ELECTROSPINNING OF LOW SURFACE ENERGY QUATERNARY AMMONIUM SALT CONTAINING POLYMERS AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract: Antibacterial agent (quaternary ammonium salt) containing perfluorinated polymers had been synthesized at different agent concentrations. The polymers were dissolved in solvent mixture and electrospun, to increase effective surface area, at 12 kV which had resulted nanofibers with diameters as low as 40 nanometers and fluffy structures. The resultant electrospun webs' biocidal activities relative to the solution cast film samples and other biocides had been tested with *Escherichia Coli* bacteria containing aqueous medium. The potential application fields of the product may be antifouling applications at air filtering, marine industry and antibacterial applications in medicine.

Keywords: Electrospinning, Low-surface energy, Antibacterial, Antifungal.

1. INTRODUCTION

Quaternary ammonium compounds (QACs) have a large variety of usage areas from cosmetics to clothes softeners, but especially they are known to be good disinfectants. In proper concentrations, they are very effective against fungal attack [1]. QACs, having cationic nitrogen structure, have the general formula of $R_4N^+X^-$. In a quaternary structure, nitrogen atom is covalently bonded to four groups and the positive charge is balanced by a negative counterion.

QACs antibacterial ability is resulted from their amphiphilic structure and surfactant property which was first reported by Dogmak [2]. The antimicrobial action of the QACs is based on their damaging surfactant-like interaction with the membrane (cytoplasmic) of bacteria resulting the loss of the membrane permeability. At convenient concentrations, they can cause cell leakage and the death of the cell [1].

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. Quaternary compounds are widely used because of their non-toxic and non-irritant property [3].

Quaternary ammonium compounds are not affected from the protein concentration of the environment and don't loose their affectivity on bacteria over the course of time [4]. The effectiveness of the agent against microorganisms is directly related to its area of contact with the microorganism's medium. Especially, if it has been attached into a non-dissolving phase, such as solid phase, and in contact with a non-solvent fluid, such as flowing air.

Electrospinning, or electrostatic fiber spinning, is a novel fiber manufacturing process to produce sub-micron, or nanometer, diameter polymeric fibers. Although its idea was first published by Formhals in 1934 [5] and some of preliminary studies were carried by Baumgarten [6], its reputation had risen in 90's due to increased interest to nanotechnology and relative ease of fiber fabrication by electrospinning [7, 8].

Electrospun fibers have several outstanding features such as very high surface to volume ratio, flexibility in surface functionality, and enhanced mechanical properties [9]. These impressive properties make the electrospun fiber mats recently emerging candidate for filtration, membrane, composite applications, tissue templating, biomedical applications such as protective clothing, medical prosthesis and wound dressing, electrical and optical applications and nanoscale tube fabrication [9].

The electrospun fibers are generated by subjecting polymeric solution (or melt) in a glass syringe to an electric field of several kilovolts to tens of kilovolts. The liquid droplet at the capillary tip of the syringe, which is kept by surface tension of the liquid, is distorted as the intensity of the electric field between the tip and ground increase. Previously hemispherical droplet is elongated, and turned to a conical shape known as the Taylor cone. Further increase of the attractive force of electric field results generation of a charged jet of fluid that is channeled to the ground. The discharged jet of fluid travels in a random pattern with continuous instable whipping motions. Meanwhile, the solvent in the traveling wet fiber evaporates and the charged fiber becomes thinner. The fibers are deposited on the grounded collector in a random, nonwoven manner.

The typical diameters of the collected dry fibers are ranged from several nanometers to a few micrometers. The thickness of the electrospun fibers differs depends on the fluid properties, such as the fluid's viscosity, conductivity, dielectric constant, surface tension, polymer's molecular weight and process operating parameters, such as flow rate of the solution, jet current, applied electrical potential and tip to ground distance [9, 10, 11]. Ambient parameters such as temperature, humidity and air velocity around the spinning chamber is also important [12]. Huang et al. stated that in open literature fifty different polymers, some in several different solvents, have been successfully electrospun to nanometer thickness nonwoven mats [12].

In this study, we synthesized a terpolymer consisting of a backbone with two kinds of functional side chains, one with quaternary structure and the other with perfluorinated structure for hydrophobic nature. Then, we investigated the effect of electrospinning on the antibacterial activity. Bulky structures could have difficulties to confront with microorganism membrane so the best way is to increase encountering chance of microorganism and quaternary structure, as every phenomenon occurs on the surface [13]. Therefore, we increased surface area by turning the quaternary structure containing polymers into nanofibers by electrospinning.

