TEMPERATURE AND HUMIDITY EFFECTS ON CHITOSAN FIBERS DURING ELECTROSPINNING

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Approval of the Graduate School of Natural and Applied Sciences, Atılım University.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Atılım University.

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ABSTRACT

TEMPERATURE AND HUMIDITY EFFECTS ON CHITOSAN FIBERS DURING ELECTROSPINNING

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During this research, a natural biodegradable polysaccharide, chitosan (CS), is employed to prepare nanofibers. The temperature and humidity results of the electrospinning process on chitosan fibers are examined within ideal parameters, and these parameters embrace 7 cm for the distance between the needle and collector. In addition, the flow rate was fixed at 2 µl / min, while applied voltage was at 16.5 kV. The process of obtaining a polymer solution was administered by dissolving chitosan powder in trifluoroacetic acid (TFA) on a hot plate ranging between 50 – 55°C with a magnetic stirrer until a homogenous solution was procured. Specific examinations of the chitosan fibers were conducted by SEM, ATR-FTIR, and DSC. Scanning Electron Microscopy (SEM) was employed to observe the morphology of chitosan fibers, while Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) was employed to investigate the chemical structural analyses of the chitosan fibers. Differential Scanning Calorimeter (DSC) was done in order to show the melting enthalpy and its temperature changes for different cases of temperature and humidity. The average fiber diameter, pore size distribution, and percentage porosity values were found via ImageJ program (Version, 1.52a) using SEM photographs taken from SEM analysis. The average fiber diameter was determined to be in the range of (260 ± 2.20 – 442 ± 2.72 nm), whereas the pore size distribution (0.039 ± 0.004 – 0.063 ± 0.007 µm) and the porosity was in the range of (49 – 60 %). According to the results of SEM analysis, the fiber diameter decreased with increase in temperature, the pore size distribution and the percentage porosity of chitosan fibers were increased by raising the temperature. In contrast, the average fiber diameter increased after raising the humidity, including the pore size distribution and porosity. ATR-FTIR results showed that the chemical structure of chitosan has the same composition for various cases of the ambient parameters. Thermal properties of chitosan fibers were studied by DSC and the results showed that the variation of temperature and humidity in the production of chitosan fibers leads to change in the melting enthalpy and its temperature.

Key words: Electrospinning, Chitosan, Temperature, Humidity, Nanofibers

ÖZ

ELEKTROEĞİRME PROSESİ BOYUNCA SICAKLIK VE NEM PARAMETRELERİNİN KİTOSAN FİBERLER ÜZERİNE ETKİSİ

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Bu çalışmada, doğal biyobozunur polisakkarit, kitosan (CS), kullanılarak nanofiberler elde edilmiştir. Elektroeğirme işleminin sıcaklık ve nem parametrelerinin kitoson fiberler üzerine etkisi optimize edilmiş parametrelerle araştırılmıştır. Bu parametrelerden iğne ve toplayıcı arasındaki mesafe 7 cm'dir. Ek olarak, akış hızı 2 µl/dk iken, uygulanan voltaj ise 16,5 kV'dir. Kullanılan polimer çözeltisi, kitosan polimerinin trifloroasetik asit (TFA) içerisinde 50 – 55°C'de sıcak plaka üzerinde manyetik karıştırıcı ile homojen çözelti elde edilene kadar karıştırılması ile elde edilmiştir. Kitosan fiberlerinin karakteristik özellikleri SEM, ATR-FTIR ve DSC ile tayin edilmiştir. Kitosan fiberlerinin morfolojik özellikleri Taramalı Elektron Mikroskopisi (SEM) ile, kimyasal yapı analizi ise Zayıflatılmış Toplam Yansıma - Fourier Dönüşümlü Kızılötesi Spektrometresi (ATR-FTIR) ile incelenmiştir. Farklı sıcaklık ve nem parametreleri için erime entalpisi ve sıcaklık değişimleri Diferansiyel Taramalı Kalorimetre (DSC) ile belirlenmiştir. Ortalama fiber çapı, gözenek boyutu dağılımı ve yüzde gözeneklilik değerleri, SEM analizinden alınan fotoğraflarla ImageJ programı (Versiyon, 1.52a) kullanılarak tayin edilmiştir. Ortalama fiber çapı (260 ± 2.20 – 442 ± 2.72 nm), gözenek boyutu dağılımı (0.039 ± 0.004 – 0.063 ± 0.007 µm) ve yüzde gözeneklilik ise (% 49 – 60) aralığında bulunmuştur. SEM analizi sonuçlarına göre sıcaklık artışı ile; ortalama fiber çapı azalmış, gözenek boyutu dağılımı ve yüzde gözeneklilik ise artmıştır. Buna karşılık nem artışı ile ise; ortalama fiber çapı, gözenek boyutu dağılımı ve yüzde gözeneklilik artmıştır. ATR-FTIR sonuçları ile, kitosanın kimyasal yapısının çeşitli ortam parametreleri için aynı bileşime sahip olduğu görülmüştür. Kitosan fiberlerinin termal özellikleri DSC ile araştırılmış, sonuçlar kitosan fiberi üretimindeki sıcaklık ve nem değişikliğinin erime entalpisi ve sıcaklığında değişime yol açtığını göstermiştir.

Anahtar kelimeler: Elektroeğirme, Kitosan, Sıcaklık, Nem, Nanofiberler

To My Family

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LIST OF ABBREVIATIONS

CS	Chitosan
TFA	Trifluoroacetic Acid
PCL	Poly (Ɛ-Caprolactone)
PVP	Poly (Vinyl Pyrrolidone)
PVA	Poly (Vinyl Alcohol)
PVC	Poly (Vinyl Chloride)
DMF	Di-Methyl Formamide
MC	Methanol-chloroform
PEO	Polyethylene Oxide
PDLA	Poly (D-L-Lactic Acid)
PS	Polystyrene
PE	Polyethylene
SEM	Scanning Electron Microscopy
ATR-FTIR	Attenuated Total Reflection Fourier Transform Infrared
DSC	Differential Scanning Calorimeter
T (°C)	Temperature
RH (%)	Relative Humidity
Tm	Melting Temperature
Tg	Glass Transition Temperature
Tc	Crystallization Temperature

CHAPTER 1

INTRODUCTION

Tissue engineering procedures mainly include three key parts: cells, biochemical or mechanical stimuli and scaffolds. Scaffolds ordinarily work as the foundation for several procedures to support tissue production. Despite a broad variety of scaffold substances being available, scaffolds of the polymeric are generally used as carriers for bioactive factor delivery and promoting tissue growth. Considering the nanofibers of the polymer being suited for several applications, these nanofibers are employed in tissue engineering; moreover, to restore a difference of tissues in trials. The demand for nanofibers is described in several studies concentrating on their application, interaction with cells and production [1]. Electrospinning offers a unique method for producing nanofibers from polymer solutions, which can be used for advanced application in tissue scaffolding and chemical and biomedical sensors [2]. The process of electrospinning considers a broadly utilized technology that uses electrical powers to fabricate polymer fibers with diameters varying from 2 nm to many micrometers [3], [4]. Electrospinning has gained popularity in the last 10 years due in large part to an increased interest in nanoscale properties and technologies [5]. Despite the apparent simplicity of the electrospinning principle, the process itself is quite complicated because many parameters influence the properties of the acquired nanofibrous structures [6]. These parameters can be merged into three groups, i.e. solution parameters, processing parameters, and ambient parameters [7]. The polymers used in the process are classified into two types: natural and synthetic. In comparison with their synthetic counterparts, natural polymers generally have better biocompatibility and hence are more suitable for the human body. However, to convert a natural polymer into submicron or nanometer fibers through electrospinning is usually more difficult than producing a synthetic polymer, and for this reason, relatively few studies addressing electrospinning of some natural polymers can be found [8]. In this study, the natural polymer material, chitosan was used to produce nanofibers. Chitosan is a linear polymer of α (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose and is easily derived by N-deacetylation to a varying extent that is characterized by the degree of deacetylation and is consequently a copolymer of N-acetylglucosamine and glucosamine [9].

Previous studies have mostly concentrated on synthetic polymers, which are easier producing nanofibers than natural polymers [10]. Also, some of the other studies used process and solution parameters, but more attention was given to process and solution parameters studies than ambient parameters [11]. In this study, the ambient parameter was used in addition to the process and solution parameters. The other difference is for studies in most of the previous research, more than one polymer was used to produce nanofibers [12]. In contrast, this study used one polymer.

The main goal of this work is to study the effect of the temperature and relative humidity on chitosan fibers (taking 8 wt.% for the concentration of chitosan) and prepare the chitosan fibers under different circumstances of temperature and humidity. In addition, the purpose of investigating these effects of the temperature and relative humidity on the fibers is to obtain continuous free bead-less fibers.

CHAPTER 2

LITERATURE REVIEW

2.1. Electrospinning

2.1.1. History of Electrospinning

Electrospinning is an old technique which was first observed in 1897 by Rayleigh, studied meticulously by Zeleny on electrospraying [13], and patented by Formhals in 1934 [14]. Taylor’s work on electrically driven jets laid the foundation for electrospinning [15]. The term “electrospinning,” which derives from “electrostatic spinning,” has been in common use relatively recently (since around 1994), but its origin can be traced back more than 60 years [16]. From 1934 to 1944, Formhals published a series of patents outlining an experimental setup for the production of polymer filaments utilizing an electrostatic force [17]. The first patent (US Patent Number: 2116942) on electrospinning was issued for the fabrication of textile yarns and a voltage of 57 kV was utilized for electrospinning cellulose acetate using acetone and monomethyl ether of ethylene glycol as solvents [16]. This process was patented by Antonin Formhals in 1934 who was subsequently granted related patents (U.S. Patents 2116942, 2160962 and 2187306) in 1938, 1939, and 1940 [18]. Formhals' spinning process consists of a movable thread collecting device to collect threads in an elongated state, like that of a spinning drum in conventional spinning [19]. About 50 patents for electrospinning polymer melts and solutions have been filed in the past 60 years [20]. Vonnegut and Newbauer in 1952 invented a simple device for electrical atomization and produced streams of highly electrified uniform droplets of about 0.1 mm in diameter [21]. After that, Drozin in 1955 examined the dispersion of a series of liquids into aerosols under high electric potentials [22], and Simons in 1966 patented an apparatus for the production of non-woven fabrics that were ultra-thin and very light in weight with various patterns using electrical spinning [23]. In 1971, Baumgarten invented an apparatus to electrospin acrylic fibers with diameters in the range of 0.05 – 1.1 μm [24]. Since the 1980s, particularly recently, the electrospinning process has regained more attention probably due to a surging interest in nanotechnology, as ultrafine fibers or fibrous structures of various polymers with diameters down to submicrons or nanometers can be easily assembled with this process [17]. The surge in popularity of the electrospinning process can be understood by the fact that over 200 universities and research institutes worldwide are studying various aspects of the electrospinning process and the fiber it produces. Also, the number of patents for applications based on electrospinning has grown in recent years [16]. Some companies such as eSpin Technologies, NanoTechnics, and KATO Tech are actively engaged in reaping the benefits of the unique advantages offered by electrospinning, while companies such as Donaldson Company and Freudenberg have been using this process for the last two decades in their air filtration products [25].

