

**Fabrication of Electrospun PVA Nanofibers Incorporated with Silver
Nanoparticles: Antibacterial Application**

Mahsa Zarian Deroo

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By: Mahsa Zarian Deroo

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_____	Chair
Dr. M. Z. Kabir	
_____	Examiner, External
Dr. C. Mulligan (BCEE)	To the Program
_____	Examiner
Dr. A. J. Al-Khalili	
_____	Supervisor
Dr. M. Kahrizi	

Approved by: _____

Dr. W. E. Lynch, Chair

Department of Electrical and Computer Engineering

_____ 20_____

Dr. C. W. Trueman

Interim Dean, Faculty of Engineering
and Computer Science

ABSTRACT

Fabrication of Electrospun PVA Nanofibers Incorporated with Silver Nanoparticles: Antibacterial Application

Mahsa Zarian Deroo

PVA nanofibers incorporated with silver nanoparticles produced from electrospinning have higher surface area to volume ratio, which could be useful for many environmental health and safety purposes such as water disinfection. Although there are several works done regarding the silver nanoparticles and its application as a bactericidal agent, there are no reports available on immobilized silver nanoparticles incorporated in PVA nanofibers and their application against *Escherichia coli* using growth inhibition in liquid medium, which is the primary cause of many serious diseases associated with contaminated water. We have used electrospinning technique to fabricate PVA/AgNO₃ nanofibers and also investigated the effect of several operational parameters such as applied voltage, collection distance, flow rate and some solution parameters like concentration and conductivity on average fiber diameter. Structures were characterized using SEM, TEM and EDX spectroscopy.

The antibacterial tests were performed by growing *E.coli* in liquid LB medium supplemented with pure PVA and PVA/ AgNO₃ and results confirmed the antibacterial activity of immobilized PVA nanofibers incorporated with silver nanoparticles while pure PVA showed no inhibition of bacterial growth. Decreasing the ratio of PVA/ AgNO₃ by increasing the silver content enhanced the antibacterial efficiency of the fabricated nanofibers.

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LIST OF ACRONYM

AFM	Atomic Force Microscope
AgNO ₃	Silver Nitrate
CaCl ₂	Calcium Chloride
°C	Celsius
DC	Direct Current
DI	De-Ionized Water
DMF	Dimethylformamide
E.Coli	Escherichia Coli
EDX	Energy-Dispersive X-ray Spectroscopy
FE-SEM	Field Emission Scanning Electron Microscope
LB	Luria-Broth
MW	Molecular Weight
HV	High Voltage
NaCl	Sodium Chloride
NSF	National Science Foundation
PA6	Polyamide 6
PAMPS	Poly (2-acrylamido-2-methyl-1-propanesulfonic acid)
PAN	Polyacrylonitrile
PEG	Polyethylene Glycol
PEO	Polymer of Ethylene Oxide
PLA	Poly lactic Acid
PS	Polystyrene
PU	Polyurethane

PVA	Polyvinyl Alcohol
SiO ₂	Silicon Dioxide
STM	Scanning Tunneling Microscope
TEM	Transmission Electron Microscopy
THF	Tetrahydrofuran
UV	Ultraviolet light

Chapter 1

1. Introduction

1.1. Nanotechnology and Nanoscience

The nanoscale science began in 1905 when Albert Einstein published a paper on his experimental data about the diameter of a single sugar molecule to be 1nm. [1] But the birth of nanotechnology began in 1959, when the Nobel Prize laureate physicist Richard Feynman [2] delivered a famous talk titled “There is Plenty of Room at the Bottom”. He was the first person who proposed Nanotechnology. He suggested “someday it would be possible to put the entire 24 volumes Encyclopedia Britannica on the head of a pin” [2]. Dr. Feynman attracted his audience attention to biological cells, he pointed out that “although cells are very tiny, they are very active; they manufacture various substances; they walk around; they wiggle; and they do all kinds of marvelous things all on a very small scale”. Also, he noted that computing machines could be miniaturized to a very small scale in order to measure the properties of small-scale structures. In the 1980s, Gerd binning and Heinrich Rohrer [3] invented the Atomic Force Microscope (AFM) and the Scanning Tunneling Microscope (STM) in IBM Research - Zurich and they began to do what Dr. Feynman talked about it.

In 1986, K. Eric Drexler [4] released his famous book, titled “Engines of Creation: The Coming Era of Nanotechnology”. It was the first book about Nanotechnology. His book describes the idea of nanoscale “assembler” or in other words self-replicating which is

able to copy of itself.

According to the dictionary definition, Nano is “extremely small or one-billionth ”[5] Hence; the scale of nanoscience is nanometer [6]. Nanotechnology is based on the ability to manipulate, control and reduce atoms and molecules to scale down components, tools, systems and devices. In other words, at the small scale, materials show remarkable physical and chemical properties, which have a great potential for a variety of applications such as medicine, electronic, environment, etc. [6]

Nanoparticles are being used in several industries; the reason that nanoparticles attracted such interest is its distinct properties compared to the same material in bulk. They possess larger surface areas to volume ratios than materials in the bulk [7] so they have many applications in medical and water disinfection [8-12]. Nanoparticles constituted of several hundreds of atoms or molecules, which have at least one dimension less than 100nm [13]. Metal nanoparticles such as silver attracted a lot of attention due to their unique properties. They have been utilized in a wide range of applications and they are very effective biocides against fungi and bacteria [12,14-16].

Today worldwide, specially undeveloped countries are in need of clean drinking water; for this reason a cost effective and efficient way to purify the infected water is needed. Bacteria are the common contaminants in water, which give rise in waterborne diseases; hence antimicrobial modification of water to prevent growth of bacteria is a highly desired objective. Today the most common technique used for treatment of water is the addition of chemical disinfectants such as chlorine and chloramines as well as membrane-based water

filtration [8] however, the diseases still continue to occur due to some side effects of chlorine, such as toxicity, which raised some concerns about human health.

Disinfection of water, using metal nanoparticles is a recent method. The main drawback of using nanoparticles is that they tend to congregate and disperse from the solution, which leads to a reduction of their antibacterial efficacy. One way to stabilize them and avoid aggregation is incorporation with various polymers. Polymers have a long chain of molecules with repeating structural units called monomers [17]. They have many properties, which makes them suitable for many applications. Polymers such as polyvinyl alcohols are excellent candidates for incorporation of nanoparticles into them because of their excellent physical properties, chemical resistance, great fiber formation property and more importantly non-toxicity [18]. One way to incorporate nanoparticles into polymers is producing nanofibers, which are discussed in the following section.

1.2. Polymer nanofibers

Nanofibers according to National Science Foundation (NSF) have a measured diameter of less than a micrometer with a large surface area to volume ratio, which makes them a great candidate for many applications. Polymer based nanofibers are materials with unique properties that have larger surface areas per unit mass [19] and so by incorporation with nanoparticles they are considered for many applications such as water treatment [20-24] and wound dressing [25]. Polymeric fibers have been fabricated by several techniques such as: drawing [26-28], template synthesis [29-32], phase separation [33], and Electrospinning [18,32,34-37]. Each of these methods has advantages and disadvantages, which are summarized in Table 1.1

Table 1.1. Advantages and disadvantages of different techniques for producing polymer nanofibers [19].

Technique	Pros	Cons
Drawing	Minimum equipment required	Material limitation
Template synthesis	Fibers with different diameters could be obtained by employing different templates	Cannot produce one-by-one Continuous fiber
Phase separation	Minimum equipment required	Material limitation, time consuming
Electrospinning	Simple one-step process, continuous nanofibers	Low productivity

1.3. Literature Review

As mentioned previously, there are various ways for obtaining polymeric fibers. In the following, the first four techniques are briefly reviewed but a comprehensive review is done on the research and developments related to electrospinning and especially on electrospun polymer nanofibers containing metal nanoparticles.

Drawing: This method is very similar to dry spinning process. In this method by evaporation of solvent, long single nanofibers with the lengths over hundreds of microns are obtained [26]. Ondarcuhu and Joachim [26] demonstrated that fibers could be fabricated by extruding them one at a time from a droplet of sodium citrate solution during solvent evaporation. In their method a micropipette was dipped into a droplet, which was placed on SiO₂ surface, then it was withdrawn from the droplet and resulted in

a fiber, afterwards the produced fiber was placed on a surface. The method was repeated many times on a droplet of solution. They also mentioned that this method is just suitable for polymeric viscoelastic material, which endure strong deformation and would not tear apart during the formation of nanofibers.

Harfenist *et al.* [27] demonstrated a one-step method for fabricating suspended fibers using a sharp tip. In their work, a polymer solution droplet is applied to a sharp tip and the tip drawn fibers over an array of silicon tips. By doing it subsequently a series of suspended fibers were obtained as shown in Figure 1.1. The main drawback of this method is the gathering of the polymer solution on the tip, which leads to blocking of the tip.

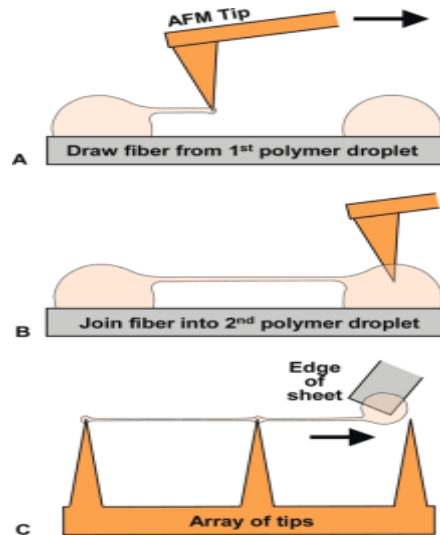


Figure 1.1. Fabrication technique for drawing polymer nanofibers. (A) Drawing fiber from polymer droplet. (B) Joining the drawn fiber into second droplet. (C) Drawing polymer fibers in parallel [27].

Nain *et al.* [28] proposed a method in which suspended fibers were fabricated within micro/nano sizes. As shown in the following schematic (Figure 1.2) they put a substrate

in contact with a droplet of polymer, which was pumped inside the micropipette, and then they moved micropipette in a specific trajectory and formed solid fibers due to the evaporation of solvent. By repeating they obtained sequential fibers.

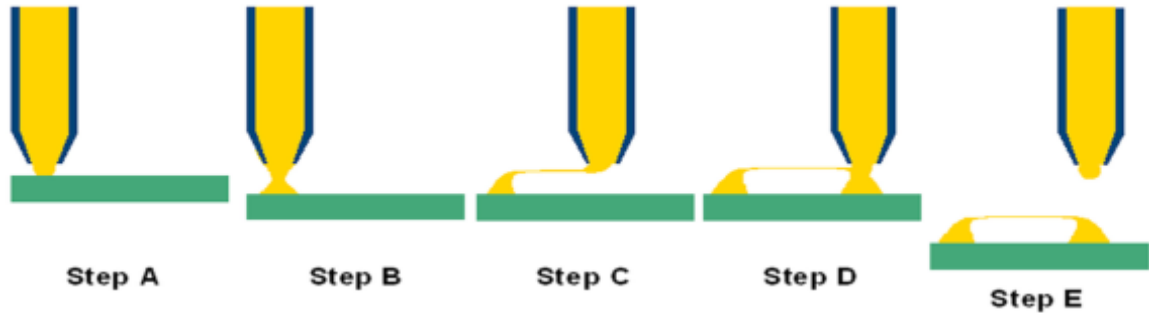


Figure 1.2. Steps in forming and drawing a single fiber. (Step A) substrate was lifted to get in touch with the droplet; (Step B) micropipette was moved at a constant height.;(Step C) moving micropipette in a specific trajectory; (Step D) substrate was touched with the micropipette to suspend the fiber; (Step E) substrate was lowered to break the fiber [28].

Template synthesis: as the name offers is the use of template to obtain fibers or hollow (tubule)[29]; Feng *et al.* [30] produced fibers with diameter in range of nano by using an anodic aluminum oxide membrane as a template, which was produced by anodization of aluminum in acid [31]. As shown in Figure 1.3, in their work, water pressure pushed water through porous membrane and resulted in protruding and solidification of the solution, which leads to obtaining nanofibers with diameters determined by porous [30]. Figure 1.4 illustrates three images taken by Scanning Electron Microscopic (SEM) from

the template, surface of PAN nanofibers and cross-sectional of the nanofibers. The main drawback is that, it cannot produce one-by-one continuous fibers [32].

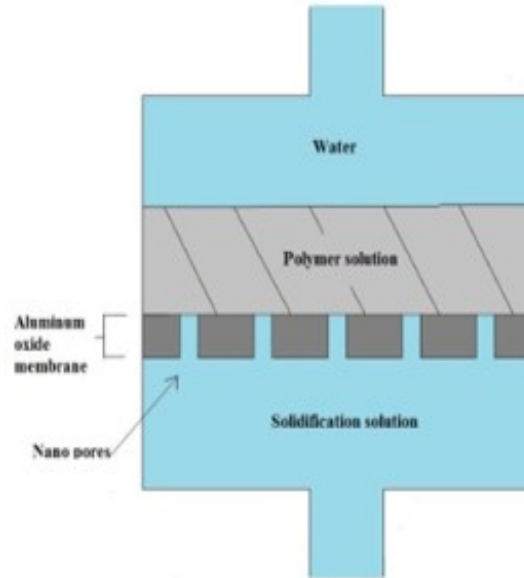


Figure1.3. Schematic of template synthesis method. By applying water pressure, polymer solution will extrude from nonporous template in the form of nanofibers [19].

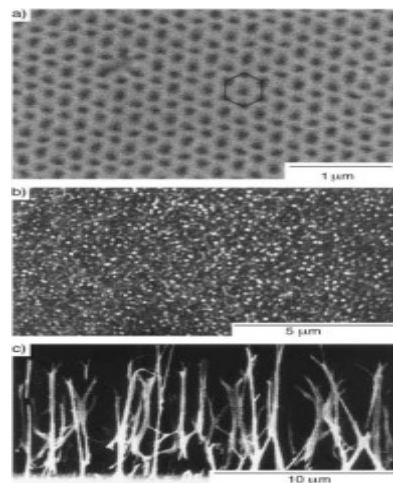


Figure1.4. SEM images of a) template; b) view from the surface of the PAN nanofibers; c) cross-sectional view of fibers [30].

Phase separation: this technique involves 5 steps as follows: dissolution, gelation, extraction, freezing and drying. According to Ma and Zhang [33], at first polymer is dissolved in solvent, then transferred into the refrigerator for gelation, next with the use of distilled water, solvent was extracted from gel and left the porous nanofibrous behind. Then the gel transferred to the refrigerator and finally the frozen gel freeze-dried under vacuum. Figure 1.5 exhibits a schematic view of the steps. The major drawback of this technique is that it is relatively time-consuming to make porous nanofibrous from solid polymer.

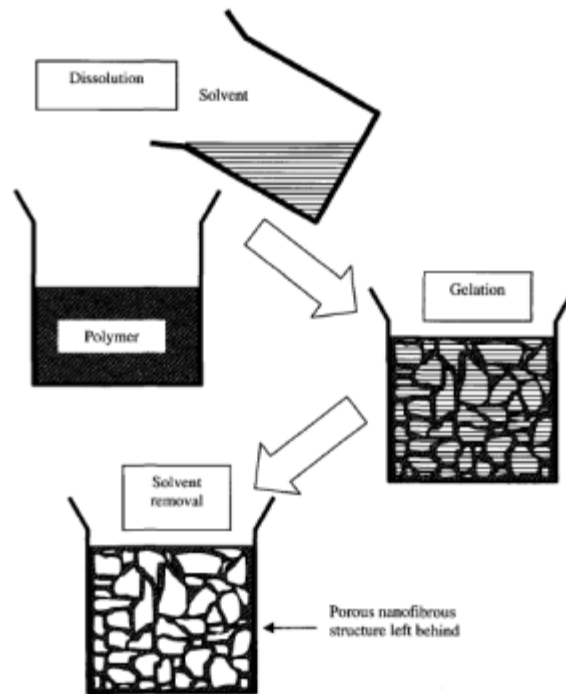


Figure1.5. Schematic of phase separation method [19].

Electrospinning: the physical phenomena relating to electrospinning goes back to 17th century when William Gilbert [38] observed a spherical drop of water which was drawn

up toward a piece of rubbed amber, deformed into a conical shape. The idea of using electrospinning, as an application to spin fibers was first put forward by William Morton [39] in his patent in 19th century. He created fibrous masses by using some sources of high tension of static electricity. Further development toward electrospinning was done by John Zeleny [40] in 1914. He designed a capillary device for observing the behavior of the liquid. However, Electrospinning process was first pioneered by Anton Formhals [41] in 1934. He is known through his patent as a father of electrospinning technique. Formhals produced threads of fibers from a cellulose acetate solution and collected them in spools using electrostatic force. Following his work, Vonnegut and Neubauer in 1952 [42] produced a fine thread of droplets by applying different voltages in the range of kilo volts to a liquid by a wire into a glass tube which was drawn down to a capillary. Knowledge about electrospinning had improved by Simons [43] in 1966 that patented an instrument and accomplished very thin and relatively continuous fibers by employing electrical spinning. The idea of electrospinning was further developed by Baumgarten [44] in 1971. He made an instrument to electrospun fibers from a polyacrylonitrile/ dimethylformamide (PAN/DMF) solution with diameters ranging from 0.05 to 1.1 micrometer. Since 1980, nanotechnology gained more attention among researchers probably due to the easy fabrication of fibers with diameters down to submicron from polymers and employment for various applications in the field of filtration, wound healing [25] and drug delivery [45-48].

A broad range of fiber architecture can be derived from electrospinning of polymer solutions including smooth fibers, porous fibers and flattened fibers just by varying the parameters. Figure 1.6 shows various fiber architecture.

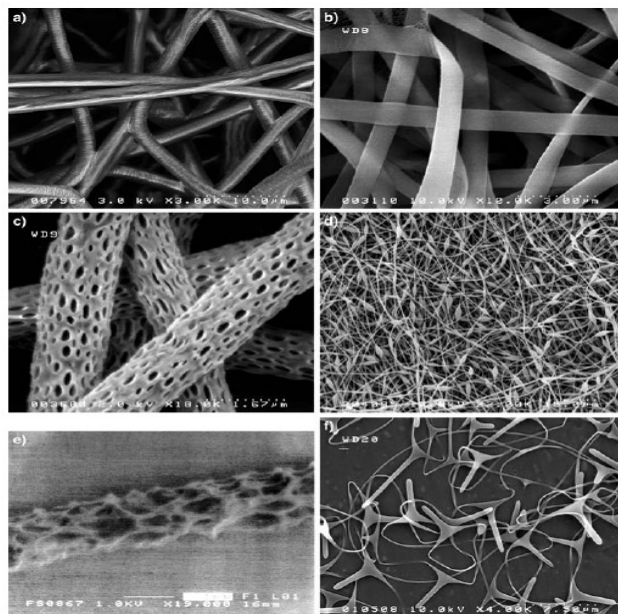


Figure 1.6. Various fiber architectures: a) Smooth circular PA6 fibers, b) Ribbon like PA6 fibers, c) Porous PLA fibers, d) PAN fibers with spindle type disturbance, e) Fractile shape structures is PLA fibers, f) Barbed PVA fibers [48].

McCann *et al.* [34] described a way for producing porous fibers by using a mineral oil as the core, and poly (vinyl pyrrolidone) (PVP) containing titanium iso-propoxide as the shell. The inner core was extracted by dipping in octane and leaving porous fibers behind. Koski *et al.* [18] produced flattened nanofibers by increasing polymer concentration and molecular weight. Varying these parameters has effect on the fiber diameter as well which will be discussed in the next chapter.

Produced electrospun nanofibers have an extremely broad range of applications due to their unique intrinsic structure and surface properties. One of the interesting applications of nanofibers is in treating large wounds such as burns. Hong [25] produced nanofibrous webs by electrospinning and used them as a wound dressing. These nanofibers webs

showed great adhesion even to moist wounds. The wounds heal fast if covered by a thin web of electrospun nanofibers especially from a biodegradable polymer. These nanowebs provide enough pores for the exchange of liquid and gasses with the surrounding environment but the size distribution of the pores are in a way that bacteria cannot pass through.

As drug delivery carriers, electrospun fibers have many advantages. Electrospinning demonstrated great potential in drug delivery applications. Drugs could be loaded on nanofibers easier than other methods. High specific surface area of the fibers and short diffusion passage length result in higher overall release rate. Changing the fiber morphology and porosity can control the release rate. Drugs such as anticancer and antibiotics can be delivered toward electrospun fibers. [45] Delivery of anticancer drugs attracted attention of the researchers in the last few years. Jing Zeng *et al.* [46] encapsulated drugs into the Poly L-Lactic acid (PLLA) fibers and observed the constant release of drugs by degradation of the fibers, which is suitable for clinical application.

Another interesting application of electrospun non-woven webs is in filtration; electrospun nanofibers have already been used as air filtration membranes, but little research had been conducted on electrospun nanofibers in water treatment due to the concern about capability of nanofibers against water pressure. Many researchers have studied incorporation of metal nanoparticles into different polymers for producing antibacterial nanofibers [20-24].

W. Son [20] prepared ultrafine cellulose acetate fibers containing silver nanoparticles and investigated the antibacterial activity of it. In their work, size and numbers of the silver nanoparticles were increased by UV irradiation for 2 hours as shown in Figure 1.7.



Figure 1.7. TEM images of the cellulose acetate nanofibers containing 0.5 wt% AgNO_3 at UV irradiation time (a) 0 min, (b) 10 min, (c) 60 min, (d) 120 min, and (e) 240 min [20].

