Kaunas University of Technology

Faculty of Mechanical Engineering and Design

The Formation and Analysis of Electrospun Materials from Nano-Microfibers with Hemp Extract

Master's Final Degree Project

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Kaunas, 2020

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Textile Engineering and Finishing (6211FX007)

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Declaration of Academic Integrity

I confirm that the final project of mine, Sandra Goroškaitė, on the topic „The Formation and Analysis of Electrospun Materials from Nano-Microfibers with Hemp Extract" is written completely by myself; all the provided data and research results are correct and have been obtained honestly. None of the parts of this thesis have been plagiarised from any printed, Internet-based or otherwise recorded sources. All direct and indirect quotations from external resources are indicated in the list of references. No monetary funds (unless required by Law) have been paid to anyone for any contribution to this project.

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Kaunas University of Technology

Faculty of Mechanical Engineering and Design

Task of the Master's final degree project

Study Programme: 6211FX007 T*extile Engineering and Finishing*

Given to the student – Sandra Goroškaitė

1. Title of the project –

The Formation and Analysis of Electrospun Materials from Nano-Microfibers with Hemp Extract

(In English)

Elektrinio verpimo būdu suformuotų neaustinių medžiagų iš nano-mikrogijų su kanapių ekstraktu formavimas ir analizė

(In Lithuanian)

2. Aim and tasks of the project –

Aim of the project- to electrospun nonwoven materials from Eudragit S-100 (ES), Eudragit E-100 (EE) and polyvinylpyrrolidone (PVP) polymers with hemp extract and investigate its properties.

Tasks of the project:

1) To form solutions from biomedical polymers with hemp extract and investigate its properties;
2) To electrospin nonwoven materials from nano-microfibers;
3) To investigate the influence of biomedical polymer solution composition on a structure of electrospun solution;
4) To investigate the release kinetics of hemp components from electrospun solution;
5) To analyze chemical composition of electrospun nonwoven materials.

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Summary

Electrospinning is one of the used techniques in textile engineering industry, that forms nonwoven mat from nano-microfibers with different kinds of properties that depends on the type of selected polymers, which is a big variety to choose. It is possible to successfully and efficiently incorporate drugs and bioactive materials into nano-microfibers formed by electrospinning technique, their release and delivery can be controlled and targeted straight into the inflammation place. Mixtures of synthetic polymers ensures stability of nano-microfibers together with mechanical properties as well as maintains biomedical properties, avoids too rapid desorbtion or degradation of the molecules. In this Master's Final Degree Project pharmaceutical Poly (methyl methacrylate) (PMMA) derivatives Eudragit S-100 (ES), Eudragit E-100 (EE) and polyvinylpyrrolidone (PVP) polymers that offer antibacterial, good mechanical properties as well as mixtures in 50/50 and 75/25 concentration dissolved in ethanol were chosen to form nano-microfibers by electrospinning technique. Furthermore, nonpsychotropic hemp extract was incorporated into the structure of nano-microfibers that has a scientifically proven great potential in a medical field and comparison of formed structures have been done during the investigation. *Cannabis Sativa L.* (Cannabigerol) is composed from more than 120 identified cannabinoid compounds, from which the most in quantity are nonpsychotropic cannabidiol (CBD) and cannabigerol (CBG) and that has anti-inflammatory and anti-bacterial properties. As hemp extract for electrospinning technique have been not investigated yet, this Master's Final Degree Project task was to form solutions from biomedical polymers with hemp extract and investigate its properties, electrospin nonwoven materials from PVP, ES, EE with incorporated hemp extract, investigate the influence of biomedical polymer solution composition on a structure of electrospun solution with Scanning Electron Microscopy (SEM), investigate the release kinetics of hemp components from electrospun solution and analyze the chemical composition of electrospun nonwoven materials. Results demonstrated no significant changes on viscosity of solution after adding hemp extract, however electrical conductivity and Ultraviolet-Visible Spectroscopy (UV-VIS) test results demonstrated values that confirm the presence of hemp extract in a polymeric solution and electrospun nonwoven respectively, also release of hemp extract was determined from one-component nonwoven. The obtained results can testify that hemp extract can be incorporated into the biomedical polymer solutions and electrospun into a nano-microfiber nonwoven that can be used for biomedical purposes.

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Kaunas, 2020. 70 psl.

Santrauka

Elektrinis verpimas yra viena iš neaustinės medžiagos formavimo technologijų tekstilės inžinerijos srityje, kurios metu suformuotos nano-mikrogijos pasižymi įvairiomis savybėmis, priklausomai nuo pasirinktų polimerų, kurių yra plati įvairovė. Į elektrinio verpimo būdu suformuotas nano-mikrogijas galima sėkmingai ir efektyviai inkorporuoti vaistines medžiagas, kurių atpalaidavimas gali būti kontroliuojamas ir nukreiptas tiesiai į uždegimo židinį. Mišiniai, sudaryti iš sintetinių polimerų, užtikrina nano-mikrogijų stabilumą, mechanines savybes, norimų biomedicininių savybių išlaikymą, taip pat išvengiama pernelyg greita molekulių desorbcija ar degradacija. Šiame magistriniame darbe nano-mikrogijų formavimui pasirinkti ir tarpusavyje tyrimų metu palyginti šie biomediciniai polimerai: polivinilpirolidonas (PVP) ir polimetilmetakrilato (PMMA) išvestiniai dariniai Eudragit S-100 (ES) ir Eudragit E-100 (EE) bei jų mišiniai 50/50 ir 75/25 koncentracijose, kurie pasižymi antibakterinėmis bei geromis mechaninėmis savybėmis. Visi išvardinti bandiniai buvo tirpinti etanolyje, Be to, psichotropinių medžiagų neturintis kanapių pluošto ekstraktas, kurio pritaikomumas medicinos srityje yra įrodytas moksliškai, buvo integruotas į biomedicininius polimerus ir buvo atliktas suformuotų tirpalų, tirpintų etanolyje bei kanapių ekstrakte, taip pat ir neaustinių medžiagų palyginimas. Tyrimams pasirinkta kanapė *Cannabis Sativa L.* (*angl. Cannabigerol*) yra sudaryta iš daugiau nei 120 identifikuotų kanabinoidinių junginių, iš kurių daugiausia yra kanabidiolio (CBD) ir kanabigerolio (CBG) pasižyminčių priešuždegiminėmis bei antibakterinėmis savybėmis. Kadangi kol kas dar nėra atlikta tyrimų naudojant kanapių ekstraktą elektrinio verpimo technologijai, šio magistrinio darbo tikslas buvo įterpti kanapių ekstraktą į biomedicininių polimerų tirpalus, įvertinti jų savybes, elektrinio verpimo būdu suformuoti nano-mikrogijų neaustinę medžiagą, įvertinti biomedicininio polimero ir tirpiklio įtaką suformuotai neaustinei nano-mikrogijų struktūrai naudojant skenuojantį elektroninį mikroskopą (SEM), ištirti kanapių ekstrakto atpalaidavimą iš neaustinės medžiagos bei išanalizuoti cheminę neaustinės medžiagos, suformuotos elektrinio verpimo technologija, struktūrą. Tyrimo metu buvo nustatyta, kad kanapių ekstraktas neturi ženklios įtakos polimerinio tirpalo klampai, tačiau tirpalo elektrinio laidumo ir neaustinės medžiagos ultravioletinių-regimosios šviesos spektroskopijos (UV-RŠ) testų rezultatai patvirtino kanapių ekstraktą esant sudėtyje, taip pat ir kinetinio atpalaidavimo tyrimo metu, CBD ir CBG atsipalaidavo iš vienkomponenčių neaustinių medžiagų. Gauti rezultatai patvirtina, kad kanapių ekstraktas gali būti integruotas į biomedicininių polimerų tirpalus ir neaustinė medžiaga, suformuota elektrinio verpimo būdu, gali būti pritaikoma biomedicinos tikslams.

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Introduction

Electrospinning is known as a technique offering high efficiency for fabrication of polymer nano-microfibers. Many of synthetic (polyurethane (PU), PVP, poly (methyl methacrylate) (PMMA), poly (ethylene oxide) (PEO) and etc.) and natural (chitosan, gelatin, collagen and etc.) polymers have already been successfully electrospun into ultrafine micro- to nano-meter range diameter fibers in recent years mostly from the solvent solution and some from the melt form [1].

Electrospun textile compared to commercial textiles materials have larger specific surface area and smaller pore size. In addition, electrospun fibers are possible to make into three-dimensional structures as they have a sizable static charge during their deposition. This allows them to become an excellent candidate for use in filtration, membrane applications, scaffolds for tissue engineering, drug delivery system, wound dressings and etc. [1].

PVP also known as povidone, is a water and other polar solvent soluble, biodegradable and biocompatible polymer. PVP is used as a binder in many pharmaceutical tablets, that is why it is usually mixed with other polymers to possess better mechanical stability [2]. PMMA derivatives such as Eudragits are largely used for drug encapsulation and in controlled oral drug delivery. Eudragits have different sensitivity to pH. In this project ES was chosen, which is soluble in pH above 7. While EE is soluble in gastric fluid and weakly acidic solutions up to pH 5 and was originally used for coating of orally administered tablets, which later degrade in stomach by normal gastric acids [3, 4, 5]. *Cannabis sativa L.* possess biological activity because of its chemical structure. The main nonpsychotropic phytocannabinoid that are present in *Cannabis sativa L.* plant are Cannabigerol (CBG) and Cannabidiol (CBD) [6].

Novel studies show that CBG has a broad pharmacological profile and can be suggested for cancer treatment, shows anti-inflammatory and analgesic properties also can be suggested for treatment of skin conditions [6]. Nevertheless, there are no studies that shows CBG potential in electrospinning technique, so all the investigations and tests are important to analyze the possibility of applying *Cannabis sativa L.* in textile nonwoven forming technology.

Hemp extract was successfully incorporated and 12 different polymer solutions from PVP, ES, EE dissolved in ethanol as well as in hemp extract and in different concentrations were prepared. Later 2 of them did not mix, due to strong electrostatic interaction between anionic groups of ES polymer and cationic groups of PVP, 2 of them electrosprayed during the electrospinning process, so no analysis of structure was done and finally, 8 of them were successfully electrospun into a nano-microfiber nonwoven material by electrospinning technique and several investigation were done for analysis of the samples.

For the polymeric solutions electrical conductivity and viscosity tests were made, while the nonwovens from nano-microfibers were characterized by Scanning Electron Microscopy (SEM) to determine the influence of hemp extract on the morphology of nonwovens made, Differential Scanning Calorimetry (DSC) to see the if there is a change in glass transition temperature of material after adding hemp extract, UltraViolet-Visible Spectroscopy (UV-VIS) to measure the optical properties of nanofibers and lastly kinetic release of hemp extract from nonwoven material tests were made.

All the research was done to analyze and understand the possibility of incorporating hemp extract into the polymer solution and create a bioactive material that could be used for biomedical purposes.

The aim of the project- to electrospun nonwoven materials from Eudragit S-100 (ES), Eudragit E-100 (EE) and polyvinylpyrrolidone (PVP) polymers with hemp extract and investigate its properties.

Tasks of project:

1) To form solutions from biomedical polymers with hemp extract and investigate its properties;
2) To electrospin nonwoven materials from nano-microfibers;
3) To investigate the influence of biomedical polymer solution composition on a structure of electrospun solution;
4) To investigate the release kinetics of hemp components from electrospun solution;
5) To analyze chemical composition of electrospun nonwoven materials.

1. Literature analysis

1.1. Basics of electrospinning process

Electrospinning is a method of straightforward production of ultrafine fibers with micro- to nano-meter range diameter and with a possibility to control morphology of the electrospun surface of a nonwoven material. Electrospinning is a technique, already reported in 1897, that produces thin (nm to μm) polymer fibers by using electrostatic forces. Ordinary setup of electrospinning technique (with syringe type electrode) is presented in the figure 1.1.1. A high electric field is applied to a polymer solution or melt which is held together by its surface tension at the tip of a syringe. After applying high voltage, mostly between 1 and 30 kV, the pendant drop of polymer solution becomes highly electrified and the induced charges are evenly distributed over the surface. By using a syringe pump polymer solution is pumped through the needle with a particular feeding rate. Upon increasing the electrical field strength (increasing applied voltage), repulsive electrical forces prevail the surface tension forces of polymer drop and the drop become a cone with angle of 49,3˚ (i.e. Taylor cone, the equilibrium shape of a dripping solution at a critical voltage). Then a critical value of applied voltage is reached, a jet from Taylor cone is formed, resulting in the ejection of a charged jet of the solution [7, 8, 9] .

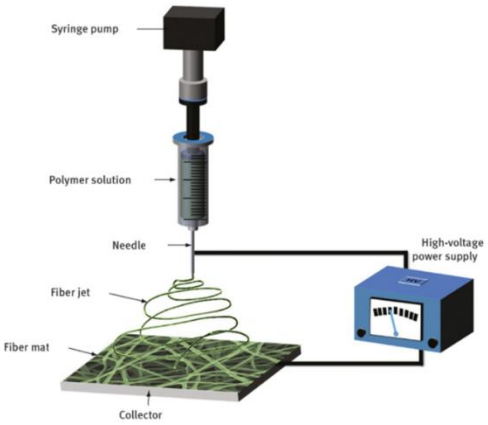


Figure 1.1.1. Set-up of electrospinning principal [9]

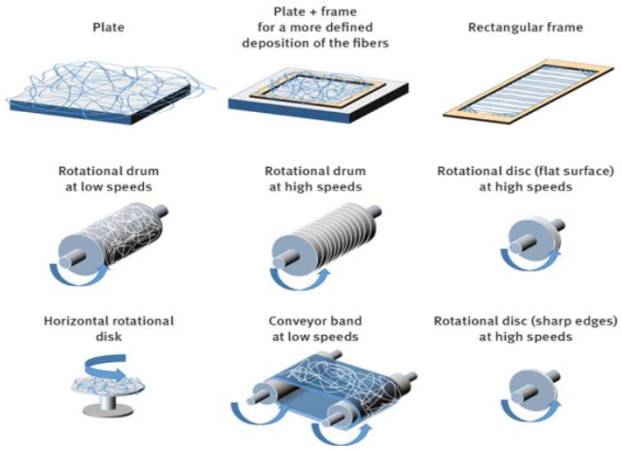


Figure 1.1.2. Schematic representation of different collector types for electrospinning [9]

Geometry (diameter undulations and branching processes) of fiber can be affected by the processes such as Rayleigh instabilities as well as the electrically driven axi-symmetric instabilities. The jet coming out of the tip in the beginning for a very short time flows in a straight path and then bending, winding, looping and curling of the jet occurs [9]. The jet is only stable at the tip, when electrostatic repulsive forces between charged elements of the polymer solution jet overcome the surface tension. This means that the jet will be lashing unstable and rapidly in the space between the tip and the collector (i.e. the electrodes). During this motion, the solvent is evaporated leaving only thin nonwoven material from nano – microfibers formed on the collector. Collectors can be stationary or rotating, generally, placed 10-25 cm away from the primary electrode, but this distance can vary, depending on the spinning situation (especially used applied voltage) and structure of electrospun nonwoven material should be formed. Examples of the different collector types are presented in figure

1.1.2. Stationary collectors can provide randomly oriented fibers, while aligned fibers can be collected on rotating collectors [8, 9,10].

