

Novel Approaches to the Development and Characterization of Antimicrobial
Conventional and Nanostructured Materials

by
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Biography

Huseyin Avci was born on June 23, 1982 in Adiyaman, Turkey. He is very lucky because he has five brothers with his mother and father; also it is one of the best things to have niece and nephew. He graduated with a Bachelor's degree in Textile Engineering from Dokuz Eylul University in Turkey and he received his undergraduate degree on July in 2005 with a GPA of 3.44, the second highest grade in the textile department.

During his work at Calik Denim, which is one of the most important textile corporation in the world, Avci was as a product and development superintendent, and cooperated with customers from France, Italy and England on preparation of the new fabric collection for each season, and also helped with the production for customer's demand. Furthermore, at Calik Denim, he became the team leader of Gabardine's Product and Development. The company sales increased more than 40% in that year period. As a result, he received financial support from Calik Denim to improve his English language.

Huseyin was also awarded a scholarship from the Turkish Government in order to pursue a master's degree in the United States. He studied English in the ELS Language Canter in Charlotte, NC and soon after was admitted to the College of Textiles at NC State University. He expects to graduate in May 2010.

ABSTRACT

AVCI, HUSEYIN. Novel Approaches to the Development and Characterization of Antimicrobial Conventional and Nanostructured Materials. (Under the direction of Dr. Richard Kotek.)

One of the most important challenges for antimicrobial materials is their production methods and adverse effects of toxicants on human and living organisms during their using and disposing. It will be one of the crucial concerns to develop models for predicting antibacterial agents, which release and/or come from antibacterial products and/or the environments during their life-cycle, behavior in the body with high-throughput toxicity-testing protocols in the near future. Therefore, efficiency and mechanism of the antimicrobial agents with minimum requirements is so important. To address the issue of antimicrobial materials and focus on gaining insights and developing new types of antimicrobial products, the present study is divided into three sections. In the first section, commonly used a 'leaching' antimicrobial agent, silver nanoparticles, and a polymer, polypropylene (PP) were selected to produce antibacterial composite fibers via the melt spinning technique. The antimicrobial composite fibers were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), MTS Q-Test/5 for tensile testing, linear tester equipment for antistatic property, and elemental content analysis. The significant *E. coli* reduction was observed while real silver content was more than 0.030% and an excellent *E. coli* reduction was observed with 0.721% silver filler content. On the other hand, we successfully incorporated zinc nanoparticles to PP fibers via melt spinning processes to obtain antimicrobial fibers. A high antimicrobial efficacy of PP/zinc composite fibers was demonstrated for the first time and the composite fibers started to exert a marked influence on the reduction of the bacteria with 0.034% zinc filler content. PP fibers with 0.3% and 0.5% Ag nanoparticles built up less charge and shorter characteristic decay time than PP

control sample. The zinc nanoparticles had agglomerated more than silver nanoparticles while 0.588% zinc and 0.721% silver filler content were compared.

In the second section of this study, we selected a 'bound' type of antimicrobial agents, an organosilicone quaternary ammonium compound, commercially known as AEM 5772 was also applied to the surface of knitted polyester fabric. Direct Red 47 dyestuff was used to develop a new method to calculate the concentration of quaternary ammonium groups, which has a very strong relationship with antibacterial activity, on the surface of polyester fabrics. We also characterized the surface of polyester fabrics via gravimetric absorbency testing system (GATS), fieldmeter, and microbiological analysis. At lower concentrations, almost all the charges present in the organic layer attached with sulfonate groups on the dye molecule, also the experimental charge density value was close to calculated value which were 1.4×10^{16} units/cm² and 1.3×10^{16} units/cm² at 0.05% aAEM 5772, respectively. We observed the reason for higher color strength values on the treated polyester surface was coming from the higher charge density.

The main objective of the third section of this work was to demonstrate and produce an eco-friendly antimicrobial nanofiber mat: PEO and PVA containing Lawsonia Inermis (henna) leaves extracts via electrospinning technique, and then characterize these nanofibers via SEM and FTIR. PVA and PEO based solutions at 2.8 wt% LI showed bactericidal effect against *S. aureus* and bacteriostatic action to *E. coli*. According to SEM images, there is no clear evidence for inconsiderable increases in the diameter of electrospun fibers with an increasing extract concentration.

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I. Introduction

1.1. Research Motivation and Objectives

A huge class of micro-organisms, which are very tiny creatures and cannot be seen by naked eye, coexists in a natural equilibrium with human body and other living environments such as animals and plants which, on the contrary, classified in various ways. They exist as single cells, cell bunches, or clusters and can be found almost anywhere on Earth, also a broad ranges of micro-organism are not harmful.

Table 1.1. Simplified classification of microorganism [1].

Kingdom	Members	Cell Classification
Animal	Rotifers	Eukaryotic
	Crustaceans	
	Worms and larvae	
Plant	Ferns	
	Mosses	
Protista	Protozoa	
	Algae	
	Fungi	
	Bacteria	Prokaryotic
	Lower algae forms	

On the other hand, pathogenic microorganisms consisting of viruses, fungi, parasites, and bacteria can proliferate rapidly and can cause adverse effects and many diseases which also can become resistant to antimicrobials [2]:

- i. coloring, discoloration, decrease in strength, deterioration, etc. of materials,
- ii. production of unpleasant odor,
- iii. increase of potential health risks and diseases.

For example; methicillin-resistant *Staphylococcus aureus* (MRSA) infections occurred by the estimating 94,360 in the United States in 2005 and 18,650 associated these infections patients died [3]. To address the issue, a large research focus on antimicrobial materials and agents, such as, National Institute of Allergy and Infectious Diseases (NIAID) becomes more important recently to fund antimicrobial researches and it exceeds \$700 million annually [4].

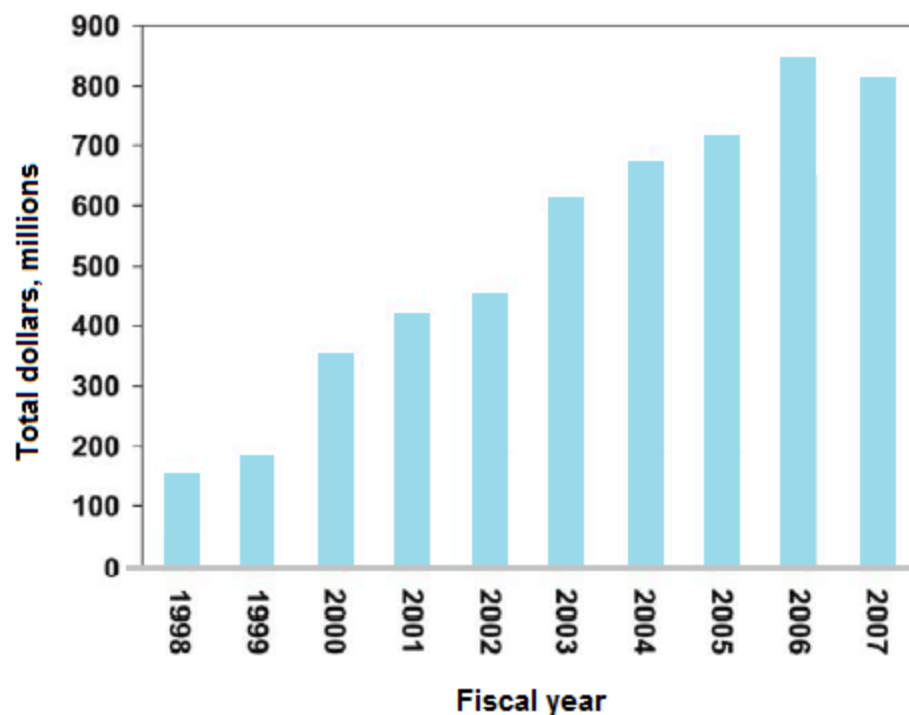


Figure 1.1. NIAID funds for antimicrobial research [4].

In addition, development of nanotechnology considered as one of the most promising technologies for the 21st century and a rapid widespread occurrence of nanomaterials which become a source of many different engineered nanoparticles in the industries and researching areas, and more than 300 nanoproducts already on the market [5]. They have unique properties, for example, high specific surface area, mobility and controlling matter at near atomic scales. However, the production, use and disposal of nanomaterials can potentially lead to some risks on air, water, soils, and organisms [6]. For example, silver nanoparticles are already in use in a wide class of consumer products including medical and

healthcare textiles, implants, and prostheses, sutures, wound healing and dressing, disinfection systems, air filters, home appliances, paints, and even food supplements, etc. During use of these products, the silver nanoparticles will be released to the environments and possibly exert toxic effects on aquatic organisms and other living creatures [6, 7].

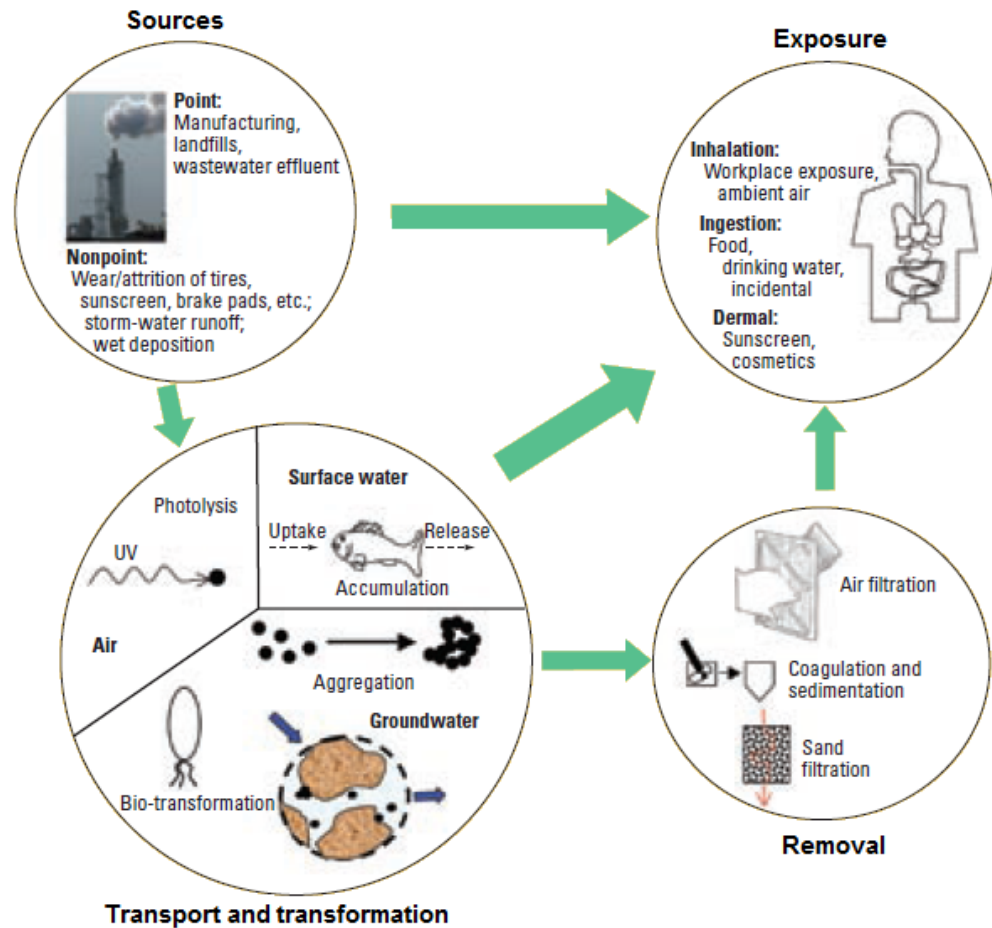


Figure 1.2. Nanomaterial releases and life-cycle [6].

Maynard et al. [5] proposed five grand challenges to develop safety nanotechnologies as shown below.

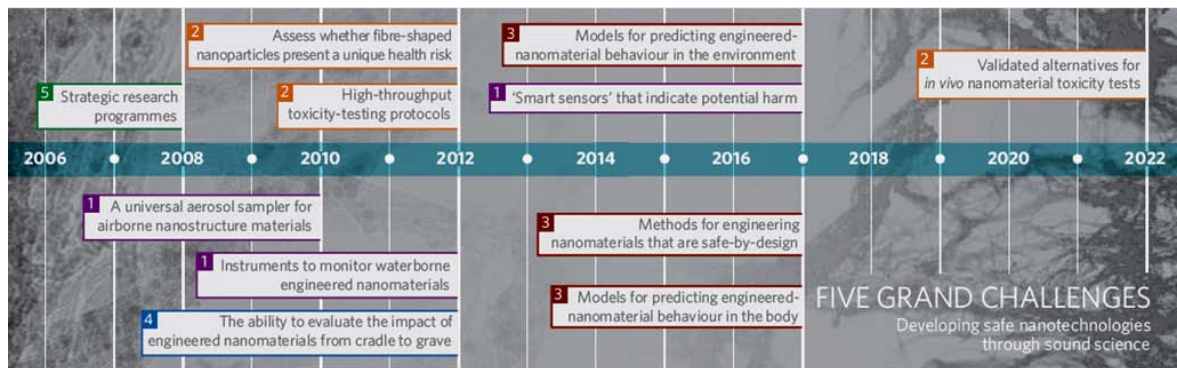


Figure 1.3. Five grand challenges for nanomaterials [5].

A new approach to produce an eco-friendly natural antimicrobial material is incorporating the active compound of plant extract with the material in order to treat humans, animals, and plant infections. The plants and their extract are still crucial in the pharmacy and medicine. People in many countries, for example, Europe, North America and Australasia now want to cure their minor health problems with ‘natural’ products as well as being an important form of treatment in many developing countries [8].

As a result, in order to be commercially viable as well as and skin and eco-friendly an ideal antimicrobial agent should fulfill the following requirements:

- a. Safety in the form, kills only bacteria.
- b. Low environmental impact.
- c. Durability.
- d. Ease of application.
- e. No adverse effects: good color fastness and fiber strength etc.
- f. Regulatory requirements; EPA (Environmental Protection Agency) registration.
- g. Low cost.
- h. ...

The primary objectives of this study are much focus on to develop efficiency and mechanism of different and commonly used antimicrobial materials with minimum requirements.

- The extrusion process was used for manufacturing composite fibers consist of a commonly used 'leaching' antimicrobial agent, silver nanoparticles, with one of the most widely used commercial polymers, polypropylene (PP), zinc nanoparticles also used, which is not used as much as silver nanoparticles. In addition, a method developed to increase the real filler content for higher amount of nanoparticles.
- The charge density of a 'bound' antimicrobial agent, an organosilicone quaternary ammonium compound (AEM 5772) which attached covalently to the surface, hence, unknown spread of the biocide into environments eliminated, was measured and calculated by a new method. The charge density is a critical factor to design a

surface to get maximum antimicrobial efficiency. Hence, the antibacterial surface can be obtained with minimum requirements.

- A new approach to produce an eco-friendly natural, Lawsonia Inermis (henna) extract, PEO and PVA based antimicrobial nanofibers obtained via electrospinning technique.

The stability and activities of antimicrobial agent can be divided two groups; i) 'leaching' and ii) 'bound' antimicrobials. They will be described in detail in the next section.

1.1. Thesis Outline

Section one provides a literature review and general information about bacteria, antimicrobial action of silver and zinc, and then determines and characterizes of PP fibers including silver and zinc nanoparticles with the examination of real filler content. In addition, their crystallization behavior, mechanical properties, antibacterial activities, antistatic properties, etc. mainly examined. Accordingly, we now pay attention the real filler content because the additives get lost during the extrusion processes and a method developed and evaluated to carry out higher metal content with polymer chips before the spinning processes.

Section two analyzes knitted 100% polyester fabrics treated with an organosilicone quaternary ammonium compound (AEM 5772) which is one of the most important antimicrobial agents. The mechanism for attaching fabric surface and mode of antibacterial action was determined. A most commonly used older method to determine the charge density also explained. The new method carried out to determine quaternary ammonium units/cm² and its relationship between K/S values and compared with other studies. Moreover, hydrophilicity and the surface potentials were investigated.

Section three presents a brief explanation of the medical plants, their trend and the popularity of 'natural' products. Afterwards, we focused on potential application of Lawsonia Inermis Linn and its definition, chemistry, and physical properties. Finally, preparation of antibacterial PVA and PEO nanofibers containing Lawsonia Inermis (henna) leaf extracts and their properties by characterizing SEM images, FTIR analysis, antibacterial activities, total phenolic compounds, and so forth were observed.

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Section 1.

Antimicrobial Nanoparticles Composite

Polypropylene Fibers

Chapter I. Introduction

A textile material can protect us either from relatively mild external or extreme environmental conditions for instance exposure to water, extreme cold, intensive heat, open fire, high voltage, propelled bullets, toxic chemicals, nuclear radiation, biological toxins or UV-rays. In addition, the textile products need to be comfortable and meet personal desire and demand. On the contrary, the microorganisms such as bacteria and fungi can live and multiply quickly on the textile materials and everywhere when moisture, pH, nutrients and temperature being suitable for bacterial growth. Hence, some harmful effects can be generated for both the textile itself and the wearer. Physical and chemical properties of the textile materials, for example, stains, decrease in strength, discoloration, unpleasant odor, reduction in fabric mechanical, and also they can consume cotton or latex. Natural fibers, in general, are more hydrophilic than most synthetic fibers therefore the microorganism can attack easily to the hydrophilic surfaces [1, 14].

Recently, there has been a growing interest in the development of medical, healthcare and hygienic clothing for a substantial market. These textiles also have some particular properties, such as, good strength, flexibility, and sometimes moisture for medical application. Antimicrobial textiles can be manufactured by two main techniques, mainly by introducing an antimicrobial agent during yarn, fabric or garment finishing processes by means of chemical or physicochemical modification and incorporation of biocide additives into fibers during melt or wet spinning processes [2]. The stability of antimicrobial textiles can be categorized as temporary or durable. In addition, biocides can be divided in two groups:

- i. 'Leaching' antimicrobial agents that are dispersed in a fiber and show controlled release mechanism, such as, silver based antimicrobial agents.
- ii. 'Bound' antimicrobial agents which are chemically bonded with fiber surfaces, for example, silicone quaternaries.

Microorganisms are very tiny creatures that cannot be seen by naked eye. They include an incredibly diverse group, mainly, bacteria, fungi, algae and viruses. Some specific types of them are pathogenic and harmful for human body, or any living system. Therefore the personal hygiene in human health would be one of the top an issue to deal with harmful conditions [3].

1.1. Research Purpose and Objectives

Polypropylene (PP) is one of the most widely used commercial polymers because it is relatively cheap, has a low density and exhibits a narrow molecular weight distribution. Fibers and yarns made from PP exhibit outstanding mechanical properties.

In addition silver is widely used in textiles because it has an excellent thermal stability and exhibits a broad-spectrum of biocidal activities. Furthermore, silver or silver based biocides have a little toxicity to mammalian cells and tissues.

PP is used in different applications [4], for instance, surgical masks, diapers, filters, burn and wound dressing, hygienic bands, also carpets, automotive interior trim, films, packaging, cover stock, cables, napkins, which are some other applications of polypropylene.

The purpose of this study is to produce antibacterial polypropylene (PP) composite fibers using nanoparticles of silver and zinc and to determine whether PP fibers containing silver or zinc nanoparticles in the range from 0.025 to 1 wt%, and also silver exchanged zeolite (1-2 wt%) concentrations can be produced via the melt-spinning technique. We also studied effect of zinc nanoparticles to antibacterial efficiency and investigated the effect of silver and zinc content on the fiber properties. The effect of the real filler concentration to antimicrobial activity was determined. In order to characterize the thermal properties and crystallization behavior of the spun fibers; PP composite fibers were examined using differential scanning calorimetry (DSC), scanning electron microscopy (SEM) to investigate the morphology of the composite fibers. Furthermore, mechanical properties of the composite fibers compared with PP control without the additives. The antibacterial activity of these fibers was determined by the percentage count reduction growth of *E. coli* and finally, the antistatic properties were examined by the linear tester instrument [5].

Chapter II. Literature Review

In recent decade, a rising interest in personal health, hygiene and infection control, created a new market for antimicrobial fibers. The antimicrobial textiles were produced in the magnitude of 30,000 tones in Western Europe and 100,000 tones worldwide in 2000. Moreover, it was estimated that the production increased by more than 15% a year in Western Europe between 2001 and 2005, hence, this is one of the fastest growing sectors of the textile industry. Sportswears, socks, shoe linings and lingerie constitute for about 85% of the total of these manufacturing materials [1].

2.1. Bacteria and Their Structures

Scientists and researchers determined that bacteria have been around for nearly 3.5 billion years. Antonie van Leeuwenhoek (1632-1723) was first to described bacteria in the Netherlands in 1674 [6]. When he examined a drop of water by a single lens microscope, he noticed tiny creatures, and then called them "*animalcules*". Christian Gottfried Ehrenberg gave it a name "*bacterium*" in 1838.

Most bacterial (Figure 2.1) species are single-celled microorganisms and they can live either independent or as parasites as in the bodies of any living creatures and everywhere [6], such as, in dirt, air, water, hot springs, snow, ice, and so on. Furthermore, bacteria can grow and divide with great rapidity under optimum conditions. It is believed that the proliferation of

bacteria under right conditions can easily double every 20-30 minutes, so, a single bacterium can reproduce over 2 million in 7 hours if assumed all off spring live. They usually live between pH 5-9, and 25-37 °C, also mutate rapidly.

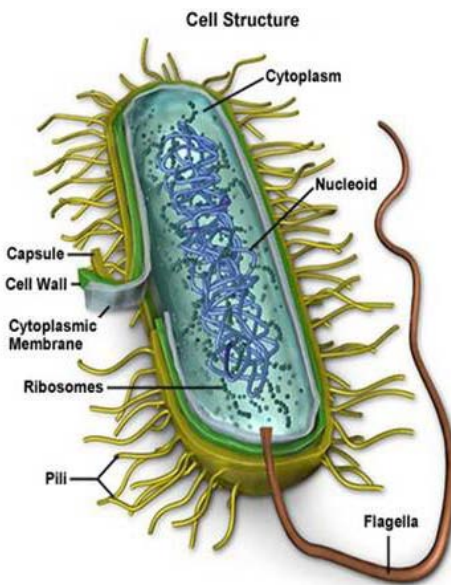


Figure 2.1. A typical bacterial cell [54].

Many classifications can be made for the bacteria according to their cell structure, metabolism, pigments, growth characteristics, and lipid composition. In general, the bacteria

are divided into two groups based on the chemical and physical properties of their cell structure and staining characteristic. Gram-negative bacteria, which are considered to be harmful for the host, are stained pink because the bacteria have a thinner layer and containing less peptidoglycan, also more structurally complex, for example, *Escherichia coli* has about 46 nm of the cell membrane thickness which including cytoplasmic membrane, periplasmic space, peptidoglycan, and outer membrane from inside to outside. On the contrary, Gram positive bacteria have a thick cell wall and are rich in peptidoglycan, for instance, *Bacillus subtilis* has approximately 45-55 nm of the thickness [7], and stains purple or dark blue by the Gram staining (see Figure 2.2).

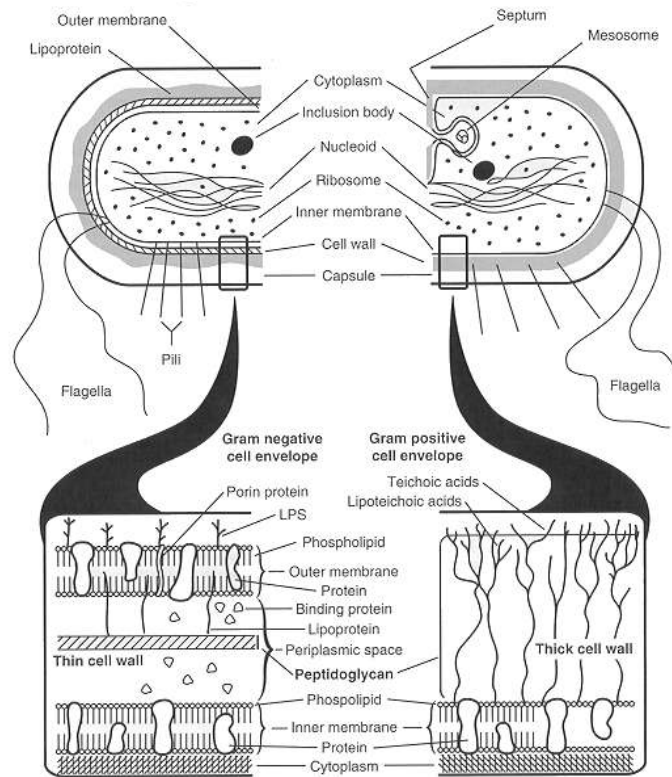


Figure 2.2. Comparison of Gram-positive and Gram-negative bacteria [8].

2.2. Types of Antibacterial Action

A biocide is explained as a material that is destructive to many different types of organisms, such as, bacteria, viruses, fungi, algae, yeast and other microbes. The bacterium typically has an outermost cell wall which is mainly composed of polysaccharides. This structure protects organic compounds present in bacteria from adverse outside conditions and maintains the integrity. Therefore, the survival and growth of the bacteria depends on the integrity of the membrane, and also the nucleic acids, which storage all genetic information and enzymes need to work together with proper state. Furthermore, the cell wall, the cytoplasmic membrane, and the cytoplasm are major part of the cellular regions and the biocide can interact with them to cause leakage of intracellular components or disturb membrane-enzyme reactions. However, the bacterial resistances have not been fully investigated; it is believed that the mechanism can be intrinsic and/or acquired. Some general biocidal mechanism of action and some examples of them are given in Table 2.1 [10].

Table 2.1. Mechanism of biocides and their effects [10].

Mechanism	Effect	Examples
Oxidation and oxidizing agent	Electron removal from macromolecules leading to loss of structure and function.	Halogens, e.g., iodine, chlorine peroxygens, e.g., hydrogen peroxide, peracetic acid.
Cross-linking and coagulation	Cause interaction cross-linking within/between macromolecules leading to clumping and coagulation.	Aldehydes, e.g., formaldehyde, glutaraldehyde alkylating agent, e.g., ethylene oxide, phenols, alcohols.
Transfer of energy	Transfer of energy to various macromolecule bonds and structures leading to loss of structure and function.	Heat radiation, e.g., UV light, γ -radiation.
Other structure-disrupting agent	Specific macromolecular structure disruption and loss of activity.	Acridines surfactants, e.g., quaternary ammonium compounds, metals, e.g., silver, copper.

Biocides, which are mainly silver, triclosan, poly(hexamethylene biguanide) (PHMB) and quaternary ammonium compounds, can be divided into two groups (Figure 2.3) [9].

2.2.1. Bactericidal Activity

The effect of agent leads to the death or elimination of the bacterial cells.

2.2.2. Bacteriostatic Activity

The bacteriostatic agent can interact with nucleic acid of bacteria to prevent and disturb the cell reproduction, hence, the proliferation of the bacteria population inhibited.

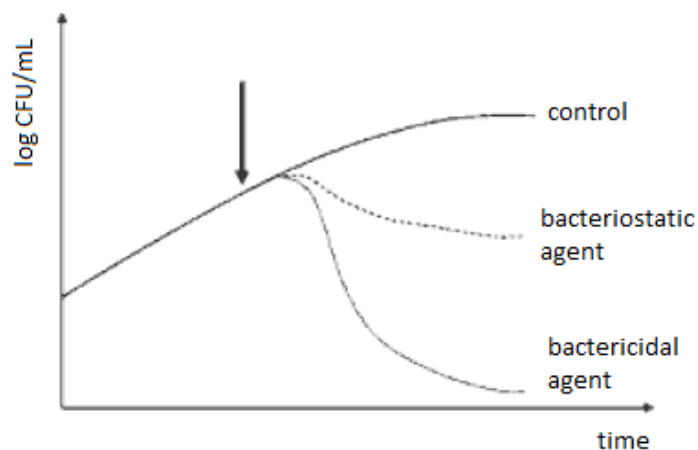


Figure 2.3. Changes in bacterial growth after addition of an antibacterial agent (the arrow indicates that starting of application time) CFU colony forming unit [9].

It has been observed in the Figure 2.3 that bacteriostatic and bactericidal agents have different degree of activity against bacteria which lead to the death or inhibit the microbial growth. In general, the mode of action depends on characteristic features of the agents and the mechanism of the polycationic antibacterial compounds usually occurs as six steps [9]:

- i. Adsorption onto the bacterial cell surface.
- ii. Diffusion through the cell wall.
- iii. Binding to the cytoplasmic membrane.
- iv. Disruption of the cytoplasmic membrane.
- v. Release of cytoplasmic constituents such as K^+ ion, DNA and RNA.
- vi. Death of the cell.

2.2.3. Antibacterial Action of Silver and Zinc Nanopowder

Antibacterial activity of silver has been known since ancient times. The first written information about silver was that of the Egyptian Gabir ibn Hayan as-Sufi around 950 A.D. [11]. At similar time Avicenna (960-1037), who was a legendary philosopher and physician, determined the medical benefits of silver and described the poisoning effect of silver based materials. Then, following the discovery of bactericidal effects of silver as antimicrobial, antifungal and partially antiviral properties made one of the most promising materials for future application. On the other hand, there is an idiom “born with a silver spoon in their mouth” that means the person had an affluent upbringing. It can be seen that silver widely accepted a metal in the human life. The historical summary of the medical uses of silver is shown in Table 2.2.

Table 2.2. Historical summary of the medical uses of silver [11].

4000 BC, Egypt	Earliest craft silver
Alexander the Great	Silver goblets for drinking water disinfection
1884	Credés Prophylactic
1893	Von Nägeli's theory of oligodynamic metals; various practical applications; Höllenstein, silver protein combination; silver-arsenic compounds for syphilis treatment
1950	Introduction of antibiotics
1965	Silver nitrate compresses
1968	Silver-sulphadiazine for burns
1980	Vitacuff (subcutaneous cuff) catheter coating

Silver forms several inorganic and a few organic salts, such as, silver chloride, silver fluoride, silver iodide, silver nitrate, silver acetate, silver sulfide, silver perchlorate, silver benzoate, and silver diethyl dithiocarbamate, on the contrary, it can react with dilute nitric acid and hot sulfuric acid [13]. Nowadays, silver is still used as an antimicrobial agent in various applications such as medical and healthcare textiles, implants, and prostheses, also sutures, wound healing and dressing, disinfection systems, water treatments, air filters,

laundry additives, home appliances, paints, and even food supplements, etc. Silver medically demonstrates to kill over 650 disease-causing organisms in the body and Minimal Inhibitory Concentration (MIC) values is between 0.05–0.1 mg/L against *E. coli*, moreover, the silver nanoparticles incorporated into the membrane of the bacterial cells and some of them penetrated into the cells as shown in Figure 2.4 and 2.5 [45]. As mentioned in the introduction it can be incorporated by finishing processes and added to a polymer or a polymer solution accordingly prior to melt spinning or electrospinning processes [1, 2, 4].

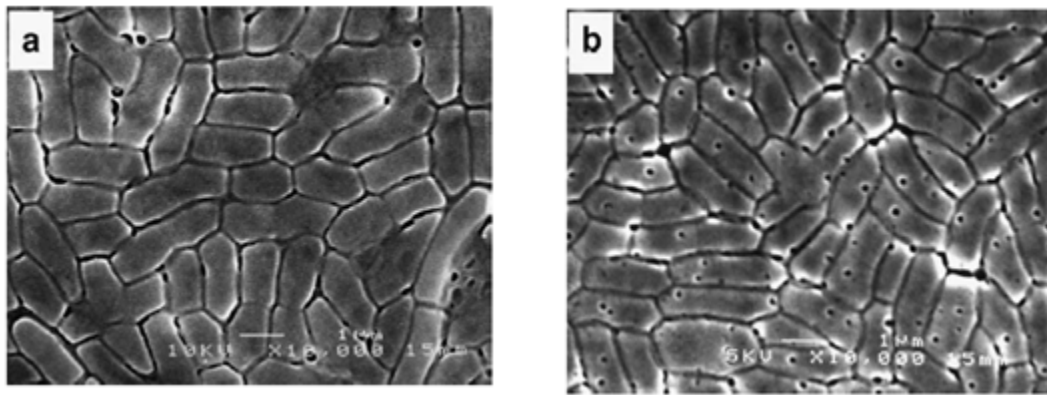


Figure 2.4. SEM images of native *E. coli* cells (a) and cells treated with $50 \mu\text{g cm}^{-3}$ of silver nanoparticles for 4 hr (b).

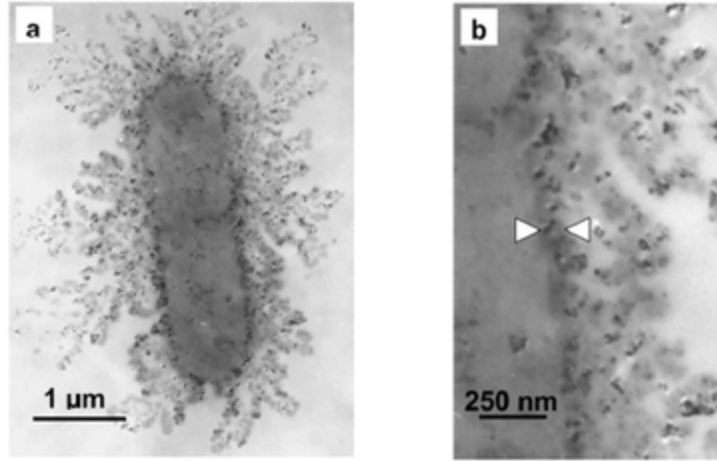
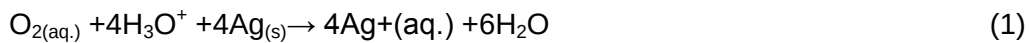


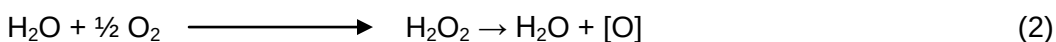
Figure 2.5. TEM images of *E. coli* cell treated with $50 \mu\text{g cm}^{-3}$ of silver nanoparticles for 1 hr (a) and enlarged view of the membrane of this cell (b).

Despite the fact that, especially, there is no clear mechanism of toxicity for silver nanoparticles [15]. On the other hand these particles cause the damage to the cell wall by oxidative stress or interaction of Ag^+ ions with protein and enzymes. Silver metal particles can be converted to Ag^+ ions in the presence of water and oxygen, and this relationship is determined by Equation (1). In addition the silver ion release rate need to have at least $9.5 \times 10^{-4} \text{ mgL}^{-1} \text{cm}^{-2} \text{day}^{-1}$ to kill all *E. coli* and *S.epidermidis* within 24 hours. It was demonstrated that polyamide 6 should have at least 0.06 wt% of silver nanoparticles [16].



On the other hand; Gram positive bacteria contain three to twenty times more peptidoglycan than Gram-negative bacteria which negatively charged so it is speculated that some Ag⁺ ions attached to the cell walls and prevent more Ag⁺ ions to reach the plasma membrane [50]. Furthermore, it is known that Gram negative bacteria are more resistant to antibiotics; Ag⁺ ions are therefore a suitable antibacterial agent against the bacteria.

Silver can prevent some oxidative enzymes, for example, yeast alcohol dehydrogenase, the respiratory chain of *E. coli* and membrane vesicles uptake succinate, therefore metabolite efflux and interfering with DNA replication are formed [47]. Silver ions attach to sulfur-, nitrogen- or oxygen containing functional groups that present in bacteria, and react with sulphhydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins to denaturation of protein, hence, cause the death of the bacterial cells (bactericidal activity of Ag) and also inhibit the cell reproduction (bacteriostatic activity of Ag). The metal ions can accelerate the rate of the chemical reaction to production of oxygen radicals to cause oxidize the molecular structure of the microorganism as shown in the Equation 2 [46].



Zeolites are crystalline aluminosilicates that basically consist of SiO₄⁴⁻ and AlO₄⁵⁻ tetrahedra. Cation exchange is a very important property of the zeolite [49]. The typical zeolite structure is as follows:



Where x and y represent the total number of tetrahedra in a basic cell.