Prashant and Pradeep [22] coated polyurethane (PU) foams with silver nanoparticles and used it as a water filter. In their work PU foams showed a strong antibacterial activity against bacteria. In another work, Gangadharan [23] developed a method by containing macroporous methacrylic acid copolymer beads with silver nanoparticles and used it for water disinfection. The copolymer beads showed a great inhibition zone against bacteria. Up to now, obtained results revealed the potential use of nanofibers for many applications.

1.4. Objective of the Research

The present work is an attempt to study and investigate the fabrication of fine polyvinyl alcohol nanofibers containing silver nanoparticles for possible application in water disinfection. The present study identifies the parameters such as concentration, voltage, collection distance, flow rate and conductivity, which govern electrospinning process. The analyses were done to observe the structure and diameter of nanofibers, number and size of the nanoparticles using different equipment such as scanning electron microscope, energy dispersive x-ray spectroscopy and transmission electron microscope respectively. In order to investigate the possibility of antibacterial activity of produced nanofibers, they were exposed to gram-negative bacteria. For this, a number of plates were prepared and samples were shaken into bacteria at two contact times and then placed in petri dishes to observe the inhibition growth of bacteria. It is anticipated that this work provides a relatively comprehensive instructions to fabricate antimicrobial fine PVA nanofibers with silver nanoparticles.

1.5. Organization of this thesis

In the next chapter, Chapter 2, electrospinning apparatus and parameters are presented. Experimental procedure to fabricate PVA nanofibers and a brief overview on the characterization and measurement techniques such as SEM, TEM and EDX are provided in Chapter 3. Chapter 4 discusses the experimental results and characterization of the fabricated samples. Chapter 5 demonstrates the application of produced nanofibers as an antimicrobial agent. Conclusion, contribution and future work are presented in Chapter 6.

Chapter 2

2. Electrospinning process and parameters

2.1. Objective of the chapter

As the title implies, this chapter describes electrospinning process and parameters. Electrospinning is a unique and cost effective method for fabricating novel fibers with diameter in the range of nanometer. There are some factors that involve on fiber morphology and diameter, which are classified into three groups. The first group is operational conditions such as electric potential, flow rate, collection distance and diameter of the needle. The second group is the intrinsic properties of the solution such as molecular weight of polymer, concentration, viscosity and conductivity; and the third group is ambient parameters, which include temperature of surroundings and humidity. By changing these parameters, fibers with different diameters are obtained. This section is vitally important to observe different parameters of electrospinning and also to understand the characterization of electrospun nanofibers in order to see their potential application.

2.2 Electrospinning of nanofibers

Recently, electrospinning has attracted attention because it is a unique and very flexible technique, which allows manufacturing of long nanofibers in the range of nanometer. Also as mentioned earlier, electrospinning yields long nanofibers with different morphologies such as smooth fibers, ribbon like fibers, and odd shaped fibers [48].

2.2.1 Description of Apparatus

Electrospinning apparatus consist of two main components as follows: High voltage DC power, and syringe pump. In addition, there are other components such as a charged solution, a container for the solution such as a syringe, a pipette and a metal collector. The basic principle of this process is that an electric charge in the range of kilo volt is applied to a solution, which held by its surface tension, to produce fibers from liquid. The capillary tube and collector typically serve as electrodes and the distance between them is set in the range of 6cm to 20 cm. A syringe pump is used to control the flow rate and the syringe filled with polymer is used to pump the solution into the needle. The needle is connected to the positive charge and the collector is attached to the ground. Figure 2.1 shows the schematic of the electrospining set-up.

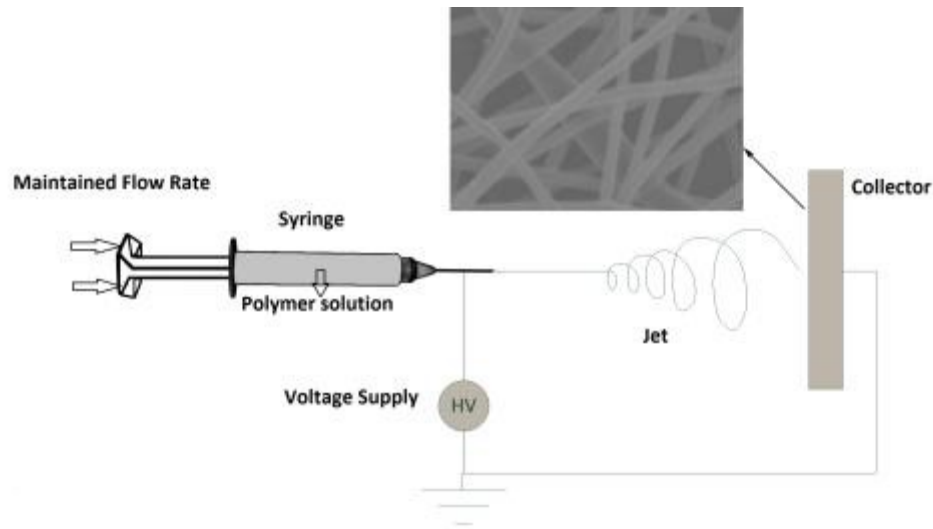


Figure 2.1. Schematic diagram of electrospinning apparatus

2.2.2 Formation of Taylor Cone

Electrical forces caused by the high potential electrical field are a driving force on fluid. If no electric field is applied, the fluid will protrude slightly from the spinneret and some drops will be seen. When fluid is subjected to an electric field, the negatively charged ions are attracted to the opposite direction, while the positive charged ions tend to be repelled down toward the spinneret tip. By increasing the intensity of the electric field, positively charged ions move closer to the collector and alter the form of drop into conical shape known as Taylor cone [49]. When voltage exceeds a critical value, the electrical forces overcome the surface tension, resulting in ejection of the jet from the cone towards the collector. As the jet accelerates in the air, the solvent evaporates and polymer fibers are left behind. Figure 2.2 illustrates Taylor cone formation.

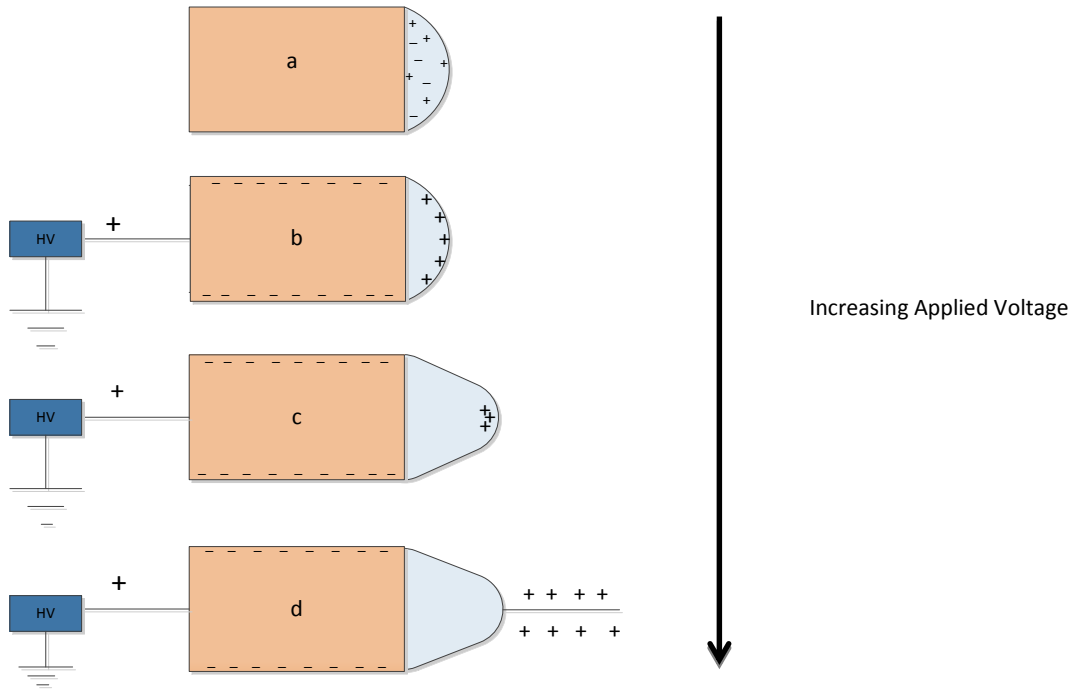


Figure 2.2. Schematic of Taylor cone formation at different stages. **a)** Before applying voltage, the liquid forms a suspended spherical shape which is caused by surface tension and then drops slightly from the capillary orifice **b)** By applying voltage to the solution, the shape of the solution deforms and negatively charged ions are attracted to the wall of capillary while positively charged ions tend to be repelled down from the orifice. **c)** As the voltage increases, the collector attracts the charges and the shape of the drop changes into a cone known as Taylor cone [49]. **d)** Finally, when the voltage passes the critical value, the repulsion force between the same charges overcomes the surface tension of the liquid and results in an eruption and acceleration of the jet from the tip of the needle towards the collector.

2.2.3 Formation of Bending Instability

Initially, the jet moves in a linear trajectory, but then it begins to whip out spontaneously in a three-dimensional way or in other words bend into a complex path [50] due to the repulsive electrostatic forces between the charges that causes the jet to move in a spiral

shape. Secondly, as the circular shape grows, the jet becomes thinner and secondary instability develops [51]. Figure 2.3 illustrates the start of bending instability. In Figure 2.4, it can be seen that the jet path was straight but then at a particular distance it bends into a coil and after some turns, the coil was transformed into smaller one and then by evaporation of solvent the nanofibers left behind.

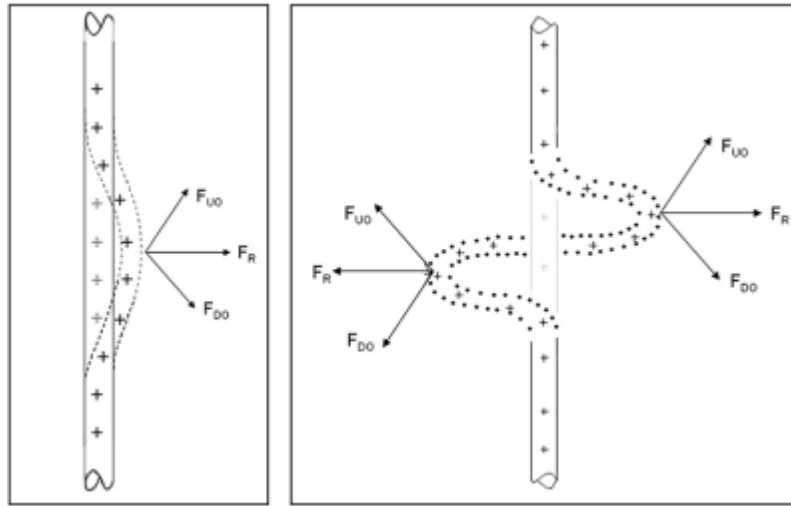


Figure 2.3. Electrospinning bending instability. (A) The charges were forced down (F_{D0}) and up (F_{U0}) due to the repulsive forces between the uniformly distributed charges and resulted in F_R . (B) F_R grew exponentially with respect to the straight jet and the repulsion of the adjacent charges lead to elongation of bending of the jet [50].

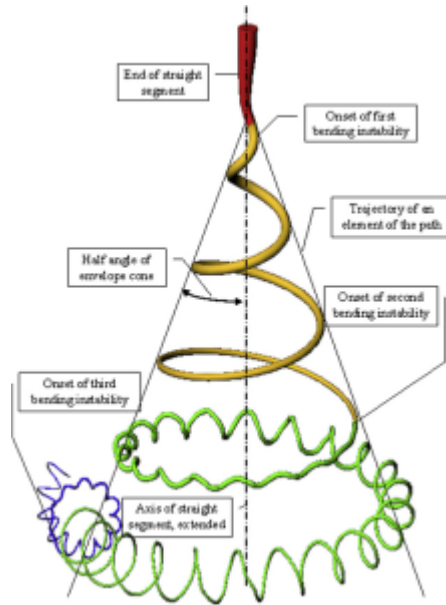


Figure 2.4. Schematic of electrospinning jet that contains three bending instabilities. At first jet moves in a straight path but afterwards it undergoes a series of electrically driven bending instabilities due to the mutually repulsive electrostatic forces and at last jet solidifies by evaporation of the solvent [50].

2.3. Operational conditions

As long as the polymer sample is susceptible to nanofiber formation through electrospinning, some of the targets could be achieved, including: production of fibers with consistent and controllable diameters, producing defect-free fibers, and also collecting the continuous nanofibers. Perhaps the most important quantity in electrospinning is the fiber diameter. Various parameters are involved in determining the diameter of the electrospun nanofibers, which will be discussed in the following.

Applied voltage: The initiation of electrospinning requires high voltage, which creates charges on the solution, and then the repulsive forces in the charged solution must overcome the surface tension in order to start the electrospinning process [19]. As it was mentioned before in section 2.2.2, at this point the droplet is deformed into a cone and in

order to observe a stable cone, an applied voltage than threshold is required [19]. According to Zhang *et al.* [35] the variation in the applied electrical voltage showed remarkable effect on the diameter distribution of the fibers, however, it did not have a notable influence on the diameter of the electrospun fibers. Increasing the voltage could change physical appearance of fibers. It was reported by Zong *et al.* [36] that unlike the low voltage, there was a great tendency in formation of highly beaded structures when the voltage was high. Figure 2.5 illustrates the beaded structures of PDLA (poly (D,L - lactic acid)) membranes when the voltage is very high.

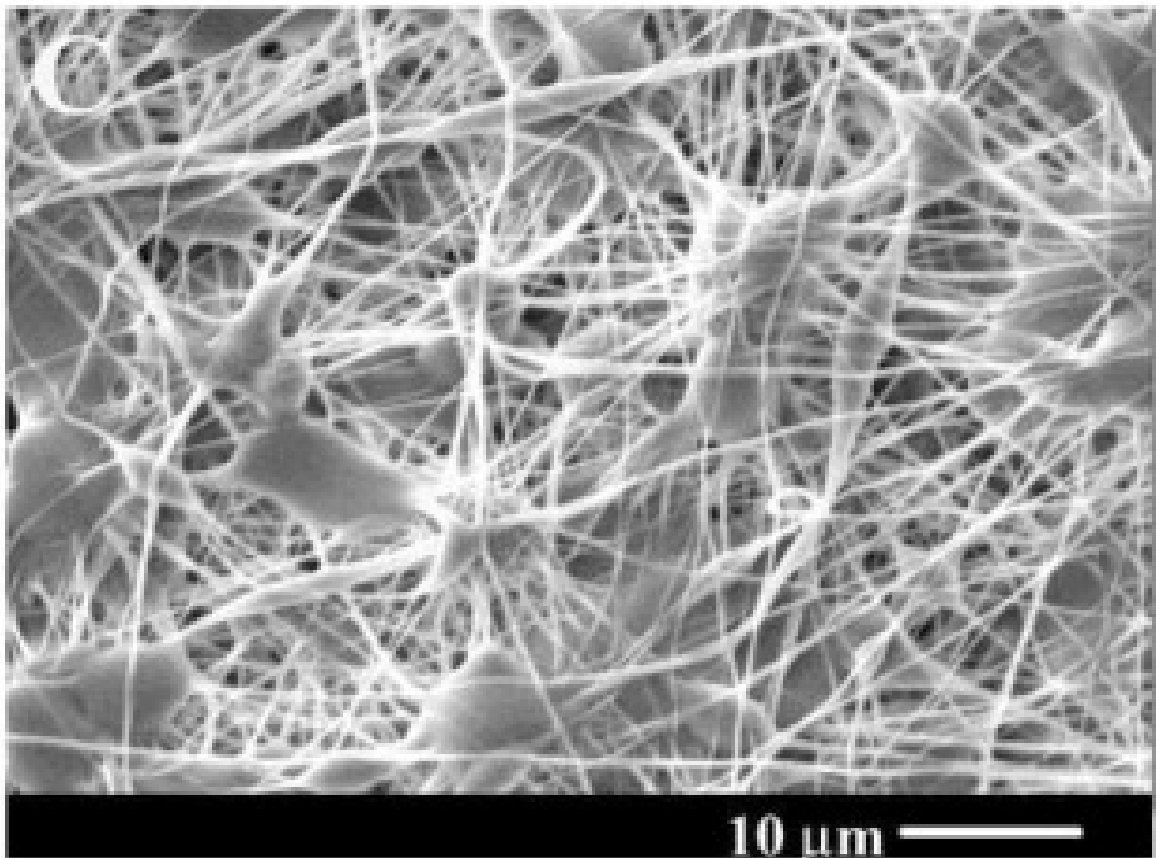


Figure 2.5. Effect of high voltage on the electrospun PDLA membranes [36].

Flow rate: It will indicate the amount of solution available for electrospinning process [19]. The precise flow rate adjustment results in achieving a stable Taylor cone during the electrospinning. Chowdhury [53] obtained flattened web-like nanofibers at high flow rate as shown in Figure 2.6, he also concluded that lower flow rates are more desirable for observing Taylor cone. In another study, Zhang [35] investigated the effect of flow rate and observed by increasing flow rates, fiber morphology changed from fine fibers to beaded fibers.

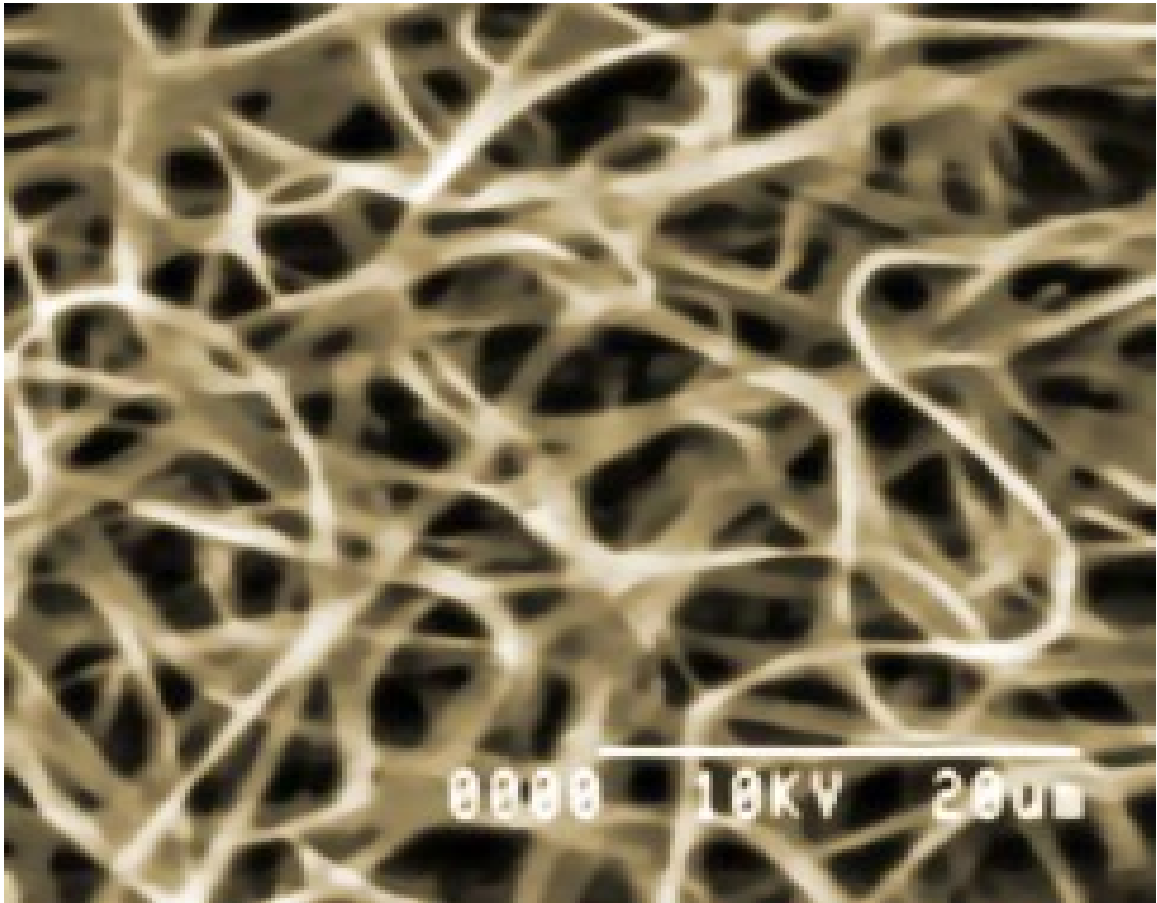


Figure 2.6. Flattened Web-like morphology of electrospun Nylon 6 as the result of high flow rate [53].

Collection distance: It refers to the distance between the tip of the needle or pipette and the collector. The material chosen for collector should be highly conductive in order to create potential difference between the source and collector, and also allowing more fibers to be deposited on the collector. Depending on the solution properties, increasing and decreasing the collection distance may or may not have impact on the fiber deposition and formation [19]. Buchko and Shen [55] reduced the deposition distance between tip to collector and observed wet fibers hitting the collector and excess solvent caused fiber to merge together (Figure2.7).