2. EXPERIMENTAL

2.1. Materials

The monomers; commercial grade methylmethacrylate (MMA) was purified by double passing through alumina powder packed column before use, and perfluoro alkyl ethyl acrylate (PFAEA, Fluowet AC 812), kindly supplied by Clariant, was used without purification. The quaternary ammonium salt containing monomer was synthesized with the reaction of dimethyl coconut amine and vinyl benzyl chloride, supplied from Fluka, in distilled water. Other commercial biocides, Nipaguard BPX and 2-bromo-2-nitropropane-1,3-diol was, also, supplied by Clariant and used as received. Azoisobutyronitrile (AIBN, Fluka) was used as polymerization reaction initiator and the solvents Tetrahydrofuran (THF) and Dimethylformamide (DMF) was used without purification.

2.2. Synthesis of Vinylbenzyl-Dimethylcocoammonium Chloride (VBDCC) Monomer

The reaction of quaternary ammonium salt containing monomer was carried out in distilled water environment at 50°C with vinyl benzyl chloride and dimethyl coconut amine as the reactants, in the presence of Na_2CO_3 as catalyst (Figure 1). The reaction was stopped when the opaque color of the mixture in the flask turned to clear, this took approximately 30 minutes. The water in the monomer reactor was removed under vacuum at room temperature.

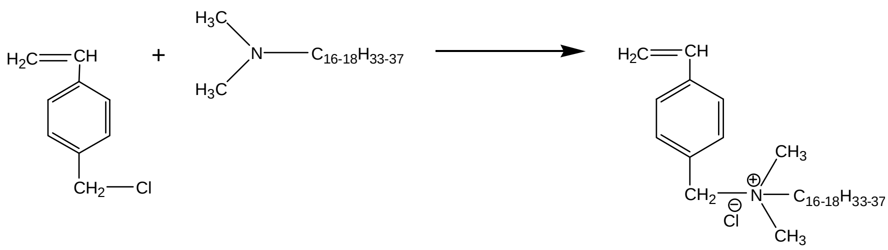

Figure 1. Reaction scheme for Vinylbenzyl-dimethylcocoammonium chloride synthesis.

2.3. Synthesis of Terpolymers

For the synthesis of vinylbenzyl-dimethylcocoammonium chloride - MMA - perfluoro alkyl ethyl acrylate terpolymer, polymerization reaction was carried in THF with AIBN as initiator at 70°C (Figure 2). There were five different sets, named as quat-free, 1%, 5%, 10% and 25%, according to the molar ratio of the quaternary monomer in the polymer. The compositions of the terpolymers are presented in Table 1.

Table 1. The molar compositions of the synthesized terpolymers.

	VBDCC (% molar)	**MMA (% molar)**	**PFAEA (% molar)**
Quat-free	0	90	10
1% polymer	1	89	10
5% polymer	5	85	10
10% polymer	10	80	10
25% polymer	25	65	10

The reaction conditions for the terpolymers, [AIBN] / $[M]_{total} = 1.02 \times 10^{-3}$, reaction temperature 70°C and reaction time was 24 hours. After the reactions were completed in the corresponding interval, the products were first dissolved in chloroform and then precipitated in *n*-hexane (solution/precipitator = 1/10). The terpolymers were obtained by filtration, and dried in vacuum for 12 hours at room temperature.

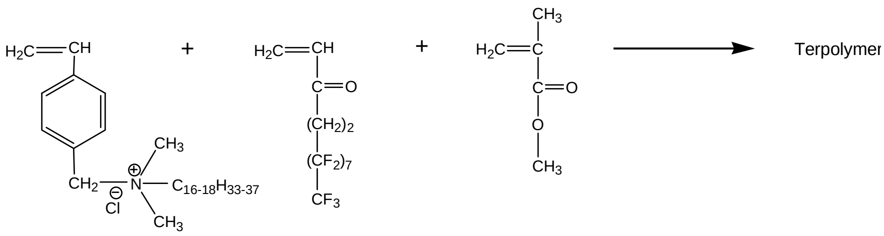

Figure 2. Terpolymerization reaction scheme.

