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FINAL MASTER THESIS

Master in Chemical Engineering

HYBRID MICROFIBERS BASED ON POLYLACTIC ACID/ HYDROXYPROPYL-METHYL-CELLULOSE LOADED WITH CURCUMIN

Report

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Abstract

In this study, a polymer matrix based on microfibers obtained by the electrospinning technique has been prepared. Microfibers are characterized by their composition of polylactic acid (PLA, hydrophobic polymer) and hydroxypropyl-methyl-cellulose (HPMC, hydrophilic polymer). Thus, we can characterize the fibers as a hybrid blend of PLA and HPMC. These fibers were loaded with curcumin, an active molecule with antitumor and antibacterial properties and hydrophobic nature. To obtain the matrices, we proceed to study the solubility of the polymers and establish the compatible solvents for the optimization of the electrospinning process. The different matrices prepared were characterized by the morphology of their constituent fibers. Finally, the curcumin release from the fiber matrices is studied. The dual character (hydrophobic/hydrophilic) of PLA/HPMC microfibers allows the loading of any drug, with the assurance of its compatibility and uniform distribution in the fibers, and therefore this could be considered as a general drug delivery system.

Resum

En aquest estudi s'ha preparat una matriu polimèrica basada en microfibres obtingudes mitjançant la tècnica d'electrospinning. Les microfibres es caracteritzen per la composició d'àcid polilàctic (PLA, polímer hidròfob) i hidroxipropilmetilcel·lulosa (HPMC, polímer hidròfil). Així, podem caracteritzar les fibres com una barreja híbrida de PLA i HPMC. Aquestes fibres es van carregar amb curcumina, una molècula activa amb propietats antitumorals i antibacterianes i naturalesa hidrofòbica. Per a l'obtenció de les matrius es va estudiar la solubilitat dels polímers i establir els dissolvents compatibles per a l'optimització del procés d'electrospinning. Les diferents matrius preparades es van caracteritzar per la morfologia de les fibres que les componen. Finalment, es duu a terme l'estudi de l'alliberament de curcumina a partir de les matrius de fibres. El caràcter dual (hidròfob/hidròfil) de les microfibres de PLA/HPMC permet la càrrega de qualsevol fàrmac, amb la garantia de la seva compatibilitat i distribució uniforme a les fibres, per la qual cosa es podria considerar com un sistema general d'alliberament de fàrmacs.

Resumen

En este estudio se ha preparado una matriz polimérica basada en microfibras obtenidas mediante la técnica de electrospinning. Las microfibras se caracterizan por su composición de ácido poliláctico (PLA, polímero hidrófobo) e hidroxipropilmetilcelulosa (HPMC, polímero hidrófilo). Así, podemos caracterizar las fibras como una mezcla híbrida de PLA y HPMC. Estas fibras se cargaron con curcumina, una molécula activa con propiedades antitumorales y antibacterianas y naturaleza hidrofóbica. Para la obtención de las matrices se procedió a estudiar la solubilidad de los polímeros y establecer los disolventes compatibles para la optimización del proceso de electrospinning. Las diferentes matrices preparadas se caracterizaron por la morfología de las fibras que las componen. Por último, se lleva a cabo el estudio de la liberación de curcumina a partir de las matrices de fibras. El carácter dual (hidrófobo/hidrófilo) de las microfibras de PLA/HPMC permite la carga de cualquier fármaco, con la garantía de su compatibilidad y distribución uniforme en las fibras, por lo que podría considerarse como un sistema general de liberación de fármacos.

Appreciations

First and foremost, I would like to thank my supervisor Prof. Luis Javier del Valle for giving me the opportunity to do my master's thesis project in the PSEP research group, for his guidance, inspiration, and time invested in explaining and helping me with understanding the scientific background of the project and the use of all the equipment necessary for the experiments.

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Lastly, I want to express my biggest appreciation to my family and friends, for their unconditional love and support throughout my academic formation.

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Glossary

% v/v – Volume concentration

% w/v – Weight-volume concentration

C_3H_6O – Acetone

C_2H_6O – Ethanol

CH_2O_2 – Formic acid

$CHCL_3$ – Chloroform

D – Needle-collector distance

DDS – Drug delivery system

FIB – Focused Ion Beam

HPMC – Hydroxypropyl-methyl-cellulose

LA – Lactic acid

LAB – Lactic Acid Bacteria

Mw – Molecular weight

OC- Optimal Conditions

OM – Optical microscope

PBS – Phosphate buffered saline

PE – polyethylene

PLA – Polylactid acid

PP – polypropylene

PS – polystyrene

R – Flow rate

TFE – 2,2,2-Trifluoroethanol

Tg – Glass transition temperature

Tm – Melting temperature

UV – Ultra-violet

V – Voltage

Vc – Critical voltage

1. Introduction

1.1. Objective

The present project has been developed having as a general purpose the preparation of PLA/HPMC scaffolds that incorporate curcumin. These mats of microfibers are produced by the electrospinning process, and they could be considered as a general drug delivery system due to their dual character (hydrophobic/hydrophilic).

1.1.1. Specific Objectives

The following specific objectives are stablished on purpose of achieving the general objective of the project:

1. The establishment of the optimal conditions to prepare PLA/HPMC/Curcumin electrospun fibers. Typically, the solvents are optimized as well as the electrospinning operational parameters (e.g. collector distance, flow rate, voltage).

2. The morphologic characterization of the fibers obtained under the optimal conditions. Morphology is evaluated by optical and FIB microscope observation and then the fiber diameters are measured with the Image-j program.

3. To load the scaffolds with curcumin and analyze the drug release in PBS and PBS/ Ethanol solutions.

1.2. Polylactide (PLA)

Polylactide (PLA) or Poly(lactic acid) is a thermoplastic biopolymer synthesized from lactic acid which can be derived from plant or animal sources like corn starch, sugar cane, kitchen waste, and fish waste. The fact that it is derived from biomass resources distinguishes it from most other plastics that are produced by distilling and polymerizing petroleum. Despite this major difference, PLA can be made using the same equipment used to produce petrochemical plastics, which makes PLA manufacturing cost-effective. Polylactic acid is the second most produced bioplastic, following thermoplastic starch, has similar properties to polypropylene (PP), polystyrene (PS), or polyethylene (PE), and is also recyclable, compostable, and biodegradable. [1],[2],[3],[8]

1.2.1. Production of PLA

The monomer of PLA, lactic acid (2-hydroxypropanoic acid, CH3–CH(OH)–COOH), is industrially produced by the anaerobic fermentation of organic products, such as potatoes, wheat, corn, sugar beets, and sugarcane molasses, using Lactic Acid Bacteria (LAB). It can also be produced from cellulosic products like cotton or agricultural waste, by converting lignin, xylan, glycan, and arabinan into LA through fermentation. Another possible source of LA is whey, which comes from dairy by-products. Whey contains a lot of lactose which can be made into LA through microbial fermentation. [1],[4],[5]

There are two main ways to produce PLA from lactic acid as a monomer (Fig. 1.3.1.1). The first one is the polycondensation of lactic acid, which is the conventional process for making PLA, and it is carried out under high temperature and high vacuum. A solvent is used for the extraction of the water produced by the condensation reaction. The final product of this process tends to have low to intermediate molecular weight (Mw 10,000-20,000) because of difficulties in removing water and impurities. The second method is the ring-opening polymerization of a cyclic dimer of lactic acid. This method results in a polymer with higher molecular weight and is performed in milder conditions. The production of lactide from lactic acid can create three different stereoisomers, the L-lactide, the D-lactide, and the meso-lactide (Fig. 1.3.1.2). Each form of lactic acid can result in different PLA properties, affecting the molecular weight, the crystallinity, the melting temperature, the toughness, and other polymer characteristics. [6]

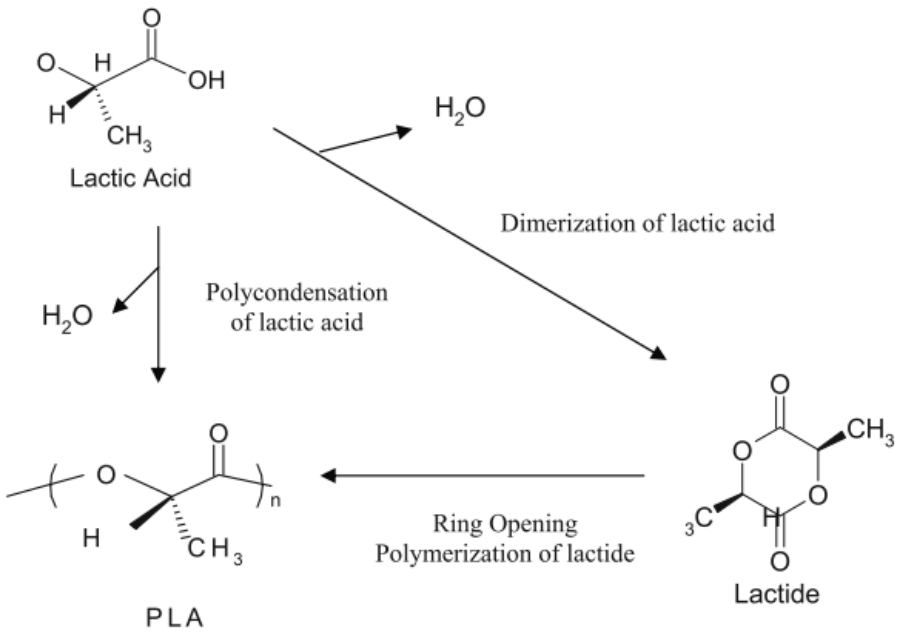

Fig. 1.3.1.1. *Polymerization routes for producing PLA [6]*

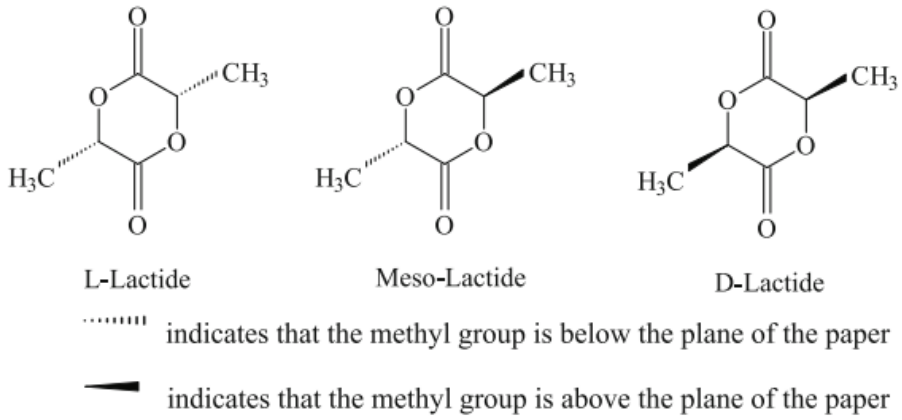

***Fig.1.3.1.2**. Different forms of lactide isomers [6]*

1.2.2. Properties of PLA

In order to select a biomaterial, the physical, chemical, and biological properties should be considered. They should not evoke an inflammatory or toxic response, should be easy to metabolize and cause the least possible side effects. Molecular weight, polydispersity, crystallinity, thermal transition, and degradation rate are also some of the key parameters. [14]

PLA's properties depend on its component isomers, the processing temperature, the annealing time, and molecular weight. The stereochemistry and thermal history influence PLA's crystallinity, and therefore, its properties in general. Crystallinity is a very important property of polymers that influences the hardness, tensile strength, modulus, stiffness, crease, and melting points. As a result, when selecting a polymer for any application, its crystallinity is of great importance. [8]

PLA crystals can grow in 3 structural positions (α, β, γ) and they are characterized by different cell symmetries and helix conformations, which are developed through different thermal and mechanical treatments. The α form can develop upon melt or cold crystallization, the β form grows upon mechanical stretching of the more stable α form, and the γ form develops on hexamethylbenzene substrate, as reported.[12] PLA properties can be controlled by using special catalysts of syndiotactic and isotactic content with different enantiomeric units. PLA with more than 90% of PLLA tends to be crystalline, while the lower optically pure is amorphous. When the PLLA amount is decreased there is also a decrease in the glass transition temperature (Tg) and the melting temperature (Tm) of PLA. [8],[14]

The Mw can significantly impact the properties of PLA, such as degradation, mechanical strength, and solubility. PLA with high Mw has a complete resorption time of 2 to 8 years. Inflammation and infection can be caused by this prolonged existence in vivo in some organs. [13] Therefore, for biomedical applications the production and use of low Mw PLA are desirable. Low Mw PLAs are used for drug delivery and have a weak retarding effect. They hydrolyze rather quickly into lactic acid, reducing the possibility of material buildup in the tissue. [8]

PLA is soluble in chloroform, methylene chloride, dioxane, acetonitrile, 1,1,2-trichloroethane, and dichloroacetic acid. Lactic acid-based polymers are hydrophobic, so they are not soluble in water. Also, PLA is neither soluble in alcohols such as methanol, and ethanol, nor in propylene glycol and unsubstituted hydrocarbons. [8],[14]

1.2.3. Degradation of PLA

Biodegradation refers to the breakdown of organic matter by living organisms, such as bacteria and fungi. In the context of plastic, biodegradation refers to the ability of certain types of plastic to break down and be absorbed by the environment, rather than persist as litter or waste.

One of the key advantages of PLA is that it is biodegradable. However, the biodegradation of PLA is not as straightforward as it may seem. For PLA to biodegrade, it must be exposed to certain conditions, such as heat, moisture, and the presence of microorganisms. These conditions are not always present in the environment, especially in landfills, where most plastic waste ends up.

In a landfill, plastic is typically buried and covered with layers of soil, which prevents the access of oxygen and light. Without these essential factors, microorganisms cannot thrive and biodegrade the plastic. As a result, PLA and other types of plastic can remain in landfills for hundreds of years, leaching toxic chemicals and contributing to pollution. However, when PLA is exposed to the right conditions, it can biodegrade in a matter of months or years. In a composting environment, for example, microorganisms can break down the polymer chains of PLA, reducing it to water, carbon dioxide, and biomass. Composting is a controlled process that involves the decomposition of organic matter under specific conditions, such as temperature, humidity, and the presence of oxygen. [8,14]

Composting is not the only way to promote the biodegradation of PLA. In some cases, PLA can biodegrade in the natural environment, such as in soil, water, or in the ocean. However, the rate of biodegradation depends on various factors, including the type of microorganisms present, the availability of nutrients, and the environmental conditions.