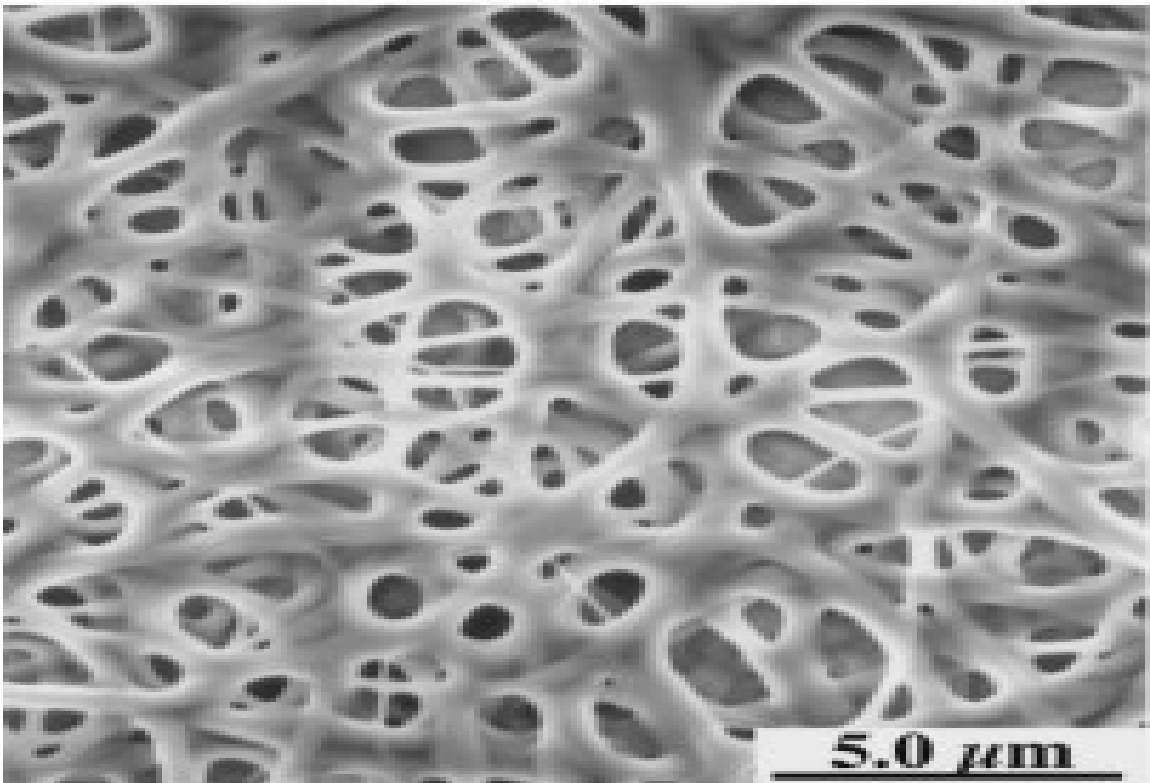


Figure 2.7. Formation of flat fibers from electrospun nylon at low collection distance [55].

Solution Temperature: An increase in temperature has influence on many polymer solutions, which decrease the viscosity and increase the evaporation of solvent. In other words, there is an inverse relationship between temperature and viscosity. At high temperature, Mit-uppatham *et al.* [56] observed no distinction between the appearances of as-spun PA-6-32 fibers, but fibers with smaller diameter were obtained as shown in Figure 2.8. The reason for obtaining fibers with small diameter is due to the reduction in viscosity of the solution, which led further stretching of the solution.

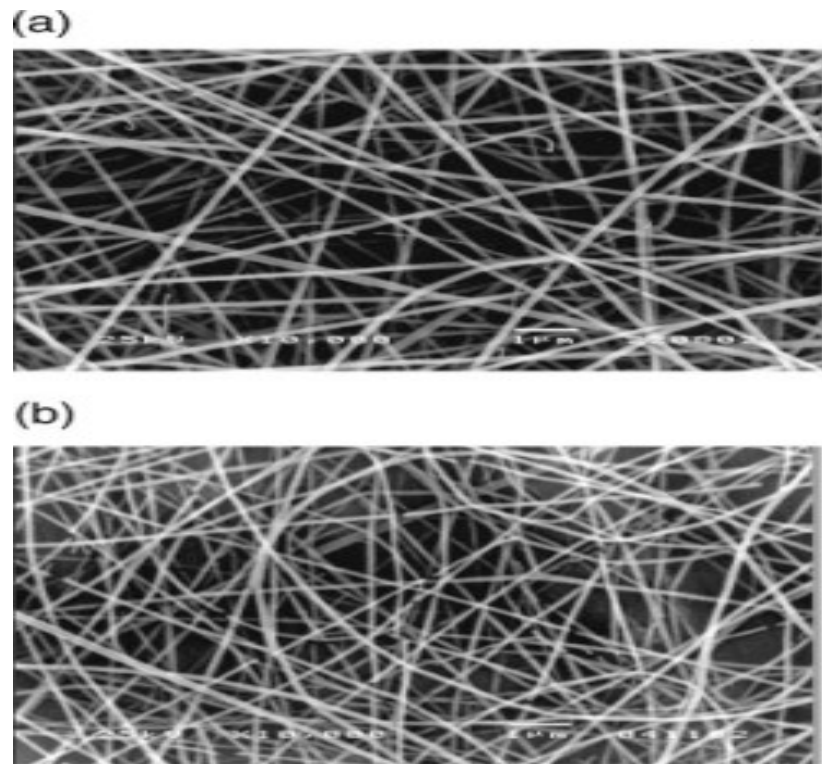


Figure 2.8. SEM images of as spun PA-6-32 at temperature of a) 30°C and b) 60 °C [56].

Needle diameter: The greater or smaller internal needle diameter may or may not have correlation with fiber diameter. Macossay *et al.* [57] found that average fiber diameter is independent from internal needle diameter; in contrast it was reported by Ho Wang Tong *et al.* [58] that increase in the internal diameter cause an increase in the diameter of obtained fibers; however in today's experiments the internal needle diameter is constant at 18G (inner diameter of 0.84mm). According to Mo *et al.* [59] fiber morphologies were changed by varying internal diameter of needle from beaded- structure to non-clogging structures.

2.4. Ambient Conditions

As mentioned earlier, electrospinning is governed by many parameters; one of them is the ambient parameter. It includes temperature of surroundings and humidity, which have affect on the diameter of electrospun fibers. These conditions have to be designed precisely in order to achieve desirable fibers.

Temperature: the variations in temperature have notable effect on average fiber diameter. Dao and Jirsak [60] examined the effect of temperature of surrounding environment of spinning area and found that fiber diameters are temperature dependent and by increasing temperature, average fiber diameter decreased. In another study, Vrieze *et al.* [61] observed that decrease in ambient temperature will result in reduction of evaporation rate of the solvent and longer solidification time of the jet and all lead in decrease in average fiber diameter.

Humidity: the variations in humidity play a notable role in determining morphology of fibers during electrospinning. Casper *et al.* [62] demonstrated randomly distributed pores by increasing the humidity as shown in figure 2.9. Uppatham [56] also investigated that at high moisture in the air, the appearance of fibers were like small circular pores; and further increasing in humidity caused the greater sizes of the pores. In addition, humidity has effect on the evaporation of the solvent, for instance; Baumgarten [44] investigated that low humidity during electrospinning caused the removal of the solvent from needle quicker than the evaporation of solvent and lead to the blocking of needle tip.

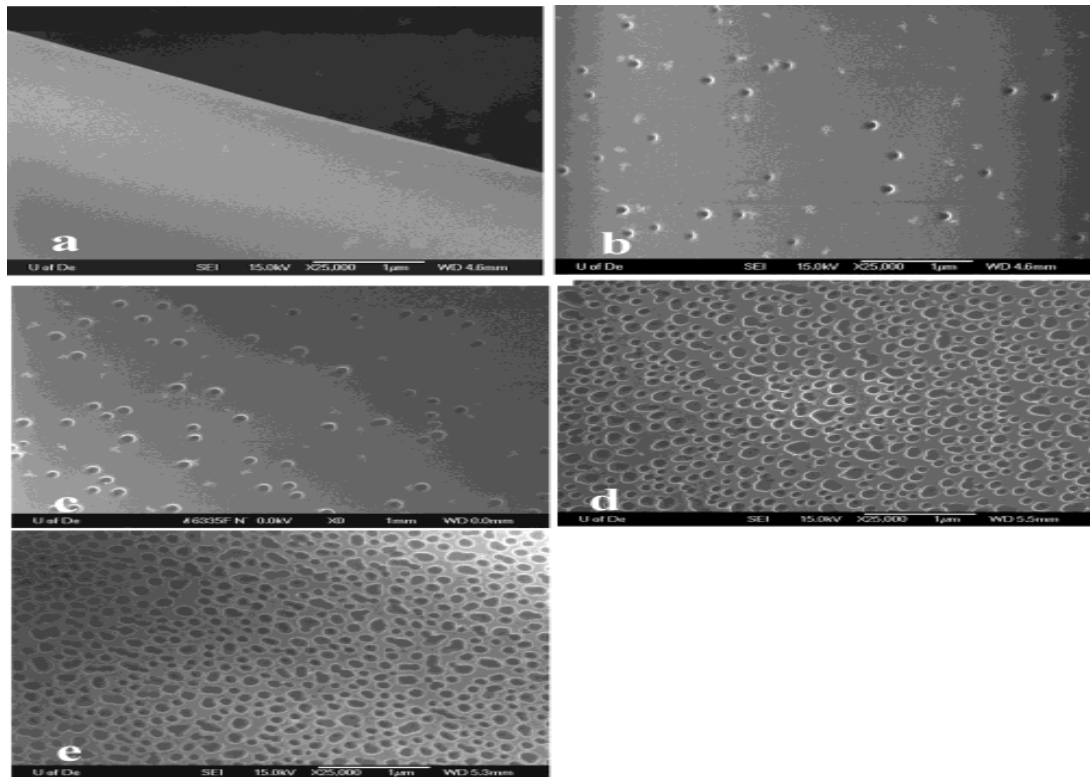


Figure 2.9. Scanning electron microscope images of PS/THF fibers at different humidity [62].

2.5 Polymer Solution Parameters

The characteristics of electrospun fibers are also determined by solution viscosity or molecular weight, conductivity and concentration. The properties of polymer solution have a significant effect on diameter of the resultant fiber.

Molecular weight: it determines the length of polymer chains and influences the viscosity of the solution at a given concentration. By dissolving a higher molecular weight polymer in a solvent, solution with higher viscosity is obtained which leads to the greater degree of entanglement of polymer chains and therefore it prevents the formation of drops at the needle tip during the electrospinning. But it should not be neglected that when the viscosity is too high, the syringe pump cannot pump the solution through the needle properly and electrospinning process fails [36]. Moreover, Jacobs *et al.* [63] observed that at low viscosity (low molecular weight) it is probable to find more circular beads on the collector while at higher molecular weight, but of the same concentration, beads started to disappear and fibers with spindle like morphology as illustrates in figure 2.10 were formed which indicates the stretching of the polymer solution. They also observed that by increasing molecular weight of PEO, average fiber diameter increased as well. It can be concluded that by decreasing the viscosity of the solution (lower molecular weight) the evaporation rate of the solvent increase, hence, fibers are dry when they deposit on the collector and their shape is more like spherical beads. On the contrary, increasing molecular weight leads to reduce of the evaporation rate and hitting of wet fibers on the collector plate with flat shape [19].

Variations in molecular weight have an influence on fiber diameter, Koski *et al.* [18] observed a broader range of fiber diameter distribution and increase in average fiber

diameter, by increasing the molecular weight of the polymer solution. This could be because of the solution resistance for stretching [19] therefore the molecular weight of the polymer plays a significant role during electrospinning.

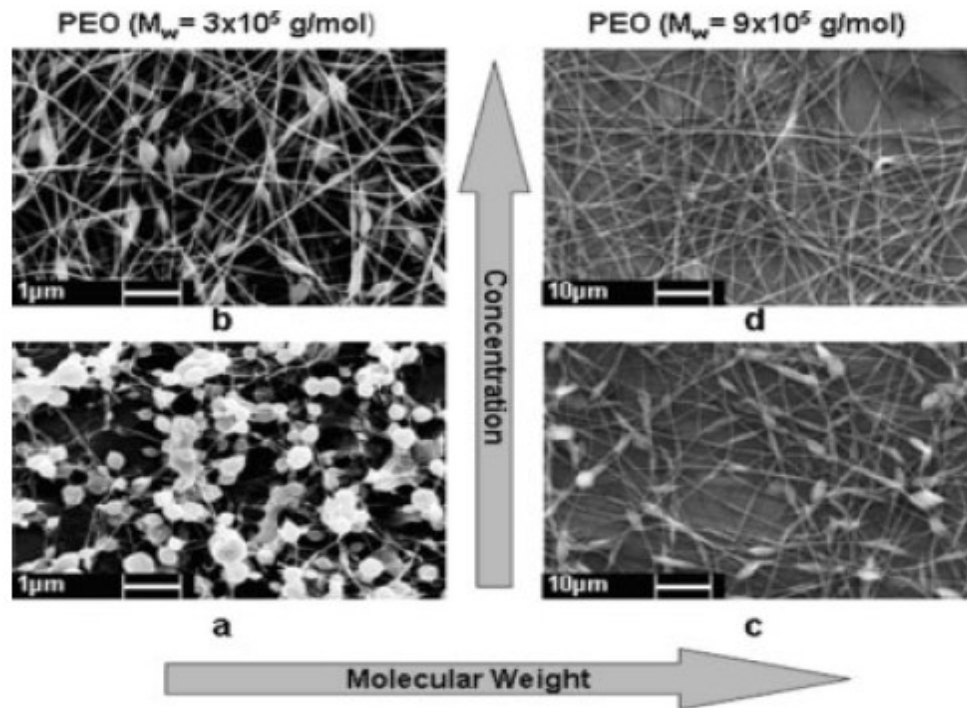


Figure 2.10. Effect of molecular weight on the appearance of electrospun PEO nanofibers [63].

Conductivity: The first step in gaining successful electrospinning process is the counteraction of the repulsive forces against the surface tension, which needs enough charges in the solution. Functional groups on polymer backbone, type of solvent, and ionic species that exist in the solution contribute to the conductivity of the polymer solution. In polymer solutions with higher conductivities more electrical charges will be carried out by electrospinning jet [19] and also more smooth fibers will be formed. Kim *et al.* [64] studied the effect of adding ionic salts (NaCl and CaCl₂) to Poly (2-acrylamido-2-

methyl-1-propane sulfonic acid) (PAMPS) solution and they observed that polymer solution with ionic salts resulted in bead-free uniform fibers. Zong *et al.* [36] arrived at similar results that increase in charges will make the diameter of fibers smaller due to the increased in the stretching of the solution. They also observed that by adding various salts to the solution, beads become smaller and their appearance are more like a spindle. It can be concluded that highly conductive polymer solutions carry more charges, which increase repulsive forces on the jet and leads to fibers with smaller diameters.

Solution concentration: the most influential parameter, which affects the fiber diameter in electrospinning, is the polymer concentration. The major influence of variation of concentration on the resulting diameter comes from the corresponding variation in viscosity of the polymer jet. When viscosity is high, fibers with larger diameters are obtained [65]. However, as it was mentioned in section 2.5.1, there is also a maximum upper value for polymer concentration, above which the solution is so viscous that electrospinning process fails [36]. Zhang *et al.* [35] studied the electrospinning of PVA nanofibers and noticed that with increasing the polymer concentration, the morphology changed from beaded fibers to uniform fibers and the diameter also increased. Concentration was also found to have a significant effect on the morphology of the fibers. At high concentrations, fibers demonstrated curly, wavy and straight structures with identical knots with regular distances. In lower viscosity solutions, formation of fibers with beads and thinner diameters was favored. Beads are usually denoted as defects since they disturb the properties of the nanofibers and decrease the surface area to volume ratio.

Chapter 3

3. Fabrication and Characterization Equipment

3.1. Objective of the Chapter

The objective of this chapter is to describe the materials and methods and process parameters that are used in electrospinning of PVA/ AgNO₃ nanofibers. Fibers were obtained by electrospinning of PVA/ AgNO₃ solution with various concentrations, conductivity, voltages, flow rates and distances between two electrodes at a constant temperature and time. In the following, equipment that were used to obtain information about morphology and diameter of the nanofibers, are introduced.

3.2. Materials and Devices

Electrospinning experiments were carried out using Poly Vinyl alcohol (PVA) with the molecular weight (Mw) of 85,000-124,000, 99% hydrolyzed granules and silver nitrate (99.998%), which were purchased from Aldrich Canada and used as received. De-Ionized water was used as a solvent to prepare the solution. The chemical structure of PVA is shown in Figure 3.1.

The experimental setups for this work consisted of high voltage supply, a syringe pump, a plastic tip, a 5 ml syringe that were positioned horizontally on a syringe pump and a collector plate. Figure 3.2 and 3.3 show PVA and silver nitrate (AgNO_3) powders before solving in deionized water respectively.

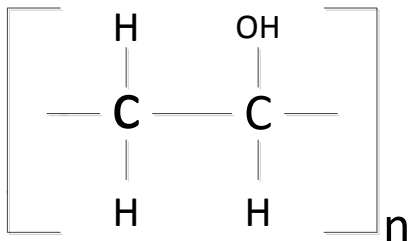


Figure 3.1. Chemical Structure of PVA which is represented by $(\text{C}_2\text{H}_4\text{O})_n$ formula.



Figure 3.2. Poly (vinyl alcohol) powder



Figure 3.3. Silver nitrate powder

3.2.1 Preparation of PVA/AgNO₃ Solution

Glassware was used to prepare solutions using following method. They were washed and cleaned with deionized water (DI). Different amounts of PVA were weighed according to the desired concentration and then poured into the vials. Parafilms were used to further seal the vials to maintain the concentration constant. Next, PVA were dissolved in Deionized water (DI) at a specific temperature and time to form a spinning solution. A magnetic stirrer was used to make solution uniform and homogenous for 3 hours. The amount of PVA required for obtaining weight percentage of polymer solution was calculated by the following equation:

$$WP\% = \frac{M_p}{(M_p + M_s)}$$

WP%= Weight Percent

M_p= mass of polymer (g)

M_s=mass of solvent (ml)

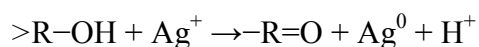
Preliminary experiments were conducted to investigate the limits of parameters of electrospinning. The range, which was reported by Li [32] and Zhang [35] was used as an initial experiment. Hence PVA with various concentrations from 5wt% to 12wt% were prepared at constant time and temperature of 3 hours and 70°C respectively. Figure 3.4 shows PVA solution before adding AgNO₃. When the solution was cooled down to room temperature, 1wt% of silver nitrate was added to the as-prepared PVA solution, then heated and gently stirred for 6 hours in a dark room to avoid the reduction under light.



Figure 3.4. Poly (vinyl alcohol) solution

3.2.2 Reduction of AgNO_3

Changes in color of solution shows reduction of silver nitrate. Hence, to test this reduction, solutions were observed by camera at different time intervals of 0 h, 2h, 4h, 6h and results are shown in figure 3.5. It was seen that the color of PVA/ AgNO_3 solution changed significantly from white to brown. It is widely known that PVA has active -OH groups, which absorbs Ag^+ ions, and coordination of Ag^+ ions with the lone pairs of electrons of hydroxyl group of PVA result in formation of Ag nanoparticles [66]. A reaction of Ag^+ with PVA can be expressed by following formula, Where R=O presents a monomer in the PVA.



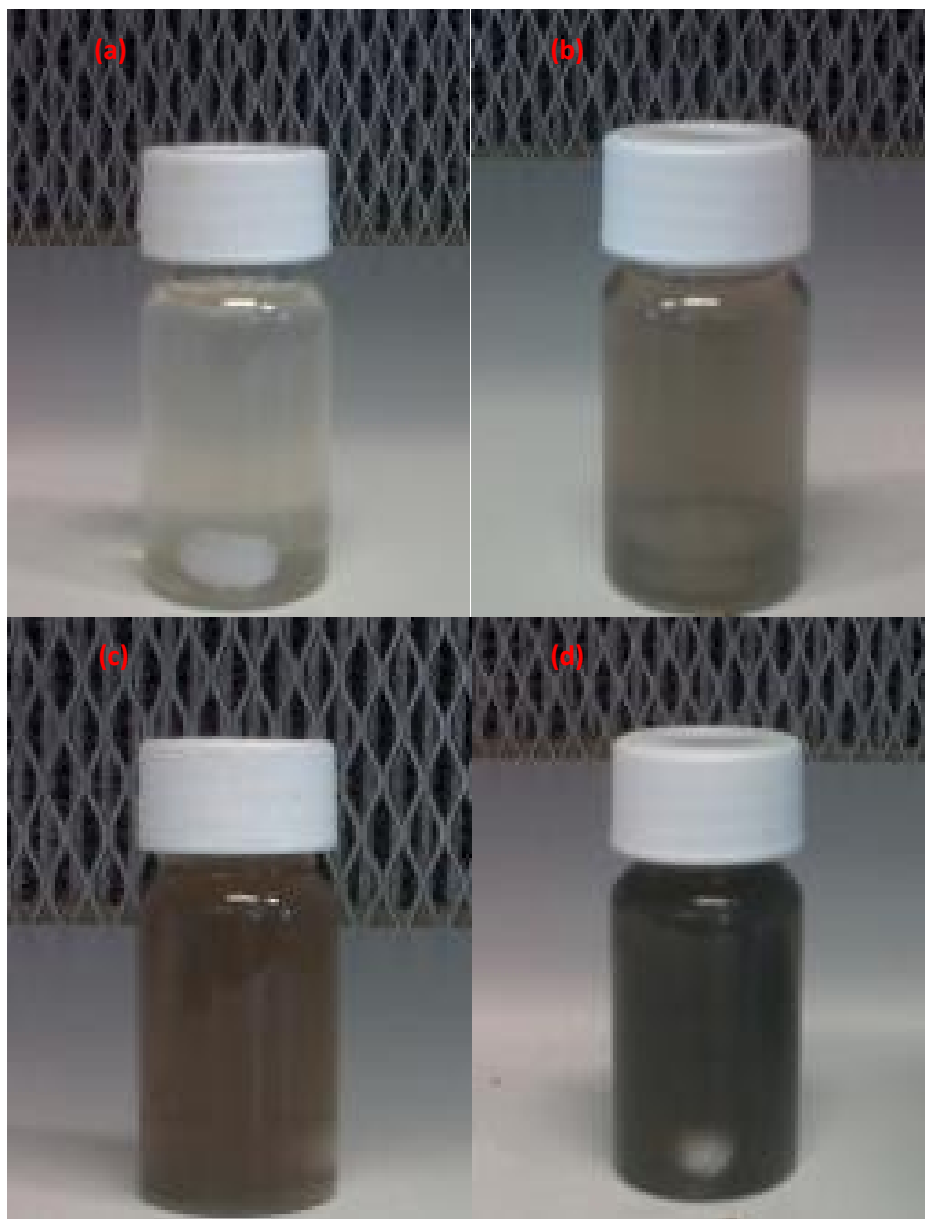


Figure 3.5. Test for reduction of AgNO_3 into silver nanoparticles at aqueous solution of PVA/ AgNO_3 (9/1wt %) at time intervals of a) 0 hours; b) 2 hours; c) 4 hours; d) 6 hours

3.3 Electrospinning Setup and Process

The electrospinning setup of PVA/AgNO₃ in these experiments is different from electrospinning of PVA. When AgNO₃ is added to the solution, different electrospinning setups needed [67]. Silver or silver oxide is sensitive to metallic parts, and will block the needle; instead a micropipette and a graphite lead were used. In this experiment electrospinning set-up consisted of a high voltage supply (Glassman High Voltage Inc.) syringe pump (PHD 2000 infusion, Harvard apparatus), a collector, a syringe, a micropipette tip and a graphite lead. At first syringe was filled up of about 5ml PVA/AgNO₃ and then mounted on an insulator stand and then placed into a chamber on a syringe pump to better govern the evaporation rate of the solvent. Micropipette was connected to the syringe and graphite lead was inserted into it.