The aluminum tetrahedron has a negative charge. It is compensated by the M^{+} cation and not bound by a covalent bond, hence, can be very easily replaced by other elements.

Silver ions can be incorporated with in a zeolite carrier by the ion-exchange with an alkaline earth metal ion within crystal aluminosilicate [48]. It has low toxicity for humans and ensures durable efficiency against the bacteria. There are two mechanisms to explain the bactericidal action of silver zeolite [48]:

- i. Released the silver ions from the zeolite by itself, also it is dependent on the concentration of cations in the solution [50].

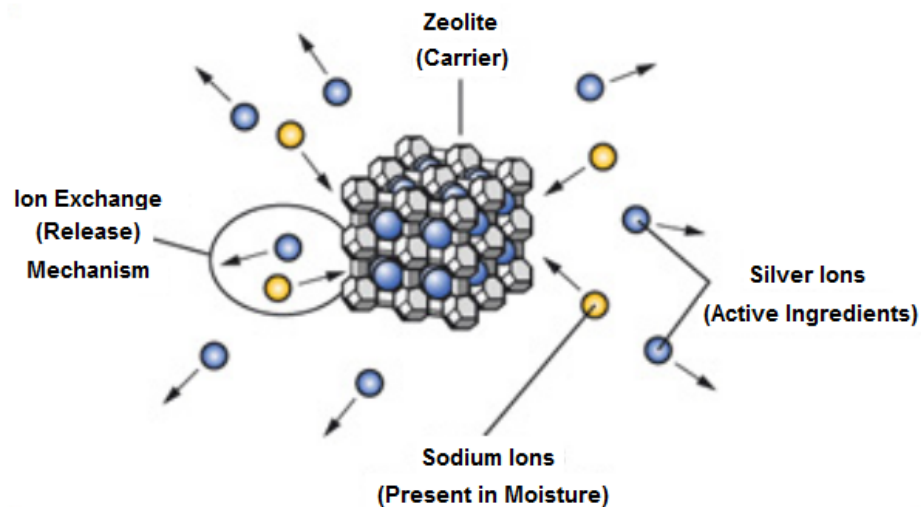


Figure 2.6. The patented multi-faced zeolite crystal carrier [51].

- ii. Reactive oxygen species are produced from the silver in the matrix because Inoue et al. [52] demonstrated that the bactericidal activity depended on dissolved oxygen because Ag-zeolite activated to the oxygen for the formation of superoxide anion, hydrogen peroxide and hydroxyl radical to contribute to the activity.

Antibacterial actions of zinc-based materials, either alone or in compounds, are also exhibited. They show a detrimental effect on bacterial cells which combined with cellular proteins to inactivate and coagulate cytoplasmic proteins, hence, causing damage or death of to the cell. However, zinc ion concentration between 10^{-5} - 10^{-7} M which is the most suitable condition in order to grow for most microorganisms in vitro, on the other hand, high zinc ion concentration acts as an adverse effect on the cell growth and proliferation, it can also exhibit bacteriostatic and bactericidal effects, especially, in combination with other ions, for example, fluoride or antibacterial agents [17, 18]. Söderberg et al. [19] determined effect of zinc ion concentration on Gram-positive and Gram-negative bacteria that Gram-positive bacteria became the most susceptible group and Gram-negative aerobic bacteria were usually not effected even at the highest concentrations of zinc oxide used (1024 µg/mL). In addition, zinc pyrithione has bactericidal, notably Gram-positive bacteria, fungicidal and algicidal activities which has been used in a variety of different products, for instance, soaps, cream and spray, also as a preservative in adhesive coating, typically, antifungal activity which against *Pityrosporum ovale* (dandruff) is well known, also can inhibit eczema-related and fungal skin infections [20]. Its mechanism are still are largely unknown and it is believed that the compound disrupts function of DNA interaction.

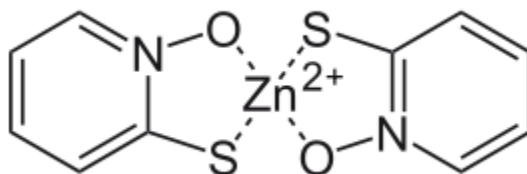


Figure 2.7. Zinc pyrithione [20].

2.3. Methodologies and Characterization of Polypropylene Including Silver and Zinc

With a rising interest in personal health and hygiene, studies employing different strategies have been performed to prevent bacterial infection. All of these studies serve a different purpose and targets a different group of bacteria and/or pathogens. One strategy developed to prevent the infection, also for this purpose, polypropylene (PP) consisting of different additives and their particles size are described by different researcher in the following sections.

Jeong et al. [2] explored the effect of additives particle size on the antibacterial properties so that manufactured polypropylene with micro- or nano sized silver powders as antimicrobial compounded fibers via conventional twin-screw mixer. In general, synthetic fibers are more resistance than natural fibers; however, a microorganism can attack the main chain of some polymers, for example, PP which contains methyl group. In this study, two different size of silver powders which were about 100 nm and 1 μm were used as a particular weight percentages incorporated with PP to get thin films. Based on the SEM images, the average

diameter of the silver particles was 30 nm with good dispersibility; however, there were some agglomerations because of interaction forces. The compound, which containing 0.1% silver nanoparticles, exhibited excellent antimicrobial effect (99.9 % reduction) against both a Gram-positive microbe (*Staphylococcus aureus*, ATCC 6538) and a Gram-negative microbe (*Escherichia coli*, ATCC 25922). On the contrary, the micron-sized silver should be more than 0.5 wt% in the compound to show good antimicrobial activity.

Dastjerdi et al. [21] investigated the properties of bioactive continuous PP filament yarns containing Ag/TiO₂ is that the surface of silver nano-particles coated by titanium dioxide to get good dispersibility into samples. Firstly, the materials were mixed in a twin screw extruder to obtain master batch, then, a pilot plant melt spinning machine utilized at different draw ratios to obtain continuous filament yarns. Mechanical and structural properties and also antibacterial activities evaluated. In the SEM pictures, the nanofiller well dispersed therein, except a sample containing 1.00 wt% of nano-filler which agglomerations were examined. The crystallinity was slightly decreased when compared with pure sample. In addition; tenacity, modulus, and tensile properties were improved, also maximum antibacterial activity observed for the sample including 0.75 wt% of nano-filler which probably corresponded to the minimum of crystallinity in this sample.

Broda et al. [22] studied the influence of the additives which two organic pigments and two flame retardants on the structure of PP fibers. It has been observed that the effect of additives on fiber structure became more dominant at low take-up velocities that a highly crystalline structure was manufactured. The pigments acted as a nucleating agent, and heterogeneous nucleation occurred to cause formation of crystalline structure. However, the

flame retardants did not act as a nucleating agent but increased viscosity during spinning. It provoked highly orientation and ordered structure, thus, crystalline structure was formed. Adversely, test results confirmed the effect of additives on fiber structure at high take-up velocities were less prominent.

Joshi et al. [23] prepared PP composite filaments with nano-clay reinforcement to develop high tenacity and modulus monofilaments. The modified nanoclay particles incorporated to PP with varying weight percentages of nanoclay as well as varying the spinning and drawing conditions. The composite demonstrated improved tensile strength, modulus, thermal stability and reduced elongation at break and the crystallinity was increased via 0.5 wt% modified clay. On the other hand, there was no any improvement in tensile strength for the filaments including unmodified clay but modulus and thermal stability were enhanced.

Houshyar et al. [24] determined the effect of fiber concentration on the mechanical and thermal properties of fiber reinforced PP composites. In this work, poly(propylene-co-ethylene) (PPE) matrix was reinforced by PP fibers. Strong interfacial interaction between PPE and PP occurred without any surface treatment. The storage, tensile, also flexural modulus improved of about four times than that for the pure PPE with increasing the fiber concentration, and the maximum increasing observed at 50% fiber concentration. Furthermore, the melting temperature of the composite increased without any significant changing in the crystallization temperature; but different fiber concentration in the composite did not affect to change the melting temperature.

Jeong and co-workers have incorporated antibacterial nano-sized silver powder into polypropylene by co-extrusion of polypropylene (PP) and PP/Ag master-batches [25] to

produce permanent antibacterial activity. The produced samples including silver nanoparticles in core and in sheath-part demonstrated poor and significant antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia*; respectively. Moreover, the crystallinity of the samples was nearly the same as the virgin polypropylene sample.

On the contrary; Dastjerdi et al. [26] prepared silver/zinc-loaded nanocomposite PP yarns, then the samples were drawn and textured, lastly weft knitted. Tenacity and modulus of modified samples were improved, and there was no significant change in crystallinity observed between –loaded and –unloaded samples. Furthermore, the modified fabrics and modified PP fibers demonstrated very good permanent antimicrobial efficiency against *S. aureus*.

An alternative method of antibacterial material is incorporated zinc-based agents to materials. However, it is not used very common substance in general textiles as well as in wound dressing as silver.

Atmaca et al. [17] used zinc acetate in order to show antibacterial efficiency against *S. aureus*, *S. epidermidis* which was better than *P. aeruginosa*.

Vigneshwaran et al. [27] obtained cotton fabrics coated with 2% zinc oxide nanoparticles using acrylic binder, hence, uniform and very thin coating also produced. In this work, UV blocking properties which was about 75%, air permeability, and strength properties were demonstrated. In addition, Kathirvelu [28] and co-workers have showed the antibacterial performance of ZnO nanoparticles on the surface of both woven and knitted cotton and polyester/cotton blended fabrics.

Chapter III. Materials and Experimental Methods

3.1. Introduction

In order to inhibit and kill the bacteria, antibacterial fibers containing 'leaching' or 'bound' antimicrobial agents are often used in biomedical applications. It is very important that the type of antimicrobial agent used is appropriate for its target application.

Electrical conductivity is a measure of a material's ability to conduct an electric current which displayed in SI units of Siemens per meter ($\text{S}\cdot\text{m}^{-1}$). If a substance is a conductor, for example a metal that it has high conductivity and a low resistivity. A semiconductor substance has usually intermediate conductivity and it is affected by different conditions such as electric fields, specific frequencies of light, temperature and composition of the semiconductor material.

The degree of filling materials of the semiconductor substance can change the conductivity, for instance, the conductivity of water is highly dependent on its concentration of dissolved salt or some other chemicals which can make ionize in the solution. A measure of the conductivity of water is often reported as "Specific Conductance", which is carried out at 25 °C.

Silver is the best electrical conductor of any known material as well as thermal conductor which is about 63.01×10^6 ($\text{S}\cdot\text{m}^{-1}$) at 20 °C [30].

Siemens = 1/Ohms, or Ohms = 1/Siemens [31].

Electrical conductivity of zinc is $16.60 \times 10^6 \text{ (S}\cdot\text{m}^{-1})$ [32].

Static charge dissipation is based on surface properties, such as if the surfaces are conductive, the extra charge can dissipate to ground very easily, however the surfaces keep the charge when they are insulators. Most of the polymers are not 100% crystalline and their surfaces are not homogenous. They might have also some impurities. The generation mechanism and location of tribocharge on low energy surfaces (polymers) is still under investigation. The theory of surface states, which examines the behavior of electrons at solid surfaces, could not explain well electrostatic problems in polymer product manufacturing processes and parameters, such as, effect of processing speed. To understanding correlation between electrostatic generations, dissipation properties, static charge potential (voltage), charge amount (coulomb), their variations with time and the polymer surfaces that statistical experimental responses are usually used to determine relationship between them [5]. Furthermore, resistivity is another important factor for static charge accumulation and decay properties with time.

The focus of the present study is to incorporate PP with silver and zinc nano-metals, and also silver substituted zeolite via the melt spinning technique. In general, nanosilver particles are smaller than 100 nm and contain 20-15,000 silver atoms [44]. The silver nanoparticles, with high specific surface area, show notable and unusual physical, chemical and biological properties. Resultant fibers characteristic and properties were examined by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), the linear tester

instrument; MTS Q-test/5 universal testing machine, in addition, the real metal content, static charge behavior and antibacterial properties were investigated.

On the other hand, Nylon 6 was used with silver and zinc nanoparticles to determine its electrostatic behavior and compare PP composite fibers. In addition, some other antimicrobial chemical compounds which are polydiallyl amine, colloidal Ag, quaternary ammonium compound and triclosan were applied to the surface of PP to observe their effects. The detailed information and using percentage of these chemicals will be explained in the next sections.

3.2. Materials

Fiber forming isotactic polypropylene (PP 3155) with specific gravity of 0.902 was obtained from Exxon Mobil. We were able to produce the PP yarn with tenacity of 3.8 g/denier. Silver nano-metal powder (< 100 nm) with 99.5% metals basis, FW 107.87, mp. 962 °C; zinc nano-metal powder (<50 nm) with 99+% metals basis, FW 65.37, bp 908 °C, mp 419.5 °C, d 7.14, and silver exchanged zeolite (20 mesh granular, ground to 60 mesh) were purchased from Aldrich Chemical Co. A non-ionic dispersing agent (polyethylene glycol monooleate, 100%), was obtained from Boehme Filatex Boehmelev NT. Some other fatty acid ester surfactants were obtained from the College of Textiles dyeing and finishing pilot plant.

3.3. Experimental Techniques

3.3.1. The Spinning Process

Hill's bicomponent melt extrusion machine was used to produce antibacterial fibers as illustrated in Figure 3.1 and 3.2. PP chips and additives (silver and zinc nano-metals, and silver zeolite) were mixed at least 10 minutes in a glass container by hand-shaking. Then, 2 g/kg the non-ionic dispersing agent was added to the mixture and mixed approximately for 30 minutes and placed in a pressurized oven at 100 °C for 1 hour. Final mixing was carried out for 10 minutes to pour into hopper tanks under nitrogen pressure which is guided through an automatic pressure control booster pump at a constant 8 rpm rate and metered with geared pumps. On the other hand, the surfaces of the PP fibers were coated by the antimicrobial chemical compounds according to following steps: After pure PP fibers were emerged from spinneret, the surfaces of the fibers were treated by chemicals, which are polydiallyl amine, colloidal Ag, quaternary ammonium compound and triclosan, with 2 g/L concentration via the finish applicator. Then the bobbins were dried at 90 °C for 5 minutes and at 160 °C for 10 minutes in a pressurized oven.

The extrusion process consisting of a two screw extruders at 400 psi and the screw diameter was 1 inch with L/D = 24:1 which have three static mixers with gradient temperatures. The first and zone for the extruders was set at 220 °C, the second zone at 230 °C, and third zone at 240 °C and the spin pack at 255 °C temperatures were adjusted for PP composite fibers. Polyamide 6 (PA6) was carried out with the first and zone for the

extruders was set at 240 °C, the second zone at 250 °C, and third zone at 260 °C and the spin pack at 260 °C temperatures.

The spin pack was designed as a 288 filament/yarn in order to manufacture multifilament fibers and the spinneret with 1 mm in diameter and 40 mm in length which also has filtration screen to capture of any impurities.

The fibers were cooled at room temperature and drawn at 810 m/min for first set of PP fibers, which have lower nanoparticles content, and 900 m/min for PA6, also the surface treated with Lurol PP L 425 lubricant. The draw ratio was adjusted to 3 for PA6 and 4.05 for the first set of PP fibers. PP/0.5% and PP/1% Ag, also PP/0.5% and 1% Zn fibers were second set of fibers and produced at a lower take-up speed that was 620 m/min with 2.07 draw ratios.

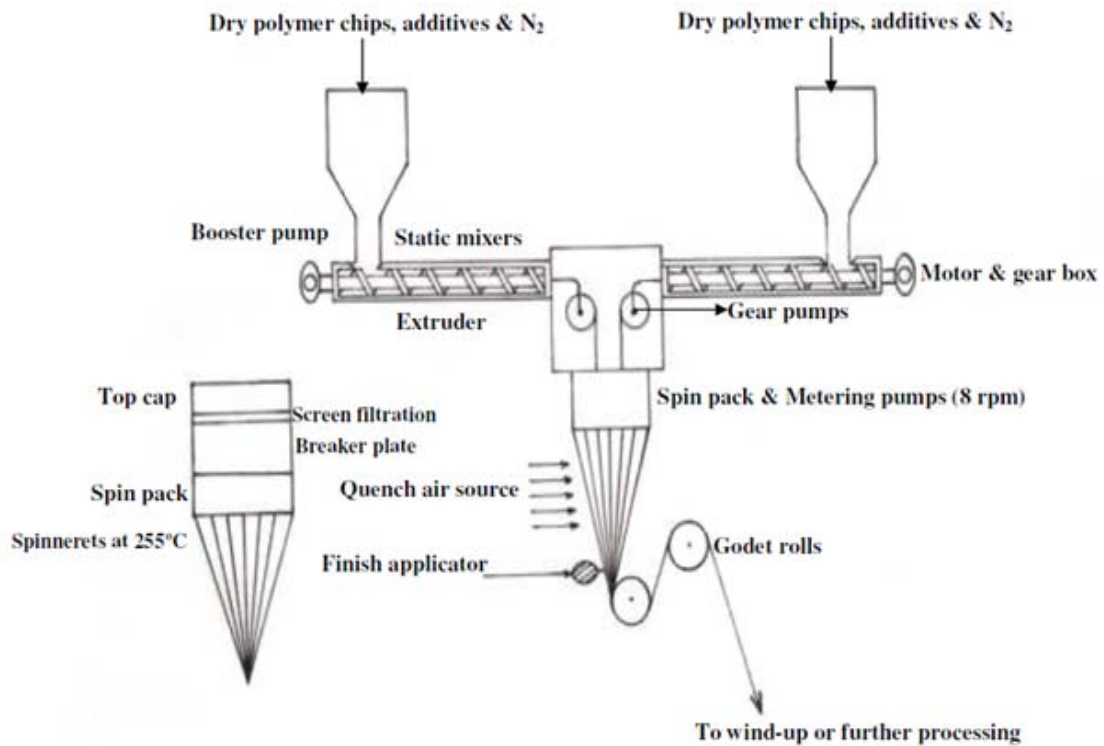


Figure 3.1. Schematic of the melt extruder spinning process [Hills Inc., Pilot fiber spinning].



Figure 3.2. Bicomponent pilot machine.

Basic Specifications [12]:

Fiber Extrusion up to 350 °C.

Extruders, various sizes:

3/4" (18mm) to 1 1/4" (32mm)

24:1 and 30:1 l/d

Screw includes Maddox mixing section.

Direct connection of extruder to spin head.

Extruder screw removal from rear of extruder.

Metered polymer stream to spinneret.

Variable quench air flow rate.

Even air flow distribution from quench.

Throughput range (with 2.92 cc / rev polymer pumps) = ~ 3 lbs / hour up to 40 lbs / hour.

The lab mixing extruder (see Figure 3.3 and 3.4) was also used in order to evaluate different process conditions to find the best one to manufacture the highest real filler content for fiber composites.



Figure 3.3. Lab mixing extruder (LME).



Figure 3.4. LME lab mixing extruder [53].

3.4. Fiber Analysis

3.4.1. Electrostatic Observation

In this study, linear tester designed (Figure 3.5 and Figure 3.6) was used to assess static charge behavior of all yarns. It is a constant tension transport machine and includes a charge pin, two potential probes connected to voltmeters, and computer system for representing of data acquisition of the two potential probes based on time (second).

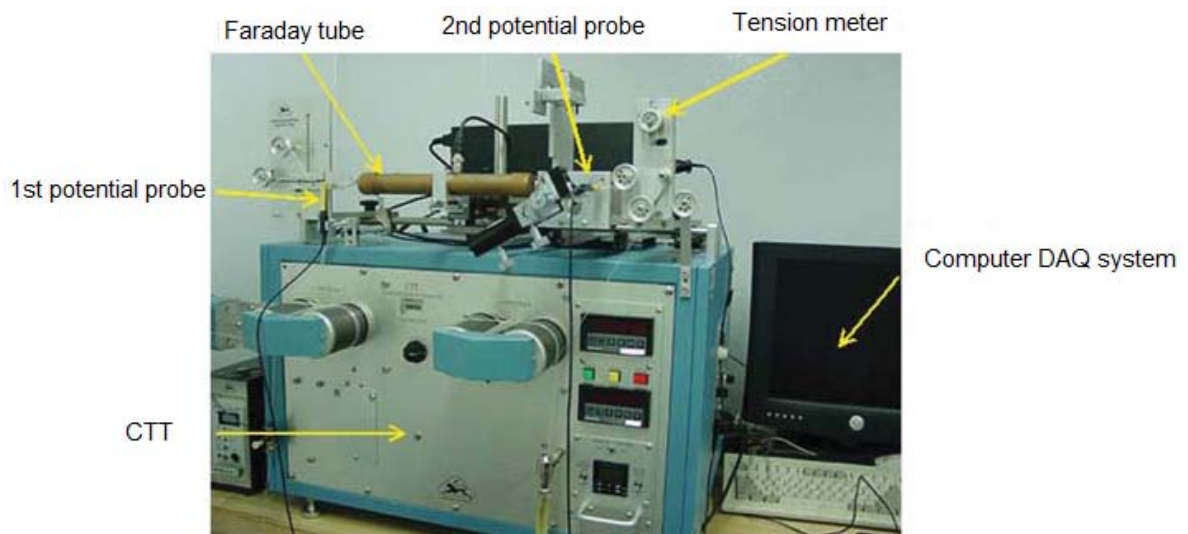


Figure 3.5. The linear tester [5].

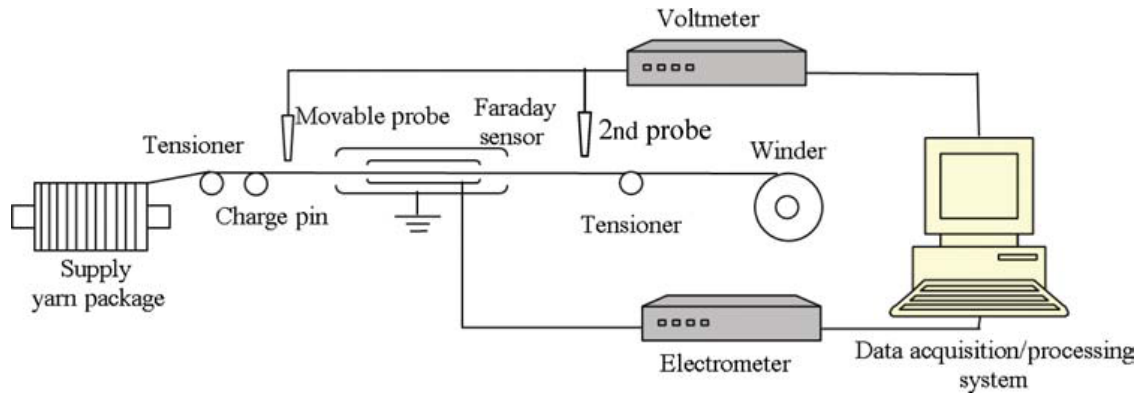


Figure 3.6. Schematic of linear tester equipment [5].

The correlation between initially generated charge potential and characteristic dissipation time can be explained by a decreasing exponential function:

$$V = V_0 e^{-t/t^*} \quad [5] \quad (1)$$

Where: V (Volt) is the amount of electrostatic potential on the yarn surface after time t (second) from the beginning of initial charge; V_0 (Volt) is the electrostatic potential at the point of separation (initial charge potential at $t = 0$ second) and t^* (second) is the characteristic decay time of the yarn under test conditions.

The yarn conditioning and tests were performed at the normal conditions (20 ± 5 °C and 60% maximum humidity) in a special environmental room protected from external weathering and

controlled by air controller and insulated panels. Usually, the yarns were conditioned for 24 hours at 21 °C and relative humidity of 43% prior to testing. These conditions were suitable for static test because if we tested nylon 6 yarns under the textile standard condition of 65% RH, there may be no charge at all.

The testing system consists of two potential probes and one stainless steel friction pin. The first probe was very close to the pin, 3.5 cm, and the second one was 41 cm from the pin (Figure 3.7). The filament ran against the friction stainless steel pin and then winding in front of the potential probe. The probe recorded the charge potential on the yarn with the potential detected on the two probes, data collection rate 100 data/sec, and we then calculated the characteristic decay time and initial generated potential at the yarn/pin separation point (Equation 1). The rollers and all guides of the system were cleaned by alcohol before each experiment because contamination has very important effect on the static charge and decay time.

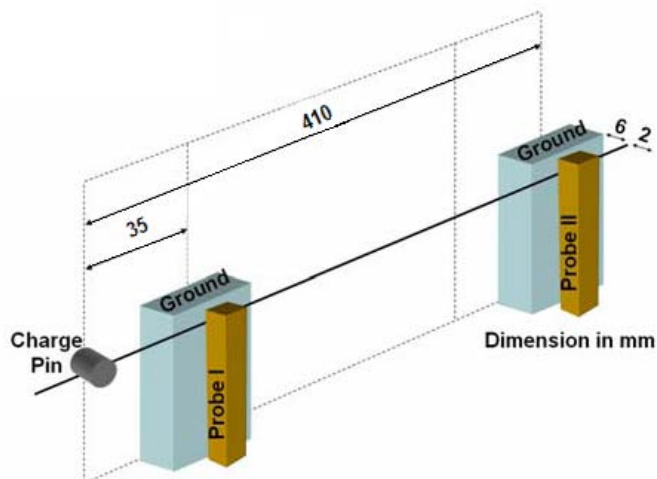


Figure 3.7. The charge pin and probes.

The yarn winding speed was setup as 40 m/min, and the yarn tension was adjusted as 50 gf for nylon 6 and 40 g for PP yarns based on their dissipation factors to support the yarn on the device. Nylon 6 yarns had denier of 600, so there was about $50/600 = 0.08$ gf/denier tension adjustment for all nylon 6 samples. The PP yarn denier was 128 denier, so there was about $40/128 = 0.3$ gf/denier tension adjustment for all the PP samples.

3.4.2. Differential Scanning Calorimetry (DSC)

A Perkin Elmer Diamond DSC Model 7 was used with a cooler under nitrogen atmosphere to investigate some properties about the materials. A 3-5 mg of the sample was sealed and

encapsulated in a non-volatile aluminum pan and all samples was heated from -40 °C to 200 °C at a heating rate of 30 °C /min. The resulting plot gives the heat flow as a function of temperature and ΔH obtained which is the enthalpy of transition, hence, melting temperatures (T_m) of the nanocomposite fibers and the apparent fusion enthalpies can be easily determined from the area of the endothermic peak to show crystallinity in these materials. The degree of crystallinity of PP can be calculated by the formula as shown below:

$$\text{Degree of crystallinity (\%)} = \frac{\Delta H_f \times 100}{\Delta H_f^0}$$

Where ΔH_f is the heat of fusion of PP fibers.

ΔH_f^0 is heat of fusion of 100% crystalline PP which is 209 J/g [29].

3.4.3. Tensile Testing

The fiber denier was determined by the skein method, ASTM D- 1907, after condition at 20 °C and 65% RH for 24 hours. A particular length of the yarn were wound on reels as skeins, and weighed, denier then calculated according to the following formula:

$$\{\text{Denier}\}D = \frac{[W(g) \times 9000]}{L(m)}$$

The fiber modulus and tenacity (gf/denier), and also percentage strain were investigated by a MTS Q-test/5 universal testing machine using TestWorks 4EM V4.11B software with according to ASTM-D2256. The fibers were mounted on a square holder with 5 inch gage length and a cross-head speed of 300 mm/min; in addition, 5 pounds load cell was used. The data was the mean of ten tests of replicates and collected by the digital hardware and software to evaluate.

3.4.4. Antimicrobial Analysis

A modified AATCC 100 test method [33] was adopted and performed in order to determine the antimicrobial activity of PP/nanoparticle composite fibers. *E. coli* was that the strains were grown overnight in LBG (Luria Broth (Difco) with 1% glucose) at 37 °C. One piece of fiber string, which was 20 cm and 0.31 mg, was placed in a 1.5 mL microcentrifuge tube 200µL of saline containing 10⁷ CFU/mL of organism transferred in the tube to completely covering the fiber and was incubated for 24 hours at the temperature appropriate for the inoculated culture. After 24 hours, LBG plate was spread with the supernatant using a spiral plater (Model 4000, Spiral Biotech). After incubation for 24 hours at 37 °C, the bacteria colonies were counted by an automated spiral plate reader (QCount, Spiral Biotech) using the following equation:

$$\text{Reduction Rate (\%)} = \frac{(A-B)}{A} \times 100$$

Where A = the number of bacterial colonies from untreated fabrics

B = the number of bacterial colonies from treated fabrics

One log reduction indicates that the antibacterial material was able to kill about 90% of the bacteria.

3.4.5. Elemental Content Analysis

In order to determine certain amount of the metals in the fiber composite that Varian Inductively Coupled Plasma - Mass Spectrometer (ICP-MS with Model 820-MS) was used.

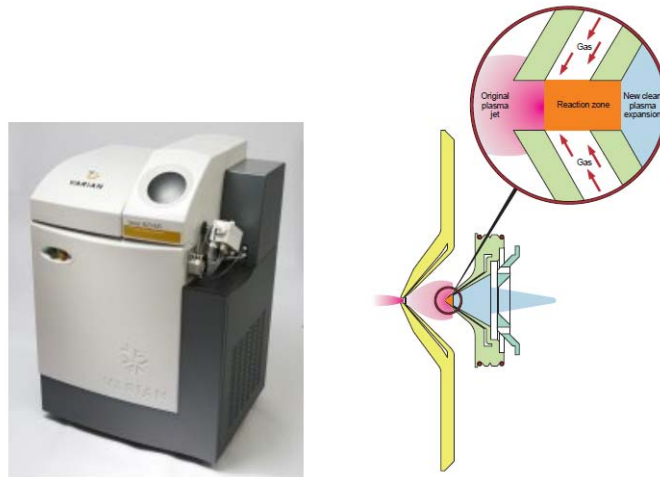


Figure 3.8. Varian 820-MS and schematic diagram of the Varian CRI system [43].

Basic Specifications:

Nebulizer micromist: 0.4 mL/min

Nebulizer Argon gas pressure: 30 psi

Nebulizer Argon gas flow: 1 L/min

Argon system gas flow: 20 L/min

RF power: 1400 watts

Spray chamber: Scotts double-pass

Most elements run with Ni non-CRI (collision reaction interface) skimmer and sampler cones in the place, and internal standard was Indium.

As presented in Figure 3.8, collision reaction interface (CRI) is that hydrogen and helium are used as CRI gases which injected into the plasma to start charge transfer, proton transfer, electron-ion reactions and ion-molecule interactions. Therefore, common polyatomic interferences in ICP-MS analysis removed, and lower detection limits in hot plasma, and also samples with complex matrices can be investigated [43].

Elemental content analysis was performed that the fibers cut in to small pieces with a stainless steel scissors; duplicate samples were weighed to the nearest 0.00001 g on to a piece of weighing paper and then carefully quantitatively transferred to a Teflon microwave digestion bombs. 5 mL of conc. HNO_3 and 4 mL of concentrate HCL (optima quality) and 1 mL of 18 Mega-Ohm DI water were added to each Teflon digestion bomb, respectively; allowed to sit over night with loose covers. The samples were then digested in a CEM Mars

5 Microwave digestion unit using Omni XP1500 Teflon digestion bombs and caps. The rack holds twelve, and the first position has a pressure and temperature monitor.

The following settings were carried out:

1. 5 min ramp to 150 °C and hold for 5.00 min
2. 5 min ramp to 180 °C and hold for 5.00 min
3. 5 min ramp to 200 °C and hold for 5.00 min

Pressure could get as high as 250 psi - pressure not controlled except by maximum limits set for each stage.

1. max pressure 500
2. max pressure 800
3. max pressure 800

1200 W power was used at 100% for all three stages.

The solutions then transferred to 125 mL Teflon sample bottles, and were diluted to 100 g with 18 Mega-Ohm DI water, and analyzed by Varian 820-MS ICP-Mass Spectrometer. After microwave digestion, there was no insoluble matter or precipitates. Two method blanks and one spiked method blank were digested in the same microwave digestion run. A third replicate of each fiber was spiked with 1 mL of 100 ppm Ag standard and carried through the same digestion procedure, Ag recovery was then calculated. On the contrary, the

samples were diluted x20 or x40 depending on concentrations to fall within the calibration range. Also, calibration curves had a linear regression of 0.9999, and Indium was used as an internal standard to correct for small differences in sample matrices.

3.4.6. Scanning Electron Microscopy (SEM)

To analyze the metal particles dispersed in the PP matrix, and the morphology of the composite fibers were observed using a field-emission scanning electron microscope which was JEOL 6400F FESEM. The fibers were placed onto specimen mounts with double sided conductive carbon tape. After placed on the mount, the samples were coated with gold/palladium to reduce charging via a Hummer IV sputter coater using Argon gas for 15 second. uniform coating obtained after several coated times, and then examined in the JEOL 6400F FESEM with 5kV beam energy. All of the images were acquired with the 4pi system and the Revolution Software attached to the JEOL. The resolution of the system is quoted at 70 angstroms at 1kV.

Chapter IV. Results and Discussion

4.1. Antistatic Properties

Polymer properties play major role on type of the static charge depending upon the tribo-electric series in literatures and experiments. For examples, the polyester and polypropylene yarns accumulate negative charges, and nylon 6 builds up positive charges after friction between the stainless steel charge pin. Moreover, based on the experiments, polypropylene had the lowest charge generation, if we compare the charge generation between nylon 6 and polypropylene. Fiber materials which are without finishing processes have not effect static dissipation time and the time for all fiber materials are between 0.03~1.20 seconds. In general polypropylene yarns with finish have more static charges than those without it.

Literature experimental results [34] which were obtained with polypropylene, polyester and nylon yarns lead to the following conclusions:

- The effect of yarn speed influences amount of accumulation of the static charge on the yarn surface, and the decay time, especially it has larger effect at the low temperature and humidity but has smaller effect at the high temperature and humidity to build the static charge. On the contrary, at high speed that static charge, which is on the surface, has more contact with the surrounding air so the dissipation time is shorter.

- Higher temperature and humidity cause to accumulate less charge, however higher yarn tension and speed lead to more charge generation but yarn tension has no effect on decay time.
- An increase in yarn tension leads to greater contact area between the sample yarn and a charge pin resulting in greater friction forces.
- Yarn tension showed a significant influence on amount of static charge when temperature and humidity were lower and yarn speed was higher.
- The degree of amorphous region and moisture regain have positive influence to the charge dissipation in air [35], also the temperature has a little bit effect on the dissipation time, and increase in temperature about 30 °C always cause to decrease the decay time.
- In general, the dissipation time has reverse relationship with relative humidity and there was the shortest at 30% RH, however no significant difference between 40% RH and 50% RH.

Table 4.1 shows lists of all nylon 6 and polypropylene yarns which were used for this experiment. All the yarns have no twist. Furthermore, this Table indicates the surface treatment of each yarn by finish compound and % finish content.

The polymer properties are [36]:

Nylon 6:

Degree of crystallinity (Typical molded) 50%

Dissipation factor - ASTM D-150; DAM, 23C, 1 MHz; 33% glass fiber is 0.022

Moisture absorption (23 °C Neat resin) 50% RH equilibrium 2.7, Saturation 9.5

Polypropylene:

Degree of crystallinity: 50-70%

Dissipation factor 0.3-1 ($\times 10^{-3}$); 1-3 ($\times 10^{-4}$)

Moisture regain (70°F, 65% RH): 0

Table 4.1. Characteristics of polypropylene and nylon 6 yarns with various additives.