PLA is primarily degraded by hydrolysis and after several months of exposure to moisture. Its degradation occurs in two stages. Firstly, the Mw is reduced by random non-enzymatic chain scission of the ester groups. Then, the Mw is further reduced until low Mw oligomers and the lactic acid are naturally metabolized by microorganisms to yield carbon dioxide and water. The degradation rate is primarily determined by the polymer's reactivity with water and catalysts. [8],[14]

1.2.4. Applications of PLA

There are numerous applications for PLA products. No toxins are contained in PLA, so it is neither carcinogenic nor toxic to the human body. This makes it perfect for biomedical applications like sutures, clips, and drug delivery systems (DDS). [1] One of the main medical applications of PLA is the production of scaffolds in tissue engineering. PLA scaffolds provide a surface that promotes the reconstruction and regeneration of human organs and human tissue. PLA is considered suitable for skin, blood vessels, bone, ligaments, cartilage, nerves, and muscle fixation due to its great chemical, physical, biomechanical, and degradation properties. [9] Precise and reproducible 3D structures of PLA-based scaffolds are fabricated by 3D printing with well-defined predetermined geometries. PLA emergence in 3D printing technology helped to develop scaffolds in various desired shapes and forms, with low production cost, corresponding biological and mechanical properties, and controlled micro/nanostructures. [1], [10]

PLA is also used in packaging, especially food packaging. The rapidly growing necessity for sustainable packaging and ecological alarms throughout the world are forcing companies to use PLA, among other biodegradable materials, in numerous products. PLA's physical properties, including thermoplasticity, high strength, processability, and non-toxicity, make it suitable to produce jars, bottles, containers, and fresh food packaging.[1]

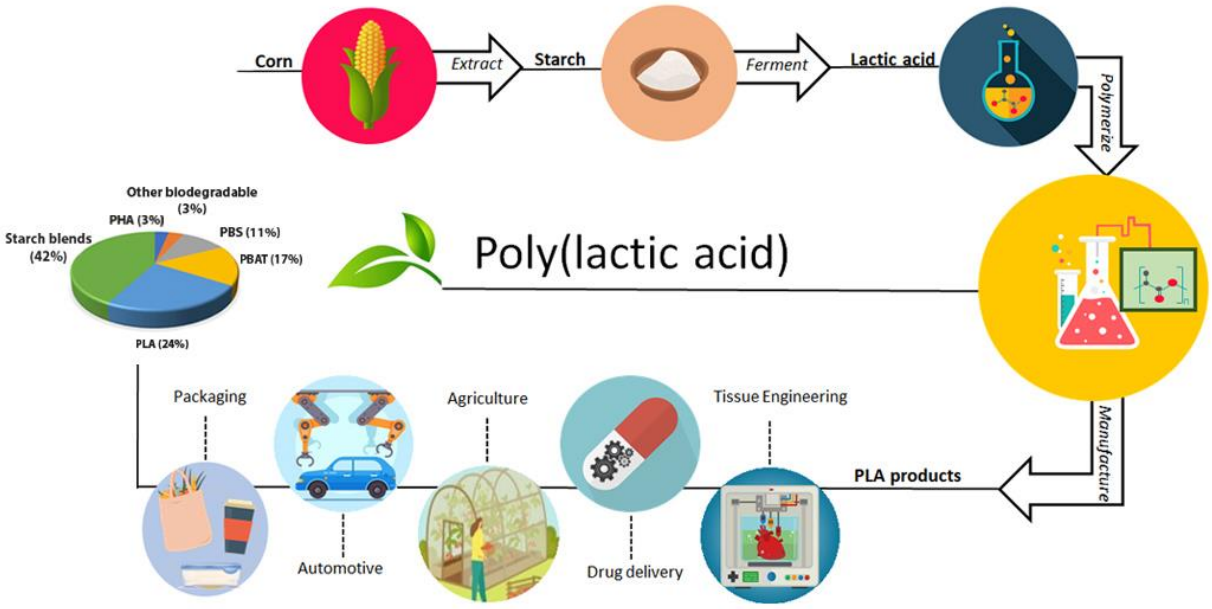

Fig. 1.3.4.1. *Graphical depiction of the raw material, process, and end products of PLA [1]*

1.2.5. Environmental impact of PLA

It is estimated that until 2021, the total world production of polymers was around 9 billion tons. Only 9-10% of these polymers are reused or recycled, only 12% are incinerated, while the rest 78-79% are accumulated in the natural environment and landfills. [11] This poses a serious threat, not only to humans but also to the environment. Plastics are responsible for environmental contamination and are toxic to our ecosystems and our health. New eco-friendly polymers, such as PLA, are now of great interest since plastics' benefits are undeniable due to their material properties. [1]

LA is a sustainable monomer derived from plants, which is then polymerized and processed into the desired end products. PLA's raw material, for example, corn, is renewable, non-polluting, and gives an end to the use of a finite supply of oil as a raw material.[6] For producing PLA, 65% less energy is necessary than producing conventional plastics and 68% fewer greenhouse gasses are released.[1] PLA's production also requires 20-50% less fossil fuel resources than the production of conventional, petroleum-based polymers. Even though in processing the raw material of PLA and through its production, fossil fuels are unavoidably used, like in all synthetic polymers, it is obvious that PLA will contribute to reducing the world's dependence on fossil fuels. [6],[7]

1.3. Hydroxy-propyl-methyl-cellulose (HPMC)

Hydroxypropyl methylcellulose, also known as HPMC, is a mixed ether CD (copolymer of cellulose) that has been synthesized to include both hydroxypropyl and methyl functional groups.

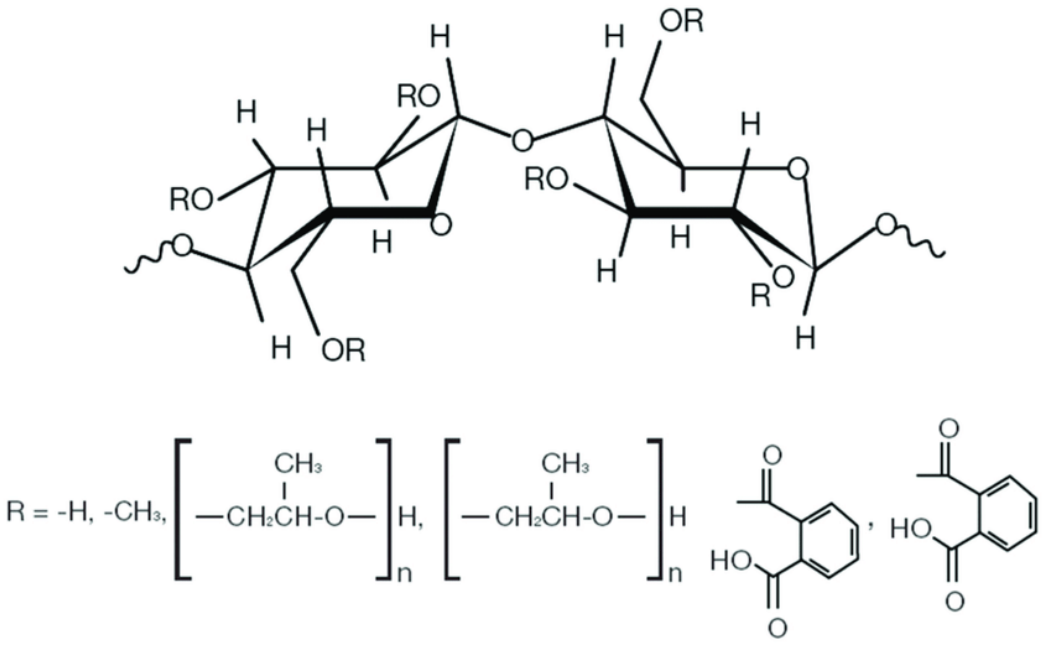

Fig. 1.3.1 *Chemical structure of hydroxypropyl-methylcellulose HPMC and their substitute groups [25]*

1.3.1. Production of HPMC

Hydroxypropyl methyl cellulose (HPMC) is a type of cellulose derivative that is commonly used in a variety of industries, including pharmaceuticals, food, cosmetics, and construction. It is a white, odorless, tasteless powder that is highly soluble in water and has excellent thickening, emulsifying, and film-forming properties. HPMC is synthesized from cellulose, which is a natural polymer found in plants. Cellulose is made up of glucose units that are linked together by chemical bonds. To create HPMC, cellulose is first treated with an alkaline solution to break down the chemical bonds. This process is known as hydrolysis. Next, the cellulose is treated with propylene oxide and then with methyl chloride, which adds hydroxyl and methyl groups to the cellulose molecule, respectively. This process is known as etherification. The resulting product is HPMC, which is a cellulose derivative with a higher molecular weight and improved water solubility compared to cellulose. [24]

1.3.2. Properties of HPMC

HPMC has a number of unique properties that make it an ideal choice for use in numerous industries. It is non-toxic, non-irritating, and non-allergenic, making it safe for use in a variety of applications. It is also resistant to bacteria and fungi, which makes it ideal for use in products that require a long shelf life. HPMC also has a high degree of flexibility, which allows it to be used in a wide range of applications. It can be used in products that require a wide range of viscosities, from very thick to very thin. It is also resistant to temperature changes, making it suitable for use in products that will be exposed to extreme temperatures. [24], [25]

In addition to its many uses, HPMC is also environmentally friendly. It is biodegradable and can be easily broken down into its component parts, which makes it an attractive alternative to synthetic polymers. Despite its many benefits, there are also some potential drawbacks to using HPMC. It is not as stable as some other cellulose derivatives, and it can break down over time if it is exposed to high temperatures or strong acids. It is also relatively expensive compared to some other thickening agents, which may make it less appealing for use in some applications. [23], [25]

1.3.3. Degradation of HPMC

One important aspect to consider when discussing the environmental impact of HPMC is its biodegradability. Biodegradability refers to the ability of a material to break down and be naturally assimilated into the environment through the action of microorganisms. This process helps to reduce the amount of waste in the environment and can help to mitigate the negative effects of pollution. [25], [26]

HPMC is classified as a biodegradable polymer, meaning that it can be broken down by microorganisms under certain conditions. However, the rate of biodegradation can vary depending on the specific conditions and the type of microorganisms present. In general, HPMC has been shown to biodegrade more slowly than some other biodegradable polymers, such as polylactic acid (PLA). [26]

One factor that can impact the biodegradability of HPMC is the presence of oxygen. In the presence of oxygen, HPMC can be more easily broken down by microorganisms. However, in the absence of oxygen, the biodegradation process can be slowed down. This is because the microorganisms that are responsible for breaking down HPMC require oxygen to carry out their metabolism. [26], [27]

The pH of the environment can also impact the biodegradation of HPMC. In general, HPMC tends to biodegrade more readily at neutral pH levels. However, it has been shown to biodegrade more slowly at lower pH levels, such as those found in acidic environments. [26], [27]

1.3.4. Applications of HPMC

One of the most common uses of HPMC is as a thickening agent in the pharmaceutical industry. It is used to increase the viscosity of liquids, such as syrups, suspensions, and emulsions, which makes them easier to swallow and improves their stability. HPMC can also be used to improve the flow properties of powders, making them easier to handle and process. [24]

In the food industry, HPMC is used as a stabilizer, emulsifier, and thickener. It is commonly found in products such as ice cream, cheese, and bakery products. It helps to improve the texture and consistency of these products and extends their shelf life by preventing the separation of ingredients. [23],[25]

HPMC is also used in the cosmetics industry as a thickening agent, emulsifier, and film former. It is found in products such as lotions, creams, and gels, and helps to improve their stability and consistency. [24]

In the construction industry, HPMC is used as a binder and thickener in products such as paint, plaster, and cement. It helps to improve the flow properties of these products, making them easier to apply and more durable.

1.4. Electrospinning

We can define the word ‘nanofiber’ by splitting it into the terms “nano” and “fiber”. Fibers are defined by the textile industry as a natural or synthetic filament that may be spun into yarn, such as cotton or nylon. From a geometrical perspective, a "fiber" is characterized as a thin, elongated, threadlike item or structure. Technically speaking, "nano" refers to scales that are one billionth of a unit. Generally, nanofibers have a diameter between 50 and 300 nanometers. [16]

A method for creating fibers with diameters as thin as a few tens of nanometers is electrospinning. In electrospinning, a drop of polymer solution held at the end of a capillary by its surface tension is subjected to a high electrostatic voltage. The liquid's surface is warped into the Taylor cone, a conical shape. A steady liquid jet is released from the tip of the cone once the voltage reaches a critical point, and the electrostatic force overcomes the surface tension of the solution. As the jet passes through the air, the solvent evaporates, leaving behind ultrafine polymeric fibers that are collected on an electrically grounded target. Solution variables such as concentration, viscosity, surface tension, conductivity, and solvent vapor pressure, as well as process parameters such as solution feed rate, applied voltage, and nozzle-to-collector distance, and ambient parameters such as humidity and temperature, all have an impact on the electrospinning process. Compared to

commercial non-woven textiles, electrospun mats have a greater specific surface area and smaller pore size. They are useful in many different applications, such as semi-permeable membranes, scaffolds for tissue engineering, and drug delivery systems. [15], [19]

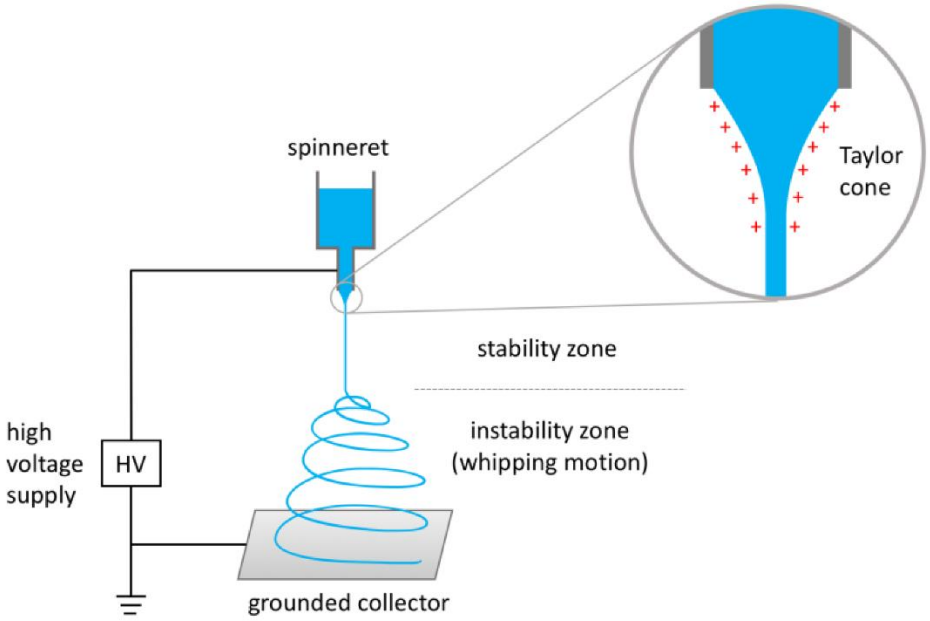

***Fig. 1.4.1**. Diagram of the electrospinning method [19]*

1.4.1. Physics of electrospinning

When the electric field/voltage is gradually increased, the drop's surface turns convex at a particular voltage, the Vc (critical voltage). When V_c is reached, the jets (electrospinning) and sprays (electrospraying) begin, which are represented by Equation (1), a relationship identified by Taylor:

$$V_c = 4\frac{H^2}{L^2}\left(ln\frac{2L}{R} - \frac{3}{2}\right)(0.117\pi\gamma R) \quad (1)$$

where:

H: the separation between capillary and the collector

L: the length of capillary

***R**:* the radius of capillary

γ: the surface tension

A similar relationship for the potential necessary for the electrospraying of charged pendant drops of solutions from the pendant in a capillary tube was developed by Hendrick:

$$V = 300\sqrt{20\pi\gamma r} \quad (2)$$

where:

V: the required voltage

γ: the surface tension

r: the radius of the pendant drop

Viscosity and conductivity are essential components of the electrospinning process but are not included in the equation (2). The use of applied voltage and the surface tension gives a representative equation only for slightly conducting, medium- to low-conductivity solutions. [20]

1.4.2. Parameters of electrospinning

Electrospinning is a straightforward method that doesn't require complicated equipment. The size, porosity, and homogeneity of the fibers are influenced by the processing parameters, solution and ambient parameters. These parameters could be arranged in three main groups as shown in ***Table 1.4.2.1.*** Although each of these characteristics has been explored in detail, not all researchers will agree with the findings of any given study. A parameter modification in one polymer may result in a completely different outcome in another polymer. The finished fibers are the product of a combination of several parameters, and none of the parameters act independently throughout the electrospinning process.

Solution properties	**Equipment variables**	**External/ambient parameters**
Polymer type (molecular weight, structure)	Flow rate	Humidity
Polymer concentration	Distance between the needle tip ant the collector	Temperature of the solution
Viscosity	Electric potential	Air velocity in the electrospinning chamber
Conductivity	Geometry	

Density of surface charge	Type of collector
Surface tension	
Dielectric constant of the solvent	

Table 1.4.2.1. *Parameters affecting the electrospinning process and produced fibers.*

The strength of the applied DC voltage is the main factor affecting fiber synthesis. The applied DC voltage affects the size of the fiber, bead formation, and the absence of jet production. A pendant drop and a cone are first generated at the capillary's tip in the presence of a low electric field. The amount of the drop reduces as the applied voltage is raised progressively until just a cone is left at the capillary's tip. If the voltage is raised even higher, no visible Taylor cone forms on the needle's blunt tip, and fiber creation begins from within the needle, as shown in *Figure 1.5.1*. The charge is transferred to the grounded collection plate by the electrospun fibers, which completes the circuit. When conductivity, the dielectric constant, and the flow rate through the pump stay constant, a rise in current implies that the mass of the fibers generated is increased.