The electrospinning technique has several important advantages:

- easily achieved production of very thin fibers with large surface area;
- produced fibers are easy to functionalize;
- controlled porosity of electrospun material;
- possibility to create a 3D structure.

However, the processes of electrospinning should be done in chambers which have a ventilation system, because some solvents of polymer may emit unpleasant or even harmful smells. Moreover, to generate the electrospinning, a voltage in the range of several to several tens of kV (to 30 kV then is used syringe type, to 80 kV rotating drum type electrospinning setup) is necessary. It is important to be careful and avoid touching any of the charged jet while manipulation [10]. Also, during electrospinning process it is very important to control environmental humidity and temperature.

Ideal targets for the process of electrospinning on the polymers that can be electrospun into nano - microfibers would be:

- the diameters of fibers must vary in narrow intervals of margins error;
- the fiber surface must be without defects or defect-controllable (without beads, spots of polymer solution);
- it must be possible to collect continuous single nano - microfibers [1].

Nano-microfibers can be obtained by several different methods, such as drawing out, molecular self-assembly, thermally induced phase separation, but electrospun nano-microfibers are most widely adopted because of diversity of the possible application areas. Electrospun nano-microfibers are flexible to spin into a variety of different shapes and sizes as well as to form a structure of controlled porosity for each specific application [11, 12]. Moreover, electrospinning technique allows to functionalize the nano-microfibers during preparation. It can be done by incorporating viruses, bacteria, enzymes, drugs, catalysts, metal nanoparticles, nanotubes and nanowires [9].

1.2. Electrospinnability of polymer solution

The electrospinning process is influenced by many parameters determining the structure of electrospun nonwoven material and diameter of the obtained fibers [13]. Process, systemic, solution and physical parameters are listed in table 1.2.1.

Depending on the particular application area, properties required for the material must be optimized. It might, for instance, be necessary that the polymer would be hydrophobic or hydrophilic, that it would possess a high stiffness and strength, would be biocompatible and biodegradable, or that it would display piezo- and pyro-electrical properties [14].

Deitzel with co-authors [1] used the poly (ethylene oxide) (PEO) polymer, which was dissolved in High Performance Liquid Chromatography (HPLC) grade water to obtain solutions with concentrations ranging from 4 to 10 wt%. From the experimental study it was found that the morphology of the produced nano-microfibers is strongly influenced by parameters such as feed rate

of the polymer solution, the electrospinning voltage, and the properties of solution such as concentration, viscosity and surface tension.

Table 1.2.1. List of parameters influencing electrospinning which determine the structure of electrospun nonwoven material and diameter of obtained fibers [13]

Process Parameters	Systemic Parameters	Solution Parameters	Physical Parameters
Applied electric field	Polymer type	Viscosity of polymer	Relative humidity
Flow rate of polymer solution	Molecular weight	Solvent concentration	Temperature
Collection plate	Architecture of molecule	Solution conductivity	Air velocity
Distance between electrode and collector	Solvent used	Solvent concentration	-

According to a power law relationship, solution concentration has been found to most strongly affect electrospun fiber diameter - fiber diameter increase with increase of concentration of the PEO solution. The same results were estimated in other research work done by Stepanyan and co-authors with Polyamide 6 (PA 6) polymer [11]. In this study authors concluded that diameter of fibers is mainly influenced by solution viscosity, evaporation rate and current. During the experiments of PA 6 nanofiber it was indicated two different nanofiber morphologies - at relatively low viscosity (below 3000 mPa) round fibers were observed. However, if viscosity of solution increased beyond 3000 mPa, flat ribbon-like structures were obtained. Moreover, Young Jun and co-authors [15] in their research concluded that appropriate control of the electrospinning process makes it possible to prepare nano-microfibers of required structure from various polymers. In figure 1.2.1 results of the Brunauer, Emmett and Teller (BET) technique are presented. Increasing the concentration of polymer solution, fiber diameter increases and average pore sizes of the nonwoven increases as well. These results lead to wider pore size distributions and reduction of specific surface area of the nonwoven [15].

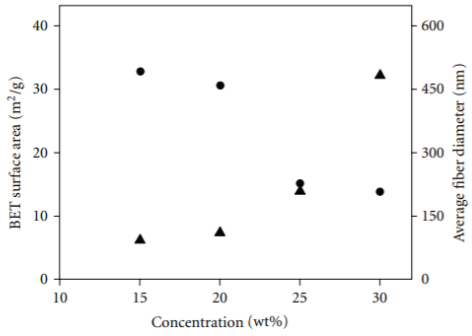


Figure 1.2.1. BET technique for analyzing the surface area and average electrospun Polyamide 6 nanofibers diameters dependability on a polymer concentration (• BET surface area; ▲ average fiber diameter) [15]

The structure of electrospun materials depends also from the solvent type. Xiaoli with co-authors [16] studied the electrospinnability of poly(lactic-co-glycolic acid) (PLGA) solutions. The aim of the research was to estimate the influence of PLGA molecular weight and solvent type on the physical properties of electrospun fibers. Moreover, viscoelasticity, surface tension and evaporation rate of the PLGA solutions were characterized to explain the electrospinning process. Tetrahydrofuran (THF), dimethylformamide (DMF), chloroform (CHL) and their combinations were used as a solvent to dissolve PLGA. PLGA was dissolved in DMF-THF or DMF-CHL (40:60, 50:50, 60:40 v:v) at concentrations of 10%, 20% and 30% (w/v), respectively. The surface tension of the polymer

solutions was measured by the pendant drop method and results showed that increased concentration of polymer significantly enlarges the surface tension of PLGA polymer solutions (1.2.2 a.). While comparing surface tension at the same polymer concentration, surface tension of PLGA solutions increased with an increase in amount of the DMF (1.2.2 b.), because of the higher initial surface tension than THF and CHL. Furthermore, polymers that were dissolved in DMF-CHL had a higher surface tension than the ones dissolved in the DMF-THF.

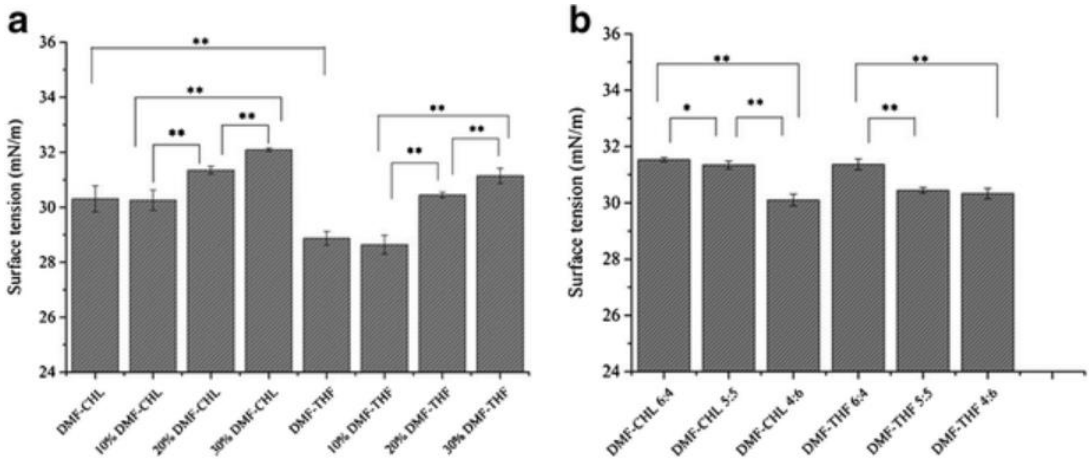


Figure 1.2.2. The surface tension of different polymer solutions. a) polymer solutions at different concentration; b) polymer solutions in different solvent compositions at the same concentration (20% w/v) [16]

The evaporation rate of pure solvents was straightforward, it is seen in a figure 1.2.3 a). While in polymer solutions as the concentration of PLGA polymer is increased, evaporation of the solvents becomes slower (figure 1.2.3 b) [16].

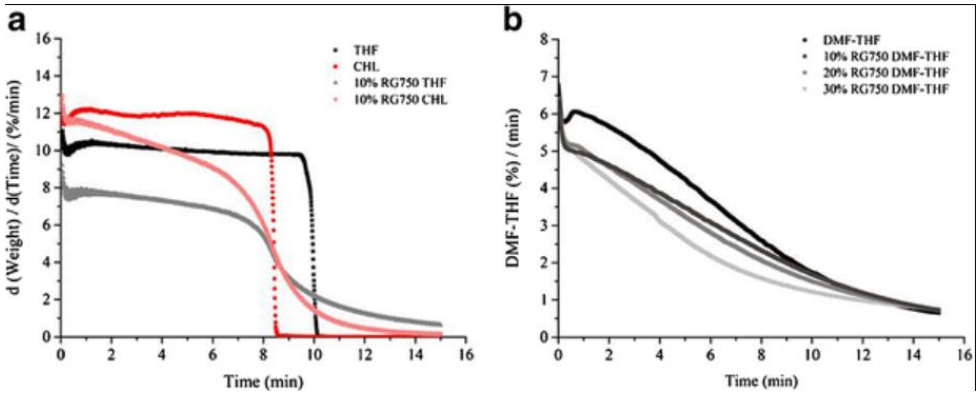


Figure 1.2.3. Evaporation rate of different solvents and polymer solutions. a) pure solvents and polymer solutions dissolved in the same solvent; b) polymer solutions at different concentration [16]

Solvent type is also important for properties of electrospinnability of solution. Polymer solutions that dissolved in DMF-CHL showed higher storage modulus (G') than the solutions in DMF-THF. This means, that higher storage modulus can help to create a more stable extensional flow in the spinning process, because material has higher elastic response when deformed. Higher elasticity is obtained by using DMF-CHL and result in more uniform fibers. Diameter of the fibers of DMF-CHL was larger and more uniform than from DMF-THF (figure 1.2.4).

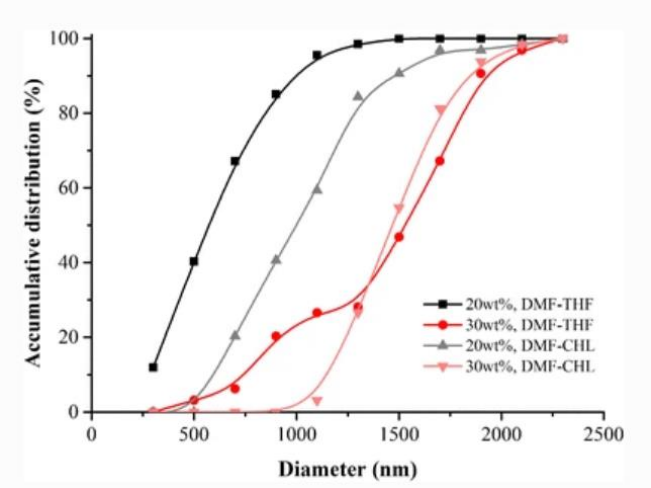


Figure 1.2.4. The fiber diameter distribution in different solvents at varied concentrations [16]

With an increase of polymer concentration, also viscosity and surface tension of the solution increases. At the same time polymer concentration, change of both viscosity and surface tension of the polymer solutions, depends on a change in solvent composition. Uniform fibers can be produced only when viscosity of polymer solutions can overpower the effect of surface tension. Most importantly researchers agree on a theory, that higher molecular weight at higher concentration can form uniform fibers [16].

Researchers Nunez et al. [17] used two types of solvents – ethanol and dimethylformamide (DMF) and different mixtures of them both for making a morphological characterization of PVP fibers. To obtain an electrospun material of homogeneous fibers, weight percent, feed rate, applied voltage and distance from the needle's tip to collector were adjusted. Results showed that concentration affected diameter of electrospun nano-microfibers the most, having a directly proportional relation. Even though the jet was stable for each one of the solutions tested, only three of them gave flawless fibers: ethanol with 8% wt PVP (3 ml/h feed rate, 15 cm distance to collector, 6 kV voltage), DMF with 14% wt PVP (0.5 ml/h feed rate, 20 cm distance to collector, 6 kV voltage), and ethanol with DMF in a weight ratio 70/30 and 8% wt PVP (5 ml/h feed rate, 17 cm distance to collector, 6 kV voltage). The results were 40.08 MPa, 8.8 MPa, and 32.78 MPa, for the elastic modulus for electrospun fibers with ethanol, DMF, and ethanol-DMF, respectively. Being the value of ethanol the greatest, for better mechanical properties, researchers concluded that ethanol must be used as a solvent in PVP solutions [17].

In addition, flow of current influence electrospinning process. Generally, current flows from a high-voltage power supply to a polymer solution which becomes highly electrified and the induced charges are evenly distributed over the surface. Electrostatic forces cause a distortion of polymer solution and spherical droplet deforms into a Taylor cone and form nano-microfibers. Value of applied voltage varies from polymer to polymer. If the value of applied voltage is higher, it attributes to the stretching of the polymer solution together with charge repulsion within the polymer jet, so fiber diameter decrease. However, for each polymer applied voltage has a certain critical value, and if it goes beyond - formation of beads or beaded nano-microfibers will occur [18].

