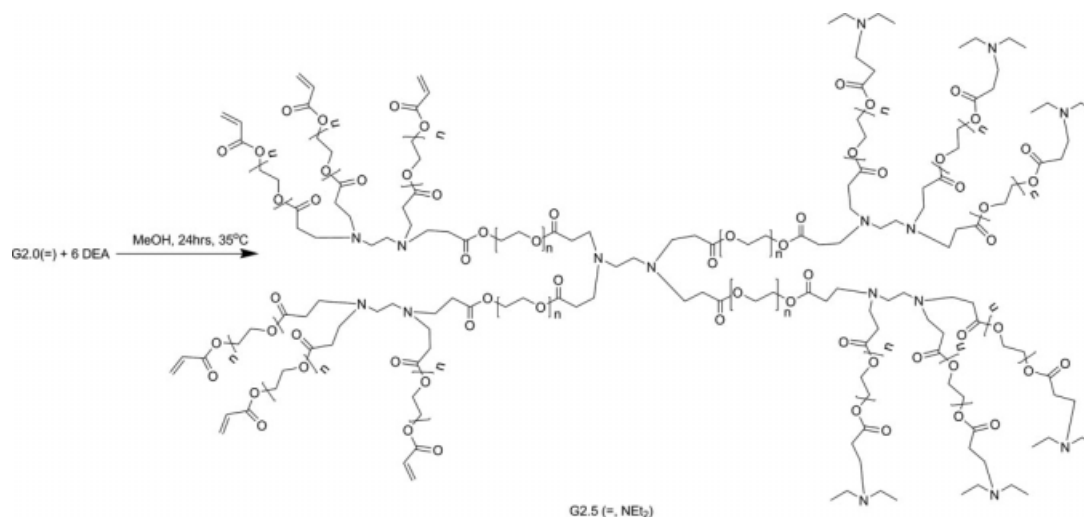
ATR-FTIR spectrums of functionalized dendrimers and quaternary ammonium dendritic copolymer networks were recorded on Perkin-Elmer spectrum one spectrometer.

Nuclear magnetic resonance spectroscopy

A Bruker AC 300 spectrometer at a frequency of 300 MHz was used for recording ¹H NMR of various dendrimers in CDCl₃ solvent. Tetramethyl silane was used as an internal standard.



Scheme 3 Synthesis of G2.0 (=).



Scheme 4 Synthesis of G2.5 (=, NEt₂).

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) studies on various quaternary ammonium dendritic copolymer networks were carried out using TA instruments DSC Q 200 system. Vacuum-dried samples were loaded into the DSC system and the thermograms were obtained in the temperature range of -50 to 200°C under the nitrogen atmosphere at the heating rate of $10^{\circ}\text{C}/\text{min}$.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) studies of all vacuum-dried quaternary ammonium dendritic copolymer networks were carried out on Perkin–Elmer TGA-6 system. Vacuum-dried samples were loaded into the TGA system and the thermograms were obtained in the temperature range of 50 to 750°C under the nitrogen atmosphere at the heating rate of $10^{\circ}\text{C}/\text{min}$. Relative thermal stability of the samples was evaluated in terms of initial decomposition temperature (IDT) and final decomposition temperature (FDT).

Scanning electron microscope studies

The surface characteristics of synthesized quaternary ammonium dendritic copolymer networks were studied using ZEISS EVO series EVO 50 scanning electron microscope (SEM). Samples were dried under vacuum overnight and images were taken after coating them with silver to provide conduction.

Gel content

Gel content of the quaternized dendritic copolymer network was determined by immersing the dry and

weighed amount (1 g) of copolymer network in chloroform at room temperature for 24 h. Sample was then taken out, dried at 60°C and weight of the sample was noted. Gel content was calculated using the following equation.

$$\text{Gel content (\%)} = \frac{W_i - (W_i - W_f)}{W_i} \times 100$$

where W_i and W_f are the weights of the dried copolymer networks before and after immersing in chloroform, respectively.