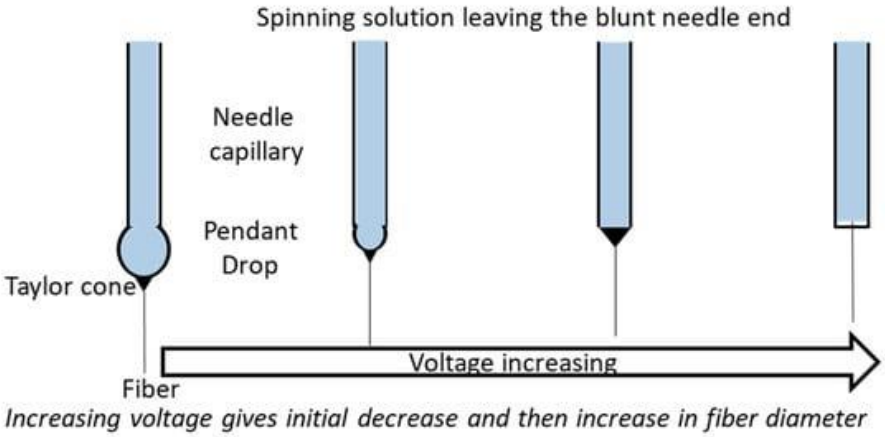

Figure 1.4.2.2 *The effect of applied electric field on Taylor cone formation (dark colored tip)*

The size, shape, and porosity of electrospun fibers are all directly influenced by the polymer flow rate. Studies report that increasing flow rate results in decreasing fiber diameters. [20], [21] However, below a certain flow rate the electric field may not be sufficient to maintain the Taylor cone and drops can appear due to gravitational force. [20]

Another aspect that significantly influences the size and morphology of the nanofibers is the distance between the capillary and the collector. It is important to optimize this distance because it could be the difference between electrospraying and electrospinning. When using the traditional method of electrospinning, an effective spinning distance is typically thought to be between 10 and 20 cm. The fiber diameter typically decreases when increasing the distance between the Taylor cone and the collector. In addition, decreasing the distance can also lead to the formation of non-homogeneous and elongated beads. [20], [22]

When choosing the concentration of the solution for electrospinning, the viscosity and surface tension should be considered, which affect a solution's spinnability. When a solution has a low concentration (low viscosity, <1 poise), surface tension prevails, and droplets rather than continuous fibers develop. Higher concentrations (viscosity > 20 poise) make it impossible to sustain and control the flow of the solution. Each of the three variables—viscosity, polymer concentration, and molecular weight—relates to the others and cannot be evaluated separately. The molecular weight of the polymer affects the viscosity of the solution, which has a significant impact on fiber shape. Also, when the viscosity is low, fibers cannot be formed, and when the viscosity is high, the electrospinning process is difficult to perform because it requires a stronger electric field.

1.4.3. Commercialization Challenges of Electrospinning

Even though many scientific domains have extensively documented the advantages of electrospinning, there is still a pressing need to apply manufacturing in an effective manner. The electrospinning procedure still faces several difficulties that need to be resolved. These include (a) manufacturing on a large scale; (b) accuracy and repeatability across all processes of fabrication; and (c) safety and environmental issues of electrospinning. Low output per spinneret, clogging of the spinneret tip, inter-jet interference, recovery of vaporized solvents used in the process, and fiber alignment over a wide area of significant thickness are the main difficulties in mass producing electrospun fibers. The solution concentration is kept to a minimum, with solvent accounting for more than 70% of the solution mass, to generate electrospun nanofibers free of any morphological flaws. Because of this, only a small portion of the fluid that flows through the spinneret contributes to the mass of the nanofiber that is created. Additionally, there is a maximum feed rate allowed per nozzle, and greater rates may cause the solution to drip from the nozzle, particularly if the nozzle is positioned in the center. This is because the nozzles' insufficiently strong electric fields cause insufficient solution drawing. [20]

The solvent used in the electrospinning process is a further significant issue. This problem is crucial for both manufacturing safety and the quality of the final product because solvent residues may get trapped inside electrospun nanofibers. When using large-volume, solvent-based electrospinning for

biomedical and pharmaceutical applications, precise control over solvent residues becomes essential. However, the possibility of solvent residues and solvent recovery should be eliminated when using solvent-free spinning. The application of numerous different active polymeric materials for the creation of nanofibers is currently restricted due to the lack of cost-effective and dependable electrospinning technologies. Moreover, it is highly challenging to produce identical scaffolds, especially between research groups, which limits the utilization of electrospun fiber mats for tissue engineering. Additionally, it can be challenging to guarantee the consistency of the fibers with particular morphologies and qualities when using customized electrospun fibers. [20]

1.5. Curcumin

Curcumin is a natural polyphenol found in the spice turmeric, which is commonly used in Indian and Middle Eastern cuisine. It is known for its bright yellow color (Figure 1.6.1) and has been used for centuries in traditional medicine for its numerous health benefits.

Fig. 1.5.1. *Turmeric whole and in powder*

1.5.1. Curcumin properties

One of the main properties of curcumin is its strong antioxidant activity. Antioxidants help to neutralize harmful free radicals in the body, which can cause damage to cells and contribute to the development of chronic diseases such as heart disease, cancer, and neurodegenerative conditions. Curcumin has been shown to have a stronger antioxidant activity than vitamin C or vitamin E, and it can help to protect against oxidative stress and inflammation in the body. [29], [30]

Curcumin also has anti-inflammatory properties, which make it a popular choice for the treatment of conditions such as arthritis, asthma, and irritable bowel syndrome. It has been shown to reduce

inflammation in the body by inhibiting the production of certain enzymes and cytokines, which are involved in the inflammatory process. [30]

In addition to its antioxidant and anti-inflammatory effects, curcumin has also been shown to have potential in the treatment of cancer. It has been shown to induce cell death in cancer cells, and it may also inhibit the growth and spread of cancer cells. It has also been shown to have a protective effect against radiation-induced DNA damage, which may make it a useful adjuvant treatment for cancer patients undergoing radiation therapy. [29], [31]

Curcumin has also been studied for its potential in the treatment of neurological conditions such as Alzheimer's disease and Parkinson's disease. It has been shown to improve memory and cognitive function in animal models of these conditions, and it may also have a protective effect on brain cells. Studies also suggest that it has antidepressant and anxiolytic effects, which may make it a useful treatment option for individuals with mental health conditions such as depression and anxiety.

Curcumin has a low bioavailability when taken orally, which means that it is not well absorbed by the body. This has limited its use as a therapeutic agent in clinical trials, as higher doses are required to achieve therapeutic effects. To overcome this issue, various formulations of curcumin have been developed, including nanocurcumin, which is a highly bioavailable form of curcumin. [29], [31]

In conclusion, curcumin is a natural polyphenol with numerous health benefits. It has strong antioxidant and anti-inflammatory properties, and it has shown potential in the treatment of cancer and neurological conditions. While its low bioavailability has limited its use as a therapeutic agent, various formulations have been developed to overcome this issue. Further research is needed to fully understand the therapeutic potential of curcumin and to determine its optimal dosage and administration.

1.6. Drug Delivery Systems (DDSs)

In medical therapy, controlled release is an effective method of drug delivery. It can increase patient convenience, balance delivery kinetics, and reduce toxicity and side effects. In a controlled release system, the active ingredient is first loaded into a carrier or materials science device, after which it releases in vivo at a controlled rate, either injected or not.

Electrospun nanofibers have demonstrated several benefits as a potential medication delivery method. The electrospinning technology makes it very simple to perform the drug loading, and the high applied voltage has little impact on the drug activity. The overall release rate of the nanofiber

drug system is higher compared to the bulk material (e.g., film) due to the high specific surface area and short diffusion passage length. The morphology, porosity, and composition of the nanofibers can be adjusted to precisely control the release profile. Biodegradable polymers like PLA, PCL, poly(Dlactide) (PDLA), PLLA, and PLGA as well as hydrophilic polymers like HPMC, PVA, PEG, and PEO are the main sources of nanofibers for drug release systems. [16]

1.6.1. PLA Based DDSs

PLA has been used for continuous drug release for different time periods including prolonged administration of many medical agents such as anesthetics, vaccines, contraceptives, narcotic antagonists, local peptides, and proteins. There are three different ways that polymeric drugs can be released: erosion, diffusion and swelling. For PLA, the device was subsequently eroded as a result of the randomly occurring hydrolytic ester cleavage of ester bonds. The hydrolytic byproducts of this degradation process are subsequently converted into non-toxic byproducts, which are excreted by regular cellular function and urine. Numerous medications, including those for psychosis, restenosis, hormones, dermatotherapy, protein and antitumor drugs were encapsulated using PLA and their copolymers in the form of micro-or nanoparticles. Utilizing solvent evaporation technique, PLA particles were created and discovered to be excellent candidates for the construction of drug delivery systems. It has been discovered that the mechanical stability and degree of crystallinity of PLA in the formulation can adjust the difficulty of controlled drug release. [8]

1.6.2. HPMC based DDSs

Electrospun nanofibers (NFs) made with cellulose and its derivatives have been investigated as viable candidates for use in the pharmaceutical industry. For instance, several studies look into the use of electrospun fiber mats as delivery systems and present dosage forms with advantageous and controllable dissolution characteristics. HPMC is a hydrophilic polymer, so the HPMC matrix swells when it comes into touch with water or biological fluids, allowing the fluid to seep into it. This allows the drugs to diffuse out of the matrix. [15], [18]

The environmental benefits of cellulose as a biomaterial are what is primarily driving this interest in cellulose-based NFs. Since cellulose is a plentiful and renewable resource that can be found almost everywhere in the world, it is an affordable raw material for a variety of uses. The challenges associated with electrospinning cellulose are mostly caused by the material's several drawbacks, one of which being its resistance to interacting with common solvents. So, selecting the right solvent system is crucial. [15]

Because of its ease of use, wide availability, strong biocompatibility, biodegradability, and outstanding film-forming potential, HPMC is one of the most used excipients in pharmaceutics. HPMC has both hydrophilic hydroxypropyl and hydrophobic methyl groups, resulting in a polymer with surface-active characteristics. As a result, HPMC is extremely beneficial in avoiding the re-crystallization of amorphous versions of poorly water-soluble medicines such felodipine and indomethacin. HPMC was widely utilized as a hydrophilic carrier in traditional industrial procedures to manufacture commercial items such as tablets, films, gels, and capsules. Furthermore, numerous advanced nanotechnologies have researched the use of HPMC as a carrier in the production of novel nano DDSs such as nanogels and electrospun nanofibers [28].

2. Materials and methods

2.1. Chemical Products

- The PLA used in this research was *PLA 4032D* from *Natureworks®* which contains 98% L-lactic isomer.
- The HPMC used was the *H7509 (hydroxypropyl)methyl cellulose* from *Sigma Aldrich*.
- The curcumin used was the *C1386* from *Sigma Aldrich*
- The chloroform used was from *Fisher Chemical,* stabilized with amylene.
- The PBS used was the D5652 from *Sigma Aldrich*

The solvent used to get PLA mats was chloroform ($CHCL_3$). The polymer concentration selected was 13,6 % w/v of PLA. For the reference material, 1,36 g of PLA was dissolved in 10 mL of $CHCL_3$ and placed in the orbital chamber (80 rpm) overnight at 37 °C.

The solvent used to get PLA/HPMC mats was chloroform ($CHCL_3$), acetone (C_3H_6O) and trifluoroethanol or TFE (CF_3CH_2OH). The polymer concentration selected was 13,6 % w/v of PLA/HPMC. For the reference material, 1.345g of PLA was dissolved in 6 mL of $CHCL_3$ and 0.136g of HPMC was dissolved in 5 ml of Chloroform - Acetone- TFE (3.2 ml- 0.8 ml- 1ml). The two solutions were placed in the orbital chamber (80 rpm) overnight at 37 °C.

The solvent used to get PLA/HPMC/Curcumin mats was chloroform ($CHCL_3$), acetone (C_3H_6O) and trifluoroethanol or TFE (CF_3CH_2OH). The polymer/drug concentration selected was 13,6 % w/v of PLA/HPMC/Curcumin. For the reference material, 1.275 g of PLA was dissolved in 6 mL of $CHCL_3$ and 0.150g of HPMC was dissolved in 5 ml of Chloroform - Acetone- TFE (3.2 ml- 0.8 ml- 1ml). The two solutions were placed in the orbital chamber (80 rpm) overnight at 37 °C. Once the HPMC was dissolved, 0.075g of curcumin was added to the HPMC solution. The mix of the two solutions was done just before the electrospinning process.

The polymer and solvent concentrations are summarized in the ***Table 2.1.1***.

Solution	**PLA**	**PLA/HPMC 9:1 (w/w)**	**PLA/HPMC/Curcumin (85%/10%/5%)**
Concentration w/v %	13,6	13,6	13,6

Solvents	Chloroform	Chloroform - Aceton- TFE	Chloroform - Aceton- TFE
Solvents concentration %	100%	83.7% - 7.3% - 9%	83.7% - 7.3% - 9%

Table 2.1.1. *Polymer and solvent concentrations of each solution*

2.2. Electrospinning setup

The electrospinning setup is composed by the following elements:

1. Collector

The collector is the electric component that collects the fibers, and the top surface is covered with aluminum foil. It could be adjusted in height and position within the protective box. To generate the electric field, it is connected to one of the electrodes. The use of a security box is necessary due to the high voltage required.

2. High Voltage Supply

A high voltage supply is a device that creates a voltage difference between the needle and the collector, promoting fiber formation and deposition on the collector. The device used was the *ES30-5W Gamma High Voltage Research*, with a voltage range of 0 to 30 kV and an intensity range of 0 to 750 mA.

3. Pump

The pump is an electronic device that allows the user to dose and regulate the injection of the polymer solution into the needle at a constant rate. The injection pump used is the KDS 100 legacy syringe pump.

4. Syringe

The syringe is where the polymer is kept. It should be inert and have no effect on the nature of the solution contained.

5. Needle

The needle is the component of the setup through which the solution flows. It is linked to the power supply device's other electrode. It should be blunt to facilitate the formation of Taylor's cone. The needle used was the *BD Microlance™ 3*, 18G 1 1/2", 1.2*40mm, with its tip cut straight.

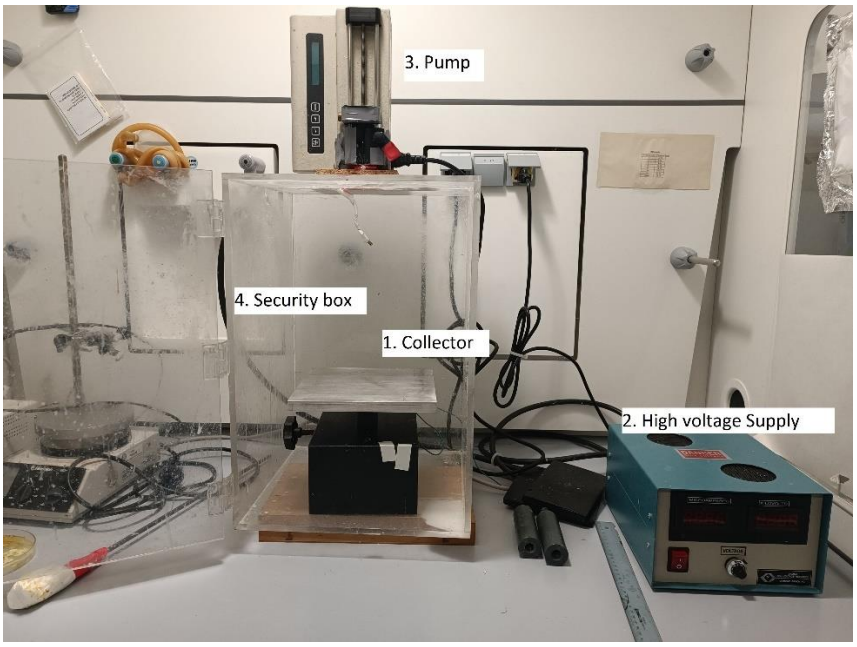

Fig. 2.2.1. *Photograph of the equipment used for the electrospinning.*

The polymer solution is prepared at the desired concentration the day before and placed in the orbital chamber overnight at 37 °C. Before the electrospinning is performed, 100 µL of formic acid is added to the 10mL solution. After filling a 10 mL plastic syringe with the polymer solution, electrospinning is performed between the needle connected to the anode and the static collector connected to the cathode. The high voltage supply is used to apply direct current. The syringe pump is used to control the flow. The polymer jet is gathered on aluminum foil until a 1-1.5 g mat is obtained. All electrospinning procedures are carried out at room temperature.