Material	Linear Density (Denier)	No. of Filaments	Finish Compound (%)	Method
Nylon 6 control	616.00	288	-	-
Nylon 6-1	606.00	288	0.1% Ag	Extrusion
Nylon 6-2	592.00	288	0.025% Zn	Extrusion
PP control	128.00	288	-	-
PP-1 (1)	239.10	288	0.3% Ag	Extrusion
PP-2 (2)	247.10	288	0.5% Ag	Extrusion
PP-Lub1	129.10	288	PP/ Polydiallyl Mw 1000 (2 g/L)	Finishing
PP-Lub2	128.30	288	PP/Colloidal Ag (2 g/L)	Finishing
PP-Lub 3	125.70	288	Mixture: 50% Lub1& Lub2 (2 g/L)	Finishing
PP-Lub4	128.80	288	The quaternary ammonium compound* (2 g/L)	Finishing
PP-Lub5	125.20	288	Mix tri quaternary silicone+ colloidal Ag (2 g/L)	Finishing
PP-3 (3)	218.16	288	0.1 % Irgaguard 1000	Finishing

*3-(trimethoxysilyl) propyloctadecyl dimethyl ammonium chloride, 72% active

Table 4.2 and 4.3 show the chemical names and active ingredients for various additives and the electrostatic results of the all yarns, respectively.

Table 4.2. Chemical names and active ingredients for various additives.

Chemical Name	Active Ingredients
Polydiallyl amine Mw 1000	Diallyl amine
Colloidal Ag	Silver*
Quaternary ammonium compound**	3-(trimethoxysilyl)propyloctadecyl dimethyl ammonium chloride**
Irgaguard B 1000	Triclosan

* It contains various concentrations of ionic silver, silver colloids, ionic silver compounds or silver proteins in purified water.

** 3-(trimethoxysilyl) propyloctadecyl dimethyl ammonium chloride, 72% active.

Active Ingredients

- Diallyl amine can be used as an intermediate for ion exchange resins, pharmaceuticals, water soluble polymers, herbicides, rubber chemicals, polymerization initiators and cross-linking agents. Its formula is $C_6H_{11}N$ and structure:

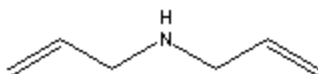


Figure 4.1.1. Structure of diallyl amine.

- 3-(trimethoxysilyl)propyloctadecyl dimethyl ammonium chloride is a trifunctional monomer and it has very good antibacterial action. The chemical structure is [39]:

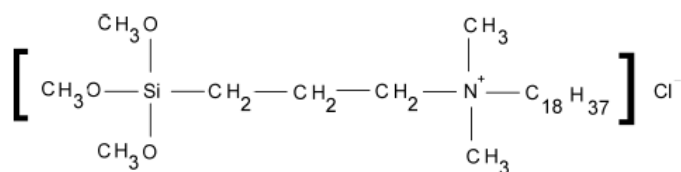


Figure 4.1.2. Structure of 3-(trimethoxysilyl)propyloctadecyldimethyl ammonium chloride.

- Triclosan (IUPAC name: 5-chloro-2-(2,4-dichlorophenoxy)phenol is used as an antibacterial and antifungal agent. It has been used in a broad spectrum of professional and consumer products including hand soaps, surgical scrubs, shower gels, deodorants, healthcare hand washes, toothpastes and mouthwashes. The chemical structure is [40]:

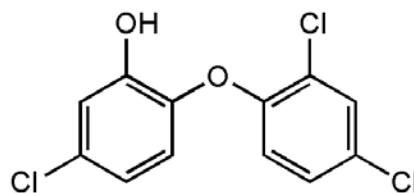


Figure 4.1.3. Structure of triclosan.

- Colloidal silver does not have a scientific meaning but is determined by a generic term that covers silver particles, silver ions and water [41].

In addition, differences between solutions, colloids, and suspensions as shown below:

solutions < 10^{-9} m (less than 1 nm)

colloids = 10^{-9} m to 10^{-6} m (1 nm to 1000 nm)

suspensions > 10^{-6} m (greater than 1000 nm)

Table 4.3. Electrostatic testing results.

Material	Finish Compound (%)	First Probe Potential (volt)	Second Probe Potential (volt)	Calculated Initial Potential (volt)	Characteristic Decay Time (second)
Nylon 6 control	-	9521	3701	10309	0.82
Nylon 6-1	0.1 % Ag	5892	3316	6361	1.23
Nylon 6-2	0.025 % Zn	5121	3085	5423	1.47
PP control	-	-1023	-855	-1041	1.25
PP-1 (1)	0.3 % Ag	-347	-220	-348	0.51
PP-2 (2)	0.5 % Ag	-175	-111	-213	0.16
PP-Lub1	PP/ Polydiallyl amine Mw 1000 (2 g/L)	-1357	-660	-1473	0.73
PP-Lub2	PP/Colloidal Ag (2 g/L)	-550	-179	-618	0.5
PP-Lub3	Mixture 50% Lub1& Lub2 (2 g/L)	-2912	-1514	-3110	1.1
PP-Lub4	Quaternary ammonium compound* (2 g/L)	-3162	-1600	-3371	0.9
PP-Lub5	Mixed quaternary ammonium compound*+ colloidal Ag (2g/L)	-3001	-1561	-3190	0.86
PP-3 (3)	0.1% Irgaguard B 1000	95	17	111	0.33

* 3-(trimethoxysilyl) propyloctadecyl dimethyl ammonium chloride, 72% active

As shown in Table 4.3, all results confirmed nylon 6 samples have accumulated more static charge than polypropylene.

Electrical conductivity of silver is approximately 3.8 times higher than zinc. For both samples of nylon 6 with additives that silver causes to increase the dissipation time as well as zinc but nylon 6-1 (0.1 %Ag) had lower dissipation time than nylon 6-2 (0.025 %Zn)'s dissipation time even though silver content of the polymer is 4 times higher than zinc content. In addition, it can be seen that the effect of silver and zinc nanoparticles were main reason to build up less charge than the control samples (Figure 4.2), on the contrary, the nanoparticles show higher decay time (Figure 4.3).

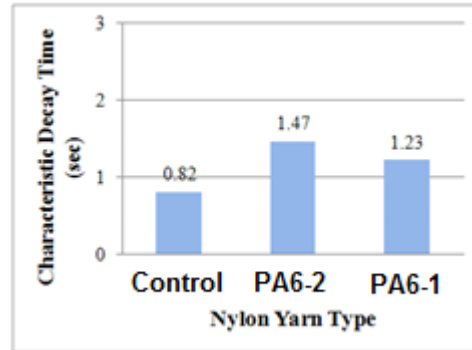
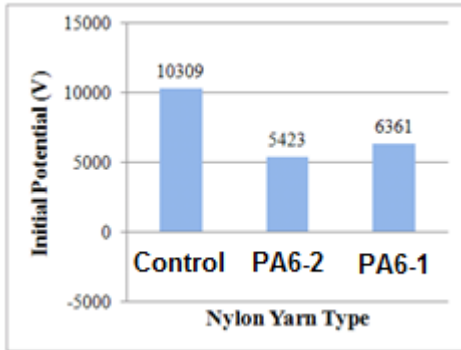


Figure 4.2. PA6 yarns initial potential. **Figure 4.3.** PA6 yarns characteristic decay time.

Figure 4.4, 4.5 and 4.6 give potential detected for different nylon 6 yarns.

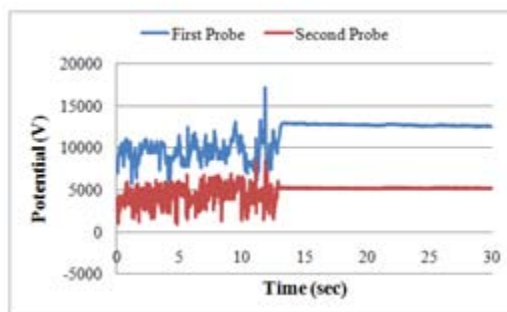


Figure 4.4. Potential detected on PA6 control yarn.

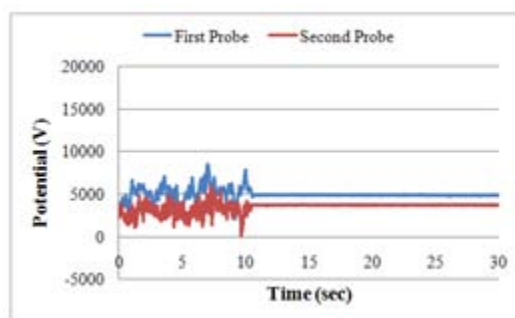


Figure 4.5. Potential detected on PA6-2 yarn.

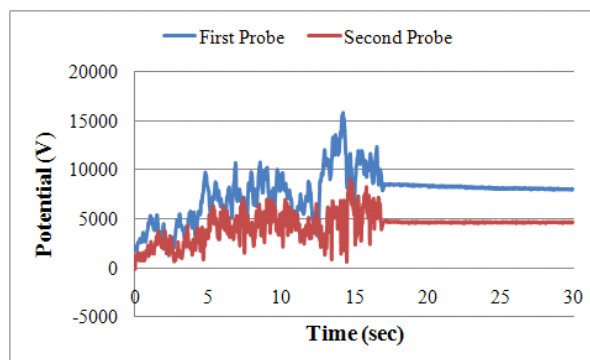


Figure 4.6. Potential detected on PA6-1 yarn.

Compared to nylon 6 control and PP control samples, which were without finish compound, the nylon 6 yarns had lower decay time than the PP sample (Figure 4.8). This result demonstrates perhaps that a degree of amorphous region and moisture regain has a greater effect on dissipation factor because the degree of crystallinity of nylon 6 (Typical molded): 50% and moisture absorption of PA6 at 23 °C neat resin at 50% RH equilibrium 2.7, and saturation 9.5. And degree of crystallinity of PP is 50-70% and moisture regain is zero [36], therefore the higher degree of amorphous region and higher moisture regain have positive effect on the charge dissipation in air [35] when we compared PA6 and PP.

Static charge generation of all PP yarns is shown in Figure 4.7. A higher content of silver nanoparticles can decrease characteristic decay time and prevent static charge accumulation on polypropylene yarns; moreover, we observed similar results for colloidal silver. On the other hand, it was shown that all chemicals and additives which have already

been used for the tests play a significant role on the characteristic decay time and the charge generation of PP yarns and shorter decay time was observed (Figure 4.8).

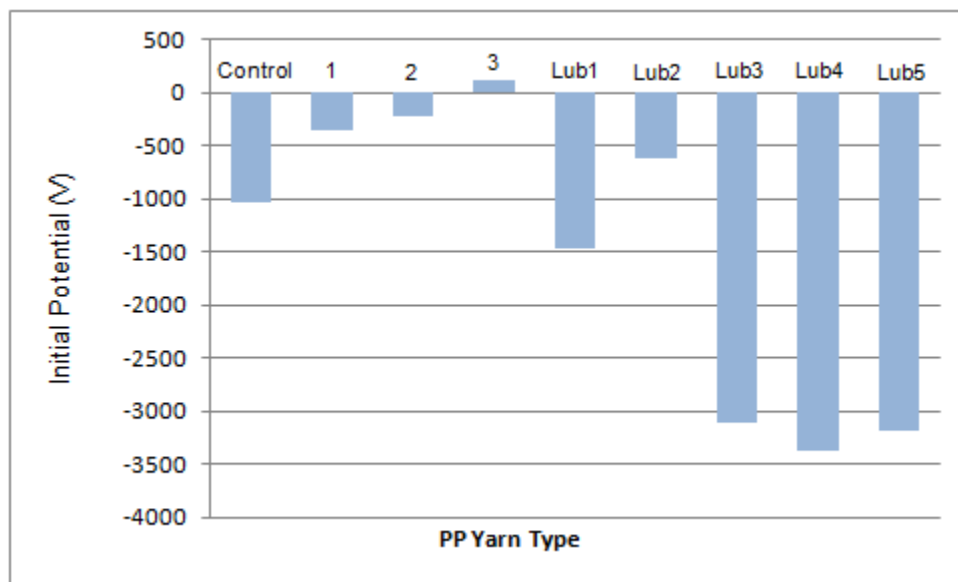


Figure 4.7. PP yarns initial potential.

Based on the hypothesis of correlation of chemical structure and electrical conductivity is that conducting polymers, which is also called conjugated polymers, have a backbone of contiguous sp^2 hybridized carbon centers. It allows carbon atoms to form double bonds [37]. In our study, we observed that the decay time was shorter when we carried out finish compounds which have double bonds to the polymer surface, and this meant static charge decayed faster. This result indicates that after silver, we can obtain the second group of best

decay time treated the polymer surface with polydiallyl amine. Interestingly; the surface of PP-3 accumulated lowest positive charge and characteristic decay time. Triclosan has rings with double bonds, we can assume that during the friction between stainless steel and the yarn oxidation occurred because of double bond, therefore, a very low amount of positive charge might be generated and dissipated in the shortest time [38].

Mixture of polydiallyl amine and colloidal silver showed higher charge generation and characteristic dissipation time. This result indicates that the chemicals blocked their activities. On the other hand, it is known that quaternary ammonium compound can increase electrical conductivity. In this study, the compound gave better dissipation time and built up more static charge than the control samples. However, combination of the compound and colloidal silver showed similar results as the compound had (Figures about potential detected on PP yarns are given in Appendix A).

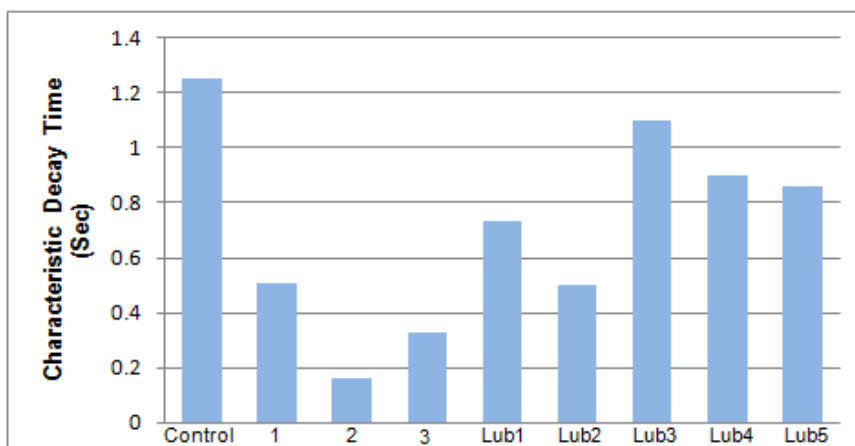


Figure 4.8. PP yarns characteristic decay time.

4.2. Differential Scanning Calorimetry (DSC)

The heat capacity is one of the most important properties for any material and its knowledge is necessary for many applications. Melting point, glass transition temperature, the percent of crystallinity are related to heat capacity.

In order to compare the control sample with the composite fibers, nano zinc and silver and also silver zeolite were used to estimate and compare new properties. DSC thermogram of all the filaments are shown in Figure 4.9, 4.10, and 4.11, also Table 4.4 gives a summary of the melting points, the percent of crystallinity of samples, and ΔH . There was a negligible relationship between the melting point of PP filaments with an increase of the nano-silver content from 0.05% to 0.1%; however, the melting point decreased when the silver content increased from 0.5 to 1%. PP/0.5% Ag yarn had the highest degree of crystallinity. Yeo et al. [25] proposed that the silver can sometimes function as a nucleating agent and accelerate crystallization of PP; hence, the crystallinity is not always decreased. On the contrary, it can be seen that the crystallinity of PP/1% Ag yarns is close to PP control.

On the other hand, the melting points (T_m) of fibers with zinc nanoparticles were lower than that of the pure PP filaments. The highest zinc content, PP/1% Zn, leads to a more dominant decrease of the melting point and degree of crystallinity. Most of the time that this phenomenon indicates the degree of crystallinity of PP composite fibers decreases with increasing the metal content because the metals did not act as nucleating agents in the polymer matrix but as impurities and reduced the rate of the polymer crystallization rate [2]. Interestingly; silver zeolite, exerted little acceleration on the crystallization of PP composite

fibers when used at the lower concentration of 1% but greater zeolite amount of 2% caused a decrease of fibers degree of crystallinity.

Table 4.4. Thermal properties of PP fibers.

Sample	Onset Melting Point (°C)	Peak Melting Point (°C)	ΔH^0_f (J/g)	Degree of Crystallinity (%)
PP Control	157.26	169.10	89.52	42.83
PP/0.05% Ag	156.25	168.76	86.06	41.18
PP/0.1% Ag	156.81	168.88	85.56	40.94
PP/0.5% Ag	153.21	166.47	93.47	44.72
PP/1% Ag	152.93	160.65	86.96	41.61
PP/0.05% Zn	157.99	168.54	86.43	41.35
PP/0.1% Zn	158.15	167.66	87.40	41.82
PP/0.5% Zn	153.99	164.46	81.583	39.03
PP/1% Zn	154.65	159.75	80.393	38.47
PP/1% Ag zeolite	158.88	169.43	90.19	43.15
PP/2% Ag zeolite	157.88	170.72	85.49	40.90

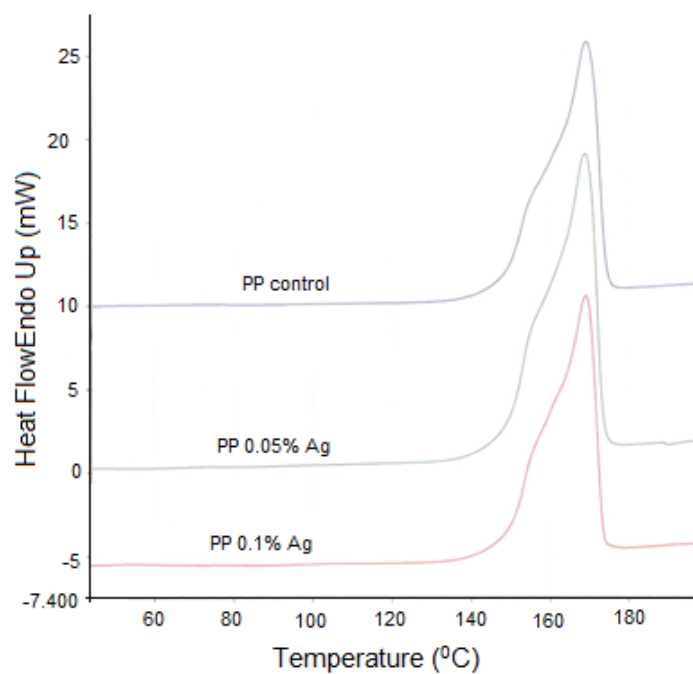


Figure 4.9. DSC curves for PP/Ag composite fibers.

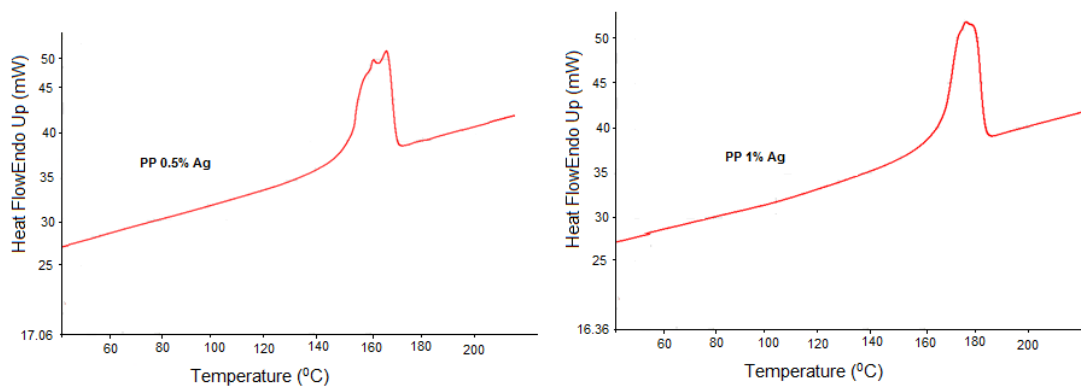


Figure 4.9. Continued.

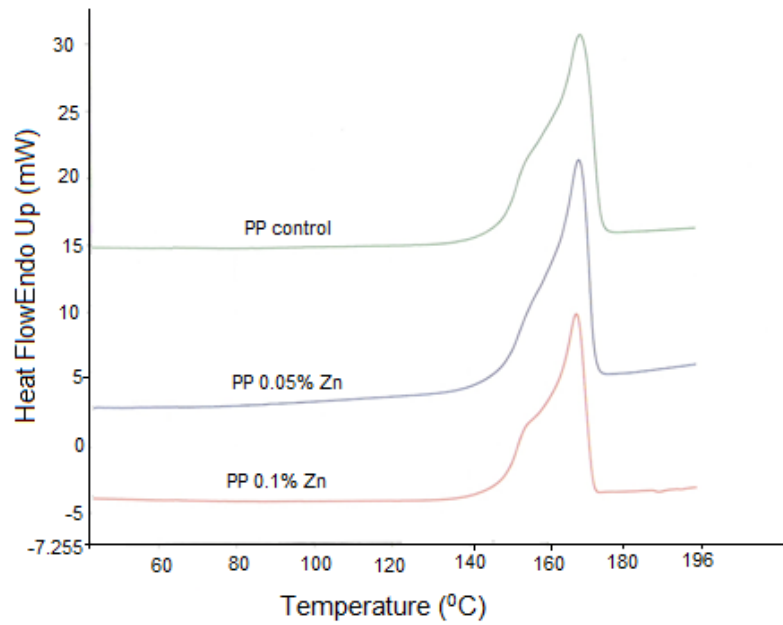


Figure 4.10. DSC curves for PP/Zn composite fibers.

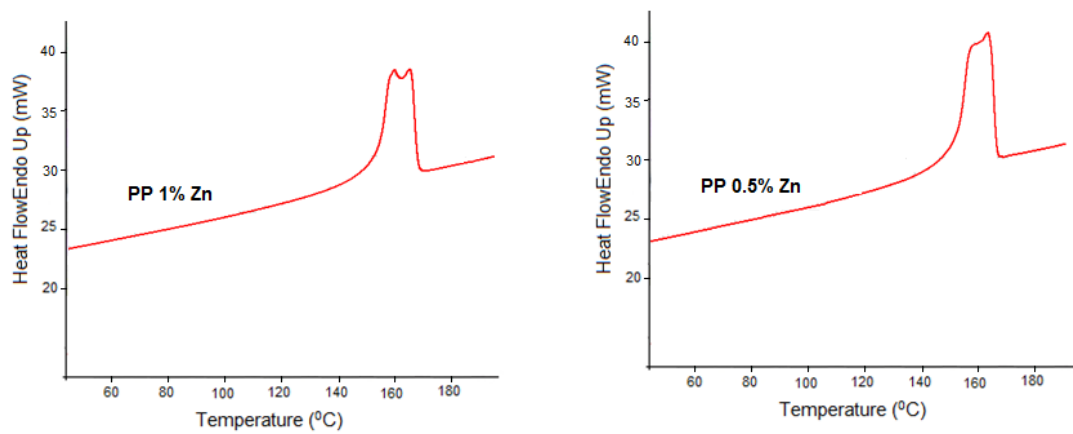


Figure 4.10. Continued.

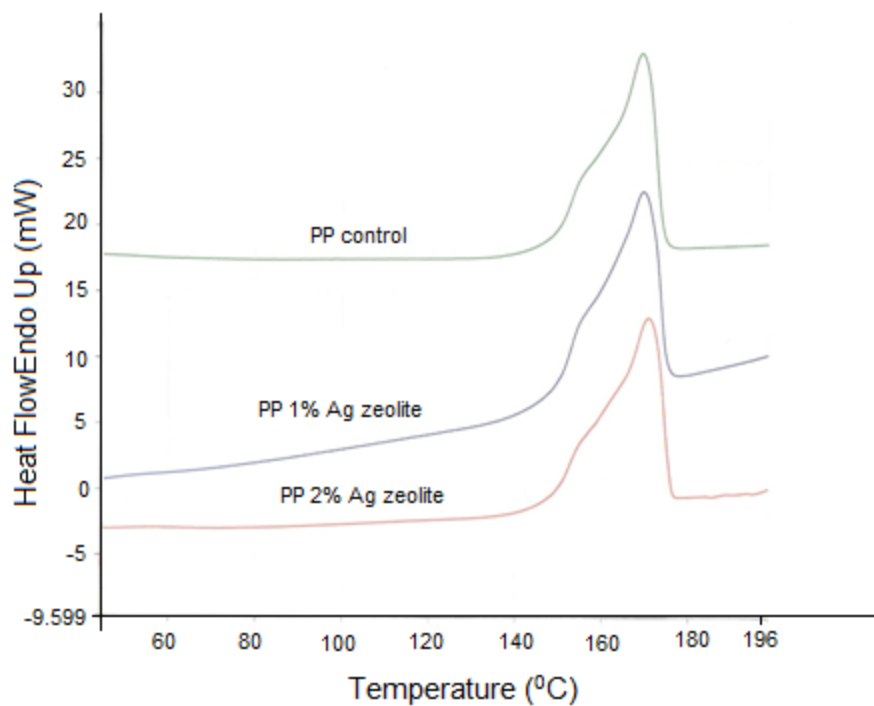


Figure 4.11. DSC curves for PP/Ag zeolite composite fibers.

4.3. Yarn Mechanical Properties

The mechanical properties of the composite fibers are one of the most crucial features that remain difficult to predict because of its complex dependence on many factors. Molecular orientation and crystallinity are some important factors that effect to the tensile properties.

The mechanical properties of the fibers, such as tensile strength, elongation at break, strain, fiber modulus and fiber tenacity, also denier were measured to evaluate the effect of the

filler, take-up speed and draw ratio in the polymer fibers. First set of the composite fibers, which as shown below, were produced at higher take-up speed (810 m/min) with 4.05 draw ratio. PP/0.5% and PP/1% Ag, also PP/0.5% and 1% Zn yarns were produced at a lower take-up speed (620 m/min) and draw ratio (2.07) to see its effect on the physical properties. The highest tenacity of 3.99 ± 0.12 g/denier and the lowest tenacity of 3.62 ± 0.23 g/denier were obtained for those first set of PP fibers including silver nanoparticles. Generally, the composite fibers were not significantly affected as the weight percentage of silver increased to 0.1%; and the initial values were nearly maintained after compounding with the silver particles. PP/1% silver zeolite composite fibers did show any significant change in effect on the tensile properties.

On the other hand, the fiber tenacity decreased as the amount of silver content increased to 0.5%. This effect can be caused by a lower take-up speed of 620 m/min with a lower draw ratio of 2.07 and higher amount of silver content. The fiber breaking elongation increased because during the test the fibers were not as oriented as those spun at higher draw ratios. The mechanical properties of semi-crystalline polymers are mainly affected by their molecular weight, the nature of the crystal phase, such as α and β forms, crystallinity, spherulite size, the microstructure and the degree of molecular orientation in the direction of the fiber axis [42]. The fiber tenacity decreased below than 2 gf/denier when the amount of silver increased to 1% Ag; on the contrary a similar fiber modulus was observed when we compared 0.5 and 1% Ag.

Table 4.5. Physical properties of produced fibers containing silver nanoparticles (first set).

Sample	Denier	Peak Load (gforce)	Strain (%)	Fiber Modulus (gf/denier)	Fiber Tenacity (gf/denier)	Std. Dev. for Fiber Tenacity (gf/denier)
PP control	409.6	1494.89	68.32	34.63	3.65	0.26
PP/0.025% Ag	407.4	1549.68	70.43	30.99	3.80	0.18
PP/0.05% Ag	404.2	1463.92	64.04	31.34	3.62	0.23
PP/0.1% Ag	409.0	1509.18	67.05	32.35	3.69	0.37
PP/1% Ag zeolite	289.30	1155.50	65.12	31.39	3.99	0.12

Table 4.6. Physical properties of produced fibers containing silver nanoparticles (second set).

Sample	Denier	Peak Load (gforce)	Strain (%)	Fiber Modulus (gf/denier)	Fiber Tenacity (gf/denier)	Std. Dev. for Fiber Tenacity (gf/denier)
PP control	321.1	1147.54	199.63	28.74	3.57	0.33
PP/0.5% Ag	247.0	549.16	142.32	25.52	2.22	0.06
PP/1% Ag	409.6	689.14	149.13	25.49	1.68	0.06

On the contrary, the first set of PP composite fibers including a lower amount of zinc filler content did not have any unfavorable changes on their mechanical properties with 810 m/min take-up speed and 4.05 draw ratio. However, as expected, the second set of PP composite fibers, which were produced at 620 m/min take-up speed and 2.07 draw ratio and showed lower fiber modulus and fiber tenacity.

The fiber tenacity and peak load of PP/0.5 and 1% Zn composite fibers were higher than those of containing 0.5 and 1% Ag.

Table 4.7. Physical properties of polypropylene fibers containing zinc nanoparticles (first set).

Sample	Denier	Peak Load (gforce)	Strain (%)	Fiber Modulus (gf/denier)	Fiber Tenacity (gf/denier)	Std. Dev. for Fiber Tenacity (gf/denier)
PP control	409.6	1494.89	68.32	34.63	3.65	0.26
PP/0.025% Zn	397.5	1522.33	83.13	42.16	3.83	0.26
PP/0.05% Zn	398.7	1570.63	90.79	43.53	3.94	0.10
PP/0.1% Zn	402.2	1613.94	84.78	48.11	4.01	0.28

Table 4.8. Physical properties of polypropylene fibers containing zinc nanoparticles (second set).

Sample	Denier	Peak Load (gforce)	Strain (%)	Fiber Modulus (gf/denier)	Fiber Tenacity (gf/denier)	Std. Dev. for Fiber Tenacity (gf/denier)
PP control	321.1	1147.54	199.63	28.74	3.57	0.33
PP/0.5% Zn	409.6	1133.53	196.33	19.75	2.77	0.11
PP/1% Zn	409.6	1139.91	209.17	19.03	2.78	0.06

4.4. Real Filler Content and Antibacterial Efficacy

A proper metal content is essential for the antimicrobial efficacy of the composite fibers. During extrusion processes a part of the filler can get lost, hence, the real metal content is usually smaller than the calculated metal content. The metal particles should be quite well distributed in the polymer matrix to prevent aggregation and agglomeration. In general, much lower metal content was observed while high metal content before extrusion (the calculated metal content) carried out because a higher amount of nano-metals disappeared during the extrusion processes and the filtration stage. In order to prevent and make minimize the amount of losing part of the metals that some process methodologies were developed and evaluated via the LME lab mixing extruder, and the best method was then performed the spinning experiments. In Table 4.9, the effect of heat pretreatment and/or

nonionic dispersing agent incorporated metal nano-silver particles into polypropylene was determined. These pretreatment methods described in detail in the section of 'The Spinning Process'. All samples which containing the isotactic polypropylene chips with nano-metal silver in an amount of 0.5% was mixed as in the previous section, and then the following treatment was performed as explained by Table 4.9; finally they proceeded via LME lab mixing extruder. On the other hand, using a powerful mixer for the mixing of solids and solids with liquids before the extrusion process that can gives a better dispersion provides much higher levels of certain metals in the last product.

It indicates that all pretreatments had a good effect on the real metal content and 3A is the best method to increase the real metal content which was about 37%. Therefore, method 3A was applied in order to get higher certain metal content.

Table 4.9. The effect of the pretreatments on the real metal content.

Sample No	Pretreatment	First Analysis (%)	Second Analysis (%)	Third Analysis (%)	Std. Dev.	Mean (%)
1A	Only 0.5% Ag	0.26649	0.25568	0.27553	0.00994	0.26649
2A	0.5% Ag with 2g/kg the dispersing agent	0.24800	0.33567	0.37337	0.06432	0.31901
3A	0.5% Ag with 2g/kg the dispersing agent and then heated at 100 °C for 1 hour	0.35082	0.33941	0.40302	0.03392	0.36442
4A	0.5% Ag and heated at 100 °C for 1 hour	0.28968	0.27872	0.29376	0.00778	0.28739

The amount of metal nanoparticles in fibers has a strong relationship with antibacterial effect and the percentage reduction in *E. coli* growth. It generally increases with an increase of a metal content in fibers. Table 4.10 demonstrates the results of antimicrobial evaluation. Polypropylene itself is resistant to bacteria, moulds and fungi because of its chemical

structure and little moisture regain (<0.1%), also a very small amount of a catalyst used during producing of PP. On the other hand, the composite fibers containing a very small real amounts of silver of 0.030% and zinc nanoparticles of 0.034%, exhibited 88.41% and 93.45% *E. coli* reduction, respectively. *E. coli* reduction increased to ~ 90.7% when the real silver content increased above 0.08%, and an excellent *E. coli* reduction was observed after 5 consecutive washings when the zinc real content was 0.096%. Interestingly, PP/0.5% Zn composite showed an increased *E. coli* reduction (99.99%) after 5 times rinses, and also an excellent antibacterial efficiency was observed after 5 consecutive washings for PP/1 % Zn. It might be speculated that most of the zinc nanoparticles deposited inside the fiber because 'leaching' antimicrobial agents that were dispersed in the fibers and showed controlled release mechanism after the washing processes. In addition, an excellent *E. coli* reduction was observed with 0.721% silver and 0.588% zinc filler content.

Table 4.10. Microbiological analysis of composite fibers.

Sample	Real Metal Content (%)	<i>E. coli</i> Colonies Reduction (%)
PP control	-	<20
PP/0.1% Ag	0.030	88.41
PP/0.5% Ag	0.081	90.68
PP/1% Ag	0.721	99.99
PP/0.1% Zn	0.034	93.45
PP/0.5% Zn	0.096	99.99*
PP/1% Zn	0.588	99.99

* *E. coli* reduction observed after 5 water rinses

4.5. Surface Observation of the Composites

The surface of the composite fibers was observed by the SEM which reveals that, in fact, some of the silver nanoparticles had agglomerated into clusters because of attractive interaction forces. Most of the silver nanoparticles have diameters between 50 and 100 nm, in addition, some larger particles were observed.

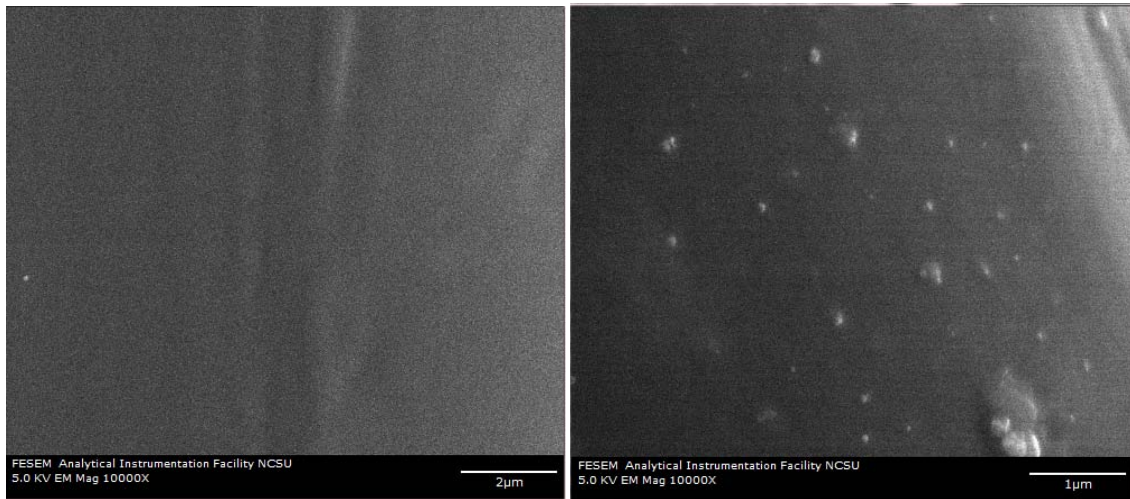


Figure 4.12. SEM image of PP control (left), PP + 0.721% Ag silver nanoparticles on the surface (right).

On the other hand, the zinc nanoparticles were agglomerated and their diameter range from approximately 150 nm to 350 nm, and also irregularly shaped.

