

# Preparation and Characterization of Polymeric Nanofibers by Electrospinning as Potential Antibacterial Materials

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**Abstract:** Quaternized PDMAEMA (qPDMAEMA) was used to prepare nanofibers by electrospinning. At first the DMAEMA monomer was quaternized using Hexyl, dodecyl and hexadecyl bromide. Then the quaternized DMAEMA was polymerized by free radical polymerization. This polymer was characterized by <sup>1</sup>HNMR, GPC, and thermal analysis (DSC and TGA). The (qPDMAEMA) was blended with PVA in different ratios (20/80, 25/75 and 50/50). The antibacterial properties of the prepared blends were examined against two strains type, the gram positive *M. luteus* and the gram negative *E.coli*. The antimicrobial activity showed that all blends with different alkyl side chain length (i.e. 6, 12, and 16) are highly active against *M. luteus* and no growth of the bacteria was observed after incubation period of 96 h, but in case of *E. coli*, the antibacterial activity is different. The blend having short alkyl side chain (6) are very active and can kill all the bacteria colonies. Blends that contain longer side chains are mostly inactive. However the blend compositions of PVA/PDMAEMA-12 (80/20 and 75/25) exhibit a good antimicrobial effect against *E. Coli*. The minimum bactericidal concentration (MBC) was obtained by determining the minimum polymer concentration at which no growth was observed. qPDMAEMA based fibers were produced using a solution blend of PDMAEMA and PVA. The quaternized PDEAMMAs/PVA blends were electrospun in ethanol. The concentration of the polymer was as high as 20% in order to get fibers. The diameter of formed fibers was found to be around 500 nm.

**Keywords:** Nanofibers, PDMAEMA, Electrospinning, Antibacterial Polymers, Free Radical Polymerization

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## 1. Introduction

Fighting against infections by microorganism remains one of the major actual challenges in different areas; this includes devices in medical uses, healthcare products, systems for water cleansing, food packaging, food storage, etc. [1, 2]. Contamination of biomedical devices such as permanent catheters or implants by bacteria is a main problem especially when a biomaterial is employed [3]. For example using of catheters for a long time can lead to serious infections as the bacteria that made these infections are highly resistant to wide-ranging antibiotics [4]. For this reason there is a need to other materials to prevent these infections. Antimicrobial agents are the materials which have potency of killing pathogenic microorganisms [5]. Antibacterial agents of low molecular weight are used for the disinfection of water, as antimicrobial drugs, as food preservatives, and for soil

sterilization [6]. However, a main drawback of using these materials is the presence of residual toxicity even when suitable amounts of the antimicrobial agent are used [7-9]; therefore, there is urgent necessity for new antimicrobial materials.

Antimicrobial polymers can play a big role to achieve the goal of killing bacteria and avoid the limitation of the low molecular weight antimicrobial agents. Generally these materials may be combined into fibers, or extruded into fibers, and used for disinfectants by contact in many biomedical applications [10].

As associated with conventional antibacterial agents, polymeric antibacterial agents have also the advantages to be non-volatile; chemically stable and hard to permeate through the skin, it also minimize the ecological harms associated with conventional antimicrobial agents by reducing the residual toxicity of the agents. Moreover, increased efficiency, selectivity, extending the lifetime of the

antimicrobial agents and handling safety are additional benefits [11-15].

Quaternary ammonium bearing polymers are famous active antimicrobial agents and are utilized many fields like cosmetics, common antiseptics, sanitizers in hospitals and disinfectants for contact lenses [16].

Some of the commonly used low-molecular-weight antibacterial agents are fluoroquinolones [17-20], quaternary ammonium salts [21-24], biguanide groups [25], and phosphonium salts [6, 26, 27]. Among these antibacterial agents, quaternary ammonium compounds (QACs) have been the most widely used agents. [6, 28-32]. They have some advantages over other antibacterial agents; they can penetrate easily cell membrane, low toxicity, good environmental stability, lack of skin irritation, low corrosive effects, and extended residence time and biological activity [33]. Common characteristics among quaternary ammonium compounds are that they possess both a hydrophobic segment and positive charge [34-36]. These compounds with a long alkyl chain have the ability to kill microorganisms like bacteria, fungi, and molds by interacting with their cell membranes [33, 37, 38]. It is generally accepted that the positively charged QACs are adsorbed via electrostatic interaction onto the negatively charged cell surface, and then the long lipophilic chain promotes diffusion into and/or through the wall of the cell [38]. The long alkyl chains, especially as multiple groups acting in concert along the polymer chain, disrupt the cell cytoplasmic membrane and cause the loss of cytoplasmic constituents, which leads to the death of the microorganisms [31, 38]. The antibacterial activity of the QACs is highly dependent on the chain length of the alkyl chain and the overall molecular structure. It has been shown that an increase of the length of the alkyl chain an amphiphilic compound, i.e., to 14 carbon alkyl chains, increases the antibacterial activity of the compound against both Gram-negative and Gram-positive bacteria [22, 31].

Two ways are generally employed for the attachment of these antibacterial agents to polymers [38]. The first includes the introduction of the antibacterial agents to monomers, followed by their polymerization. This method is better because the monomers could be co-polymerized with many other monomers and the composition simply could be altered. The second way, includes the pending the antibacterial agents directly onto a specific ready-made polymers. The advantage of using this method is that the specific polymers are easily modified with different antibacterial agents. Several Polycations based on quaternary ammonium salts are prepared and evaluated as antimicrobial agent, For example, the antibacterial behavior of poly-(trialkylvinylbenzylammonium chloride) against *S aureus* was evaluated [39]. It was found that the bactericidal properties were increased monotonically with molecular weight up to  $7.7 \times 10^4$  Da, the highest molecular weight tested during the study. They found also that antimicrobial activity of poly (trialkylvinylbenzylammonium chloride) was the highest with the longest chain (C12) that they investigated [34]. Yancheva and coworker [40] have prepared

polyelectrolyte complexes (PECs) between N-carboxyethylchitosan (CECh) and well-defined (quaternized) poly [2- (dimethylamino)ethyl methacrylate] (PDMAEMA). The functionalization of the amino groups of PDMAEMA allows complex formation with CECh. The antibacterial activity of CECh, qPDMAEMA, and their complexes against *Escherichia coli* were tested.