2.4. Measurements

Scanning electron microscope (SEM) analysis was carried out with a JEOL 840-A instrument. Growth curve analyses of the antibacterial terpolymer tests were performed with the aid of a Shimadzu UV-3150 Ultraviolet-Visible-Near Infrared (UV-VIS-NIR) Spectrophotometer and Innova 4330 Refrigerated Incubator Shaker. The terpolymerization reactions were controlled by 500 MHz Varian Inova Nuclear Magnetic Resonance (NMR). Molecular weights of polymers were measured by polystyrene calibrated Waters Gel Permeation Chromatography (GPC) instrument.

2.5. Electrospinning

The electrospinning setup (Figure 3) employed in this study was consisted of a high voltage (HV) power supply (GPS HV power supply

Model 2594), a collector screen connected to a grounded electrode, and a vertically located syringe controlled by a Univentor 801 Syringe Pump. The terpolymer solutions were prepared for 15 wt % polymer in THF-DMF (50/50 wt %) solvent mixture. In each of the four electrospinning processes, polymer solution was transferred into a syringe, with a 16 gauge stainless steel tip, and the flow rate of the syringe set to 5 μl/min. 12 kV voltages was applied to the polymer solution, while the distance between the syringe tip and the collector screen was kept at 10 cm.

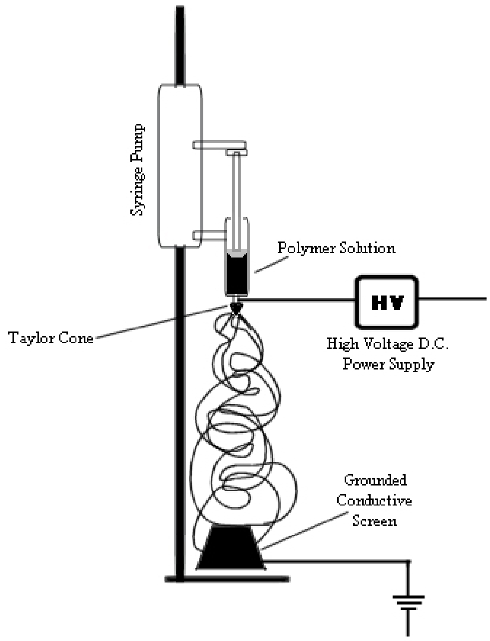

Figure 3. The electrospinning apparatus.

2.6. Biocide Activity Tests

A series of growth curve tests were carried out to investigate the antimicrobial activity of the polymers, with *Escherichia Coli* (XL1 blue), a gram positive bacteria. For this purpose, similar concentrations of bacteria solutions were prepared in falcon tubes by diluting the overnight grown bacteria, at 37^{o}C in incubator shaker, with LB Broth growth media. Electrospun polymers were put into different tubes and metal nets were placed onto the electrospun polymers in the tubes, in order to make the swimming polymer sink completely into the bacteria solution. In each experiment, one more bacteria solution was used as control group. Growth curve analysis of the bacteria was performed by recording the bacteria population in each tube in certain time intervals, by measuring the absorption via UV/Vis/NIR at 600 nm, which is the specific wavelength for *E. Coli* dissolved in water. Antimicrobial activity investigation of the quaternary monomer and other biocides, such as industrial grade Nipaguard BPX and 2-bromo-2-nitropropane-1,3-diol, were also performed.

3. RESULTS AND DISCUSSION

3.1. Characterization

Chemical structure of the synthesized quaternary monomer was confirmed by ^{1}H NMR, where peaks around 7.4~7.6 ppm are assigned to the aromatic protons of VBC, the peaks between 5 and 7 ppm are assigned to the vinylic C-H and C=H protons and the peak around 4.9 ppm corresponds to benzylic protons. The peak of methyl groups were bonded to the nitrogen cation appears around 3.2 ppm.

GPC analysis of the 1% terpolymer was performed and number average molecular weight (M_n) and weight average molecular weight (M_w) were found to be 31700 and 59100, respectively.

3.2. Imaging of Electrospun Polymers

The photograph in Figure 4 is one of the electrospun terpolymers. A fluffy structure, like a piece of cotton on the foil, can be seen on the figure. While the electrospun quat-free (0% quaternary part containing) polymer had formed a flat film on the aluminum foil, the quaternary moiety containing ones, especially 25% polymer, did not stick to the surface. Fluffy structure's amount increased with the increasing ratio of the quaternary monomer, starting from the 1% polymer to the 25%. So, the emergence of fluffy structure is related to the existence of quaternary ammonium part in the terpolymer. The quaternary moiety has restricted uniform lie of the fibers, probably because of its charged structure, and fibers heaped. However, the quat-free sample has formed a flat surface on the grounded aluminum foil.