Important to note, that distance between the metallic needle tip (in the piston/ peristaltic/syringe pump type electrospinning equipment) and the collector determines the morphology of electrospun material, because this distance has influence on the deposition time, evaporation rate and instability interval.

To obtain smooth and uniform electrospun nano-microfibers critical distance must be controlled [19, 20]. Matabola and Moutloali [19] in their research concluded that large diameter fibers formed when distance was kept small, while thinner fibers were obtained when the distance between the needle tip and collector was increased.

Selection of solvent is a significant factor during the formation process of smooth electrospun nano-microfiber. First of all, solvent has to be chosen in accordance to the used polymer that has to be completely soluble in a solvent. Secondly, solvent should have a moderate boiling point which gives information about volatility of solvent. Highly volatile solvents evaporate more during the nano-microfiber flight from the needle tip to the collector. Generally, highly volatile solvents have low boiling points and high evaporation rates and are usually avoided to be used because they cause drying of the jet at the needle tip. Secondary structures could be created from the rate of solvent evaporation from the liquid jet and determine the uniformity of electrospun nano-microfibers. Solvents of high volatility absorbs heat from the jet, lowering its temperature, and decreases the thermodynamic stability of non-solvent phase [21].

Ethanol can be used as a solvent of polymer during the electrospinning process as it offers high production rate, is patient safe and has good environmental compatibility [5, 13].

1.3. Application of electrospun materials for scaffolds

Electrospun nano-microfibrous scaffolds mimic the structure of nanofibrils of extracellular matrix (ECM) and shows a quick signaling pathway and fibroblasts are attracted to the derma layer, where collagen and several cytokines (e.g. angiogenic factors and growth factors) as such important ECM components can be excreted and damaged tissue can be repaired. This property draws a lot of attention for medical applications. Furthermore, electrospun nonwoven material is also important for cell attachment and proliferation in wound healing. Scaffolds control the rate of drug release, regulate behavior of cells, regenerate injured tissues. Scaffolds can be made of electrospun nano-microfibers from different polymer with chosen size of diameter which can vary from several nanometers to tens of micrometers [22, 23].

Electrospun fiber scaffolds have properties of big surface area-to-volume ratio, high porosity, uniform fiber size in all nonwoven material, very diverse composition (for example, hydrophobic and hydrophilic materials), flexibly assembled structure, as well as bioactive materials can be easily functionalized and made as carriers in future regenerative medicine research for drug delivery and scaffolds. Composition of fibers for scaffolds must be selected in accordance to the properties that have to be achieved in the resulting material, such as stiffness, hydrophilicity, biodegradation rate, biocompatibility and bioactivity for tissue regeneration [22, 24].

Designed scaffold should provide a surface for a cell to attach and grow on as well as to control acceptable biomechanical support during tissue regeneration and structure degradation [13]. Fiber orientation is also important to maintain the growth of cells. In the research of Junyu M. and etc [25] PLLA was electrospun into nano-microfiber material, that was produced by collecting the fibers on a stationary plate for randomly oriented nano-microfibers and on a rotating wheel for aligned. It has been reported that although osteoblast proliferation on aligned and random nano-microfibers is comparable, after 21 days a significantly higher calcium production has been detected when the cells were seeded on aligned fibers ($p=0.003$).

Cells proliferate and differentiate at a higher rate when seeded on highly porous electrospun nonwoven material. Moreover, during the in vitro studies it was concluded that cells exhibit higher cellular adhesion with decreasing pore size and an increasing of pore density [26, 27]. In a scientific research [27] nano-microstructures of biodegradable poly (L-lactide-co-ε-caprolactone) (PLCL) fabrics with different compositions (mol% in feed; 70/30, 50/50, and 30/70), poly(L-lactide) (PLL) and poly(ε-caprolactone) (PCL), while using methylene chloride (MC) as a solvent were prepared by the electrospinning technique. In figure 1.3.1 the adhesion, spreading and proliferation of human umbilical vein endothelial cells (HUVECs) on electrospun fabrics of different diameter of fibers are presented (A, B and C) and evaluated for 1 and 7 days after the seeding of the cells on the material. It was concluded that (HUVECs) adhered well on the samples and proliferated without troubles on a small diameter of 0.3 μm (A) and in 1.2 μm (B) fiber fabrics while both of which were dense fabrics. Controversially, reduced cell adhesion, restricted cell spreading, and no proliferation were noted on the large diameter fiber fabrics (7.0 μm). Furthermore, it was concluded that the decrease in the fiber diameter leads to decrease in porosity as well as pore size, but an increase in fiber density and mechanical strength.

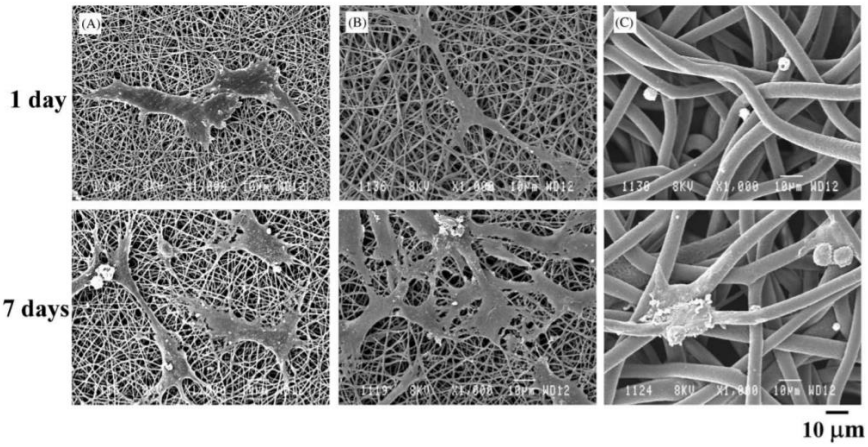


Figure 1.3.1. SEM images of the HUVECs cells cultured for 1 and 7 days after the seeding on electrospun PLCL 50/50 fabrics that had different diameter of fibers: (A) 0.3 μm, (B) 1.2 μm, and (C) 7 μm [27]

Cell attachment to electrospun nano-microfibers is a crucial factor for tissue engineering applications. Researchers Carlberg B. etc. [28] made a research on electrospun polyurethane (PU) scaffolds for proliferation and neuronal differentiation of human embryonic stem cells (hESCs). Cultivation of hESCs on a scaffold of a thickness of approximately 150 microm was successful and neuronal differentiation was observed via standard immunocytochemistry. The physical interaction between the hESCs and the fibrous electrospun PU scaffolds indicated that these scaffolds mimic ECM and provides physical support for central nervous system (CNS) tissues to recover after trauma or disease. It was estimated that cells are attached to individual fibers of nano-microstructure, as well as neurite outgrowth and connection to adjacent cells.

Not only nano-microfibrous structure, but also polymer type is very important to obtain wanted electrospun nano-microfibrous scaffolds properties. Natural polymers such as chitosan, chitin, collagen and etc. have high biological properties: biocompatibility, antimicrobial ability and cell adhering so they are widely used in biomedical applications. Important to note, these polymers have low mechanical properties which can limit their application possibilities, but in this case mentioned polymers are used with polymers, that demonstrate better mechanical properties. Natural polymers have high hydrophilicity, although produced scaffolds have not shown enough physical properties in

aqueous biological environments. This means it is recommended to modify or blend with other synthetic polymers. Most of the synthetic biopolymers have low biological properties in comparison with natural fibers, but they can offer high mechanical properties and high electrospinnability.

In another research, PCL, chitosan (Cs), poly(vinyl alcohol) (PVA) blend was combined to obtain nanofibrous scaffolds which were applied to cutting and burn wounds as a tissue matrix [29]. PCL:Cs:PVA nanofibrous scaffolds were successfully electrospun in 2:1:1.33 blend ratio. PCL in this blend reduces hydrophilicity and preserves nonwoven material integrity in aqueous media. Also, higher physical properties of blends were reached because of adding PCL as it results in better cell attachment. The obtained electrospun nano-microfibers from blended synthetic and natural polymers succeeded to reach targeted and unique combinations of structural, biochemical and mechanical properties that cannot be achieved by any single polymer. The scaffolds were used in two - acellular and cell-seeded forms. Scaffolds were applied on excisional cutting and burn wounds on the skins of dorsum rat. Wounds which were treated with acellular scaffolds demonstrated bigger scrubs areas as treated with cell-seeded ones. It was found that from pathological results, nanofibrous scaffolds can absorb liquid from the wounds due to nanoporous structure and hydrophilic nature of polymers. Moreover, scaffolds could also help oxygen ventilation which is important for faster and better healing. It is known that chitosan has antimicrobial properties and it was useful for reducing the number of inflammatory cells in the wound [29].

1.4. Application of electrospun materials for drug delivery

Electrospun nano-microfibers can be used for drug release controlling devices in accordance to the selection of polymer as well as porosity, morphology and geometry of the nano-microfibers. Electrospun fibers for future biomedical application as drug carriers will be a promising technique, especially for postoperative local chemotherapy. Furthermore, it was successfully achieved that electrospun nano-microfibers can show different drug release control profiles, such as immediate, smooth, pulsatile, delayed, and biphasic release. It is important to understand that control of the dosage of the released drug is a very crucial factor for treatment – especially for anti-cancer drugs, because it can affect and cause harm to normal and healthy tissues that are around cancer cells [7, 5, 30, 31].

Electrospun materials for drug delivery systems were initially created to ease the administration of medicines. In addition to various potential applications of electrospun textiles, drug delivery application attracts the most attention is known as one of the most promising uses. High loading capacity, high encapsulation efficiency, simultaneous delivery of diverse therapies, cost-effectiveness and ease of operation are the key features that make a high potential for electrospinning used in drug delivery [7, 5].

There are two possible ways to incorporate drug (drug nanoparticles) into the nano-microfibers. First is the direct method, when drug (drug nanoparticles) are dispersed directly in the solution that is going to be electrospun. Another one is indirect method when surface treatment is applied on a nano-microfibrous structure as drug cannot be easily and uniformly dispersed in the solution [30].

There are polymer related parameters, that are observed to affect the drug release from nano-microfibers. That is polymer composition, crystallinity and molecular weight of polymer. In a research of Chou and Woodrow [32] mechanical properties of electrospun PCL and poly (D,L-lactic-

co-glycolic) acid (PLGA) fibers in different blending ratios and in presence and absence of a hydrophilic drug tenofovir (TFV) were investigated. Results showed that with increasing loading of TFV up to 40 wt% in a structure, there are only minimal effects on elastic modulus and tensile strength of the 20/80 PCL/PLGA electrospun nano-microfibers, because of offsetting drug-polymer interactions. However, in vitro studies of the drug release rate showed that increasing amount TFV in a structure, significantly decreased nano-microfiber mechanical properties, although drug release rates were higher (figure 1.4.1).

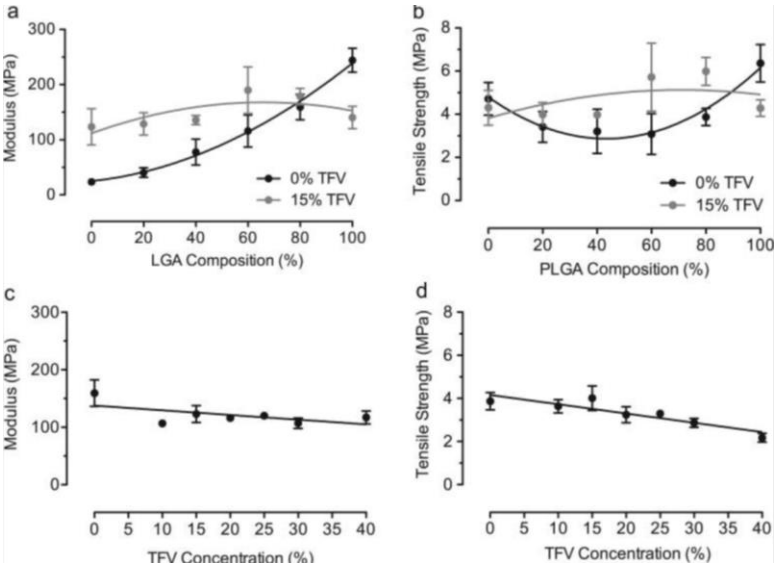


Figure 1.4.1. Mechanical properties of PCL/PLGA fibers (black dots) and PCL/PLGA fibers loaded with 15 wt% of TFV (grey dots): a) Young‘s modulus, b) tensile strength. Mechanical properties of 20/80 PCL/PLGA fibers with loaded up to 40 wt% TFV: c) Young‘s modulus, d) Tensile strength [32]

Additionally, mechanically stretched nano-microfibers demonstrate faster release rate of a drug than the non-stretched nano-microfibers. For example, 20/80 PCL/PLGA sample released around 70% of drug in 96 hours after being stretched to level of failure, while in comparison almost same amount of drug release was detected in 240 hours for unstretched sample (figure 1.4.2). This can be explained in two reasons: first, reduction of fiber diameter, because applied stretching increased the speed of release as fiber diameter is known to have influence and possibility to affect drug release. Another possible reason is that smaller diameter of nano-microfiber leads to decreased distance of diffusion for drug particles that are encapsulated in the core of nanofibers.