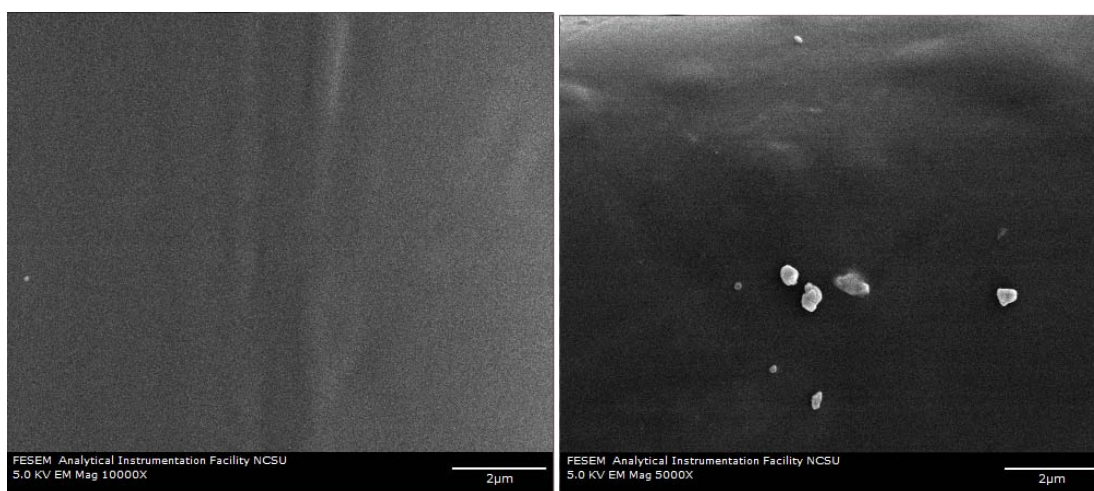


Figure 4.13. SEM image of PP control (left), PP + 0.588% zinc nanoparticles on the surface (right).

Chapter V. Conclusions

Silver and zinc nanoparticles were successfully introduced to PP via melt spinning processes to produce antimicrobial composite fibers.

For the first time zinc nanoparticles were incorporated to PP via melt spinning processes to produce antimicrobial composite fibers. Furthermore we improved a method to increase real metal content during the extrusion processes. Zn nanoparticles are relatively cheap and showed a very good *E. coli* reduction. An excellent antimicrobial efficacy was also documented for silver particles in PP fibers. Zinc maintained and showed an increase in *E. coli* reduction after 5 consecutive washings. There is limited information about mechanism and usage of zinc based antibacterial materials. This is a promising area for antibacterial research with a further investigation of antimicrobial mechanism of zinc and effects of human body systems.

A simple blending of silver or zinc nanoparticles with polypropylene chips is not a very efficient method to introduce these metals into PP fibers. We recommended using a twin screw extruder and producing a concentrate of these particles prior to compounding with polypropylene. Such approach should assure a good dispersion and high content of these additives in polypropylene fibers and improve fiber properties.

Our detailed studies on PP/Zn and PP/Ag fibers indicated that the antibacterial efficiency, antistatic and mechanical properties, crystallization and melting temperature have a strong relationship with the real filler content.

The findings summarized below represent our contributions and main conclusions:

- Antibacterial efficiency increased with increasing real filler content, for example real silver content has to be more than 0.030% and zinc filler content has to be more than 0.034% to see starting significant *E. coli* reduction. An excellent *E. coli* reduction was observed with 0.588% zinc and 0.721% silver filler content.
- 0.025% silver and 0.1% Ag nanoparticles caused to accumulate less charge than the control samples; however, they showed longer decay time for nylon 6.
- On the contrary, 0.3% and 0.5% Ag nanoparticles built up less charge and shorter characteristic decay time for PP when we compared with PP control. It was demonstrated that all chemicals which have already been used for the tests and their chemical structure play a very important role on the characteristic decay time and the charge generation of PP yarns and they showed shorter decay time than PP control sample.
- There is a complex relationship between the polymer degree of crystallinity, melting point and nanoparticles content because the additives can possibly act as nucleating agents in the polymer matrix or act as inclusions at a higher metal content.
- The zinc nanoparticles agglomerated more than silver nanoparticles.

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Appendix

Appendix A: Potential determination of different PP composite yarns via the linear tester.

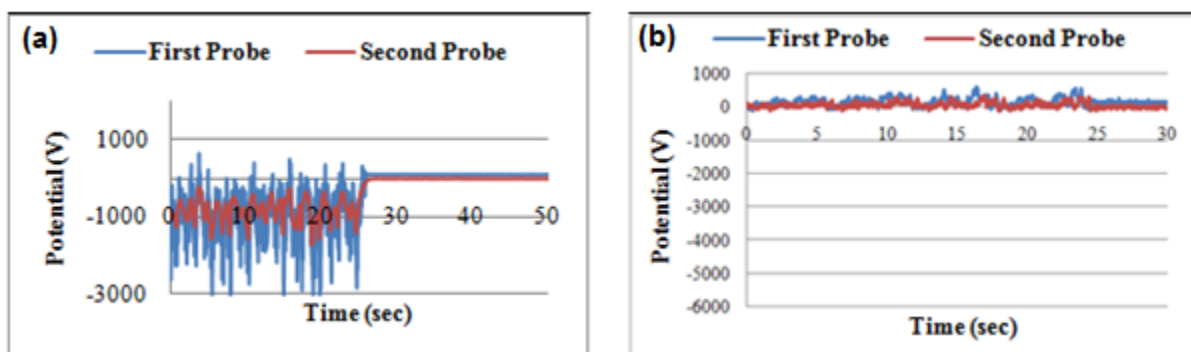


Figure A.1. Potential detected on PP control yarn (a), and on PP-3 yarn (b).

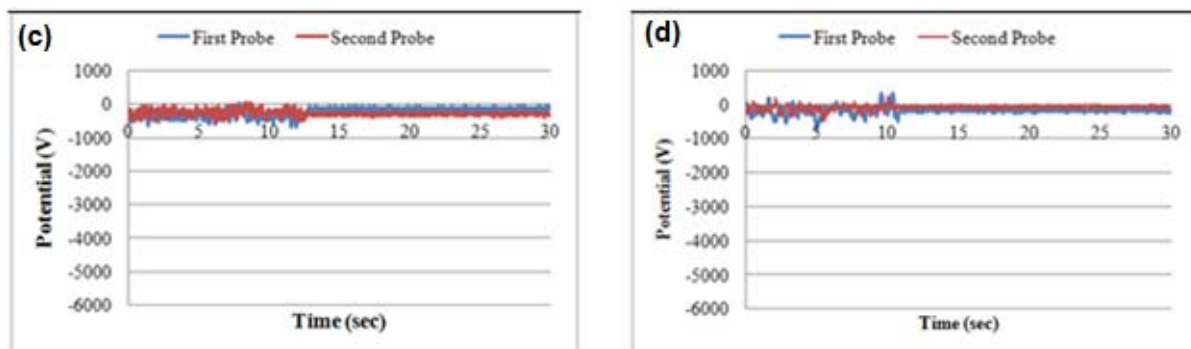


Figure A.2. Potential detected on PP-1 yarn (c), and on PP-2 yarn (d).

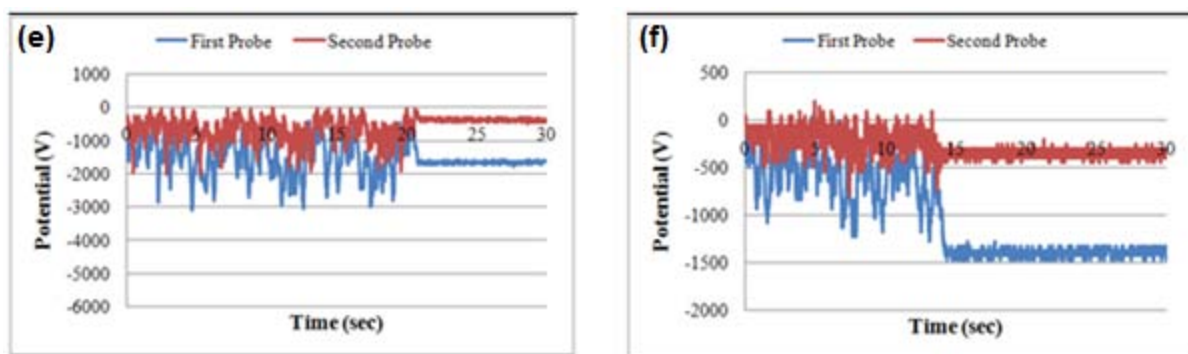


Figure A.3. Potential detected on PP-Lub1 yarn (e), and on PP-Lub2 yarn (f).

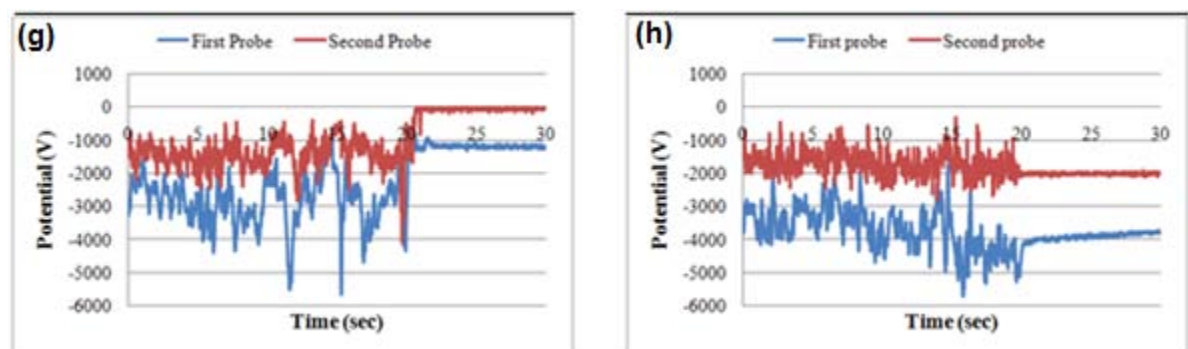


Figure A.4. Potential detected on PP-Lub3 yarn (g), and on PP-Lub4 yarn (h).

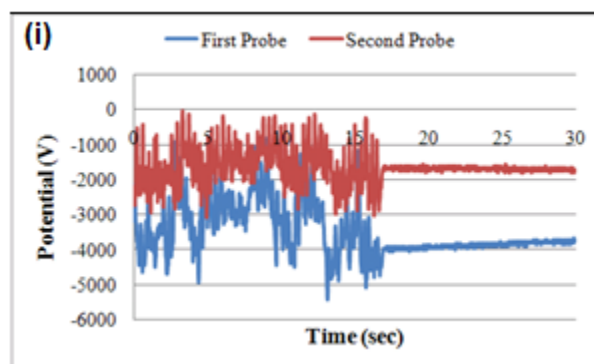


Figure A.5. Potential detected on PP-Lub5 yarn (i).

Section 2.

Characterization and Polymerization of 3-(trimethoxysilyl)-propyldimethyloctadecyl Ammonium Chloride on PET Surfaces

Chapter I. Introduction

A quaternary ammonium compound is one of the most important antimicrobial compounds which consists of a positively charged nitrogen atom covalently bonded to four carbon atoms and a halogen counter ion and can be represented as follows [12, 13]:

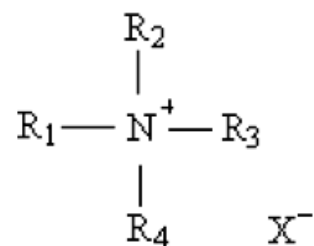


Figure 1.1. Chemical structure of the quaternary ammonium compound [13].

R₁, R₂, R₃, R₄ are alkyl groups which can be similar or different, substituted or unsubstituted, saturated or unsaturated, branched or unbranched, cyclic or acyclic, might have ether, ester or amide linkages, and they can be aromatic or substituted aromatic groups [13].

In addition, natural quaternary ammonium salts such as choline or N,N,N-trimethylethanolammonium salts are water soluble, essential nutrients occurring in living cells.

In order to show antimicrobial activities an alkyl chain usually has eight to twenty two, even number of carbon atoms with a counter ion [12, 14].

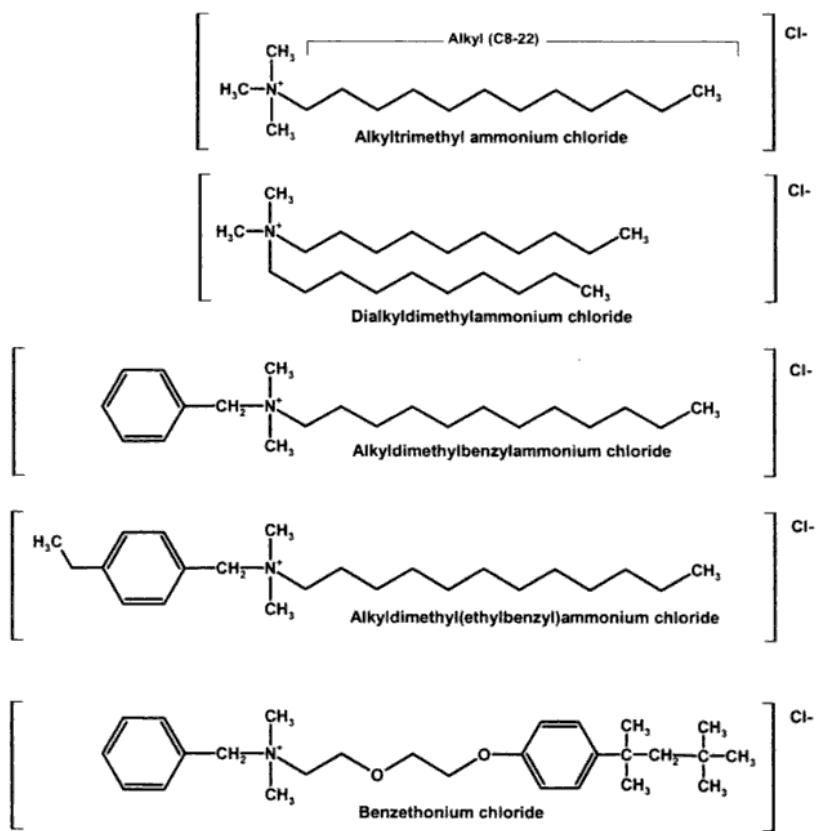


Figure 1.2. Chemical structure of common quaternary ammonium chloride compounds with antimicrobial activity [12].

On the other hand, an organosilicone quaternary ammonium compound is used as an antimicrobial agent (Figure 1.3) against a broad range of microorganism that is chemically bonded to a variety of surfaces.

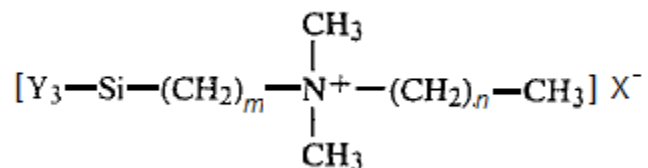


Figure 1.3. An organosilicone quaternary ammonium compound [15].

Where $m+n$ is 16 to 19, m is 1 to 6 and n is 13 to 17 or $m+n$ is 20 to 23, m is 4 to 11 and n is 9 to 17; X is a halogen, and Y is a hydrolysable radical or hydroxyl group [15]. It can be used in the manufacturing process of antimicrobial materials, such as, air filters/filter materials, bed sheets, blankets and bedspreads, men's underwear and outerwear, socks, nonwoven disposable diapers, and so forth.

1.1. Research Purpose and Objectives

The world population and the spread of diseases are increasing gradually. Furthermore, a number of bacteria resistant to antibiotics is rising and can also cause serious health problems. Furthermore, the widespread use of biocides as additives in commercial products, especially textiles, is harmful to the environment not only because of the chemicals used in the treatments but also because the treated textiles are not reusable. To obtain permanent antimicrobial efficiency without releasing them to the environment, biocidal molecules must be attached covalently to the surface. Hence, a promising mechanism which is based on physical interactions can be manufactured with a reusable and durable surface against harmful pathogens.

The positively quaternary ammonium charged groups are able to kill the bacteria upon contact which is a broader coverage of various architectures [10]. The charge density (moles of quaternary ammonium /cm²) is a critical factor to design a surface to get maximum antimicrobial efficiency. Therefore, the minimum surface requirement should be determined.

The aim of this study was to use Direct Red 47 dyestuff to develop a new method to determine the concentration of quaternary ammonium groups on the surface of polyester fabrics treated with 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride and calculate the charge density. The relationship between K/S (color strength) values and the charge density was determined and compared with other studies. Moreover, the antibacterial efficiency, hydrophilicity and the surface potentials were investigated.

Chapter II. Literature Review

3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (AEGIS AEM5772) is an organosilicone quaternary ammonium compound or a silane quaternary ammonium salt which has antibacterial properties [1]. It is known that the quaternary compound has very strong antimicrobial action against Gram positive and Gram-negative bacteria, fungi, algae, and yeast. The quaternary compound is included in a water/methanol mixture in an amount of 72.1% by weight of the solution. The chemical structure of the compound is as follows:

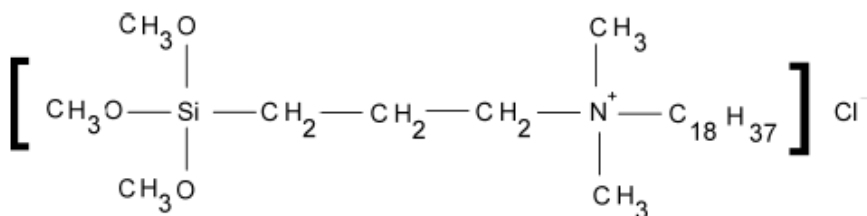


Figure 2.1. Chemical structure of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride [2].

The organofunctional silane has hydrolysable groups on the silicon atom therefore under right conditions it can be hydrolyzed to transform silanols which can bind to each other and the surface. The chemical bonding on the surface is formed by two steps, first; ion exchange

process, the cation of the silane quaternary ammonium compound replaces protons from water on the surface – water generate negative electrical charge at the interface between water and the surface, second; the compound has silicon functionality so it is able to provide polymerization and covalent bonding is occurred between surface and the chemical (Figure 2.2) [2, 3].

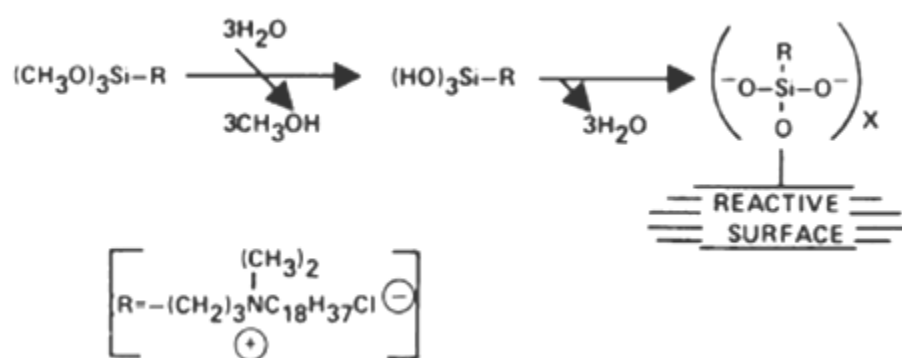


Figure 2.2. Mechanism of hydrolysis and subsequent condensation of the compound with reactive surfaces [3].

The result of the polymerization, the compound oriented away from the surface. Positive charge at the N atom in solution gives rise to detrimental effects on microbes, such as, damage to cell membranes, denaturation of proteins and disruption of the cell structure [4]. Due to the fact that the bacteria cell generally includes functional groups containing sulfur-, nitrogen- or oxygen [5]. That's why this new structure on the surface also cause to disrupt

the cell membrane of microbes by a physical process so that this effect leads to either the death or inactivation of the bacterial cells (see Figure 2.3). On the other hand, because of the ionic interaction between highly positive charge on the surface and the cell membrane which has divalent cations to neutralize the membrane components that cause the cell attached on the surface. Hence, the highly positive charge causes to remove the structural cations from the membrane. Therefore, the membrane loses its integrity, then the cell death occurs [10]. Furthermore, the method described to obtain a nonleaching antibacterial surface which maintains its antimicrobial ability as long as the compound is bound to textiles [4].

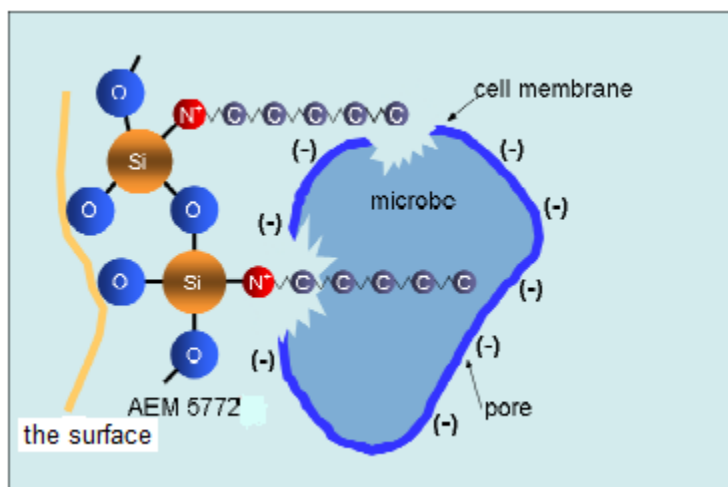


Figure 2.3. The antimicrobial substance AEM 5772 is a quaternary ammonium which disrupts the cell membrane of microbes by a physical process (Henricsson et al., 2007).

Tigler, Kügler and Russell et al. [9, 10, 16] used a very similar technique to determine the density of quaternary ammonium groups on the surface. The treated surface was immersed in a solution of fluorescein disodium with 1% distilled water for between 5 and 10 minutes. The negative dye molecules attach strongly to the quaternary ammonium groups. The unreacted molecules then removed by washing with distilled water. Afterwards, the treated sample placed in 3 mL of 0.1% solution of cetyltrimethylammonium chloride in distilled water to desorb the dye via an orbital shaker. The absorbance of the resultant solution was measured at 501 nm after adding 10% of a 100 mM aqueous phosphate buffer, pH 8.0. The density of the dye bound to the quaternary ammonium groups was calculated taking a value of $77\,000\text{ M}^{-1}\text{ cm}^{-1}$ for the extinction coefficient with assuming a 1:1 fluorescein to accessible the quaternary ammonium group ratio.

Chapter III. Materials and Experimental Methods

3.1. Materials

Five knitted 100% polyester fabrics treated with different concentration of AEM 5772 from AEGIS ENVIROMENTS was provided together with an untreated knitted 100% polyester fabric (Table 3.1).

Table 3.1. Knitted 100% polyester fabrics treatment with AEM 5772.

Description
100% PES Test fabrics
Untreated
0.05% aAEM from AEM 5772
0.15% aAEM from AEM 5772
0.30% aAEM from AEM 5772
0.15% aAEM from AEM 5772 + 4% AMMS-1
0.15% aAEM from 3.6% aAEM

Finish free, clean knitted polyester fabrics without any impurities were placed in the solution of AEM 5772, which diluted by distilled water to obtain different concentration such as 0.05%, 0.15%, etc active AEM from AEM 5772.

The fabrics were then proceeded towards the padder and wet pick up was adjusted approximately 100% and determined via Equation (1).

$$w_{pu} = \frac{w_f - w_o}{w_o} \quad (1)$$

Where:

w_{pu} : wet pick up

w_f : weight of fabric after treatment

w_o : initial fabric weight

After treatment the substrates were dried and polymerization was carried out at 100 °C for 5 minutes, eventually washed and rinsed with de-ionized water repeatedly to remove any unbounded chemical.

3.2. Experimental Methods

3.2.1 Microbiological Analysis

ASTM E2149-01, which is a dynamic shake flask test and a quantitative method, was used for quantitatively determining the antimicrobial activity in test tubes with 1.0 g sample, 50 mL 0.3 mM KH_2PO_4 , 1×10^5 *Escherichia coli*/mL, 0.01% Q2-5211 wetting agent. The samples were shaken at room temperature for one hour. After one hour, the samples were removed and the remaining solution was filtered. Then, another Q2 buffer with a 10^5 concentration of *E. coli* was added to this solution. The solution was shaken for one to test for leaching. In order to rinse the samples and remove any substance that was leaching off, the samples were shaken in sterile centrifuge tubes with Q2 buffer solution. After 20 minutes, the buffer was poured off and fresh buffer was added. This was repeated a total of five times. The samples were then retested in the same manner as they were initially. Finally, the concentration of colony forming units of the bacteria after 1 hour was compared with the value of the colony at beginning time to calculate the percent bacterial reduction.

3.2.2. Dyeing Experiments

C.I. Direct Red 47 (C.I. 14985) was used in this work. The chemical structure of this dyestuff is shown in Figure 3.1.

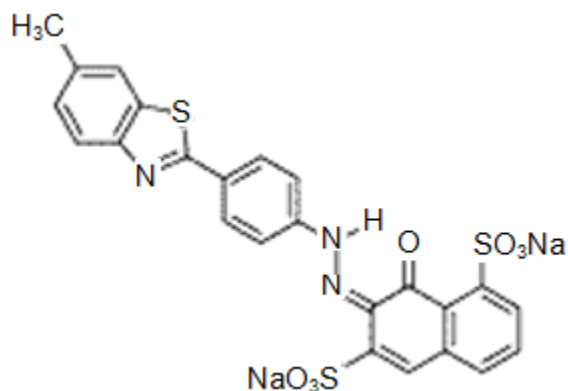


Figure 3.1. Chemical structure of Direct Red 47 dyestuff.
(Society of Dyers and Colourist and AATCC, 2009)

This experiment was conducted using a Werner Mathis AG type CH-8155 laboratory dyeing machine with a liquor ratio of 40:1. The initially dye bath was adjusted to 1 g/L the dyeing. At the beginning, appropriate amounts of de-ionized water were added to each stainless steel beakers, then, particular of dry dyestuff powders were added to each tube and agitated via glass rod for 10 minutes. Finally, the fabric samples were placed into the baths. The temperature was increased from about 25 °C to 100 °C at 1 °C /min, and then the process was completed at this temperature for 10 minutes. The fabrics were then removed immediately from the baths and 10 mL of the dyebath solutions were placed in sealed glass containers for Cary 3E UV-Visible Spectrophotometer to analyze the concentrations. Table 3.2 outlines the experimental design for the dyeing procedure.

Table 3.2. Experimental design for the dyeing procedure.

Sample No	100% PES Test Fabrics	Weight (g)	Liquor (mL)	The Dyestuff (g)
1	Untreated	4.22	168.80	0.17
2	0.05% aAEM from AEM 5772	4.50	180.00	0.18
3	0.15% aAEM from AEM 5772	5.11	204.40	0.21
4	0.30% aAEM from AEM 5772	5.26	210.40	0.21
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	5.05	202.00	0.20
6	0.15% aAEM from 3.6% aAEM	4.45	178.00	0.18

At the end of the dyeing process the fabric samples were washed fresh tap water at approximately 30 °C for 10 minutes three times, successively. Then, the fabric samples were dried at 55 °C for 15 minutes via Yamato model DKN 812 constant temperature forced convection oven.

3.2.3. K/S Data Analysis

The dyed fabrics were left to stand for 24 hours at room temperature, and then analyzed using a X Rite Spectrometer model SP64 instrument equipped with Color iControl® software to obtain K/S data.

3.2.4. Determination of Dye Content on 100% Polyester Fabrics

Dye concentration on the fabrics and absorbance were determined via a Cary 3E UV-Visible Spectrophotometer with preparation of diluted the dye solutions. Furthermore, the standard Beer-Lambert Law calibration curve was created for each dye in order to determine the amount of the dye on the fabrics. Standard calibration curve were developed for each dye in de-ionized water and the following known concentrations: 0.11556 g/L, 0.05578 g/L, 0.03347 g/L, and 0.011156 g/L (see Figure 3.2).

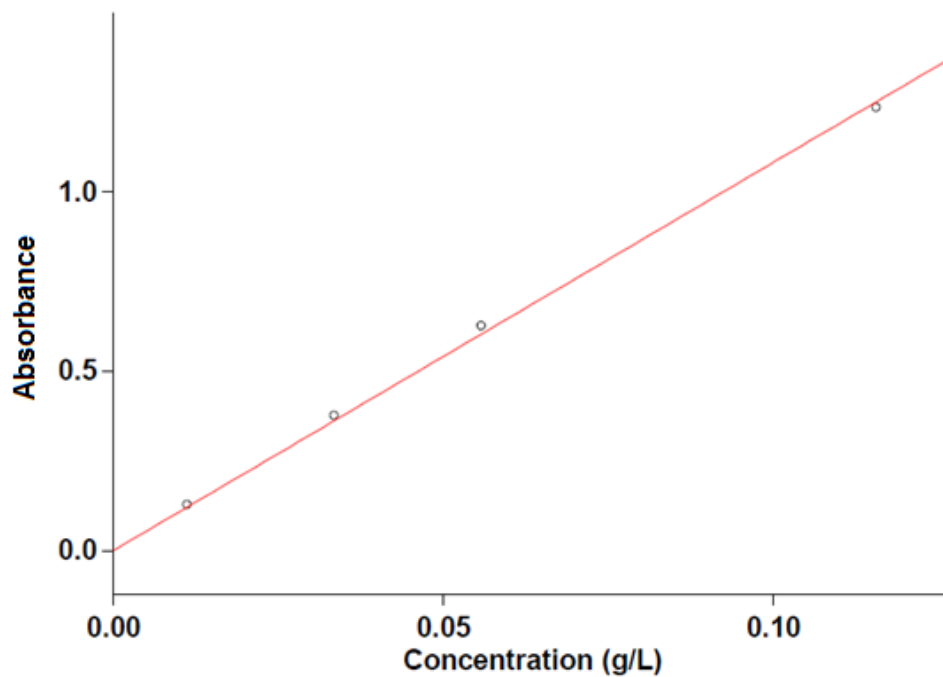


Figure 3.2. Correlation between the dye concentration and absorbance.

The percent dye exhaustion values were calculated by Equation (2).

$$\% \text{ Exhaustion} = \left[\frac{C_1 - C_2}{C_1} \right] \times 100 \% \quad (2)$$

Where C_1 represents the dye concentration before dyeing, C_2 is the dye concentration after dyeing.

3.2.5. Determination of Hydrophilicity

AATCC test method 79-2000 Water Drop was used for the determination of the hydrophilicity.

AATCC test method 79-2000 performed as a drop of water from plastic dropper is about 1 inch above the fabric that is dropped onto fabric and evaluation was the average time taken for the drop to absorb into the fabric is measured in minutes. The test was carried out before washing and after 5x, 10x, 20x, 30x, and 40x washing cycle. The washing was performed in accordance with the wash procedure of AATCC test method 61-2A and AATCC test method 135. AATCC test method 61-2A is accelerated laundering which was carried out at $49^{\circ}\pm 3^{\circ}\text{C}$ with 50 steel balls and 0.15% AATCC standard reference detergent. Furthermore, AATCC test method 135 was performed via regular home washing machine that parameters were 9 minutes cycle, cool temperature, small load, dried 40 minutes by home dryer, low heat and added 46 g AATCC liquid reference detergent. All tests were performed and kept in the conditioned laboratory at 24 hours in the standard atmosphere laboratory condition of 70°F (21°C), 65% RH. On the other hand, The Gravimetric Absorbency Testing System (GATS) was used to measure the demand wettability of 'Sample' 1 –untreated- and 'Sample' 5 which had the best hydrophilic properties in comparison with the other fabrics to compare previous methods. This test determines the ability of the material to take up distilled water, in grams, spontaneously in the direction perpendicular to its plane during 999 seconds. The diameter of the sample were cut a circular shape of 3.5 inch diameter (62 cm^2) and weighed. A water filled reservoir connects with the porous test plate which has 2 mm diameter hole

and the fabrics on (see Figure 3.3). The sensor automatically weighs the amount of water absorbed by the fabric; hence, the maximum absorbent capacity is determined.

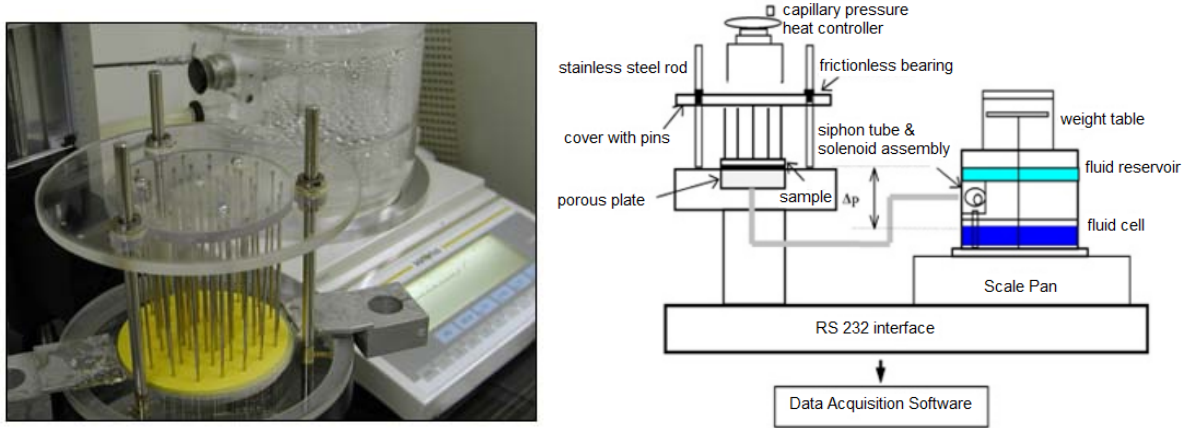


Figure 3.3. GATS instrumentation [11].

3.2.6. The Surface Potential Assessment

Electric field is defined as the electric force (F) per unit charge (q). The relationship between the electric force and the charge was determined from Equation (3). It is displayed with SI units of Newton per coulomb (N C^{-1}) or, equivalently, volts per meter (V m^{-1}).

$$F = qE \quad (3)$$

Where E is called electric field strength and is determined by the magnitude and locations of the other charges.

Charge is always difficult to measure directly so detection and measurement of the electric field from the charged material that relate some factors such as humidity, temperature, etc. Monroe electronics Fieldmeter model 177 (Figure 3.4) and the probe type model 1036E (Figure 3.5) were used in order to determine the surface potential.



Figure 3.4. Monroe electronics fieldmeter.



Figure 3.5. The fieldmeter sensor.

There are some specifications for model 1036E [6]:

- **Analog outputs:** +/- 10kV
- **Sensor range:** +/- 20kV
- **Accuracy:** Better than 1%
- **Sensitivity:** 0.025% of full scale
- **Response speed:** 150 ms from 10% to 90% of full scale (typical)
- **Operating temperature range:** -30 to 100 °C
- **Weight:** 3lbs, 6oz (1.5 kg)
- **Dimensions:** 21/16" x 23/4" x 6" (5.2 x 7.0 x 15.2 cm)

Fieldmeter determines the electrostatic field (voltage per unit distance) by the small hole at the front of a grounded probe. It is known that a uniform electric field is created between the charge and the grounded surface (see Figure 3.6). The probe is placed on the grounded surface so electrical field occurs between these two surfaces depend on distance, d . The distance need to be controlled for accurate results with calibrated distance and the sensor are needed.

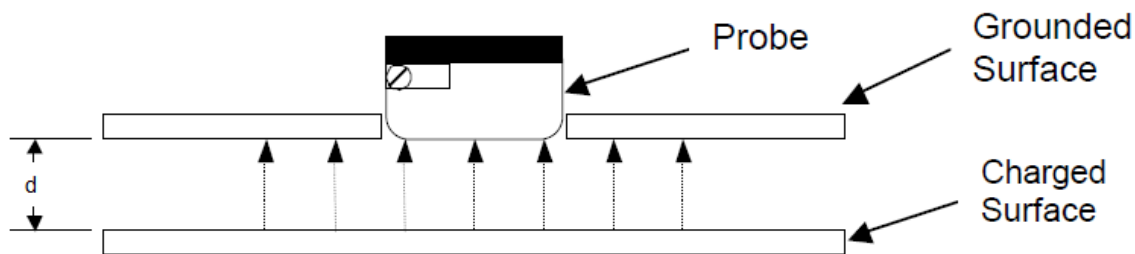


Figure 3.6. Schematic illustration of probe, grounded and charged surface.