The Scanning Electron Microscope (SEM) images had displayed one more important distinction between QAC containing and not containing polymers (Figure 5): Fiber diameter distribution. The quat-free sample had uniform diameter distribution in the range of 150-250 nm. However, the 25% QAC containing sample showed a diameter distribution between 40-800 nm, where one group was between 400-800 nm and a second group was 40-120 nm thickness. Similar occurrence had observed by Bognitzki et al. [14], where they used tetrabenzylammonium chloride (TEBAC) salt. Due to fiber diameter decrease, average fiber diameter (AFD) significantly decreases. So, effective surface area has increased per volume. This is important for tuning of the material properties.

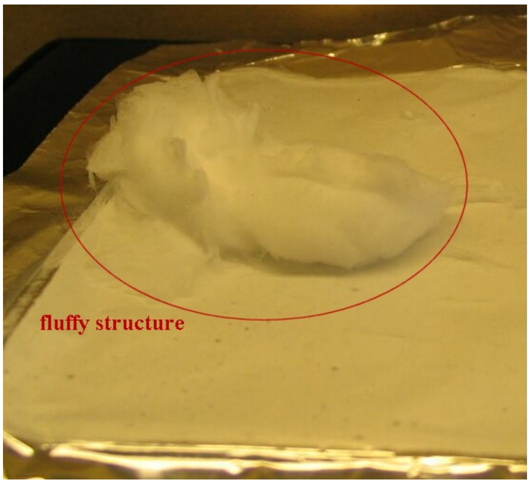

Figure 4. Photograph of an electrospun polymer in fluffy structure.

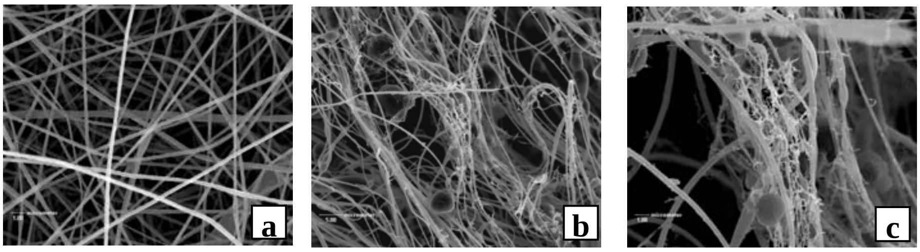

Figure 5. SEM images of (a) Quat-free polymer, (b) 25% terpolymer in fluffy structure, and (c) enlarged image of the same zone in b.

3.3. Testing the Biocidal Activity

The preliminary bacterial tests showed that 25% terpolymer (electrospun) has the most effective antibacterial property, which led to further biocide activity tests performed only with 25% terpolymer. Antimicrobial affectivity investigation of the quaternary monomer and other reference biocides Nipaguard and Bronopol was also performed (Figure 6).

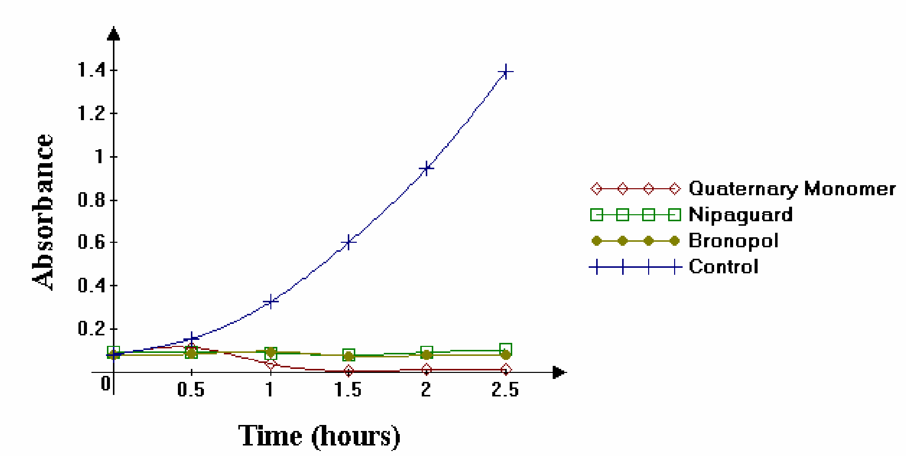

Figure 6. Tests with the quaternary monomer and other biocides.