Swelling studies

Swelling studies of the dendritic copolymer networks are important because they will be used as water disinfectant and they should not contaminate the water upon degradation due excessive swelling. Water absorption capacities of the quaternized dendritic copolymer networks were determined by immersing the dry copolymer sample (1 g) in distilled water at room temperature. The samples were taken out from water at various time intervals and weighed after blotting out the excess water from the surface with filter paper for 10 sec. They were put back in water immediately after weighing. The percent water absorption of the synthesized copolymer networks were calculated using the following equation.

$$\text{Percentage swelling} = \frac{W_s - W_d}{W_d} \times 100$$

where W_s and W_d are the weights of the copolymer networks in the swollen and dry states, respectively. The experiment was repeated thrice for each specimen and average values are reported.

Leaching studies

Two grams of dried quaternized dendritic copolymer network was kept in 50 mL distilled water for a period of 30 days and aliquot of 5 mL was taken out after every 5 days interval and scanned by UV-Visible spectrophotometer in the range of 190–800 nm wavelength. After 30 days polymer was taken out dried in vacuum oven and weight was noted.

Evaluation of antimicrobial properties of quaternary ammonium dendritic copolymer networks

Nutrient agar plates were prepared by dissolving 20 g of Luria broth along with 20 g of bacterial agar in 1 L water and pH was adjusted to 7.0. The content was then sterilized by autoclaving at 15 lbs pressure (121°C) for 30 min. The antibacterial activity of quaternary ammonium dendritic copolymer network was evaluated against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) by colony count method.³¹ The effect of total number of nitrogen atoms present in the dendritic copolymer network on antibacterial activity was also studied by taking 0.05 and 0.1 wt % of various copolymer networks in 10 mL of bacterial contaminated water with initial count of 2000 CFU/mL at a fixed contact time under constant shaking. Then 100 μ L aliquots were withdrawn and laid over the nutrient agar plates using sterile glass spreader, incubated for 24 h at 37°C, and viable cell count was noted. A negative control, which contains no quaternary ammonium compound was also included in the experiment.

Evaluation of antimicrobial efficiency of G2.5 (=, NEt₂): EGDMA QHCl for repetitive use

G2.5 (=, NEt₂): EGDMA QHCl (0.1g) dendritic copolymer network was kept in contact with 10 mL of bacterial solution (2000 CFU/mL) separately with *E.coli* and *S.aureus* bacteria for 2 min and then 100 μ L aliquot was withdrawn and laid over the nutrient agar plate using sterile glass spreader. Rest of the solution was completely removed and fresh 10 mL of bacterial solution (2000 CFU/mL) was added and same procedure was followed for 10 cycles and each time 100 μ L aliquot was withdrawn and laid over the nutrient agar plate. All plates were incubated for 24 h at 37°C along with a positive control and the growth of the bacteria was examined by colony count method.

RESULTS AND DISCUSSION

Synthesis of functionalized dendrimer of various generations

Functionalized dendrimer of various generations were successfully synthesized by Michael addition

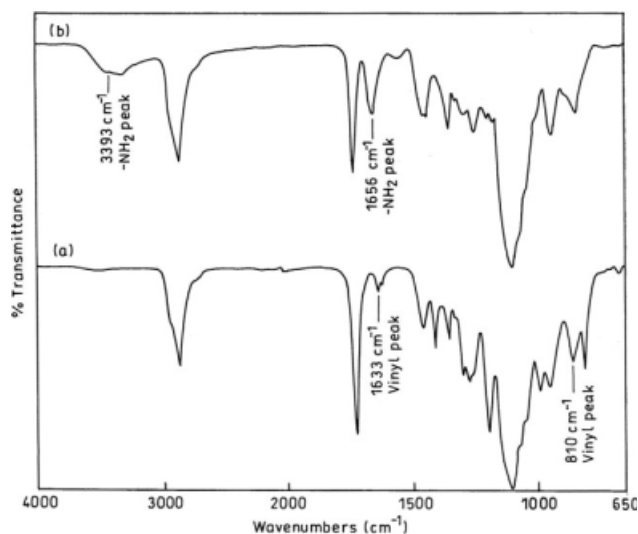


Figure 1 a) IR spectrum of G1.0 (=) b) IR spectrum of G1.5 (NH₂).

reaction. All synthesized dendrimers were light yellow in color except G1.0 (=), which was colorless. They were highly viscous in nature and it was observed that as the generation increased the viscosity of the dendrimer also increased.