2.3. Optical Microscope

The optical microscope makes it simple to determine the best parameters for the electrospinning process. Some fibers are collected in a coverslip, which is then placed in a specimen holder and observed under the *Nikon Eclipse Ei* optical microscope. The micrographs were taken with the digital camera *Moticam Pro 252B*.

However, this method is inefficient for measuring fiber diameter because light disperses on the fibers and casts shadows, and it would lead to inaccurate diameter measurements. As a result, FIB analysis is performed on all mats produced.

Fig. 2.3.1. *The Nicon Eclipse Ei optical microscope used for the fiber optimization.*

2.4. Focused Ion Beam (FIB) microscope

The FIB instrument is nearly identical to the scanning electron microscope (SEM), except that instead of electrons, it uses ions to interact with the sample. Ions are heavier than electrons and, through the sputtering process, can eject atoms from the sample surface. The ion beam can be controlled with nanometric precision, allowing the FIB instrument to perform nanolithography, selective milling and ion-beam assisted deposition, 3D tomography, TEM lamella preparation, microfabrication, and elemental analysis directly on the sample surface.

A *Focus Ion Beam Zeiss Neon 40 instrument (Carl Zeiss, Germany)* is used in this study for obtaining photos used for the fiber diameter measurements. 6 samples were used for this analysis, 1 sample of each electrospun fiber mat and one sample of each fiber mat after it was put in water overnight to study the possible change in the diameter. A conductive layer is applied to the samples during preparation. A *Balzers SCD-004 Sputter-coater* is used to coat samples with a thin carbon film of around 5-7 nm thickness. This coating prevents the formation of an electric charge in the sample as a result of the polymer's low conductivity and the interaction of this charge with the incident ion beam. Images would become distorted if this occurred. As a result, this coating is critical for optimal

image acquisition. Furthermore, because all polymers melt when exposed to high voltage ion beams, this coating serves as a shield.

Some of the most important properties of this kind of microscopy are:

- ✓ Three-dimensional appearance of the images
- ✓ Easy sample preparation
- ✓ Ease to focus.

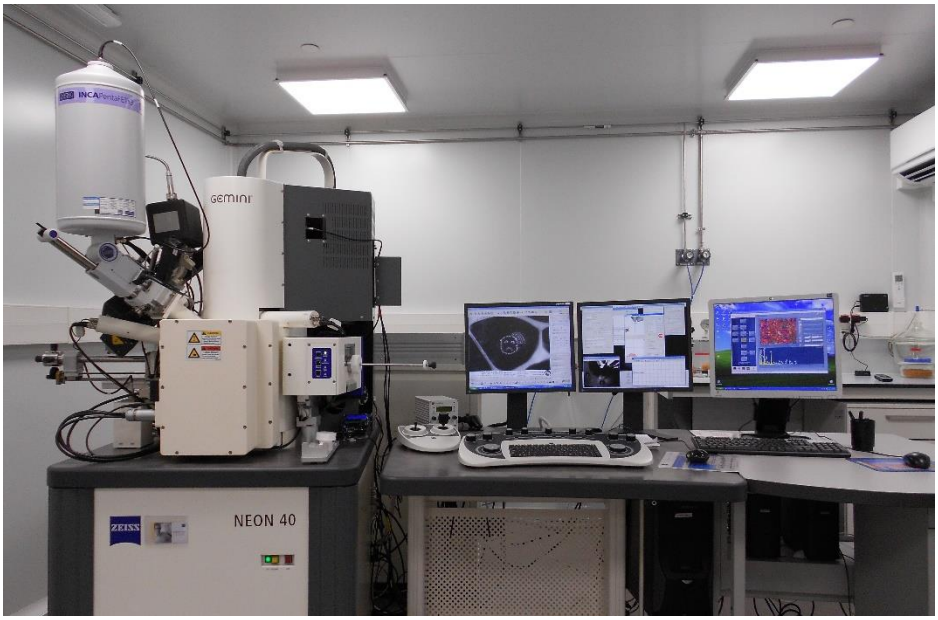

Fig. 2.4.1. *The Neon40 Crossbeam™ workstation is used in this study*

2.5. Drug release/ UV-Vis spectrophotometer

The procedure followed to study the drug release is described below.

- First, the PBS and PBS/Ethanol solutions were prepared. For the PBS, 9.6g was added to 1L of water along with 0.3g of Sodium azide (NaN_3) to prevent microbial contamination. Half of this solution was mixed with ethanol 10% v/v.
- Then, 6 samples of the PLA/HPMC/Curcumin matrix (with the aluminum foil) about 1X1 cm dimension were prepared.
- The weight of the samples was taken.

- Then, 3 samples were put in plastic sample bottle-tubes containing 10ml PBS and the other 3 were put in 10ml PBS/Ethanol.
- They were all put in the spinning machine shown in ***Figure 2.5.1.*** and 1ml samples were taken after 1h, 2h, 4h, 6h, 8h, 24h, 48.5h.
- The fluid (1ml) was replaced with PBS or PBS/Ethanol right after each sample was taken.
- The samples were put in 4°C until their UV-Vis spectra were recorded from 700-200 nm.

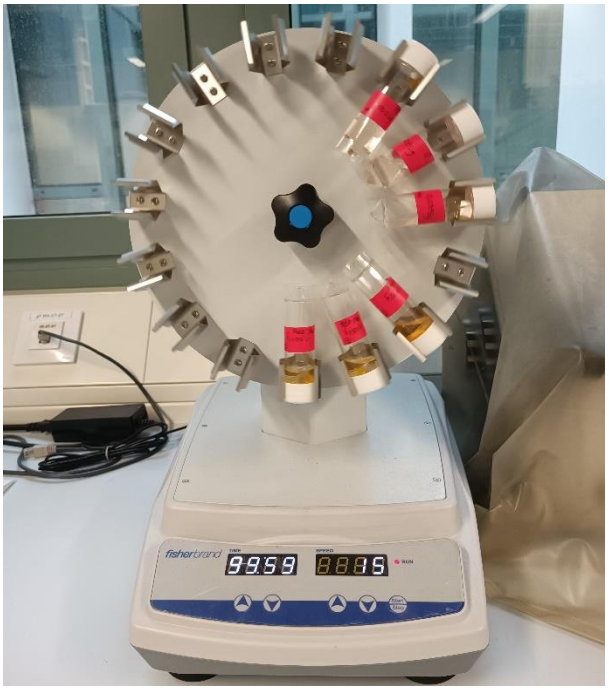

Fig. 2.5.1. *The spinning machine used in the experiment with the samples on it.*

UV–Vis spectra were recorded using a UV-Vis Cary 100 Bio spectrophotometer (Agilent, Santa Clara, USA).

- For calculating the drug release, the standard curves for each solution (PBS and PBS/Ethanol) were obtained using samples with known concentrations of curcumin.
- The UV-Vis spectra for the following concentrations of curcumin were recorded (0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.1, 0.13, 0.16, 0.19, 0.2 μg/ml)
- The data collected was gathered in the Excel program and the highest absorption of each sample was plotted with the curcumin's concentration.
- From each diagram the equation of the standard curve was obtained
- These equations were then used to calculate the concentration of curcumin in the samples taken from the 10ml PBS and PBS/Ethanol tubes.

The UV-Vis spectra for the remaining drug in the matrices were also obtained.

- The matrices were first put it 1ml $CHCl_3$ so that the matrix would completely dissolve, and the aluminum foil was removed and put to dry in order to measure the weight.
- Then 1ml of PBS/Ethanol was added to the dissolved matrix samples.
- The solution separated in two phases and the top phase that constituted of the PBS/Ethanol and some curcumin was removed and its spectra was recorded.
- The bottom phase still had much curcumin, so its spectra was also recorded.
- With the use of the standard curves' equations the concentrations of the remaining curcumin in the 6 matrices were approximately calculated.
- This way, the percentage of curcumin released through the time was calculated.

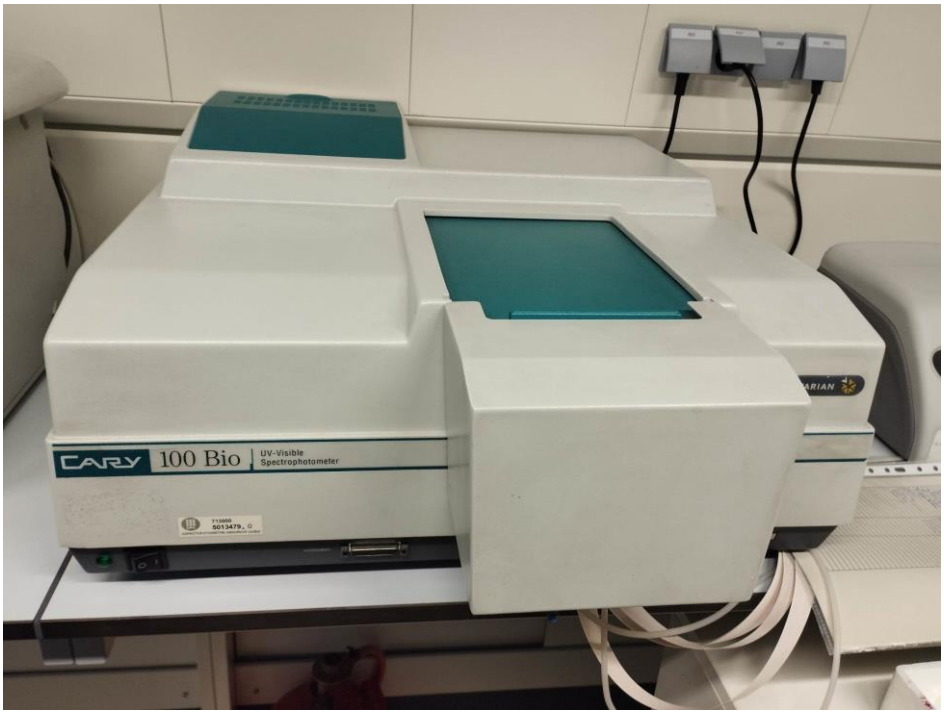

Fig. 2.5.2. *The UV-Vis Cary 100 Bio spectrophotometer used in the study.*

3. Results and discussion

3.1. Optimal preparation of electrospinable polymer solutions

The solutions for electrospinning were created in different ways depending on the compatibility of the polymers with the available solvents. The solubility tests performed with the different materials utilized during the development of this project are summarized in the table below.

Solvent	PLA	HPMC
Water	Insoluble	Soluble
Chloroform	Soluble	Soluble
Acetone	Partially soluble	Soluble
TFE	-	Soluble

***Table 3.1.1**. Solubility tests for solvent selection.*

The exact proportions and methods for producing electrospinable solutions are described in detail in paragraph 2.1.

3.2. Optimization of the electrospinning conditions

As mentioned before in paragraph 1.4.2, it is challenging to create the ideal electrospinning circumstances since they depend on a variety of variables, including, for example, the ambient conditions. In order to create the optimal conditions (OC), 3 factors were modified before getting the desired mat. These variables are the needle-collector distance, the applied voltage, and the flow rate. In ***Table 3.2.1*** the optimal operational parameters for electrospinning each sample are listed.

Solution	PLA	PLA/HPMC	PLA/HPMC/Curcumin
V- Voltage (KV)	12	17	17
R- Flow rate (ml/h)	2.3	2,3	2,3

D- Needle-collector distance (cm)	18	15	11

Table 3.2.1. *The optimal conditions (OC) for electrospinning each solution*

3.2.1. Optical microscope analysis

Following, the photos obtained with the optical microscope are presented, each time by changing one parameter and keeping the other two constant. The 3 parameters for this optimization are the voltage (KV), the flow rate (ml/h) and the needle-collector distance (cm).

3.2.1.1. PLA matrix

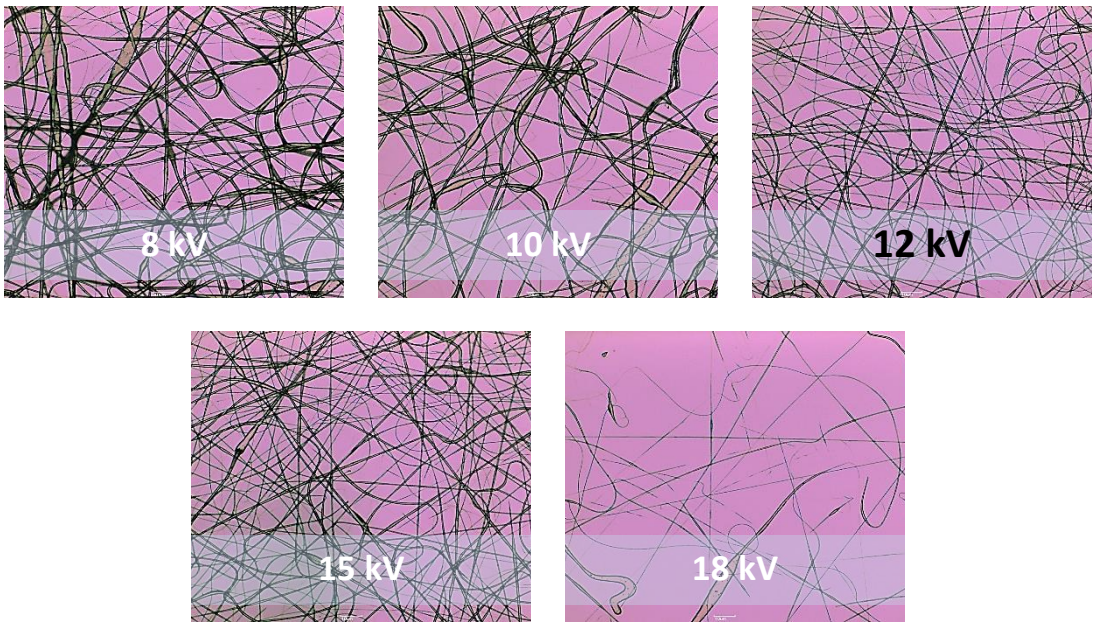

Fig. 3.2.1.1. *Optimization of the electrospinning conditions of PLA with OM analysis. The flow rate and the distance between the needle and the collector are constant (OC) and the voltage is changed.*

- Under 8 kV there was no electrospinning, only dripping. From 8-10 kV we can observe that the fibers have bigger diameters than are not consistent. From 15-18 kV the Taylor-cone formation wasn't ideal which affects the fiber produced as we can observe in the last photo (18kV).

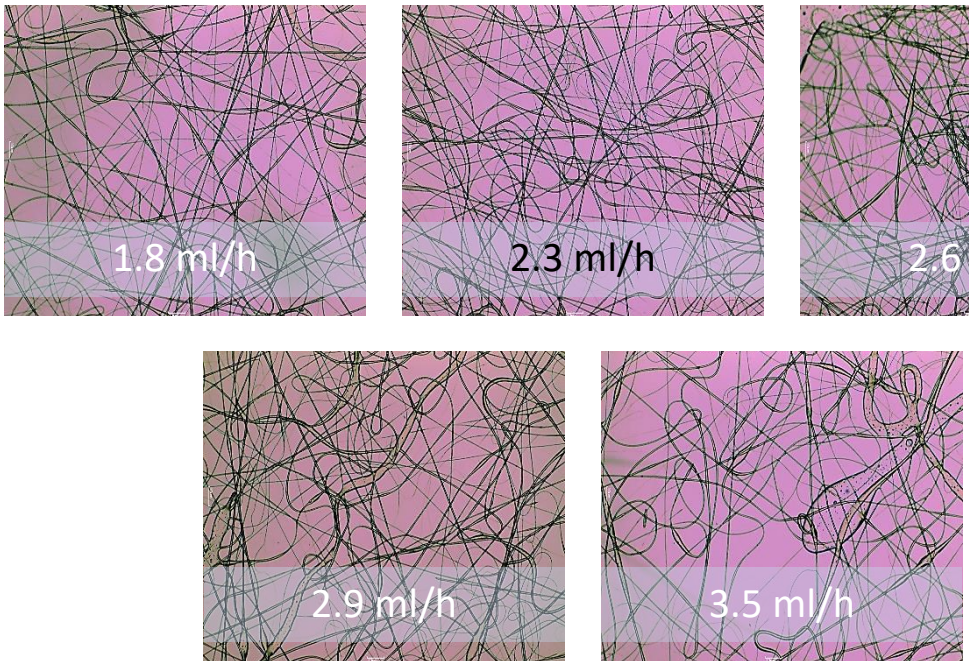

Fig. 3.2.1.2. *Optimization of the electrospinning conditions of PLA with OM analysis. The voltage and the distance between the needle and the collector are constant (OC) and the flow rate is changed*

- For flow rate values between 1.8-2.6 ml/h there is no big difference observed in the electrospinning process and the fiber produced. Over 2.6 ml/h no consistency of fiber diameter and drop formation is observed.