Antimicrobial nanowires of quaternized PDMAEMA were also established. Using atom transfer radical polymerization (ATRP), DMAEMA was grafted on silicon nanowires arrays (SiNWAs), and quaternized using benzyl chloride. The graft density on the modified nanowire arrays was much higher than on analogous smooth silicon, leading to higher bacterial adhesion on the nanowire arrays ( $34.6 \pm 0.39 \times 10^6$  vs.  $5.0 \pm 0.15 \times 10^6$  cells/cm<sup>2</sup>) [41]. In another study, poly [2-(dimethylamino)ethyl methacrylate] (PDMAEMA) was grafted onto the bromine-functionalized multi-walled carbon nanotubes (MWNTs) using ATRP method. The PDMAEMA-functionalized MWNT clearly showed an antibacterial effect against *E. coli* as well as *S. aureus* [42].

In this paper, we used electrospinning technique to prepare antimicrobial nanofibers. The polymer used is quaternized PDMAEMA. At first the DMAEMA monomer was quaternized using Hexyl, dodecyl and hexadecyl bromide. Then the quaternized DMAEMA was polymerized by conventional free radical polymerization. This polymer was characterized by NMR, GPC, and thermal analysis (DSC and TGA). The antibacterial properties of the prepared copolymers were examined against two strains type, the gram positive *M. Luteus* and the gram negative *E.coli*. The nanofibers were prepared from a solution of PDMAEMA and polyvinyl alcohol in ethanol.

## 2. Materials and Methods

### 2.1. Materials

2- (Dimethylamino) ethyl methacrylate (DMAEMA), Poly (vinyl alcohol, Mw 89,000-98,000, 99+% hydrolyzed) and Water soluble initiator 2,2'-Azobis (2-methylpropionamide) dihydrochloride (V50) were supplied from Sigma-Aldrich. 1-bromohexane, 1-bromododecane, 1-bromohexadecane were purchased from Acros and used as received. The used solvents were commercial reagent grade and distilled before use.

### 2.2. Quaternization of DMAEMA with Different Alkyl Halide

In a round flask equipped with a magnetic stirrer, a condenser and a thermometer, 0.1 mole of DMAEMA (1), 0.11 mole of alkyl halide namely; hexyl bromide, dodecyl bromide, or hexadecyl bromide, a small amount of hydroquinone, and 25 ml of acetonitrile were charged. The reaction mixture was stirred at 40°C for 48 h., cooled and filtered to obtain the final product (DMAEMA-6, DMAEMA-12, DMAEMA-16). The obtained quaternary ammonium salts were washed several times with dry diethyl

ether and dried under vacuum at room temperature.

### 2.3. Instrumentation

$^1\text{H}$  NMR spectra were recorded on a Bruker AV-300 spectrometer. Molecular weight distributions (MWDs) of the polymers were determined by gel permeation chromatography (GPC) in a set-up comprised a Knauer pump equipped with three NOVEMA columns (particle size  $10\mu\text{m}$ , dimension  $8.00 \times 300.00\text{mm}$ , porosity  $30 \text{ \AA}$  calibrated with poly (2-vinylpyridine) standards and a differential refractive index detector using  $0.1 \text{ N NaCl}$  acidified with  $1\%$  trifluoroacetic acid (TFAc) as eluent with a flow rate of  $0.2 \text{ mL min}^{-1}$ . Thermal Gravimetric Analysis (TGA) was done by using Mettler thermal analyzers having 851 thermogravimetric modules. Thermal stability was determined by recording TGA traces in nitrogen atmosphere (flow rate =  $50 \text{ mL min}^{-1}$ ). A heating rate of  $10^\circ\text{C min}^{-1}$  and a sample size of  $10\text{-}12 \text{ mg}$  was used in each experiment. The samples were heated from room temperature to  $800^\circ\text{C}$  at a heating rate of  $10^\circ\text{C/min}$ . Differential scanning calorimetry (DSC) Mettler Thermal Analyzer having 821 DSC module was used for thermal analysis of the polymers. DSC scans were recorded in nitrogen atmosphere (flow rate =  $80 \text{ ml min}^{-1}$ ). The samples were heated in the first heating cycle from  $-100^\circ\text{C}$  to  $100^\circ\text{C}$  at a heating rate  $20^\circ\text{C/min}$ . The samples were cooled again to  $-100^\circ\text{C}$  with cooling rate  $-20^\circ\text{C/min}$  and again heated in the second heating cycle till  $100^\circ\text{C}$ . A VHX digital microscope (Keyence) was used to provide optical images.

### 2.4. Synthesis of Quaternized Homopolymers

In a Schlenk flask was added  $0.05$  mole of the DMAEMA-6 monomer and  $15 \text{ mL}$  water. The reaction mixture was purged with argon for 20 minutes,  $0.005$  mole of water soluble initiator V50 was added and then placed in a preheated oil bath at  $75^\circ\text{C}$  for 10 h. After cooling, the reaction mixture was poured into large amount of acetone to get the polymer. The polymer was collected by filtration dried at  $40^\circ\text{C}$  in vacuum oven for 72 h to give a conversion of  $56\%$  of yellowish solid. Similarly DMAEMA-12 and DMAEMA-16 with conversion of  $53\%$  for both.

### 2.5. Antibacterial Assessment

To prepare one liter of the bacterial solution,  $5 \text{ g}$  of peptone digest and  $3 \text{ g}$  of meat extract were dissolved in  $1\text{L}$  of deionized water and stirred for 5 minutes on a stirrer plate. The whole solution was sterilized at  $80^\circ\text{C}$  for 20 min in an automated autoclave. After cooling, *E. coli* colony was added to the solution using wire loop and incubated at  $37^\circ\text{C}$  with shaking for 24h. The bacteria count was expressed in Colony forming Unit (CFU)/mL and was determined by preparing different concentrations of bacteria solution by diluting the original bacteria solution with potassium phosphate buffer ( $50\text{mmol/L}$   $\text{pH}=7$ ). Then  $100 \mu\text{L}$  of each diluted bacteria solution was cultured on agar plate and incubated for 72 h at  $37^\circ\text{C}$ , after growth the bacteria colonies was counted manually then multiplied by the dilution factor to give the