Synthesis of dendritic copolymer networks

Various generations of dendrimers were successfully copolymerized with EGDMA by free radical redox polymerization method. Addition of comonomer (EGDMA) was required because pure G1.0 (=), G2.0 (=), and G2.5 (=, NEt₂) showed resistant to redox polymerization because tertiary nitrogen atoms inhibit free radical polymerization.³² Synthesized dendritic copolymer networks were light yellow in color. It was observed that as the generation of the dendrimer increased the mechanical stability of the synthesized dendritic copolymer network decreased while intensity of the yellow color increased probably because of increase in nitrogen content and crosslink density.

Attenuated total reflectance fourier transform spectroscopy

The ATR-FTIR spectrum of G1.0 (=) [Fig. 1(a)] showed characteristic peaks of ester $\text{C}=\text{O}$ at 1723 cm^{-1} , $\text{C}=\text{C}$ at 1633 and 810 cm^{-1} , $\text{C}-\text{O}$ of carbonyl at 1270 cm^{-1} and $\text{C}-\text{N}$ at 1453 cm^{-1} . Whereas in the spectrum of G1.5 (NH₂) [Fig. 1(b)], the stretching vibration of $\text{C}=\text{C}$ group at 1633 cm^{-1} and 810 cm^{-1} disappeared and characteristics peaks of NH_2 were observed at 3393 cm^{-1} and 1656 cm^{-1} , which confirmed the completion of Michael addition reaction. In the spectrum of G2.0 (=) [Fig. 2(a)] $\text{C}=\text{C}$ peak at 1633 cm^{-1} and 810 cm^{-1} reappeared, which indicated the formation of G2.0

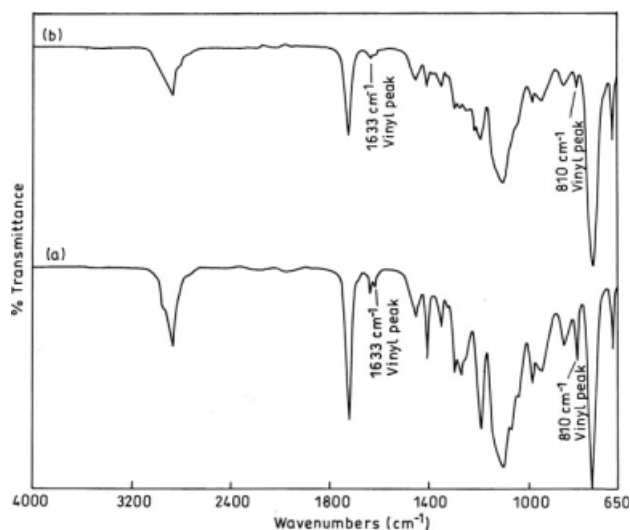


Figure 2 a) IR spectrum of G2.0 (=) b) IR spectrum of G2.5 (=, NEt_2).

(=) with double bonds and it showed complete conversion of amino groups. Figure 2(b) showed intensity of double bond peaks at 1633 cm^{-1} and 810 cm^{-1} decreased, which indicated the partial reaction of double bonds with DEA to form G2.5 (=, NEt_2). Figure 3(a–c) are the IR spectra of G1.0 (=): EGDMA QHCl, G2.0 (=): EGDMA QHCl and G2.5 (=, NEt_2): EGDMA QHCl dendritic copolymer networks, respectively. Appearance of a new peak at 946 cm^{-1} indicated the quaternization of the copolymer network due to the formation of N^+Cl^- .