As shown in Figure 3.6 aluminum foil was attached on a thin piece of glass in order to be removed easily and used as a collection device. Then it was placed on the collector and negative voltage (Power design Inc.) of 2kV was connected to it. The syringe pump controlled the flow rate of the solution. Applying voltage to the graphite lead, which was positioned into micropipette, charged the solution as shown in Figure 3.7. Figure 3.8 illustrates the electrospinning setup.

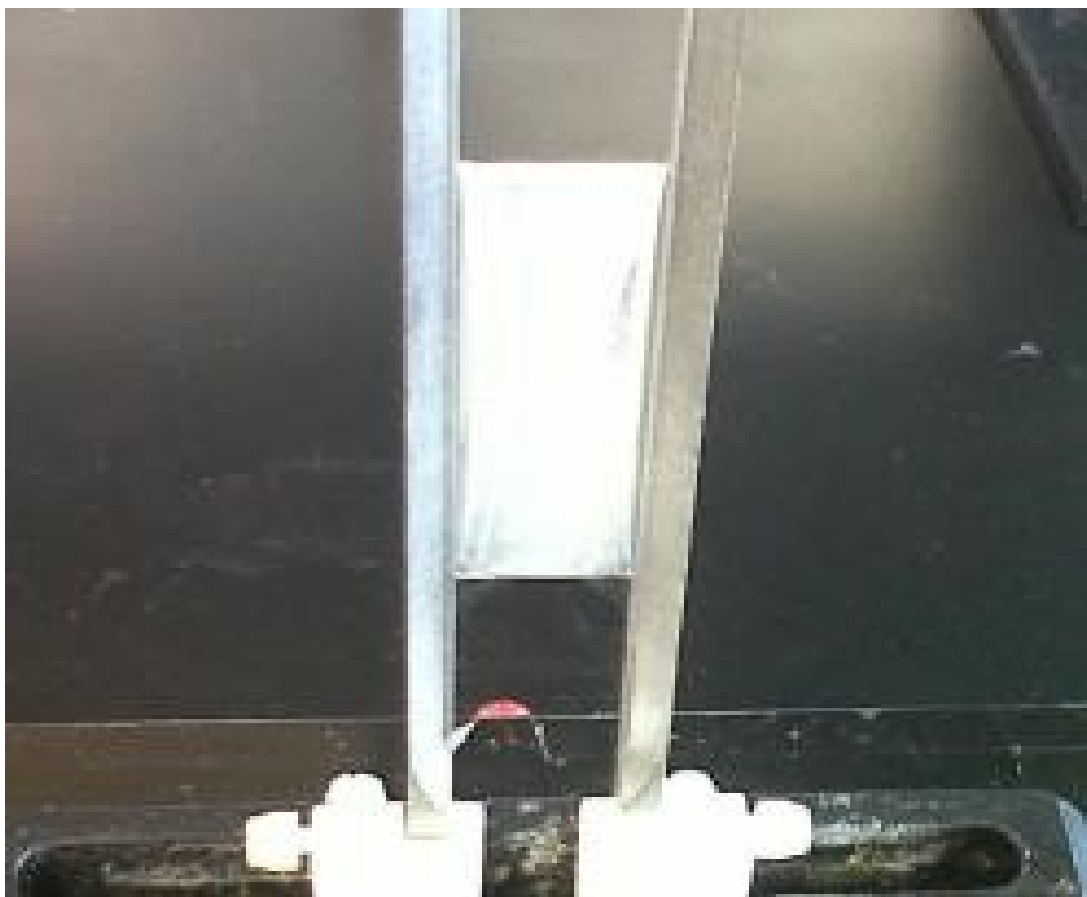


Figure 3.6. Use of aluminum substrate as a collection device



Figure 3.7. Applying voltage to the graphite lead



Figure 3.8. Electrospinning set-up

3.4 Experimental Design

In order to investigate the effect of various parameters of electrospinning on average fiber diameter and obtain desired fibers, several preliminary experiments were conducted. Each run was done for 15 minutes to ensure that enough fibers are collected (if any) on aluminum foils. In all of the experiments temperature was set to 35°C. Subsequently, when the experiments was done, aluminum foils were removed from the collector and samples were placed into a vacuum oven at an ambient temperature for 24 hours to ensure elimination of the solvent.

3.4.1 Solution Concentration

In order to determine the lower and upper limit of solution concentration, several experiments were done by varying the amount of polymer concentrations and holding other parameters constant. As it was mentioned in section 2.5, at lower concentrations, polymer solution tends to form drops and breaks the jet into droplets because of low viscosity while at higher concentration due to the higher viscosity of the solution, electrospinning process fails, hence there should be a minimum and maximum values for polymer concentration. Experiments were done in several runs with various polymer concentrations (Table 3.1). The results show that at higher concentrations than 10wt% (viscosity too high, causing the solution dried up at the tip of the micropipette), or lower than 7wt% (Viscosity too low, producing only droplets) no fiber was generated. So our experiments showed that electrospinning was only successful for the polymer concentrations between 8wt% to 10wt%.

Table 3.1. Summary of PVA concentration used in experiment.

Run	1	2	3	5	6	5	6	7
Polymer	5wt%	6wt%	7wt%	8 wt%	9wt%	10 wt%	11 wt%	12wt%
Concentration								

3.4.2 Applied Voltage

As it was mentioned earlier, in order to obtain fibers, a minimum and maximum value of voltages are required. To determine lower and upper limit of voltage, series of experiments were conducted which are summarized in table 3.2. Voltages of more than 18kV caused deformation of solution drops into the shape of Taylor cone and formation of fibers while below that, just drops at the tip of the needle were observed and as a result no fibers were obtained. Also, at voltages of 26kV and higher, Taylor cone was not stable and few fibers, mostly merged, were seen. A possible reason that high voltages make the Taylor cone unstable could be due to the fast movement of the jet causing to repel more solution down the tip of the needle, leading to less stable cone. Based on our studies, fiber formation will be optimum when the solution is delivered to the collector at the output voltages in the range of 18kV to 24kV.

Table 3.2. Summary of electrospinning voltages used in experiment.

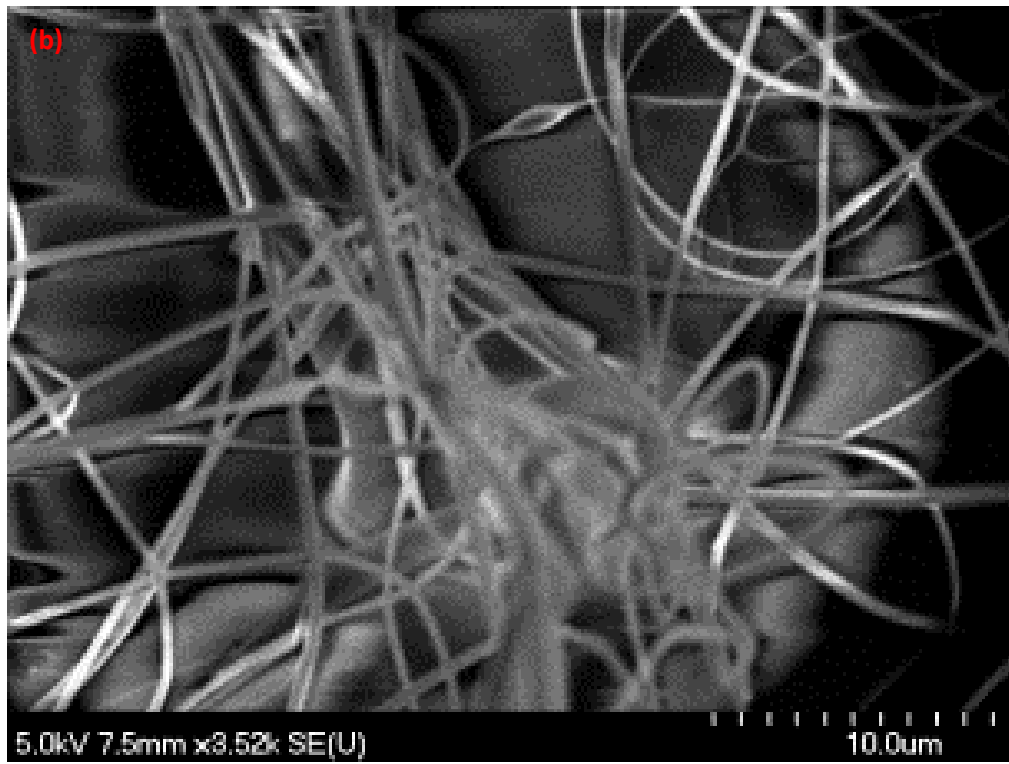
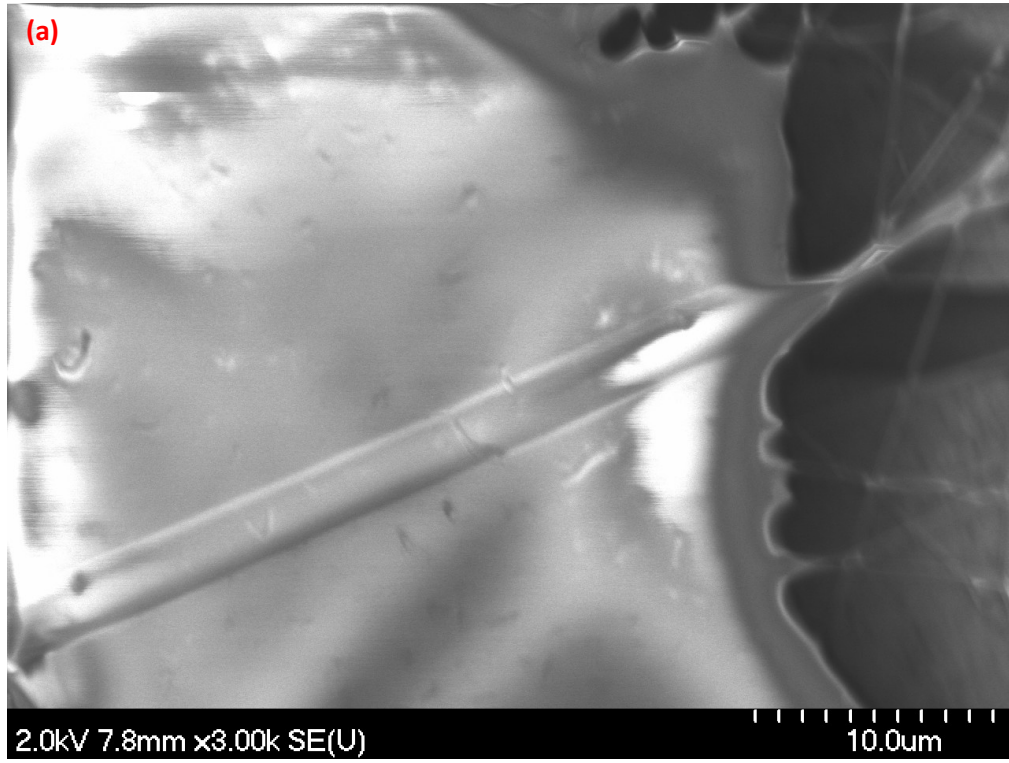
Run	1	2	3	5	6	5	6	7	8	9
Voltage	12 kV	14 kV	16 kV	18 kV	20 kV	22 kV	24 kV	26 kV	28 kV	30 kV

3.4.3 Collection Distance

In these experiments, the distance from tip to collector was varied from 2 cm to 24 cm (Table 3.3) to find the lower and upper limit of this parameter. It was noticed that, by placing collector at distance of 10cm and lower from pipette orifice, some fibers were seen but most of them got conglutinated. The reason would probably be due to the decrease in flight time and lack of enough time for excess solvent evaporation when the jet reached the collector (Figure 3.9). By placing the collector at a distance of 24cm, the spun fibers failed to collect on the collector. It was found that the optimum collection distances should be between 14cm and 20cm.

Table 3.3. Summary of electrospinning collection distances used in experiment.

Run	1	2	3	4	5	6
Distance	2cm	6cm	10cm	14cm	20cm	24cm



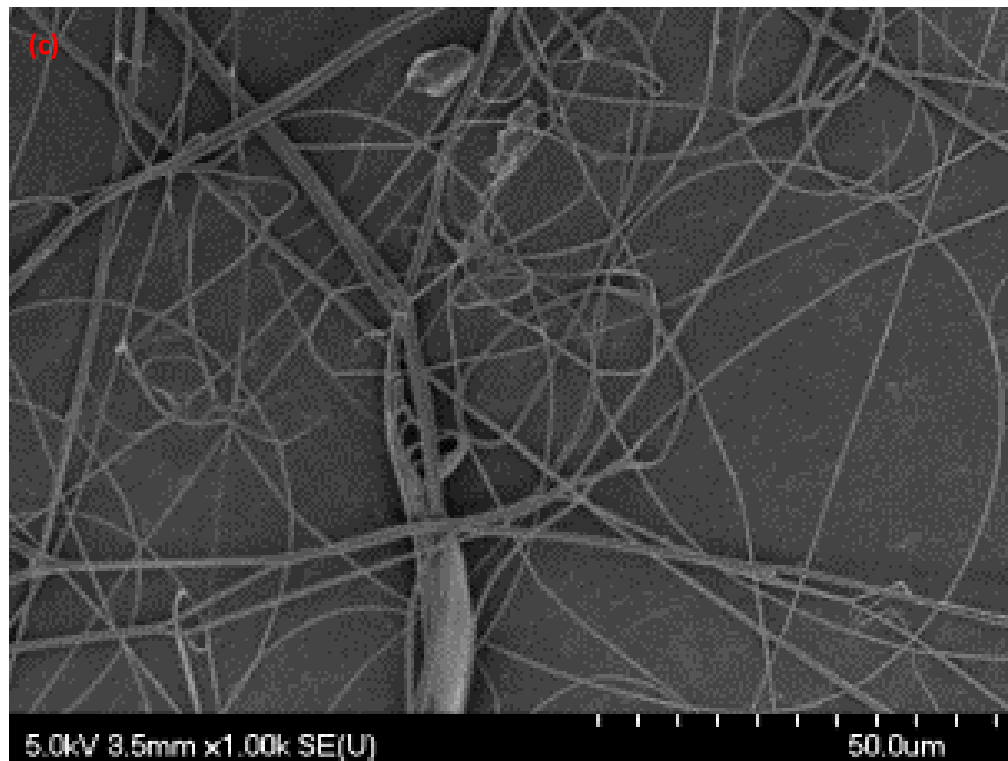


Figure 3.9. Fibers conglomeration at distances of a) 2cm; b) 6cm; and c) 10cm.

3.4.4 Solution Flow Rate

As mentioned earlier, in order to maintain a stable Taylor cone during electrospinning process, solution flow rate has to be adjusted. To discover the lower and upper limit of solution flow rate several experiments were carried out by altering flow rate from 0.03 ml/h to 0.8ml/h (Table3.4) while maintaining other parameters constant. At very low flow rates no Taylor cone was observed and solution evaporated at the pipette tip and prevented electrospinning. Low flow rates require an increase in voltage in order for electrospinning to take place. By increasing the flow rate too high, fibers got

flattened and merged. It could be because of ejection of more solution from the tip and consequently the solvent takes a longer time to evaporate and as a result fibers merge together. In accordance with obtained results, the optimum solution flow rates were determined to be between 0.1ml/h to 0.4ml/h.

Run	1	2	3	4	5	6	7	8
Flow rate	0.03	0.06	0.08	0.1	0.2	0.4	0.6	0.8
	ml/h	ml/h	ml/h	ml/h	ml/h	ml/h	ml/h	ml/h

Table 3.4. Summary of electrospinning flow rates used in experiment.

3.4.5 Solution Conductivity

It is known that the conductivity of the solvent is low due to the lack of free ions [19]. Polymer solutions with higher conductivities carry more electrical charges, which increase repulsive forces on the jet, and thus decrease the average fiber diameter. In order to detect the upper and lower limit of conductivity, experiments were done by adding different amount of silver nitrate (Table3.5). At all three conductivities fibers were formed. Here the amounts of the silver nitrate in the solution are from 1wt% to 6wt%.

Table 3.5. Various ratios of solutions used to fabricate nanofibers containing silver nanoparticles. The initial concentration (wt%) of PVA was 9wt%.

Sample	Ratio of silver nitrate to PVA concentration (wt%)
Sample 1	1/9
Sample 2	3/9
Sample 3	6/9

The examples of optimum conditions of electrospinning process are summarized in Table 3.6.

Table 3.6. The example of optimum conditions of electrospinning process.

Run	Concentrations of PVA (wt%)	Concentrations of Silver nitrate (wt%)	Voltage (KV)	Flow rate (ml/h)	Distance (cm)
1	8%	1	20	0.2	14
2	9%	1	20	0.2	14
3	10%	1	20	0.2	14
4	9%	1	20	0.2	14
5	9%	3	20	0.2	14
6	9%	6	20	0.2	14
7	9%	1	18	0.2	14
8	9%	1	20	0.2	14
9	9%	1	22	0.2	14
10	9%	1	24	0.2	14
11	9%	1	20	0.1	14
12	9%	1	20	0.2	14
13	9%	1	20	0.4	14
14	9%	1	20	0.2	14
15	9%	1	20	0.2	20

3.5 Equipment Characterization

The morphologies of the electrospun PVA/AgNO₃ nanofibers were determined using Field Emission Scanning Electron Microscope (FE-SEM, Hitachi S-4700) and Transmission Electron Microscope (TEM, JEOL JEM-2100F). Before using the SEM microscope, a small section of the aluminum foil holding nanofibers was placed on the SEM sample holder and was gold coated using Hummer VI. Energy-dispersive X-ray spectroscopy (EDX) was used for obtaining detailed information about chemical composition of materials. Transmission Electron Microscope was employed to observe the dispersion and size of silver nanoparticles on nanofibers. This section introduces the working mechanism of this equipment.

3.5.1 Scanning Electron Microscope (SEM)

SEM is a microscope that is routinely used in nanotechnology. It provides a high-resolution image as small as 10 nm by using high beam energy of electrons to scan the surface of the sample [68]. Hence, at higher magnification, closely spaced features can be examined. Table 3.7 provides the comparison between the resolution of the SEM and other techniques.

Method	Limit	Resolution
Transmission Electron Microscope	Diffraction of electrons	0.1 nm
Field ion microscope	Atomic size	0.3 nm
Scanning Electron Microscope	Diffraction of electrons	30 nm
Optical Microscope	Diffraction of light	300 nm
Eye	Retina	60000 nm

Table 3.7. Resolutions of various measurement techniques [68].

In SEM, when primary electrons hit the sample, secondary electrons would be excited and then leave the specimen. The ejected electrons go through the Second Electron Detector (SED), which generates analog signal. Afterward the converter converts the analog signal into digital and then transfers it into computer [69]. Figure 3.10 illustrates a schematic of a typical SEM apparatus. The sample-electron interactions result in producing different signals such as: secondary electrons, backscattered electrons and characteristic X-ray, which are detectable by special detectors. The generated signals reveal information about morphology, chemical composition and topography of the sample [69]. A picture of SEM S-4700, which was used in this research, is depicted in Figure 3.11.