2.1.2. Electrospinning Process

Electrospinning is a method in which materials in solution or melt are formed into nano - or micro - sized continuous fibers (Figure 2.1) shows a schematic illustration of the basic setup for electrospinning.

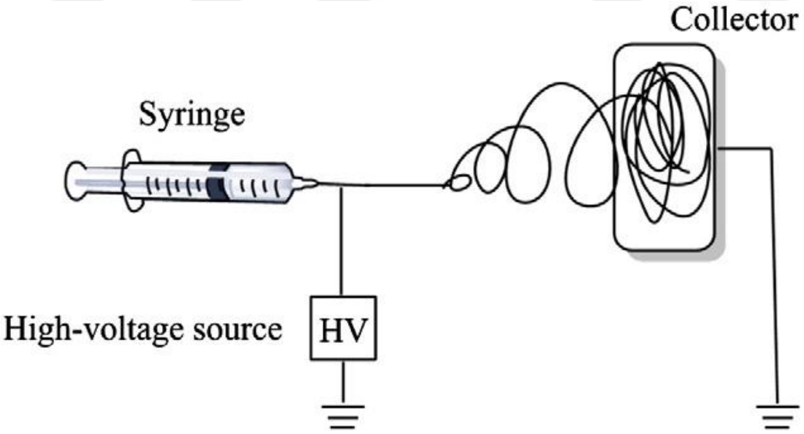

Figure 2.1 Schematic illustration of the basic setup for electrospinning [26].

It consists of three main components: a high-voltage source, a syringe pump, and a collector [26]. The electrospinning technique can be regarded as a variant of the electrostatic spraying (electrospraying) process, as both techniques use high voltage to induce the formation of liquid jets [27]. Small droplets or particles are formed as a result of the break-up of the electrified jet in electrospraying, whereas a solid fiber is formed as the electrified jet is elongated in electrospinning and this process employs electrostatic forces to stretch the solution or melt it as it solidifies [28]. The process of electrospinning in general occurs within the atmosphere of a room which implies the temperature and humidity [27]. The electrostatic forces transforming the liquid cause the formation of the droplet to convert from a rounded meniscus into the Taylor cone, a phenomenon observed due to the electrostatic repulsions among similar charges toward the liquid [15]. At this crucial stage, the applied electric range eventually converts more apparently and succeeds the surface tension of the liquid beginning with a jet of the solution stretched from the top of the Taylor cone. Also, as the jet moves through the air, the current changes from ohmic to convective because of the charges transferring to the surface of the fiber. Furthermore, an unbalanced vortex of the jet occurs in the gap between the tip and the collector [7].

2.1.3. Types of Electrospinning

2.1.3.1- Basic Needle Based Electrospinning

Generally, there are vertical and horizontal set ups and these are the two standards for electrospinning as shown in Figure 2.2 [29].

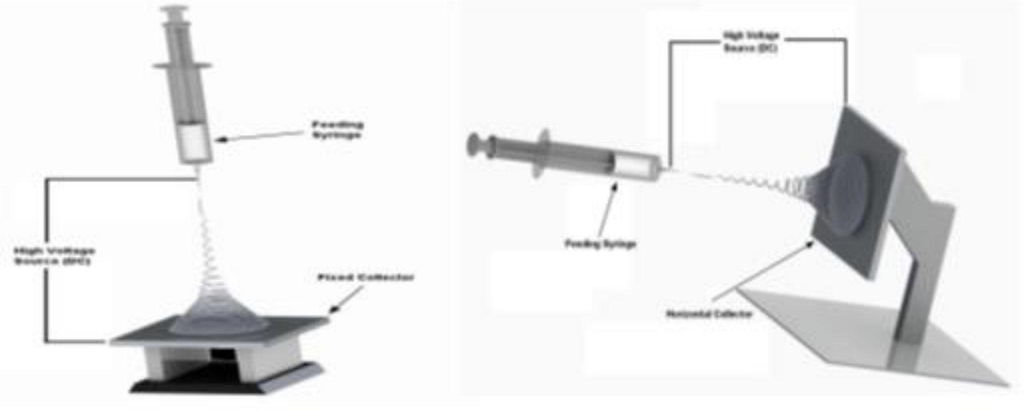

Figure 2.2. The vertical and horizontal setup for electrospinning [7].

There are many types of basic needle based and some of these are:

2.1.3.1.1- Multi-axial Electrospinning

Modern attempts in electrospinning focus on changing the fibers with structural characteristics like core-sheath, hollow, porous, as well as tri-axial-channel threads for use in different applications. the Figure 2.3(a, b) shown the setup for multi-axial electrospinning [30].

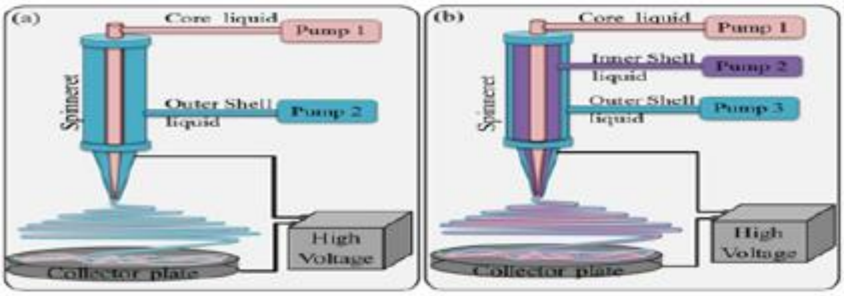

Figure 2.3. Multi-axial electrospinning with different setup: (a) Co-axial electrospinning & (b) Tri-axial electrospinning [30].

2.1.3.1.1.1- Coaxial Electrospinning

Core-sheath threads are created by coaxial electrospinning, where a coaxial spinneret is formed outside and the inner needle is generally utilized as shown in Figure 2.3(a). Coaxial electrospinning could generate threads from several solution partners, core-sheath, hollow, and practical fibers that may include particles. A hollow fiber is created using coaxial electrospinning usually by a temporary substance as the core and the original material as the shell. Depending on the post-spinning method, oil is usually used as the substitute material as it is almost always easier to remove it than different higher molecular weight substances [30].

2.1.3.1.1.2- Tri-axial Electrospinning

In triaxial electrospinning, three polymer liquids are poured into a composite Taylor cone utilizing a spinneret as shown in Figure 2.3(b). Triaxial fibers could be produced with differing hydrophobicity and mechanical force [30].

2.1.3.1.2- Bi-component Electrospinning

The picture for the bi-component side by side electrospinning is shown in Figure 2.4.

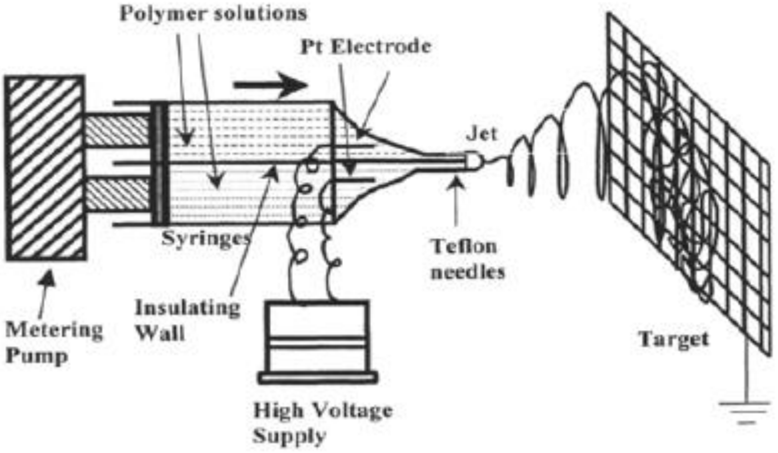

Figure 2.4. The setup of electrospinning for side by side bi- component [26].

where the two plastic syringes, each including a polymer liquid, are side-by-side. A typical syringe pump controlled the flow rate of these two polymer solutions. The platinum electrodes immersed in each of these solutions were connected in parallel into a high voltage DC supply, and the free tips of the Teflon needles connected to the syringes were adhered together [31]. The reason for the side-by-side bi-component thread is that the single thread can show the characteristics of all of the components of the fiber [29].

2.1.3.1.3- Multi-needle Electrospinning

The most direct approach to raising productivity is increasing the number of needles, which is connected to multi-needle electro-spinning as shown in Figure 2.5 [30].

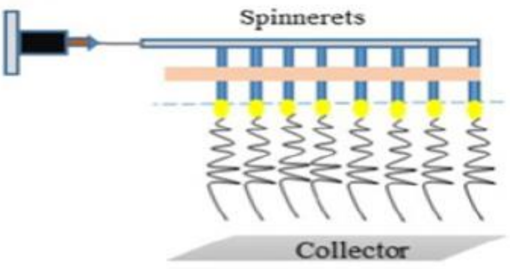

Figure 2.5. Multi-needle based electrospinning [30].

Here the polymer solution is applied through multiple needles joined into a high voltage supply, and the syringe pump is used to pump the spinning solvent to the spinneret setup; also, other spinning solutions could be injected individually into two different sets within the same multiple spinneret setups. Due to the large mass of the spinning solvent transfer, a high voltage is required for continuous electrospinning. The disadvantages of this process include blocking at the top of the needles and cleansing of multiple needles, unsettled electric field force and the difference in fiber size distribution. However, even though a high flow rate (1–18 mL/h) could be obtained in multi-needle methods, the repulsion of adjacent jets in multi-needles is still an issue [30].

2.1.3.1.4- Electroblowing/Gas-assisted/Gas jet Electrospinning

Electroblowing is a method which mixes electrostatic nanofiber products (electrospinning) beside airflow around the spinneret as shown in Figure 2.6 [32].

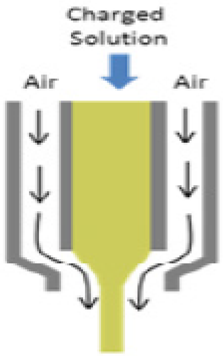

Figure 2.6. Gas jet electrospinning [34].

Also, the tangential powers of the flowing air working on a drop of mixture add to the creation of the Taylor cone and production of the nanofiber [32]. With the extra elongating force supplied by the gas stream, small diameter fibers are created [33]. A combination of forces of the electric field is applied and the airflow augments the performance of the electrospinning method, and airflow accelerates the vaporization of solvent from the solution. This is the only method in which hyaluronic acid could spin in its native mode [30].

2.1.3.1.5- Magnetic Field Assisted Electrospinning

Juan A. Gonzalez Sanchez et al. described the formation of polymeric nanofibers, including magnetic nanoparticles, using electrospinning supported by a magnetic field [35]. Figure 2.7 shown the set up for magnetic field assisted electrospinning.