approximate actual count. At this stage the approximate bacteria count was  $10^{11}$  CFU/mL. Concentration of the bacteria solution is adjusted to  $10^8$  CFU/mL by dilution with blank solution. For *M. Luteus* one liter of Tryptic Soy Broth solution was prepared by suspending  $30 \text{ g}$  of the media in  $1\text{L}$  of deionized water and sterilizing in automated autoclave at  $80^\circ\text{C}$  for 20 min, *M. Luteus* colony was added to the solution and incubated at  $37^\circ\text{C}$  for 24 h with shaking. After the incubation period the bacteria count was about  $10^{10}$  CFU/mL. Concentration of the bacteria solution is adjusted to  $10^8$  CFU/mL by dilution with blank solution. A blank bacteria solution (i.e. solution contains only nutrients) and bacteria solution containing  $10^8$  CFU/mL are accompanied in all investigations as a negative and positive control respectively. This is an important step to observe any contamination coming from the surroundings. The minimum bactericidal concentration (MBC) was obtained by determining the minimum polymer concentration at which no growth was observed.

### 2.6. Preparation of Nanofibers

Nanofibers were prepared by electrospinning technique. One can see from Figure 1 that, the instrument set up includes a syringe to load polymer solutions for the electrospinning. A driver to control the pressure on the syringe and consequently the injection speed of the polymer solution through a capillary can be controlled. By creating a high intensity electric field with an anode connected to the capillary and grounded with a cathode connected to a metal plate (collector) placed with a X distance below the capillary. The distance between the capillary and the metal plate is variable, so that a proper distance between the capillary and the metal plate for electrospinning of different polymers can be optimized. In this work, nanofibers were fabricated from the polymer solutions at room temperature.  $2 \text{ mL}$  syringe with a needle with inner diameter of approximately  $0.3 \text{ mm}$  was placed  $20 \text{ cm}$  above a surface of aluminum foil was used. A voltage difference of  $20 \text{ kV}$  was applied [43].

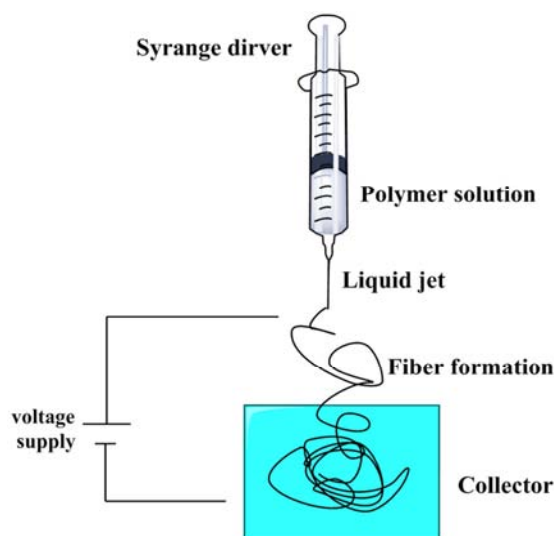


Figure 1. Sketch of Electrospinning instrument.

### 3. Results and Discussion

#### 3.1. Polymer Synthesis and Characterization

At first DMAEMA was quaternized with different alkyl halide, namely, Hexyl bromide, Dodecyl bromide and finally hexadecyl bromide. The quaternization process was done in chloroform at 40°C in presence of small amount of hydroquinone. <sup>1</sup>HNMR of the quaternized monomer revealed the success of the quaternization process, where the vinyl

protons  $-CH_2$  appeared at 5.5-6.1 ppm. The hydrocarbons protons (8-12) are assigned at 1.75 ppm to 1 ppm. Protons on the carbons attached to nitrogen atom are assigned at 3.4 ppm (atom No 7), 3.3 ppm (atoms No 5,6). Protons of methyl group  $-CH_3$  (atom No 2) are assigned at 2 ppm. Protons of atom No 3 appeared at 4.5 ppm, while those of atom No. 4 appeared at 3.7 ppm as shown in Figure 2. By the same way the other two quaternized monomers are assigned as represented in Figures 3 and 4.

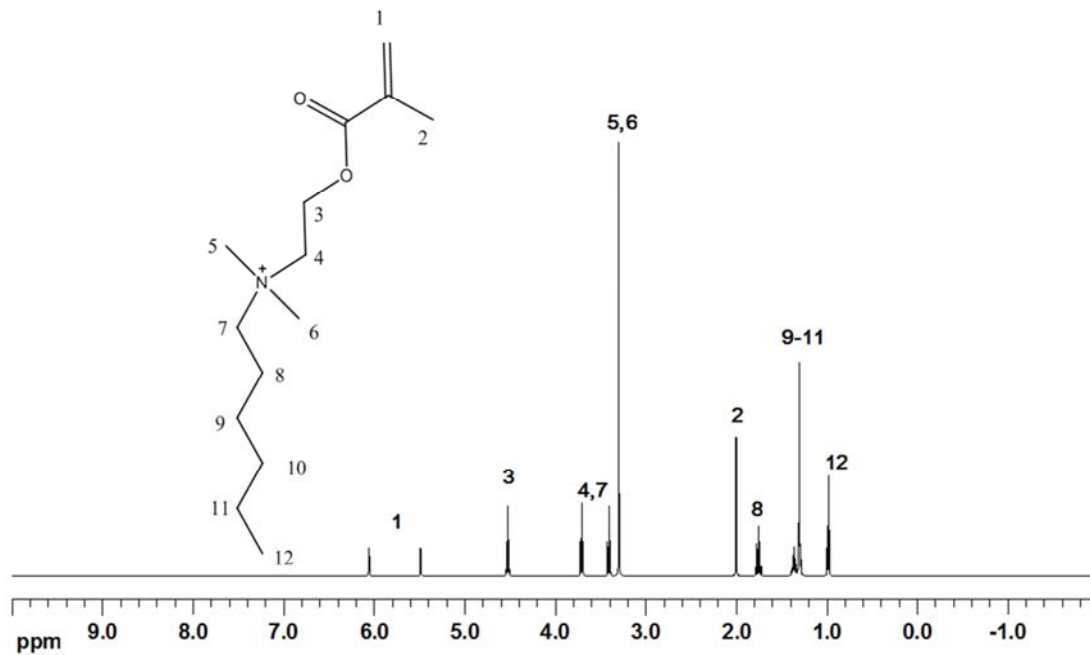


Figure 2. <sup>1</sup>HNMR of DMAEMA quaternized by Hexyl bromide.