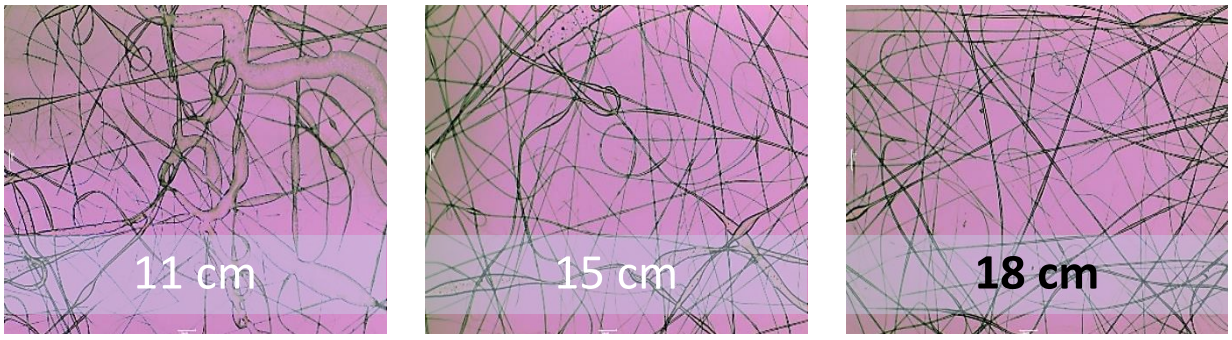

Fig. 3.2.1.3. *Optimization of the electrospinning conditions of PLA with OM analysis. The voltage and the flow rate are constant (OC) and the distance between the needle and the collector is changed*

- It is easily observed in the figure 3.2.1.3 than the distance between the needle and the collector affected the produced fiber and especially between 11-15cm the fiber diameter is much bigger and not consistent.

3.2.1.2. PLA/ HPMC matrix

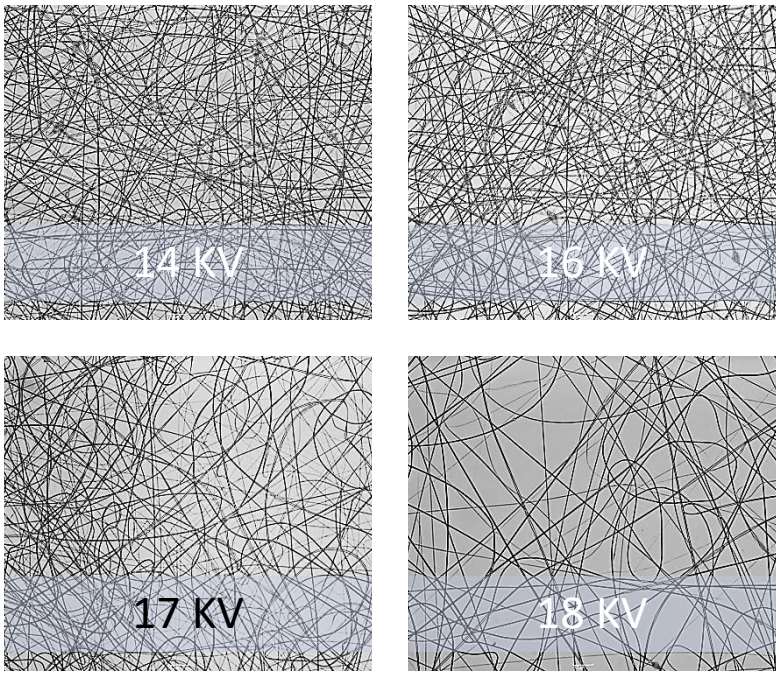

Fig. 3.2.1.4. *Optimization of the electrospinning conditions of PLA/HPMC with OM analysis. The flow rate and the distance between the needle and the collector are constant (OC) and the voltage is changed.*

- The voltage didn't have a significant effect on the PLA/HPMC solution between 14-18 kV but for V<14 kV dripping was observed and for V>19 kV there was no Taylor-cone formation.

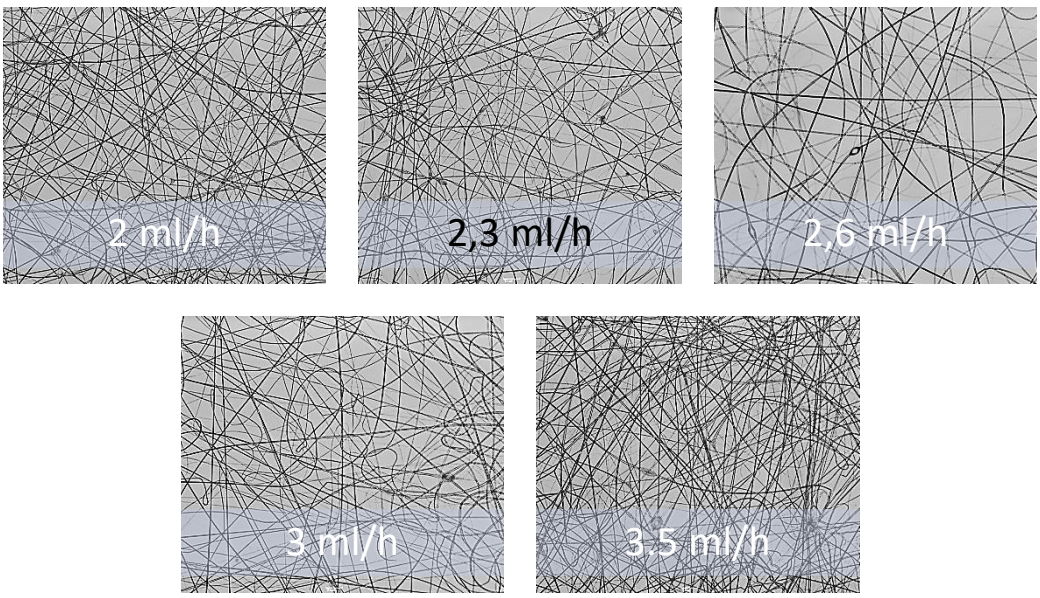

Fig. 3.2.1.5. *Optimization of the electrospinning conditions of PLA/HPMC with OM analysis. The voltage and the distance between the needle and the collector are constant (OC) and the flow rate is changed*

- The flow rate also could be adjusted at any value between 2-3 ml/h with no significant effect on the fiber. Over 3 ml/h, accumulation of the solution at the tip of the needle was observed.

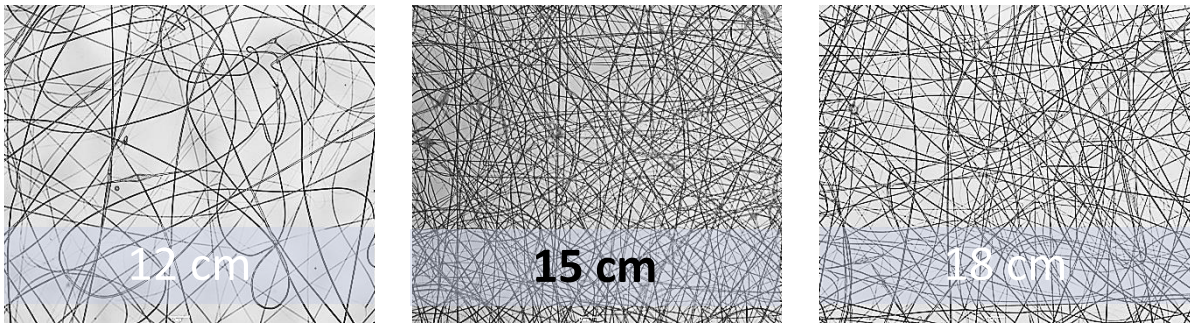

Fig. 3.2.1.6. *Optimization of the electrospinning conditions of PLA/HPMC with OM analysis. The voltage and the flow rate are constant (OC) and the distance between the needle and the collector is changed.*

The solutions for elecrospinning can't be prepared many days in advance because needles will be produced instead of fibers, as shown in ***Figure 3.2.1.7***.

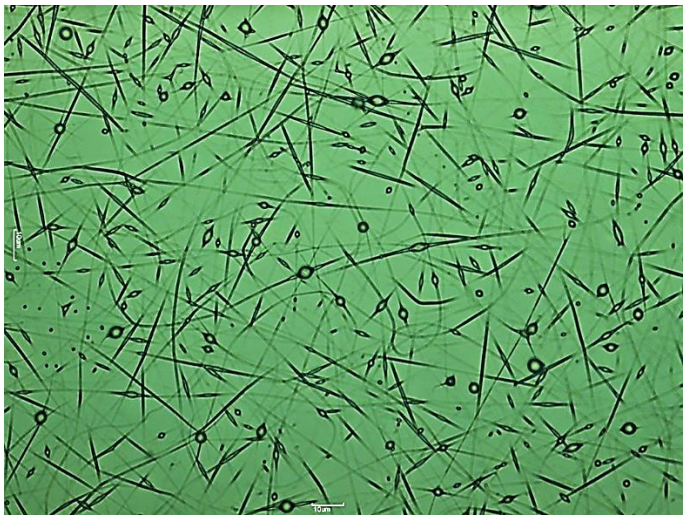

Fig. 3.2.1.7. *PLA/ HPMC electrospun solution after 5 days of its preparation*

3.2.1.3. PLA/HPMC/Curcumin matrix

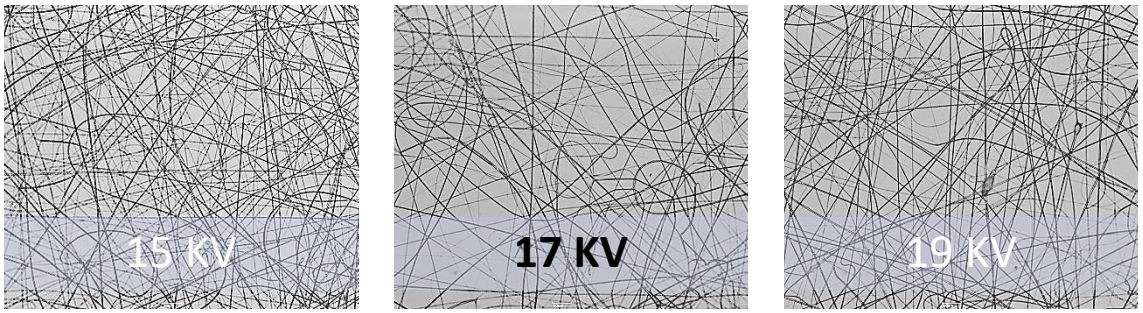

Fig. 3.2.1.8. *Optimization of the electrospinning conditions of PLA/HPMC/Curcumin with OM analysis. The flow rate and the distance between the needle and the collector are constant (OC) and the voltage is changed.*

- The voltage for electrospinning the PLA/HPMC/Curcumin solution had to be over 14 kV or there was dripping. For V > 18 kV the formation of beads is observed.

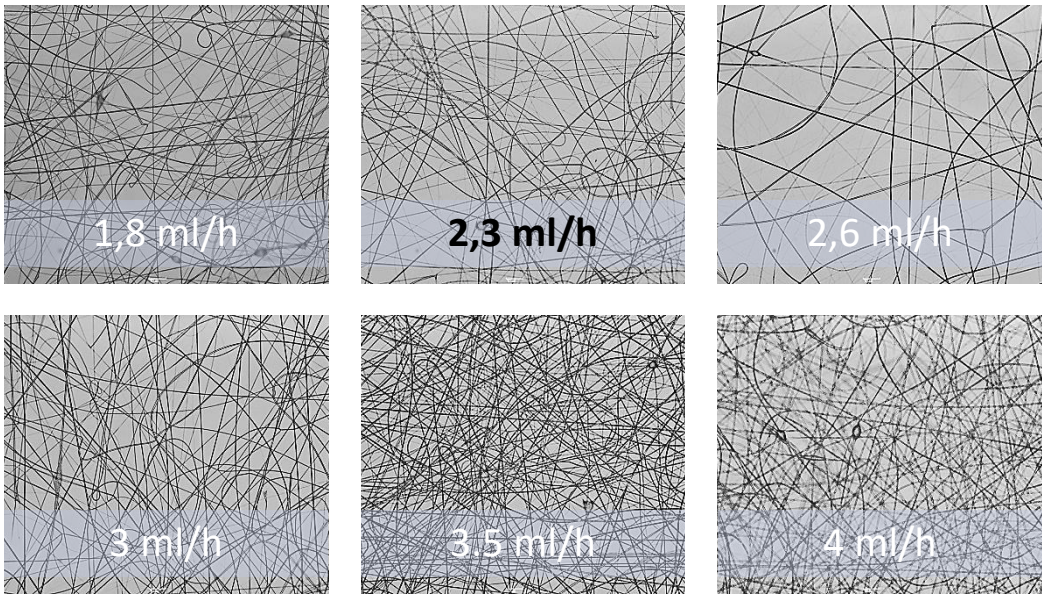

Fig. 3.2.1.9. *Optimization of the electrospinning conditions of PLA/HPMC with OM analysis. The voltage and the distance between the needle and the collector are constant (OC) and the flow rate is changed.*

- Between 1.8-2,6 ml/h flow rate a few beads are observed. For R > 2.6 ml/h there was accumulation of the solution at the needle tip.

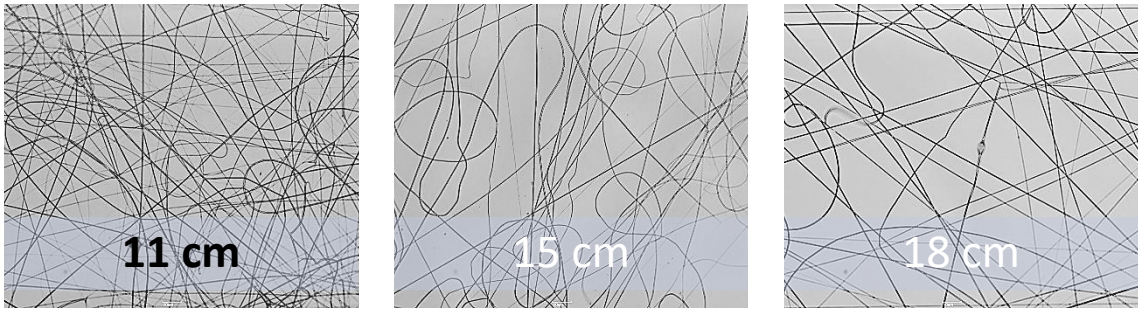

Fig. 3.2.1.10. *Optimization of the electrospinning conditions of PLA/HPMC with OM analysis. The voltage and the flow rate are constant (OC) and the distance between the needle and the collector is changed*

- There were no significant problems with the electrospinning process by altering the needle-collector distance from 11-18 cm but the Taylor cone formation was better at 11cm in long term.

3.3. Fiber morphology

FIB was used to study the morphology of electrospun fibers. This way we could obtain highly magnified pictures of the fibers, allowing the observation of features such as roughness, porosity, and the presence of breaks. The figures below show comprehensive FIB pictures of the PLA, PLA/HPMC and PLA/HPMC/CURCUMIN matrices in different magnifications. It could be observed that the PLA mats have a rough surface with many pores. The PLA/HPMC mats show a less rough surface with some pores and PLA/HPMC/curcumin mats also have pores but fewer and the surface is smoother.