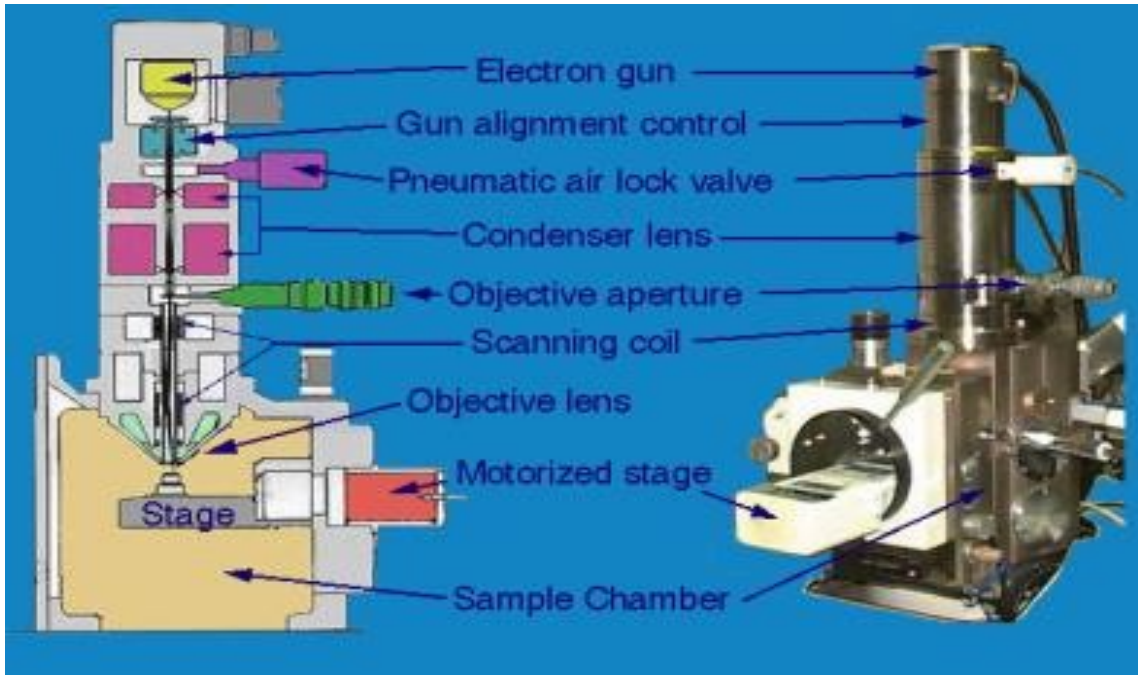


Fig.3.10. Schematic of SEM apparatus [70].



Fig.3.11. Hitachi SEM S-4700 [71].

Before inserting specimen into SEM chamber following tips should be considered to sustain optimum performance:

- Gloves should be worn to avoid greasing the vacuum.
- Samples should be conductive to avoid agglomeration of electrostatic charges [72] if not; special preparation is required such as partially attaching a conductive tape or sputter coating, which will be explained later.
- For keeping specimen fixed on the sample holder a sticky tape should be used.
- All samples must have suitable size to fit into chamber hence a sample height gauge must be used as shown in Figure 3.12.

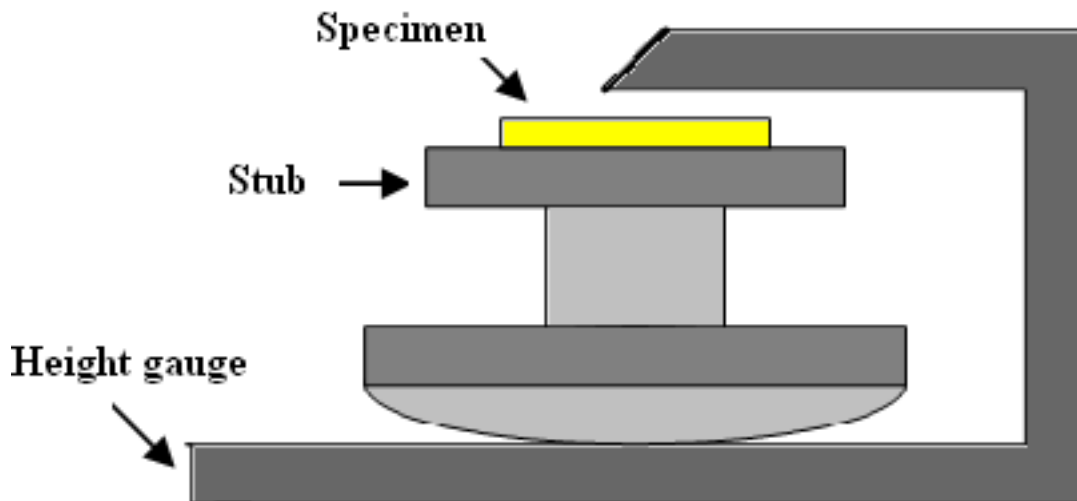


Figure 3.12. Specimen height adjustments [72].

3.5.2 Energy-dispersive X-ray Spectroscopy (EDX)

EDX is used to obtain information about elemental analysis of different materials. EDX system composed of an x-ray detector, software analyzer and liquid nitrogen [69]. EDX detects the x-ray spectrum, which was emitted by bombarding the sample with an electron beam to identify the elements of the sample. When a beam of electrons hits the sample, it excites electrons in the inner orbital and ejects the electron to a higher state while leaves an electron hole behind. Then an electron from a higher state fills the hole and the energy difference between the inner and outer shell is released as x-ray to balance this difference. Afterward, the EDX detector collects the x-ray spectrums, which were emitted, from the sample and because each atom has a sole atomic structure, EDX automatically recognize the elements [69].

3.5.3 Gold Sputtering

In case of non-conducting materials, a sputtering machine is used to deposit the surface of the specimen with a range of target materials such as gold or palladium in few nanometers for viewing it in scanning electron microscope. This machine works in such a way that specimen is attached on the stub and placed on sample platen in a cylindrical plasma chamber which is vacuum with a very low-pressure argon gas. When the target bombards sample, a pink colored discharged glow will be observed inside the plasma chamber. Sputter coating is useful for reducing damage to SEM and increasing conduction [73]. Figure 3.13 illustrates Hummer VI sputter coating machine that used in this research.



Figure 3.13. Hummer VI sputter coating machine [74].

3.5.4 Transmission Electron Microscope (TEM)

TEM is a type of microscope, which has capability of imaging microstructures of materials with a magnification of 1,000,000 times, with the resolution smaller than 1nm. TEM has the ability to analyze the elements, determine the crystal structure and orientation as small as 1 nm [75].

In TEM microscope, a source of electrons travel through the sample and interact with it, and then will form an image. Typically the resulting image could be recorded on an imaging device such as CCD camera or fluorescent screen [75]. Image of the TEM

JEOL JEM-2100F equipment which was used in this research is depicted in figure 3.14.



Figure 3.14. JEOL TEM model JEM-2100F [75].

Chapter 4

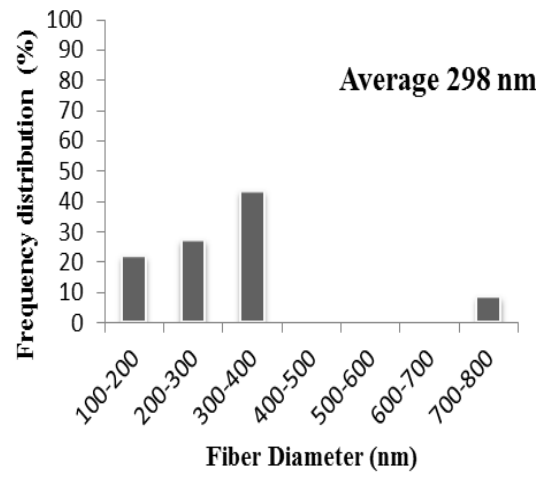
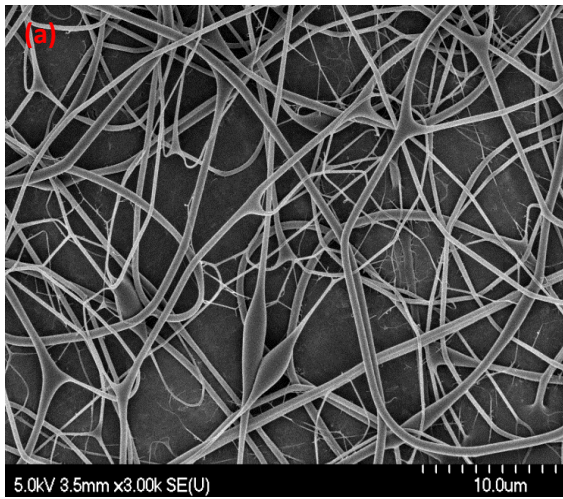
4. Experimental Results and Discussion

4.1. Objectives of the chapter

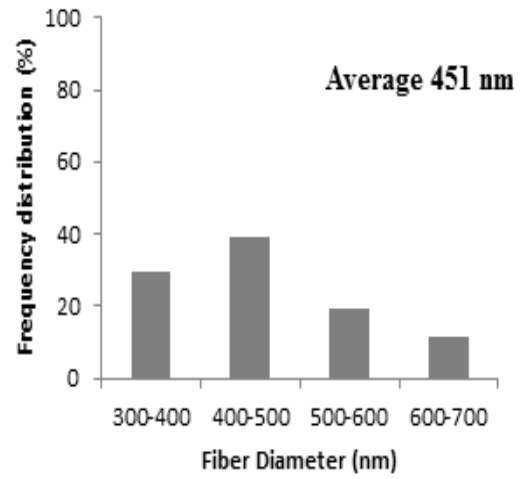
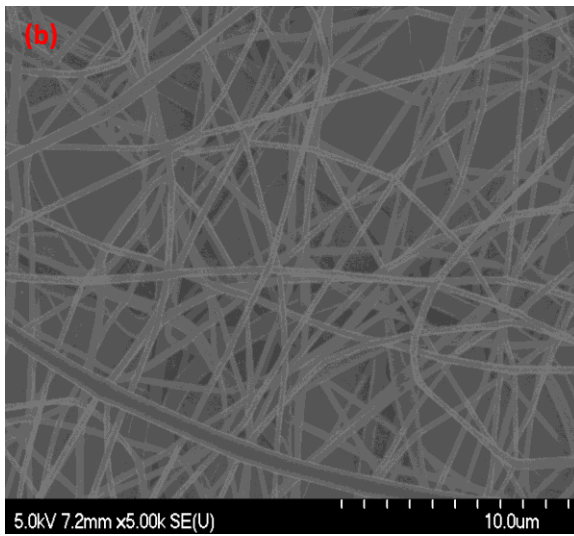
The objective of this chapter is to discuss the effects of various parameters of the electrospinning on diameters of fabricated nanofibers. For determining the effects of voltage, concentration, distance, flow rate and conductivity on fiber diameters, several experiments were carried out and the results were categorized into five groups to show each of them. The morphologies of the electrospun PVA/ AgNO₃ nanofibers were obtained using Field Emission Scanning Electron Microscope (SEM). Before Loading a sample in SEM chamber, a thin layer of gold deposited on the fiber using sputtering technique to observe better SEM images. Voltage and current of 5KV and 15 μ A were employed to take SEM photographs. The average diameter of the electrospun fibers was measured using Image analyzer software (Image J, US) from the SEM images. In order to ensure the integrity of the experiments, we fabricated 4 sets of fibers, from each set we have chosen a minimum of 20 fibers randomly and measured the diameters of all, then take the average to plot the charts, shown in Figures 4.1 to 4.6. Energy-dispersive X-ray spectroscopy (EDX) was used for elemental analysis of PVA/ AgNO₃ nanofibers. Transmission Electron Microscope (TEM) also was used to show the size of the silver nanoparticles.

4.2. Effect of Solution Concentration

To study the effect of the solution concentration, PVA/AgNO₃ solutions with different concentrations of PVA ranging from 8wt% to 10wt% were electrospun. Electrospinning was conducted under conditions of applied voltage of 20 kV, tip to collector distance of 14cm and flow rate of 0.2ml/h. In these experiments by varying concentration from 8wt% to 10wt% and holding other parameters constant, few beads were observed at concentration of 8wt% and the average fiber diameter between beads was 360, which indicates that this concentration is still low for obtaining fine fibers. The formation of the beads is due to the low viscosity of the solution, which indicates less polymer chain entanglement that results in unstable jet and forming beads on the fibers. By increasing the concentration to 9wt% and 10wt% almost beaded-free fibers were obtained. Average fiber diameter increased from 298nm to 554nm at concentration of 8wt% and 10wt% respectively. As it was mentioned previously in section 3.4.1 by increasing the concentration, viscosity increases as well which avoids the breaking off the jet and results in uniform fibers with larger diameter. Figure 4.1 represents SEM images of PVA/AgNO₃ electrospun nanofiber.



(c)



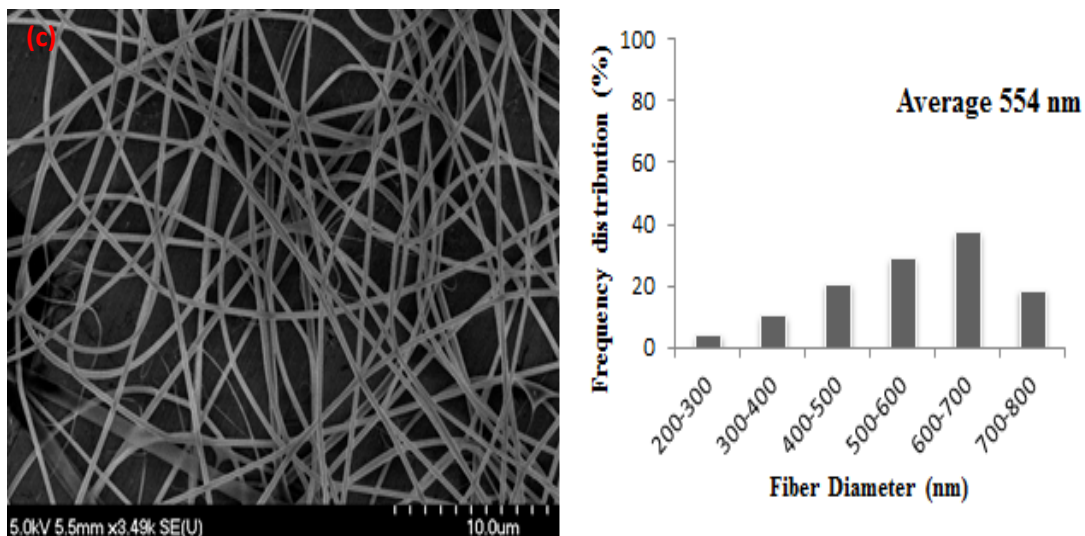
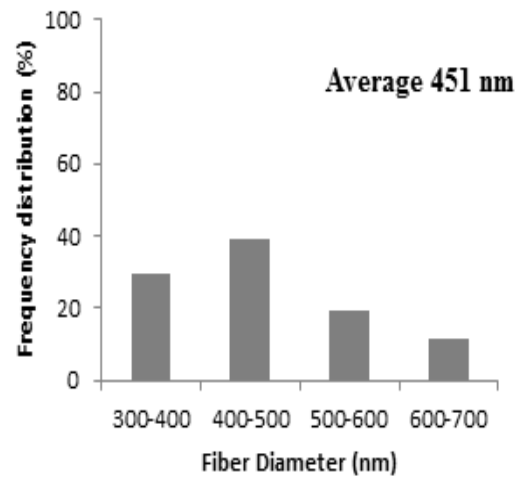
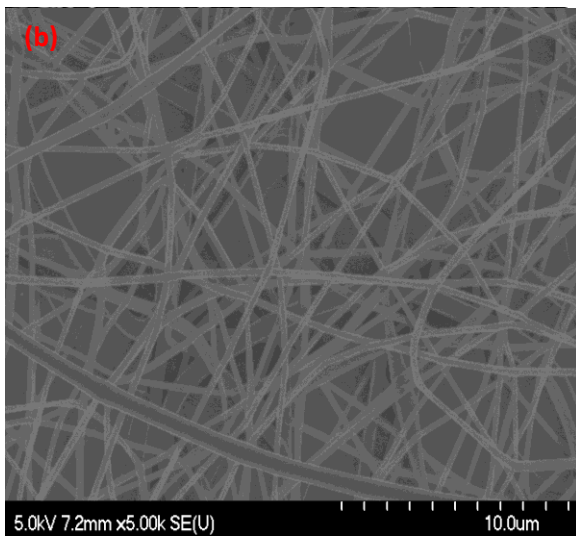
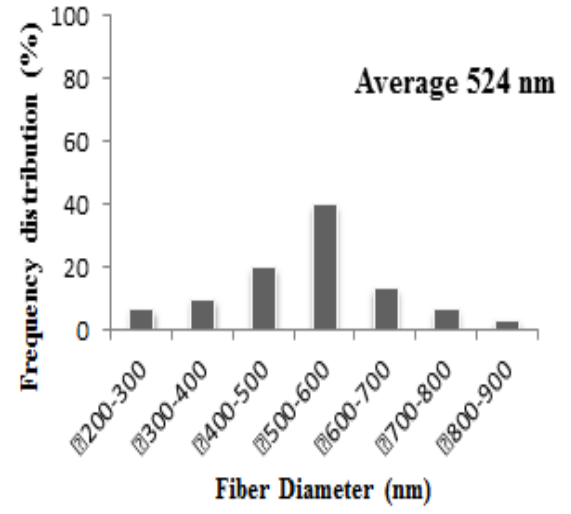
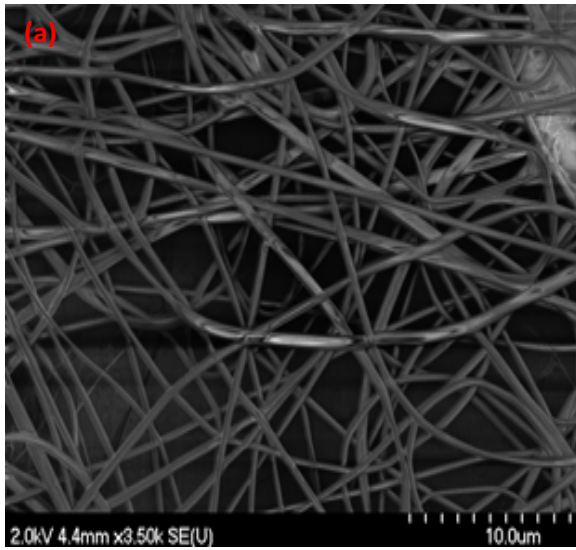


Figure 4.1. SEM images of the PVA/AgNO₃ electrospun nanofibers with various solution concentration (Voltage= 20kV, Flow rate= 0.2 ml/h, Distance= 14 Cm) PVA Concentrations of a) 8wt%; b) 9wt%; c) 10wt%.

4.3. Effect of Applied Voltage

To investigate the effect of voltage on fiber diameters, applied voltage from 18 to 24kV was varied for electrospinning of 9wt% solution whilst keeping other parameters constant. As it was mentioned in section 2.3, voltage will initiate the electrospinning process by charging the solution and movement of the ions in the solution and finally the ability of repulsive forces to overcome the surface tension. Here it has been verified experimentally that the initiation jet was at 18kV and jet was stable up to the voltage of 24kV. Figure 4.2 illustrates the SEM images of nanofibers obtained at different voltages of 18kV, 20kV, 22kV and 24kV. The average fiber diameter was found to slightly decrease from 524nm at a voltage of 18kV to 360nm at a voltage of 24kV in a constant distance and flow rate. Higher voltage induces larger electrostatic forces on the jet and result in more elongation of the jet, which favor the formation of thinner fibers.