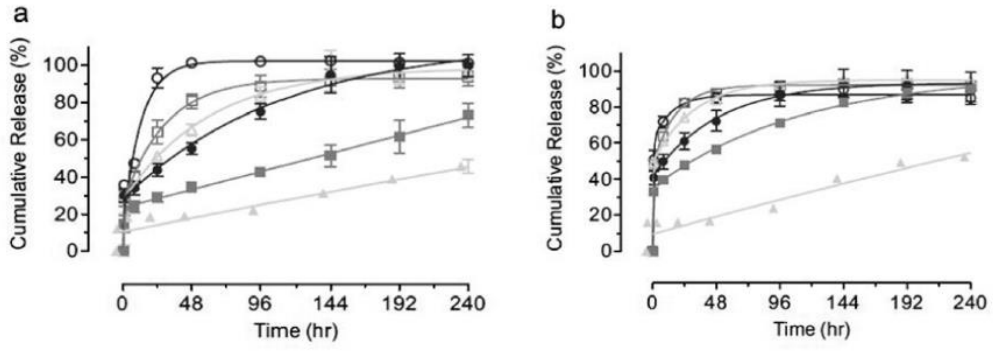


Figure 1.4.2. Cumulative release curves of PCL/PLGA fibers loaded with 15% TFV a) without mechanical stretching b) after mechanical stretching leading to failure. ○) 100 PCL, □) 80/20 PCL/PLGA, Δ) 60/40 PCL/PLGA , ●) 40/60 PCL/PLGA, ■) 20/80 PCL/PLGA, ▲) 100 PLGA [32]

Various ranges of drugs, for example, antibiotics, anticancer agents as well as proteins, aptamer, DNA, and RNA have been incorporated into electrospun nano-microfibers. Drugs such as antimicrobial, analgesics and anti-inflammatory agents have been carried using electrospun scaffolds for dentistry surgical treatment as electrospun scaffolds minimize systemic dosage of drug delivery devices. Recently implantable drugs were included into electrospun scaffolds and that helps to repair surgical sites by preventing infection and lowering the possible risk of osseointegration [7].

As it was mentioned before, in the study of Kenawy et al. [33] electrospun nano-microfibers have been successfully used to achieve different controlled drug release profiles. Nonwoven material of electrospun fiber were made either from PLA, poly(ethylene-co-vinyl acetate) (PEVA), or from a 50:50 blend of the two. To solubilize the drug, chloroform with small amount of methanol was used to electrospun the fibers. UV–VIS spectroscopy was used to determine the release of tetracycline hydrochloride from these newly created drug delivery systems. The cast films of the release profiles of tetracycline hydrochloride from electrospun material are shown in figure 1.4.3. Electrospun PEVA shows a higher release rate, because within 120 h about 65% of its drug content was released, while 50/50 concentration material released about 50% of drug content over the same period. Nonwoven of PLA fiber most probably from tetracycline hydrochloride on the fiber surfaces exhibit some instantaneous release with negligible release over 50h. This may be because PLA is partially crystalline, and this limits the diffusion of aqua from the environment into the polymer inner layers and consequently limits the diffusion of drug from the fibers. Authors of the research noted that further investigation must be done on long term release from PLA mats via hydrolytic degradation. The 50/50 blend gave about 5% release of tetracycline hydrochloride within 5 h with a smooth and regulated release thereafter. A 50:50 blend with 25 wt% tetracycline hydrochloride releases drug much more rapidly than the 5% sample, and it is suggested that this is due to surface-segregated tetracycline that quickly dissolves. It can be concluded that drug release from electrospun PEVA and 50/50 PLA/ PEVA mats is relatively smooth for about 5 days.

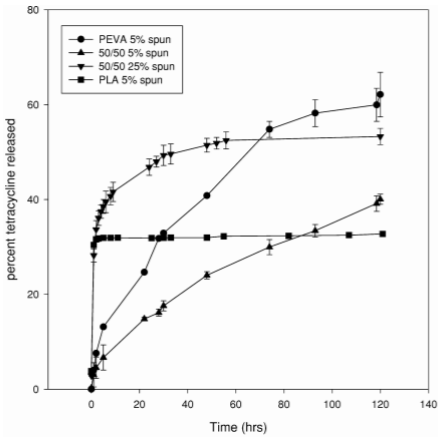


Figure 1.4.3. Percentage release of tetracycline HCL from electrospun mats vs. time [33]

From researches [30, 31] it is concluded that parameters affecting the drug release from created nano-microfiber structure are diameter and porosity of electrospun nano-microfibers as well as fiber's alignment. Generally smaller the diameter of nano-microfiber is, faster the drug release process, because of higher surface area and dissolution rate in a smaller diameter nano-microfiber. Furthermore, porosity of the nano-microstructure is considered as the most important factor of controlled drug release. Thicker nano-microfiber structure has very high porosity and it is considered

as likely to release the drug faster than lower porosity material. Another important parameter is alignment of nano-microfibers that can affect the rate of drug release. Random pattern of the nano-microfibers in a structure tend to uptake more water, so it would release the drug faster [30, 31].

There are also drug-related parameters that have influence on the release rate from nano-microfibers. To begin with, the lower molecular weight of the drug is, the higher is the release rate from the structure. Considering amorphous and crystalline forms of drug it can be concluded that crystalline form of the drug gets deposited on nano-microfiber surface and causes burst release, while amorphous form of the drug can be deposited deeper inside the structure and can be released in a sustainable and controlled manner. When amount of the loaded drug increases, release rate increases respectively, suggesting that drug remained encapsulated and trapped inside the fibers [34].

There are particularly important factors, for example, drug loading, stability of active ingredients, initial burst release, industrial scale-up that still are great challenges which must be analysed more to bring this technique into primary drug delivery technologies. Irregular distribution of the drug in the nano-microfiber structure often causes initial burst effect, which is the most unwanted property of the drug delivery system, as release of drug must be controlled. Either synthetic or natural polymers that are the backbone of nano-microfibers causes the amorphization of crystalline drug particles. Furthermore, viscosity and surface tension of the polymer solution may be negatively affected by drug loading, making it unsuitable for electrospinning [21].

1.5. Application of electrospun materials for wound healing

Wound dressing materials are one of the applications of electrospun materials for biomedical applications. In order to obtain a suitable environment for healing, a range of dressing types have been explored, starting from films, foams, to hydrogels, hydrofibers. All types of dressing should absorb excess exudates, offer thermal insulation, limit gaseous and fluid exchanges, avoid allergic reactions, should be created without toxic components, have a soft feeling, and most importantly be sterile to not to support microbial growth. Correctly chosen biopolymers used for electrospinning process could significantly enhance healing of wounds compared with the conventional dressing materials, such as gauze. New modern bandages can be made with incorporated bioactive ingredients, such as antimicrobial, antibacterial and anti-inflammatory substrates that can be released from the structure at a controlled range and addressed to the direct place of the wound [35].

Electrospun nonwoven materials from nano-microfiber based dressings made by the electrospinning technique have few very important advantages between other wound dressing materials, which is the reason why this application is attracting so much attention of researchers nowadays. This material has very high surface area-to-volume ratio, porosity, it is able to mimic the ECM structure and have good biocompatibility [36].

The pore size below 1 μm blocks the wound from the bacterial penetration using aerosol particle capturing mechanisms, but at the same time allowing O_2 permeability for wound to heal. Different kind of antibacterial substances, for example iodine, Ag nanoparticles and others can be incorporated into nano-microfiber dressings and help to lower the possibility of infections. Additionally, vitamins, minerals and growth factors can be included as active compounds in the material to enhance normal skin growth and reduce a scar of tissue [21].

In case of a wound, wound dressings must be replaced periodically to keep the wound sterilized, which can cause some secondary injuries of a tissue that was adhered to dressing and during the replacement was pulled off. This situation reduces the speed of the healing process and is a potential problem of nano-microfiber based dressings as they have biomimetic structures. In accordance to this, thermosensitive polymers can be used [37].

Li et al. [37] for the investigation of a research chose to create a thermosensitive nano-microfiber material that would be loaded with ciprofloxacin (CIF) (antibiotic for broad spectrum of bacteria) as antibacterial wound dressing. CIF was loaded in thermo-responsive electrospun fiber mat made of poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA) and (poly(L-lactic acid-co-ε-caprolactone)) P(LLA-CL). CIF is widely reported as extremely effective against *E. coli* and *S. Aureus* bacteria growth and in this research with obtained results it was demonstrated that after 3 days of CIF inhibition against *E.coli* and *S. Aureus*, growth was lowered by 87.5 ± 6.2 % and 84.6 ± 2.8% respectively. Open excision type wound of 3 cm x 3 cm were created to the depth of tissues on the upper backs of rats. Inhibition zones after 24 h of all the fibers loaded with CIF (S5-S8) are similar, and this indicates comparable antibacterial effects, while inhibition zones cannot be seen in the groups made without CIF. After incubation for 72 hours, indication zones became slightly larger and this can be concluded as the antibacterial activity lasts for at least three days. For the analyzing the ability of the fibers to heal the wounds, figure 1.5.1 represents the in vivo studies results after 0, 5, 14 and 21 day after the operation. It is seen that for groups that were treated with gauze (a), gauze soaked with CIF (b), and 0.9% CIF solution (c) thicker scrubs and bleeding of the wound can be seen and this can be caused by local inflammation. Furthermore, these groups show slower wound healing process than those treated with (PDEGMA/P(LLA-CL)/CIF and P(LLA-CL)/CIF. To conclude, electrospun material from PDEGMA/P(LLA-CL) loaded with CIF can be a potential wound dressing material with antibacterial properties [37].

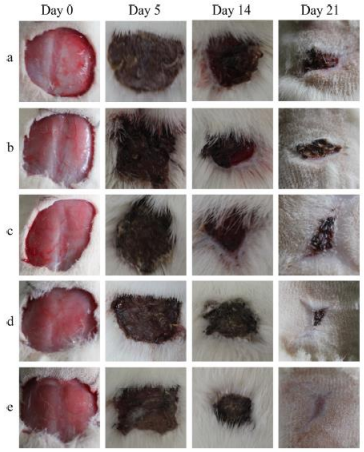


Figure 1.5.1. Images of skin wounds of rats after the treatment for 0,5, 14, and 21 days after the operation. The wounds were treated with a) commercial gauze alone; b) commercial gauze soaked with 0.9% CIF solution; c) CIF solution; d) P(LLA-CL)/CIF; e) (PDEGMA/P(LLA-CL)/CIF [37]

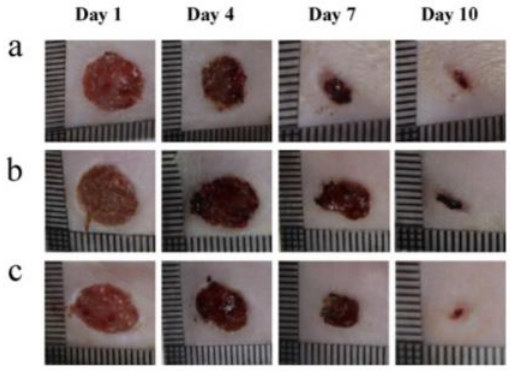


Figure 1.5.2. Appearance of the wounds during treatment for healing at 1, 4, 7 and 10 day after the treatment with a) 30% LZ loaded CS-EDTA/PVA nano-microfiber mats; b) gauze (negative control); c) gauze (positive control) [36]

In the research [36], blend of chitosan–ethylenediaminetetraacetic acid (CS 2 wt%–EDTA) at a weight ratio of 30/70 and polyvinyl alcohol(PVA) solution (10 wt%) was made by electrospinning

and electrospun material for wound healing were produced with 10, 20 and 30 wt% of lysozyme (LZ) from chicken egg whites. The wound healing activity was analyzed and measured by in vivo studies using male Wistar rats. After the investigation it was concluded that lysozyme loaded CS–EDTA nanofiber mats increased the speed of wound healing of a rat after comparison with gauzes in negative (treatment is not expected to have results) and positive (treatment is expected to have results) control (figure 1.5.2). It is seen that wound healed during all ways of treatment in 10 days. However, figure 1.5.3 represents that healing process in wound areas was different. In the first 1 to 5 days the healing effect that created 30% LZ loaded CS–EDTA/PVA nano-microfiber mat was better than the one of the gauze ($p < 0.05$). This may happen because LZ was rapidly released from the nano-microfiber material, that is why the healing process was accelerated from the beginning by deactivating bacteria.

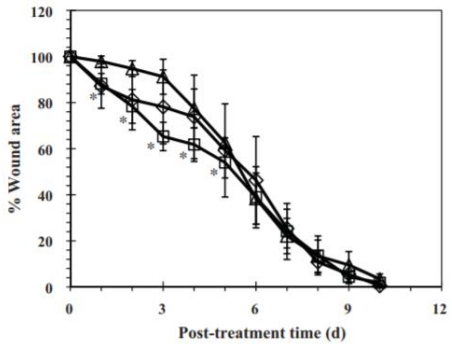


Figure 1.5.3. Wound healing tests of □) 30% LZ loaded CS-EDTA/PVA nanofiber mats; Δ) gauze (negative control); ◊) commercial antibacterial gauze dressing (positive control) [36]

To conclude, this experiment proved that lysozyme loaded CS-EDTA nano-microfibers have a potential and can be applied for wound healing [36].

1.6. Electrospun nonwoven materials from PVP and PMMA

PVP also known as polyvidone or povidone, is a water-soluble, biodegradable polymer. PVP is made after the polymerization of a cyclic amide monomer (N-Vinylpyrrolidone) and due to the carbonyl group in the amide ring has high polarizability (figure 1.6.1) [38].

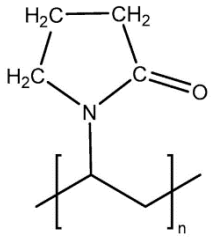


Figure 1.6.1. The PVP polymeric unit [38]

In a dry state PVP is a light flaky hygroscopic powder and absorbs up to 40% of water by its weight. Meanwhile in solution it shows excellent wetting properties and can easily form films. This PVP property make it good as a coating or as an additive to coatings [38].

PVP is not only soluble in water, but also in other polar solvents, for example, in various alcohols, such as methanol or ethanol, as well as in deep eutectic solvent formed by choline chloride and urea. Biodegradability is one of the most important properties of biocompatible polymers. The application

of biodegradable polymers is versatile and very successful in controlled drug delivery which includes implants, drug-eluting stents, microspheres, polymeric scaffolds, nanoparticles. PVP is used in many pharmaceutical tablets as a binder as it can easily pass through the body when is administered orally. Furthermore, for trauma victims PVP can be used as a blood plasma expander [2].

PVP mixed with iodine forms a complex called povidone-iodine and this compound possesses disinfectant and antibacterial properties. Povidone-iodine is equally safe and effective as talc and can be more preferred because of easy availability and low cost. It can increase the solubility of drugs in liquid and semi-liquid dosage forms also as an inhibitor of recrystallization. Povidone-iodine complex can be used in different products, for example, ointment, pessaries, solutions, liquid soaps and surgical scrubs [2].