3.3.1. PLA matrix

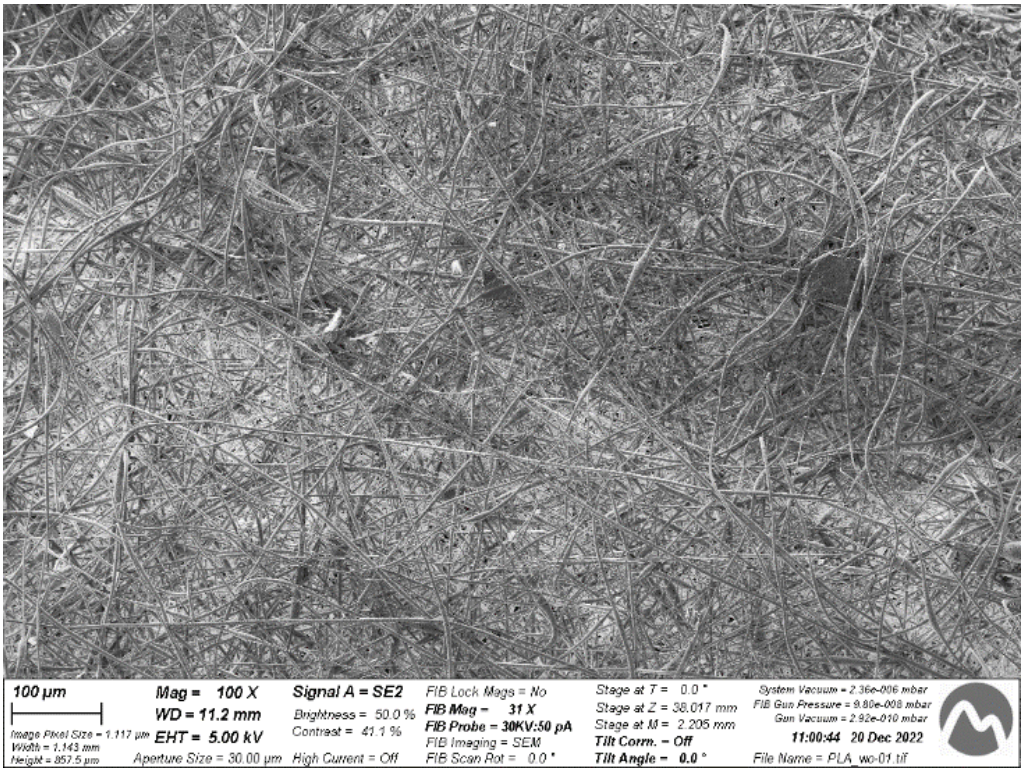

Fig. 3.3.1.1 *FIB picture of the PLA matrix in 100X magnification*

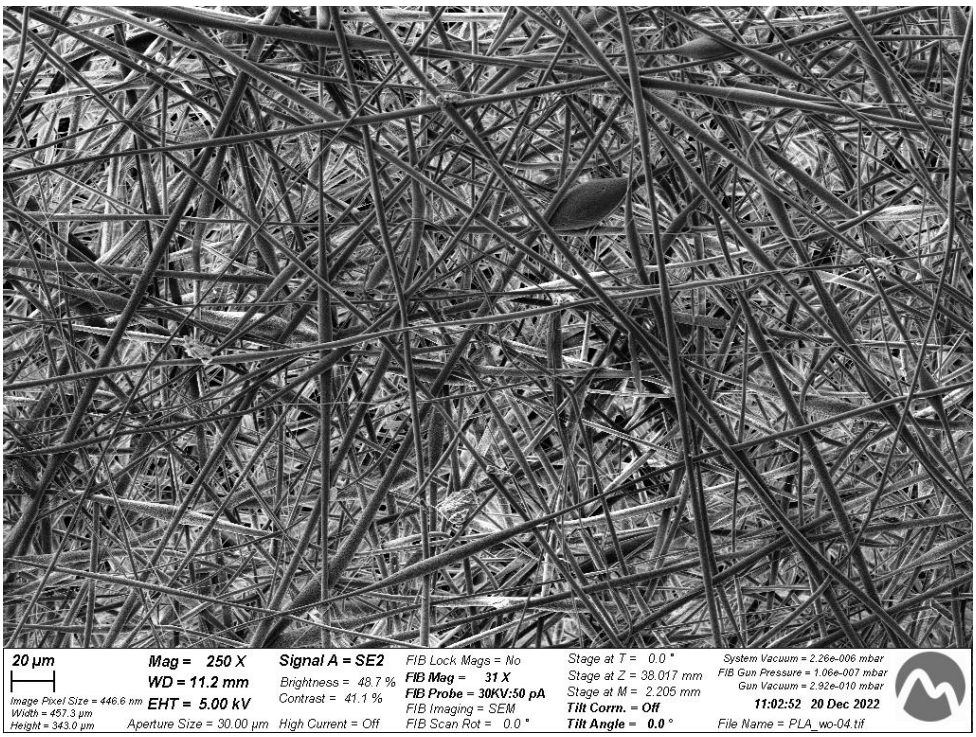

Fig. 3.3.1.2 *FIB picture of the PLA matrix in 250X magnification*

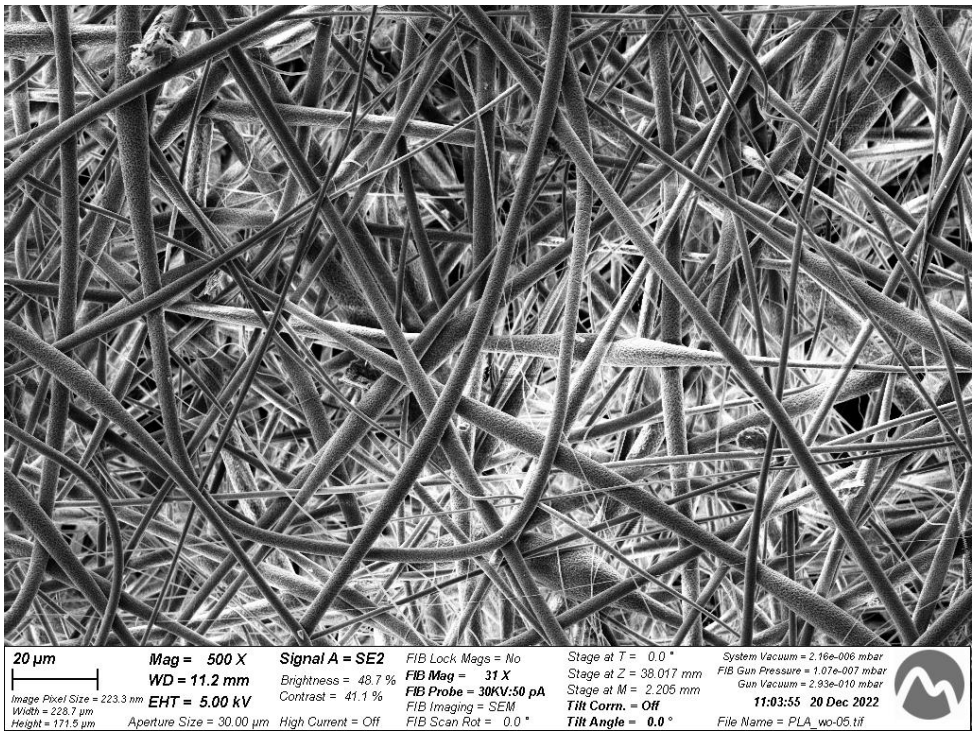

Fig. 3.3.1.3. *FIB picture of the PLA matrix in 500X magnification*

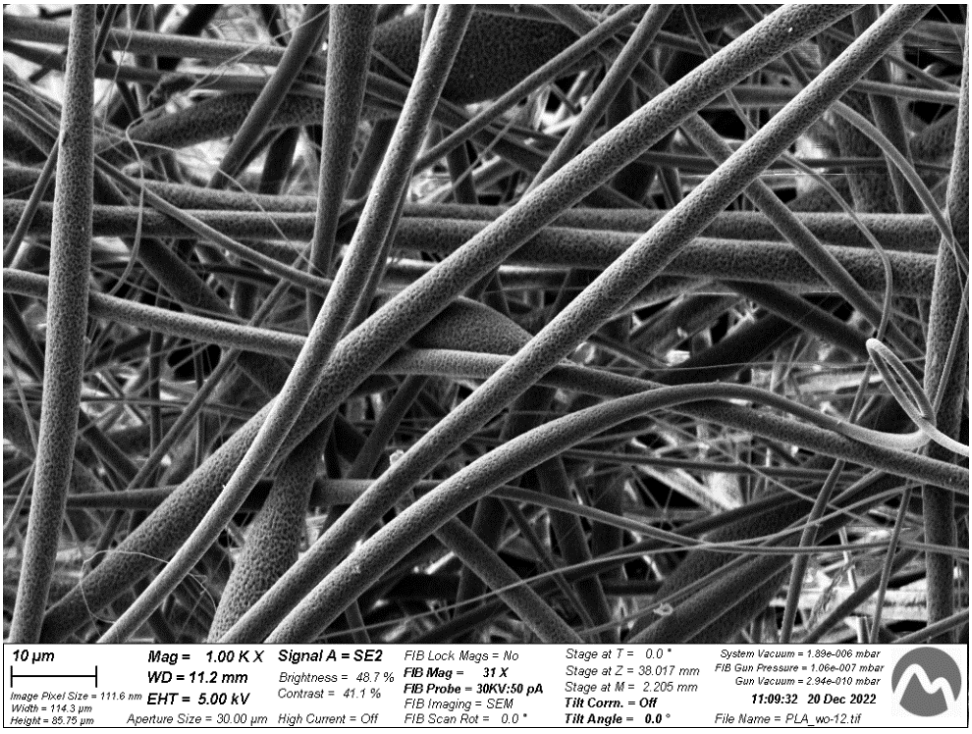

Fig. 3.3.1.4 *FIB picture of the PLA matrix in 1000X magnification*

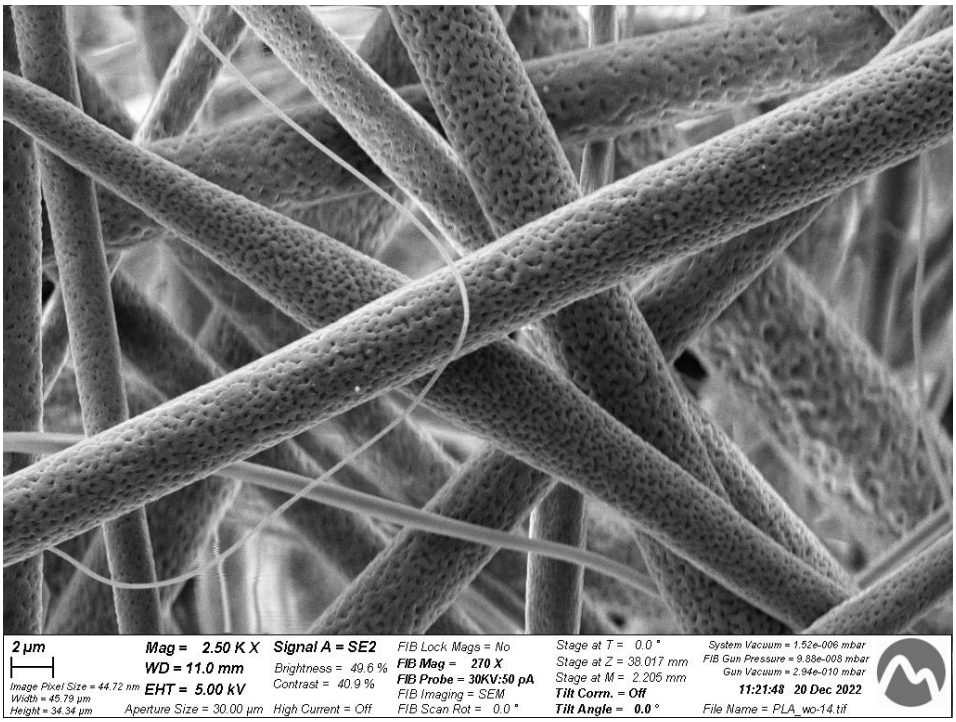

***Fig. 3.3.1.5**. FIB picture of the PLA matrix in 2500X magnification*

3.3.2. PLA/HPMC matrix

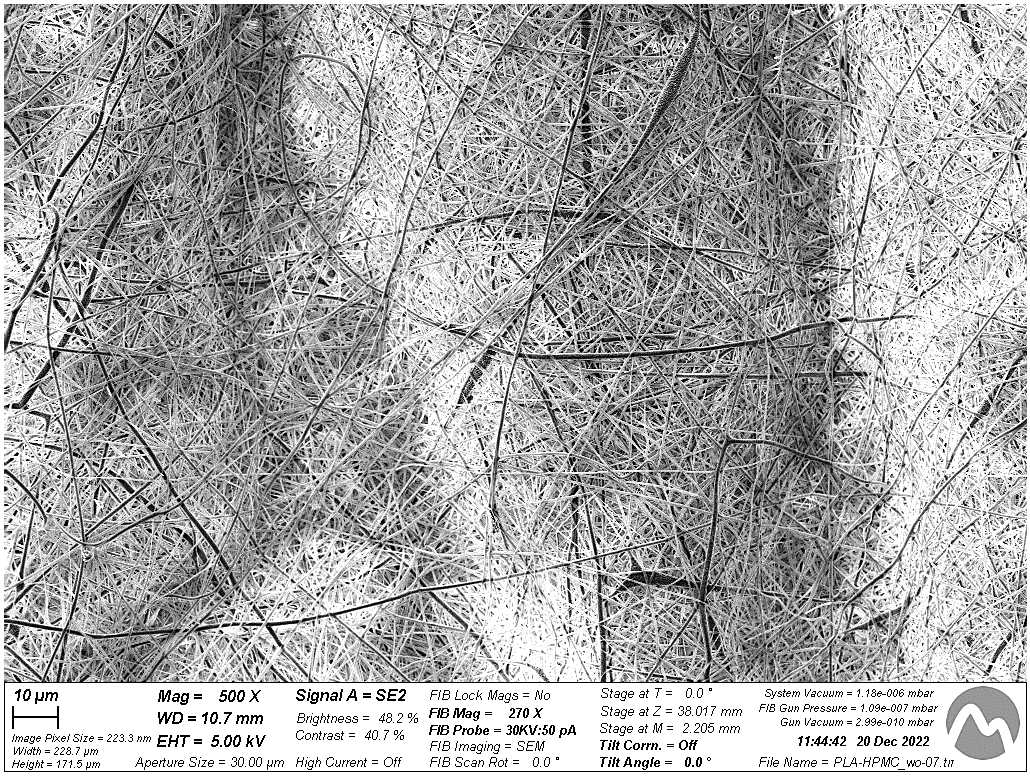

Fig. 3.3.2.1 *FIB picture of the PLA/HPMC matrix in 500X magnification*

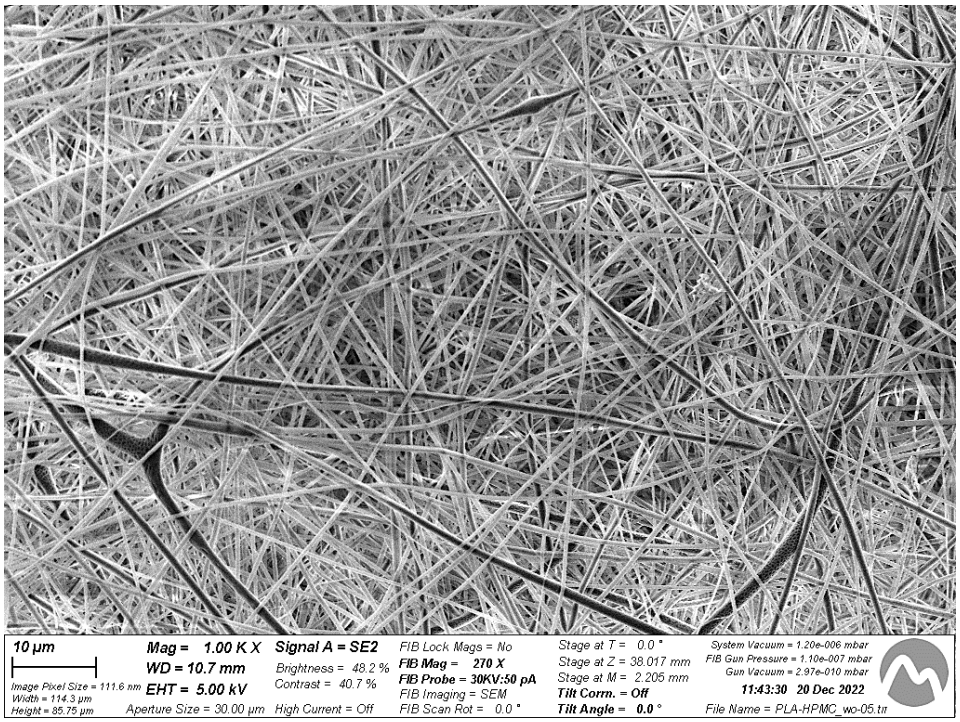

Fig. 3.3.2.2. *FIB picture of the PLA/HPMC matrix in 1,000X magnification*

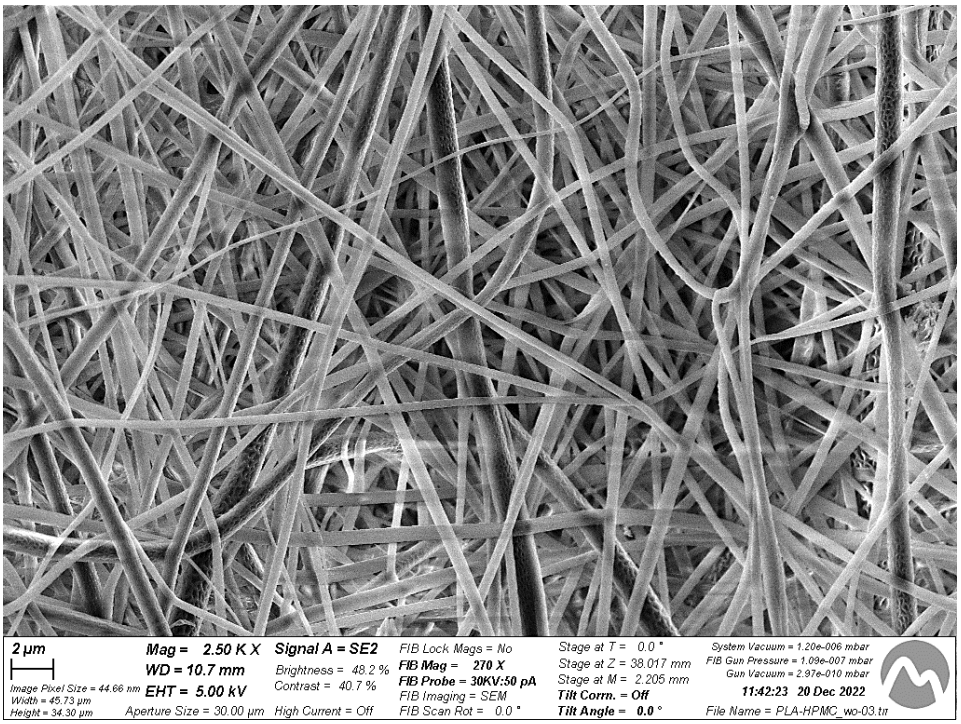

Fig. 3.3.2.3. *FIB picture of the PLA/HPMC matrix in 2,500X magnification*

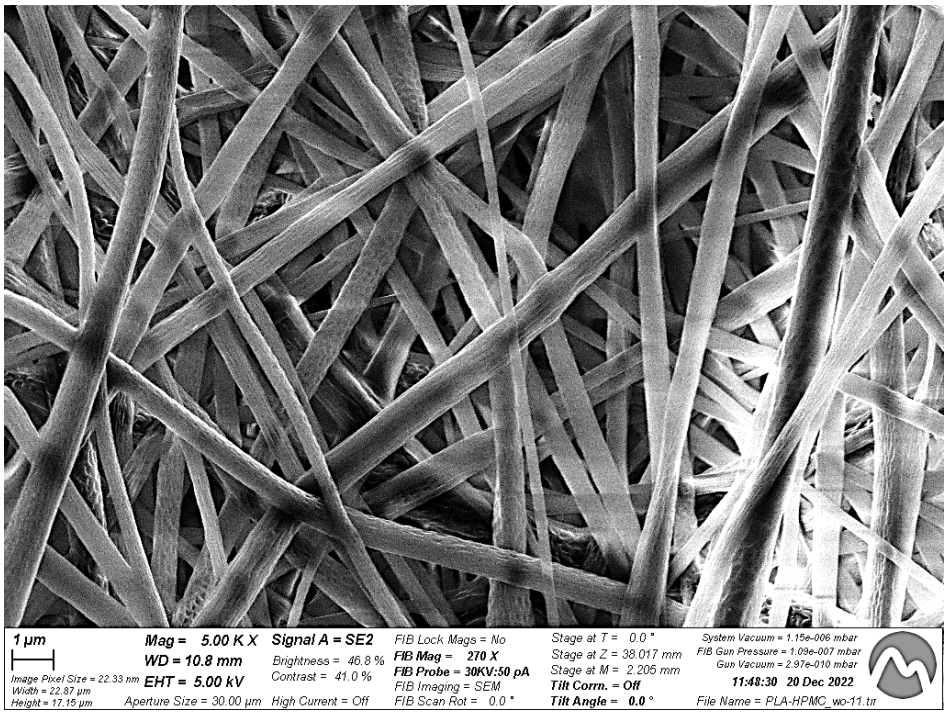

Fig. 3.3.2.4. *FIB picture of the PLA/HPMC matrix in 5,000X magnification*

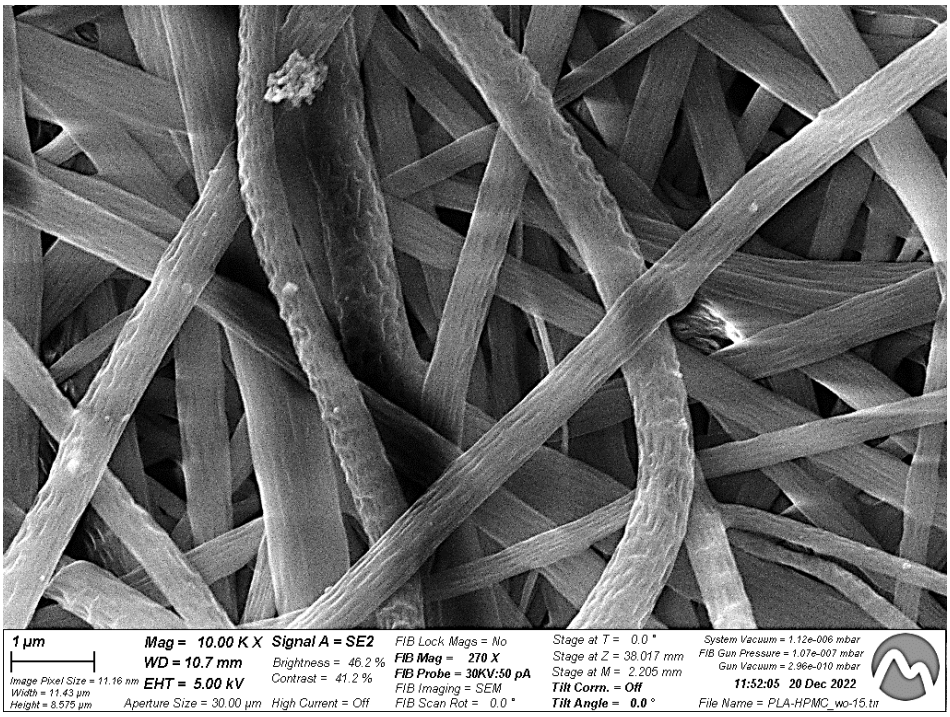

Fig. 3.3.2.5. *FIB picture of the PLA/HPMC matrix in 10,000X magnification*