Nuclear magnetic resonance (^1H NMR) spectroscopy

Figure 4(a) is the ^1H NMR spectrum of G1.0 (=), from which all expected signals appeared as follows: 5.8–

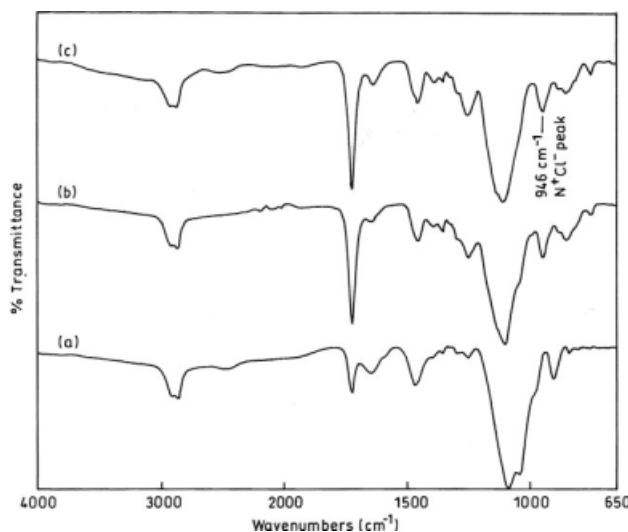


Figure 3 a) IR spectrum of G1.0 (=): EGDMA QHCl b) IR spectrum of G2.0 (=): EGDMA QHCl c) IR spectrum of G2.5 (=, NEt_2): EGDMA QHCl.

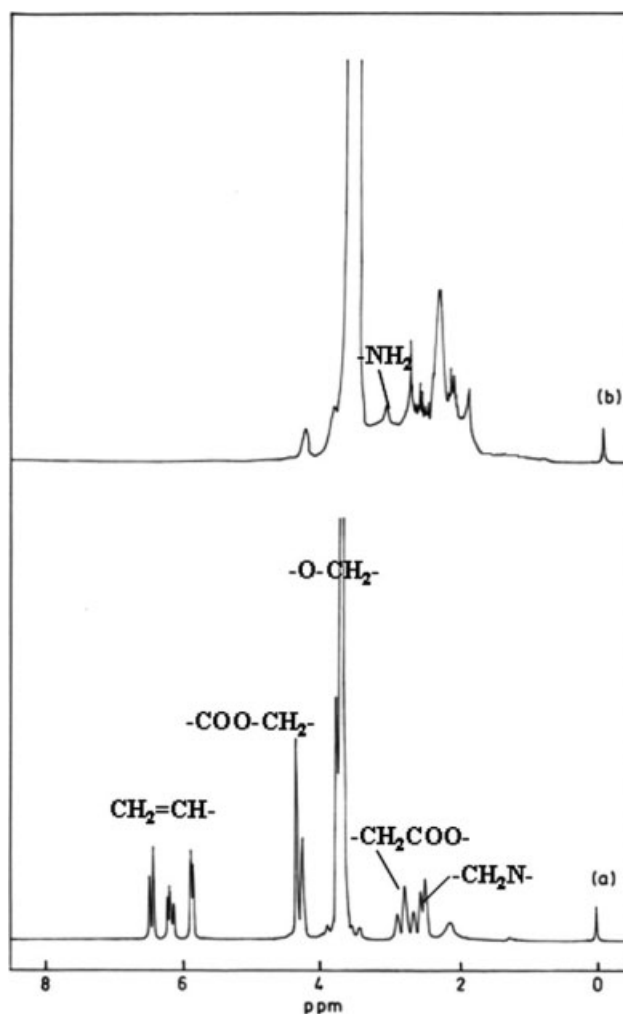


Figure 4 a) ^1H NMR spectrum of G1.0 (=) b) ^1H NMR spectrum of G1.5 (NH_2).