For the last few decades PVP has been used in wound dressing as it is able to form hydrogel by cross-linking upon radiation treatment. To improve mechanical properties, dozens of polymers have been used to form blends with PVP. Chitosan (CS) is believed to be a good candidate for preparing PVP blends as it interacts with PVP by forming hydrogen bonds between amino and hydroxyl groups of CS and carbonyl group of PVP [39].

Blends of PVP with other polymers for producing a biomedical product is a very common thing in electrospinning technique. In a scientific research [40] blended mixtures of PCL with PVP were dissolved in chloroform/ethanol compound. PCL is hydrophobic, non-toxic and tissue-compatible synthetic polymer, which is used for electrospinning nano-microfiber scaffolds, as well as for drug delivery, wound dressings and devices for bone fracture. However, PCL alone can show relatively slow biodegradation rates because its ester linkages may hinder bio-absorption rates and strong hydrophobicity negatively affects the adherent cells‘ migration and morphology. For these reasons, blending a strongly hydrophilic and biocompatible PVP into a polymeric compound, enhances hydrophilicity and accelerates biodegradation rates of the electrospun nano-microfibers and can be used to fabricate scaffolds.

In scientific article, made by Frontera P. et al. [38], electrospinning was used for preparing functional coating for adsorption heat pump systems, which have eco-friendly properties and are sustainable systems for refrigeration and cooling. PVP is a polymer that shows good water affinity, however in adsorbing material the structural stability is a key issue. The aim of this work was to preserve the hybrid organic-inorganic nature of nano-microfibers polymeric structure to ensure high flexibility and mechanical strength to coatings. Scientists presented the coating with novelty consisting three principal features: material based on PVP and modified with tetraethyl orthosilicate (TEOS), layers of nonwoven nano-microfibers that make coating structure and coating technique based on the electrospinning. PVP is easily spinnable and has high water adsorption capability, while TEOS increases thermal stability of microfibers. In this case, different coatings were obtained by the electrospinning of PVP solutions with added different quantities of 0, 5, 8, 13, 18, 24 wt. % of TEOS to increase coating stability to water adsorption/desorption cycles. The results showed that pure PVP coating (Mf-0 in figure 1.5.4) shows regular-shaped nano-microfibres of few millimeters length and micromeric diameters. With high TEOS concentration of 18 wt. % and 24 wt. %, nano-microfibres were formed irregularly and the average diameter increased. When TEOS concentrations were in the range of 5 to 13 wt.%, hybrid nano-microfibres demonstrated both good water affinity and good short-term thermal stability. Furthermore, Scanning Electron Microscopy (SEM) images of coatings

showed that layers of nano-microfibres have a high surface area as well as high permeability. Both of these properties are advantageous for adsorption systems [38].

In the research of Matysiak W. et al [41] scientists produced composite nano-microfibers from PVP as a polymer matrix doped with the iron oxide (Fe_2O_3) (30% wt.) because of its potential of photocatalytic activity due to visible-light responsive compound using a combined method of sol-gel technique and electrospinning process. After morphology examination with SEM equipment it was seen that Fe_2O_3 doping caused almost 3 times decrease in nano-microfiber diameter as average diameter of PVP nano-microfibers was 565 nm, while PVP/ Fe_2O_3 was 165 nm. Change of the average diameter leads to higher surface area of nonwoven. After UV-VIS spectroscopy there was indicated a slight increase in absorption level in the range of visible light wavelengths from 310 nm to 560 nm in a composite nano-microfibers with a doping with Fe_2O_3. This result lead to conclusion that this composite nano-microfiber can be used in photocatalytic processes in this range of visible light and can be used in UV light protection shields.

Karayegen G. et al. [42] used PVP as a polymer for electrospinning that resultant nonwoven material would be used in novel biomedical applications, such as tissue regeneration. The aim of investigation was to create aligned nano-microfibers, because in ordinary electrospinning technique diameter of nano-microfibers are chaotic and random due to the chaotic behavior of polymer jet. By using finite hollow cylinder focusing electrodes for electrospinning, external electrostatic field is created that decreases whipping instability of the polymer jet. The result that researchers obtained was decreased radius of the nano-microfiber dispersion on the collector. Furthermore, authors used conductive parallel electrodes placed through jet trajectory which resulted in aligned nano-microfibers that can be used in tissue engineering applications.

S. Huang with coauthors [43] had investigated electrospun PVP /Cellulose Nanocrystal (CS) /Silver Nanoparticle ($AgNO_3$) fibers. It was estimated that insertion of CS and/or $AgNO_3$ cause the increase of electrospun polymer conductivity. Consequently, thinner electrospun fibers were formed. Insertion of CS in electrospun solution increase tensile strength of the electrospun material comparing with electrospun material from pure PVP. $AgNO_3$ improve antibacterial properties of electrospun material.

Electrospun nonwoven materials from PMMA derivatives such as Eudragit are largely used for drug encapsulation and in controlled oral drug delivery. Eudragits are methacrylate-based polymers with different sensitivity to pH. Eudragit S-100 (ES) (figure 1.6.2) is soluble above pH 7. Eudragit L100 (EL) and ES families of pH-sensitive polymers are used for formation of nanoscale fibers and nowadays is widely explored and received significant attention for targeted drug delivery [3].

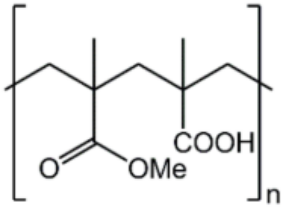


Figure 1.6.2. Structural formula of ES [3]

In the research study [3] factors influencing the release of active ingredients (AI) from ES nano-microfibers were analysed. For monolithic fibers benzoic acid, 1-naphthoic acid, 1-naphthylamine, and 9-anthracene carboxylic acid were used as AI. Coaxial fibers were made out of the core (ES and

AI) and shell (ES). Coaxial fibers were analysed with Scanning Electron Microscopy (SEM). From the images it was seen that coaxial fibres are, in general, smooth and homogeneous, with a diameter of around 550-700 nm. On close inspection of TEM images it is seen coaxial fibers have separate core and shell compartments.

Nano-microfibers have a very large surface area-to-volume ratio. Making fibers from ES does not prevent AI release at pH 1. Important to note, that AI is able to diffuse through the polymer matrix to reach solution and thermodynamic solubility is the driver for this to happen. Significant release at pH 1 with all of the monolithic fibers, except those that contained 9-anthracene carboxylic acid, was established. In this case, molecular weight of the AI and its acidity/basicity are important for controlling release from such materials. For the coaxial fibers, when AI is only present in the core, the acidity/basicity appears to be a less important factor, while molecular weight and diffusion through the polymer matric are the rate limiting steps in release [3].

Karthikeyan et al. [44] found that blend fibers of zein (a plant protein) and ES loaded with pantoprazole (acid labile drug) and aceclofenac gave 25% release of the latter after 2 h immersion in 0.1 M HCL. This electrospun blend was developed to deliver two different classes of drugs simultaneously that would compensate the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs). Zein is hydrophobic, its susceptibility to proteolytic degradation is reduced, as well as it is able to withstand gastric pH which occurs in stomach. For these reasons zein is a perfect carrier for oral drug delivery of aceclofenac in chronic therapies [45]. In the scientific research [44] K. Karthikeyan et al. investigated various concentrations of zein and ES ranging from 5 to 40% and voltage potential varying from 10 to 25 kV to develop fine nano-microfibers by electrospinning and avoid electrospraying. Researchers estimated that minimum necessary concentrations for electrospinning was 20% (w/v) of zein and 10% (w/v) of ES at a potential of 25 kV. Lower concentration lead to electrospraying, while higher concentration restricted free falling of fibers because of its high viscosity. Worth to mention, that change in polymer concentration leads to different values of surface tension, electrical conductivity and viscosity of the final product.

The drug release from ES was mostly influenced by erosion of polymer at a pH above 7. Because of the presence of zein, release was assisted by the diffusion of molecules from fibers to solution. Usage of two different characteristics polymers, control the release of each drug and guarantee a successful release of its ingredients. Gastric irritation caused by non-steroidal inflammatory drugs can be significantly reduced by the release of pantoprazole together with aceclofenac, as well as lower the side effects [27]. It is reported by Kumari et al. that polymer based nano drug delivery systems protects the drugs from degradation in the acidic stomach fluids and reduce its side effects [46].

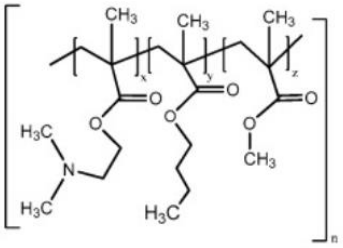


Figure 1.6.3. Structural formula of Eudragit E-100: Poly(butyl methacrylate-co-(2- demethylaminoethyl) methacrylate-co-methyl methacrylate) 1:2:1 [47]

Casian T. and co-authors [5] developed electrospun amorphous solid dispersion (ASD) with meloxicam (MEL) and evaluated what kind of effect of polymeric matrix is received on fiber morphology, as well as other properties, for example, dissolution-permeation, stability and mechanical properties. Meloxicam was selected as the active pharmaceutical ingredient (API) that has low solubility and high permeability. In the results researchers discussed that selection of MEL as a polymeric matrix for the formulation of electrospun ASDs, greatly influenced productivity of the process as well as fiber properties. In accordance of developing EE based electrospun fiber, ethanol-based solution could be used, and it increased the productivity. In this case, the drug-polymer intermolecular interactions enabled the complete solubilization of MEL. EE based tablets disintegrated in contact with water in less than 15 s [5].

1.7. Application of biologically active materials in electrospinning

Biologically active materials as propolis, *Calendula officinalis*, aloe, oregano are derived from renewable sources. They can be applied as a protective barrier into wound healing product as a wound covering material to prevent from infection and help to heal the wound. Combination of synthetic polymer, for example, polyethylene oxide (PEO), polyvinylpyrollidone (PVP) and others with biologically active material in one product offer excellent microenvironment for wound healing. Furthermore, synthetic polymers control the mechanical, degradation, morphological properties as well as make the electrospinning process more flexible of wound healing and other porous fiber materials considering the final form of use and needed result [48, 49, 50].

E. Adomavičiūtė et al. [51] investigated the formulation of an electrospun hydrophilic biocompatible polylactic acid (PLA) nonwoven material with propolis ethanolic extract (PEE) and silver nanoparticles (AgNPs) for wound dressing material with antiseptic and antimicrobial activity. Researchers concluded that presence of ethanol and PEE in PLA polymer solution improved the process of electrospinning by forming denser electrospun nonwoven material. Furthermore, increase of the amount of ethanol in the solution resulted in bigger average diameter of fibers, it increased from 168 ± 29 nm to 318 ± 30 nm and 370 ± 30 nm when respectively 10 wt% and 20 wt% ethanol was added. Positive antimicrobial and antifunginal activity were confirmed for all the PLA samples with AgNPs and PEE. FTIR was done in accordance to confirm the presence of functional groups of PEE compounds in PLA microfibers and it was verified. However, only trace quantities of propolis phenolic acids were released from PLA based electrospun material. This could be explained by in vitro testing as propolis ability to preserve the viability of keratinocytes, which constitute 90% of cells of epidermis, the outermost layer of skin [51]. In accordance to this scientific research, it can be concluded that FTIR test is necessary to check the presence of bioactive material in the structure of nonwoven.

In other research study of Pedram Zahra R. et al [50] another bioactive material *Calendula officinalis* (*C. officinalis*) was loaded into PCL/Zein/Gum Arabic (GA) composites and scaffolds were formulated. Studies show that *C. officinalis* extracts have antibacterial, antiseptic, antifungal, antiviral, blood coagulation properties, so it is an essential medicinal plant that is commonly used in a treatment of various kinds of skin damages, for example, burns, bruises, cuts, rashes and etc. Its extract can be used in drug delivery systems, wounds dressing, and tissue engineering made by electrospinning technique [52, 53]. Authors used three methods of electrospinning during the research: suspension electrospinning, when *C. officinalis* extract was directly added in the PCL/ZEIN/GA solution, two-nozzle electrospinning, where layers of PCL/ZEIN/GA and PCL/*C.*

officinalis were prepared by two syringes and multilayer electrospinning, where layer-by-layer scaffold was fabricated of PCL/ZEIN/GA and PCL/*C. officinalis* nanofibrous mats. Investigation results showed that PCL/Zein/GA scaffolds with the increasing amount of *C. officinalis* extract had made influence on fiber diameters as well as in the research [51] – average size increased from 449.2 ± 242.3 to 550.9 ± 231.1 nm. At the same time, strength of the scaffold decreased due to poor mechanical properties of increased quantity of natural polymer. However, no significant change occurred in elongation at break with more and less *C. officinalis* in the scaffolds. Foremost, PCL/Zein/GA/*C. officinalis* scaffolds were observed as having strong antimicrobial efficacy against gram-negative bacteria, as well as small inhibition zone against *S. aureus* (gram-positive) bacteria. This can lead to the important conclusion, that *C. officinalis*-loaded PCL/Zein/GA nanocomposite scaffolds have excellent antibacterial activity and can be a desirable biomaterial for skin regeneration [50].

Furthermore, some of the natural fiber plants, such as hemp, jute, flax, sisal, bamboo and others, are observed to maintain antibacterial activity against several pathogenic bacteria. For example, hemp (*Cannabis sativa L.*) has various cultivation purposes, such as medicine, recreational drugs as well as food. Cannabis has a very complex chemistry due to the vast number of its constituents and their possible interaction with each other. Hemp contains cannabinoids, amino acids, protein nitrogenous compounds, glycoproteins, enzymes, sugars, hydrocarbons, simple alcohols and etc. The total number of natural compounds found in hemp is higher than 500 [54, 55].