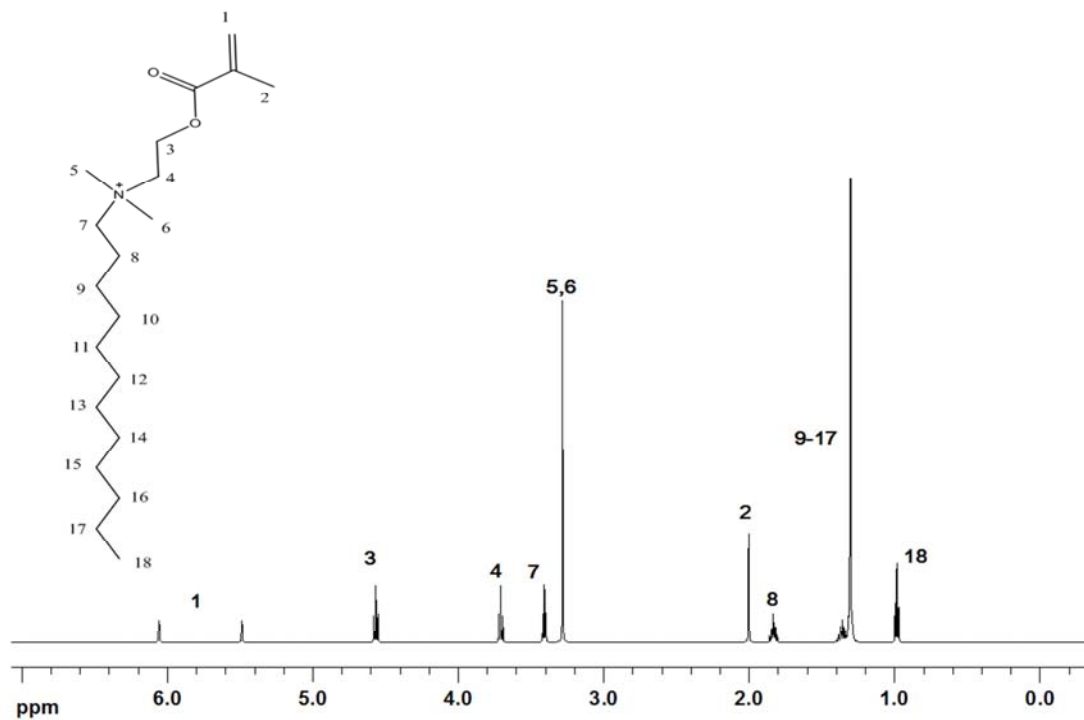


Figure 3. <sup>1</sup>HNMR of DMAEMA quaternized by dodecyl bromide.

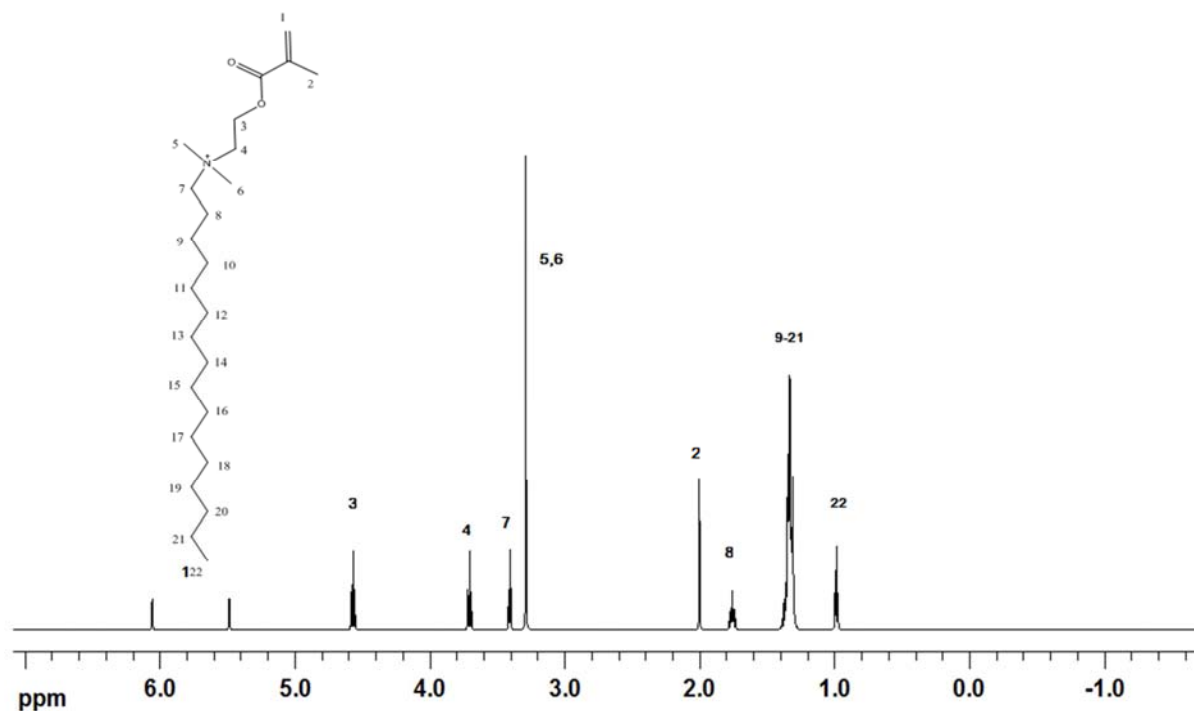


Figure 4.  $^1\text{H}$ NMR of DMAEMA quaternized by hexadecyl bromide.

The  $^1\text{H}$ NMR of the different polymers are shown in Figures 5-7. The peak assignment was done easily and there is no significant difference between the monomers and their corresponding polymers. Important is the disappearance of the vinyl protons indicates a success polymerization. The different quaternized PDMAEA were also further subjected to GPC analysis in order to measure the molecular weights and the polydispersity (PDI). GPC Elugrams are represented in Figure 8. The GPC Elugrams are of mono-modal curves,

the PDIs results are in the same range of polymers obtained by conventional radical polymerization. The molecular weights of the polymers are of average values and it is observed a slight decrease in their values as the hydrophobic chains in creased. TGA and DSC analysis revealed a good thermal stability of the polymers and the no clear effect of the quaternization on the Tgs of the prepared polymers as shown in Figures 9 and 10. Table 1, shows the results of GPC and thermal analysis.