6.4 ppm ($\text{CH}_2=\text{CH}-$); 4.2 ppm ($-\text{COO}-\text{CH}_2-$); 3.6 ppm ($-\text{O}-\text{CH}_2-$); 2.75 ppm ($-\text{CH}_2-\text{COO}-$); 2.45 ppm ($-\text{CH}_2-\text{N}-$). Figure 4(b) is the ^1H NMR spectrum G1.5 (NH_2), it showed absence of vinyl peaks at 5.8–6.4 ppm and presence of $-\text{NH}_2$ peak at 3.2 ppm. Thus, the Michael addition reaction during the synthesis of dendrimer was completed by this procedure. Figure 5(a) confirmed the reappearance of double bonds and formation of G2.0 (=) through the reappearance of vinyl peak in the region 5.8–6.4 ppm. Decrease in intensity of vinyl peak in region 5.8–6.4 ppm and appearance of $\text{CH}_3-\text{CH}_2-\text{N}-$ methyl peak at 1.02 ppm confirms the formation of G2.5 (=, NEt_2) [Fig. 5(b)]. The ^1H NMR spectra of various generations of synthesized dendrimers were very similar due to the layer-by-layer structure of the dendrimers and similar amino functional groups.

Differential scanning calorimetry

The DSC thermograms of various quaternary ammonium dendritic copolymer networks are shown in

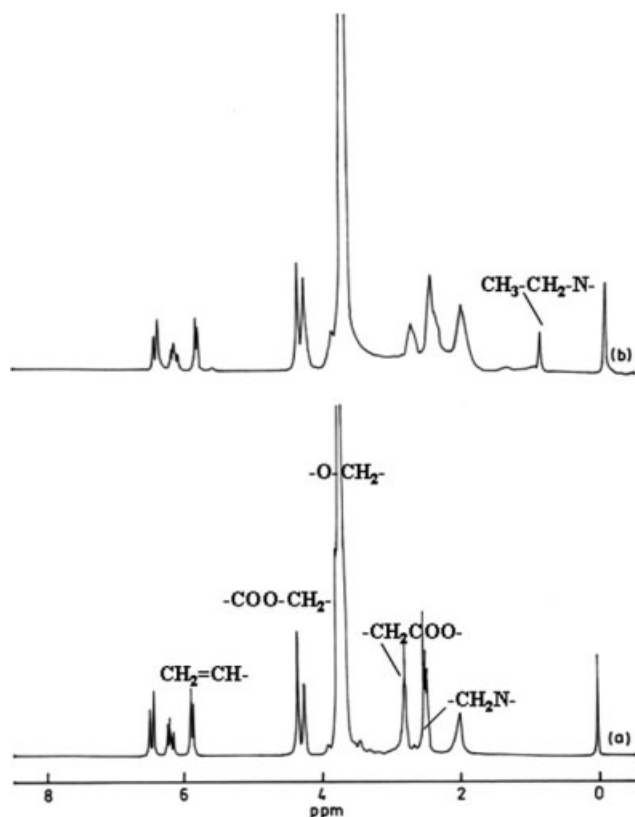


Figure 5 a) ^1H NMR spectrum of G2.0 (=) b) ^1H NMR spectrum of G2.5 (=, NEt_2).

Figure 6. It was found that T_g of dendritic copolymer network decreases from 26°C to 18°C while going from G1.0 (=): EGDMA QHCl, to G2.5 (=, NEt_2): EGDMA QHCl. G2.0 (=): EGDMA QHCl showed T_g of 25°C and pure PEGDA : EGDMA (70 : 30) copolymer showed a T_g of 125°C . Kim and Webster³³ proposed that the glass transition (T_g) of hyperbranched

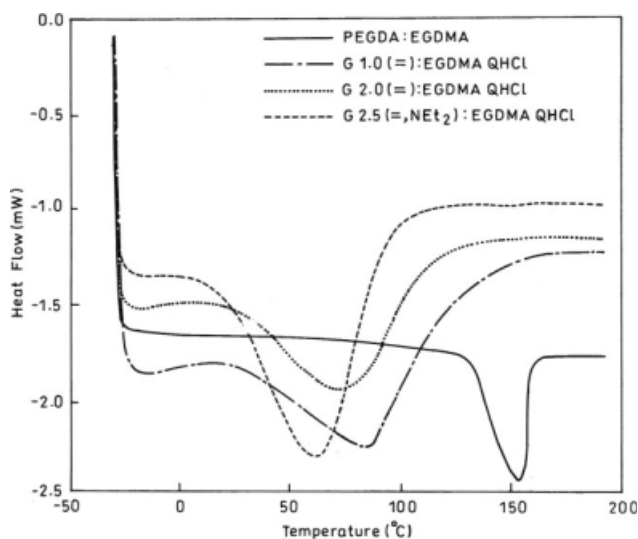


Figure 6 DSC curves of various quaternary ammonium dendritic copolymer networks.