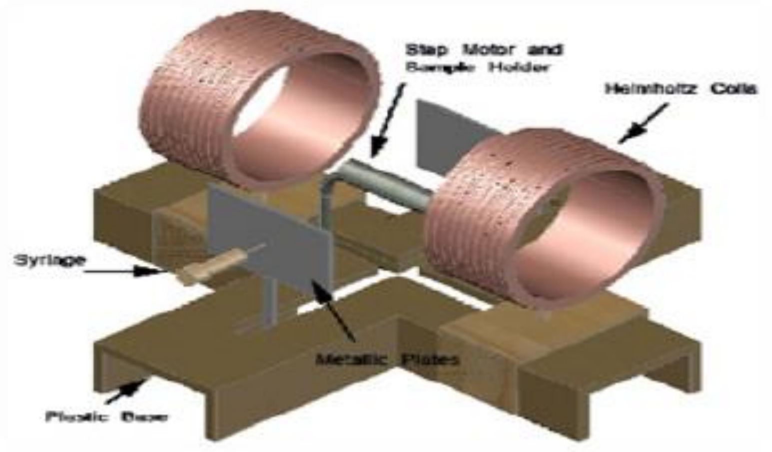

Figure 2.7. The set up for magnetic field assisted electrospinning [35].

A magnetic field was employed in situ through the electrospinning process. For this purpose, a combination of Helmholtz Coils, all having 200 turns, providing a current ranging from 1 A to 3 A, were utilized. The coils were parted with a distance equal to the radius of the annular loops (10.5 cm). A step machine was used to rotate the specimen holder during deposition. By observing the electrospinning method, it can be seen that with the utilization of the electromagnetic field via fiber deposition, the polymer stream results were more fixed to the target. The nanofibers produced had a diameter ranging from 100 nm to 700 nm [35].

2.1.3.1.6- Conjugate Electrospinning

The system used in conjugate electrospinning is depicted in Figure 2.8.

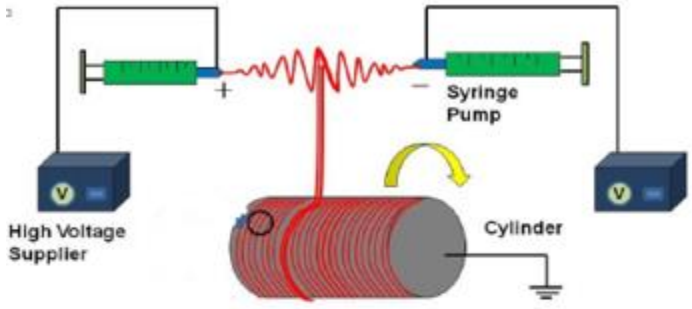

Figure 2.8. The conjugate electrospinning [104].

It contains two or three very high voltage power supplies with different polarities, two or three spinnerets, and a collector drum. Two or three programmable pumps are employed to control the transfer rate of solutions. Power supplies are connected with spinnerets, sequentially. Spinnerets are arranged in reverse positions on the identical horizontal line. Syringes independently deliver the solution to two or three spinnerets. The target is a rotating drum controlled by a stepping motor. The fibers of the two or three in reverse become charged electrospinning spinnerets received and elongated via the drum collector at a fixed speed. The nanofiber assemblies, which can be generated by this technique, are dehydrated below vacuum at room temperature [36].

2.1.3.1.7- Centrifugal Electrospinning

During centrifugal electrospinning, the force contributing to the stretching of the solution droplet into fibers is a mixture of centrifugal power and electrostatic energy [37]. Figure 2.9 shown the setup of centrifugal spinning.

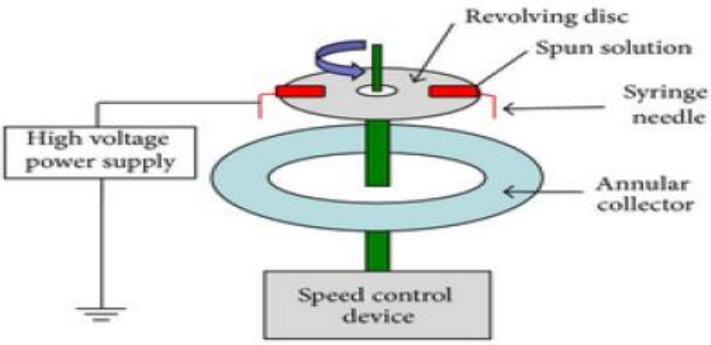

Figure 2.9. Centrifugal spinning[105].

Externally, with the application of high voltage, the simple centrifugal spinning of fibers needs the spinneret to revolve at thousands of rpms. However, in centrifugal electrospinning, the revolution speed can be decreased by 50%. The electric field is provided to stretch the jets to minimal dimensions below the simultaneous drying of the solvent, transmitting a dry nano-fibrous cover on the substrate. With the introduction of the centrifugal strength, a lower voltage is needed in the surface tension of the liquid to succeed in instigating electrospinning. The combination of mechanical revolution and decreased energy makes this a very efficient method for forming aligned nanofibers. Multiple nozzles may be placed around the axis of rotation to raise the generation rate of centrifugal electrospinning [37].

2.1.3.2 - Needleless Electrospinning

Although conventional electrospinning methods based on the use of a syringe create nanofiber layers in quantities of around 0.1 – 1 gram per hour, needleless electrospinning provides the possibility of industrial-scale manufacture of nanofibers [38].

2.1.3.2.1- Bubble Electrospinning

Bubble-electrospinning was discovered in 2007 [26]. Bubble electrospinning is considered one of the smoothest and most efficient techniques that has the potential for wide-scale creation of continuous nanofibers with a diameter scale from many nanometers to micrometers [39]. Figure 2.10 shown Setup for classical bubble spinning.

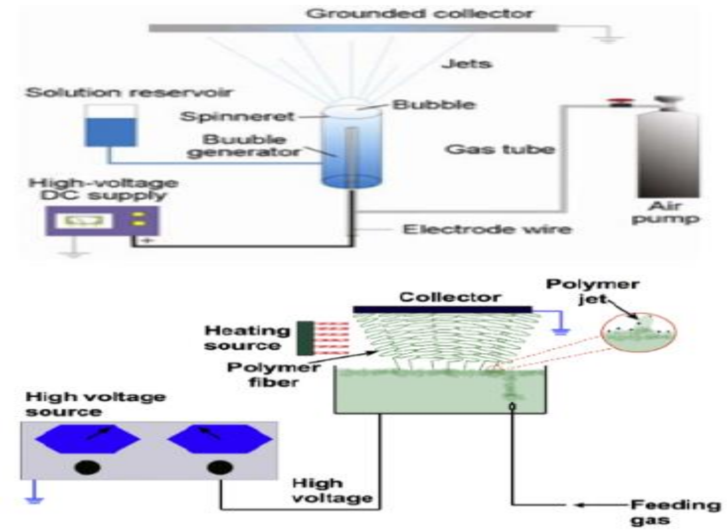

Figure 2.10. Setup for classical bubble spinning. (a) Single bubble electrospinning & (b) Multi-bubble electrospinning [45].

2.1.3.2.2- Two Layer Fluid Electrospinning

A two-layer method, the lower layer having a ferromagnetic stopper and the top layer a polymer solution, is aimed at a standard magnetic area provided by a strong magnet or a coil and the setup for this process as shown in Figure 2.11 [40].

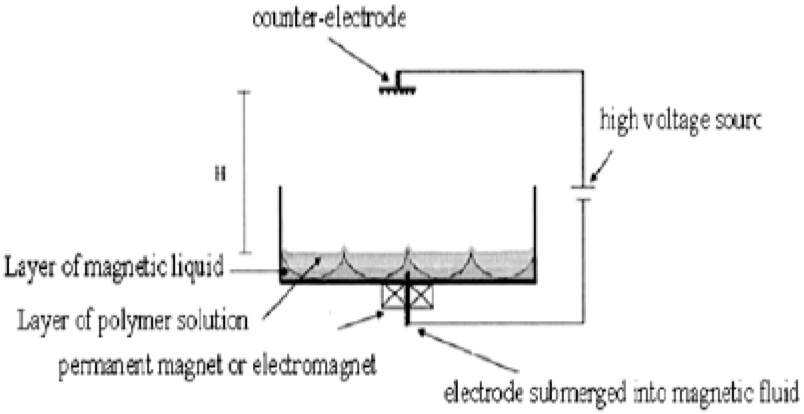

Figure 2.11. Electrospinning of two layer fluid [41].

As a result, even upward spikes of magnetic suspension confused these interlayer interfaces, as accurately as the free surface of the highest polymer layer. When a standard electric field is used in addition to this, the confusions of the free surface convert sites of jetting in an upward direction. Many electrified jets support energetic elongation by electric area and turning instability, the solvent volatilizes, and solidification nanofibers drop on the upper counter-electrode, as in a simple electrospinning method. Despite this, the creation rate is determined to be higher [40].

2.1.3.2.3- Splashing Electrospinning

Solution storage is used to supply a spinning liquid to a mineral roller electrospinning spinneret [41]. Figure 2.12 shown the setup for splashing electrospinning.

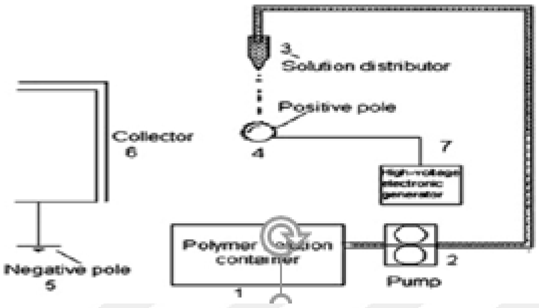

Figure 2.12. Setup for splashing electrospinning [41].

The polymer liquid droplets were sprayed on the surface of a metal roller via a liquid distributor, which became a hole in the bottom. While the voltage was applied, liquid droplets adhering to the surface of the metal roller spinneret discharged and elongated below the electric force to produce nanofibers. This setup intended to create the possibility of doing electrospinning with enhanced fiber production rate [41].

2.1.3.2.4- Melt Differential Electrospinning

By Melt differential electrospinning, fibers with a diameter of less than one micrometer can be created at a yield of 10 – 20 g/h using a needleless nozzle [30]. Figure 2.13 shown the setup for differential electrospinning (inner cone and out-cone nozzle).

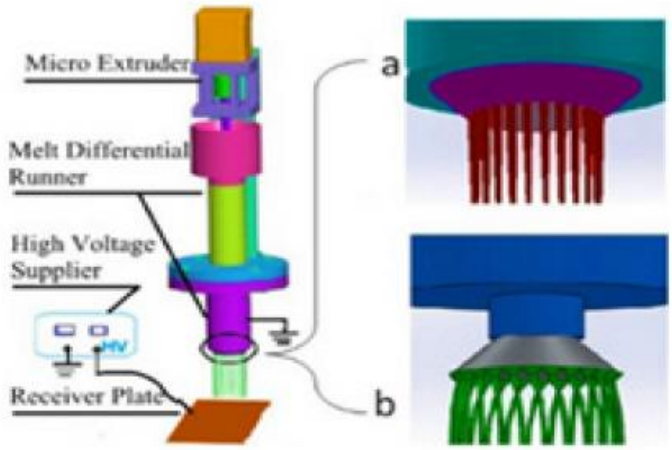

Figure 2.13. Setup for differential electrospinning (inner cone and out-cone nozzle)[106].