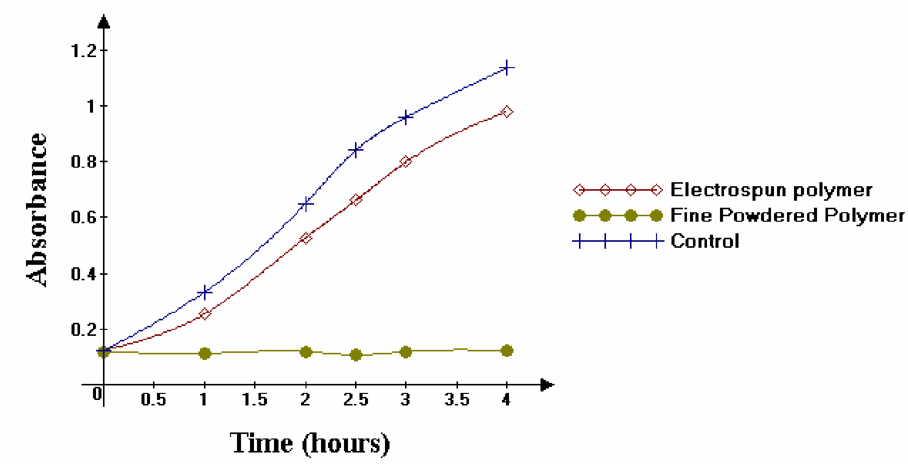

Figure 7. Comparison of the 25% electrospun polymer with the same composition but in the fine powdered form.

Water soluble quaternary monomer was detected to be quite effective against bacterial attack. However, similar effect could not be observed with the electrospun, water non-soluble terpolymer (Figure 7). Obvious success of the fine powdered polymer when compared with the electrospun one led to further studies on hydrophobic property of the polymer. 25% QAC containing non-fluorinated polymer was synthesized and additional antibacterial tests were performed.

Figure 8 represents the surviving bacterial population versus time, while testing with non-fluorinated polymer. The graph shows that both electrospun and non-spun non-fluorinated polymers had a marked biocide efficiency. Consequently, it was determined that the fluorinated, electrospun polymer showed less biocide activity because the hydrophobic property prevented the bacteria solution from entering the inside of the fiber ball and interacting with the single fibers; so the electrospun fiber ball behaved like a big solid polymer having much smaller surface to volume ratio than expected.

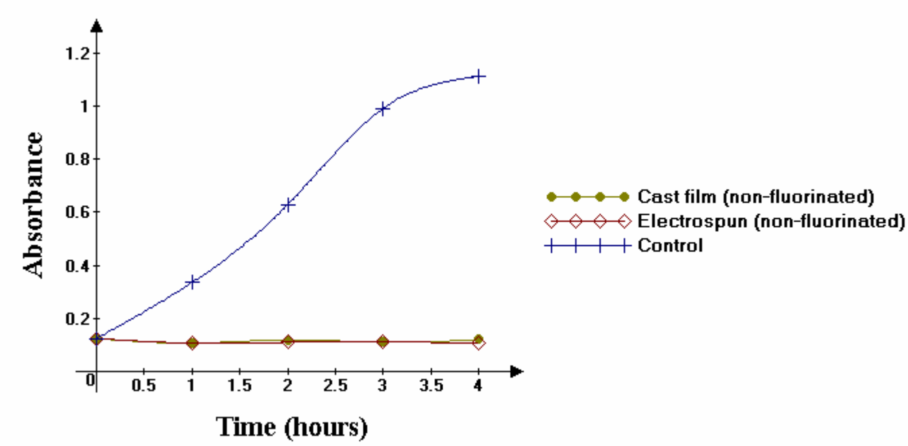

Figure 8. Tests with non-fluorinated polymer

4. CONCLUSIONS

In the present study, synthesis of quaternary ammonium salt containing polymers was performed and their antibacterial activity was observed. The electrospinning of QAC containing polymers resulted in fluffy structures, probably due to the presence of charge. The presence of salt (here QAC) resulted very thin fibers as well as normal fibers. The effective concentration of quaternary monomer in the terpolymer for biocide activity against *Escherichia Coli* bacteria was found to be 25% molar in the polymer. Antibacterial activity of fluorinated terpolymer electrospun was detected to be weak, due to its low surface tension. So, the bacteria containing liquid could not penetrate inside and contact with the interior electrospun fibers. This had decreased the effective surface for applications. Fluorinated polymer may not give effective results for liquid applications, but may show enhanced properties at air filter applications due to its non-wetting property and smaller average fiber diameter. Finally, present study proves adjustable characteristic of electrospun fibers and their ability to be easily tunable according to specific needs.

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