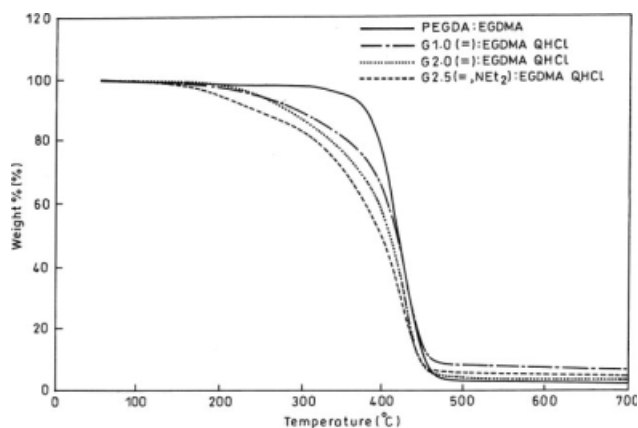


Figure 7 TGA curves of various quaternary ammonium dendritic copolymer networks.

polymers involve some transitional motion of the dendrimer, not just the segmental chain motion usually assumed for linear polymers. Dendritic polymers are a special case of polymers, where every monomer unit is branched and thus tends to reduce intermolecular chain entanglement, which causes lowering of the T_g . According to Jayakannan et al.,³⁴ as the branching of dendritic copolymer increases, T_g decreases gradually due to the increase in free volume at lower branching levels.³⁵

Thermogravimetric analysis

TGA thermograms of various quaternary dendritic copolymer networks are shown in Figure 7. Thermograms of dendritic copolymer networks showed clean single step degradation. IDT of G1.0 (=):EGDMA QHCl was 216°C while it decreased to 176°C in the case of G2.5 (=, NEt_2): EGDMA QHCl. G2.0 (=): EGDMA QHCl showed an IDT of 200°C . Pure PEGDA: EGDMA (70 : 30) copolymer started decomposing only at 330°C . Similar kind of observation was reported by Pan et al.,³⁶ where on increasing the number of generation of the dendrimers, the thermal stability of the compound decreases. The lower thermal stability on increasing the hyperbranching may be attributed to the steric hindrance of these branched derivatives, which under the effect of heat and the increasing mobility of the branches become more labile to thermal degradation.³⁷

Scanning electron microscope studies

The SEM photographs of different quaternized dendritic copolymer networks are presented in Figure 8. Pure PEGDA: EGDMA (70 : 30) copolymer and G1.0 (=): EGDMA QHCl showed no porosity, whereas G2.0 (=): EGDMA QHCl and G2.5 (=, NEt_2): EGDMA QHCl showed pores structure with average pore size of $20\ \mu\text{m}$.