The method is defined as follows: firstly, the provided polymer melt is placed onto the surface of umbellate (cone-like) nozzles. Next, the melt is film-coated regularly over the umbellate circumferential surface. When the applied high voltage exceeds a critical level, multiple self-organized jets around the top of the umbellate nozzle move toward the receiver plate. This method is given the name MD-ESP because of the melt flow crossing into tens of minor Taylor-cones as a result of their self-organization [30]. A high voltage is administered directly to the collector of the needle or nozzle, and this enables the separation of the heating operation and electrodes [42].

2.1.3.2.5- Gas Assisted Melt Differential Electrospinning

The hot air helping melt differential electrospinning is a modern technique. The equipment consists of six significant parts, as in Figure 2.14 [43].

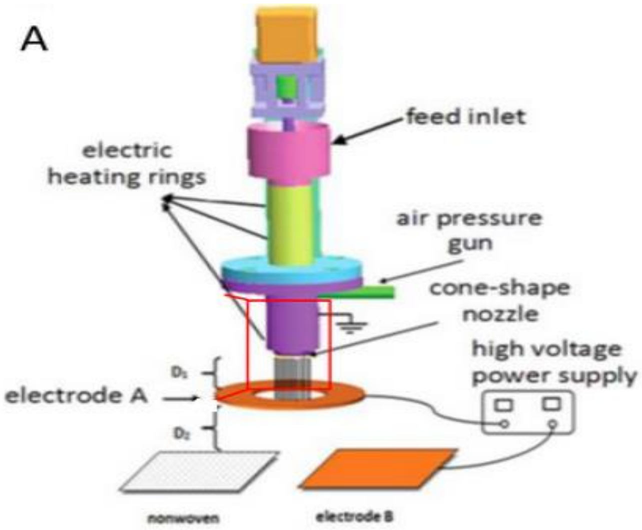

Figure 2.14. Setup for gas assisted melt differential electrospinning [34].

Power supply of high-voltage, heating mode, an atmosphere pressure gun, a copper annular ring functioning as electrode A, a needleless cone-shaped nozzle, and a collecting machine. The function of heated air could be summarized into three significant parts: maintaining heat of runner, accelerating checking of flying jets and keeping the environmental temperature higher than the softening case, which will improve the activity time of electric energy and thin fiber diameter. The single features of the device are the production of multiple Taylor cones near the base of the nozzle, producing a high throughput; the airflow is driven via an air pressure gun more reinforced than the elongating power working on the jets. Also importantly, it could enable the flying jets, with the pressure variation between the airflow and the atmosphere, to stop the polymer jets from being pulled by the inner cycle of the electrode [43].

2.1.3.2.6- Rotary Cone Electrospinning

Bingan, Lu et al. described a needleless electrospinning structure using an electriferous rotating cone like the spinneret. The production throughput of this method was about 10 g/min, which was many thousand times higher than that via the traditional electrospinning method, with a single needle as the spinneret. A schematic of the high-throughput electrospinning method is shown in Figure 2.15 [44].

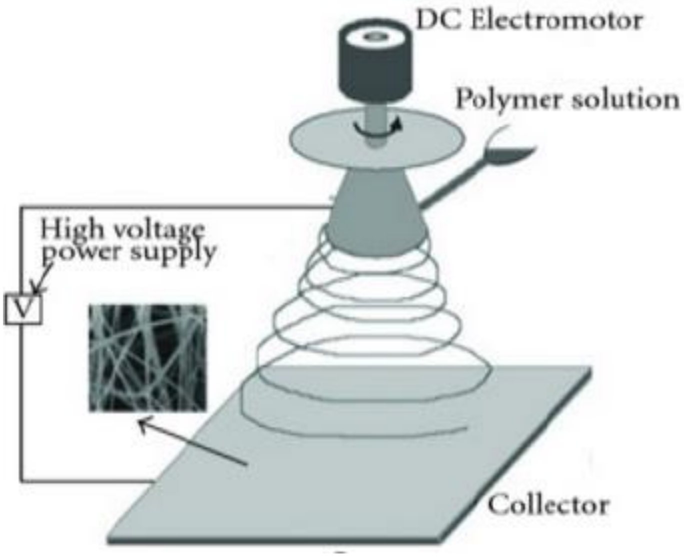

Figure 2.15. Setup for rotary cone electrospinning [45].

It contains four main parts: a high-voltage power supply, a mineral cone, a direct-current (DC) electromotor, and a receiver. When using an aluminum conveyor as the collector, the electrospun nanofiber layer was very high. When one of these PVP solutions is conveyed to the surface of the cone, it can be powered with positive charges directly. The charged solution droplet would jet along the rotating surface below the coactions of gravitation, the moment of inertia, and the electric power. Once the fluid droplet reaches the lower end of the cone, the electrospinning process will be initiated [44].

2.1.3.2.7- Rotating Roller Electrospinning/ Nano Spider Technology

Nanospider is an electrospinning process which needs a high-voltage electrostatic range to generate an electrically energized stream of polymer liquid or melt and the Figure 2.16 shown rotating roller electrospinning [46].

Figure 2.16. Rotating roller electrospinning [47].

The innovative approach of the Nanospider depends on the probability of producing nanofibers of a thin layer of solution polymer. Under these conditions, Taylor cones (the reference of nanofiber) are formed on a rotating roller surface and dipped in a polymer solution. Due to the Taylor jets being adjacent to each other, along the whole length of the roller, this excellent idea produced many advantages, like high productive ability. This popular method for the generation of polymeric nanofibers is used on an industrial level. This is a versatile and straightforward method for the production of ultrathin fibers of a variety of materials that involve polymers. Also, Nanospider can prepare a wide variety of polymers in diameters of 50 – 300 nm within non-woven webs [46].

2.1.3.2.8- Edge Electrospinning

N M Thoppey et al. demonstrated Edge electrospinning consisting of a fluid-filled bowl, high voltage power supply with a positive polarity and a concentric, circular grounded collector and the Figure 2.17 shown setup for edge electrospinning [38].

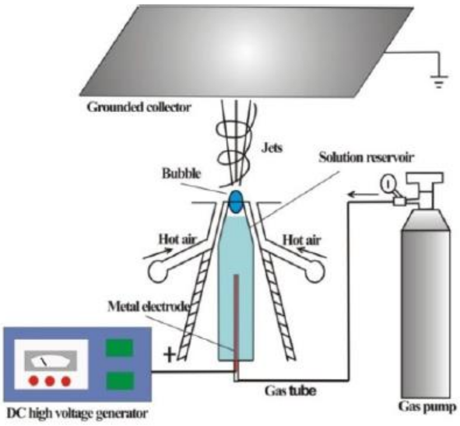

Figure 2.17. Setup for edge electrospinning [107].

Various jets are opened spontaneously from the polymer solution directly through the lip of the bowl, exactly as inside the bowl's depths, which then transfer to the edge and prepare to make approximately equally spaced spinning sections near the bowl circumference. The bowl itself works as the origin of the polymer solution, alternately of gravity-assisted fluid flows. A brief high voltage period aids in creating the jets; consequently, the voltage is decreased to a lower working rate for steady electrospinning and nanofiber generation. The obtained fibers demonstrate properties related to those fabricated below ideal needle electrospinning requirements. This easy bowl-spinning program applies to a diversity of polymers in aqueous and non-aqueous based on solvent methods and polymer solvents having differing viscosities. The receiver surface is coated with conductive aluminum foil to remove the electro smoothly spun mat specimens [38].

2.1.3.2.9- Blown Bubble Electrospinning

A polymer solution membrane is generated by a mineral ring rotating during the polymer-solvent, and the membrane is elongated by blowing air to create a bubble below a high electronic area. Setup for blown bubble electrospinning as shown in Figure 2.18 [29].

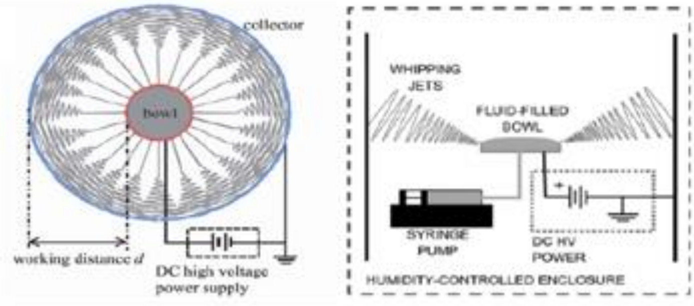

Figure 2.18. Setup for blown bubble electrospinning [108].

While the bubble is broken, many jets are discharged and accelerated via the electric field to produce micro/nanofibers. The completed membrane toward the ring is stretched forward gradually to create a hemisphere by a current of blowing air. While an electric field is near, the blowing bubble is twisted into a protuberance-induced upward-directed shape. During this deformation, surface thickness of the bubble decreases to about 1/2 – 1/10 of the membrane depth. Once the electric range exceeds [29].

2.1.4. Parameters of Electrospinning

It is essential to know the electrospinning operating parameters because these parameters affect fiber morphologies. It is much simpler and more reasonable to obtain required and desired fiber diameters and morphologies by control of those parameters. The typical stages in electrospinning of a polymer to nanofiber are:

a) The diameters for the fibers’ necessity have been compatible plus controllable.
b) The fiber outside should be impurity-free and controllable.
c) Continuous free nanofibers must be collectable.

Fiber diameter is with the numerous essential quantities needed in electrospinning. A complex challenge is to get unity of that fiber’s diameters. The appearance of distortions like beads and pores is the main problem [17].

The parameters of the electrospinning process can be classified into three parts:

Solution Parameters: concentration, viscosity, conductivity, molecular weight, surface tension. Process Parameters: applied electric field, tip to collector distance, feeding or flow rate. Ambient Parameters: humidity and temperature of the surroundings.

2.1.4.1. Solution Parameters

2.1.4.1.1. Concentration

When a solid polymer is dissolved in a solvent, the liquid viscosity is proportionate to the polymer concentration as high viscosity leading to big fiber diameter. Also, higher polymer concentrations will affect the outcome in larger nanofiber diameters. The lowest level of concentration is needed in the electrospinning for fiber production to occur. In very low level of concentrations, electrospray happens instead of electrospinning, because of the lower viscosity. Moreover, considerable surface tensions appear in the solution [48]. Through low solvent concentrations, a fusion of fibers plus beads is achieved. When the concentration rises, the form of the bead turns from spherical to spindle-like. Eventually, uniformity of fibers with increased diameters is produced [16]. An optimum liquid concentration should be achieved, as at a very low level, beads are generated, whereas, at a very high level of concentration, the production of continuous fibers is prevented because of the inability to manage the flow of the solution [49].

2.1.4.1.2. Molecular Weight

Molecular weight is considered another critical parameter that influences the morphology of the electrospinning nanofiber; likewise, it affects viscosity, surface tension and conductivity. In general, the molecular weight displays the entanglement of polymer strings in solutions, particularly the solution viscosity. Preservation of the set concentration, using the very low molecular weight of polymer leads to the creation of beads more than fibers, raising the molecular weight will produce smooth fibers, whereas using a polymer with extremely high molecular weight outcomes in electrospinning fibers leads to them possessing very wide diameters [12], [55], [56].