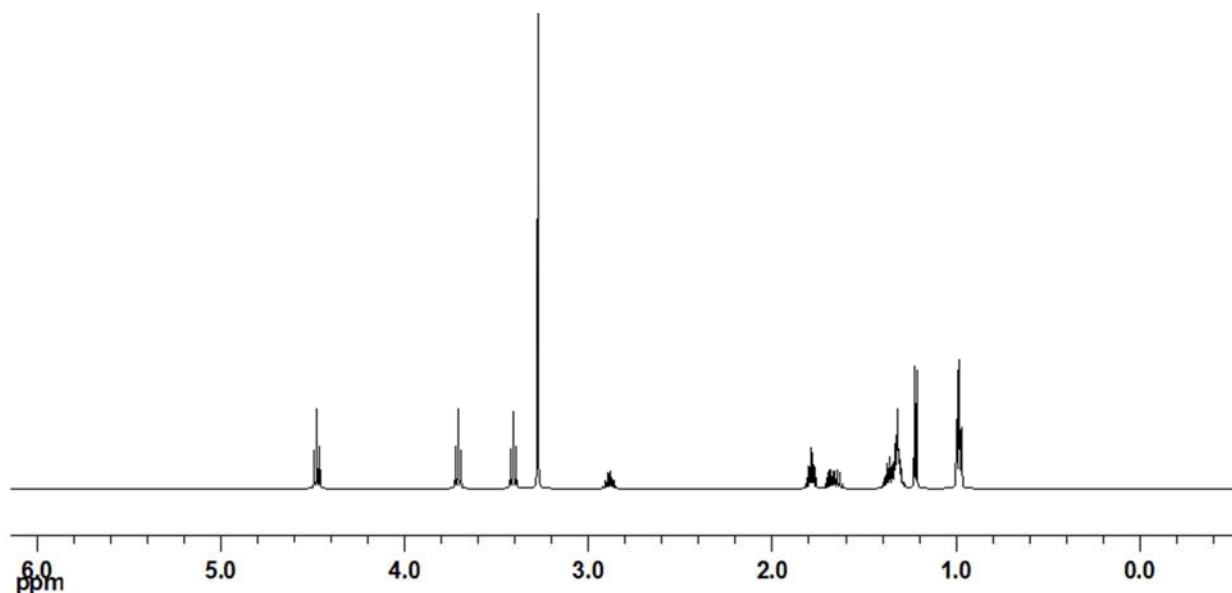
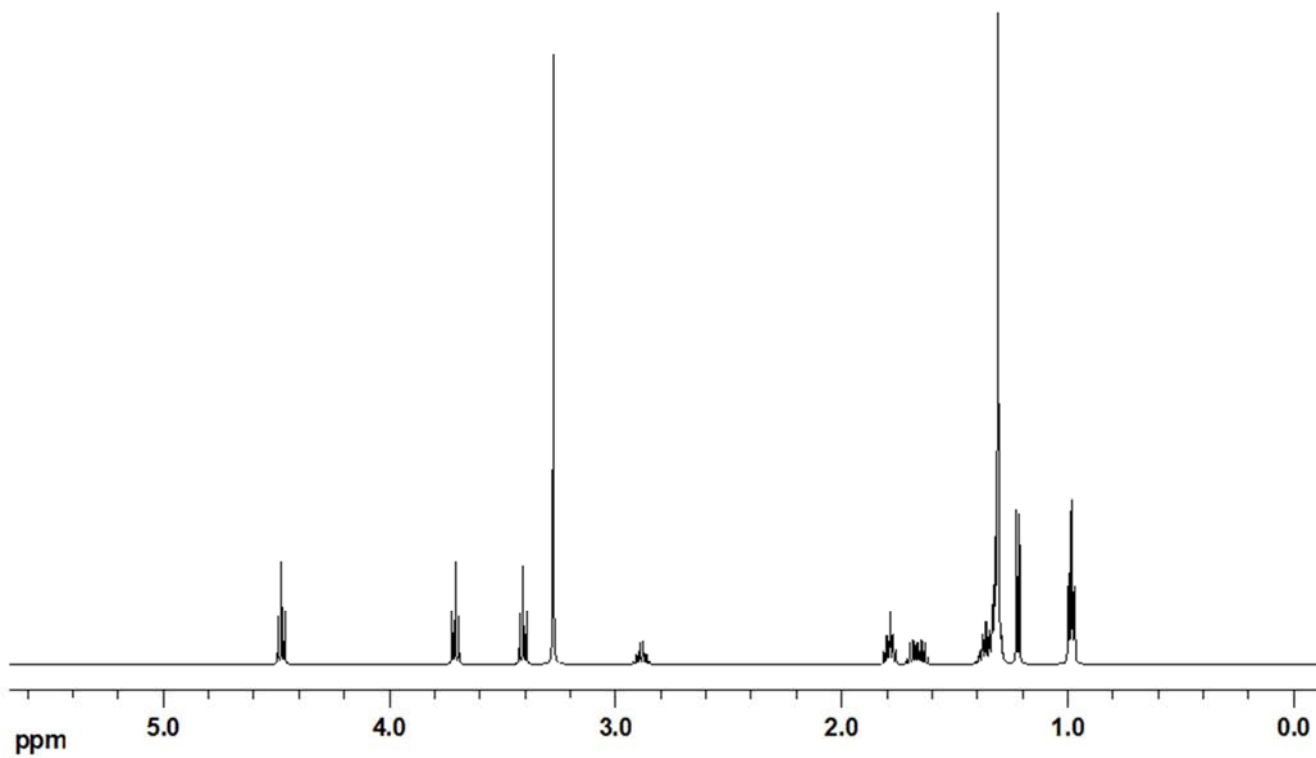
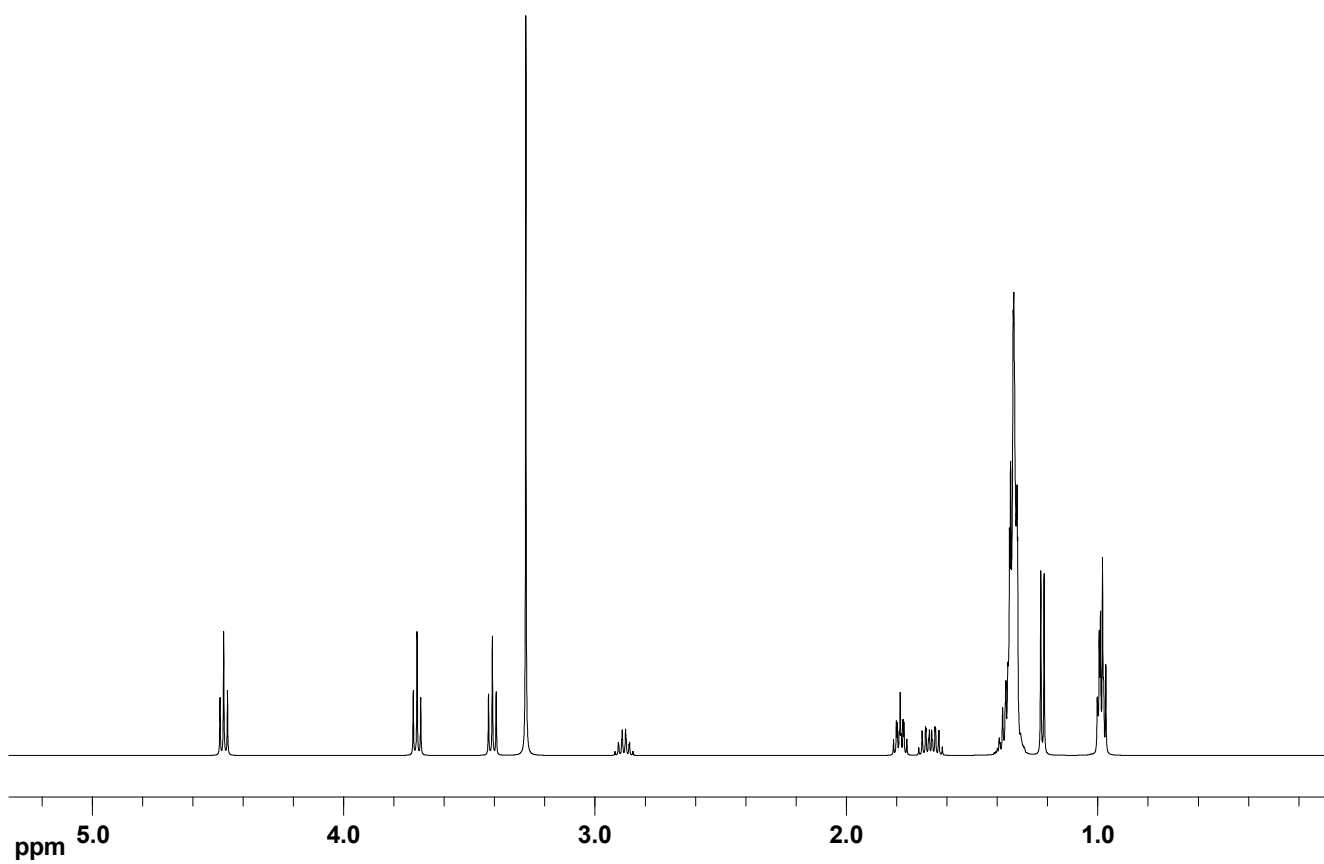