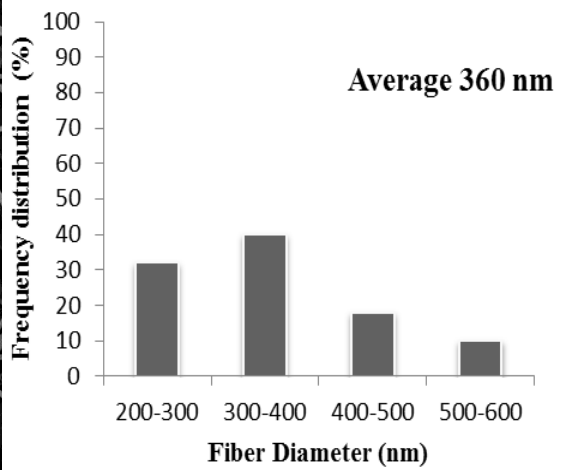
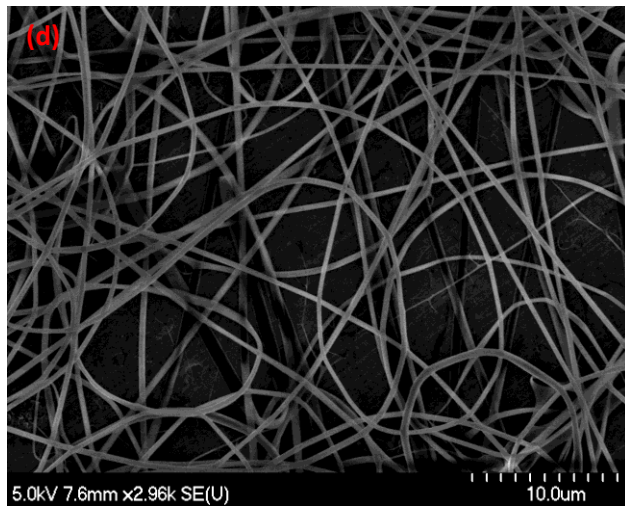
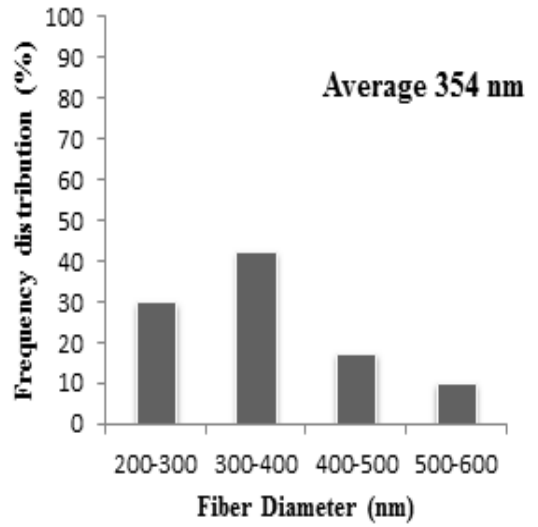
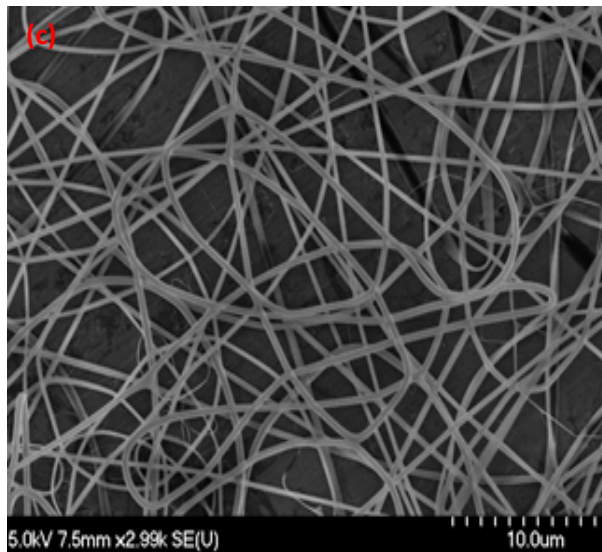
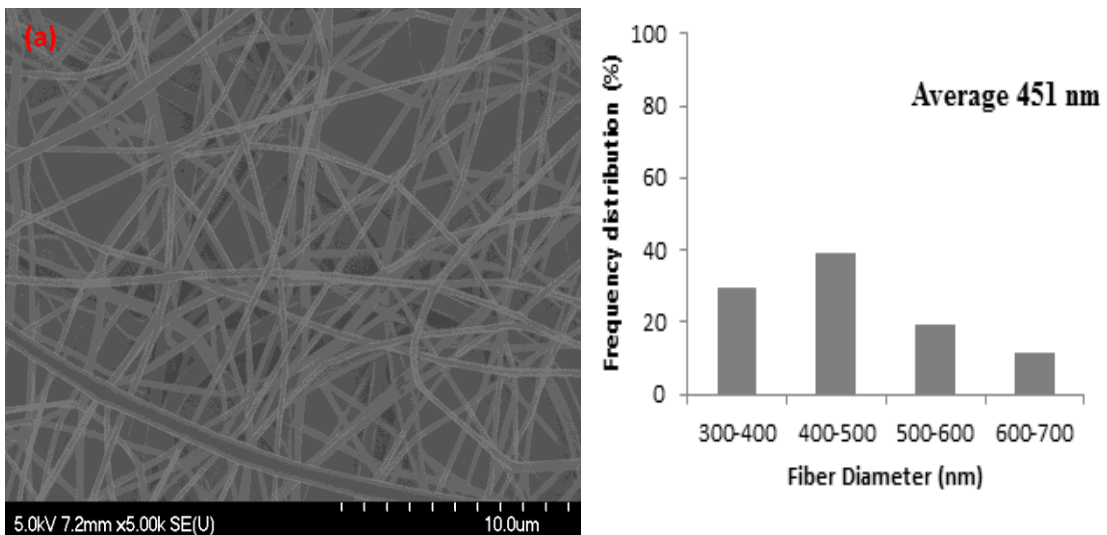


Figure 4.2. SEM images of PVA/AgNO₃ nanofibers from aqueous solution at various voltages of a) 18kV; b) 20kV; c) 22kV; d) 24kV.

4.4. Effect of Collection Distance

To investigate the effect of collection distance, series of experiments were carried out with constant voltage, flow rate and concentration at various distances. A voltage of 20kV was applied to the solution with concentration of 9wt% via alligator clip attached to the graphite lead. Flow rate was adjusted to 0.2 ml/h and collector was placed at 14cm and 20cm of micropipette tip. According to SEM images (Figure 4.3) it could be noticed that by altering the distance between tip to collector, the average fiber diameter have a slight decrease trend from 451 nm at distances of 14cm to 380nm at distance of 20cm. Possibly the larger distances increase flight time of the jet, stretching of the solution so the solvent had enough time to evaporate completely. Dried fibers stretched and deposited on the collector, which resulted in reducing the diameter.



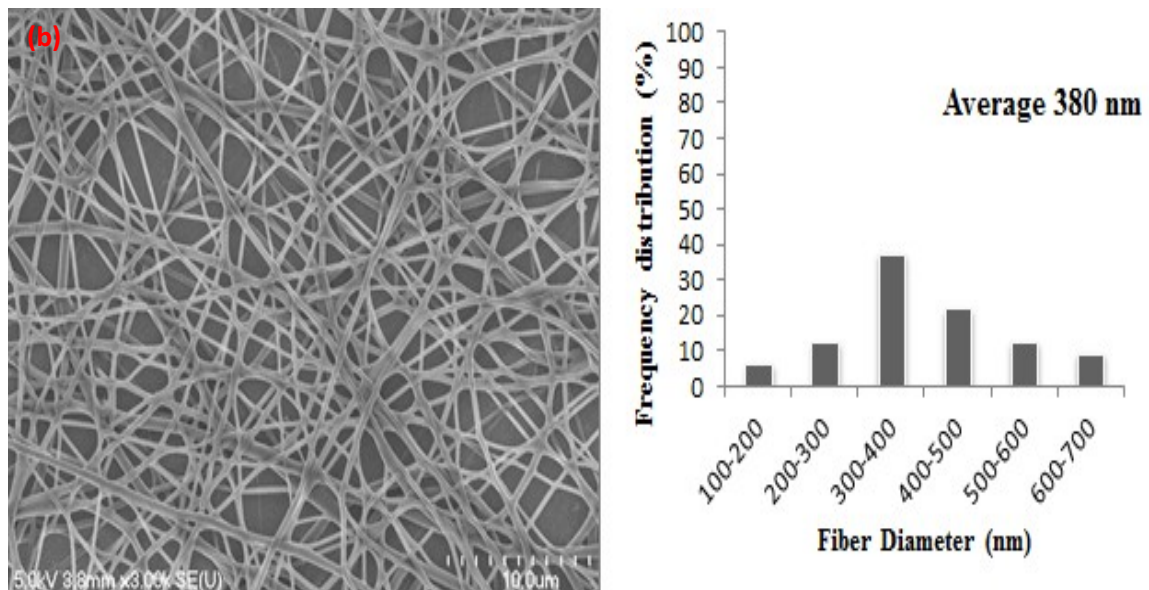
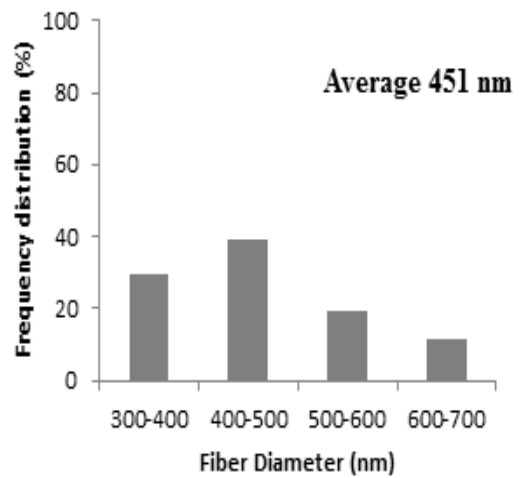
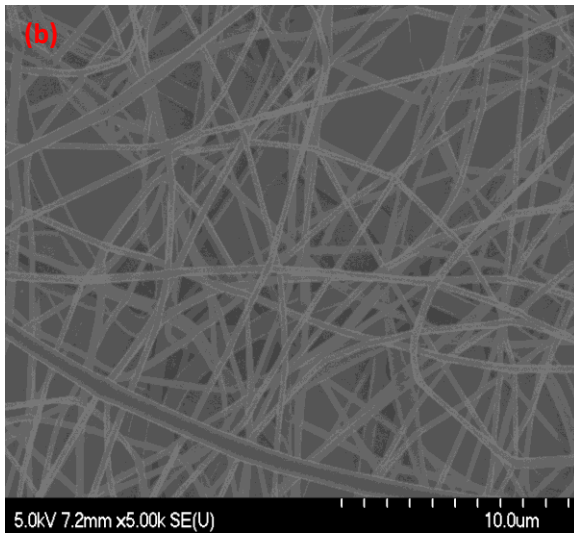
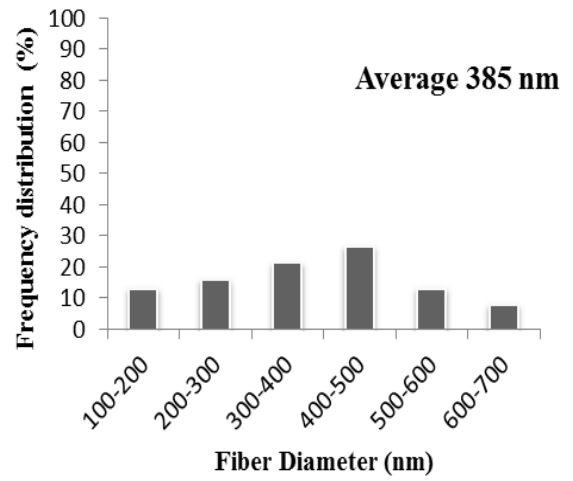
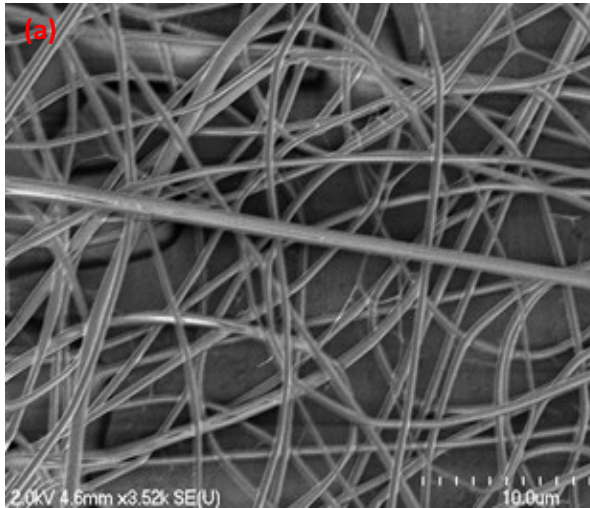


Figure 4.3. SEM images of PVA/AgNO₃ of electrospun nanofibers from aqueous solution at various distances of a) 14cm; b) 20cm from micropipette tip.

4.5. Effect of the Flow Rate

To examine the effect of flow rate on diameter of as spun PVA/AgNO₃ nanofibers, some experiments were carried out. At first a concentration of 9% of PVA solution was poured into syringe and then a spinning voltage of 20kV and flow rates from 0.1ml/h - 0.4 ml/h were applied to the solution. Tip-to-collector distance was held at 14 cm. The average fiber diameter was determined by SEM images, which is shown in Figure 4.4. By adjusting different flow rates and keeping other parameters constant, it was denoted that, fiber diameters have been increased slightly from 385 to 516nm at flow rate of 0.1ml/h and 0.4ml/h respectively. At low flow rates probably the solvent had adequate time for evaporation before depositing on the collector resulted in longer stretching of

the jet to produce thinner fibers. Increasing the flow rate causes to generate fibers with larger diameters because more solution was drawn from pipette tip.



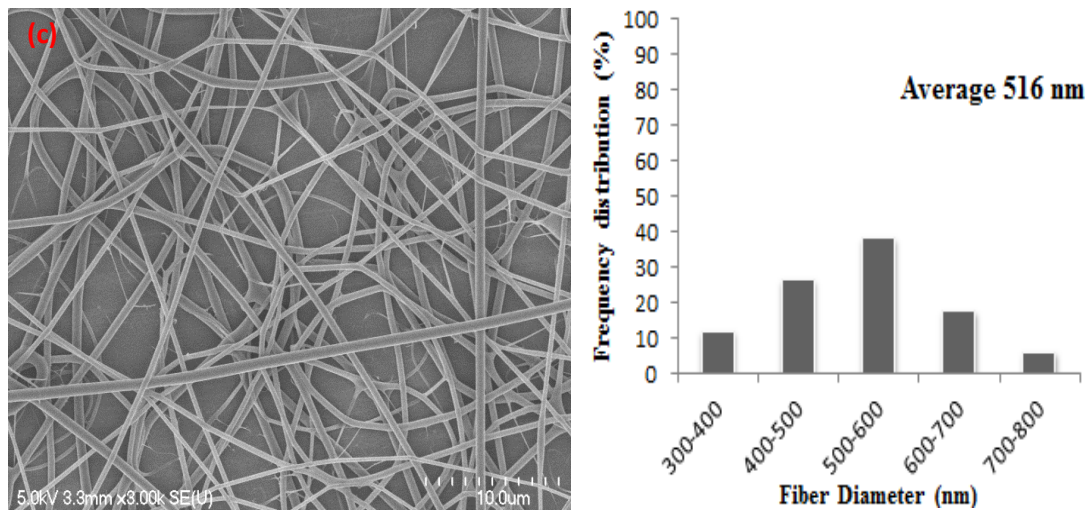
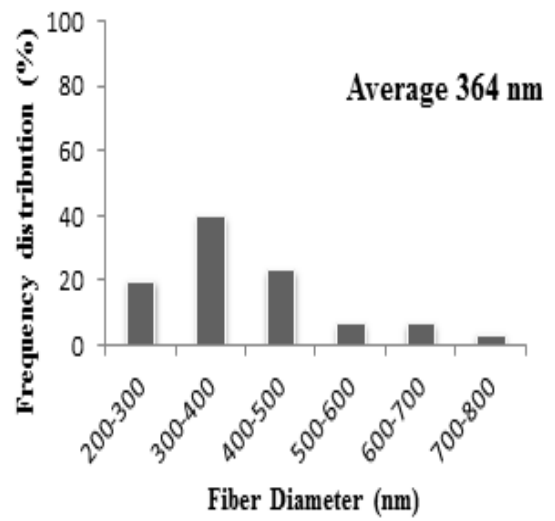
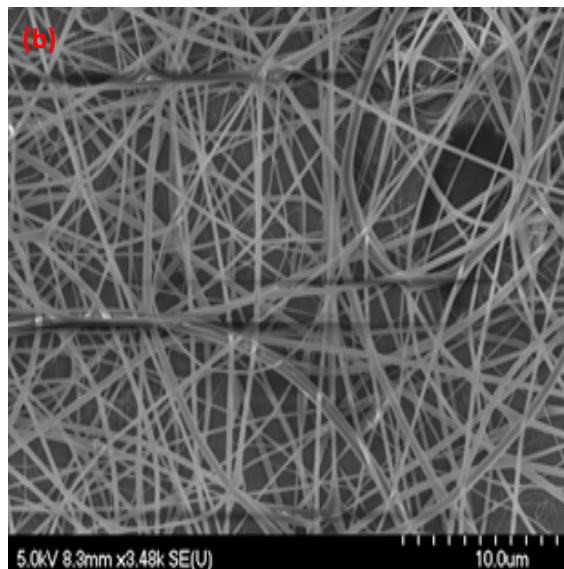
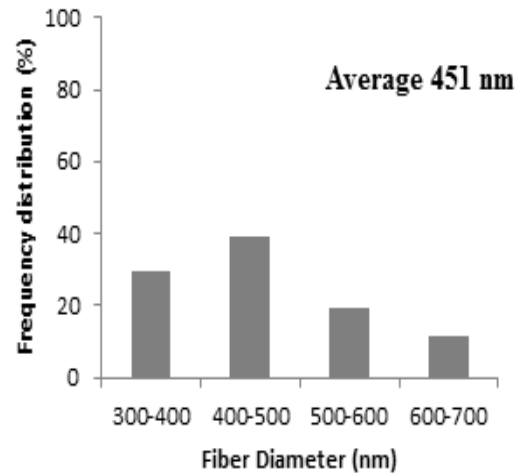
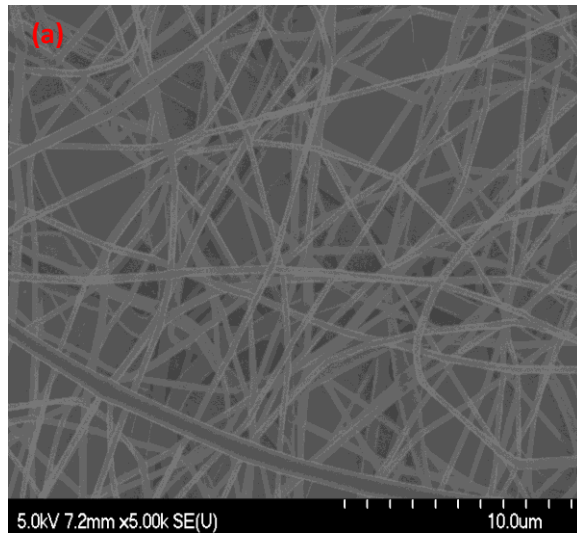


Figure 4.4. SEM images of PVA/AgNO₃ of electrospun nanofibers from aqueous solution at various flow rates of a) 0.1ml/h; b) 0.2ml/h; c) 0.4ml/h.

4.6. Effect of Solution Conductivity

To observe the effect of conductivity, PVA solution with concentration of 9wt% containing 1wt%, 3wt% and 6wt% of AgNO₃ were prepared. The electrospinning setup was consisted of 20 kV voltage, 0.2 ml/h flow rate and collection distance of 14cm. Figure 4.5 exhibits the SEM images of PVA/ AgNO₃ electrospun nanofibers. Here the electrospun fibers from the 9wt% of PVA containing 6wt% of AgNO₃ have the smallest average diameter around 232 nm while fibers from 1wt% and 3wt% of AgNO₃ have the average diameter of around 451nm and 364 nm. The results show that as the conductivity of the solution increases the average diameter of the fibers decreases. Increasing the conductivity of the solution, increasing the number of charges ejected

from the electrospinning jet, resulting in stretching the solution and consequently generating thinner fibers. Results showed that adding various amounts of silver nitrate into PVA solution resulted in gradual decrease in the average diameter of the uniform fibers.



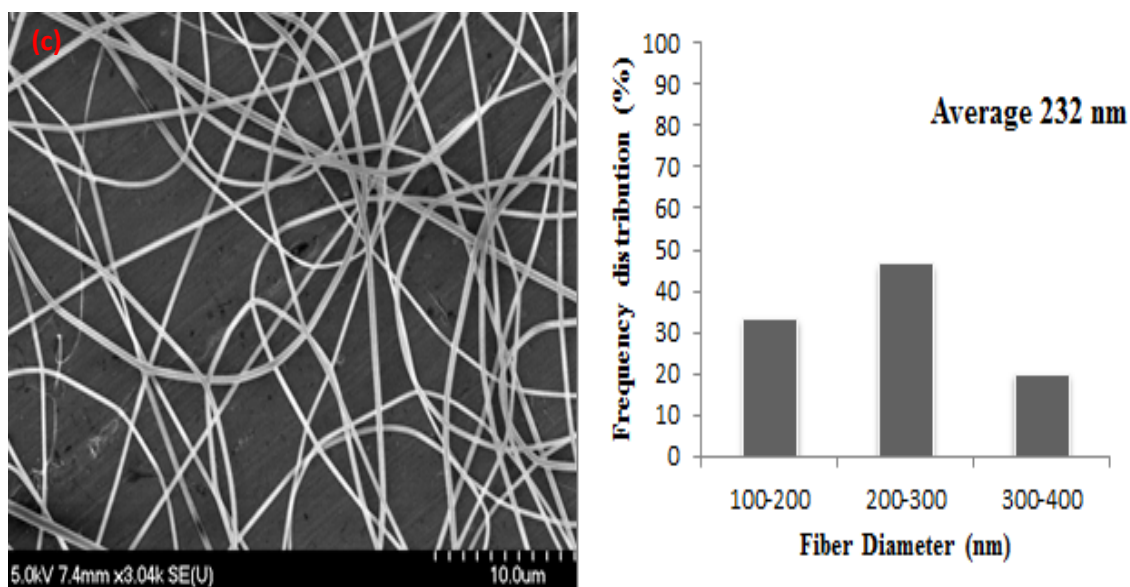
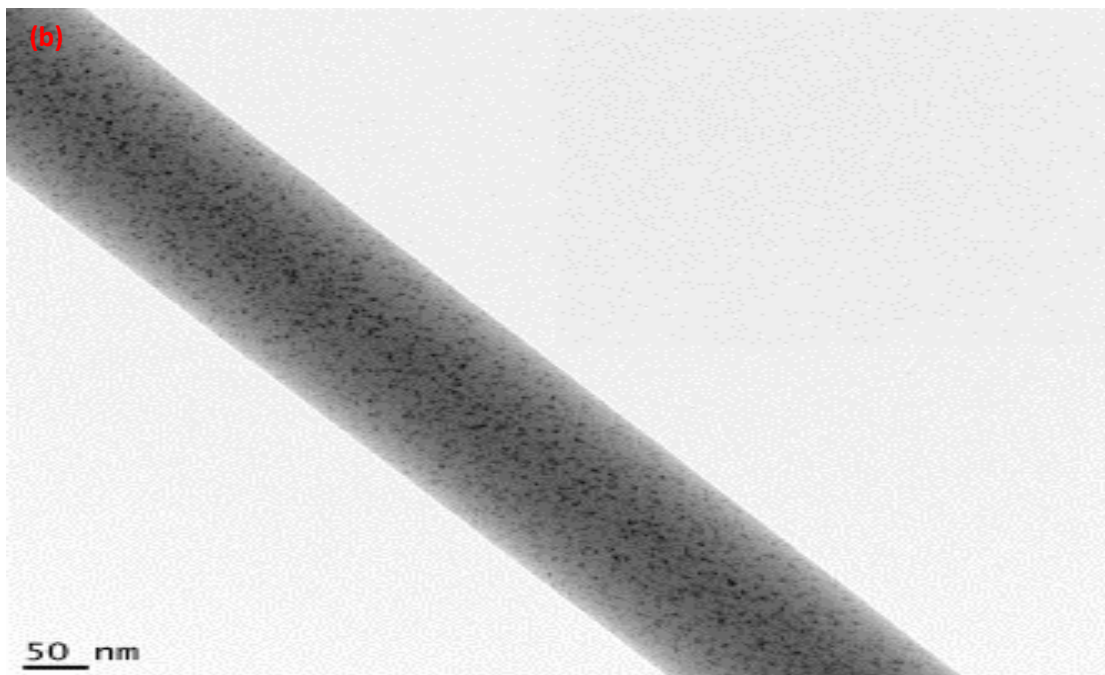
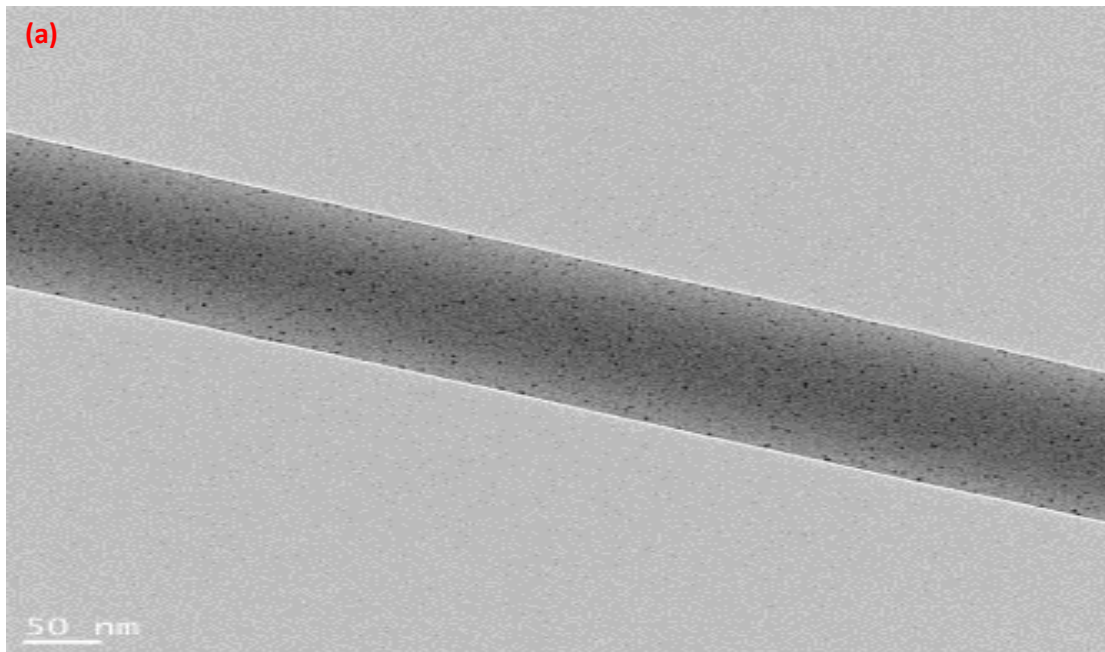


Figure 4.5. SEM images of PVA/AgNO₃ nanofibers containing a) 1wt%; b) 3wt%; c) 6wt% of silver nitrate, here by increasing the amount of silver nitrate, conductivity increased which leads to gradual decrease in average fiber diameter.