3.3.3. PLA/HPMC/Curcumin matrix

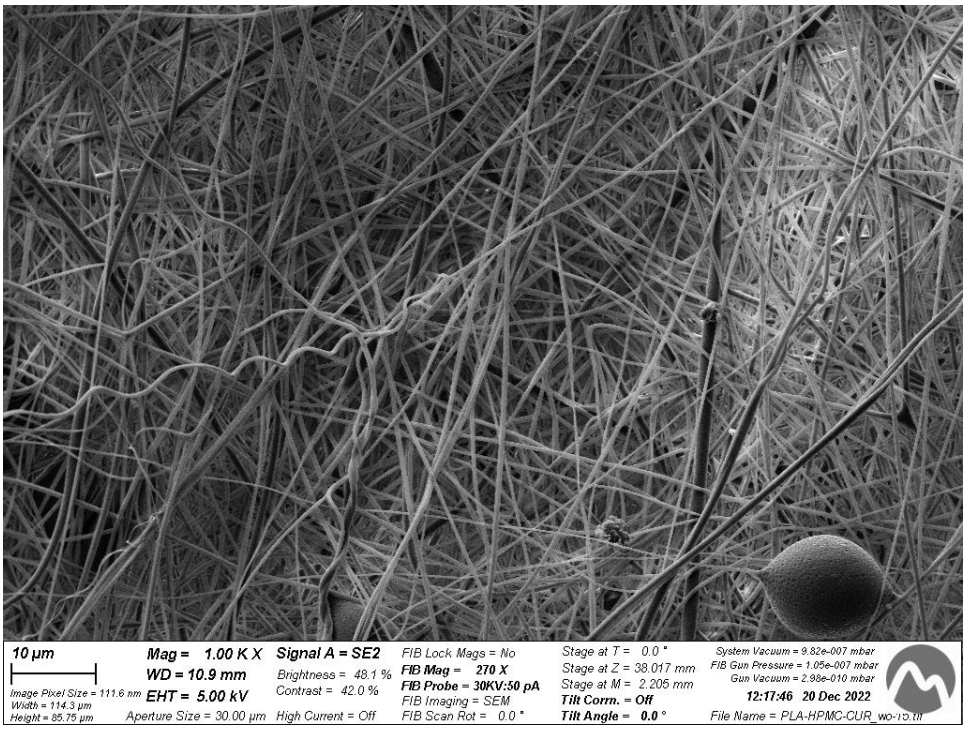

Fig. 3.3.3.1. *FIB picture of the PLA/HPMC/Curcumin matrix in 1,000X magnification*

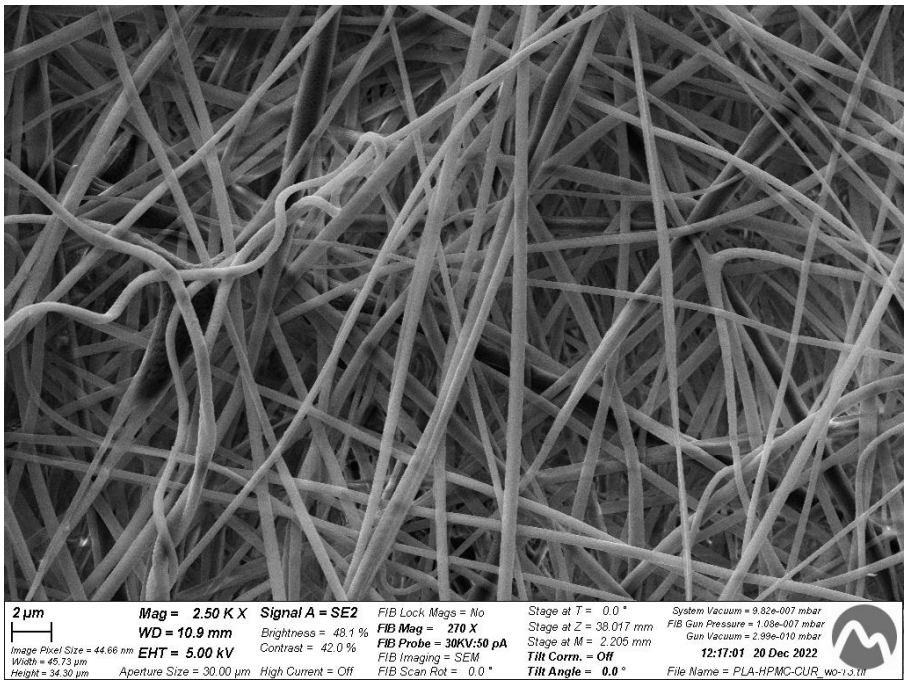

Fig. 3.3.3.2. *FIB picture of the PLA/HPMC/Curcumin matrix in 2,500X magnification*

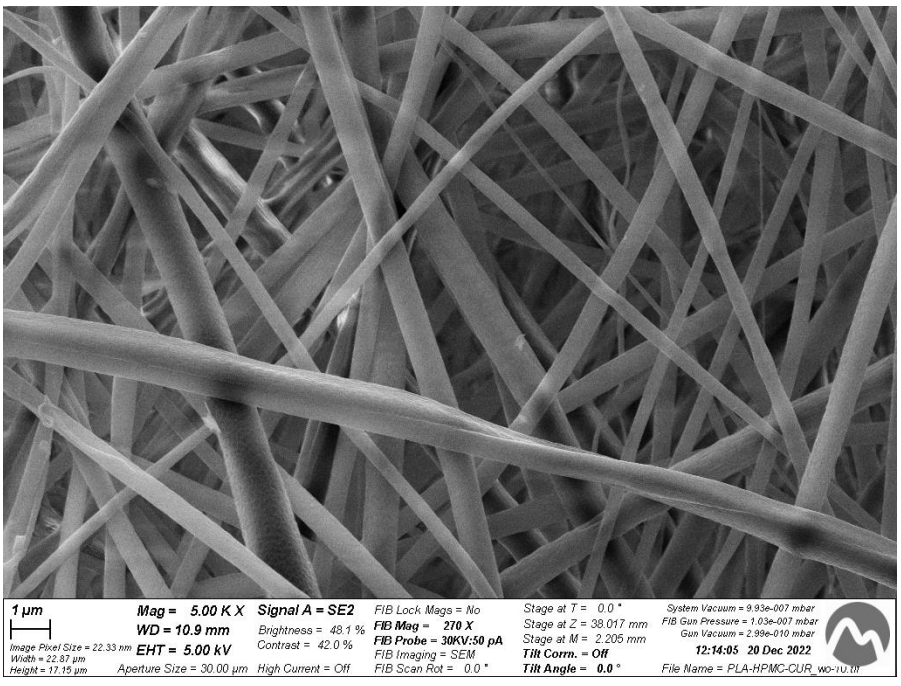

Fig. 3.3.3.3. *FIB picture of the PLA/HPMC/Curcumin matrix in 5,000X magnification*

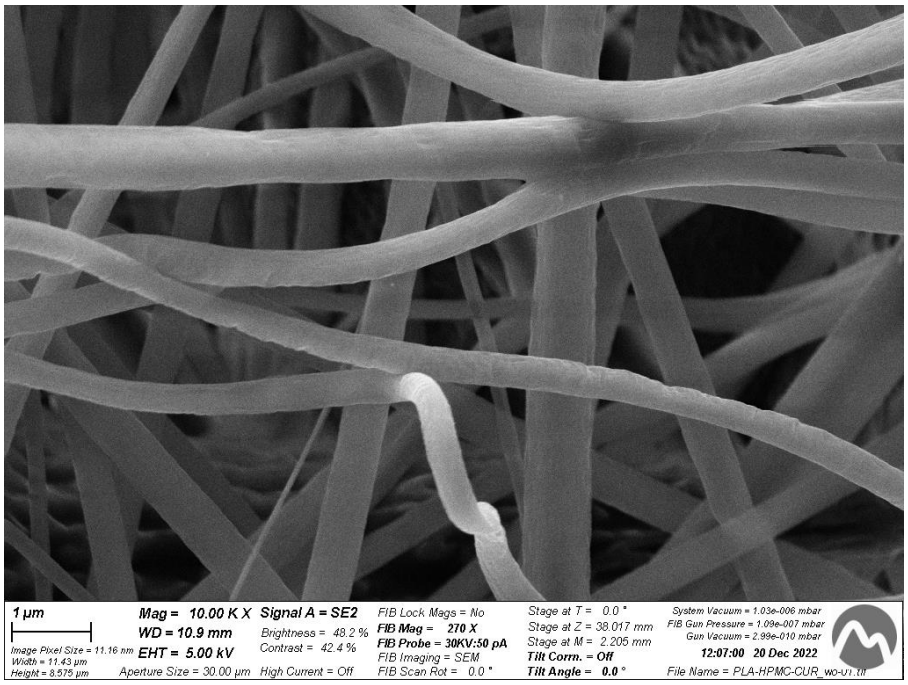

Fig. 3.3.3.4. *FIB picture of the PLA/HPMC/Curcumin matrix in 10,000X magnification*

3.4. Fiber diameter

FIB images were used to determine the fibers' diameter distribution in order to express the homogeneity of the fibers in the scaffold for each compound. For that purpose, diameter measurements were repeated many times for each sample. Around 100-120 measurements were taken from different fibers captured in 7-10 photos of different zones of the mat and the average value of the fibers' diameter is presented in ***Table 3.1***. In this research the program *Image J* was used for the diameter measurement. The obtained data was then treated with the Excel program.