Figure 5.  $^1\text{H}$ NMR of PDMAEMA quaternized by hexyl bromide.



*Figure 6.* <sup>1</sup>H NMR of PDMAEMA quaternized by dodecyl bromide.



*Figure 7.* <sup>1</sup>H NMR of PDMAEMA quaternized by hexadecyl bromide.

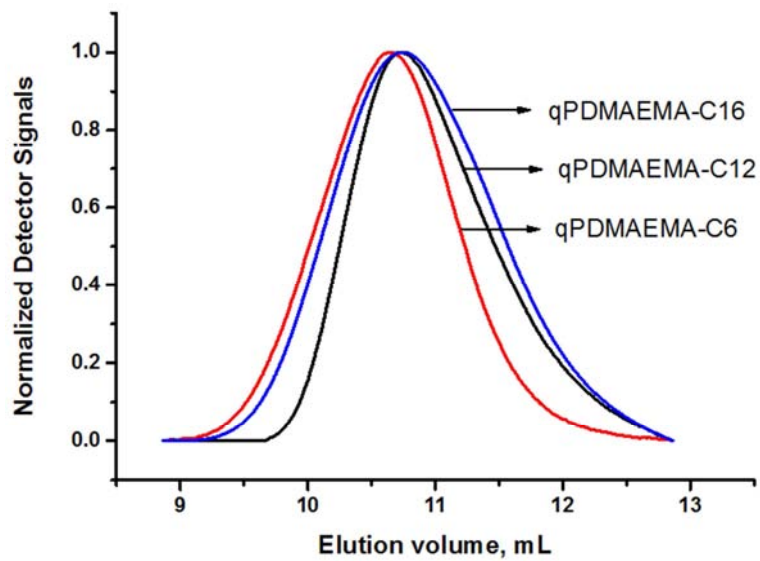


Figure 8. GPC Elugrams of the prepared polymers.

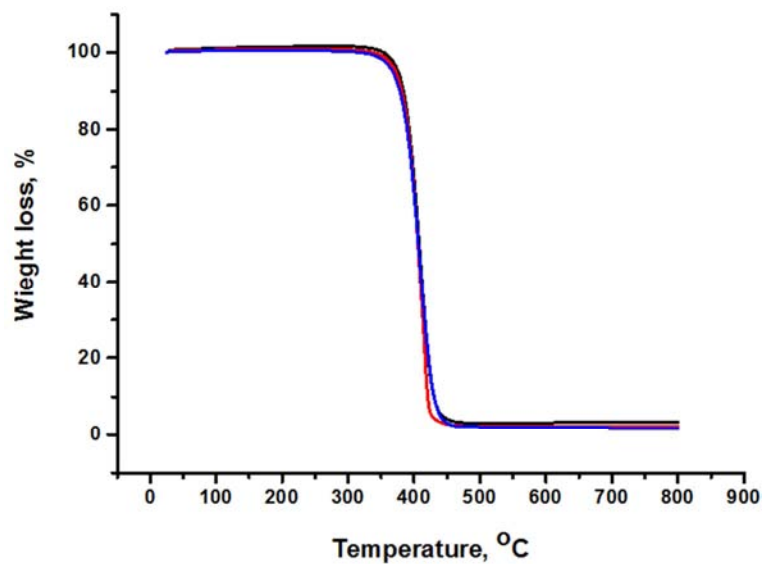


Figure 9. TGA curves of the prepared Polymers.