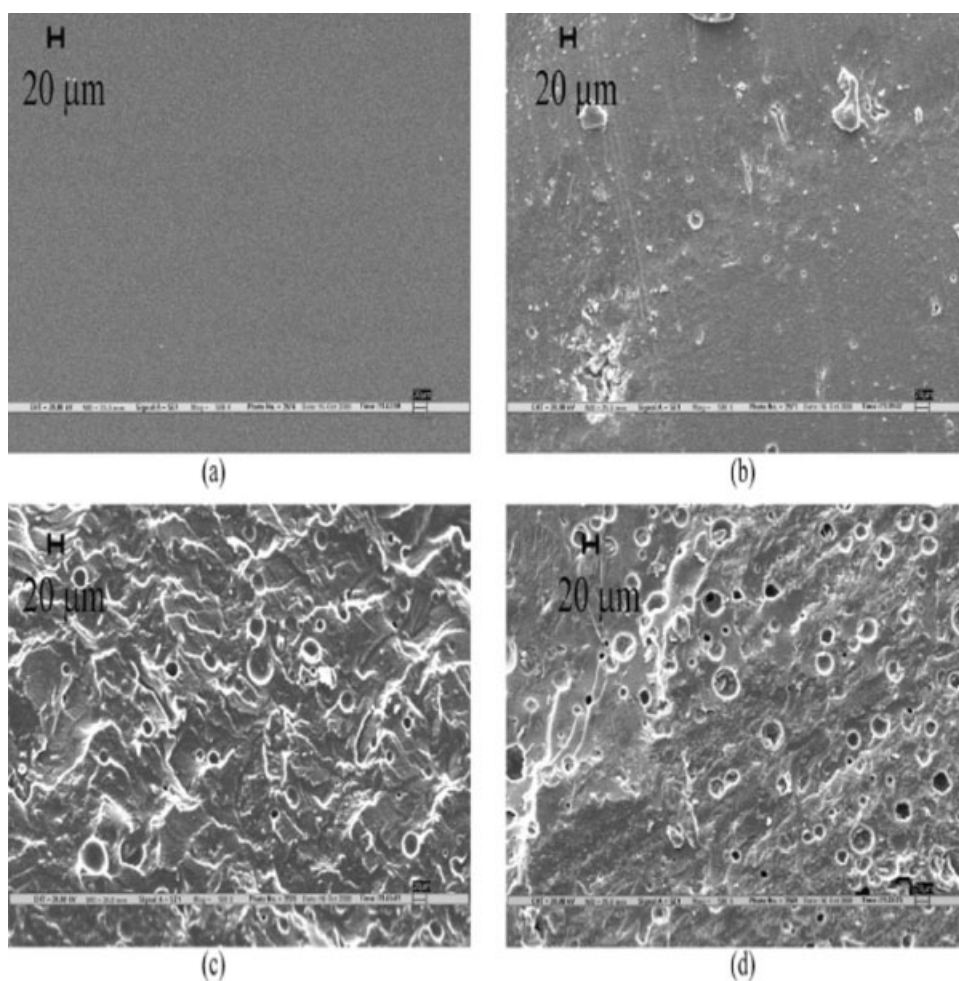


Figure 8 SEM photographs of a) PEGDA: EGDMA b) G1.0 (=): EGDMA QHCl c) G2.0 (=): EGDMA QHCl d) G2.5 (=, NEt_2): EGDMA QHCl.

Gel content

Gel content of G1.0 (=): EGDMA QHCl, G2.0 (=): EGDMA QHCl, and G2.5 (=, NEt_2): EGDMA QHCl were evaluated as 97, 96, and 94%, respectively. All the dendritic polymer networks were highly insoluble in chloroform as seen by gel content measurement but their values decreased slightly from G1.0 (=): EGDMA QHCl to G2.5 (=, NEt_2): EGDMA QHCl.

Swelling studies

The swelling behaviors of the quaternary ammonium dendritic copolymer networks are shown in Figure 9. All the dendritic copolymer networks were hydrophilic in nature but swelling increased from G1.0 (=): EGDMA QHCl to G2.5 (=, NEt_2): EGDMA QHCl due to increase in the amount of polyethylene glycol in the dendritic copolymers. Increased amount of charge repulsion experienced by the quaternary ammonium groups may also be responsible for the increase in percentage swelling with increase

in generation. The maximum percentage of swelling was observed with G2.5 (=, NEt_2): EGDMA QHCl (36%).

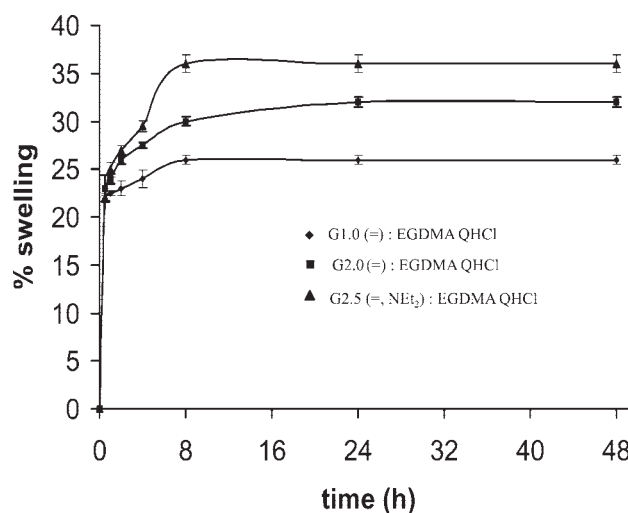


Figure 9 Percentage swelling of different quaternary ammonium dendritic copolymer networks.