2.1.4.1.3. Solution Viscosity

In the electrospinning process, it is necessary to arrange the ideal value for solution viscosity, which is required to get the best result. While using too low viscosity leads to no production of fiber, on the other hand, too high viscosity results in complexity in the ejection of jets of polymer solution. Additionally, viscosity is particularly crucial to fiber morphology. There are three essential factors which are related to each other and those factors are: viscosity, polymer concentration and polymeric molecular weight. Usually, the solution viscosity can be changed by varying the level concentration of the polymer for the solution. Each viscosity scale for various polymers used for electrospinning is distinct from the other. İncreasing concentration or solution viscosity results in electrospinning nanofibers which include a larger and more uniform diameter. The dominant factor is Surface tension. When the dose in viscosities is low, the result which you obtain will have fibers with beads or beaded fibers [12], [57].

2.1.4.1.4. Surface Tension

Various surface tensions are obtained using different solvents. Decreasing the surface tension causes the generation of nanofibers with no beads, as proposed in previous study [52], except that low surface tension tends to not always produce typical electrospinning requirements. It is significant in controlling the higher and lower boundaries on which other parameters are set. Yang et al. studied the influence of surface tensions on poly (vinyl pyrrolidone) (PVP) electrospun nanofiber morphology using ethanol, DMF as well as MC-like solvents. Moreover, they noted that various solvents might produce different surface tensions. They got smoother nanofibers by decreasing the surface tension and preventing the concentration getting set instead of the beaded composition at higher surface tensions [53].

2.1.4.1.5. Conductivity/Surface Charge Density

Solution conductivity is normally prepared by polymer type, solvent used, and the behavior of ionized salts. Usually, higher conductivity of solutions leads to producing a smaller diameter of electrospun nanofibers, increasing the conductivity of the solution and the surface charge density of the solution by the addition of salts to the polymer solution [16], [54]. In the case of the addition of salt, there are many polymers used for increasing the solution conductivity such as polyamide 6, polyacrylic acid, collagen type IPEO, polyethylene oxide (PEO), etc. Zong et al. have produced fibers with beadless, smaller diameter poly (D-L-lactic acid) (PDLA) nanofibers ranging between 100 into 200 nm via the addition of ionic salts like KH_2PO_4, NaH_2PO_4 and NaCl. They determined the influence of ions on the composition compared to fiber structure obtained without adding salt [55].

2.1.4.2. Process Parameters

2.1.4.2.1. Applied Voltage

Applied voltage is the critical factor in electrospinning as the threshold voltage necessary for the required charged jets has to be drawn from the Taylor cone. Next, the start voltage is transferred, so the fiber production occurs, causing the necessary adjustments on the solution simultaneously with the electric field and starting the electrospinning method [16], [51]. The influence of applied voltage on the electrospinning method has been widely studied. Reneker et al. have noted that applied voltage does not have a critical influence on fiber diameter in the electrospinning of polyethylene oxide [56]. Zhang et al. proved that there is also more polymer ejection by higher voltages, making it easy for the production of fiber diameter on a larger scale. They studied the impact of voltage on fiber morphology, even diameter distribution by (PVA) poly (vinyl alcohol) /water solution [57]. Some other researchers stated that the increase in applied voltage promoted the reduction of fiber diameter; also, they explained that fiber diameter reduced by increasing voltage because of the increase in electrostatic repulsive power on the charged jet. Bead creation was furthermore discovered through higher voltage [54], [58]. Yördem et al. additionally said this applied to influencing fiber diameter; moreover the level of importance changes in polymer solution concentration and also the tip to collector distance [59].

2.1.4.2.2. Feed Rate /Flow Rate

The feed rate is another process for the polymer in a syringe, and the flow rate parameter influences the material transfer rate inside the syringe and on the jet velocity, which is affected directly in the process. A slower flow rate is sustained to allow enough time for the solvent to evaporate. A minimum feed rate should be present. The high feed rate also appeared in the beaded fiber composition [16].

2.1.4.2.3. Distance Between the Tip and the Collector (Tip-To-Collector Distance)

The tip-to-collector distance bears in mind the parameters which are affected, although its influence is less critical on fiber morphology if these nanofibers are compared with the different process parameters. In order to get the evaporation for the solvent from the solution of the polymer, the ideal distance should be selected [27].

2.1.4.2.4. Collectors

Generally, the collector of nanofibers should be selected and modelled as a conductive substrate. The material which is used to collect the nanofiber is aluminum foil whereas, wire mesh, conductive paper or cloth, parallel or gridded and rotating rod are additionally used [16].

2.1.4.3. Ambient Parameters

The other parameter also affected in the morphology of nanofibers in the electrospinning process is called ambient parameters, which means the temperature and relative humidity. Using high temperature leads to fibers with decreased diameter, whereas using low relative humidity, the solvent may be completely dry. Additionally, using higher humidity leads to small pores appearing on the fiber surface [27]. Most of the process and solution parameters in this study are based on previous study [60].

2.1.5. Applications of Electrospinning

Recently, researchers have started to seek different applications for electrospun fibers. At the same time, these provide various advantages like large surface to volume ratio, high porosity. Moreover, improved physio-mechanical features as the process, treatment of process parameters and the solution can be simply done to make the required mechanical strength and fiber morphology. Additionally, the electrospun fibers are needed for a small quantity; also, the process of electrospinning itself is varied as fibers can be formed into any configuration using a wide variety of polymers in previous studies [52], [61]. Electrospinning nanofibers are widely used in biomedical features, like tissue engineering scaffolds, in drug delivery, wound healing, in immobilization of enzymes, filtration, as affinity membranes, small diameter vascular graft implants, biotechnology, healthcare, defense and security, environmental engineering, generation and energy storage and in various ongoing research [62], [63], [64], [65], [66]. In addition, the electrospun fibers have penetrated many industrial fields like in protective clothing, electrical and optical application, composite application and energy harvest and storage applications [27]. The electrical and optical applications were produced in some of the studies using conductive nanofibers in order to apply these nanofibers in sensors, actuators and batteries, etc [67]. The nanofibers are used in composite application so that the mechanical properties of the nanofibers will be better than the microfibers of the material itself. As a result, the structural properties of these compounds are strengthened. In other studies, various materials have been used, such as carbon fibers or nanotubes [17]. Bergshoef et al. created the nanocomposite by using an epoxy matrix with Nylon-4,6 nanofiber membranes during the process of electrospinning. Strength and stiffness of the nanocomposite were estimated to be significantly more powerful than the source of the matrix film [68]. The energy harvest and storage applications were generated by electrospun nanofibers and these penetrated many applications like solar cells, as a good application for potential in dye-sensitivity and in fuel cells. The nanofibers are prepared as an alternative catalyst and these are an important component of fuel cells. Also, other applications such as lithium ion batteries, etc [27]. The applications of electrospinning as shown in Figure 2.19 and Table 2.1.

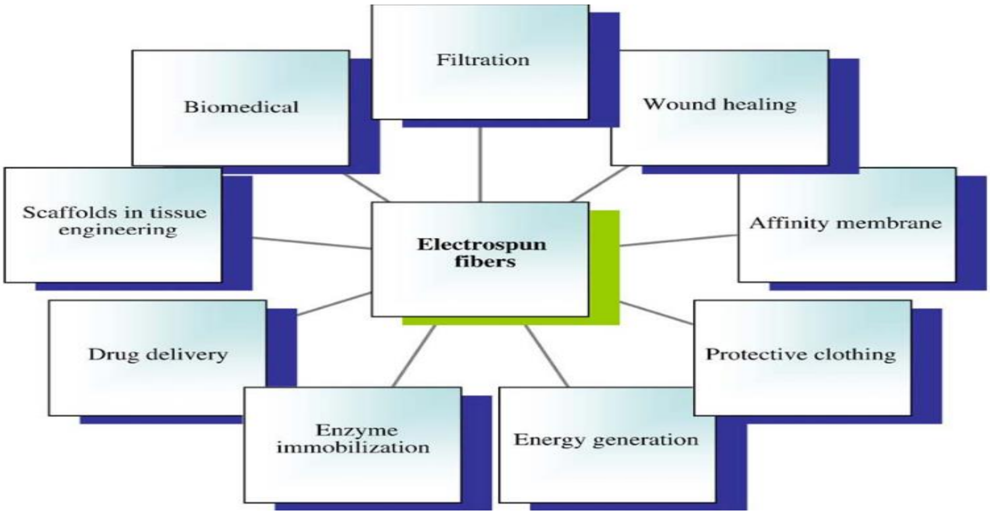

Figure 2.19. Applications of electrospinning [16].

Table 2.1. The applications of electrospinning [16].

Field	**Application**
Tissue engineering	Tissue engineering is deemed a multidisciplinary area that is directing both life sciences and the principles of engineering towards the amelioration of biological replacements. Moreover, for renovation, maintenance or development for the function of tissue.
Scaffolds for tissue engineering	The process of electrospinning has instantly become the most commonly used process in the preparation of nanofibrous scaffolds. The materials which are used for preparation of nanofibers scaffolds are generally natural polymers due to their ability for promoting biocompatibility - moreover bio-functional motifs like alginate, collagen, hyaluronic acid, chitosan, silk protein, starch and fibrinogen. Furthermore, others because of their blending capability with manufactured polymers can develop the overall scaffold for cytocompatibility. Consider the scaffolds for tissue engineering as part of tissue engineering in addition to dressings for wound healing and drug delivery.

In filtration	Polymeric nanofibers have been used in air filtration for more than ten years. For filtration, the structural and channel elements for a filter should correspond to the range of the droplets or particles, and that is to be entrapped in the screen of the filter. Therefore, we can take advantage of the unusual characteristics for electrospun membranes, including minute diameter for fibers. It has been observed that the electrospinning is increasing due to the difficulty of supplying solutions for the elimination of particles that are foreign in the range of the submicron.
As biosensors	Biosensors commonly include the transducer and bio-functional membrane, which are widely used for clinical, environmental and food items. Membranes of nanofibers that are electrospun gained important consideration in the application of sensors due to their unique wide surface area, which is the most desirable and common feature for developing the sensibility of conductometric sensors, as the larger surface area will consume more of the gas analyzed and also change the conductivity of sensor more significantly. The extensive material used is Silk fibroin membrane-based biosensors, which is for analyzing different materials, for example, hydrogen peroxide, glucose, and uric acid. There are polymers considered as sensing interfaces such as polypyrrole, nylon-6, polyaniline etc.
Protective clothing applications	Generally, protective clothing must possess some requisite characteristics, for instance, breathable fabric, lightweight, water vapor permeability and air, support toxic chemical protection, also insolubility in all solvents. The nanofiber membranes, which are electrospun are considered as possible contenders in protecting cloth features, due to their high porosity (breathable nature), large surface area, lightweight, resistant to dangerous chemical agents in aerosol formation, high filtration performance and for their capacity to neutralize