Matrix	Average diameter (µm)	Average diameter (µm)
	no water	**after water**
PLA	3.302 ±1.146	3.417 ±1.169
PLA/HPMC	0.440 ±0.188	0.547 ±0.181
PLA/HPMC/Curcumin	0.574 ±0.202	0.591 ±0.218

Table 3.4.1*. The average value of the fiber diameter of each matrix, before and after submerged in water overnight.*

The difference between PLA fiber diameter and PLA/HPMC, PLA/HPMC/Curcumin diameters is mainly because of the use of different solvents.

3.4.1. PLA matrix

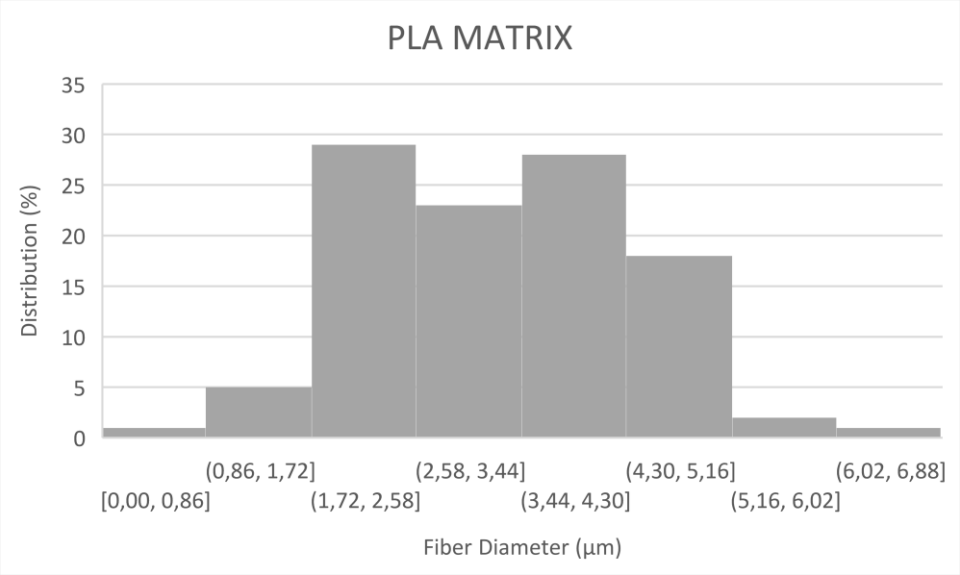

Fig. 3.4.1.6. *Graph of the fiber diameter distribution of the PLA matrix*

3.4.2. PLA/HPMC matrix

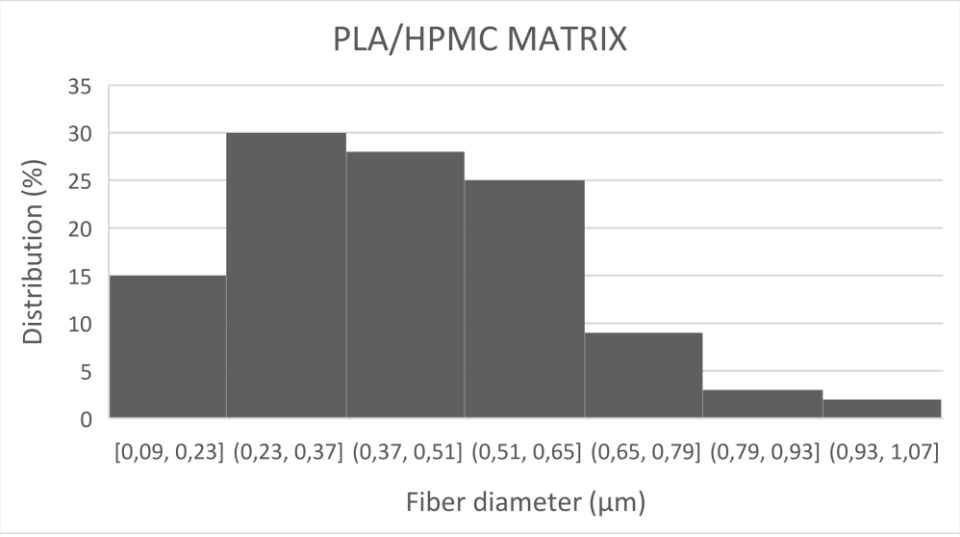

Fig. 3.4.2.6. *Graph of the fiber diameter distribution of the PLA/HPMC matrix*

3.4.3. PLA/HPMC/Curcumin matrix

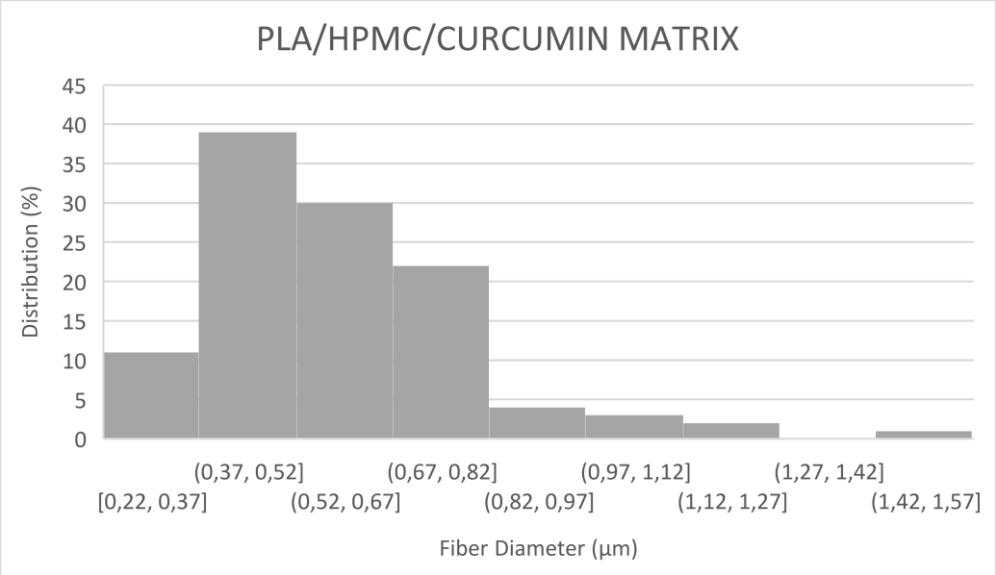

Fig. 3.4.3.5. *Graph of the fiber diameter distribution of the PLA/HPMC/Curcumin matrix*

A unimodal distribution can be observed in all the produced mats, which leads us to believe that the samples were created under optimal conditions.

3.5. Drug release

As described in paragraph 2.5, the results from the spectrophotometry of the samples with known concentrations of curcumin were used to create the standard curves in order to calculate curcumin's concentration during its release in PBS and PBS/Ethanol. The results can be observed in the diagrams below.

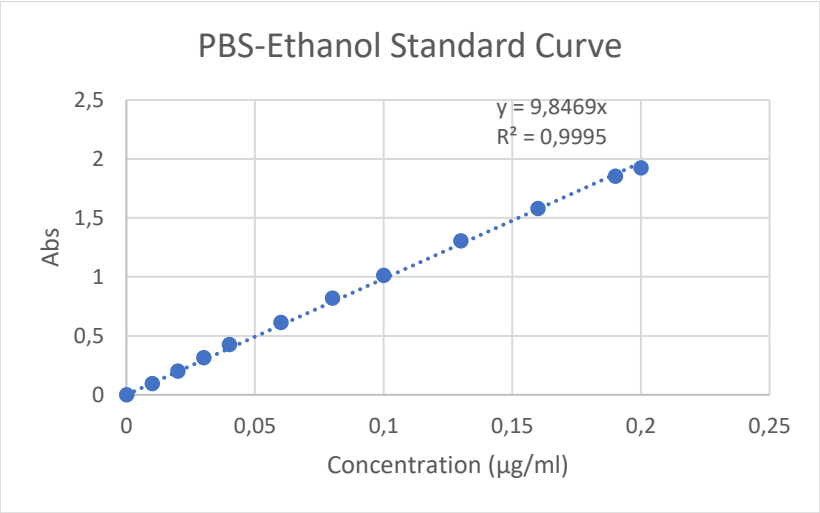

Fig. 3.5.1. *Diagram of PBS-Ethanol standard curve*

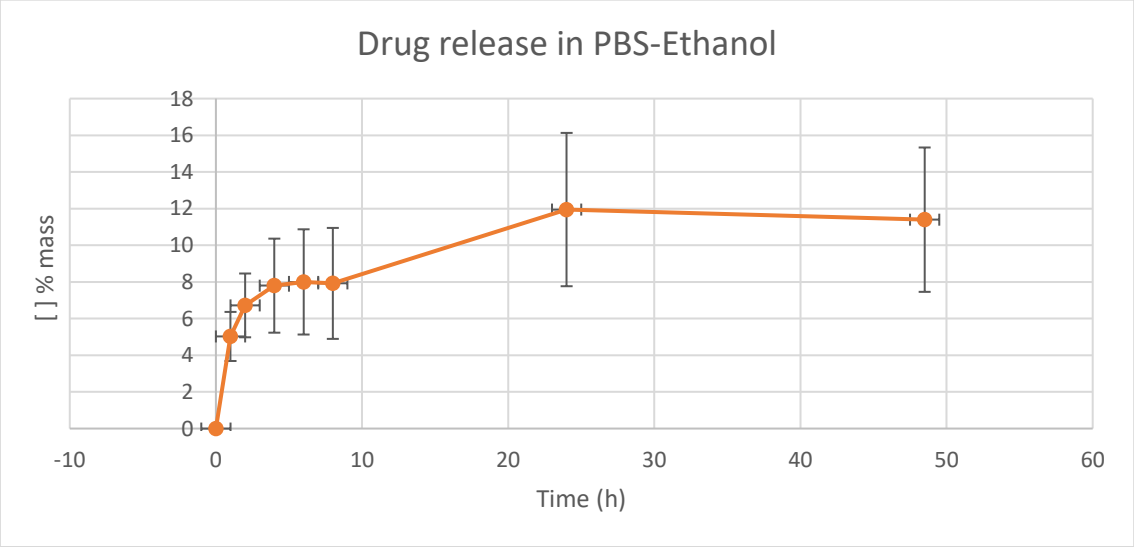

Fig. 3.5.2. *Diagram of the curcumin's release through time in PBS-Ethanol*

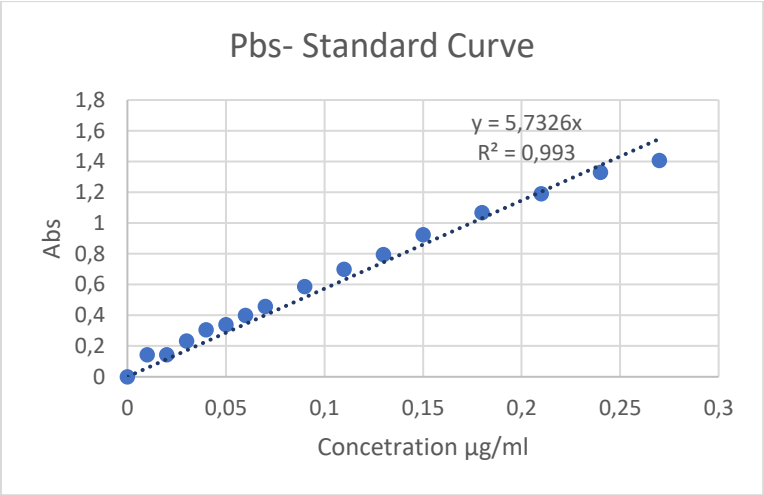

Fig. 3.5.3. *Diagram of PBS standard curve*

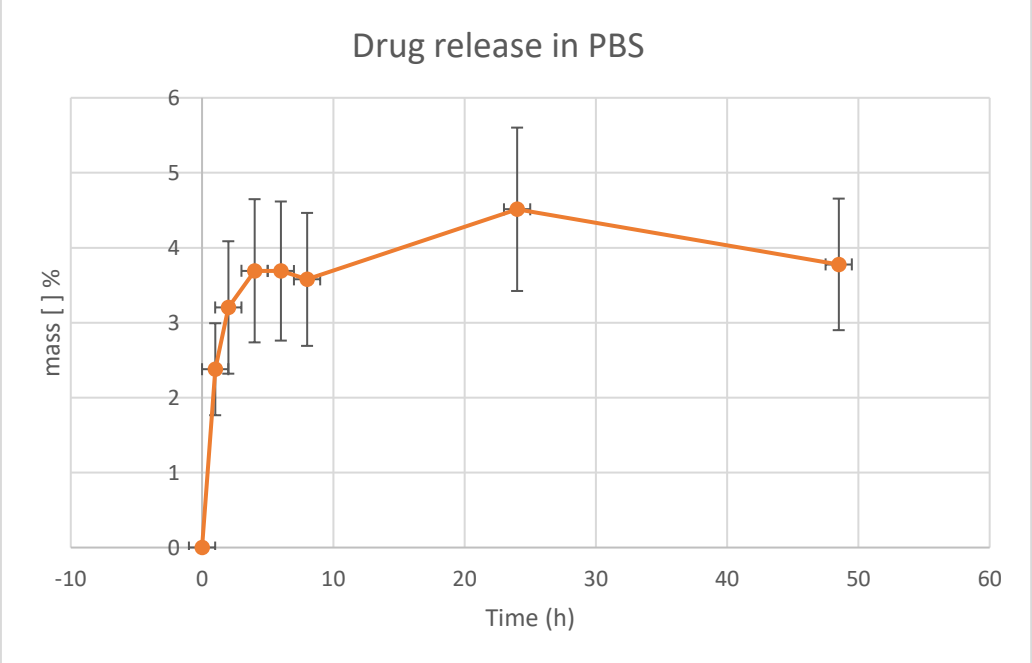

Fig. 3.5.4. *Diagram of the curcumin's release through time in PBS*

It is very noticeable that the drug release was a lot higher in PBS-Ethanol than in PBS alone. The reason for that is the increase in the polarity of the solution which affects the curcumin's release because of its hydrophobicity. PBS can't simulate exactly human body fluids; the solutions' osmolarity and ion concentrations are comparable to those of the human body but not its

polarity. So, with ethanol added, the results are more comparable to the release in the human body.

Conclusions

1. The operational parameters (Voltage, Flow rate, Needle-Collector distance) of the electrospinning process vary depending on the polymer solution (viscosity, concentration, Mw, etc). The elecrospinnable solution must also be carefully adjusted to achieve the optimal quality for the electrospinning process.

2. The use of different solvents in PLA solution and the PLA/HPMC, PLA/HPMC/Curcumin solutions concluded to a significant difference in the fibre diameter.

3. The PLA scaffold shows a typical rough fiber surface. On the contrary, the addition of HPMC and Curcumin to the PLA solution leads to a smoother fiber surface.

4. The submerse in water of the 3 scaffolds (PLA, PLA/HPMC/PLA/HPMC/Curcumin) did not reduce or affect the fibre diameter. This must be due to the fact that the two polymers were well blended and did not separate in water even though PLA is hydrophobic and HPMC is hydrophilic.

5. The drug release of curcumin is greater in PBS-Ethanol (9:1 v/v) than in PBS alone. This is because of the increase in the solution's polarity and because of curcumin's hydrophobicity. The PBS-Ethanol solution simulates the human body fluids better in this aspect.

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