Moreover, from a chemical point of view, the main active ingredients are meroterpenoids and they are specific of *Cannabis sativa L.* plant. Usually, the highest yield of cannabinoids presents in *C. sativa* herb are cannabidiol (CBD) and cannabigerol (CBG). CBD has been shown to possess antioxidant and anti-inflammatory activity, in addition to its anticonvulsant, anxiolytic, neuroprotective, and antibiotic properties. Moreover, CBG indicates powerful anti-inflammatory, antimicrobial and analgesic properties.

Cannabigerol (CBG) is a phytocannabinoid that is present in Cannabis plant, usually in extremely low concentration, because it is just a short-life precursor of tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabichromene (CBC). This means that CBG can rapidly synthesize into its metabolites and it becomes difficult to extract CBG from the Cannabis. Therefore, CBG received attention only recently, when genetic techniques became available to allow inactivation of mentioned synthases.

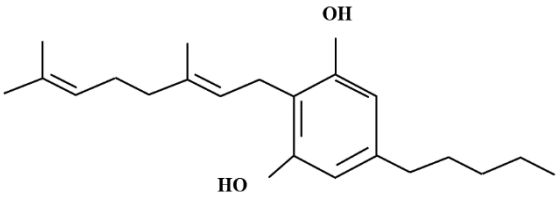


Figure 1.7.1. Structural formula of CBG [56]

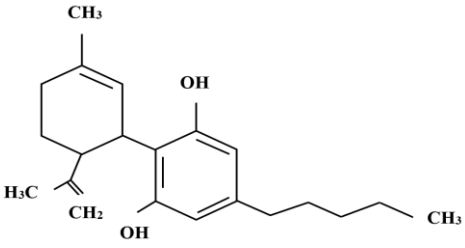


Figure 1.7.2. Structural formula of CBD [57]

The main constituent of the Cannabis plant, THC, has a psychotropic effect and this limits the possibility to test and find a possible therapeutic use. However, nonpsychotropic phytocannabinoids, such as cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG) are seen as potential

therapeutic agents that can help to cure severe conditions, such as cancer, mental disorders or inflammation [6].

Researchers Lone T.A. and Lone R.A. [58] underlined five major cannabinoids from hemp: cannabidiol (CBD), cannabichromene (CBC), cannabigerol (CBG), Δ^9 – tetrahydrocannabinol (Δ^9-THC) and cannabinol (CBN) and observed their antibacterial activity against a variety of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Investigators concluded that *Cannabis sativa* generally is very effective to killing pseudomonas aeruginosa, vibro cholerae, *Cryptococcus* neoforms, *Candida albicans*, a fungi that causes aspergillosis and oral and genital infections.

Novel studies show that CBG has a broad pharmacological profile as a nonpsychotropic phytocannabinoid and can be further investigated for chemical engineering with the aim to develop more potent drugs. Scientists already proven that CBG possess antiproliferative effects and can be suggested for cancer treatment, shows anti-inflammatory and analgesic properties, can affect mood disorders, particularly depression, can be suggested for skin conditions, eating disorders, sex hormonal dysregulations, glaucoma, bone healing and etc [6].

Experimental evidence proves a great potential of phytocannabinoids as antitumor drug and demonstrate lack of harsh side effects compared to the conventional chemotherapeutic drugs and this strongly supports their use. In the research article made by Borrelli F., et al. [56] investigation was done, whether CBG protects against colorectal tumorigenesis. As CBG is a safe to health and non-psychotropic cannabinoid (CB) derived from Cannabis, it interacts with a specific transient receptors (TRP) that are involved in carcinogenesis. Athymic nude mices were used after 1 week of acclimation period to inoculate subcutaneously colorectal cancer (CRC) cells. Tumor volumes in all the animals that were divided into 4 groups (1 control group, others treated with different dosage of CBG) were assessed on the 10th day after the inoculation and average size of the tumor reached 604±39 mm^3. Then different doses of CBG was given as a treatment: 1 mg/kg, 3 mg/kg and 10 mg/kg. CBG in exact quantities was given to the mices every day for the whole duration of experiment (5 days). Tumor size was measured every day and tumor volume was calculated in accordance. After 5 days of administrating the drugs, tumor volume in the control group was 2500 ±414 mm^3, while in the 3 mg/kg CBG-treated group was 1367 ±243 mm^3 and this means a 45,3 % hinder of tumor growth (figure 1.7.3).

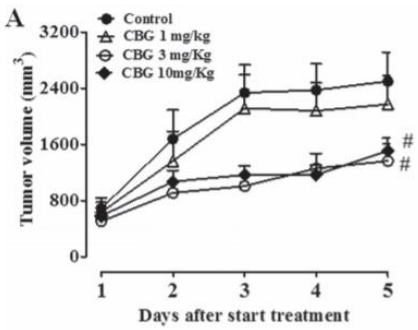


Figure 1.7.3. The effect of CBG in 3 different quantities (1 mg/kg, 3mg/kg and 10 mg/kg) on tumor volume of the athymic female mice [56]

It was concluded that CBG hinder the growth of colorectal cancer (CRC) cells mainly by the pro-apoptotic mechanism and reduces the speed of development and growth of colon carcinogenesis in vivo. This inhibitory action of CBG on the tumoral cell spread is associated to reactive oxygen species

(ROS) overproduction. Authors made a hypothesis that CBG may be a promising anti-CRC treatment as a therapeutic agent [56].

While in the research of Ligresti A. et al. [59] five plant nonpsychotropic cannabinoid compounds were tested for breast cancer treatment. Athymic mice was used for the investigations and human MDA-MB-231 breast carcinoma was injected into them. CBD, CBG, cannabichromene, CBD acid and THC acid were used as a treatment for the curing the breast cancer cells. Results showed that tumor cell growth was prohibited the most with the uptake of CBD, so CBD has a greatest potential in treatment of breast cancer.

Cannabinoids have physiological and behavioral effect on the cannabinoid receptor (CB2), which is present in the brain and spinal cord composed by wide range of immune cells. Cannabinoids acts on CB2 receptors that are composed of immune cells and modulate cell migration in the course of inflammation. Furthermore, it has recently been shown that cannabinoids can reduce inflammation by promoting apoptosis (elimination of old, unnecessary or unhealthy cells without releasing harmful substances into the surrounding area) in immune cell populations [60, 61]. Cannabinoid receptors (CB1 and CB2) and the endocannabinoid system were discovered only recently, so the research in this field expanded very quickly. CB1 mainly has influence on the cells of the central nervous system as well as in the periphery. However, CB2 is mostly expressed on the immune cells. The mechanisms through which cannabinoids influence immunosuppression can be categorized into four pathways: apoptosis, inhibition of proliferation, suppression of cytokine and chemokine production and induction of T regulatory cells (T regs) [60].

In the investigation [62] the effect of CBD on the apoptosis of thymocytes and EL-4 cells (lymphoma cancer cells) was characterized on the mice of 4-5 weeks age. Treatment of 4-8 mM CBD increased apoptosis significantly in both immature and immortalized T cells (lymphocyte immune cells that protects body from pathogens and cancer cells). Preliminary data showed that thymocytes cultured in vitro underwent spontaneous apoptosis. The apoptotic rate was approximately 10% at 1 hour in culture and 30% at 24 hours in culture. Treatment of thymocytes with 4-16 μM of CBD for 12 hours enhanced the hypodiploid apoptosis significantly in a concentration-related manner.

Treatment of thymocytes with CBD (4–16 μM) for 12 h markedly enhanced hypodiploid apoptosis in a concentration-related manner (figure 1.7.4, A).

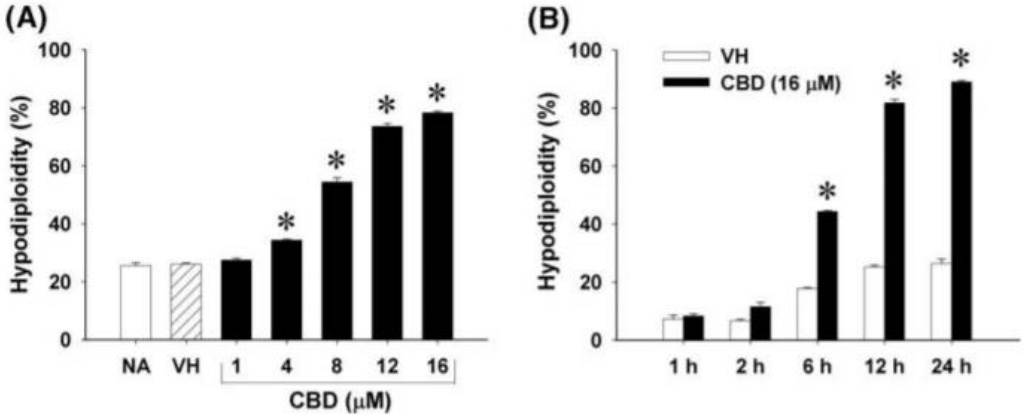


Figure 1.7.4. The effect of CBD on the production of reactive oxygen species (ROS) in thymocytes and EL-4 cells. (A) Thymocytes preloaded with DCF-DA (20 μM) for 20 min, (B) Thymocytes treated with CBD (16 μM) and/or VH (0.1% ethanol) for 0.5–6 h [62]

Maximum rate of apoptosis reached 78.3 ± 0.6% in the CBD 16 μM group. The apoptosis process was also time-related as a gradual and significant increase of the apoptic rate was detected from 6 h after CBD (16 μM) treatment in thymocytes (figure 1.7.1, B).

Furthermore, CBG possesses antibacterial properties and acts against gram-positive bacteria, mycobacteria. Additionally, CBG can be used against various methicillin-resistant *Staphylococcus aureus* strains of current clinical relevance [63]. Anti-inflammatory and anticancer properties aroused extremely high interest in CBG as a possible competitor in the developing of novel drugs to prevent, control and treat illnesses when pathological inflammations and abnormal cell proliferations shows a sign of upcoming threat [6].

Nevertheless, a number of studies are done to show CBG and CBD potential medical use, it is clear that extensive work is needed to prove clinical benefits, efficacy and demonstrate them. There is a lack of information CBG and CBD potential application in electrospinning technique, only Y. Ahn and etc. [64] had investigated electrospinnability of lignocellulosic biomass from hemp dissolved by IL (1-ethyl-3-methylimiazolium acetate [C2min][OAc] and estimated that lignin content increased diameter of nano-microfibers and diameter distribution was more uniform.

So, all the investigations and tests are important to analyze the possibility of applying CBG and CBD in textile technology.

Summary. In conclusion, electrospun materials from hydrophilic polymers and bioactive materials have a high potential for biomedical applications and it was shown in this literature analysis by citing several tens of researches. However, there was not found any research of incorporating hemp extract into the electrospun nano-microfiber, while medical advantages and superior properties of nonpsychotropic cannabinoids of *Cannabis Sativa L.* alone has been already analyzed and proven in literature and in practice. Therefore, any new research and investigation about incorporating hemp extract into the polymeric structure and electrospinning a nonwoven has a great scientific importance and requires further investigation. PVP as a hydrophilic and good mechanical property ensuring polymer that has been used in a lot of blends with other polymers was chosen as there are enough of literature to understand its advantages, as well as PMMA derivatives as Eudragit S-100 and E-100 are often used for drug encapsulation and in controlled oral drug delivery. So, this property is profitable for controlled hemp release to the targeted place of the inflammation. Incorporation of cannabis in electrospun material with possibility of full or partial release would result in a material for wound healing in mouth. For the analysis of the samples it is important to understand the viscosity of the liquid solution as it has big influence on electrospinnability as well as electrical conductivity. In order to analyze the structure of the electrospun sample SEM pictures must be taken and influence of incorporated hemp extract into the solution result on changed size of the diameter can be evaluated. Furthermore, from the SEM pictures it can be seen if nano-microfibers were formed, or solution just electrosprayed. During the FTIR test chemical structure of the molecule is displayed in the screen and functional groups of each component of the nano-microfiber can be seen by the value of the transition peak. DSC analysis should be done to evaluate if incorporation of hemp extract changed chemical structure of the polymer enough to change its glass transition temperature. While UV-VIS should make analysis of change of the color of the electrospun samples with and without hemp extract. Finally, kinetic release test is the most important, because it shows how much hemp was released from the material and this means how much drug can be released to the inflammation place- so determines the actual potential for using this material in biomedical application.

2. Methodology

2.1. Materials

To accomplish the investigations polymers and solvents were purchased. Methacrylate-based polymers Eudragit S-100 (ES) and Eudragit E-100 (EE) were supplied from Evonik Nutrition & Care (Germany) and contained Methacrylic Acid and Methyl Methacrylate Copolymer (1:2) and Amino Methacrylate Copolymer, respectively. Polyvinylpyrrolidone – PVP was purchased from Sigma-Aldrich Chemie GmbH (Germany) of the molecular mass of 1,300 000 mW. Nutritional rectified ethanol was purchased from Vilniaus Degtine (Lithuania) and contained 96% of alcohol as declared by the producer.

2.1. Preparation of hemp extract

The hemp plant was collected from experimental cultivation of hemp *Cannabis sativa* L. infolrescences (*Santicha 23)* cultivar (THC≤0.01%) carried out in Lithuania. They were harvested during September 2018. After drying, hemp plant was stored at 4 °C prior to extractions. The final moisture content of the herb was 7.37 ± 0.3%. Standards for GC analysis: cannabigerol (>98%) and cannabidiol (>99.8%) were purchased from THC Pharm GmbH (Frankfurt, Germany). All other solvents and reagents used in analytical determinations were Sigma–Aldrich Co., UK [65].

1 g of dried powdered hemp plant material was extracted with 5 mL ethanol for 4 hours at a temperature 95 C. The extract was passed through 0.22 μm diameter membrane filter.

2.2. Preparation of electrospun solutions

In this research 12 solutions were prepared for electrospinning. PVP, ES and EE were dissolved in ethanol and hemp extracts with concentration of 8 wt%. Bicomponent solutions were formed mixing PVP with ES solution in proportions of 50/50 and PVP with EE solutions in proportions of 50/50 and 75/25. Contents and codes of solutions prepared for the electrospinning are shown in table 2.2.1.