	chemical agents unaccompanied by air impedence. Also, water vapor permeability for clothing.
Energy generation applications	The polymeric conductive membranes also possess potential for characteristics such as electrostatic scattering, corrosion protection, electromagnetic intervention covering, photovoltaic machines, creation of small electronic machines or devices, in particular, Schottky junctions, actuators, and sensors, etc - the flow for electrochemical reactions is commensurate to the electrodes of the surface area. The membranes, which are conductive nanofibers, are also considered quite proper for usage in the area of porous electrodes with the aim of improving high execution batteries. Furthermore, polymer electrolyte membrane fuel cells (PEMFCs) because of their large porosity, also inherit a sizeable entire surface area.
In immobilizatio-n of enzymes	Immobilization of enzymes as inert, unresolved substances is the active region of study in promoting the method of this function and effect of enzymes on bioprocessing features. The action of immobilized enzymes gives various benefits, namely reusability, finer control reaction, also higher stability than the soluble ones. Nanofibrous membranes contribute improvements, for example, they can be prepared within various structures. Well-aligned arrays or non-woven mats also are further conveniently recovered. Moreover, they are more active than other carbon nanotubes or nanoparticles.
As affinity membrane	Some works have studied electrospun nanofiber mesh to get an application for affinity membrane; also, for this the surface should be functionalized previously as well as ligands. In almost all cases, the ligand particles must be covalently linked on the membrane in order to inhibit and stop the ligands leaching.
In cosmetics	The polymer nanofibers have also been used with or without different additives in the act of skincare masks in terms of a

	cosmetic for the medication of skin cleansing, skin healing, medical and other therapeutic properties. The nanofibers which are electrospun and used for skin masks, become advantageous because of the high surface area, which promotes better usage, including also the transfer rate which speeds up the additives into the skin. The cosmetic skin mask by using the electrospun nanofibers can be put on gently. The electrospun nanofibrous cosmetic skin mask can be put on painlessly and can even go to three-dimensional topography for the skin directly in order to contribute to healing or care for the skin.

2.2. Polymers

The word polymer originates from the Greek roots poly (many) also meros (part). The name, therefore, indicates “many parts, thus denotes a molecule prepared via the recurrence of some straightforward unit named a mer [69]. Others see them as polymers that appear naturally or biopolymers, and also synthetic polymers which in particular had better be taken alone. Other designations of these large molecules are considered as “giant molecules.” However, the same basics are used for every polymer. If one overlooks the end uses and the diversity among all polymers, in addition to fibers, plastics and rubbers, elastomers are found primarily via the intermolecular, also intramolecular forces within the molecules and most of all by their size and the individual molecule sequentially. Moreover via functional groups, enabling access to these forces [70].

2.2.1. Natural Polymers

One of the most trusted, fastest growing areas for polymer chemistry is natural polymers. The human body is composed of vast numbers of polymers like RNA, DNA, proteins, also polycarbohydrates. These are linked to ageing, consciousness, movement, power, etc, i.e., for all of the traits that enable the human body to stay alive and healthy. Various medical, health and biological enterprises are curious about substances which are, even in the slightest part, polymeric. There is an ever-increasing importance for molecular biology, for instance chemistry which is applied to natural systems. Hence, knowledge of polymeric principles is beneficial to those aspiring to work in the relevant fields. In physics, there is no variation in the study, behavior or experimentation on natural or synthetic polymers; moreover, information techniques appropriate for use in synthetic polymers are just as suited to natural polymers. At the same time, the chemistry and physics which deal with synthetic polymers is complex. Even the chemistry and physics which is related to natural polymers is too complex due to a number of relevant factors. These are represented by (1) the fact that different natural polymers consist of a variety of repeating units, usually, the same ; (2) very often dependent on the natural polymer which is in the surroundings; (3) the matter of the exact structure of the natural polymer which is found in the nature of the environment; also (4) the importance of form and size of the polymer is one of the basics found in natural polymers, rather than in industrial polymers [71]. There are many natural polymers such as chitosan, gelatin, collagen, silk fibroin etc [7].

2.2.2. Synthetic Polymers

Synthetic polymers are described as being artificially created in labs. These are additionally recognized as human-made polymers. There are some examples synthetic polymers; polystyrene (PS), polyethylene (PE), poly (vinyl chloride) (PVC), polyamides (nylon), Teflon, synthetic rubber, epoxy, and several others [72]. Synthetic polymers are usually obtained from petroleum oil under a controlled condition, also formed of carbon-carbon chains as their base. The chemical bonds between the monomers mutate and make them durable as a result of mixing both heat and pressure in the presence of a catalyst. The catalyst is used to start the chemical process or to speed it up among the monomers. Today, synthetic polymers are utilized in the design of millions of applications. These applications, which can be classified into different categories, include thermosets, synthetic fibers, and thermoplastics [73].

2.2.3. Chitosan

Chitosan (CS) is a polysaccharide biomaterial formed of distributed β-(1→4) -linked D-glucosamine and N-acetyl-D-glucosamine. The composition of the natural polymer or biopolymer is displayed in Figure 2.20. Generally, chitosan can be generated from chitin by taking partial deacetylation, where the chitin is obtained from the derivatives of the exoskeleton of crustaceans, fungi cell walls, and insects [74], [75]. The structure of the CH which possesses the amino group appeared differently in the CH chitin structure. The amino groups' number can be inferred depending on the degree of deacetylation [74]. The chemical structure of chitosan as shown in Figure 2.20.

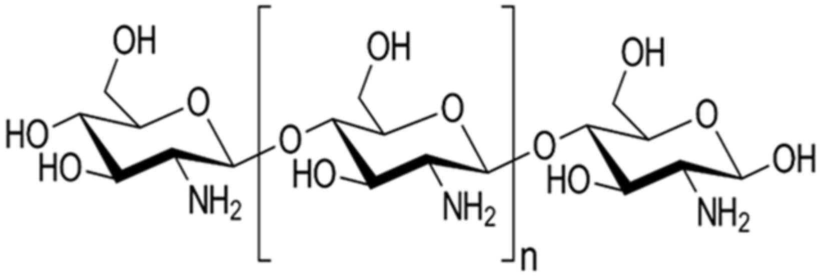

Figure 2.20. Chemical structure of chitosan [109].

Chitosan is one of the most common polysaccharides on earth and is considered one of the natural copolymers as well as cationic [75]. The electrospinning process of chitosan is a process that has been frequently used of late to produce fibers with a diameter of ~100 nm. Nevertheless, the process of producing fibers for chitosan from its aqueous solution is difficult and considered problematic, because of the powerful hydrogen bonds and high viscosity [76], [77]. Consequently, chitosan has garnered wide attention lately, due to the discovery of various ways to develop the fiber structure of chitosan. Many researchers have used anhydrous solvents to prepare the solution in order to produce chitosan fibers. As an example of this, harmful solvents were used to dissolve pure chitosan like trifluoroacetic acid in some of previous studies [78], [79], also hexafluoro propanol [80]. And simultaneously, chitosan combinations of different polymers, like polyacrylamide, polyethylene oxide (PEO), polyvinyl alcohol (PVA), and poly (Ɛ-caprolactone) (PCL) [78], [81], [82], [83], [84].

2.2.3.1. Properties of Chitosan

Chitosan is one of the most basic polysaccharides - other polysaccharides mostly occur naturally like dextrin, agar, cellulose, alginic acid, pectin and carragenas, which are acidic and natural. Chitosan has several properties, including solubility in different media, solution, polyelectrolyte behavior, viscosity, and polyoxysalt creation. Furthermore, it has the capability to produce metal chelations, films, optical also structural characteristics [85]. The pKa value of the amino group is around 6.5, and that leads to considerable protonation inside the neutral solution, rising with increased acidity (lower pH) and %DA-value. That produces water-soluble chitosan as well as a bioadhesive, which easily adheres to the surfaces carrying a negative charge [9], [86], [87]. Chitosan improves transportation for a polar drug through epithelial outsides and is biodegradable and biocompatible [88]. As for the physical, chemical and biological properties, they are as follows : for physical, by appearance and powder fineness, which are off-white powder and finer than 120 mesh size, respectively [89]. However, for the chemical properties, they are reactive amino groups; chelates have many transitions for metal ions and linear polymine. For the biological properties, chitosan is biocompatible, and that means biodegradable to normal body constituents, safe and non-toxic also a natural polymer. Furthermore, for other properties like accelerating the formation of osteoblast responsible for bone generation which binds to mammalian and microbial cells aggressively, hemostatic, has a regenerative influence on connective gum tissue, fungistatic, central nervous system depressant, anticholesteremic, immunoadjuvant, antitumor, and spermicidal [9].

2.2.3.2. Applications of Chitosan

Because of its physical and chemical characteristics, chitosan is used in a wide array of applications and products, varying from cosmetic products and pharmaceutical to plant protection as well as in water treatment. In various applications, the diverse properties of chitosan are needed. These characteristics vary with the molecular weight and degree of acetylation as well [9]. Table 2.2 outline the major applications for chitosan.

Table 2.2. The major applications of chitosan [90].

Field	Application
Cosmetics & toiletries	Maintain skin moisture, Treat acne, Improve suppleness of hair, Reduce static electricity in hair, Tone skin Oral care (toothpaste, chewing gum).
Food & beverages	Not digestible by human (dietary fiber) Bind lipids (reduce cholesterol) Preservative, Thickener and stabilizer for sauces. Protective, fungistatic, antibacterial, coating for fruit.
Water & waste treatment	Flocculant to clarify water (drinking water, pools), Removal of metal ions, Ecological polymer (eliminate synthetic polymers), Reduce odors.
Biopharmaceutics	Immunologic, antitumoral, haemostatic and anticoagulant, healing, bacteriostatic.

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1. Materials

Chitosan (CS) (high molecular weight, MW= 310000 – 375000 g/mol), Trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich.

3.2. Preparation of Chitosan Solution

Chitosan solution (8 wt.%) for electrospinning process was prepared by dissolving chitosan powder in the solvent TFA and heating the solution until a homogeneous mixture with a temperature range of between 50 – 55°C was obtained. Stirring first, start with 5 – 10 min using 200 rpm and then adjust the stirrer to 400 rpm. Continue the process around 25 min until you get the complete dissolution. The concentration used in this study and the method of preparation and ratios are from accrediting our previous research [60].

3.3. Optimization of Electrospinning Process Parameters

Chitosan solution was collected in a 10 ml syringe. Throughout the electrospinning process, the distance between the needle and collector was 7 cm, whereas the flow rate was fixed at 2 µl /min. The flow rate and the distance between the needle and collector were optimized according to our previous study [60]. Meanwhile, the voltage was obtained after several gradual experiments between (15 – 25 kV), and we observed the best products when using 16.5 kV with the naked eye (the appearance of Taylor cone was clear and the progress of the electrospinning occurred smoothly). When using a high voltage power supply in order to apply 16.5 kV voltage, the solvent inside the syringe ejects outside from the tip of the needle. The fluid jet stretches in a straight line to a certain distance, after that bends and then follows a looping and spiraling formation in order to generate a cone. The fluid jet quickened towards the destination, the solution evaporated, and nanofibers settled on aluminum foil. All electrospinning experimental sets were carried out at different temperatures (20°C, 25°C, 30°C, 35°C, 40°C) and humidity (30%, 40%, 50%, 60%).

The variations of temperature and relative humidity occurred as shown in the Table 3.1.

Table 3.1. The temperature and relative humidity values used in this work.