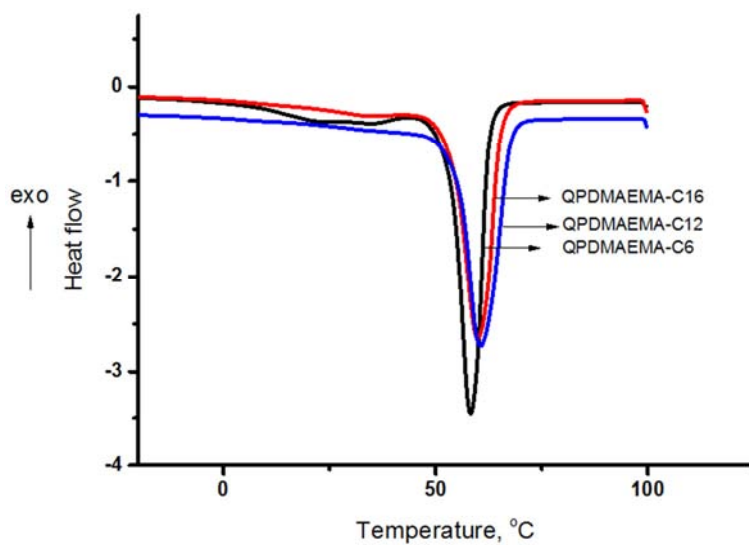


Figure 10. DSC curves of the prepared Polymers.

**Table 1.** GPC and thermal analysis data of the prepared Polymers.

Polymer	Mn	PDI	Tg, °C	5%
DMAEMA-06	12000	2.1	5	95
DMAEMA-12	10800	2.3	6	93
DMAEMA-16	10600	2.1	5	94

The prepared polymers were mixed with PVA with different portions as illustrated in Table 2. These blends were then subjected to electrospinning

**Table 2.** qPDMAEMA/PVA blend for Electrospinning.

sample	PVA%	PDMAEMA-6	PDMAEMA-12	PDMAEMA-16
B1	80	20	---	---
B2	75	25	---	---
B3	50	50	---	---
B4	80	---	20	---
B5	75	---	25	---
B6	50	---	50	---
B7	80	---	---	20
B8	75	---	---	25
B9	50	---	---	50

The antibacterial effect of the nine samples was tested against two strains type, E.coli and M. luteus.

### 3.2. Antibacterial Assessment

The antibacterial activities of the samples (B1-B9) were determined by testing these polymers against E. coli and M. luteus using both dilution and spread plate method. 20 mg of each polymer was added to 5 mL of bacteria solution to give concentration of 4 mg/mL and incubated at 37°C for 24 hours with shaking, after that 100  $\mu$ L of each solution was spread on agar plate and then incubated 37°C for 96 hours. The results are shown in Table 3. A blank bacteria solution (i.e. solution contains only nutrients) and bacteria solution containing 10<sup>8</sup> CFU/mL are accompanied in all experiments and investigations as a negative and positive control respectively. This is an important step to observe any contamination coming from the surroundings. From this table it is clearly seen that all Blends comprising different alkyl side chain length (i.e. 6, 12, and 16) are highly active against M. luteus and no growth of the bacteria was seen after incubation period of 96 h, but in case of E. coli, the antibacterial activity is different. The blend having short alkyl side chain (6) are very active and can kill all the bacteria colonies. Blends that contain longer side chains (B4-B9) are mostly inactive; this indicates that hydrophilicity/hydrophobicity balance and chain length influence the antimicrobial properties. This behavior could be attributed to the change in both charge density and conformation of the polymer, which accordingly may affect the manner of interaction with the cytoplasmic membrane [44]. Ikeda et al. studied poly (trialkylvinylbenzylammonium chloride) and discovered that the antimicrobial activity was the highest with the longest chain (C12) that they investigated [34]. However Panarin et al [45] discovered that the antimicrobial activities of their polymers did not exhibit changes with dissimilar chain lengths; it seems that each kind

of polymer has its own properties that make it an active antimicrobial agent. Rationalization of the association between antibacterial properties and alkyl chain lengths has been argued. This may be due to (1) dual binding sites on the surface for which the relative binding affinities at each site differ for long and short alkyl substituents or (2) different aggregation behavior for long and short hydrophobes [46]. However the blend compositions of PVA: PDMAEMA-12 with blend ration 80:20 and 75:25 exhibit a good antimicrobial effect against E.coli. The antibacterial activity of these two blends against E.coli is greatly enhanced as the number of the colonies is drastically decreased from 10<sup>8</sup> to 1000 and 5000 CFU/mL respectively as shown in table 3.

**Table 3.** Antibacterial activity of PDMAEMA/PVA blends with different long alkyl chains against M Luteus and E coli using broth and dilution test method.

Sample	Result	
	M. luteus	E. coli
-ve control	No Growth	No Growth
+ve control	10 <sup>8</sup> CFU/mL	10 <sup>8</sup> CFU/mL
B1	No growth	No growth
B2	No growth	No Growth
B3	No growth	No Growth
B4	No growth	1000 CFU/mL
B5	No growth	5000 CFU/mL
B6	No growth	Growth
B7	No growth	Growth
B8	No growth	Growth
B9	No growth	Growth