Five knitted 100% polyester fabrics treated with different concentration of AEM 5772 from AEGIS ENVIROMENTS with an untreated polyester fabric were used during the test which have same construction and yarn properties. The test was carried out to find out the surface potential for each fabric before and after washing. The washing process performed in a beaker for 30 minutes at room temperature with methanol. Methanol in a baker was replaced by fresh methanol after each ten minutes. Finally, they were dried under vacuum at room temperature.

Chapter IV. Results and Discussion

4.1. Evaluation of the Antibacterial Effects

Table 4.1 demonstrates the results of antibacterial assessment. It can be seen that there were excellent antimicrobial efficiency, although at very low loading of 0.05% aAEM 5772. The samples still had superior antimicrobial ability after 40 times washing cycles via AATCC 135. On the other hand, the antimicrobial performance fluctuated markedly after 20 times washing cycles by AATCC 61-2A.

Table 4.1. Antibacterial evaluation of the samples by ASTM E2149-01 test method.

100 % PES Test fabrics	Initial	5x AATCC 61-2A	5x AATCC 135	10x AATCC 61-2A	10x AATCC 135	20x AATCC 61-2A	20x AATCC 135	30x AATCC 61-2A	30x AATCC 135	40x AATCC 61-2A	40x AATCC 135
Untreated	0%	96%	0%	79%	0%	99.98%	6%	17%	6%	5%	61%
0.05% aAEM from AEM 5700	99.9%	99.99%	99.99%	99.99%	99.96%	99.99%	91%	99.99%	99.0%	99.99%	70%
0.15% aAEM from AEM 5700	99.9%	99.99%	99.99%	99.99%	99.96%	99.99%	90%	99.99%	77%	99.99%	99.6%
0.30% aAEM from AEM 5700	99.9%	99.99%	99.99%	99.99%	99.98%	99.99%	86%	99.99%	97 %	99.99%	98.7%
0.15% aAEM from AEM 5700 + 4% AMMS-1	99.9%	99.99%	99.99%	99.99%	99.99%	99.99%	96%	99.99%	99.98%	99.99%	99.7%
0.15% aAEM from 3.6% aAEM	99.9%	99.99%	99.99%	99.99%	99.99%	99.99%	96%	99.99%	88%	99.99%	99.91%

4.2. Degree of Uptake of the Dyes

To determine amount of the direct dye absorption after dyeing gives an indication of the charge density on the treated surfaces and explain that the structure was able to access to

the dye molecule. In addition, this relationship between the dye molecule and the surface which demonstrates positively charged quaternary nitrogen atoms are accessible with negatively charged the sulfonate groups in the case of direct dyes [2]. The amount of the dye was determined after standard calibration curve was developed for each dye. UV absorbance of Direct Red 47 dye solutions was measured at $\lambda_{\text{max}} = 523 \text{ nm}$ with a Cary 3E UV-Visible Spectrophotometer (Figure 4.1).

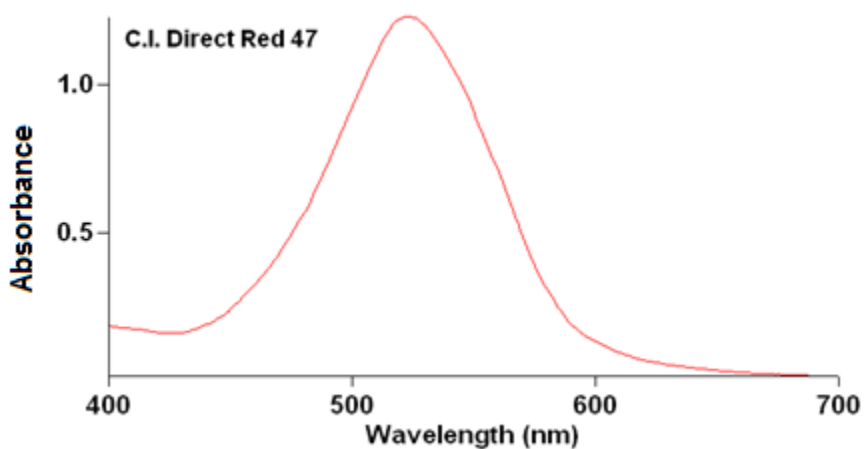


Figure 4.1. C.I. Direct Red 47.

The remaining Direct Red 47 dye solutions were diluted 50 times via de-ionized water because of higher concentration, then analyzed the UV absorbance, and the result is shown in Table 4.2.

Table 4.2. Effect of different concentration of AEM 5772 on dye molecule absorbance for Direct Red 47.

Sample No	100% PES Test Fabrics	Concentration (g/L)	F Readings
1	Untreated	0.017752	0.1919
2	0.05% aAEM from AEM 5772	0.016987	0.1837
3	0.15% aAEM from AEM 5772	0.015226	0.1646
4	0.30% aAEM from AEM 5772	0.013950	0.1508
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	0.009411	0.1017
6	0.15% aAEM from 3.6% aAEM	0.015343	0.1659

4.2.1. Determination of the Dye Concentration on Fabric Surfaces

The direct dye uptake for the each fabric surface and the remaining dye were calculated from Equation (1). Table 4.3 represents the actual amount of dye (g/L) on the fabrics surface and of the remaining solutions by adding diluted factor. The amount of the dye in moles on the fabric surface is also represented in Table 4.3.

Molecular weight of the direct dye can be calculated from Figure 3.1, and the total dye in grams on the surface can also be calculated from Table 4.2.

C.I Direct Dye 47;

Atomic weight (g/mol) [7]:

Carbon Atomic Weight: 12.01115; **Hydrogen Atomic Weight:** 1.0079; **Nitrogen Atomic Weight:** 14.0067; **Sulphur Atomic Weight:** 32.064; **Sodium Atomic Weight:** 22.9898; **Oxygen Atomic Weight:** 15.9994; **Silicone Atomic Weight:** 28.086; **Chlorine Atomic Weight:** 35.453.

$24x \text{ (carbon)} + 15x \text{ (hydrogen)} + 3x \text{ (sulfur)} + 7x \text{ (oxygen)} + 2x \text{ (sodium)} = 557.5535 \text{ g/mol.}$

Table 4.3. Actual amount of dye (g/L) on the fabrics surface and of the remaining solutions by adding diluted factor.

Sample No	100% PES Test Fabrics	Solution (g/L)	Surface (g/L)	Mole
1	Untreated	0.887600	0.112400	0.000034
2	0.05% aAEM from AEM 5772	0.849350	0.150650	0.000049
3	0.15% aAEM from AEM 5772	0.761300	0.238700	0.000088
4	0.30% aAEM from AEM 5772	0.697500	0.302500	0.000114
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	0.470550	0.529450	0.000192
6	0.15% aAEM from 3.6% aAEM	0.767150	0.232850	0.000074

A mole has 6.0221415×10^{23} , which is also known the Avogadro constant, atoms or molecules of the pure material, hence we can determine either the number of atoms or molecules on the fabric surfaces. On the other hand, it is believed that all the dye molecules spread on two faces of the fabric and then the polymerization has occurred. There are two theories about interaction between the sulfonate group, which come from the direct dye, and nitrogen atom, which come from an AEM 5772. First theory is that **each sulfonate group** attached one **nitrogen atom**, and a single dye molecule has two sulfonate groups (1). Second theory is that **only one sulfonate group on a molecule** is able to be attached **one nitrogen atom** on the fabric (2). In addition, we need to subtract the "A Number of Nitrogen Atoms" found on the 'Sample' 1 from all other samples because the dyestuff adsorbed indirectly by the surface (see Table 4.4).

Table 4.4. The total number of nitrogen (1) and (2) on the surface.

Sample No	100% PES Test fabrics	A Number of Nitrogen Atoms (1)	A Number of Nitrogen Atoms (2)
1	Untreated	2.049289×10^{19}	1.024644×10^{19}
2	0.05% aAEM from AEM 5772	$0.879621 \times 10^{19*}$	$0.439811 \times 10^{19*}$
3	0.15% aAEM from AEM 5772	$3.220555 \times 10^{19*}$	$1.610277 \times 10^{19*}$
4	0.30% aAEM from AEM 5772	$4.825123 \times 10^{19*}$	$2.412561 \times 10^{19*}$
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	$9.502274 \times 10^{19*}$	$4.751137 \times 10^{19*}$
6	0.15% aAEM from 3.6% aAEM	$2.427439 \times 10^{19*}$	$1.213719 \times 10^{19*}$

*: based on the assumption the sulfonate group directly bonded to the nitrogen atom.

Moreover, the charge density (units/cm²) was calculated by the surface area. The total number and density (units/cm²) of chlorine for each surface, which is the counter ion for the nitrogen cation, also were demonstrated based on the molecular weight of AEM 5772 from Figure 2.2 and the fabric weight in grams (Table 4.5).

Table 4.5. Nitrogen density for each surface.

Sample No	100% PES Test Fabrics	Nitrogen Density (units/cm²) (1)	Nitrogen Density (units/cm²) (2)	The Surface Area (cm²)
2	0.05% aAEM from AEM 5772	1.418744x10 ¹⁶	0.70937177x10 ¹⁶	620
3	0.15% aAEM from AEM 5772	3.833994x10 ¹⁶	1.91699702x10 ¹⁶	840
4	0.30% aAEM from AEM 5772	6.853868x10 ¹⁶	3.42693395x10 ¹⁶	704
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	16.160330x10 ¹⁶	8.08016497x10 ¹⁶	588
6	0.15% aAEM from 3.6% aAEM	3.371443x10 ¹⁶	1.68572153x10 ¹⁶	720

From Figure 2.1 and 2.2 it is clear that the numbers of counter ions are related with nitrogen in order to maintain electric neutrality therefore calculated total number of chlorine should be same as nitrogen numbers. It is known that a mole has 6.0221415×10^{23} atoms or molecules of the pure material. A method of calculating the total number of Chlorine (TNC) ions per cm^2 is that:

$$\text{TNC} = \left[\left\{ A1\% \times \frac{g}{\text{cm}^2} \right\} / \frac{g}{\text{mol}} \right] \times \text{Avogadro constant}$$

Where A1% indicates the active agent on the fabric surface.

AEM 5772 {3-(Trimethoxysilyl) Propyldimethyloctadecyl Ammonium Chloride 72.1%}.

Molecular weight of active the Si-Quat = [{2x(Oxygen)} + {1x(Silicone)} + {23x(Carbon)} + {49x(Hydrogen)} + {1x(Nitrogen)} + {1x(Chlorine)}] = 435.189 g/mol.

And;

The Polyester fabric: 0.0195 g/cm^2

Calculation the total number of the chlorine per cm^2 onto 'Sample' 2 surfaces:

$$\text{TNC}_2 = \left[\left\{ 0.05\% \times \frac{0.0195}{1} \right\} / \frac{435.189}{1} \right] \times 6.0221415 \times 10^{23} = 1.3492 \times 10^{16}$$

Other chlorine atoms were calculated in the same manner and the results are shown in Table 4.6.

Table 4.6. Relation between nitrogen and chlorine density.

Sample No	100% PES Test Fabrics	Nitrogen Density (units/cm ²) (1)	Nitrogen Density (units/cm ²) (2)	Chlorine Density (units/cm ²)
2	0.05% aAEM from AEM 5772	1.418744x10 ¹⁶	0.70937177x10 ¹⁶	1.349204 x10 ¹⁶
3	0.15% aAEM from AEM 5772	3.833994x10 ¹⁶	1.91699702x10 ¹⁶	4.047612 x10 ¹⁶
4	0.30% aAEM from AEM 5772	6.853868x10 ¹⁶	3.42693395x10 ¹⁶	8.095225 x10 ¹⁶
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	16.160330x10 ¹⁶	8.08016497x10 ¹⁶	4.047612 x10 ^{16a}
6	0.15% aAEM from 3.6% aAEM	3.371443x10 ¹⁶	1.68572153x10 ¹⁶	4.047612 x10 ¹⁶

^a: Effect of a specific durable wetting agent on nitrogen density.

It can be seen that **each sulfonate group** on a molecule is able to be attached one nitrogen atom on the fabric because it is more closer quaternary ammonium units/cm² (1) than (2). Interestingly, during increasing the aAEM that each sulfonate group on a molecule lose its

efficiency to make bond with each nitrogen group, most probably only **one sulfonate group on a molecule** attached **one nitrogen group**.

Russell et al. [9] prepared antimicrobial poly(quaternary ammonium) –PQA- compounds polymer brushes which obtained by surface initiated atom transfer radical polymerization of poly (2-dimethylamino) ethyl methacrylate (DMAEAM). Afterwards, polyDMAEAM was quaternized with alkyl bromides to get the antimicrobial activity on surface. The surface charge density for biocidal surfaces has been accepted greater than $1\text{--}5 \times 10^{15}$ quaternary ammonium units/cm², but the surface charge density if more than 3×10^{15} , the cells are killed within the short time, and it does not depend on the density and chain length of PQA compound. Furthermore, it is believed that if the surface is more than 5×10^{15} charges/cm², it can kill at least monolayer of *E. coli*.

Kügler et al. [10] obtained grafted quaternized poly(vinylpyrrolidone) chains on glass surfaces varied the charge density within organic layer between $\sim 10^{12}$ and $\sim 10^{16}$ charges/cm². ‘Outer-layer charge density’ (OLCD) should be $\sim 10^{14}$ charges/cm² for *E. coli* and *S. epidermidis* in low division conditions of the bacteria, however, for the high division conditions that it needs to be $\sim 10^{13}$ charges/cm² for *S. epidermidis* and $\sim 10^{12}$ charges/cm² for *E. coli*.

4.3. K/S Data

The Kubelka-Munk equation was used in order to determine K/S values which is related to the concentration of the dye molecules on the surface. The equation is [8]:

$$K/S = \frac{(1 - R)^2}{2R}$$

Where R is reflection factor for a sample of infinite thickness, K shows absorption coefficient, S represents scattering coefficient. If spectral reflectance R_λ is considered, which is ideally, there is a linear relationship with colorant concentration. Figure 4.7 and 4.8 provide the K/S values and $\lambda_{\max}$ obtained from equilibrium exhaustion experiments.

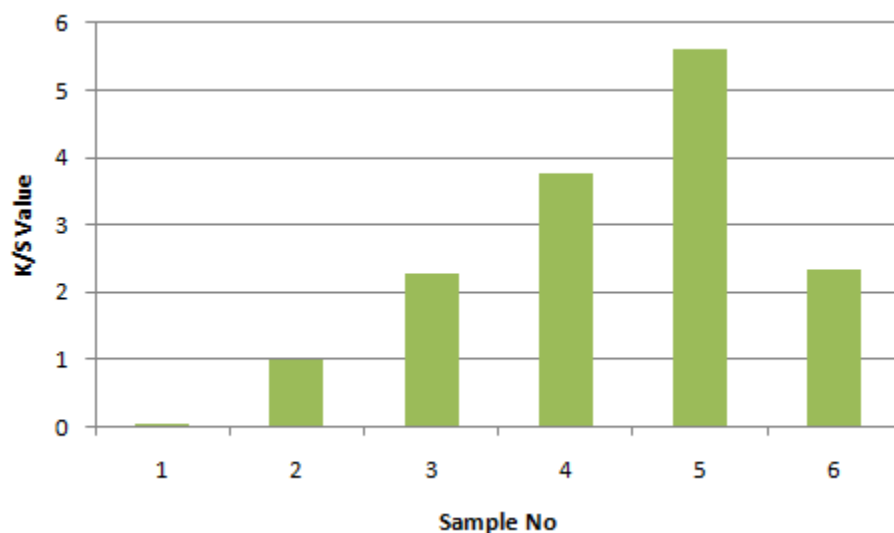


Figure 4.2. K/S values for exhaustion equilibrium of C.I. Direct Red 47.

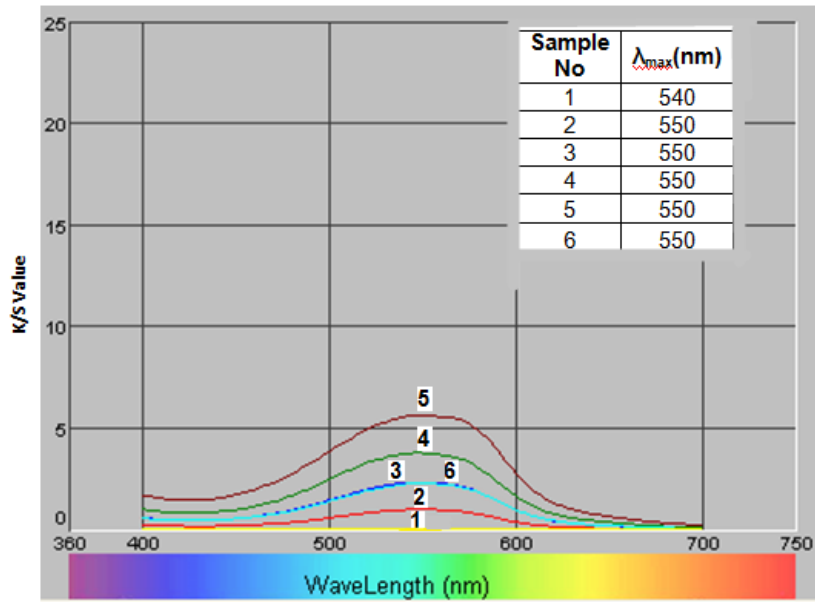


Figure 4.3. λ_{max} vs. K/S values.

The Figure 4.2 confirmed that there is a positive relationship between the nitrogen densities, which come from AEM 5772, and K/S values.

In addition, it is clear from Table 4.7 that a strong correlation between lightness (L) and K/S values.

Table 4.7. Effect of concentration of AEM 5772 on color absorbance for C.I. Direct Red 47.

Sample No	L*	a*	b*
1	91.23	4.72	-1.86
2	66.08	24.93	-18.13
3	54.37	27.74	-19.50
4	46.72	29.06	-18.43
5	40.06	26.82	-16.29
6	54.15	28.60	-19.39

a*: x-axes; b*: y-axes

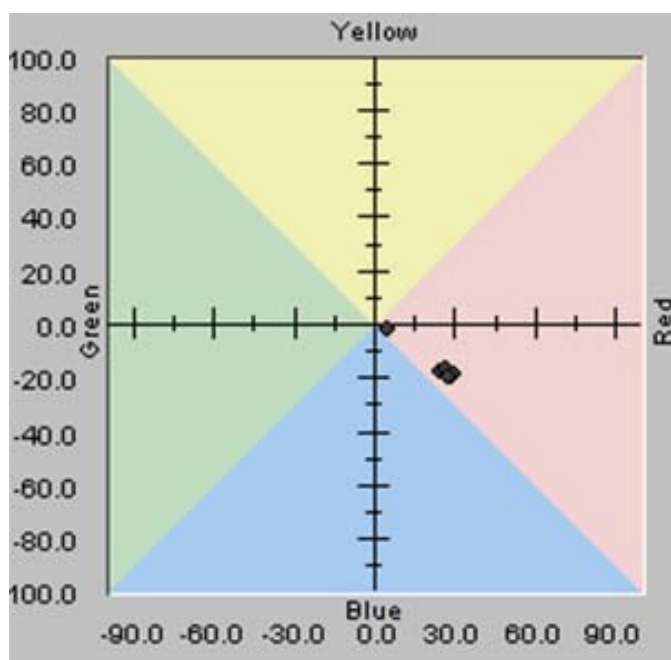


Figure 4.4. Visual representation of the fabric surfaces.

4.4. Hydrophilicity

It is known that polyester is hydrophobic in nature and as result polyester fabrics can be quickly dried. In addition, after polymerization of AEM 5772 at 100 °C (Figure 2.2), the hydrophobic coating film and a crosslinked polymer was formed. Based on to AATCC test method 27-2000 the wetting time for the polymerization of AEM 5772 on the polyester surface was determined (see Table 4.8).

Table 4.8. Water drop test (in minutes).

100 % PES Test fabrics	Initial	5x AATCC 61-2A	5x AATCC 135	10x AATCC 61-2A	10x AATCC 135	20x AATCC 61-2A	20x AATCC 135	30x AATCC 61-2A	30x AATCC 135	40x AATCC 61-2A	40x AATCC 135
Untreated	>10	>10	Instant	3.0	Instant	Instant	Instant	>10	Instant	>10	Instant
0.05% aAEM from AEM 5700	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
0.15% aAEM from AEM 5700	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
0.30% aAEM from AEM 5700	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
0.15% aAEM from AEM 5700 + 4% AMMS-1	Instant	2.75	Instant	1.0	Instant	2.75	Instant	1.0	Instant	2.75	Instant
0.15% aAEM from 3.6% aAEM	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10

Polyester fiber with inherent hydrophobic property and the hydrophobic coating films are the factors that produce a very hydrophobic surface. However, 'Sample' 5 (0.15% aAEM from AEM 5772 + 4% AMMS-1) which is very hydrophilic because it was made out with a durable wetting agent. AATCC test method 61-2A is more effective washing than AATCC test

method 135 so during the washing of the fabrics the wetting agent may get lost. Thus, the hydrophilicity determined by AATCC test method 61-2A normally is lower than AATCC test method 135. On the contrary, the hydrophilicity of 'Sample' 5 was confirmed by the Gravimetric Absorbency Testing System (GATS) compared with the untreated fabric which of amount absorbed against time as shown in the Figure 4.10 and 4.11.

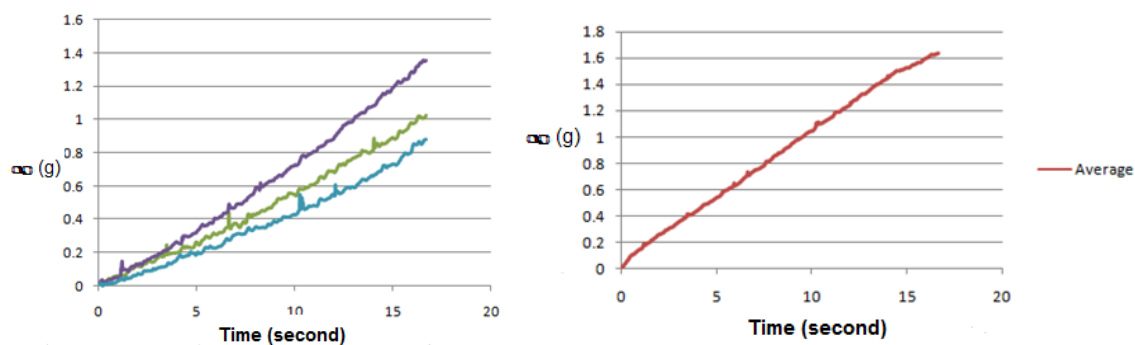


Figure 4.5. GATS result of untreated fabric (the number of experiments was three).

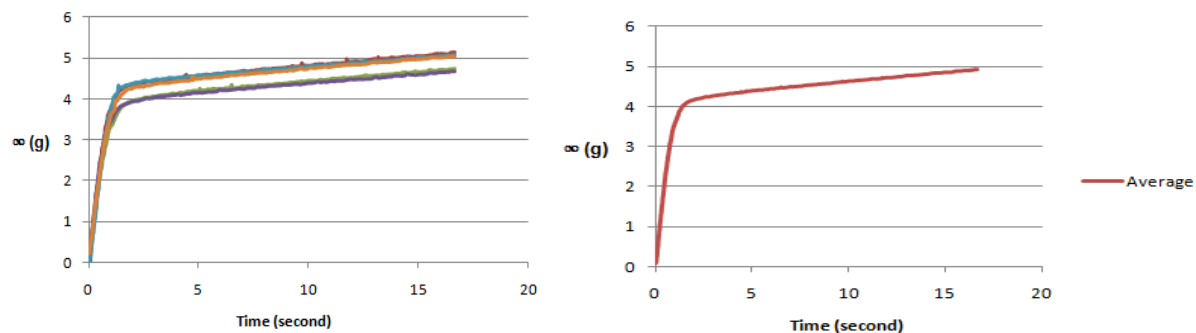


Figure 4.6. GATS result of untreated fabric (the number of experiments was five).

4.5. The Surface Potential

All five different samples with an untreated sample were tested. Tables 4.9 and 4.10 determine the results for six fabrics. It can be easily realized that after and before washing, there is no big difference between the surface potential of samples. However 'Sample' 5 is slightly different than others. It is possible that after the washing with methanol, the durable wetting agent was removed thus the surface potential was changed accordingly.

Table 4.9. Effect of polymerization on the surface potential (*before washing*).

Sample No	100% PES Test Fabrics	Measured Potential (kV)	Correct Potential (kV)	Surface Potential (kV)
1	Untreated	1.57	0.31	1.14
2	0.05% aAEM from AEM 5772	1.92	0.38	1.38
3	0.15% aAEM from AEM 5772	1.81	0.36	1.31
4	0.30% aAEM from AEM 5772	3.95	0.78	2.81
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	-0.13	-0.03	-0.05
6	0.15% aAEM from 3.6% aAEM	2.15	0.42	1.55

Table 4.10. Effect of polymerization on the surface potential (*after washing*).

Sample No	100% PES Test fabrics	Measured Potential (kV)	Correct Potential (kV)	Surface Potential (kV)
1	Untreated	2.43	0.48	1.74
2	0.05% aAEM from AEM 5772	2.55	0.50	1.83
3	0.15% aAEM from AEM 5772	2.23	0.44	1.60
4	0.30% aAEM from AEM 5772	2.23	0.44	1.60
5	0.15% aAEM from AEM 5772 + 4% AMMS-1	4.51	0.89	3.20
6	0.15% aAEM from 3.6% aAEM	2.48	0.49	1.78

Chapter V. Conclusion

The goal of our work was to investigate correlation between calculated quaternary ammonium units/cm² and measured quaternary ammonium units/cm², also to determine its relationship between K/S values the antibacterial efficiency, hydrophilicity and the surface potentials.

A new method was developed using Direct Red 47 dyestuff to measure the charge density of polyester fabric treated with AEM 5772. The experimental charge density value of 1.4×10^{16} units/cm² was close to calculated value of 1.3×10^{16} units/cm² at 0.05% aAEM 5772. Hence, almost all the charges present on the surface were detected by sulfonate groups on the dye molecule at lower concentrations.

On the other hand, Kügler et al. [10] explained OLCD 'outer-layer charge density' that there was no simple relationship between the thickness and the charge density. Fluorescein dye molecule was not able to attach all the charges present in the organic layer because hydrophobicity of the backbone of the polymer chains hinders the penetration of the dye molecule. It is become more important for thicker organic layer.

In our method which involved dyeing of treated with AEM 5772 polyester fabric that calculated quaternary ammonium units/cm² and measured quaternary ammonium units/cm² are close to each other and more than $\sim 10^{16}$, although 0.05% aAEM was used. The dyeing was performed under dynamic and active conditions with a relatively long time, more than 75 minutes, such as, the fluorescein complexation method, which used by Tigler, Kügler and Russell et al., performs 5-10 minutes under immobile conditions. Therefore, there is many more probable situations that the charges present in the layer on the surface

were interacted with the direct dye. Furthermore, this method is much easier, simpler and more accurate than that of Tigler et al.

A higher charge density or concentration of the quaternary ammonium groups on the polyester surface leads to a higher color strength values.

On the contrary, some other direct dyes can be used with a different number of sulfonate groups and size to get better experimental value of charge density at higher concentration of charges present on the surface. It will may conclude better direct dye type to elicit the minimum surface requirements.

Establishing a correlation between K/S values and surface charge density is recommended to determine the minimum surface requirements for desired antimicrobial activity.

It can be seen that relationship between the lightness value, surface quaternary ammonium density, estimation of chlorine ion and microbiological analysis (40 x AATCC 135) that which have very similar tendency.

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Section 3.

Preparation of Antibacterial PVA and PEO Nanofibers Containing Lawsonia Inermis (henna) Leaf Extracts

Chapter I. Introduction

Because of an increasing earth population and the environmental pollution, much focus has been placed in the recent years to find new health and hygiene related products with the minimum negative effect on the environment [1]. To address these growing concerns for the mankind and the environment, the development of new antibacterial agents has become one of the most important research areas to combat some pathogens, such as Gram-positive and Gram-negative bacteria, fungi, algae, yeast, and some microorganism which cause serious human infections. It is important to mention that pathogens have the ability to develop resistance against the commonly used antibiotics because of inappropriate use, side effects of the antimicrobial agents, and over prescription [2-3]. Thus, a disease-causing microbe will become more resistant and as a result:

- a) more powerful antibiotics must be used with the increased the risk for the side effects,
- b) the problem may become more severe and more difficult to treat,
- c) some serious infections may even require hospitalization, or a longer treatment time.

In addition, assessing the risks associated with the use of synthetic and metal-based antimicrobial agents in commercial products requires a detailed understanding of the materials mobility, biocompatibility, and biodegradability in a physiological environment. The additive toxicity also becomes an important issue when the compound is actually released to the environment.

The medical plants have been known for centuries as antifungal, antibacterial and antiprotozoal activities that could be used either systematically or locally which containing volatile oils, polyphenols and alkaloids as active constituents. Especially, during the second half of the 20th century, the researchers have been developed and investigated antimicrobial activity of several medicinal plants as an alternative form of health care because there are some advantages, such as, excellent therapeutic potential, and lessening the intensity of side effects which are often associated with synthetic antimicrobials [4-5].

1.1. Research Purpose and Objectives

It could be argued that the discovery, development and clinical explanation of natural antimicrobials will be the most significant medical advances of the 21st century. It is estimated that the world has 250,000 to 500,000 species of plants; and small percentage (1 to 10%) of these species are used as foods by both humans and other animals [8]. Hippocrates, who lived about in the late fifth century B.C., mentioned about 300 to 400 medicinal plants. In addition, nowadays, the researchers demonstrate that some of these can be used for medicinal purposes.

A new approach to produce an eco-friendly natural antimicrobial material is incorporating the active compound from plant extract within a material in order to treat humans, animals, and other infections. The plants and their extract are still crucial in the pharmacy and medicine. People in many countries in Europe, North America and Australasia now want to cure their minor health problems with 'natural' products as well as being an important form of

treatment in many developing countries [6]. Furthermore, thousands of published scientific papers which are about the antimicrobial activities of natural products are demonstrating the bacteriostatic and bactericidal effects, for instance, the PubMed database (data from 1975 to 2005) showed about 1360 the scientific and medical articles, Napralet database, which is the world's largest natural product database, demonstrates 58,850 plant species, 6,550 species had explained experimental antimicrobial activity, and 4,000 species used to treat infectious disease, and also the majority of these plants were effective against a range of bacteria and fungi [7].

Henna or Hina (*Lawsonia Inermis*, syn. *L. alba*) which is in the genus *Lawsonia* and belongs to the family Lythraceae, is a small shrub and widely cultivated and used in many oriental countries in Africa, Middle Eastern, Southern Asia and northern Australasia [8, 3, 10].

In this work, we investigate the *Lawsonia Inermis* (henna) leaf extracts for preparation of antibacterial poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) nanofibers via electrospinning technique. The previously work used the henna extract [3] to obtain disposable antibacterial products as well as for treatment of albinism, skin abrasion, burns, smallpox, leprosy boils, wounds, some mycotic infections, cancers, also scalp and hair infections [10]. The electrospun PEO and PVA based fibers can be used as a medical disposal product. PEO or PVA are water soluble polymers, therefore electrospun web can easily release henna antimicrobial agents while in contact with microbe or the infected skin area. On the other hand, Muhammad et al. [38] demonstrated henna leaf extract was effective to inhibit and manage burn wound infection. It was better than some conventional wound dressing, for example, silver nitrate, which imparts stain, causes hyponatraemia or

hypokalaemia, and also mafenide and can be painful to treat the infection [1, 38]. It has known for a long time that the small fiber diameter has advantage for the high specific ratio of surface area to volume that is important for many biomedical applications. PEO is hydrophilic, biocompatible, and considered to be inert and has a low toxicity in animals and humans (Sheftel et al., 2000). PVA is a water soluble synthetic polymer, also biocompatible and non-toxic; therefore, it can be used in the manufacturing of pharmaceutical products (Lin et al., 2006).

To determine the effect of the leaf extract, the electrospun fibers properties and antibacterial activities were evaluated. The electrospun fibers were characterized by using scanning electron microscopy (SEM), and FTIR was used in order to observe the antimicrobial groups both on the electrospun mats and the extract. The antibacterial effect was evaluated by ASTM E2149-01 Section 12 test parameters, zone of inhibition (ZOI) activity was demonstrated. The obtained nano-fibrous electrospun mats were promising for antibacterial applications, as well as some other applications which already mentioned.

Electrospinning is an effective technology which has been known in the first half of the 20th century, and Reneker and coworkers have demonstrated the processes in more detail [11]. The electrospinning has been increased in popularity over the past several decades which is one the most promising method for the producing the nanofibers and a variety of morphologies can be obtained [9].

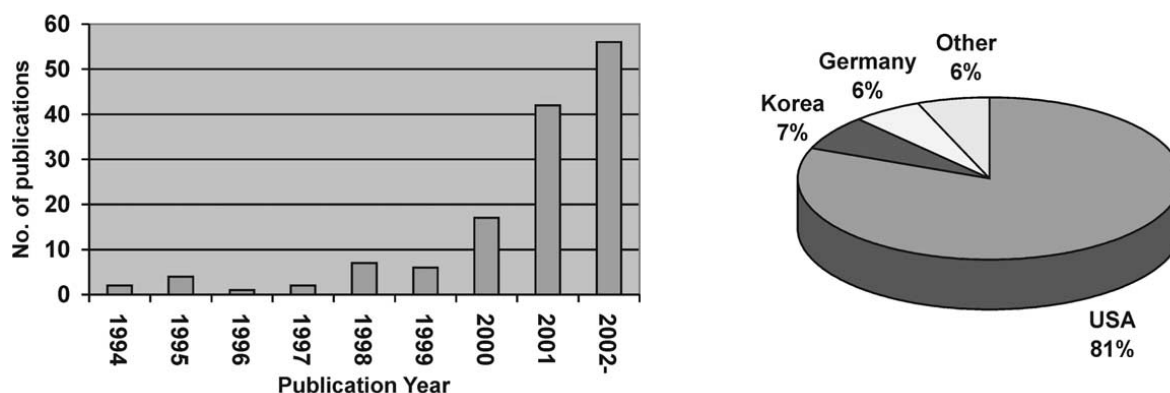


Figure 1.1. The annual number of scientific publications about electrospinning and its distributions around the world [9].

It also has some advantages (i) low cost to produce nanofibers, (ii) a high surface area to volume ratio, (iii) the ability to control pore size, (iv) ease to functionalize for various purposes, (v) superior mechanical properties, (vi) ease of process, and so on. These unique properties make this technique very attractive for many different applications.

Chapter II. Literature Review

As mentioned earlier *Lawsonia Inermis* Linn, which is popularly known as henna or mehndi, grows outdoors unsheltered, and the reported life zone temperatures higher than 11 °C (60 °F), and widely cultivated as a hedge plant. It is a small tree or large shrub which needs approximately 5 years to mature to get useful of tannins from the leaves, and higher leaf yields obtained two-year-old than one-year-old, and arid regions with the soil pH of 4.3 to 8.0 where is better than moist or wet regions [10, 12].