Temperature (°C)	**Humidity (%)**
20	30
25	40
30	50
35	60
40	–

The method of changing temperatures can be conducted by degree by setting the device to the required temperature. In contrast, the method of changing the humidity can be conducted by using a small humidifier.

3.4. Characterization of Chitosan Fibers

3.4.1. Scanning Electron Microscopy (SEM) Analysis

For an examination of the samples, and in order to see the morphology of chitosan fibers in different instances, the device that we used is (QUANTA 400F Field Emission SEM, USA) at Middle East Technical University (METU) Central Laboratory and the device shown in Figure 3.1.

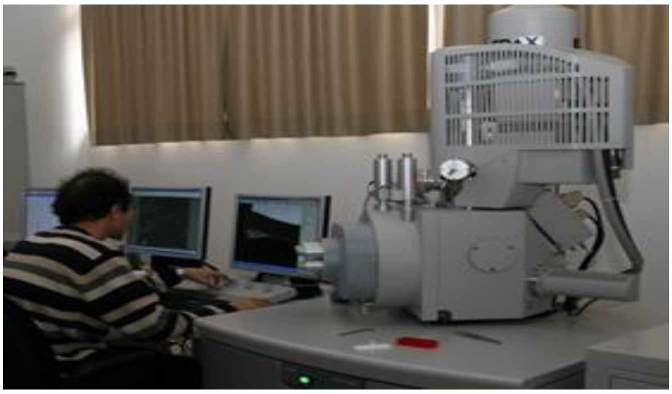

Figure 3.1. SEM Device.

The high-resolution scanning electron microscope has a resolution of 1.2 nm and the process parameters of 20 kV for accelerating voltage. The average fiber diameter, pore size distribution, and porosity values were found via the ImageJ software program (Version, 1.52a) by using SEM photographs taken from SEM analysis. Thirty measurements were conducted for each sample by using ImageJ in order to investigate the average fiber diameter. At the same time automatic measurements for the pore size distribution and porosity were also procured from the SEM photographs.

3.4.2. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analyses

The information of chemical structure of chitosan fibers was obtained using the ATR-FTIR spectrometer (Bruker Vertez 70, Germany) as shown in Figure 3.2, and this analysis took place at METU Central Laboratory with the scan range resolution of 4 cm^{-1} in the scale of 400 to 4000 cm^{-1}.

Figure 3.2. ATR-FTIR Device.

3.4.3. Differential Scanning Calorimeter (DSC) Analysis

TA Instruments DSC 250 which is found at METU Central Laboratory was used to investigate the thermal properties for chitosan fibers as shown the device in Figure 3.3. The DSC traces were in the range of 20 – 270 °C under a nitrogen environment with a heating rate of 10 °C/min.

Figure 3.3. Differential Scanning Calorimeter (DSC) Device.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Scanning Electron Microscopy (SEM) Analyses

The effects of variable humidity and the temperatures have been studied here. The humidity ranged from 30 – 60%, while the temperatures were between 20 – 40°C. As an example, the appearance of chitosan fiber samples within different humidity and temperature conditions is shown in Figure 4.1.

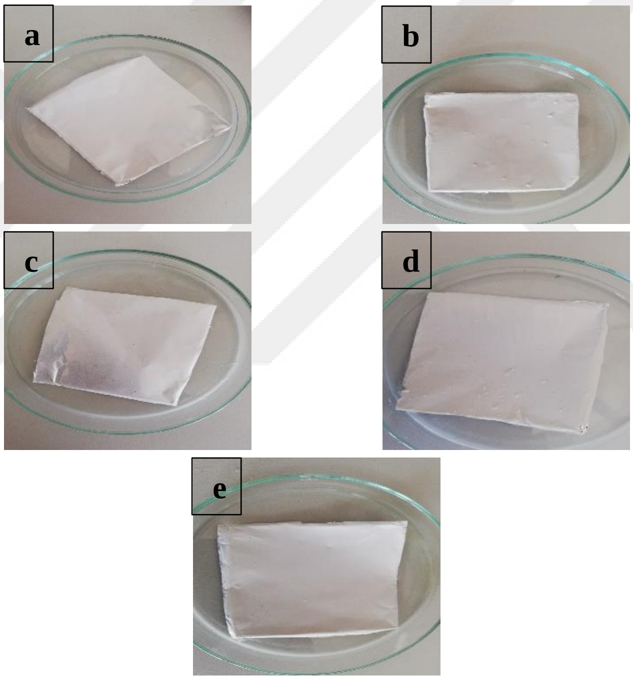

Figure 4.1. Chitosan fiber samples: (a) 20°C, 30%, (b) 25°C, 30%, (c) 35°C, 30%, (d) 35°C, 60%, (e) 40°C, 30%.

Figure 4.2 shows the SEM analyses of all conditions based on the differences in the humidity and the temperatures.

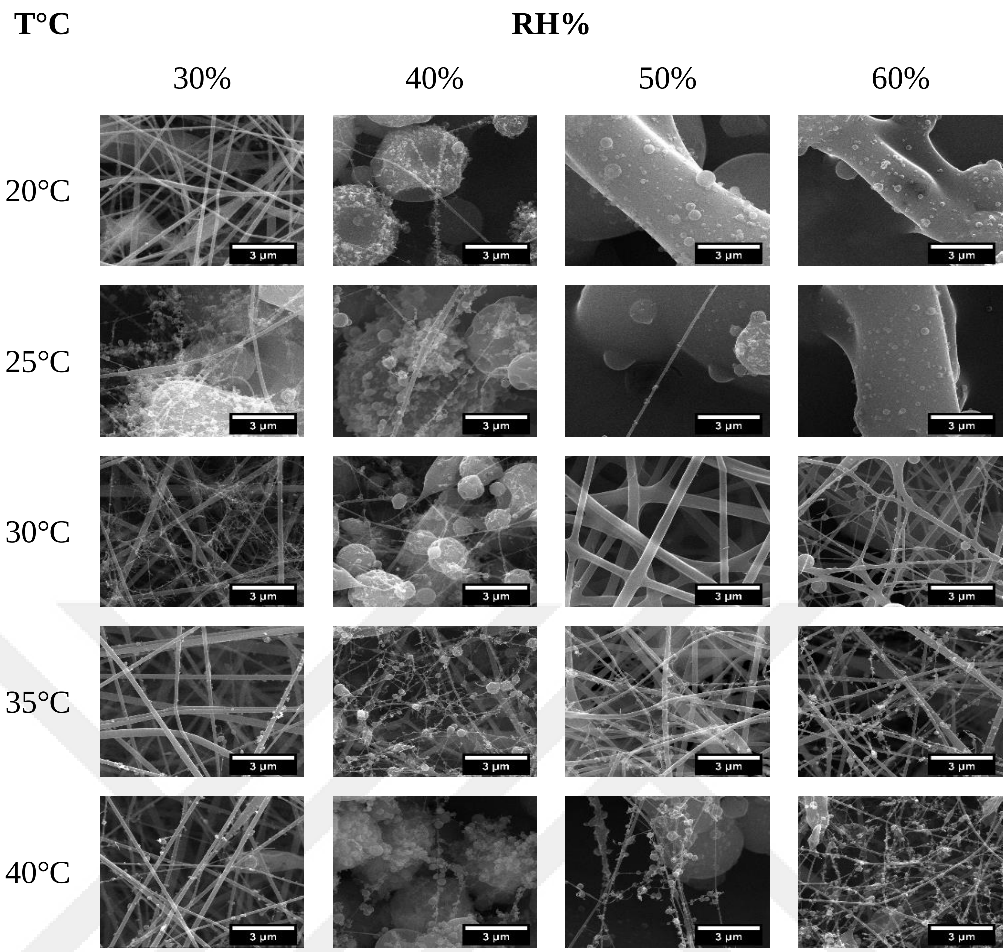

Figure 4.2. SEM Results for all cases based on the humidity and temperatures (at scale 3μm).

Table 4.1. The average fiber diameter, pore size distribution and porosity of samples with various temperature.

T(°C),RH(%)	Average fiber diameter (nm)	Pore size distribution (μm)	Porosity (%)
20°C, 30%	363 ± 2.65	0.039 ± 0.004	49
30°C, 30%	347 ± 2.14	0.043 ± 0.006	50
35°C, 30%	304 ± 2.48	0.051 ± 0.009	55
40°C, 30%	260 ± 2.20	0.063 ± 0.007	60

It can be noted from Figure 4.2 and Table 4.1 that when the humidity was set at 30%, the average diameter of fibers was decreased by increasing the temperature from 20°C to 40°C. The main reason of diameter increment is the viscosity for the solvent solution of the polymer is reduced by increasing the temperature, and this is considered one of the reasons that let the columbic forces to increase the stretch of the solvent, thereby providing thin fibers. The same results were also found in other studies [91]. Mit-Uppatham et al. who proved that a rise in temperature prompted the drop of surface tension, conductivity & solution viscosity producing polyamide-6 fiber diameter [92]. As a result of the increase in temperature, this causes an increase in the rate of evaporation and thus leads to a greater increase fiber settling on the target. Furthermore, unity for fibers and solubility allows for more stretching ability. Demir et al. cited this in their research on the electrospinning of polyurethane fibers and the affected parameters for this study [58]. Also, it has been observed that the pore size distribution and porosity increased by raising the temperature.

It's imperative to note that the pore size distribution and porosity increased by raising the temperature (Table 4.1). The reason for that may be the viscosity of solvent in high temperatures. Then the stretch of solvent increased and thus interspersed the fibers with water vapor, and that led to increased pore size distribution and porosity [92].

Table 4.2 and the photos in Figure 4.2 show the difference from the results of the previous conditions.

Table 4.2. The average fiber diameter, pore size distribution and porosity of samples with various temperature and humidity.

T(°C),RH(%)	**Average fiber diameter (nm)**	**Pore size distribution (µm)**	**Porosity (%)**
30°C, 30%	347 ± 2.14	0.043 ± 0.006	50
30°C, 50%	442 ± 2.72	0.047 ± 0.003	53
35°C, 30%	304 ± 2.48	0.051 ± 0.009	55
35°C, 50%	311 ± 2.97	0.059 ± 0.008	57

In each of the results 30°C, 30% – 30°C, 50% and 35°C, 30% – 35°C, 50%, it was observed that the diameter of fiber grew by increasing the humidity. Because of the levels of lower humidity, the rate of evaporation increased, and therefore we observed these results. Also, there is production of finer fibers, despite the needle clogging when the effect is too high. In contrast, the cause of the deposition of wet fibers combining together before drying because the relative humidity is increased. Also, these were observed in previous study [91]. Additionally, Baumgarten explained the same occurrences, but maybe the most interesting effect of relative humidity is porosity or the surface of the fiber roughness [24]. The relationship attained could be because of the lower density for a fiber that is produced at a level of higher humidity. If the range of charge repulsion through the electrospinning process in the whipping step is fixed for varying humidity differing from the fiber density, because of the surface porosity of fiber, this could match the variation in diameter of the fiber.