The mechanism of the antibacterial activity is somewhat complex, one can attribute the lethal action of the polycations containing long hydrophobic chain to a) adsorption of the polymer onto cell surface and flow through the cell wall, b) adsorption onto the cytoplasmic membrane and its disruption, releasing of the cytoplasm and death of the bacteria [47] and this is the case in our polymers. The different in activities towards M luteus and E coli might be accounted for the different structure of the cell wall of both types of bacteria under investigation. Gram +ve bacteria (M.luteus) tend to have loose cell wall, while gram -ve bacteria possess an extra outer membrane in the cell wall forming an additional barrier that complicates the penetration of the cell wall by polycations. Many other factors may influence the antibacterial activity of these kinds of polymers such as molecular weight, counter ion and hydrophobic chain length. The minimum bactericidal concentration (MBC) was obtained by determining the minimum polymer concentration at which no growth was observed (Table 4). For this purpose, different concentrations of samples B3, B6 and B9 were tested against (M luteus) starting with concentration of 500  $\mu$ g/mL, this concentration was decreased systematically by 50  $\mu$ g. For B3 it was found that at concentration of 200  $\mu$ g/mL there was no growth, while at concentration of 150 $\mu$ g/mL bacteria colonies were observed on the agar plate. This



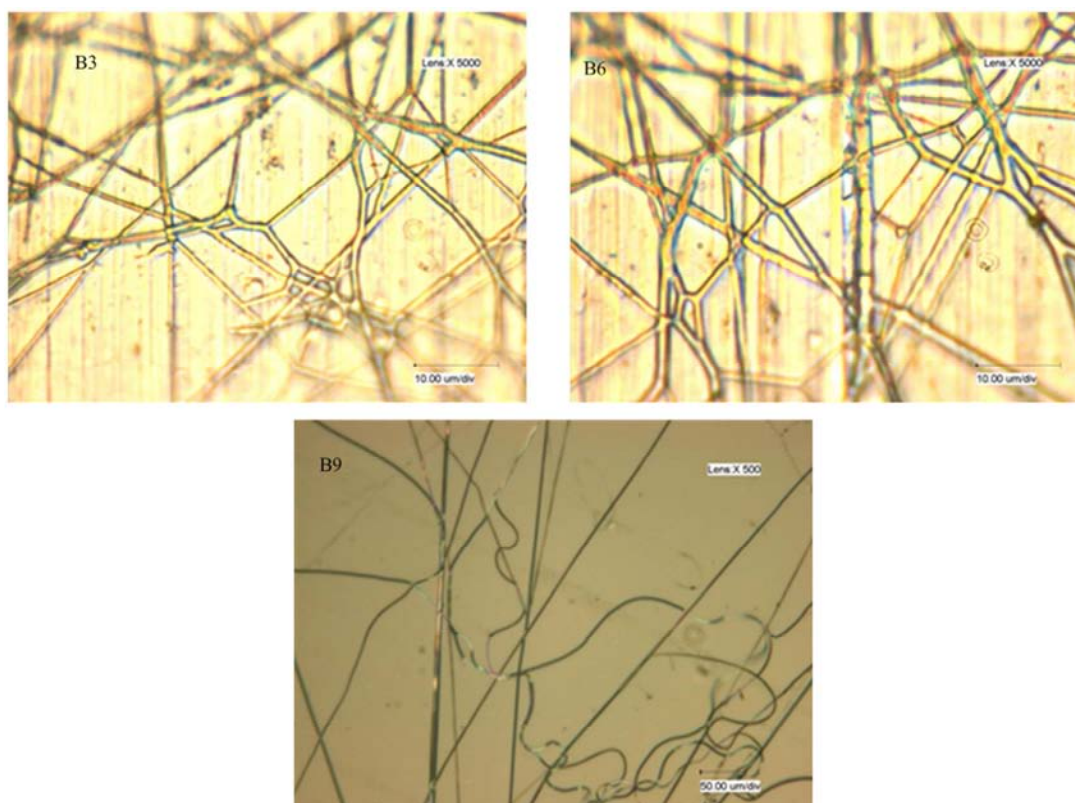
means that the MBC of B3 lies (or reclines) between 200 and 150  $\mu\text{g/mL}$  i.e.  $150 \mu\text{g/mL} < \text{MBC}_{\text{B3}} \leq 200 \mu\text{g/mL}$  for both *M. Luteus* and *E. coli*. Similarly we found that MBC for B6 is  $150 \mu\text{g/mL} < \text{MBC}_{\text{B6}} \leq 200 \mu\text{g/mL}$  and  $200 \mu\text{g/mL} < \text{MBC}_{\text{B9}} \leq 300 \mu\text{g/mL}$  for *M. Luteus*.

**Table 4.** Minimum bactericidal concentration of B3, B6, B9.

Polymer	MBC, $\mu\text{g/mL}$	
	<i>M. luteus</i>	<i>E. coli</i>
B3	150-200	150-200
B6	150 -200	---
B9	200 - 300	---

### 3.3. Fibers by Electrospinning

PDMAEMA based fibers were produced using a solution blend of PDMAEMA and PVA samples (B3, B6 and B9). The polymer chemistry of PDMAEMA makes them suitable for variety of applications. Among the promising them is the antimicrobial activity properties. The quaternized PDEAMMAs/PVA blends were electrospun in ethanol. The concentration of the polymer was as high as 20% in order to get fibers. The diameter of formed fibers (Figure 11) was found to be around 500 nm. Figure 11 shows the PDMAEMA/PVA fibers fabricated by using electrospinning.



**Figure 11.** Digital microscope images of electrospun PDMAEMA/PVA fibers.

## 4. Conclusions

1. DMAEMA was quaternized with a good yield using different alkyl bromide, then qPDMAEMA was obtained via free radical polymerization to give a conversion of about 55%.

2. The (qPDMAEMA) was blended with PVA in different ratios and tested against two bacteria strain; the gram positive *M. Luteus* and the gram negative *E.coli*. The antimicrobial activity showed that all Blends with different alkyl side chain length (i.e. 6, 12, and 16) are highly active against *M. luteus*, but in case of *E. coli*, the antibacterial activity is different. The blend having short alkyl side chain (6) are very active and can kill all the bacteria colonies. Blends that contain longer side chains are mostly inactive. However the blend compositions of PVA: PDMAEMA-12 with blend ration

80:20 and 75:25 exhibit a good antimicrobial effect against *E.coli*.

3. The minimum bactericidal concentration (MBC) was obtained by determining the minimum polymer concentration at which no growth was observed.

4. qPDMAEMA based fibers were produced using a solution blend of PDMAEMA and PVA. Because it is less toxic, the quaternized PDEAMMAs/PVA blends were electrospun in ethanol. The concentration of the polymer was as high as 20% in order to get fibers. The diameter of formed fibers was found to be around 500 nm.

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