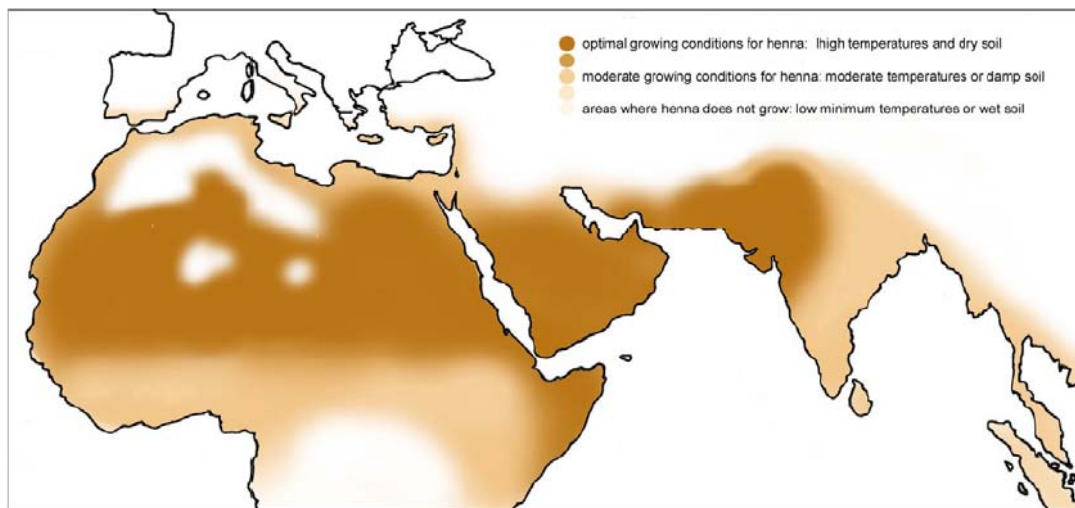


Figure 2.1. The areas potentially suitable for henna's growth [14].

In addition, the henna has lateral branches with leaves that grow in pairs and mostly opposite, broadly lance-shaped and the length of leaf is two to four centimeters, dark-green and glabrous. The flowers are white or pale pink colored, small and fragrant [3].

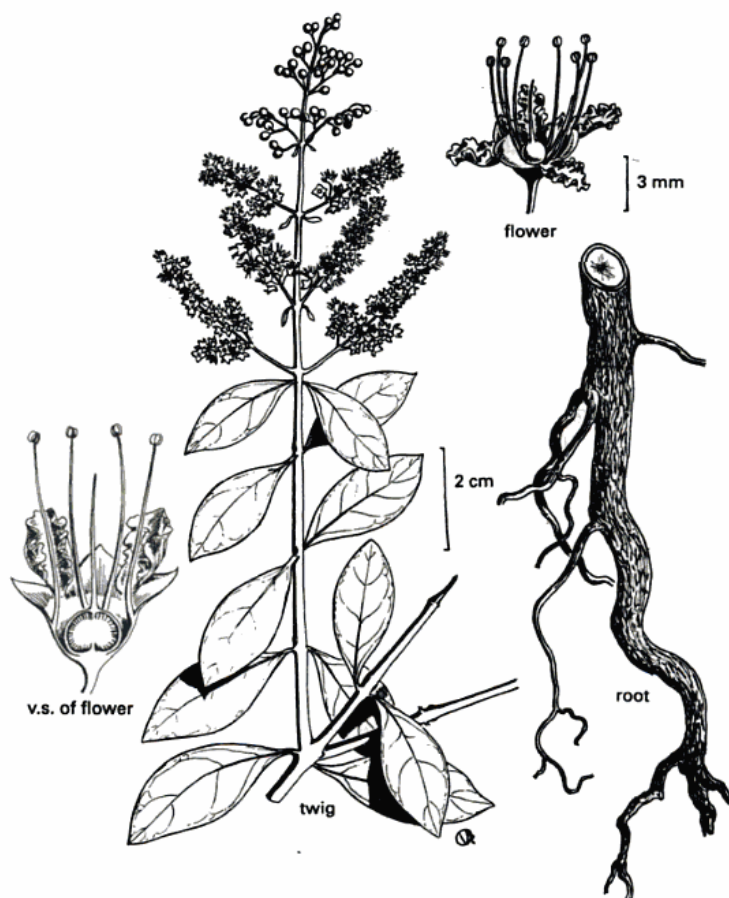


Figure 2.2. Lawsonia Inermis [13].

2.1. Potential Application of *Lawsonia Inermis* Linn.

Henna seeds, roots, leaves and flowers have many traditional and commercial uses, the most common being as cosmetics such as a dye for hair, skin and fingernails, coloring and preservative for leather and cloth, as a folk remedy against headache, jaundice, and leprosy [3], as an antifungal and antibacterial compounds, etc.



Figure 2.3. *Lawsonia Inermis* leaves, seeds and flowers [14].

The following sections provide information needed to understand the potential applications of *Lawsonia Inermis* in medical end uses and product properties.

2.1.1. Historical Discovery of Lawsonia Inermis Medicinal Properties

Herbal remedies used in the traditional folk medicine demonstrates an interesting and still largely unexplored source for the creation a development of potentially new materials in order to utilize in medical applications.

Lawsonia Inermis associated with plant family of Lythraceae and the known fossil records of Lythraceae was found in northern Latin America and 18 genera is recognized in age from lower Eocene, in addition, Indo-Malayan region is the oldest cultivation area of these genera [15]. First time, this information was written in literature by von Ettingshausen in 1879.

The various old cultures have used mostly the medical plants as a poultice and imbibed infusions. Ancient Egyptians have obtained both oil and ointment from the henna flowers in order to get the limbs supple [10]. Then, it is used in order to heal headaches, migraine, albinism, skin abrasion, burns, antibacterial, antifungal, cancers, anti-inflammatory, inhibited of some spore germination, the common urinary pathogens [3, 10, 16, 17].

2.2. Lawsonia Inermis Leaf Samples

The definition, chemistry, and physical properties of components, which are present within henna leaves by phytochemical analysis, are described below.

2.2.1. Definition, Chemistry, and Physical Properties

Henna leaves contain a variety of compounds, which can be used in the treatment of itching and skin infections in humans, as a antibacterial, antifungal and antiviral activity according to phytochemical analysis. The compounds are shown in Table 2.1 [3].

Table 2.1. Phytochemical analysis of Lawsonia Inermis leaf samples.

Constituent	Level*
Mannite	++
Tannic acid	+++
Gallic acid	++
Naphthoquinone (hennotannic acid)	+++
Crysophanic acid	+++
Anthraquinones	+++
Mucilage	+++
Sterols and/or triterpenes	±
Cyanogenic glycosides	+
Flavonoids	-
Tannis	-
Saponins	-

*: + = Low concentration, ++ = Medium concentration, +++ = High concentration, ± = traces, - = Not detectable

2.2.1.1. Mannite ($C_6H_{14}O_6$): Properties and Use

Mannite is an organic compound and one of the glucoses which obtained from plants and their exudates; also it is derived industrially from fructose by reduction [18]. It is referred as D-mannitol and manna sugar which is a colorless and very soluble in water. It is used as a sweetening agent, food additive, anticaking agent, lubricant, diagnostic aid (renal function determination) and diuretic [18]. Moreover, it is the most commonly used drug in the patients severe head injury, but its mechanism of action to the efficiency is still controversial [19].

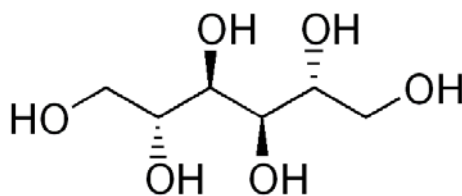


Figure 2.4. D-mannitol.

2.2.1.2. Tannic Acid: Properties and Use

Tannic acid is commonly referred to as tannins which are water soluble polyphenols. It is present in woods and many plant foods which has low nutritional value, and the compound is effective against many fungi, yeasts, bacteria, viruses, also can be used to increase shelf-

life of certain foods [20]. In addition, tannic acid that commercial extracts enriched in hydrolysable tannins, also used as antimutagenic, anticancer and antioxidant agent [25].

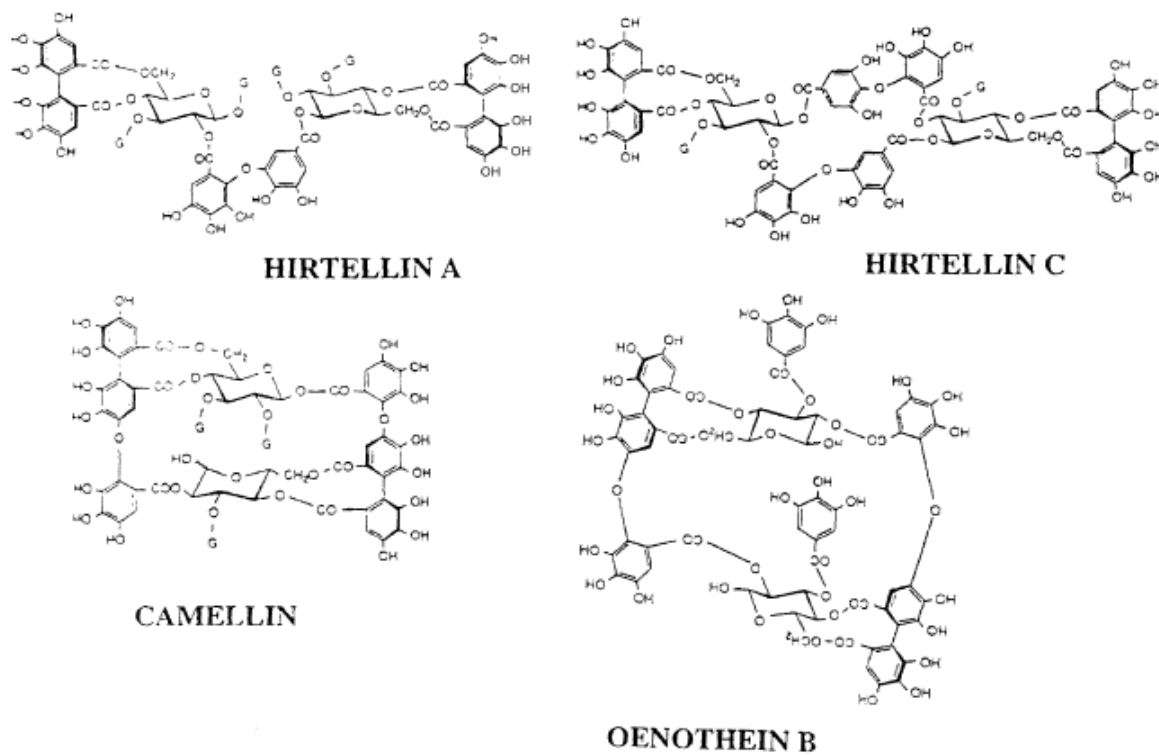


Figure 2.5. Some pharmacological tannins isolated from the plants [20].

2.2.1.3. Gallic Acid ($C_6H_2(OH)_3COOH$): Properties and Use

Gallic acid is a naturally occurring plant phenol which found in some leaves, and water solubility is limited. It can be used in processed food, cosmetics and food packing to prevent spoilage and oxidations; moreover, it shows moderate antifungal activity and some intestinal bacteria [21, 23].

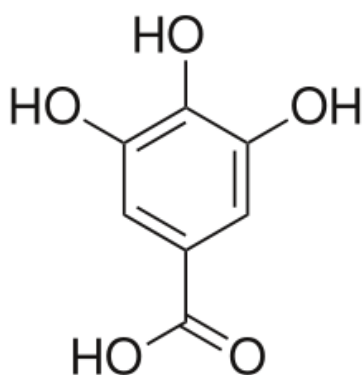
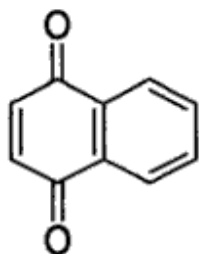


Figure 2.6. Gallic acid [21].

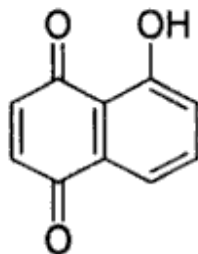
2.2.1.4. Naphthoquinone ($C_{10}H_6O_2$) (hennotannic acid): Properties and Use

Naphthoquinone (1,4-naphthoquinone) is a colorless organic compound. Lawsone (2-hydroxy-1,4-naphthoquinone), obtained from henna, and juglone, found also in walnuts. Colored naphthoquinone can have more than one OH group and is a red pigment present in sand dollars and sea urchins (see Figure 2.7 and Figure 2.8) [24]. Lawsone commonly

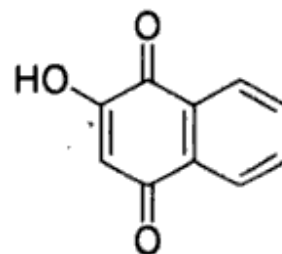
known as Natural Orange 6 and CI 75480 in industry is used as a substantive dye for keratin and an acid leveling non metalized acid dye for nylon to give orange color. On the contrary, it can react with amino acids secreted by the fingers to get finger mark on paper surfaces for forensic technology without causing irritation. In addition, lawsone is effective against oral *Candida albicans* isolated from patients with HIV/AIDS [10].



Naphthoquinone (colorless)

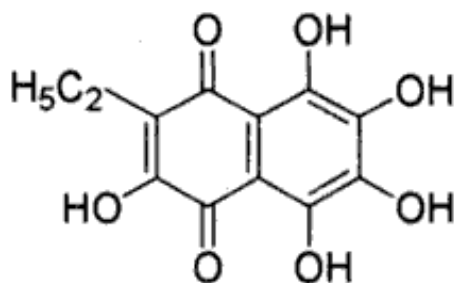


Juglone (brown)



Lawsone (reddish orange)

Figure 2.7. Chemical structure of naphthoquinone, juglone, and lawsone [24].



Echinochrome (red)

Figure 2.8. Chemical structure of echinochrome [24].

Naphthoquinone has antibacterial activities against bacteria including several species of aerobic and anaerobic organism, parasites, anti-inflammatory, fungicidal, virucidal, trypanocidal, anti-plasmodium, falciparum, the causative agent of malaria, and anti-Schistosomiasis are that investigated by researchers from around the world [10]. It has a little cold water solubility; however it is soluble polar organic solvent.

2.2.1.5. Crysophanic Acid: Properties and Use

Crysophanic acid is found in a number of plants, lichens, rumex, and is to some extent soluble in water. It is effective to treat psoriasis, and in certain cutaneous fungi, also it has antimicrobial and antifungal activities [27, 28, 40].

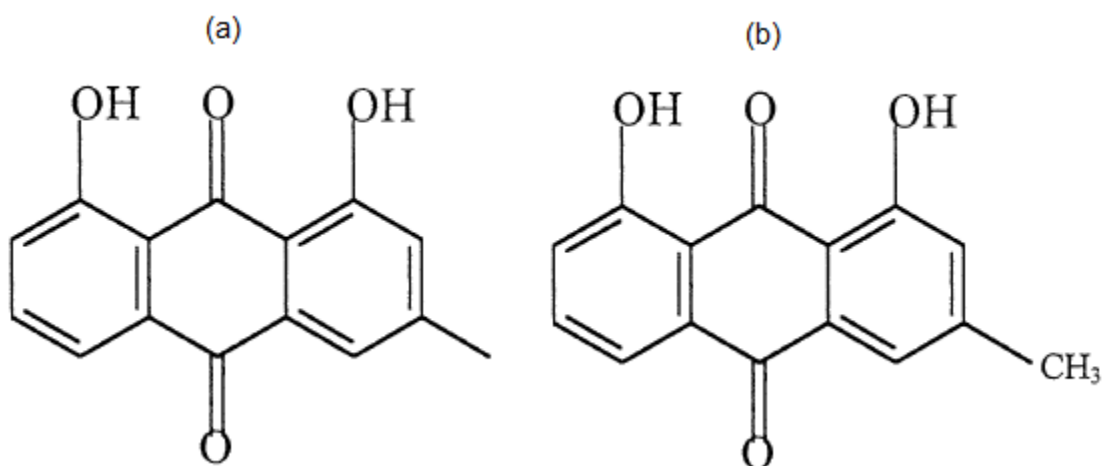


Figure 2.9. Chemical structure of (a) crysophanic acid (b) chrysophanic acid (1,8-dihydroxy-3-methylantraquinone) [28, 41].

2.2.1.6. Anthraquinones ($C_{14}H_8O_2$): Properties and Use

Anthraquinones are organic compounds and found in some plants, such as aloe latex, senna, fungi and insects which is colored compounds and partially soluble in water, but dissolve in alcohol and nitrobenzene [29]. The natural derivative has laxative effects, also it can be used as antibacterial, antiviral, and anticancer compound [29, 30].

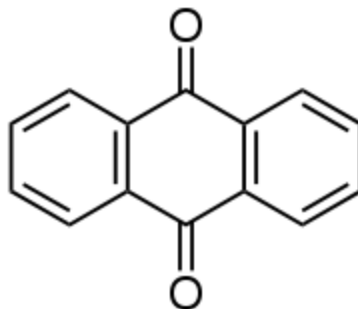


Figure 2.10. Chemical structure of anthraquinone [29].

2.2.1.7. Mucilage: Properties and Use

Mucilage, which is manufactured by most plants and some microorganisms, is thick and glutinous substance including protein, polysaccharides, and uranides [31]. It swells but does not dissolve in water which used in medicine for its emollient and demulcent properties; also it is mildly antibacterial and soothing [31-32]. In addition, Moghbel et al. (2005) demonstrated that tragacanth mucilage is promising for wound healing in rabbits.

2.2.1.8. Sterols and Triterpenes: Properties and Use

Sterol is an organic molecule and occurs naturally in tissues of animals, plants, fungi, and yeasts [33], also its structure is similar to cholesterol. Plant sterol is one of the most important components of the membranes of all eukaryotic organisms which control membrane fluidity and permeability [34]. The plant sterol named as phytosterols which are steroid alcohols and form a group of triterpenes. Plant sterol has good effect on

cardiovascular system to decrease cholesterol and the usual human diet which including approximately 200-300 mg per day of plant sterols [34].

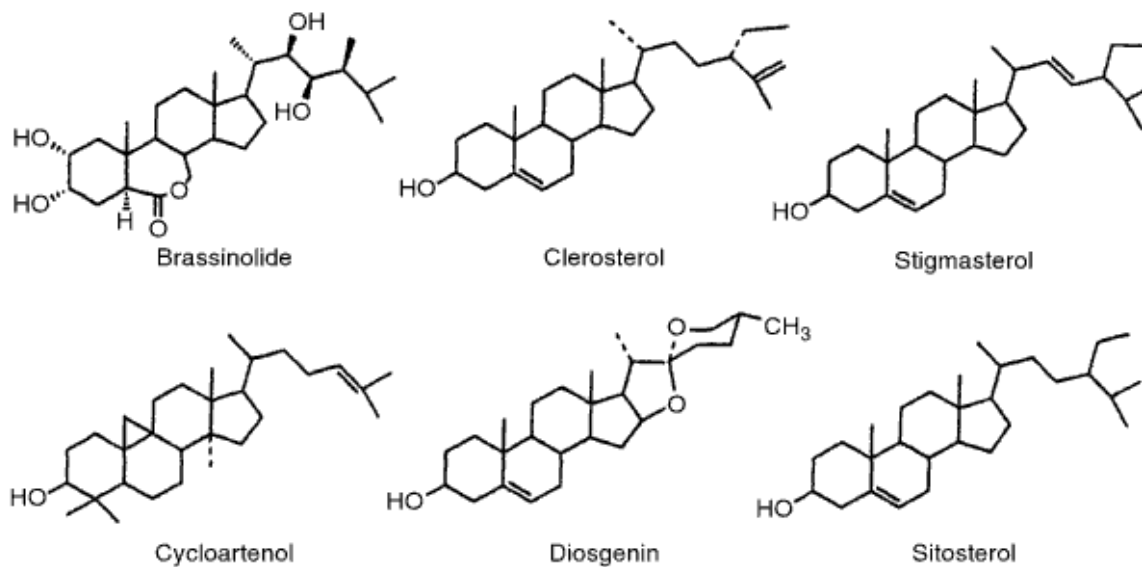


Figure 2.11. Examples of plant sterols [54].

2.2.1.9. Cyanogenic Glycosides: Properties and Use

Cyanogenic glycoside is consisting of an alpha-hydroxynitrile type aglycone and of a sugar moiety (mostly D-glucose). The plant, which has cyanogenesis to produce cyanogenic glycoside, can synthesize variation in the amount of HCN when cyanogenic glycoside

enzymatically hydrolyzed to protect itself against predators [35, 36]. On the other hand, most of plants can synthesize a small amount of cyanide associated with ethylene production. However between 3-12000 plant species, which are economical important plants such as white clover, linum, almond, sorghum, the rubber tree and cassava, are highly and sufficient cyanogenic [36].

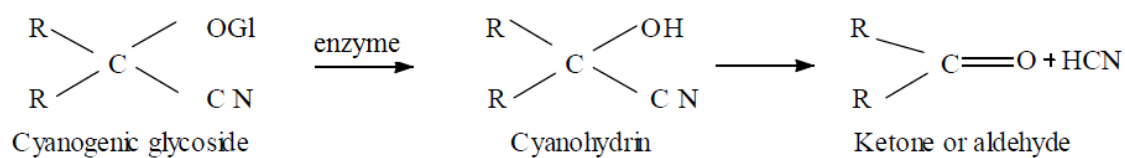


Figure 2.12. The mechanism for releasing HCN by cyanogenic plants [35].

Chapter III. Materials and Experimental Methods

3.1. Introduction

The antimicrobial activity of henna's fresh and dry leaves and seeds were observed by researchers. It is clear that crude extract of henna dry leaves demonstrate the best antimicrobial activity [3, 10, 16, 37, 38].

Table 3.1. Comparison of henna leaves and seeds antimicrobial activity at 50% concentration [37].

Micro-organisms	Henna Extract			
	Fresh Leaves	Fresh Seeds	Dry Seeds	Dry Leaves
Bacteria				
<i>Escherichia coli</i>	++	+	+	++
<i>Pseudomonas aeruginosa</i>	++	++	+	++
<i>Staphylococcus aureus</i>	+++	++	+++	+++
<i>Enteropathogenic Escherichia coli</i>	+	+	++	++
<i>Shigella sonnei</i>	+++	++	++	++++
<i>Bacillus species</i>	++	++	+	+++
<i>Klebsiella pneumoniae</i>	++	++	++	+++
<i>Salmonella species</i>	++	+	+	++
<i>Bacteriodes fragilis</i>	+	+	+	+
<i>Corynebacterium</i>	++	++	++	++
<i>Streptococcus pyogenes</i>	+	+	+	++
<i>Citrobacter freundii</i>	+	+	+	++
<i>Vibrio cholerae</i>	++	++	++	++
<i>Streptococcus Pneumoniae</i>	+	+	+	++
Yeast				
<i>Candida albicans</i>	+	-	-	+

++++ => mm zone of inhibition, +++= 31-40 mm, ++= 21-30 mm, += 10-20 mm
 -= 0 mm (no inhibitory activity detected).

Saadabi et al. [3] observed that the extract of water had a higher inhibition activity as compared to that of methanol and chloroform and reported in Table 3.2.

Table 3.2. In vitro zone of inhibition (mm) antibacterial activity of Lawsonia Inermis leaf extracts [3].

Extract type	B.s	S.a	E.c	P.a
Water	16	19	16	18
Methanol	14	16	17	16
Chloroform	14	13	14	15
Ampicillin 40 µg/mL	20	22	24	16

B.s: *Bacillus subtilis*; S.a: *Staphylococcus aureus*; E.c: *Escherichia coli*; P.a: *Pseudomonas aeruginosa*; concentration of extracts 100 mg/mL.

The focus of the present work to integrate water extract of henna leaves into poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) to obtain antibacterial nanofibers via electrospinning method. Electrospinning method is one of the effective methods to produce polymer nanofibers which have some unique properties, for example, a high surface area-to-volume ratio, high functionality, small pore sizes, high porosity, and so on. Resultant electrospun fibers then were characterized for antibacterial activity with scanning electron microscopy (SEM) and FTIR spectroscopy.

3.2. Experimental Techniques

3.2.1. Materials

Poly(ethylene oxide) (PEO), a water soluble polymer, with an average molecular weight of 900,000 g/mol, 2-Hydroxy-1,4-naphthoquinone (97%): Lawsone (Natural Orange 6) with molecular weight of 174.15 g/mol, tannic acid (ACS Reagent) with molecular weight of 1701.20 g/mol; 1,8-Dihydroxy-3-methylantraquinone: chrysophanic acid (Mw 254.24 g/mol, 98%), anthraquinone (Mw 208.21 g/mol, 97%), and 2M Folin & Ciocalteu's phenol reagent were purchased from Sigma-Aldrich Co. Poly(vinyl alcohol) (PVA), a water soluble polymer, with an average molecular weight of 127,000 g/mol and a 88% degree of hydrolysis was also obtained from the same company.

Lawsonia Inermis dry cut leaf sample, which was cultivated in Egypt, used in this study was purchased from Penn Herb Company, Ltd; Philadelphia.

Pyrex filter flask and Kimax Fritted Disc (nominal maximum pore size: 10-15 μm) was used in this study is from Fisher Scientific Inc. All materials were used as received.

3.2.2. Preparation of Solutions for Electrospinning

PVA and PEO nanofibers were spun from aqueous solutions containing water/ethanol henna leaf extract. The polymer concentration was adjusted to assure the optimum electrospinning performance.

3.2.2.1. Preparation of Henna Extract

Henna water extract were obtained by adding de-ionized water to coarsely powdered henna leaf in a conical flask and left to soak sixteen hours. Ethanol (denaturated, 92% reagent grade) was then added to extract to prepare ethanol/water (10/90 v/v) extracts. A well mixed solution was left for more eight hours to soak and final concentrations of leaf/liquid sample adjusted 25 and 75 mg/mL. Then, water of the extract was evaporated under vacuum at room temperature and the solid material was put into a desiccator to fully eliminate any moisture present. The weight of the solids was determined after first after 24 hours and then finally after 48 hours of drying. No further weight changes were observed after 48 hours. The concentrations of solid are the mean of three replicates.

The solutions were prepared using a combination of approaches based on Saadabi and Giri Dev work [1,3], because the extract of water was better as compared to that of methanol and chloroform. Ethanol is a good solvent for the compounds that were released from the leaf by water. The residue was then filtered by Pyrex filter flask and Kimax Fritted Disc (nominal maximum pore size: 10-15 μm) enforced with vacuum.

3.2.2.2. Preparation of Solution for Electrospinning

Poly(ethylene oxide) (PEO) (Figure 3.1) based solutions were prepared by dissolving 4 wt% of PEO powder samples in ethanol/water mixture (10/90 v/v) and in the extract solutions at room temperature for 24 hours with constant stirring.

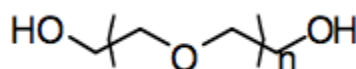


Figure 3.1. Chemical structure of poly(ethylene oxide).

In addition, poly(vinyl alcohol) (PVA) (Figure 3.2) based solutions were prepared by dissolving 7 wt% of PVA powder samples in ethanol/water mixture (10/90 v/v) and in the extract solutions at room temperature for 24 hours with constant stirring to compare the outcomes.

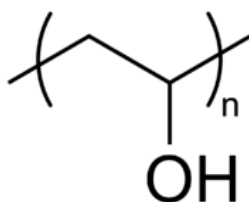


Figure 3.2. Chemical structure of poly(vinyl alcohol).

3.2.3. Electrospinning

Many traditional electrospinning setups already been used by researchers and students as illustrated in Figure 3.3. Professor Zhang of COT, NCSU allowed us to use his electrospinning set-up was to obtain electrospun fibers. The set-up included a 10 mL medical grade syringe obtained from BD Inc., a NE-1000 programmable syringe pump by New Era Pump Systems Inc., and a FC Series 120 Watt Regulated High Voltage DC Power Supply by Glassman High Voltage Inc. Also, a 14 gauge needle obtained from Vita Needle Company, Inc.

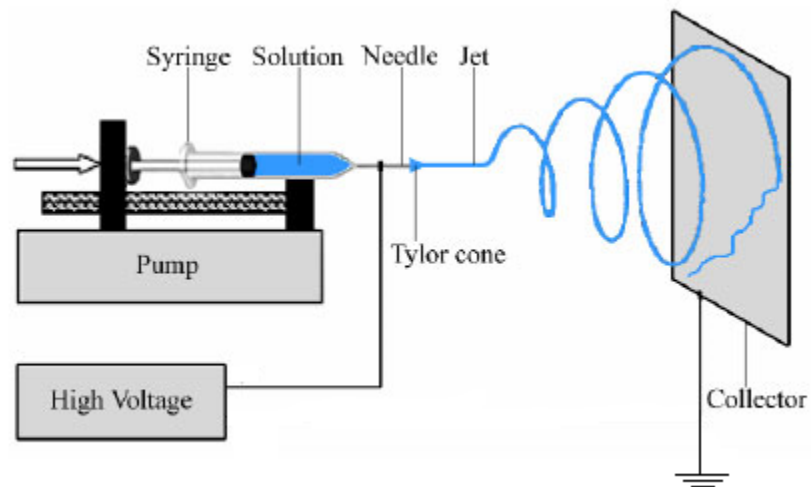


Figure 3.3. Schematic of a typical electrospinning setup.

The set-up parameters can be controlled by changing polymer flow, electric field strengths, the needle to the collector distance, and a single or parallel plate collector setup for use in multiple research projects. The collector of the set-up was covered by aluminum foil, hence, multiple samples could be produced quickly and easily removed.

3.2.3.1. Production of PVA and PEO Based Electrospun Fibers

Electrospinning was carried out at room temperature. The 14 gauge needle was attached to the syringe, and the syringe was then horizontally fixed on the infusion pump to feed the sample solution at a constant rate through the syringe to the needle tip. Afterwards, an alligator clamp fixed to a wire with a plug which connected to the collector plate to ground plate. Finally, the high voltage was applied to the 14 gauge needle via an alligator clamp and wire which was attached to the high Voltage DC Power Supply.

Since there was no any prior experience about electrospinning conditions of this particular polymer including the extract, the main focus was to find the materials and processing conditions that would successfully fabricate electrospun nanofibers. After a few several trials, the best processing conditions were determined and carried out to obtain electrospun fibers. The best experimental design for this purpose is shown in Table 3.3.

Table 3.3. Experimental design for electrospinning.

Polymers	Henna Concentration (wt%)	Polymer Concentration (wt%)	Flow Rate (μL/min)	Applied Voltage (kV)	Collector Distance (cm)
PEO	-	4	5	9	15
PEO	1.264	4	4	9	15
PEO	2.793	4	4	9	15
PVA	-	7	8	15	15
PVA	1.264	7	8	15	15
PVA	2.793	7	4	15	15

It should be noted that the different molecular weight of PVA and PEO was used for evaluating and characterization. PVA had a lower molecular weight so a higher concentration of this polymer had to be used to assure a proper electrospinning.

3.2.4. Characterization and Evaluation

Electrospun fibers were examined and characterized by using scanning electron microscopy (SEM) to analyze fiber diameter, diameter distribution and fiber alignment. Diameter distribution histograms of electrospun fibers were drawn and labeled correctly by Microsoft Office Excel 2007. The fibers diameter assessed by a basic ruler and more than fifty fibers were measured in different regions of SEM images.

ASTM E2149-01 Section 12 was used to determine zones of inhibition (ZOI) for henna leaching antimicrobial extracts prior to the electrospinning process. In addition, FTIR was used to identify match the chemical groups and classes which present on electrospun nanofibers and 100% the solid extract sample. ZOI activity demonstrated against *Escherichia coli* and *Staphylococcus aureus*.

On the other hand, high performance liquid chromatography was used to identify compounds in the leaf extract.

3.2.4.1. Total Phenolic Compounds Assay by Folin & Ciocalteu Reagent

Total phenolic compounds in the extract were determined via a Cary 3E UV-Visible Spectrophotometer with preparation of diluted tannic acid standard solutions. Dilutions of tannic acid were prepared with de-ionized water in the following known concentrations: 3.042 mg/L, 5.07 mg/L, 10.14 mg/L, and 20.28 mg/L to develop a standard calibration curve.

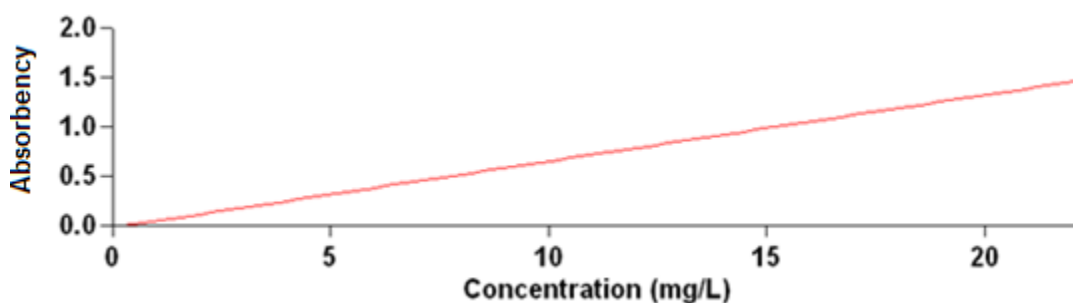


Figure 3.4. Correlation between the dilutions of tannic acid and absorbency.

The extract of henna leaf was prepared same as the procedure described in the previous section with final concentrations of leaf/liquid sample 25 and 75 mg/mL. Then, water of the extract was evaporated under vacuum at room temperature and it was put into a desiccator to keep it from picking moisture until a constant weight was observed following two weight determinations which were done in two 24 hours periods. Total phenolic compound in the dried extract was observed using Folin-Ciocalteu reagent via Cary 3E UV-Visible Spectrophotometer. A method was developed according to standard direction of Rossi [50] and Hosein [51]. The mixtures were left at room temperature for 30 minutes and then the absorbance of the solutions was read at 765 nm.

3.2.4.2. High Performance Liquid Chromatography-HPLC

HPLC is a powerful tool used by researchers to determine a compound concentration. The method allows for a much better separation of the components of the mixture as a high pressure pump generates a specified flow rate of mobile phase. In general, HPLC system consists of a mobile phase reservoirs, a pump, sample injector, a separation column, and a detector.

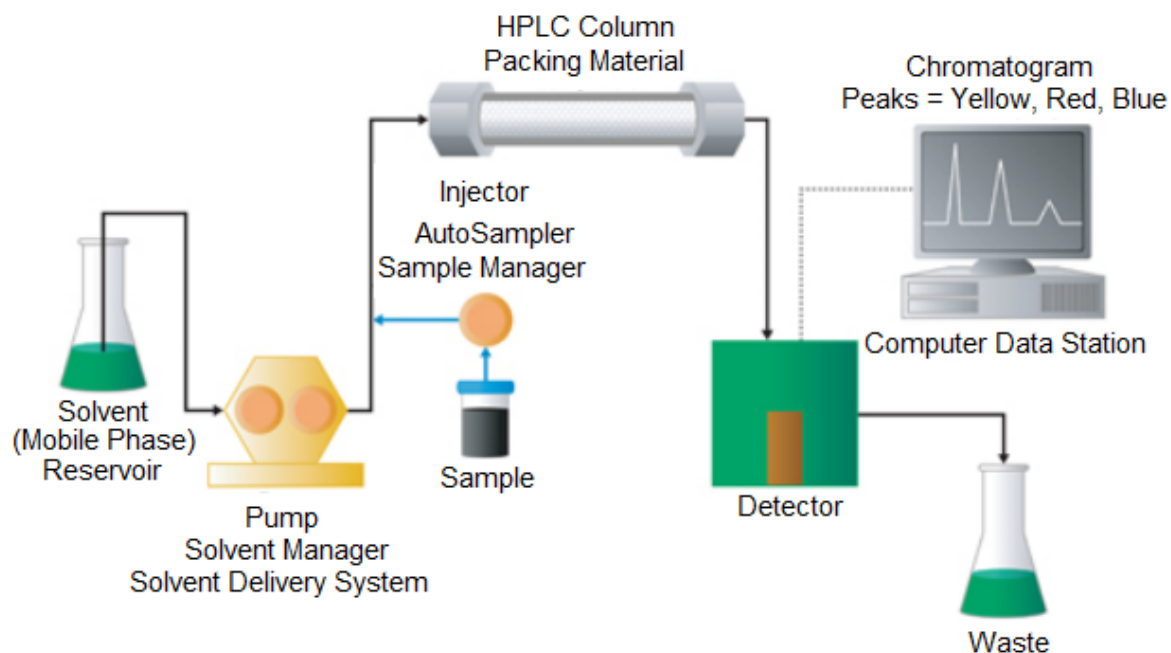


Figure 3.5. High performance liquid chromatography system [42].