4.7. Silver Nanoparticle Observations

For observing the distribution and size of the Ag nanoparticles on PVA nanofibers, samples were deposited on carbon coated copper grids via electrospinning. Figure 4.6 represents the TEM images of the Ag nanoparticles. TEM images confirm uniform dispersion of the Ag nanoparticles in the PVA/ AgNO₃ nanofibers and no aggregation of the nanoparticles were observed. It is apparent that increasing the amount of AgNO₃

has increased the number of nanoparticles. It could be clarified that nanoparticles are homogenously distributed on the nanofibers.



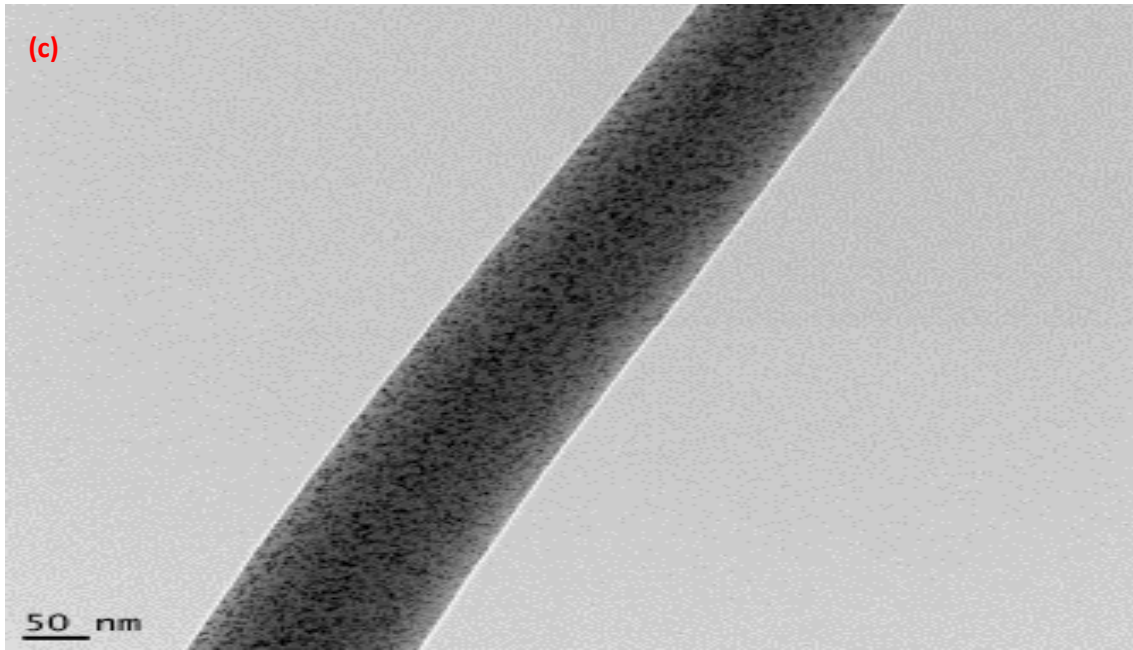


Figure 4.6. TEM images of PVA/AgNO₃ nanofibers containing a) 1wt%; b) 3wt%; c) 6wt% of silver nitrate.

4.8. Chemical Composition of the Nanofiber

Figure 4.7 show the chemical composition of nanofibers obtained using EDX elemental analysis, showing carbon, oxygen and silver as the main elements of the PVA/ AgNO₃ nanofibers on aluminum substrate.

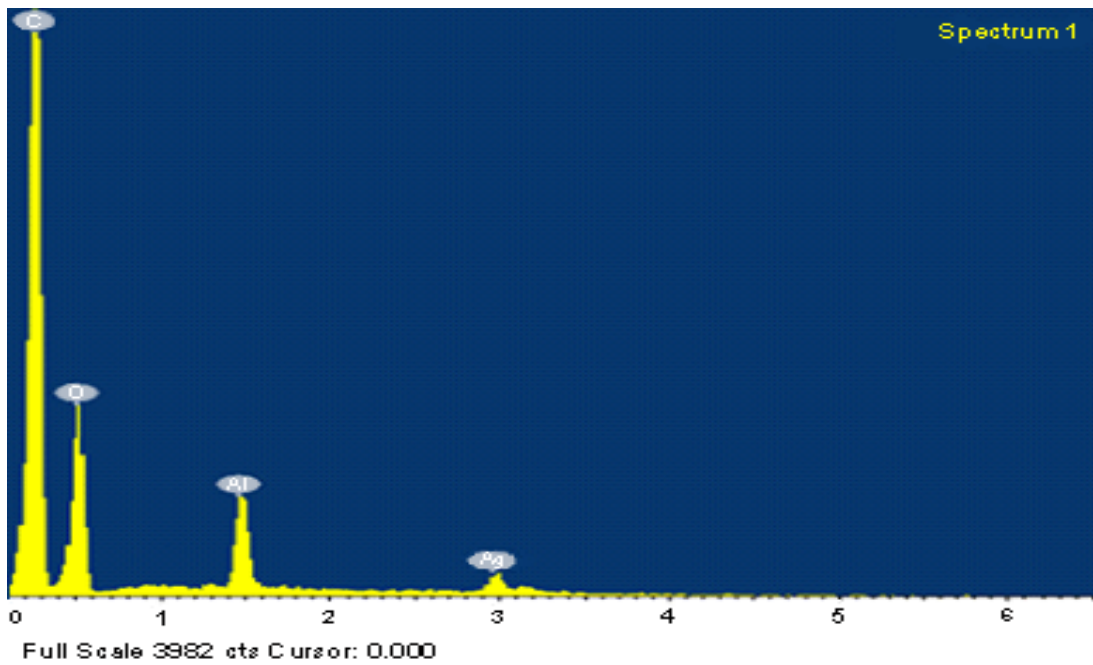


Figure 4.7. Elemental analysis of PVA/AgNO₃ nanofibers.

4.9. Conclusion

In this section using the best results of our studies described in Chapter 3, we have fabricated PVA/AgNO₃ nanofibers with diameters in the range of 230 nm to 550nm. TEM images showed that silver nanoparticles were well dispersed on the surface of the PVA nanofibers. The results showed that the concentration of the deposited nanoparticles is increased as the concentration of silver nitrate in the solvent is increased. Our calculations showed that the average concentrations of the nanoparticles content in the nanofibers are 2.6×10^{16} , 7.2×10^{16} , and 1.4×10^{17} for polymer solution containing 1wt%, 3wt% and 6 wt% of silver nitrate respectively.

Chapter 5

5. Application of the PVA/AgNO₃ nanofibers

5.1. Objective of the chapter

The ultimate goal of this chapter is to investigate the bactericidal activity of PVA/AgNO₃ and also to observe whether increasing the amount of silver nitrate in PVA solution will have any effect on inhibition growth of bacteria. In all experiments, *Escherichia coli* was selected as Gram-negative bacteria. Bacteriological tests were done in Luria Broth (LB) medium on agar plates and then their properties were evaluated employing a growth inhibition assay in liquid medium.

5.2 Studies of Antibacterial Properties

It is believed that silver compounds are toxic to a wide variety of microorganisms. Thus they are good candidates for killing bacteria [15]. Silver nanoparticles have antibacterial properties and showed biocide effect against many bacteria [15].

Nanoparticles can function with various chemical groups and in the case of silver; there are many ways to kill bacteria such as adsorption of silver into polymer [76]. The use of polymer helps the dispersion of the nanoparticles in it due to its unique morphology and long polymeric chains.

Escherichia coli (abbreviated as *E.coli*) is a Gram- Negative bacterium, which is mostly live in the digestive tract of animals and human. The outer membrane of *E.coli* is built from lipopolysaccharide (LPS) molecules that make it strong against chemical attacks. Most *E.coli* has no harm for human, except some kind that can causes poisoning and serious infection transmitted through contaminated water or food [77].

Nanoscaled silver particles with the size of 1-20 nm, get attached to the surface of *E. coli* and damage the cell by penetrating and lowering the lipopolysaccharide (LPS) molecules. Their incorporation into the membrane eventually leads to the release of silver ions in the cytoplasm of the cells. This action results in increased permeability of the cell membrane, DNA damage and finally bacterial death [78]. Figure 5.1 illustrates the interaction between silver nanoparticles and bacteria cell.

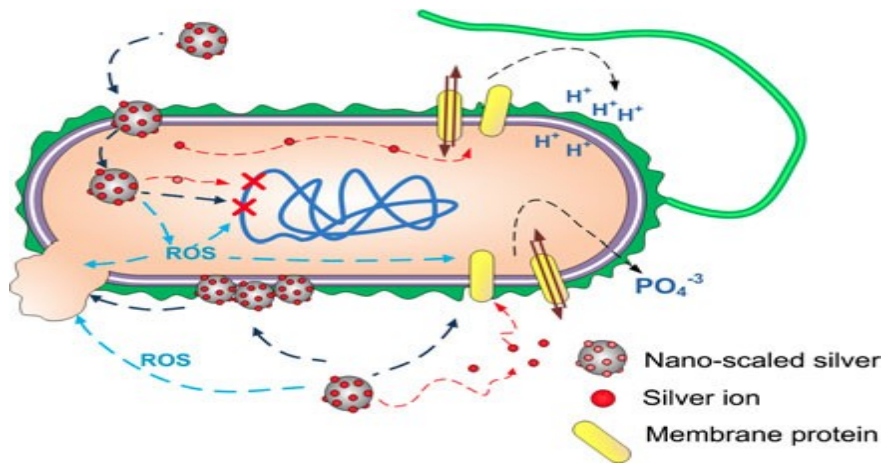


Figure 5.1. Antibacterial activity of silver nanoparticles [79].

5.3 Safety protocols of *E.coli*

As it was mentioned in section 5.2, *E.coli* is mostly live in the intestine of all warm-blooded animals and it is usually innocuous. However, it is wise to be handled with appropriate precautions while working with it, because it is possible for the cultures to be contaminated with pathogenic bacteria. The following precautions should be employed:

- A. Lab coats and safety gloves must be worn during working with bacterial cultures.
- B. For preventing any contamination all the cultures should be kept under laminar airflow with a flame sterilizer on.
- C. Ethanol was used as a disinfectant to wipe off the table. It was always kept away from the flame.
- D. All the solid wastes must be disposed in orange biohazard bags and after autoclaving must be placed in a red biohazard bag.
- E. Hands should be washed thoroughly with soap before leaving the lab.

5.4 Material and Method

The bacterial species of *E. coli* strain X11-blue was selected as gram-negative bacteria. Luria-Broth (LB) is a microbial growth medium, which is commonly used in laboratories to cultivate *E. coli* and it mainly consists of mineral, proteins and salts [77]. The following describes the steps for preparation of LB medium and agar plates.

One liter of LB medium was prepared by adding 5gr Bacto Yeast Extract, 10gr NaCl, 10gr Bacto-Tryptone and 950 ml of deionized water. XL1-Blue *E. coli* stab was plated on a fresh petri dish and was grown overnight in an incubator at 37 °C, then an isolated colony of XL1-Blue *E. coli* Strains was inoculated into 2ml of liquid LB medium, and then shaken overnight in an incubator at 37°C to grow bacteria.

Agar plates were prepared by adding 15gr agar to the solution above and then it was put into the autoclave for 50 minutes in 121°C to get a homogenous solution as shown in Figure 5.2, and then it was poured onto disposable sterilized petri dishes as Figure 5.3 represents. Petri dishes ought to be prepared at least 24 hours earlier to allow entirely solidification, and then must be placed upside down.



Figure 5.2. Well-dissolved LB medium



Figure 5.3. Preparing petri dishes with LB medium.

It is known that PVA is a water-soluble polymer; hence in order to preserve its structure against liquid medium, it is necessary to get cross-linked before placing into each test tube. There are various methods for crosslinking such as chemical cross-linking, which is not suggested due to the toxicity of the some of these chemicals. Here UV irradiation ($\lambda=360$ nm) was employed to crosslink the nanofibers for 3 hours prior to the experiments.

PVA/ AgNO₃ nanofibers containing 1wt% and 6wt% of silver nitrate was examined as a bacterium inhibitor. Pure PVA nanofibers under the same condition was used as a

blank control. In order to observe the bactericidal efficiency by the nano material containing silver, a negative control (a sample-free test) was used in absence of nanomaterial as a reference test.

For preparing four test tubes, twelve tubes were marked and *E. coli* strain XL1-Blue grown in Luria broth (LB) was added to the fresh LB medium in presence and absence of the nanomaterial. After UV irradiation of fibers, pieces of 5mm×5mm were added to the culture tubes containing bacteria and then placed into incubators and incubated at 37°C on a rotary shaker set at 250 RPM for 3 and 6 hours. At the end of the incubation period, 100 µl of the cultured samples were serially diluted (Figure 5.5) in 900µL of sterile LB medium. The serial dilutions were labeled as A (1:10 dilution), B (1:100 dilution), C (1:1000 dilution), D (1:10000 dilution) and E (1:100000 dilution). Then, 100 µL of each dilution were dispersed on a fresh petri dish as shown in Figure 5.6 and spread uniformly by a clean rod on the surface of the nutrient agar plate. For this experiment the total number of 120 petri dishes were plated by each dilution. Then, all the plates were placed into an incubator and grow overnight at 37 °C. The following day the numbers of bacterial colonies were counted on the each petri dish. We should give enough time for bacterial colonies to grow and become easily countable, usually 24 hours, otherwise tiny colonies may not be developed into larger ones and will be missed in counting process. The antibacterial efficiency of the PVA/AgNO₃ nanofibers was calculated according to the following equation:

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

Where A and B represents the numbers of colonies from the test samples and the control test respectively.



Figure 5.4. Transferring 900 μL LB medium into tube.

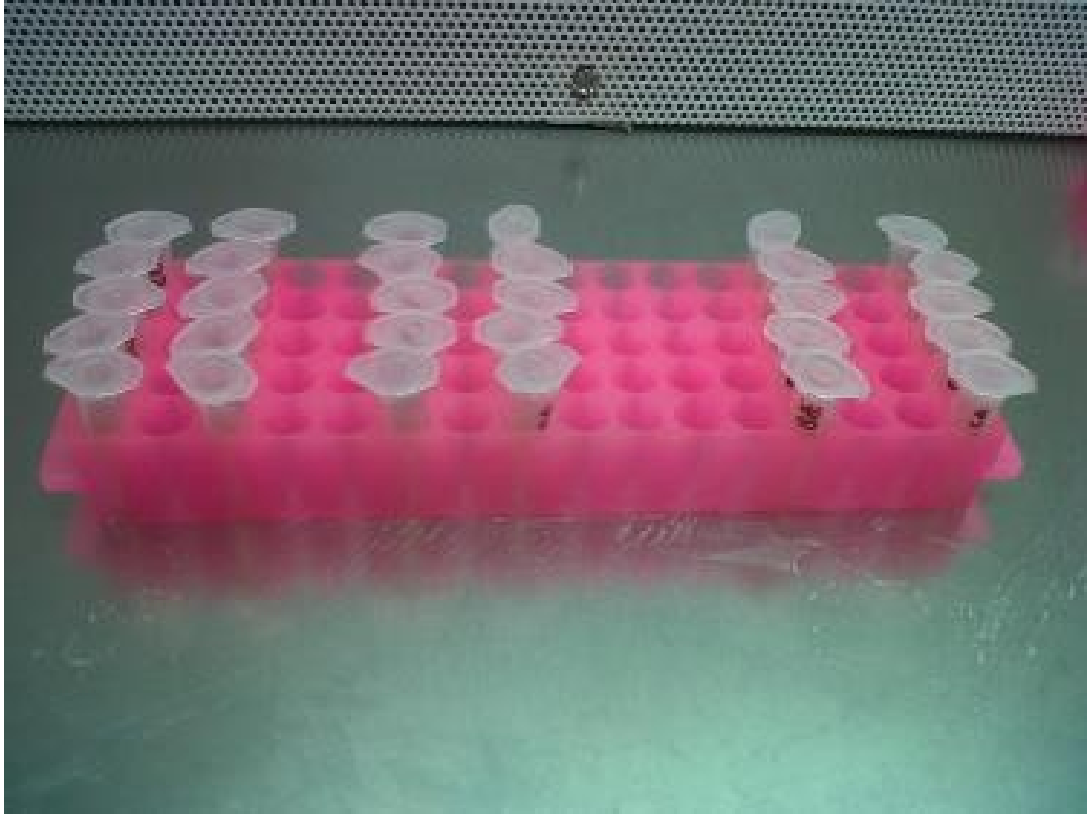


Figure 5.5. Serial dilutions for counting bacterial colonies in agar plates.