Table 2.2.1. Composition and codes of solutions prepared for electrospinning

Code of electrospun solution	Content of electrospun solution
PVP-Ethanol	8 wt% PVP/92 wt% ethanol
PVP-Hemp	8 wt% PVP/92 wt% hemp extract in ethanol
ES-Ethanol	8 wt% ES/92 wt% ethanol
ES-Hemp	8 wt% ES/92 wt% hemp extract in ethanol
EE-Ethanol	8 wt% EE/92 wt% ethanol
EE-Hemp	8 wt% EE/92 wt% hemp extract in ethanol
50/50 PVP-Ethanol/ES-Ethanol	50% (8 wt% PVP/ 92 wt% ethanol) + 50% (8 wt% ES/92 wt% ethanol)
50/50 PVP-Hemp / ES-Hemp	50% (8 wt% PVP/ 92 wt% hemp extract in ethanol compound) + 50% (8 wt% ES/92 wt% hemp extract in ethanol compound)
50/50 PVP-Ethanol/ EE-Ethanol	50% (8 wt% PVP/ 92 wt% ethanol) + 50% (8 wt% EE/92 wt% ethanol)
50/50 PVP-Hemp/EE-Hemp	50% (8 wt% PVP/ 92 wt% hemp extract in ethanol compound) + 50% (8 wt% EE/92 wt% hemp extract in ethanol compound)
75/25 PVP-Ethanol/EE-Ethanol	75% (8 wt% PVP/92 wt% ethanol) + 25% (8 wt% EE/92 wt% ethanol)
75/25 PVP-Hemp/ EE-Hemp	75% (8 wt% PVP/92 wt% hemp extract in ethanol compound) + 25% (8 wt% EE/92 wt% hemp extract in ethanol compound)

Polymeric solutions to be prepared for electrospinning were mixed on a magnetic stirrer with stainless steel heating plate (MSH basic, Yellow Line, Belgium) (figure 2.2.1). In this research option of heating was not used, only mixing of compounds by the magnet. This magnetic stirrer has a speed range from 0 to 2000 min^{-1}, heating plate surface is 125 mm diameter, stirring quantity (H_2O) is 5 l [66].

Figure 2.2.1. Magnetic stirrer with stainless steel heating plate MSH basic [66]

Compounds of PVP ethanolic and hemp extracts as well as of EE ethanolic and hemp extracts were mixing for around 3 hours at room temperature, while ES dissolved in ethanol and hemp extract in 5 hours at room temperature. The stirrer rotation speed was selected to be the same of 250 rpm for each solution. Mixtures of 50/50 PVP-Ethanol/ EE-Ethanol and 50/50 PVP-Hemp/EE-Hemp and as well as 75/25 PVP-Ethanol/EE-Ethanol and 75/25 PVP-Hemp/ EE-Hemp were mixing in the same conditions each for 5 h.

2.3. Method of solution viscosity estimation

Viscosity measurement of the electrospun solutions were made in Lithuanian University of Health Sciences with the Sine-wave Vibro Viscometer SV-10 (Japan) (figure 2.3.1).

Figure 2.3.1. Sine-wave Vibro Viscosimeter SV-10

Viscosity is measured by tuning Fork Vibration Method which enables to obtain 1 % of reading accuracy (repeatability). Viscosity is measured by detecting the driving electric current between two sensor plates at constant vibration frequency of 30 Hz. It is possible to measure the temperature of sample from 0 to 160 ˚C [67]. During the investigation viscosity of each sample was measured three times.

2.4. Method of solution electrical conductivity estimation

Measurement of conductivity was done in Faculty of Chemical Technology of Kaunas University of Technology with the Portable Multi Meter Hach HQ40d (USA) (figure 2.4.1). This two channels advanced Multi Meter is capable to measure the pH, conductivity, salinity, TDS, Dissolved Oxygen (DO), ORP and ISE for water [68]. Every solution was measured three times.

Figure 2.4.1. HQ40D Portable Multi Meter

2.5. Method of electrospinning of nonwoven materials from PVP and/or ES, EE nano-microfibers

Nonwoven materials were electrospun in Faculty of Mechanical Engineering and Design of Kaunas University of Technology on the Nanospider ^{TM} (Elmarco, Czech Republic) electrospinning machine (figure 2.5.1).

Figure 2.5.1. Nanospider™ electrospinning machine

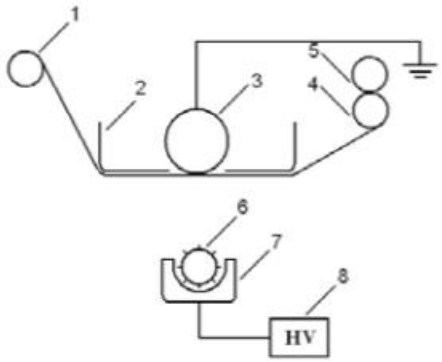


Figure 2.5.2. Nanospider electrospinning equipment: 1 – rollers of substrate material; 2 – frame of grounded electrode; 3 – 5 - rollers of support material with nonwoven mat; 6 – electrode with tines; 7 – tray with polymer solution; 8 – high voltage supply [69]

Nonwoven mats from PVP, ES and EE polymer solutions were formed using electrode with tines. As mentioned before, an electric field is necessary to be applied between a grounded electrode (3) and electrode with tines (7) (figure 2.5.2) . After applying a high voltage (in this case 50 kV and 40 kV) to a polymer solution, it becomes highly electrified and the induced charges are evenly distributed over the surface. Electrostatic forces distort polymer solution on tines from a spherical drop to a Taylor cone. The jet coming out from the electrode with tines in the beginning for a very short time flows in a straight path and then bending, winding, looping and curling of the jet occurs (figure 2.5.3). During this motion, the solvent is evaporated leaving only thin nonwoven material from nano – microfibers formed on the substrate of polypropylene material (1) made by spunbond technology (figure 2.5.4). Stationary collector – frame of grounded electrode is placed 13 cm away from the primary electrode during all electrospinning tests. Nanospider electrospinning machine offers a possibility to cover a big amount of substrate material (1) by the layer of nano-microfibers and this is the main difference from conventional (syringe) electrospinning equipment. Furthermore, in Nanospider™ a jet of polymer solution goes from bottom electrode (6) to the grounded electrode (3) (figure 2.5.2) [69].

During test was electrospun samples at:

- for 1 min at 50 kV;
- for 5 min at 50 kV;
- for 1 min at 40 kV;
- for 5 min at 40 kV.

In total 4 types of nonwoven of the same type of polymer compound, but different in thickness of nano-microfiber layers were fabricated (figure 2.5.4).

Figure 2.5.3. Electrospinning process in Nanospider

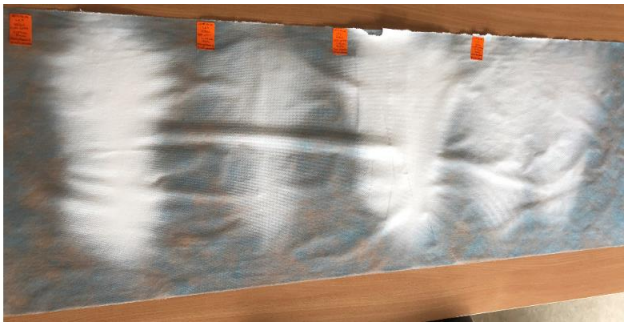


Figure 2.5.4. Electrospun Nonwoven nano-microfibers on a polypropylene (PP) substrate

During the electrospinning process, Nanospider^{TM} machine provided the information about the current that is flowing through the sample during all investigation, this means during changes of voltage and time.

2.6. Method of electrospun material structure estimation

2.6.1. Morphology estimation of nano-microfiber nonwoven

SEM pictures of electrospun nonwoven material were taken in Lithuanian Energy Institute. SEM S-3400N (Japan) (figure 2.6.1.1) does the analysis of surface structure and morphology of conductive, semi conductive and nonconductive samples. SEM has fully automatic vacuum system which can achieve $\leq 1{,}5x10^{-3}$ Pa pressure in sample chamber. 3 nm for secondary electron resolution at high vacuum and 30 kV; 10 nm at high vacuum and 3 kV. 4 nm for backscattered electron resolution at high and low vacuum and 30 kV. Machine is fully motorized, has large size 5-axis eucentric sample stage, high quality secondary and backscattered electron detectors, IR-CCD camera [70].

Material electron beam collides with the sample (figure 2.6.1.2), several types of radiation are observed. Backscattered and secondary electrons have low energy and are attracted by a detector which generates a sample image on a television screen.

5 pictures were taken for each sample in scale of 5 μm, magnification 10 000x and 50.0 μm, magnification 1000x.

Figure 2.6.1.1. SEM S-3400N

Figure 2.6.1.2. Samples prepared to be analyzed by SEM

2.6.2. Study of nano-microfiber diameter size

Measurement of the diameter of the nano-microfibers formed by electrospinning technique was done by using an imaging software NIS–Elements D 4.50.00 (Nicon Corporation) for SEM pictures. Approximately 500 nano-microfibers of each sample were measured and all the data was transferred.

2.7. Method of FTIR investigation

Fourier Transform Infrared radiation (FTIR) technique is a preferred method of infrared spectroscopy used to irradiate a sample with infrared electromagnetic radiation to excite the molecules and cause them to vibrate in a unique way. When IR radiation is passed through a sample, some radiation is absorbed by the sample and some passes through – is transmitted. The detector receives a signal and make a spectrum which represents molecular "fingerprint" of the sample. Furthermore, spectrometer can provide qualitative information such as additives or contaminants, kinetic information through the growth or decay of infrared absorptions. Interferometer measures all the frequencies simultaneously rather than splitting it into its individual spectral components as in disperse systems and produces a graph of absorbed energy by the material [71].

Measures with the FTIR spectrometer were done in collaboration with University of Boras (Sweden) during the 2019 autumn exchange semester. ThermoScientific Nicolet iS10 FTIR Spectrometer (USA) (figure 2.7.1) was used for the investigation.

Figure 2.7.1. ThermoScientific Nicolet iS10 FTIR

This spectrometer can be applied to gemstone analysis, biodiesel blending analysis, polymers and plastics, pharmaceuticals and etc. This equipment simplifies laboratory data collection, as the tests are fast, but precise [72]. Samples of electrospun material were rubbed from the substrate PP material and placed on the diamond, then spectrum was generated, and data of the spectrum of the sample was presented in a diagram on a computer screen.

Table 2.7.1. Main characteristics of the ThermoScientific Nicolet iS10 FTIR Spectrometer [72]

Metric depth	550 mm
Detector type	Fast recovery deuterated triglycine sulfate (DTGS)
Spectral resolution	7800-350 cm^{-1} optimized, mid-infrared KBr beamsplitter
Beam splitter	KBr/Ge mid-infrared optimized

FTIR investigation was done for samples:

- Pure PVP in a solid form (granules);
- Pure ES in a solid form (granules);
- Pure EE in a solid form (granules);
- PVP-Ethanol
- ES-Ethanol
- PVP-Hemp
- ES-Hemp
- 50/50 PVP-Ethanol/EE-Ethanol
- 5050 PVP-Hemp/EE-Hemp
- 75/25 PVP-Ethanol/EE-Ethanol
- 75/25 PVP-Hemp/EE-Hemp

2.8. Method of DSC investigation

Investigations with Differential Scanning Calorimetry equipment DSC Q2000 (USA) were done in collaboration with University of Boras (Sweden) during the exchange autumn semester in 2019. DSC measures heat flow associated with structure (amorphous or crystalline) of the samples, transition temperatures and changes (transitions) in structure of materials as a function of time and temperature in a controlled atmosphere. Features of this particular DSC Q2000 calorimeter are presented in 2.8.1 table [73].

Table 2.8.1. Features of the DSC Q2000 calorimeter [73]

Characteristic	Value
Temperature range	-90˚C to 400˚C
Sample weight	5-10 mg
Baseline reproducibility (Tzero)	±10 µW
Sensitivity	0.2 µW
Temperature accuracy	±0.1˚C
Temperature precision	±0.01˚C

DSC investigation was done for samples:

- PVP-Hemp;
- PVP-Ethanol;
- 75/25 PVP-Ethanol/ EE-Ethanol;
- 75/25 PVP-Hemp/ EE-Hemp.

First of all, weight of reference pan (Tzero Pan (T 190111)) and lid (Tzero Lid (T 190213)) were measured with a scale KERN ALS 120-4 (Germany) and then weight of pan and lid used for placing the sample inside was measured. Electrospun samples were scratched from the PP substrate, placed in the pan and covered with the lid and weighted. Then the lid was slightly pressed on the pan with

the Tzero Press (USA). Reference lid and pan as well as lid and pan with the sample inside were both placed into the DSC machine and the investigation started.

DSC run multiple cycles. Method includes: 1. Heating, 2. Cooling down and 3. Heating again. This consists of 3 cycles:

- Cycle 1: 25 ˚C to 250 ˚C @ 20 ˚C /min (heating 1)
- Cycle 2: 250 ˚C to 25 ˚C @ 20 ˚C/min (cooling down 1)
- Cycle 3: 25 ˚C to 250 ˚C @ 20 ˚C/min (heating 2)

Flow rate of nitrogen as pure gas was 50 ml/min. In DSC cell it provides excellent sensitivity, as its low thermal conductivity does not interfere with the heat measurement.

2.9. Kinetic release studies of hemp extract from electrospun nano-microfibers in vitro

Investigations of kinetic release were done by scientists from Lithuanian University of Health Sciences. In vitro permeation studies were conducted in modified Franz diffusion cell. During the in vitro release study of CBG and CBD from electrospun nonwoven material experiments were performed by testing the CBG and CBD dissolution of containing nano-microfibers. The buffer solution (pH 6.5) was used for the receptor phase to maintain sink condition. Approximately 20 mg of electrospun nano-microfibers were dissolved in 5 ml of buffer solution at temperature of 37 ± 0.1 ˚C. Samples of 1 ml were removed from the receptor solution at 0.25, 0.5, 1, 2, 4 hours analysis of Gas chromatography (Schimadzu GC 2010 Plus (Japan)) for determination of active compounds. Then the dissolution media was replaced by 5 mL of fresh dissolution fluid in order to maintain a constant volume.

Kinetic release investigation was done for the electrospun samples:

- PVP-Hemp;
- ES-Hemp;
- 75/25 PVP-Hemp/EE-Hemp.