Solvent reservoir in the mobile phase where the solvent should be filtered with $< 1\mu\text{m}$ pores and degassed before it is used. A high pressure pump is used to transfer the solvent with a specified flow rate into the continuously flowing mobile phase stream and an injector injects the sample into the stream that carries into the column. The column has the chromatographic packing material, which is the stationary phase, to effect the separation. A separated compound is analyzed via a detector the other end of the column during the sample migrates through the column. The detector conveys identity information to the computer data station which is a chromatogram and the waste then collects the sample. The chromatogram provides information on separation efficiency, and retention time which the

time required by the mobile phase to pass through the column (also called the breakthrough time), is a unique identifying characteristic of a given analyte [42, 43].

The Alliance chromatographic system was operated for experiments. HPLC analysis was carried out using a Waters 2695 Solvent Delivery System (Separation Module) which consists of a four bottle solvent reservoir, solvent management system, Performance Plus inline degasser, and an auto sampler, equipped with a Waters 2996 Photodiode Array Detector. HPLC separation was achieved using a Waters X-Bridge C18 column with 3.0 mm x 100 mm, 5 μ m particle size with a mobile phase flow rate of 1.00 mL/min via automatic stroke volume. Samples were injected via automatic injection through a 200- μ L syringe into a 100- μ L sample loop, and 10 μ L of sample was then injected onto the column. Data collection and peak observation were analyzed with Empower Pro software.

The mobile phases were prepared and stirred, the organic phase was then placed reservoir B, and the mixed phase was placed in reservoir A.

A: 18 M Ohm water + 0.1% trifluoroacetic acid

B: Fisher HPLC grade acetonitrile

18M ohm water used was obtained from an Elga Ultra An MK2 system.

The mobile phase gradient was prepared using an approach based on Jaziri and Bakkali's study [44].

Table 3.4. Gradient.

Flow Rate (mL/min)	Time	A	B
1	Initial	90	10
1	10	10	90
1	15	10	90
1	16	90	10
1	Hold 7 min	90	10

Reference compounds were selected according to both phytochemical analysis of Lawsonia Inermis leaf samples with their levels which are at the highest level and have antibacterial property. Their solvent systems were developed (Table 3.5) and stirred until all compounds have dissolved. In addition, the leaf extract was prepared the same as used by the electrospinning process.

Table 3.5. Solutions preparation.

Compound	Solvent	Concentration (mg/mL)
The Extract	Water: Ethanol (90/10 v/v)	34.3
Lawsone	Water: Ethanol (90/10 v/v)	1
Tannic Acid	Water	1
Chrysophanic acid	Acetonitrile	1
Anthraquinone	Acetonitrile	1

The extract and the solutions were then filtered using Whatman 0.2 μ m, 13 mm PVDF GD/X disposable syringe filters in order to analyze.

3.2.4.3. Scanning Electron Microscopy-SEM

In order to determine the diameter, the diameter distribution, the morphology and consistency of the fibers, the mats were examined under a scanning electron microscope (SEM). The observations were then evaluated in terms of positive and/or negative effect of extract and in different extract concentrations. Images were acquired from a SEM-FEI Phenom. The fibers specimens were cut approximately 1 cm x 1 cm, and then coated with a layer of gold to obtain an average uniform coating of 100 Å thickness. Later, the electrospun

mats were mounted and viewed after focusing and with suitable brightness at several magnifications.

3.2.4.4. Antimicrobial Analysis

The antimicrobial tests were performed using the ASTM E2149-01 Section 12 test parameters (AATCC 147-1998) [39].

Procedure for determining presence of 'leaching' antimicrobial:

- i. Inoculated MacConkey agar plate for *Escherichia coli* and Columbia - CNA agar plate for *Staphylococcus aureus* with confluent lawn of organism (1×10^5 CFU/mL) and allowed to dry.
- ii. An agar bore was used to produce a small hole (8 mm diameter) in the middle of the agar and plug removed.
- iii. Two sterile 250 mL flasks containing 50 mL sterile buffer solution prepared.
- iv. Test and control specimen placed in their individual flasks. Capped the flasks and placed them on the wrist-action shaker a specific time.
- v. Samples of each liquid were added to the hole (300 μ L) and the plate allowed to dry. Each plate was set for incubation at $37 \pm 2^\circ\text{C}$ for 48 hours.
- vi. Observed presence or absence of zone of bacterial inhibition surrounding 8 mm diameter hole. Presence of a zone of inhibition indicates leaching.

3.2.4.5. Fourier Transform Infrared Spectroscopy- FTIR

The electrospun fibers were analyzed by directly exposing the fiber mat to infrared beam in transmittance mode which was monitored by attenuated total reflection Fourier Transform Infrared spectroscopy (FTIR). Nicolet Nexus 470 Fourier Transform InfraRed Spectrophotometer was used for this purpose which includes a single bounce OMNI SamplerTM with Ge crystal and a diamond compression cell for identification of contaminants and small particles/fibers to examine and compare the functional groups present in surface of the electrospun fiber mats and 100% the solid extract sample. The samples were prepared as a circle with approximately 1 mm diameter and the spectra were collected with 64 scans at 4 Hz resolution and were baseline-corrected.

Chapter IV. Result and Discussion

4.1. Total Phenolic Compounds in the Extract

Polyphenols can be extracted from the plants using methanol, ethyl acetate, acetone, acetic acid, water, ethanol, etc. and a variety of combinations of these solvents [45]. Therefore, the methods need to be improved to optimize extraction efficiency for all classes of phenolic compounds in the plants because their interactions with other components with other internal structure of the plant have influence their diffusion into the extraction solvent [46]. For example, phenolic acids, anthocyanins, and flavanol monomers and oligomers, which have lower molecular weight, are well extracted with methanol; on the other hand, the higher molecular weight flavanols are better extracted with an aqueous acetone. Therefore, processes for the preparation of the extract were the same as the procedure described in the previous section for the electrospinning processes.

Phenolic compounds have been demonstrated antimutagenic, anticancer, powerful antioxidants, anti-inflammatory, fungicidal, virucidal, trypanocidal, anti-*Plasmodium falciparum*, anticancer, and so forth properties [10, 21, 22, 23, 25, 29, 30, 47].

It was observed that the total phenolic compounds in 25 and 75 mg/mL of leaf/liquid samples have similar content which are 5.2 mg and 5.0 mg tannic acid/g dried extract, respectively. This difference between extracts might be explained as the extraction efficiency increased at lower leaf concentrations because the solvent system had more chance to diffuse into the leaf compared with a higher leaf concentration. Also, many

environmental factors, time of the harvest, different plant and climate conditions can affect the total phenolic compounds in henna leaves.

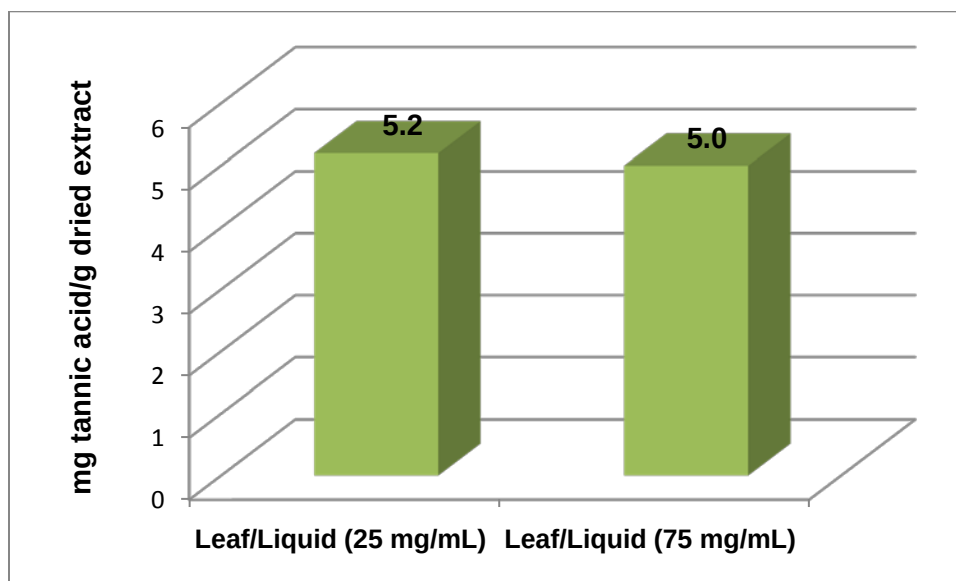


Figure 4.1. Total phenolic compounds of dried henna extract.

4.2. HPLC Assay

Very little modern research has been applied to determine compositional properties of henna leaf and its extract. The one detailed study about henna leaf was done by Saadabi et al. [3] who determined many phenolic compounds based on phytochemical analysis.

Definition, chemistry, and physical properties of these compounds were described in the previous section. The water/ethanol henna leaf extract has been analyzed using high performance liquid chromatography (HPLC). Additionally, anthraquinones, tannic acid, crysophanic acid and lawsone were selected as reference samples to determine a probability of identifying these chemicals in henna leaf extract. All these four compounds have strong antibacterial effect and they are often present in a henna leaf [3, 10].

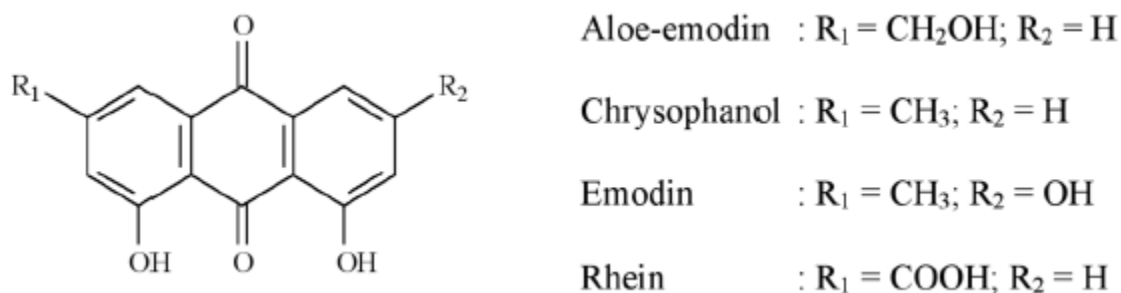


Figure 4.2. Chemical structures of anthraquinones [48].

Panichayupakaranant et al. [48] examined extraction of anthraquinones in *Senna alata* leaves via HPLC and the quantification wavelength was set at 254 nm. He also observed a variety of retention times that depended on different types of anthraquinones. See Figure 4.2.

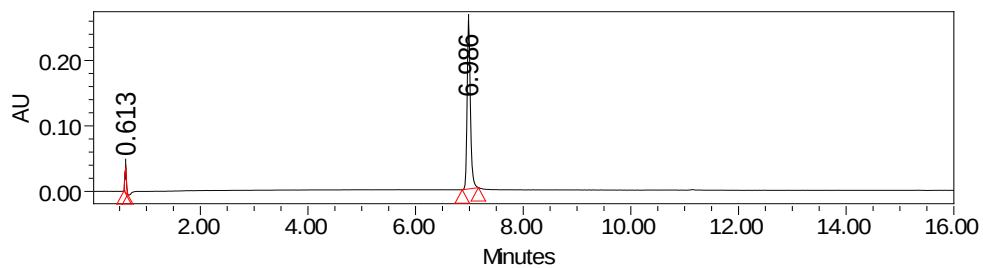


Figure 4.3. HPLC chromatogram of the anthraquinone reference.

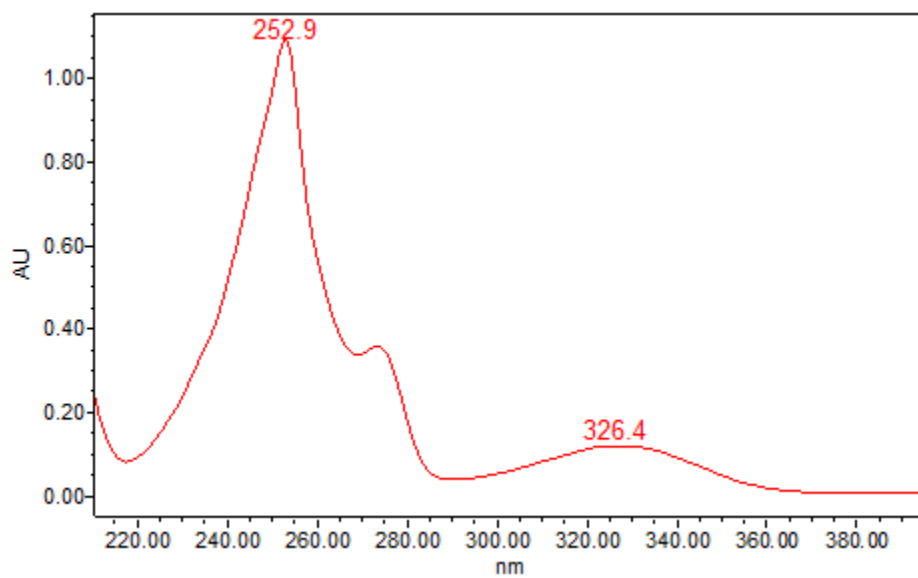


Figure 4.4. HPLC characteristic spectrum of anthraquinone reference.

Acetonitrile was used as a solvent for anthraquinone dissolution with the aid of an ultrasonic bath. Anthraquinone peaks of standard were identified by HPLC with retention times of 0.613 and 6.086 minutes and characteristic UV spectra are given in Figure 4.3 and 4.4, respectively. Chromatogram of the anthraquinone reference was taken at two points of the peaks and retention time with 6.086 minutes was a dominant peak. This compound has a good absorption at 252.9 nm, and a total run time of approximately 8 minutes was required.

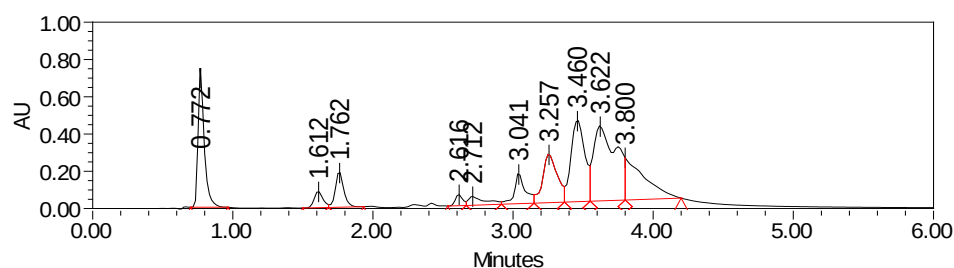


Figure 4.5. HPLC chromatogram of the tannic acid reference at 276 nm.

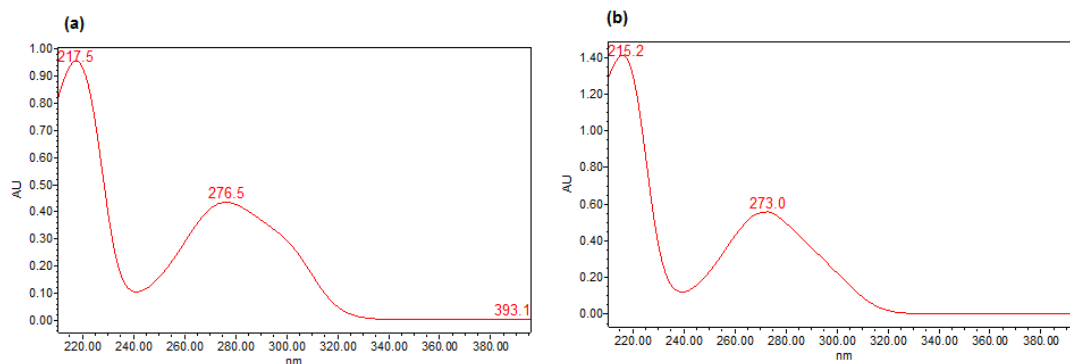


Figure 4.6. HPLC characteristic spectrum of tannic acid reference (a) at 3.629 min, (b) at 0.758 min.

Tannic acid is a typical form of hydrolyzable tannin that contains a mixture of different gallic acid esters of glucose and commercial extracts of tannic acid are enriched with hydrolysable tannins [25]. The quantification wavelength was set at 276 nm. Water was used in to dissolve tannic acid. It can be seen in Figure 4.5 the tannic acid reference is rich in different type of compounds which was an expected result because it usually represents a mixture of different gallic acid esters and polyphenols (Figure 2.5). Some different type of chemical compounds present in tannic acid yielded higher peak areas between 3 and 4 minutes retention time. Characteristic HPLC chromatograms at 3.629 minutes and at 0.758 minutes are presented in Figure 4.6, and a total run time was about 5 minutes needed to detect all type of compounds in the tannic acid reference sample.

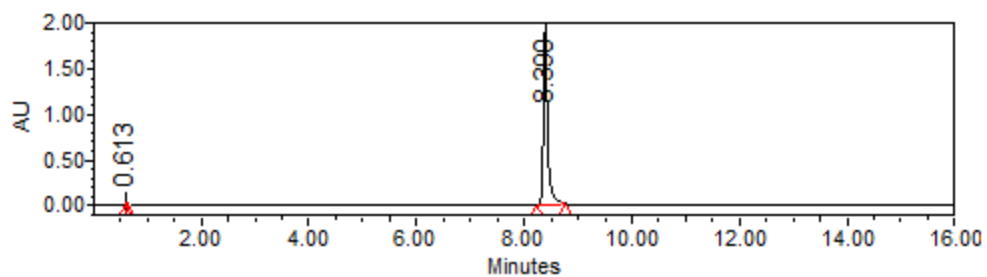


Figure 4.7. HPLC chromatogram of the chrysophanic acid reference.

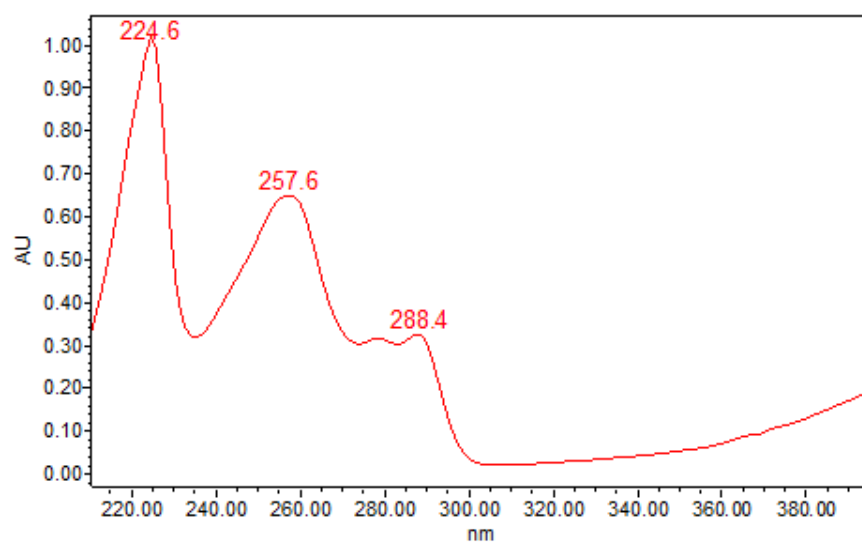


Figure 4.8. HPLC characteristic spectrum of chrysophanic acid reference.

Huber et al. [49] analyzed crysophanic acid from extraction of *Rheum palmatum* by using the wavelength of 222 nm. Crysophanic acid was dissolved in acetonitrile using an ultrasonic bath. Two points of the peaks with 0.613 and 8.300 minutes retention time were observed via HPLC analysis of crysophanic acid reference sample. Retention time with 8.300 minutes provided the highest peak areas and approximately 10 minutes needed to determine this reference sample.

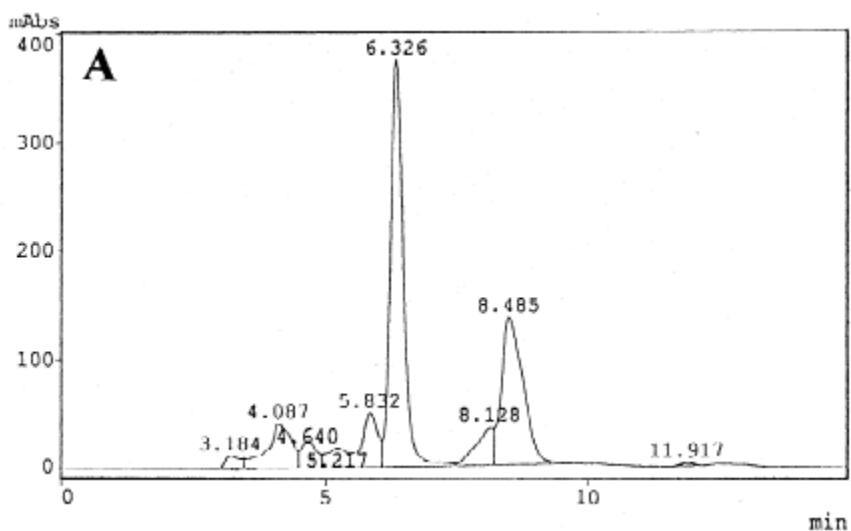


Figure 4.9. HPLC profile of prepurified extracts of *L. Inermis* (Lawsone Rt = 6.3 min) [44].

Jaziri and co-workers have cultured *Lawsonia Inermis* in vitro and found that this process depended on the iron concentration in the culture medium. Lawsone was determined in the plant using a HPLC method and the UV detector wavelength of 248 nm was used for the quantification. The characteristic retention time was determined around 6.3 minutes as shown in Figure 4.9 [44].

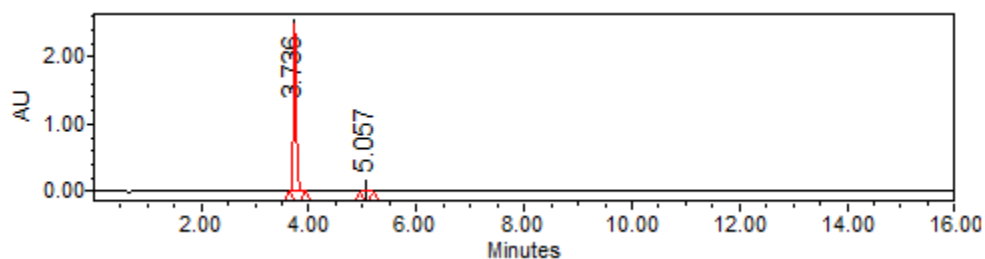


Figure 4.10. HPLC chromatogram of the lawsone reference.

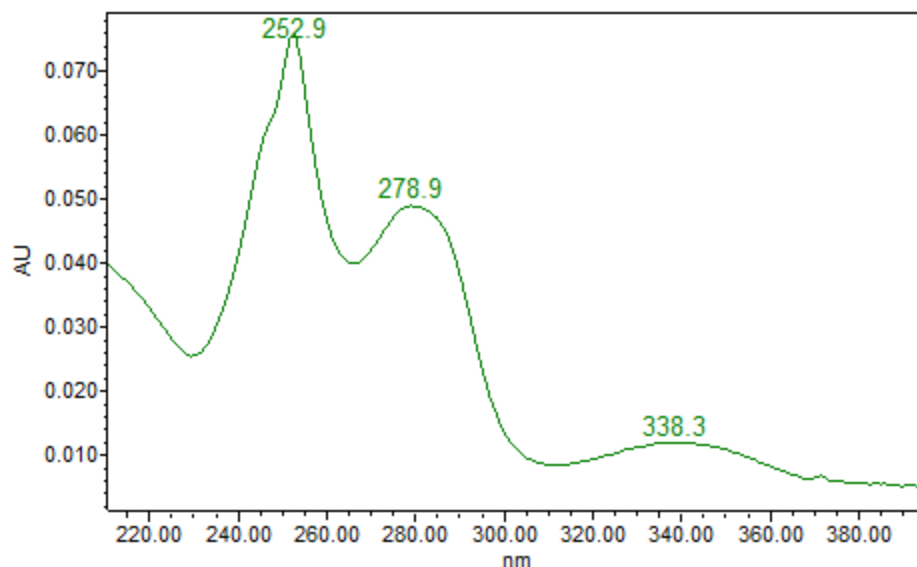


Figure 4.11. HPLC characteristic spectrum of lawsone reference.

In our studies the lawsone reference sample was dissolved in water: ethanol (90/10 v/v) solution which was the same as for PEO and PVA the electrospinning processes. The HPLC profile of lawsone reference sample is shown in Figure 4.10 and 4.11. The chromatogram was taken at two points of the peaks with 3.736 and 5.057 minutes retention time. It can be easily realized that lawsone reference sample has a different characteristic retention time, which is around 3.7 minutes, than Jaziri et al. [44] even if characteristic spectrum wavelength close to each other. The difference in response to retention time between lawsone reference sample and Jaziri et al. may be due to different forms of lawsone derivative or experimental conditions.

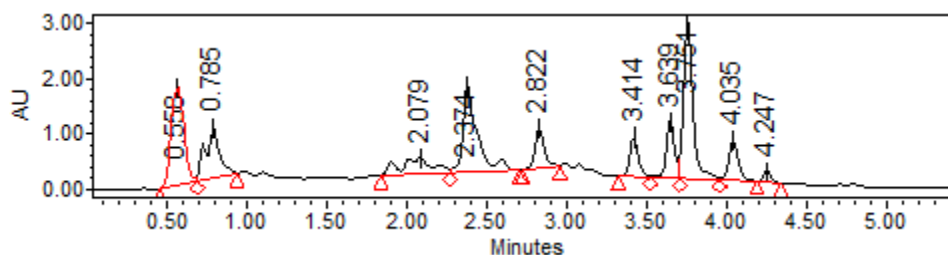


Figure 4.12. HPLC chromatogram of the extract.

Figure 4.12 HPLC chromatogram demonstrates that many polyphenols and some other compounds were present in water: ethanol henna leaf extract. Most of the chemical compounds were detected between approximately 2 and 4.5 minutes retention time and the chromatogram peak with 3.751 minutes retention time had the highest area (see Figure 4.12, 4.14, and Table 4.1) which was determined as lawsone based on the lawsone reference sample. About ten different types of compounds were detected via HPLC analysis and at least 5 minutes was needed to analyze water: ethanol henna leaf extract.

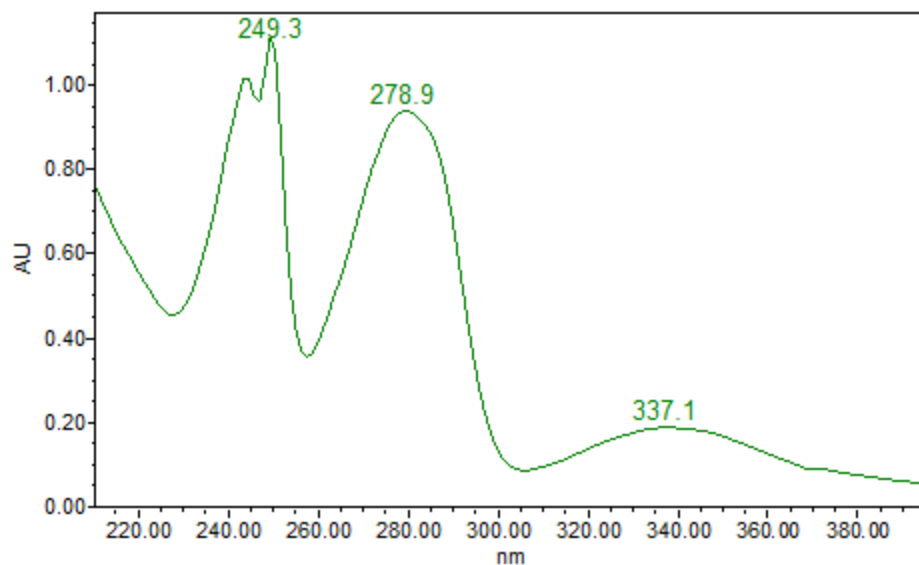


Figure 4.13. Spectrum at 3.79 minutes to get lawsone absorbance.

On the basis of HPLC analysis, only lawsone was detected in henna leaf extract (Figure 4.14) with retention time of 3.75 minutes. This compound is an active ingredient and a naturally occurring naphthoquinone (2-hydroxy-1,4-naphthoquinone) [10]. Furthermore, Figure 4.13 shows UV spectrum at 3.79 minutes for lawsone in the henna leaf extract. Other reference samples were not observed. This may be due to the wide array of structural differences of the same compound and its interaction with other compounds, also different type of solvent system [45, 46, 48] have a great effect on extract compounds level and some other reasons which were already mentioned in Section 4.1.

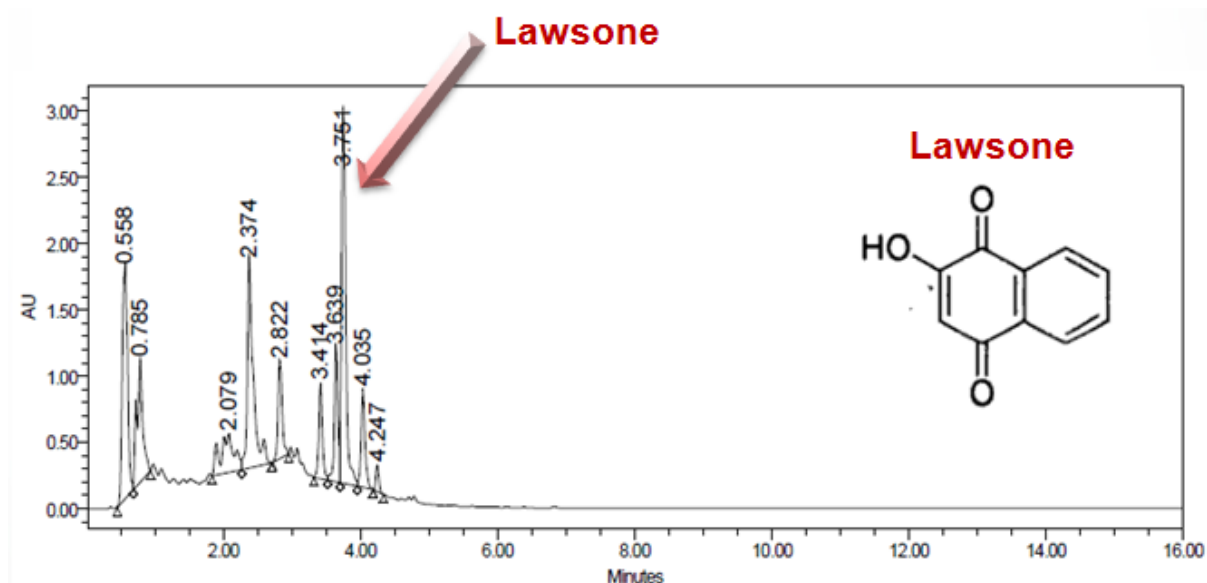


Figure 4.14. HPLC chromatogram of the extract with areas.

To determine lawsone concentration in the henna leaf extract the following procedures were performed: Water and ethanol of the extract was evaporated under vacuum at room temperature and the solid material was put into a desiccator to remove traces of water from an almost-dry sample and preserve moisture eliminate any moisture from air. The weight of the samples was determined after first after 24 hours and then 48 hours of drying until no further weight changes were observed. The concentration of extract was 34.3 mg/mL which was the mean of three replicates.

Table 4.1. Peak areas in the HPLC chromatogram for the henna extract.

Peak Number	Retention Time (minutes)	Area (%)
1	0.558	18.40
2	0.785	10.39
3	2.079	7.64
4	2.374	17.99
5	2.822	5.35
6	3.414	4.93
7	3.639	7.20
8	3.751	21.25
9	4.035	5.64
10	4.247	1.21

The concentration of lawsone in the water/ethanol extract was determined with Empower Pro software with acetonitrile as an eluent. Table 4.1 shows retention time (minutes) and area (%) for each compound. Lawsone in the henna leaf extract had a characteristic retention time of 3.751 minutes and accounted for 21.25% of the total area. Hence, its concentration in the dry extract was approximately 7.29 mg/mL. We believe that this amount of lawsone was also present present in PEO and PVA electrospun fibers.

4.3. Surface Characterization

The electrospinning process can be manipulated by a number of variables, i. e. solution properties, such as viscosity, conductivity, surface tension, polymer molecular weight, and controlled variables, for example flow rate, electric field strength, distance between tip and collector, needle tip design and ambient parameters include temperature, humidity, and air velocity which affect the structure and morphology of electrospun polymer fibers [56].

Solution viscosity, which can be controlled by the polymer concentration, is one of the most important parameter to determine fiber size and morphology of electrospun fibers. At lower concentration and viscosities, the surface tension plays a dominant effect on fiber morphology and causes the formation of beaded fibers, also droplets can be formed instead of fibers while below a certain polymer concentration is used. Furthermore junctions and bundles can be observed which indicating that the fibers on the collector are still wet [56 -57]. On the contrary, increasing polymers concentration cause increasing the solution viscosity and affect its surface tension [57], hence relatively uniform fibers with few beads and junctions can be obtained. However, if the solutions are too concentrated or too viscous, the electrospinning processes will be prevented because the solutions dried out during the flow to the tip of the needle before the jets could be initiated [56]. So many SEM images show that extremely different fiber morphologies can be observed as a result of varying degree of viscosity within the processable range. Park et al. [58] demonstrated that it is necessary for PEO solution with $[\eta]C > 10$ for “electrospinnability”. It can be seen that the variation in viscosity has very important influence on the morphological change of the electrospun fibers.

Furthermore, the most crucial process variable can be the magnitude of the electric field that a conical shape is occurred in the presence of an electric field and polymer fibers generated after a critical voltage is exceeded. Li et al. [59] examined electrospun PVA nanofibers under different spinning voltage varying from 17.5 kV to 22.5 kV that a narrow fiber diameter distribution was observed at 17.5 kV, while the broader the distribution obtained at 22.5 kV. In addition, the fiber diameter increased with increasing the spinning voltage at a constant distance from the tip to target because increasing the voltage caused to draw more solution out of the capillary. The similar result was also observed by Demir et al. [60] during producing electrospun polyurethane nanofibers. However, the fiber diameter of PEO obviously did not change as spinning voltage varied [59]. Moreover, at lower voltage or field strength bead-free fibers can be produced but higher voltage leads to the Taylor cone to recede and to form more beading morphology [56].

The electrospinning of PEO and PVA have been reported by many researchers due to various advantages of PVA and PEO. Consequently, neat electrospun PVA and PEO fiber webs as well as the electrospun PVA and PEO & fiber webs containing the henna extract were prepared to compare the apparent differences between them. The polymers solutions including the extract were individually electrospun under the optimum conditions (see Experimental) parameters to obtain nanofiber mats. In practice, a light orange coloration of the nanofiber mats was observed with the naked eye after electrospinning processes.

There were no obvious morphological differences between pure electrospun and electrospun fibers containing different concentration of LI (see Figure 4.15 and 4.17). However, it was apparent that these electrospun nanofibers are not oriented in any

preferred direction. Interestingly, some significant differences in diameter and standard deviation were observed for both set of samples Table 4.2 and 4.3 show diameter changes in the nanofibers as a function of added amount of Lawsonia Inermis.