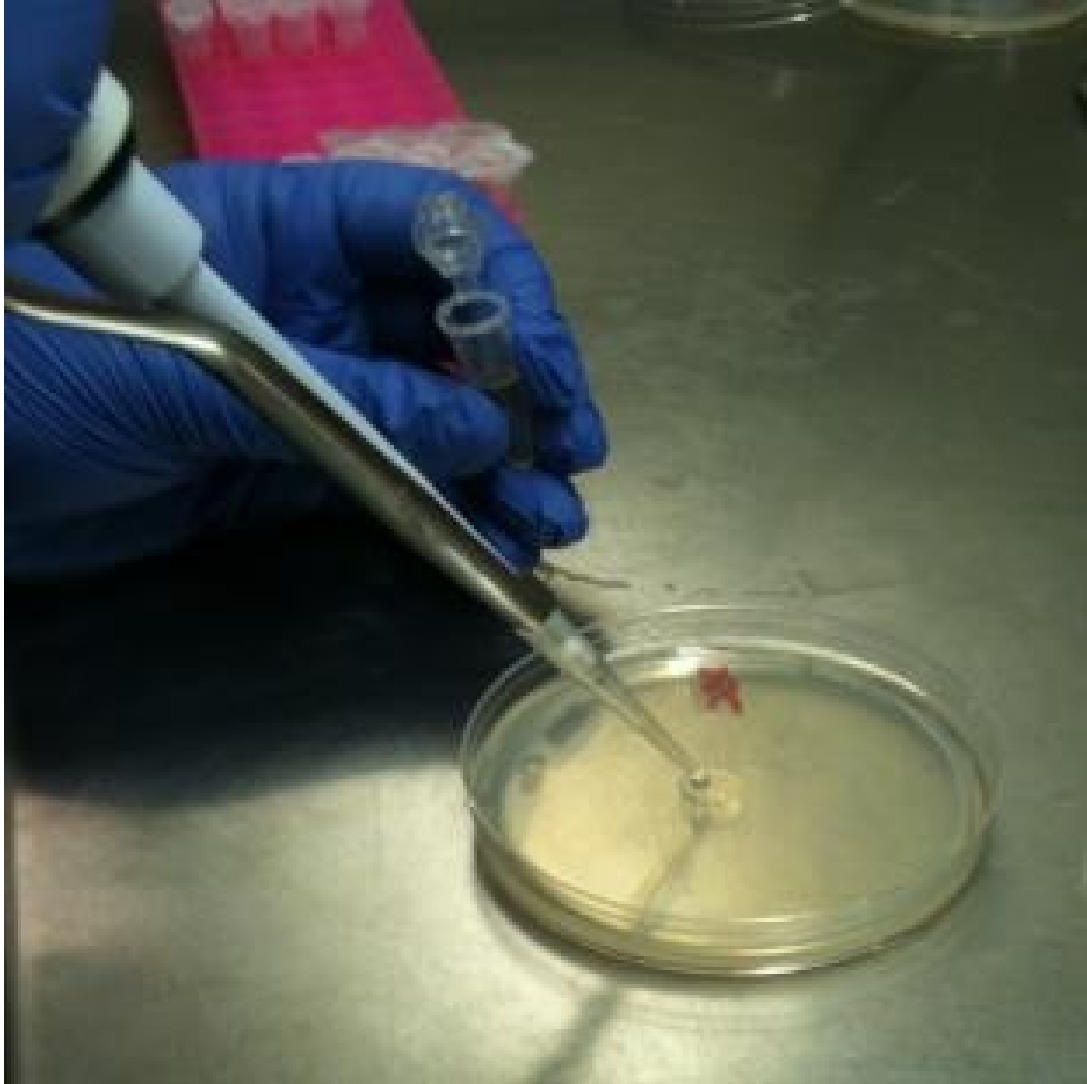
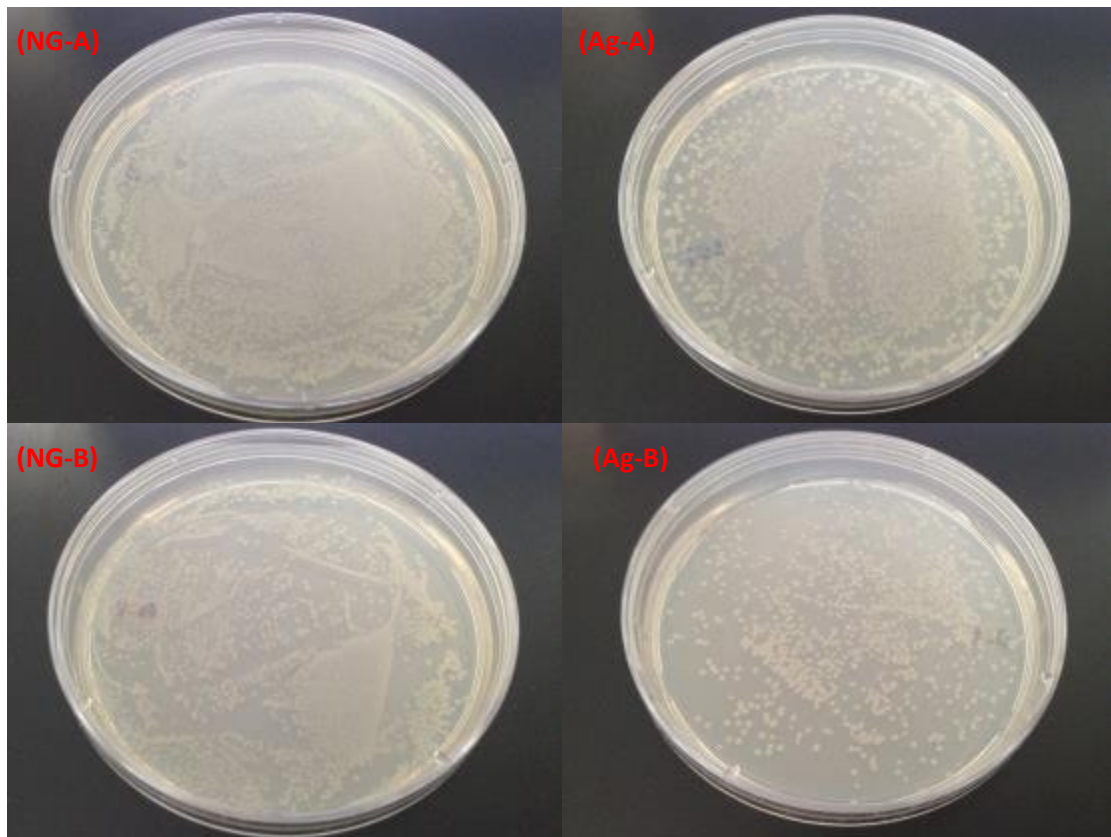


Figure 5.6. Plating diluted bacteria on petri dishes.

5.5 Results and Discussion

In this study, the antibacterial activity of pure PVA and PVA/AgNO₃ nanofibers were evaluated using a growth inhibition assay in liquid medium. In these experiments, a negative control, pure PVA nanofibers and two PVA nanofibers containing silver nanoparticles were selected to compare the inhibition platen growth of bacteria. All tests were triplicated at two contact times of 3hours and 6hours to observe their liability and accuracy. All of the dilutions were plated on nutrient agar plates however, only the last set of dilutions with much lower concentrations were compared. Figure 5.7 demonstrates that colonies, which are easily countable once the solutions are successively diluted for 5times.



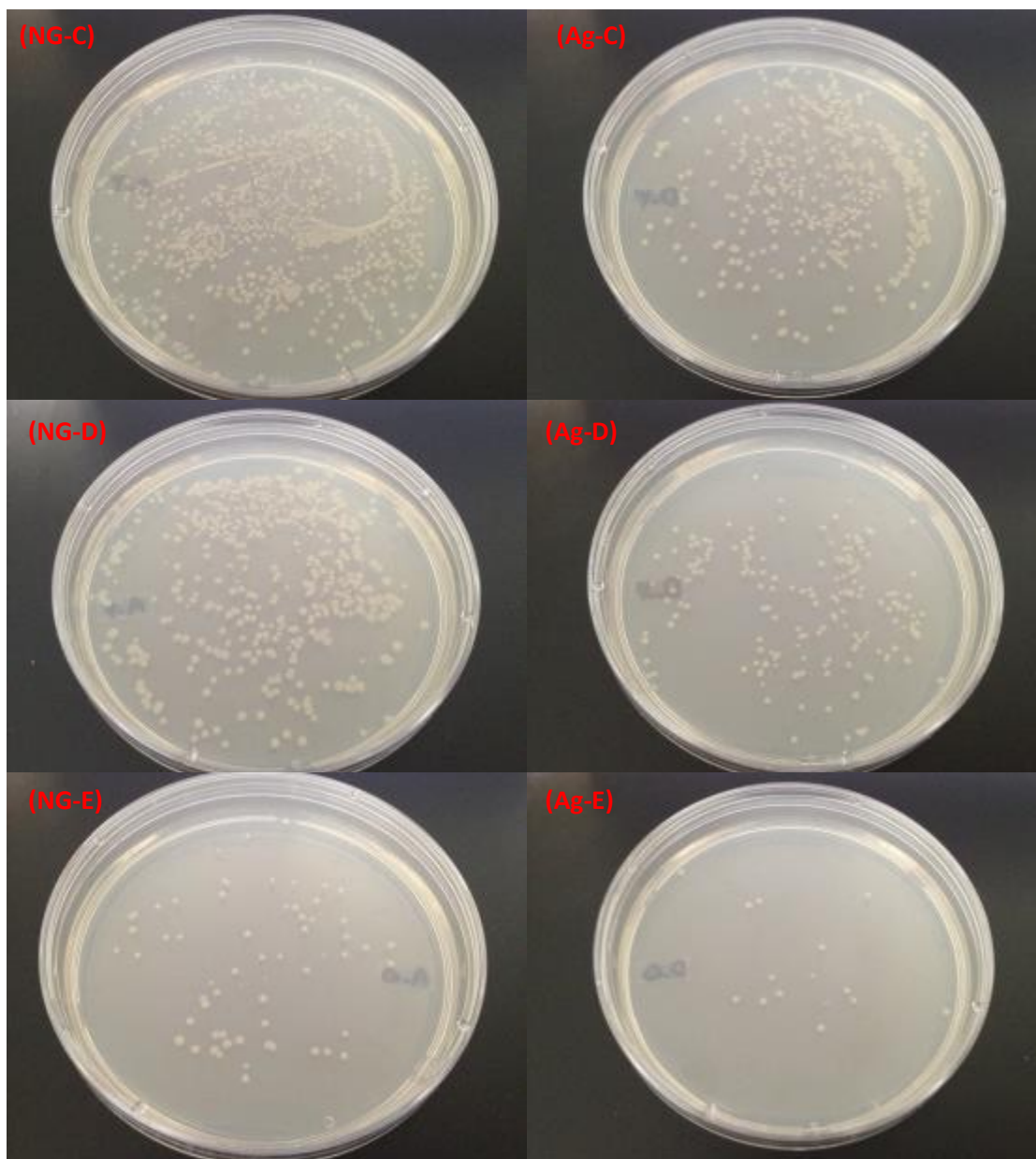


Figure 5.7. Five dilutions of negative control (NG) on the left column and PVA nanofibers containing 1wt% of silver nanoparticles (Ag) on the right column at contact time of 3hours.

For observing the antibacterial activity of pure PVA against *E.coli*, the last dilutions (1:100000) of PVA and negative control were compared, the results could be observed in Figure 5.8. It can be seen that there is no significant difference between the numbers of survived bacteria after waiting for 3 hours, which confirms the absence of bactericidal activity of pure PVA.

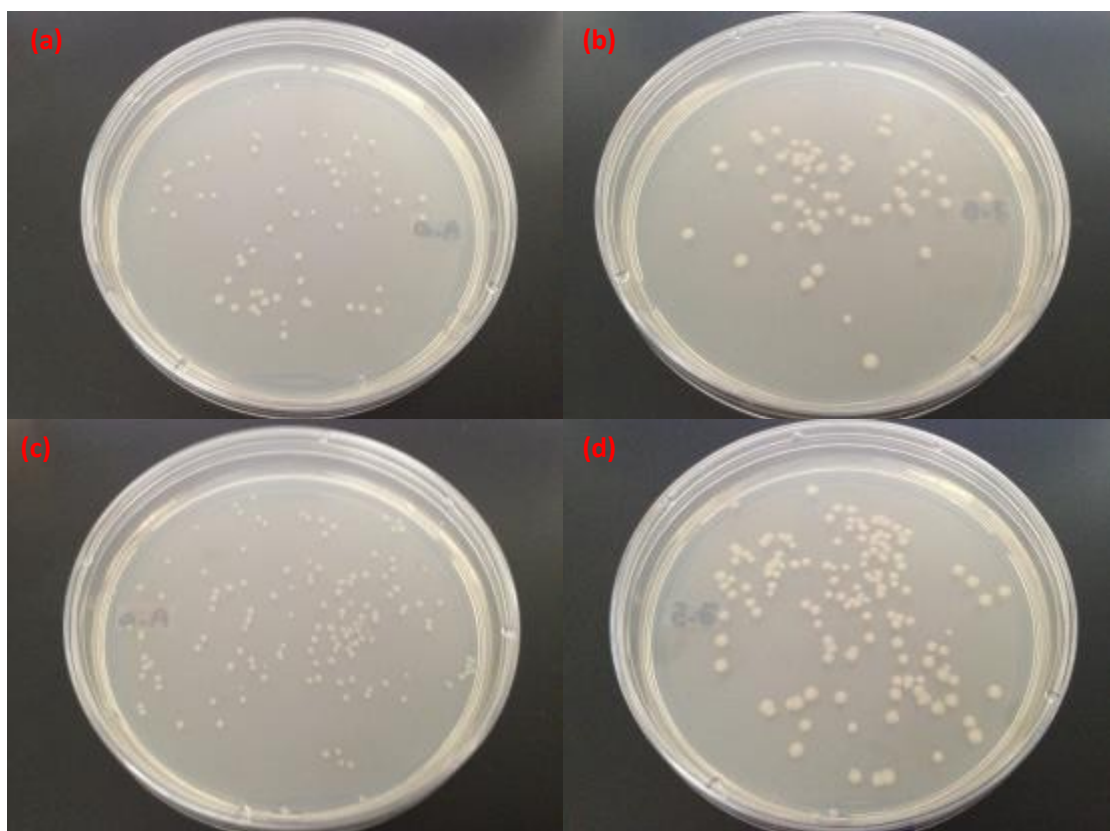


Figure 5.8. Comparison between antibacterial effects of a) Negative control; b) Pure PVA nanofibers at contact time of 3hours. c) Negative control; and d) Pure PVA nanofibers at contact time of 6hours

Figure 5.9 illustrates the survived colonies and draws a comparison between the last dilutions (1:100000) of the negative control and the PVA nanofibers containing 1 wt% and 6wt% of silver nanoparticles at the two contact times. The bactericidal activities of PVA/ AgNO₃ nanofibers are shown in Figure 5.10. The number of colonies decreased from $520(\pm 17) \times 10^5$ in the negative control to $150(\pm 30) \times 10^5$ in PVA/ AgNO₃ nanofibers containing 1wt% of silver nitrate at contact time of 3 hours, which represents 71% inhibition efficiency. For the contact time of 6hours, the numbers of survived bacteria in the negative control and PVA nanofibers containing 1wt% silver nitrate were $1160(\pm 36) \times 10^5$ and $320(\pm 37) \times 10^5$ respectively, which indicates a 72% bactericidal efficiency.

In the PVA containing 6wt% of silver nitrate the number of survived colonies was reduced. Our experiments showed that the number of survived colonies in the negative control $520(\pm 17) \times 10^5$ was reduced to $10(\pm 5) \times 10^5$ when the cells were incubated in presence of the PVA/ AgNO₃ nanofibers for 3hours, which represent 98% bactericidal efficiency. For the 6 hours contact time with the same fibers the bactericidal efficiency was also about 97%.

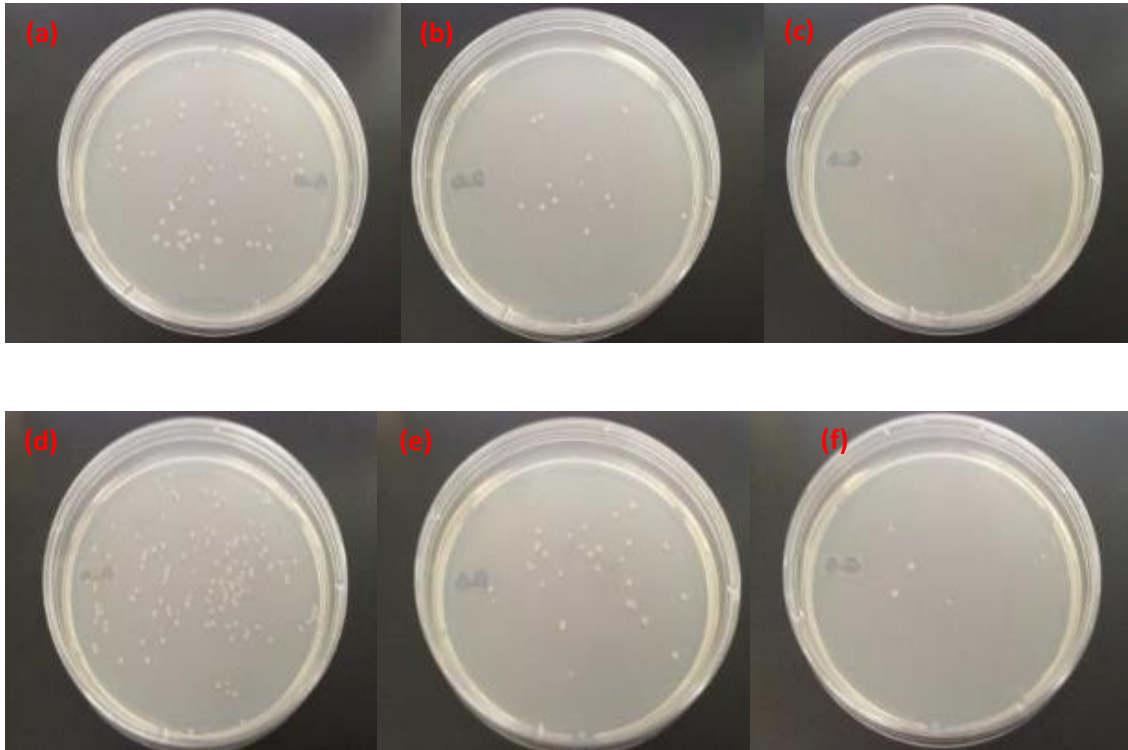


Figure 5.9. Comparison between the fifth dilution of a) negative control; b) PVA nanofibers containing 1wt% of silver nitrate; c) PVA nanofibers containing 6wt% of silver nitrate after 3hours contact time. d) Negative control; e) PVA nanofibers containing 1wt% of silver nitrate; f) PVA nanofibers containing 6wt% of silver nitrate, after 6hours contact time.

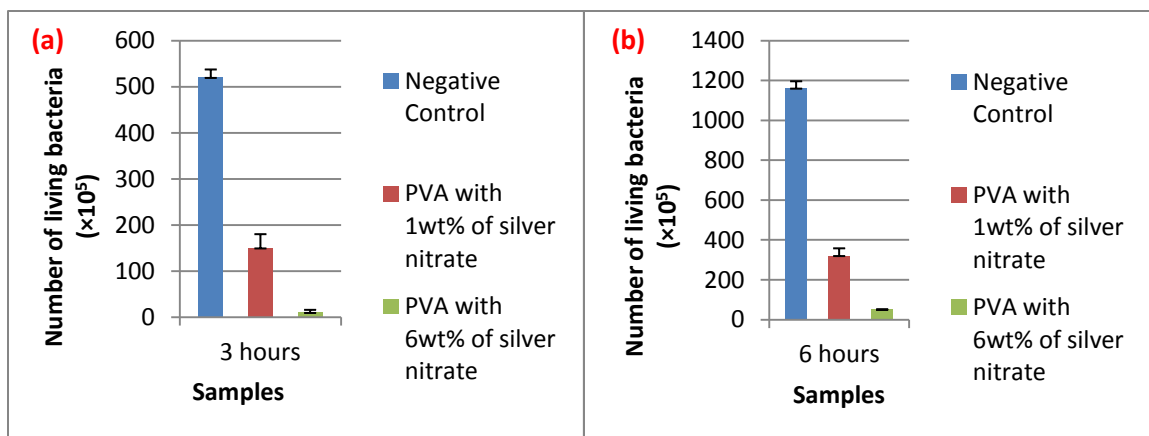


Figure 5.10. Total numbers of *E.coli* colonies grown on LB agar plates in the test samples prior to the dilutions at two contact times of a) 3 hours; b) 6 hours ($\times 10^5$). Black lines represent the standard deviation.

5.6 Conclusions

PVA nanofibers containing well-distributed silver nanoparticles were prepared by electrospinning followed by UV irradiation, and then were introduced into test tubes containing bacteria and their bactericidal activity was evaluated. Results showed that PVA nanofibers in absence of silver act like the negative control while PVA nanofibers containing low concentration of silver nitrate showed 71% growth inhibition and this number reached to 98% as the concentration of silver nanoparticles increased. The results show the ratio of the concentration of colonies died in 6 hours to the concentrations of silver nanoparticles is very low indicating, that the antibacterial effects of the device could last for a long time.

Chapter 6

6. Conclusions, Contribution and Future Work

6.1. Conclusions and Contribution

In this research, several techniques for preparing the nanofibers have been presented. Here we have fabricated and characterized PVA/AgNO₃ nanofibers by electrospinning method followed by UV irradiation to preserve the structure of the nanofibers against liquid medium. The fabricated nanofibers were used as a device for destroying bacteria. Several parameters, which affect the diameter of these nanofibers, were identified. The parameters such as voltage, flow rate; collection distance, polymer concentration and conductivity were investigated. SEM, TEM, and EDX were used to characterize the results of fabricated devices. The result of TEM confirmed the well distribution of silver nanoparticles on PVA nanofibers. For the first time these PVA nanofibers incorporated with Ag nanoparticles were examined against *E.coli* bacteria using growth inhibition in liquid medium. In this method serial dilutions were done at two contact times of 3hours and 6hours. According to our experimental results, PVA nanofibers incorporated with Ag nanoparticles exhibited strong antimicrobial activity against *E.coli*. It was shown that increasing the silver content will enhance the antibacterial efficiency of the fabricated nanofibers.

Contributions of this research are summarized as follow:

- PVA nanofibers incorporated Ag nanoparticles were fabricated using electrospinning method on aluminum substrate.
- Effect of various electrospinning and solution parameters such as applied voltage, flow rate, collection distance, solution concentration and conductivity were investigated.
- Uniform PVA/AgNO₃ nanofibers were obtained by optimizing the above parameters.
- The well dispersion of silver nanoparticles on electrospun nanofibers was confirmed using TEM microscope.
- To our best knowledge, for the first time the fabricated electrospun PVA/AgNO₃ nanofibers were examined against *E.coli* using growth inhibition in liquid medium. Our experimental results confirmed that the number of bacteria significantly reduced after increasing the content of AgNO₃ in PVA solution.
- Incorporating the fabricated device in commercialized water filters can add/enhance disinfecting parameters in the filter.

6.2. Future work

In this research the effects of several parameters on fabrication of nanofibers using electrospinning were investigated. However, effect of other parameters like types of atmosphere, surface tension and humidity on the structure of the nanofibers can be explored and this could contribute to the database used in design of the experiment.

The investigation can be carried out to study how to manipulate the size of the dispersed nanoparticles on PVA nanofibers and furthermore, the effect of nanofibers morphologies on the antibacterial activity could be studied.

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