TABLE I
Antimicrobial Efficiency of Various Quaternary Ammonium Dendritic Copolymer Networks

Quaternized dendritic copolymer network	Contact time (min)	CFU/mL observed with 0.01 g/mL copolymer network		CFU/mL observed with 0.005 g/mL copolymer network	
		<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
G1.0 (=): EGDMA QHCl	2	70	10	140	20
	5	60	No growth	130	35
	10	No growth	No growth	110	No growth
G2.0 (=): EGDMA QHCl	2	40	No growth	90	40
	5	No growth	No growth	60	No growth
	10	No growth	No growth	No growth	No growth
G2.5 (=, NEt ₂): EGDMA QHCl	2	No growth	No growth	No growth	No growth
	5	No growth	No growth	No growth	No growth
	10	No growth	No growth	No growth	No growth

Leaching studies

PEGDA, EDA, and HCl were soluble in water and showed strong absorption at 236 nm, 220 nm, and 200 nm, respectively in UV-Visible spectrophotometer. No significant absorbance was found in the entire range of wavelength from 190 to 800 nm even though sample was kept in water for a month. This confirms no leaching out of chemical components from the dendritic copolymer network in water medium. There was also no observed weight loss of the copolymer network after keeping them for one month in water.

Evaluation of antimicrobial properties of quaternary ammonium dendritic copolymer networks

Table I shows the effect of nitrogen content in the PEGDA based quaternary ammonium dendritic copolymer networks on antimicrobial properties against *E.coli* and *S.aureus*. It was observed that as the nitrogen content increased from 2 to 16 in the copolymer, the killing time of bacteria decreased. It was due to the fact that as the nitrogen content increases, the sites for quaternization in the dendritic copolymer network also increases. The higher the generation, the greater the number of quaternary ammonium groups, so more potent it should be, which results in decreasing the contact killing time of bacteria. From Table I, it was also observed that killing time decreased with the increase in copolymer amount used for contaminated water (10 mL, 2000 CFU/mL). The negative control of the experiment showed a colony count of 2000 CFU/mL. The lethal action of cationic biocides is mechanistically complex. QAC biocide's target sites are the cytoplasmic membranes of bacterial cells. Following elementary processes are responsible for the biocidal activity of quaternary ammonium dendritic copolymer networks (1) attachment of bacteria onto quaternary ammonium dendritic copolymer network, (2) destruction of cell wall, (3) release of the cytoplasmic

constituents, such as K⁺, DNA, RNA, and death of the cell.³⁸⁻⁴¹

Evaluation of antimicrobial efficiency of 2.5 (5, NEt₂): EGDMA QHCl for repetitive use

From the repetitive use studies, it was observed that there was no growth of bacteria in any of the nutrient agar plates till 10 cycles except in the negative control. These studies indicate synthesized dendritic copolymer networks acts as an efficient contact killing agent even in the repetitive use against both Gram-positive and Gram-negative bacteria.

CONCLUSIONS

A series of PEGDA dendrimers were synthesized using Michael addition reaction of PEGDA and EDA. Synthesized dendrimers were copolymerized with EGDMA and were quaternized with HCL. Quaternized dendritic copolymer network showed broad spectrum antimicrobial properties. The bactericidal properties depend on the concentration of quaternary ammonium groups present in the compound and surface porosity. G2.5 (=, NEt₂): EGDMA QHCl was found to be most potent against both Gram-positive and Gram-negative bacteria. Quaternary ammonium dendritic copolymer networks have strong potential to be used for water disinfection.

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