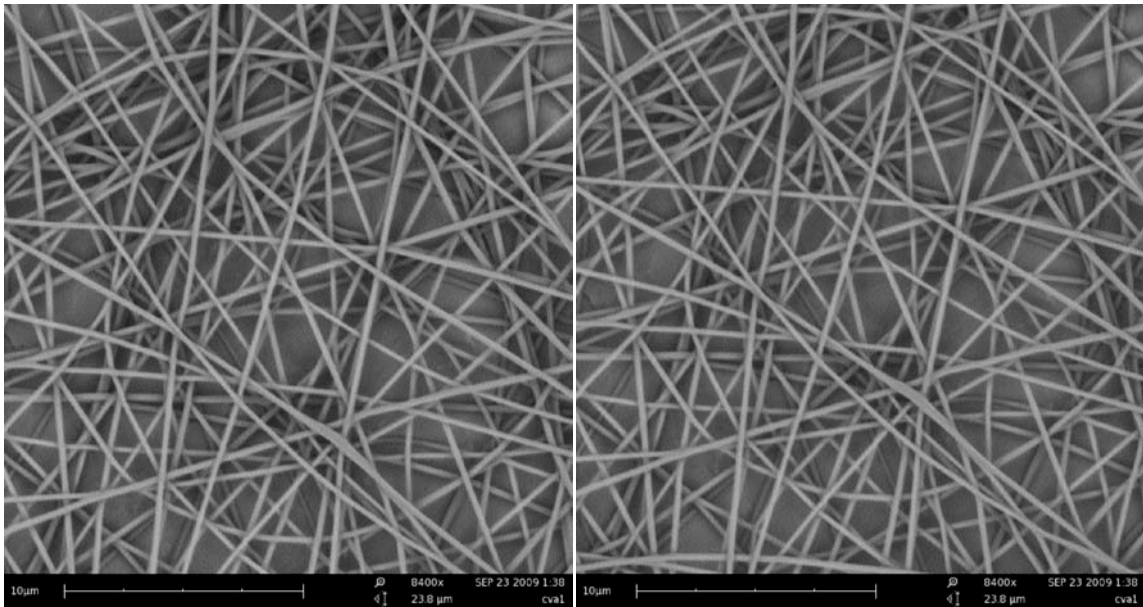


Figure 4.15. SEM image of PVA electrospun fibers.
(Scale bar = 10 μm)

The PVA nanomats had a significant number of randomly distributed as well as bound fibers per a surface unit, and therefore had film-like character and good a structural integrity.

PVA fibers appeared uniform along a fiber axis. The histogram of PVA electrospun fibers showed that most fibers diameter came around the mean diameter of 224 ± 26.06 nm.

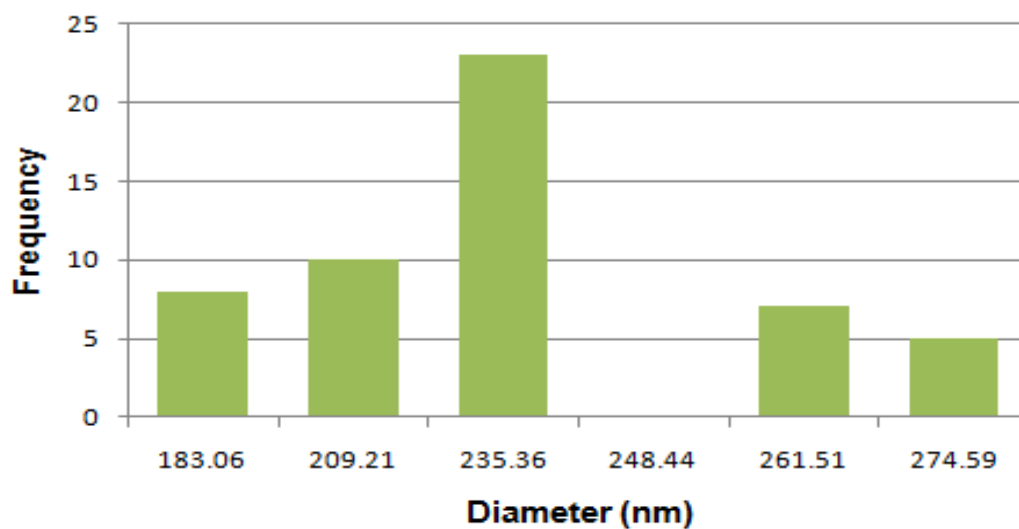


Figure 4.16. Diameter distribution histogram of PVA electrospun fibers.

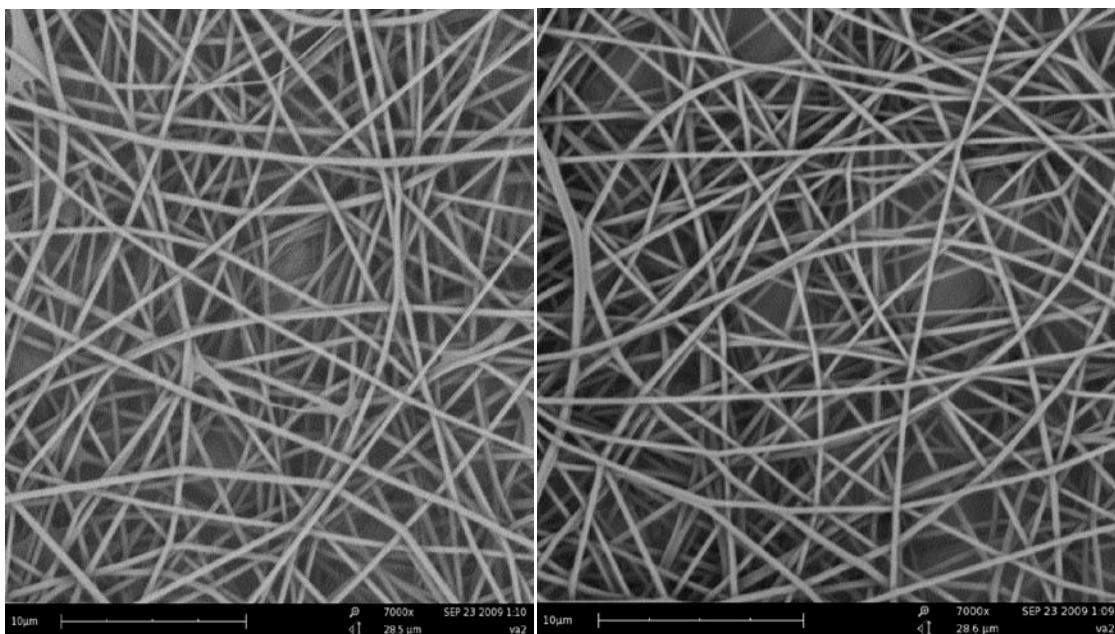


Figure 4.17. SEM image of PVA electrospun fibers containing 1.264 wt% LI.
(Scale bar = 10 µm)

SEM image of PVA unoriented fibers containing 1.264 wt% LI also appeared uniform but in detailed fiber diameter analysis revealed a higher average diameter of 257 ± 36.09 nm. A higher standard deviation of 36.09 nm was also determined. As can be seen from Figure 4.18 some fibers had a large diameter of 330 nm.

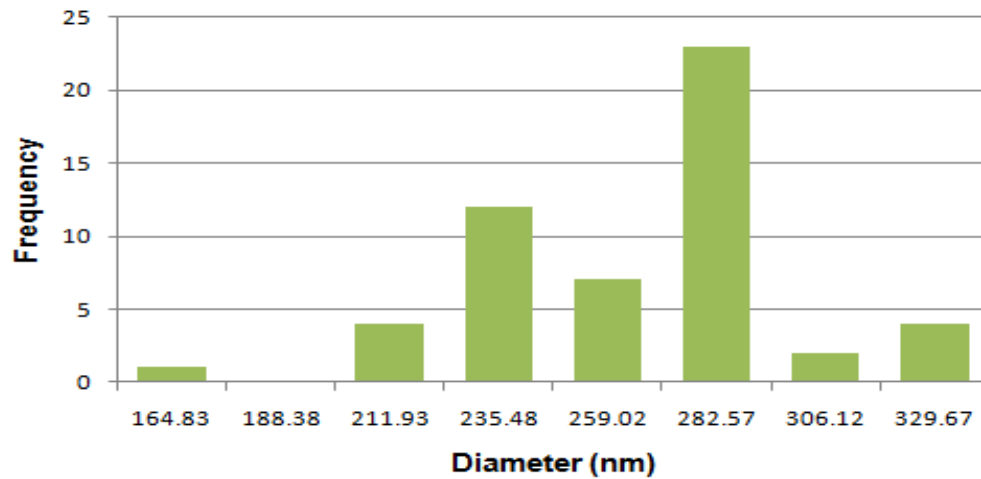


Figure 4.18. Diameter distribution histogram of PVA electrospun fibers containing 1.264 wt% LI.

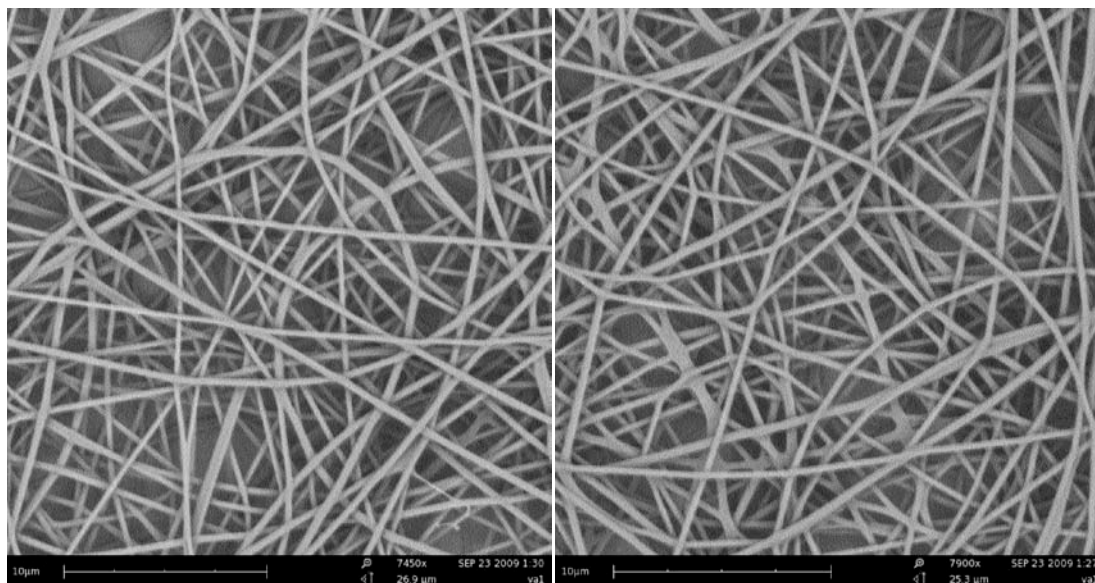


Figure 4.19. SEM image of PVA electrospun fibers containing 2.793 wt% LI.
(Scale bar = 10 µm)

PVA electrospun fibers containing 2.793 wt% LI had the highest average diameter value of 279 ± 47.77 nm and the highest standard deviation value of 47.77. These values were supported by the histogram for these fibers (see Figure 4.20). Variations in fiber diameter can also be easily seen with a naked eye from this SEM image. As demonstrated earlier this electrospun mat had also a random distribution of fibers and bond points.

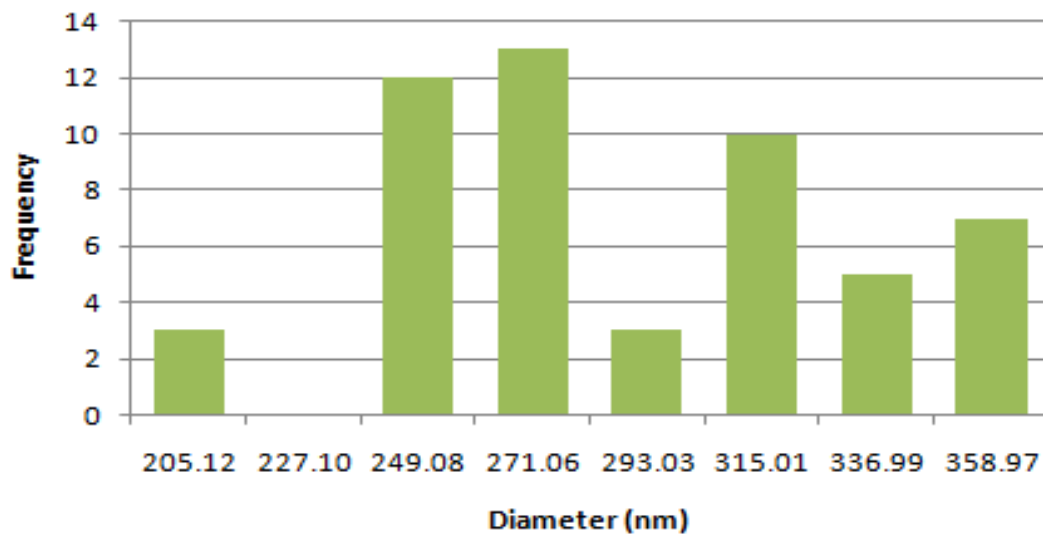


Figure 4.20. Diameter distribution histogram of PVA electrospun fibers containing 2.793 wt% LI.

Table 4.2. Diameter change in PVA electrospun fibers as a function of added amount of LI.

Material	Mean (nm)	Smallest Fiber Diameter Value (nm)	Largest Fiber Diameter Value (nm)	Standard Deviation
PVA	224	183	274	26.06
PVA & 1.264 wt% LI	257	192	329	36.09
PVA & 2.793 wt% LI	279	205	358	47.77

LI- Lawsonia Inermis leaf extract

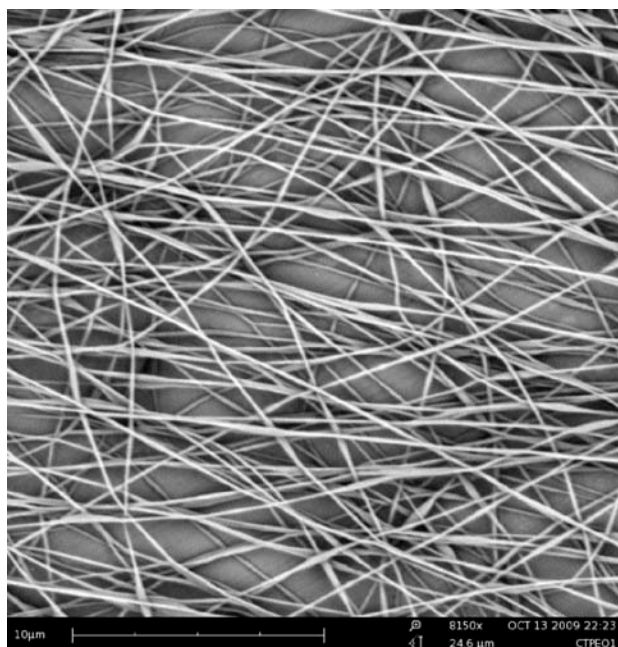


Figure 4.21. SEM images of PEO electrospun fibers.
(Scale bar = 10 μ m)

PEO electrospun fibers (Figure 4.21) looked more aligned than PVA electrospun fibers. An average diameter of 141 ± 26.48 nm was lower than PVA electrospun fibers. These fibers appeared less uniform with the standard deviation of 26.48 nm.

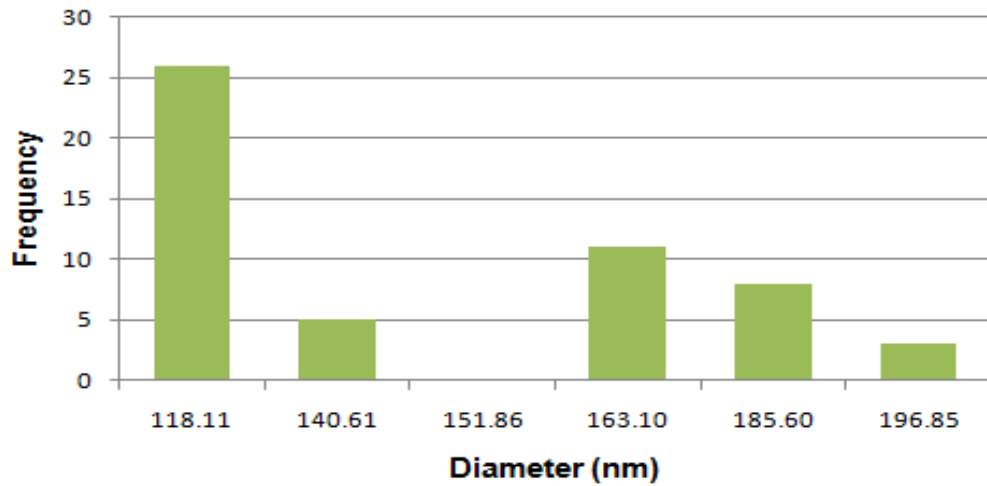


Figure 4.22. Diameter distribution histogram of PEO electrospun fibers.

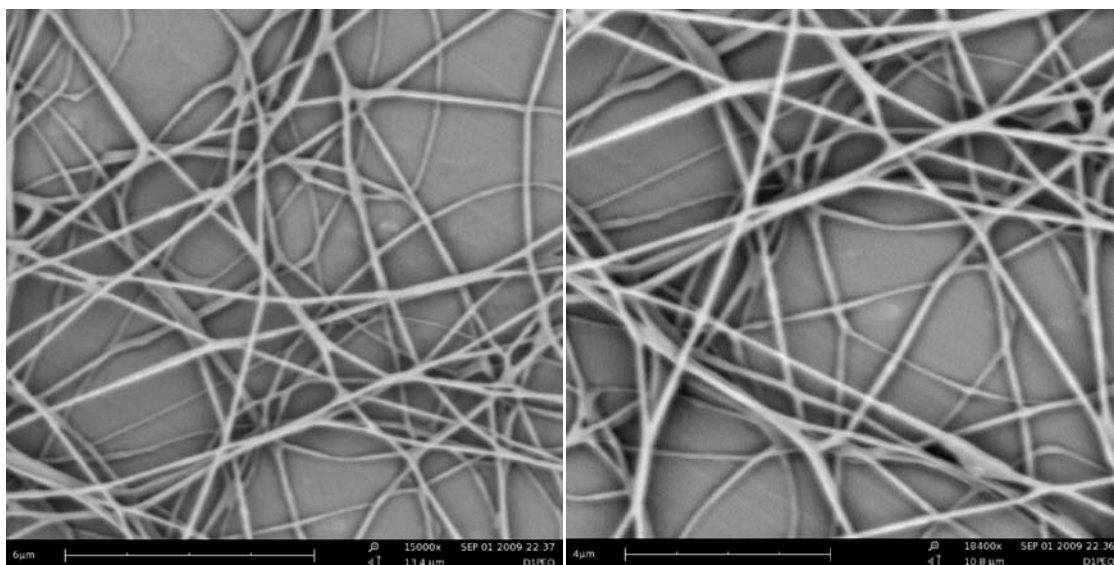


Figure 4.23. SEM image of PEO electrospun fibers containing 1.264 wt% LI.
(Scale bar = 6 μm (left), 4 μm (right))

The mat density and the appearance of PEO electrospun fibers containing 1.264 wt% LI were different from other SEM images because the electrospinning time was shorter. An average diameter of 157 ± 29.70 nm was determined for these fibers

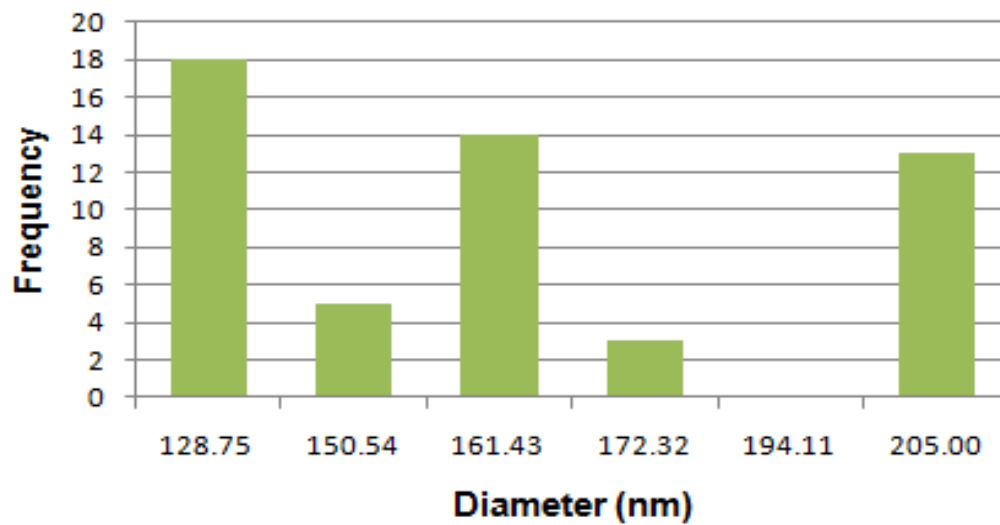


Figure 4.24. Diameter distribution histogram of PEO electrospun fibers containing 1.264 wt% LI.

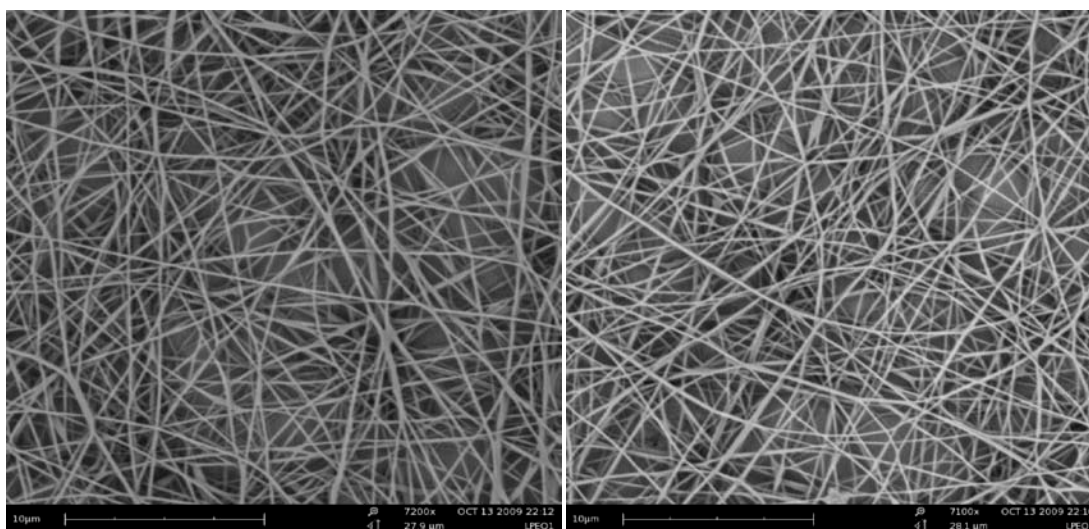


Figure 4.25. SEM images of PEO electrospun fiber containing 2.793 wt% LI. (Scale bar = 10 μ m)

Because of a longer spinning time, a SEM micrograph of PEO electrospun fibers containing 2.793 wt% LI looked similar to the PVA mats with the same LI content. A random distribution of nanofibers was observed with average diameter of 171 ± 27.70 nm.

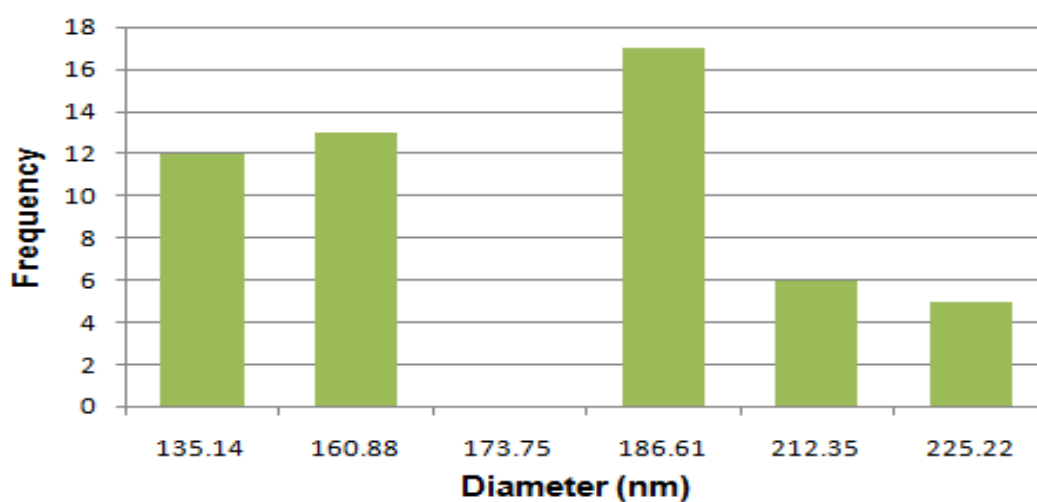


Figure 4.26. Diameter distribution histogram of PEO electrospun fibers containing 2.793 wt% LI.

Table 4.3. Diameter change in PEO electrospun fibers as a function of added amount of LI.

Material	Mean (nm)	Smallest Fiber Diameter Value (nm)	Largest Fiber Diameter Value (nm)	Standard Deviation (nm)
PEO	141	118	196	26.48
PEO & 1.264 wt% LI	157	128	205	29.70
PEO & 2.793 wt% LI	171	135	225	27.70

LI- Lawsonia Inermis leaf extract

The average diameter of electrospun PVA and PEO fibers slightly increased with an increasing extract concentration but because of high standard deviations there is no clear evidence that this effect is due to henna extract. It can be concluded that PEO and PEO fiber containing henna extract had much lower fiber diameter than those for corresponding PVA fibers.

4.4. Antibacterial Activities of the PVA and PEO-Based Solutions

Antibacterial analysis of the solutions and electrospun fibers was determined by using the zone of inhibition equation given by (Sarkar et al. 2007):

$$W = \frac{(T - D)}{2}$$

Where: **W** - width of clear zone of inhibition, mm; **T** - total diameter of test specimen and clear zone, mm; **D** - diameter of the test specimen in mm.

In general, the material is considered active against the bacteria when the zone of inhibition is greater than 6 mm [38, 55].

The release behavior of the 'leaching' antibacterial agents of the extract compound from electrospinning solution and other solution containing extract demonstrated that *S. aureus* were killed at 2.793 wt% LI, thus demonstrating a strong bactericidal effect. The experiment showed (Table 4.4) that *E. coli* survived, but could not grow to colonies onto the solution, because the extract compound inhibited the growth of bacterial cells demonstrating the bacteriostatic effect. On the contrary, the antibacterial efficiency of 1.264 wt% LI for both electrospinning solution and extract solution also showed some bactericidal and bacteriostatic effects with the ghost zones around and onto the samples observed which

indicates that colonies growing inside the zone may have acquired resistance against the henna compounds.

In addition, Habbal et al. [10] and Saadabi et al. [3] demonstrated bactericidal activities against *S. aureus* and *E. coli* with a 50 % concentration LI and 100 mg/ mL henna extract, respectively. It can be seen that their concentrations were much higher and our leaves were cultivated from Egypt but they obtained their leaves from Omani and Sudanese. As we mentioned in the previous section the amount of all possible phenolic compounds, which are mainly responsible for antibacterial activities, can be extracted via different methods and solvent system so total phenolic compounds present in the solution might be different. Also, many environmental factors, time of the harvest, different plant and climate conditions can affect the total phenolic compounds in the henna leaf. In important to mention that, the antibacterial activity of naphthoquinone products alkannin and shikonin and their derivatives was effective against Gram positive bacteria but inactive against Gram negative bacteria [10].

Table 4.4. Zone of inhibition (ZOI) data for LI and PEO and PVA electrospun mats.

Concentration of Polymer and LI	ZOI for <i>E. coli</i> (mm)	ZOI for <i>S. aureus</i> (mm)
1.264 wt% LI	1.5	5
2.793 wt% LI	2.5	12
4 wt % PEO	<1	0
4 wt %PEO &1.264 wt% LI	1	3.5
4 wt% PEO & 2.793 wt% LI	2	10
7 wt %PVA	<1	0
7 wt %PVA & 1.264 wt% LI	0.5	3.5
7 wt % PVA & 2.793 wt% LI	2	9

LI - Lawsonia Inermis leaf extract

4.5. Fourier Transform Infrared Spectroscopy -FTIR

FTIR spectra analysis proved that PEO and PVA based electrospun fibers contained the henna extract. The presence of the extract in the fibers from PVA/Lawsonia Inermis and PEO/Lawsonia Inermis can be verified by the appearance of the peaks corresponding to the pure extract.

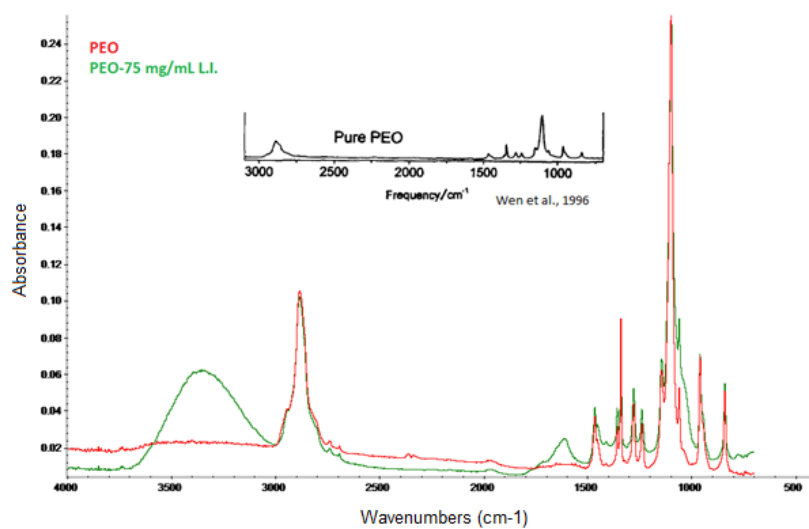


Figure 4.27. Comparison of FTIR spectra with pure PEO and PEO- 2.793 wt% LI.

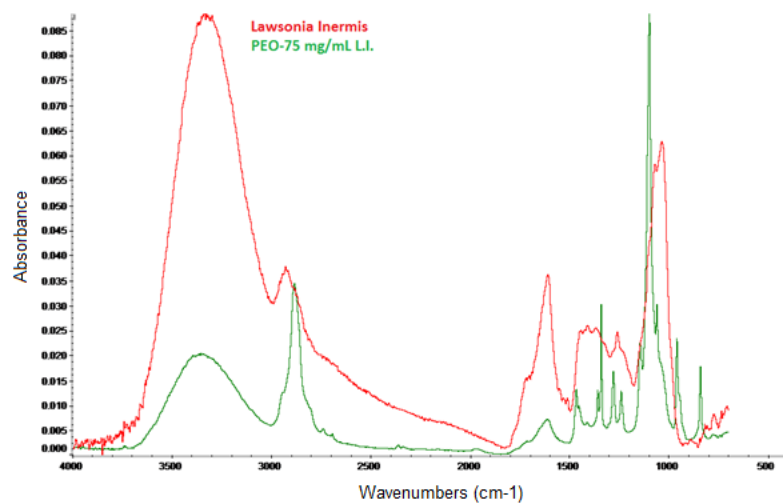


Figure 4.28. Comparison of FTIR spectra with the pure extract and PEO containing 2.793 wt% LI.

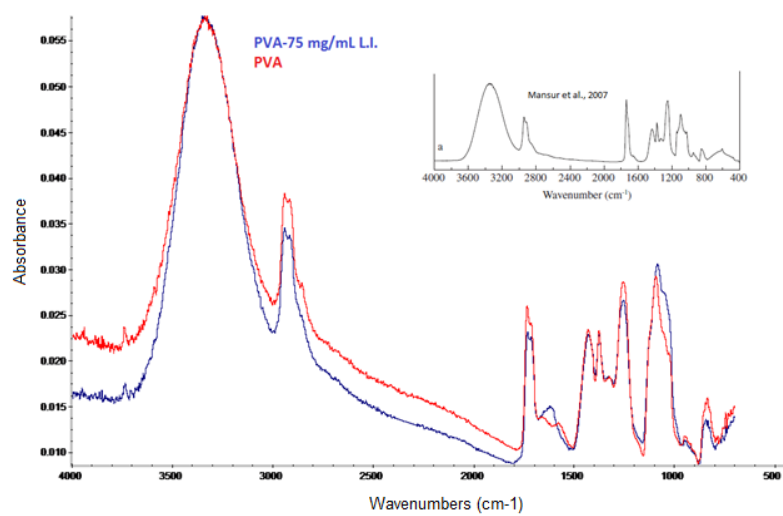


Figure 4.29. Comparison of FTIR spectra of pure PVA and PVA with 2.793 wt% LI.

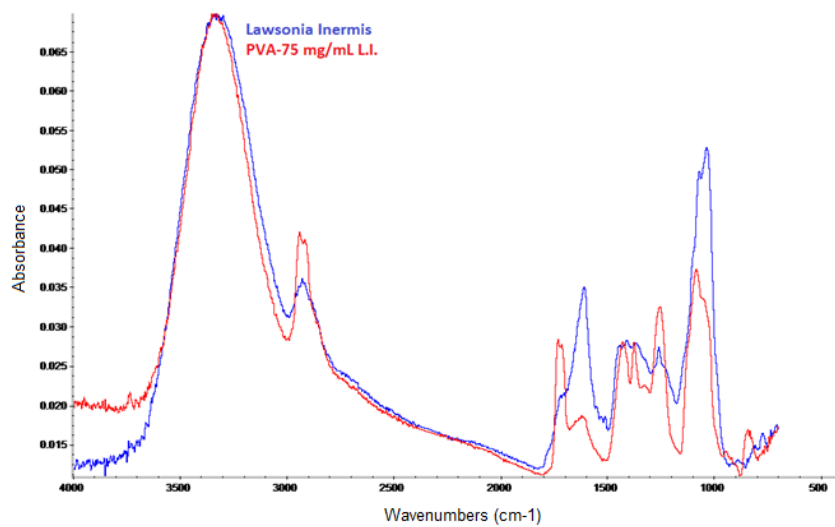


Figure 4.30. Comparison of FTIR spectra with the pure extract and PVA with 2.793 wt% LI.

Table 4.5. Functional groups and classes of the leaf extract compounds absorb in the infrared.

Wave numbers (cm ⁻¹)	Group and Class
3325.5	-OH
2930.1	-CH ₃ and -CH ₂ in aliphatic compounds
1611.5	Benzene ring in aromatic compounds, or pyridine derivatives
1412.9	C-N in primary amides
1261.7	Epoxides, or C-N in aromatic amines, or C-O-C in esters, lactones
1037.2	CH-O-H in cyclic alcohols, or CH ₂ -O-H in primary alcohols

Chapter V. Conclusion and Future Directions

Henna leaf extract was incorporated with PVA and PEO electrospun fibers. PEO and PVA based electrospun fibers containing henna extract were verified by the appearance of the FTIR peaks corresponding to the pure extract.

The present study demonstrates that 2.793 wt% LI in the PVA and PEO based solutions showed bactericidal effect against *S. aureus* and bacteriostatic action to *E. coli*. The concentration of henna leaf extract had a very strong relationship between antibacterial activities against *S. aureus* and *E. coli* bacteria. Henna leaves have a great potential to be used as a source of a potent eco-friendly antimicrobial agent.

We recommend to electrospin PEO and PVA based fibers with LI on bandages preferably made from polypropylene, polyester, nylon or polyurethane. The hydrophilic PEO or PVA layer could function and be effective as an antibacterial agent against Gram positive bacteria. The outer layer of a bandage should be hydrophobic and offer the protection against moisture and oxygen. Henna extract can easily release as an antimicrobial agents while in contact with microbe or the infected skin area. According to our work and other references, such as Habbal et al. [10] and Saadabi et al. [3], stronger antibacterial efficiency can be observed with a higher extract concentration.

The average diameter of electrospun PVA and PEO fibers slightly increased with an increasing extract concentration but because of high standard deviations, there is no clear evidence that this effect is due to henna extract.

Lawsonia Inermis dry cut leaf sample is relatively cheap and natural, and can be extracted just with de-ionized water. The extract can be easily incorporated with PVA and PEO polymers to get solutions for the electrospinning processes.

On the other hand, it would be advantageous to develop a method to extract all possible classes of phenolic compounds in henna leaf. Consequently, the maximum efficiency for antibacterial activities might be observed and it can be used to study effectively with any other treatment, such as to inhibit and manage burn wound infection, albinism, skin abrasion, etc.

Laboratories around the world have found literally so many chemicals by extraction of plants which are natural and eco-friendly and promising for use in medical applications. Furthermore, a combination of a henna extract with some other medical plant extracts can be used for improved antimicrobial efficiency and extend application areas. The key point is to find methods to incorporate the chemicals to the medical products which also need to be economically profitable.

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