In the results of pore size distribution and porosity, both of them increased by increasing the humidity (Table 4.2). The same results were observed and discussed by the other researchers in previous studies [93], [94]. During the process of electrospinning, when the atmospheric humidity inside the chamber is high, the pores on the fiber surface produced because the water and solvent evaporate when volatile solvents are used. When the humidity is increased, pore size is increasing until they assemble to create pores being large and non-uniform. Casper et al. explained that the electrospinning of polystyrene with humidity ranging between 31 – 38% was acceptable to see the production of pores on the fiber surface. They additionally noted that the size for the surface pores raised with RH%, observing an average for pore size of 85 nm through 31 – 38% and 135 nm in 66 – 72 RH% [93].

The other results can be seen in Figure 4.2 which is appeared that contain beads (for example: 20°C, 40%; 25°C, 30%; 30°C, 60%; 35°C, 40%; 40°C, 50%). This is due to many reasons. First, the difference of weather conditions outside and inside the closed chamber could affect the temperature and humidity of the test. Second, the creation of beads affected by the type of polymer because the cationic nature of chitosan allows the formation of polyelectrolyte complexes with negatively charged biomolecules as discussed in the previous study [75]. El-banna FS et al. suggest the creation of beads may be because of the solution conductivity and might significantly affect the morphology in relation to the variations of humidity; thereby the cationic nature of chitosan would promote more dendritic morphology [75].

In general, the beads appeared when the humidity is raised (because the water vapor is electrically conductive), it affects the distribution of the charge on the Taylor cone due to the creation of beads. The Taylor cone is considered the main part, which is imperative to be kept stable in order to continue the production process. The effect of increasing the humidity leads to producing a decrease of the surface charge density, which can cause defects on the fiber formation. So the interaction of the surrounding high content of water vapor in the atmosphere modified the microarchitecture of the fibers. In addition, the microstructure of electrospun fiber under air may be influenced by solvent properties and hydrophobicity/hydrophilicity polymer. These issues play a role along with humidity, and the outcomes are not always predictable as stated in previous studies [95].

4.2. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analyses

The ATR-FTIR test was utilized to observe the chemical structure and to evaluate the functional groups in the chitosan. In Figure 4.3(a, b), the infrared spectrum of chitosan for two selected samples can be seen (20°C, 30%) and (35°C, 50%).

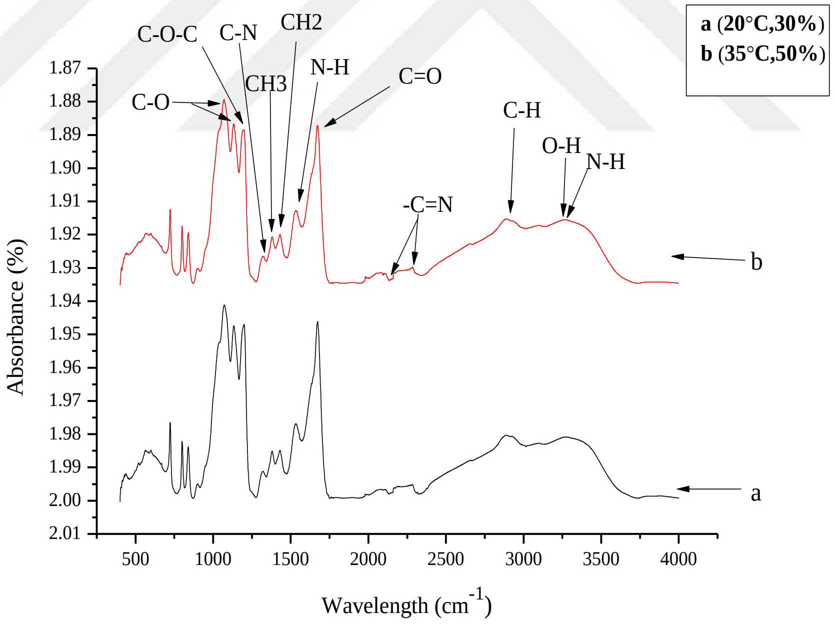

Figure 4.3. FTIR spectra of chitosan fibers.

The purpose of choosing these two different samples was to examine the change in chitosan fibers in the case of the temperature and humidity various. The group of bands peaks at 2284 and 2080 cm^{-1} in Figure 4.3(a), whereas 2284 and 2083 cm^{-1} in Figure

4.3(b) refer to nitrile group or cyanide groups (−C≡N), as the source because this band did not turn overtly by thermal ageing [96]. A strong band presented in a peak at 3263 cm^{-1} in Figure 4.3(a), and 3265 cm^{-1} in Figure 4.3(b) leads to N-H and O-H stretching, as much as the intramolecular hydrogen chains. The absorption zone at the peak 2890 cm^{-1} in Figure 4.3(a), and 2889 cm^{-1} in Figure 4.3(b), refers to C-H symmetric and asymmetric stretching. These bands are characteristics representative of polysaccharides also observed in different polysaccharide spectra, like xylan [97], glucans [98], and carrageenans [99]. The behavior of residual N-acetyl groups was affirmed via the bands at around 1673 cm^{-1} in Figure 4.3(a, b), (C=O stretching of amide I) and 1321 cm^{-1} in Figure 4.3(a), whereas 1320 cm^{-1} in Figure 4.3(b) (C-N stretching of amide III), respectively. A group at 1531 cm^{-1} in Figure 4.3(a, b) refers to the N-H bending of the primary amine [100]. The CH_2 bending and CH_3 symmetrical deformations were proved via the appearance of bands at peaks 1430 and 1379 cm^{-1} in Figure 4.3(a, b), respectively. The absorption band at 1197 cm^{-1} in Figure 4.3(a), but 1199 cm^{-1} in Figure 4.3(b) refers to asymmetric stretching of the C-O-C bond. The bands at 1131 and 1070 cm^{-1} in Figure 4.3(a), but at 1133 and 1071 cm^{-1} in Figure 4.3(b) correspond to C-O stretching. All groups are obtained within the spectra of specimens of chitosan listed by other researchers [101], [102]. Thus, from the above results, it becomes clear that the change is very slight and within the same parameters of both samples (solution and process parameters). The change is non-existent in the chemical composition when the temperature and humidity conditions change, and that is proved in the previous results.

4.3. Differential Scanning Calorimeter (DSC) Analysis

Generally, DSC was used to examine the thermal characteristics of nanofibers formed under various atmospheric conditions. The DSC was employed to estimate the energy needed (W/g) to heat any nanofiber specimen on a temperature scale from 20°C to 270°C. Two samples were selected (20°C, 30%) and (35°C, 50%) for DSC analysis in order to check the difference in thermal properties when the temperature and humidity changed. The results of the different samples, the first sample having a condition with diameter (35°C, 50%; 311 ± 2.97) (Table 4.2), the melting enthalpy for this sample is (11.842 J/g) and as shown in Figure 4.4(b).

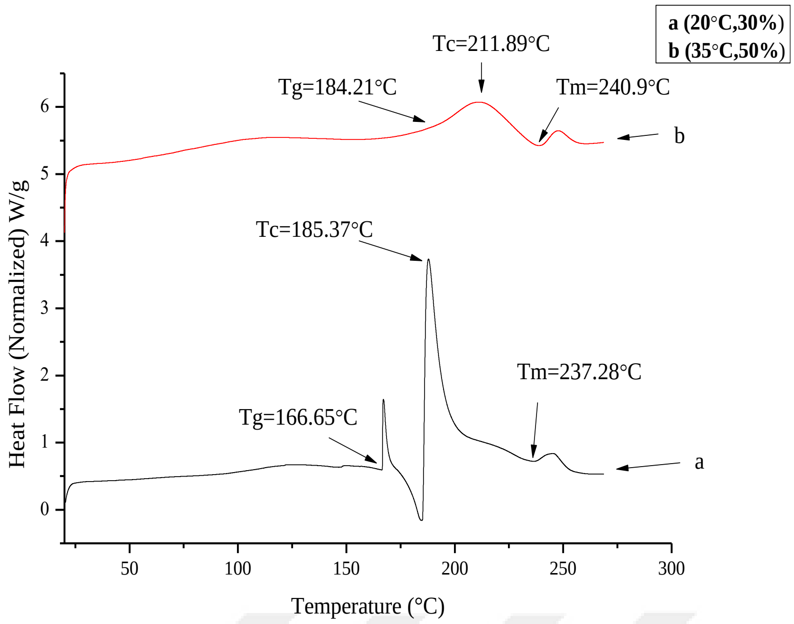

Figure 4.4. DSC curves for chitosan fibers.

In contrast, the other sample having a condition with diameter (20°C, 30%; 363 ± 2.65) (Table 4.1) the melting enthalpy for the second sample is (10.634 J/g) in Figure 4.4(a). It is important to note that the melt enthalpy values were obtained by counting the integration of the melting temperature curve from the thermograms. Therefore, these results appeared as the fiber diameter decreased, so the melting enthalpy which was represented by a broad low endothermic peak increased by increasing the temperature and humidity. These measurements suggest that the lower the crystallinity, the less ordered the molecular composition with reduced fiber diameter. Lee et al. found a similar outcome by using DSC to determine higher molecular weight PVA changed the Tm from 224.7°C to 232.7°C. This is because of the actively raised crystalline composition. The same result was discussed by Oliver Hardicka et al. for cellulose acetate changed the Tm from 227.6°C to 229.4°C [91], [103]. In addition, the previous results in Tables 4.1, 4.2 and Figure 4.4 showed that increasing the temperature and humidity leads to an increase in value of melting temperature (Tm) from 237.28°C to 240.91°C for different samples in cases of (20°C, 30%) and (35°C, 50%), respectively.

CHAPTER 5

CONCLUSIONS

Chitosan powder in the concentration of 8 wt. % was used generate chitosan fibers in the nanoscale. The experiment was conducted by dissolving the chitosan powder into trifluoroacetic acid (TFA). Meanwhile, the process parameters, process solutions and the ambient parameters were optimized for the electrospinning process. The main aim of this study was to generate continuous fibers without beads. Additionally, another goal in this work was to investigate the temperature and humidity effects on the fibers of chitosan. The conclusions of this study can be summarized below:

- SEM (Scanning Electron Microscopy) was utilized to investigate the morphology structure and the measurement of the fibers by using ImageJ software program. The fiber diameter decreased by increasing the temperature (20 – 40°C) fixing the humidity at 30%.
- The pore size distribution and the porosity of chitosan fibers were increased by raising the temperature of the electrospinning process.
- The average fiber diameter increased by raising the humidity for the conditions 30°C, 30% – 30°C, 50% and 35°C, 30% – 35°C, 50%.
- The pore size distribution and porosity at 30°C, 30% – 30°C, 50% and 35°C, 30% – 35°C, 50%, were increased by raising the humidity of the electrospinning process.
- The chemical composition of different samples was indicated by the ATR-FTIR (Attenuated Total Reflection Fourier Transform Infrared Spectroscopy) test. The change in temperature and humidity has no effect on the chemical structure of chitosan fibers in the cases of 20°C, 30% and 35°C, 50%.
- DSC (Differential Scanning Calorimeter) analysis was used to look into the thermal properties of fibers. The melting enthalpy and its temperature were altered in the cases of (20°C, 30%, and 35°C, 50%).

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