2.10. Method of UV-VIS investigation

Investigations of UV-VIS for the liquid sample of hemp extract were done with Perkin Elmer Lambda 25 spectrophotometer (USA) and for the electrospun nano-microfiber samples with Perkin Elmer Lambda 35 spectrophotometer (USA) [74] in collaboration with Faculty of Chemical Technology of Kaunas University of Technology.

Perkin Elmer Lambda 25 spectrophotometer measures the wavelength in the range of 190 to 1000 nm and can be used for liquid samples analysis (figure 2.11.2), when liquid samples are poured into the quartz glass cell and together with the reference cell is placed into the machine for the measurements. While Lambda 35 spectrophotometer measures the wavelength in the same range, but mostly for solids, pastes and powder samples (figure 2.11.3). Samples are placed directed towards the irradiation light channel and pressed with the white sphere for the control of keeping the sample in a stable position during the investigation.

Figure 2.11.1. Perkin Elmer Lambda 25/35 spectrophotometer [74]

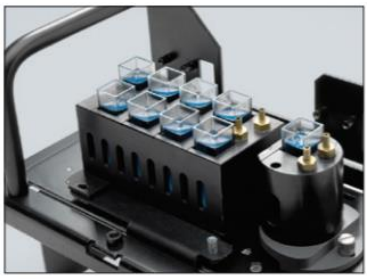


Figure 2.11.2. Quartz_ glass cells for liquid samples (Lambda 25) [74]

Figure 2.11.3. The sensitive integrating sphere for solid samples (Lambda 35) [74]

Investigation started with 2% hemp extract dissolving in ethanol and measuring the spectra of liquid solution with the Perkin Elmer Lambda 25 UV-VIS spectrophotometer and this spectra was taken as a reference for the following solid samples' spectra results.

UV-VIS investigation with Lambda 35 was done for the following solid electrospun samples:

- PVP-Ethanol
- PVP-Hemp
- EE-Ethanol
- EE-Hemp
- 75/25 PVP-Ethanol/EE-Ethanol
- 75/25 PVP-Hemp/EE-Hemp

2.11. Formulas for calculating the errors

Standard Deviation [75]:

$$S_x = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

here n - number of data points;

x_i - each of the values of the data;

$\bar{x}$ - the mean of x_i.

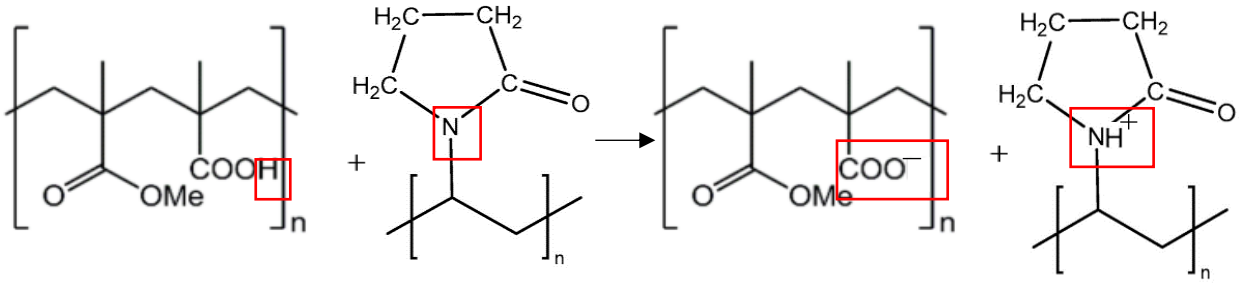
The absolute error, when sample number is <30 [75]:

$$\Delta_a = \frac{t_a * s}{\sqrt{n}}$$

here t_a – Student coefficient, for the 3 samples of a 2,483 value.

3. Results and discussion of research

In this research 12 solutions were prepared for electrospinning (see table 2.2.1). However, not all of them were possible to use for the electrospinning process, as this technique require to prepare a solution with special properties in a certain range of values of viscosity, conductivity and others that were explained before in the literature analysis part of this project. After mixing PVP-Ethanol with ES-Ethanol as well as PVP-Hemp with ES-Hemp compounds together, big bundles formed as polymers coagulated. To understand the reason why coagulation happened it was important to realize the chemistry behind it. Chemical structures of polymers, reactions between components and explanations are showed further in figure 3.1.



ES PVP ES PVP

Figure 3.1. Chemical reaction between carboxyl group in ES and nitrogen atom in PVP

During mixing of PVP and ES carboxyl groups (-COOH) of ES polymer ionize by losing protons (hydrogen cations H^+) and form a negatively charged (anionic) carboxylate groups ($-COO^-$). Meantime, PVP hydrogen cations bind by the donor-acceptor bonds to nitrogen atoms of 2-pyrrolidone heterocyclic rings of PVP molecules and form positively charged (cationic) tertiary ammonium groups ($\equiv NH^+$). Due to strong electrostatic interaction between anionic groups of ES polymer and cationic groups of PVP, the molecules of these both ionized polymers tend to form polyelectrolytic complex compounds. As a result, coagulation take place after mixing individual solutions of ES and PVP polymers. In this way, ES molecule is left with negatively charged anionic COO^- group, while PVP becomes a cation as newly formed amine has a positive charge. COO^- group in ES structure after losing hydrogen causes the coagulation during the mixing the ES and PVP polymer together.

In accordance to this, bicomponent compounds of PVP and ES with both ethanol and hemp extract were not investigated further in this research.

3.1. Analysis of the polymer solutions properties

3.1.1. Viscosity estimation of the electrospinning solutions

In accordance to the several literatures [1, 11, 16, 21, 44] which concluded viscosity of the polymeric solution is one of the most important factors of solution parameters influencing the electrospinning process and determining the structure of electrospun nonwoven material, analysis of this parameter of the samples were done during the investigation. It is reported in literature that nano-microfiber diameter of the same polymer type is mainly influenced by the solution viscosity. In scientific research [11] value of viscosity even determined the shape of the fiber – with higher viscosity flat

ribbon-like structures were obtained, instead of round fibers that were obtained with low viscosity. Furthermore, in scientific research [21] was reported that viscosity of solution must overpower the effect of surface tension of the solution so that uniform fibers would be obtained.

Viscosity of the analyzed polymer solutions with different content are presented in table 3.1.1.1. According to the data it is evident that addition of hemp extract into the polymeric compounds did not have a significant effect on the increase or decrease of the viscosity of one-component solutions prepared for electrospinning, except for PVP polymer.

Table 3.1.1.1. Results of the measurement of viscosity of prepared solutions

Code of electrospun solution	Temperature, ˚C	Average viscosity, mPa · s	Standart deviation S, mPa's	The absolute error, ±Δ
PVP-Ethanol	22,5 ~ 22,9	85,9	0,14	0,35
PVP-Hemp	23, 6 ~ 23,9	100,0	0,17	0,43
ES-Ethanol	23,8 ~ 24,0	15,2	0,12	0,30
ES-Hemp	22,9 ~ 23,1	14,4	0,21	0,51
EE-Ethanol	21,6 ~ 22,0	3,1	0,10	0,25
EE/Hemp	22,8 ~ 23,6	3,6	0,15	0,36
50/50 PVP-Ethanol / EE-Ethanol	21,2 ~ 21,4	19,6	0,29	0,72
50/50 PVP-Hemp / EE-Hemp	21,9 ~ 22,2	20,7	0,29	0,73
75/25 PVP-Ethanol / EE-Ethanol	21,3 ~ 21,8	41	0,26	0,65
75/25 PVP-Hemp / EE-Hemp	21,2 ~ 21,5	35,3	0,25	0,62

From the data (Table 3.3.1.1) it is seen that EE polymer had the lowest values of viscosity of 3,1±0,25 mPa•s and adding hemp extract into the solution did not have influence on average viscosity value, because the values changed in margins of tolerance from 3,1±0,25 mPa•s and reached 3,6±0,36 mPa•s. This means that viscosity of solution is very low, and this influence the electrospinning process, which leads to the final properties of nonwoven material. It can be concluded that by increasing the concentration of EE polymer in the solution, viscosity values would increase as well.

Highest viscosity was measured of PVP polymer solution. Value of viscosity of PVP polymer compound solubilized in ethanol was 16,4% lower than solubilized in hemp extract. For the ES polymer hemp extract did not have influence on the average viscosity, because the values changed in in margins of tolerance from 15,2±0,30 mPa•s to 14,4±0,51 mPa•s.

Analyzing the viscosity of bicomponent PVP/EE solutions is possible to notice that with increase of amount of PVP, viscosity increases too (50/50 PVP-Ethanol/EE-Ethanol has 47,8% lower viscosity than 75/25 PVP-Ethanol/EE-Ethanol samples). In conclusion, all the polymeric compounds did not have a sharp change in value of solution viscosity by incorporating hemp, except for PVP polymer. To obtain more accurate results, all samples should be investigated again to ensure the efficiency of the values.

3.1.2. Electrical conductivity estimation of the electrospinning solutions

Electrical conductivity is mentioned as one of the most important factors of the solution parameters influencing the electrospinning process and determining the structure of electrospun nonwoven material [58]. During the investigation it was also established that pure ethanol conductivity is around 1,15 ± 0,12 µS/cm, while pure hemp extract electrical conductivity is 179,5 ± 0,66 µS/cm.

Table 3.1.2.1. Measured values of electrical conductivity and temperature of the samples

Code of electrospun solution	Temperature of the sample, ˚C	Average electrical conductivity value, µS/cm	Standart deviation S, µS/cm	The absolute error, ±Δ
PVP-Ethanol	19,4	2,60	0,06	0,15
PVP-Hemp	19,4	128,53	0,19	0,48
ES-Ethanol	19,2	47,77	0,20	0,49
ES-Hemp	19,9	122,90	0,14	0,35
EE-Ethanol	19,3	8,94	0,04	0,09
EE/Hemp	20,0	155,93	0,21	0,51
50/50 PVP-Ethanol / EE-Ethanol	19,4	8,30	0,08	0,20
50/50 PVP-Hemp / EE-Hemp	19,5	151,57	0,20	0,50
75/25 PVP-Ethanol / EE-Ethanol	19,4	6,10	0,02	0,06
75/25 PVP-Hemp / EE-Hemp	18,9	155,50	0,24	0,60

From the results it can be concluded that PVP-Ethanol and EE-Ethanol samples conductivity value was 2,60±0,15 µS/cm and 8,94±0,09 µS/cm respectively, therefore after adding hemp extract the value rapidly increased by 49 times up to 128,53±0,48 µS/cm and 18 times up to 155,93±0,51 µS/cm respectively. ES-Ethanol conductivity value was higher compared with other one-component polymers dissolved in ethanol, although value of electrical conductivity increased 3 times from 47,77±0,49 µS/cm up to 122,90±0,35 µS/cm. Electrical conductivity of mixtures of pure polymers still remained in a low value, and rapid increase was seen again after adding hemp extract into the polymeric compound. 50/50 concentration bicomponent solution electrical conductivity increased by 18 times and 75/25 concentration by 26 times.

It can be concluded that hemp extract is highly electrically conductive and after mixing it with biomedical polymers the overall electrical conductivity of formed solution remains very high.

3.2. Analysis of the electrospinning process

Generally, material with low permittivity value polarizes less in response to an applied electric field compared to the material with high permittivity and stores less energy in the electric field. Permittivity can vary in accordance to the frequency of the applied field, humidity, temperature and other parameters, but it is visible that changing the voltage value, current value changes respectively.

Flow of current is another parameter mentioned in the scientific research [18] that has influence on the electrospinning process. It is explained that current flows from a high-voltage power supply to a polymer solution and caused distortion of polymer solution makes that spherical droplets deforms into a Taylor cone and form nano-microfibers. By changing the value of applied voltage, which has

a certain value for each polymer, stretching of the solution changes and if the applied voltage is too high, formation of beads can occur.

During the electrospinning process voltage of 50 kV for 1 and 5 min and 40kV for the same ranges of time were selected. At the same time current values of each polymeric compound sample were presented in the screen of Nanospider^{TM} electrospinning equipment. When voltage was higher, current value was also higher. For example, ES-Hemp at the voltage of 50 kV showed the current in the range of 0.027 to 0.041 mA, while at the voltage of 40 kV, current was in 0.010-0.018 mA value. Compounds of EE-Ethanol and EE-Hemp at 40 kV had a current value almost close to zero (from 0.000 mA to maximum 0.005 mA). From these results it can be concluded that EE and EE-Hemp samples were close to absolute dielectric permittivity.

If compare the polymer influence on the current value, then electrospinning results showed that ES-Ethanol and ES-Hemp samples had the highest current values. ES-Hemp at the voltage of 50 kV showed the current in the range of 0.027 to 0.041 mA. The lowest values were of EE-Ethanol and EE-Hemp samples with the values of 0,007 – 0,009 mA and 0,003 – 0,005 mA respectively.

Bicomponent solutions in 50/50 EE-Ethanol/PVP-Ethanol at 50 kV showed electrical current values in the range of 0,020 – 0,024 mA, while 50/50 PVP-Hemp/EE-Hemp demonstrated electrical current values in the range of 0,010 – 0,012 mA and at 40 kV 0,002 – 0,005 mA. Again, at 40 kV current value was almost close to zero (from 0.000 mA to maximum 0.005 mA).

When the influence of change in the value of current of polymers dissolved in ethanol or in hemp extract is analysed, it can be concluded, that for PVP and ES polymers, addition of hemp increased the value of current at 50 kV from 0,004 – 0,010 mA to 0,010 – 0,014 mA and from 0,020 – 0,028 mA to 0,027 – 0,041 mA, respectively. However, for EE polymer and 50/50 bicomponent compounds, the opposite results were obtained when current value was decreased by the addition of hemp extract from 0,007 – 0,009 mA to 0,003 – 0,005 mA and from 0,020 – 0,024 mA to 0,010 – 0,012 mA.

In this case, only polymer itself had a strongest influence on the current value when applied voltage is the same for each sample. However, hemp extract inclusion into the polymer solution resulted in different influence for different polymers.

3.3. Analysis of the electrospun nonwoven materials from nano-microfibers structure

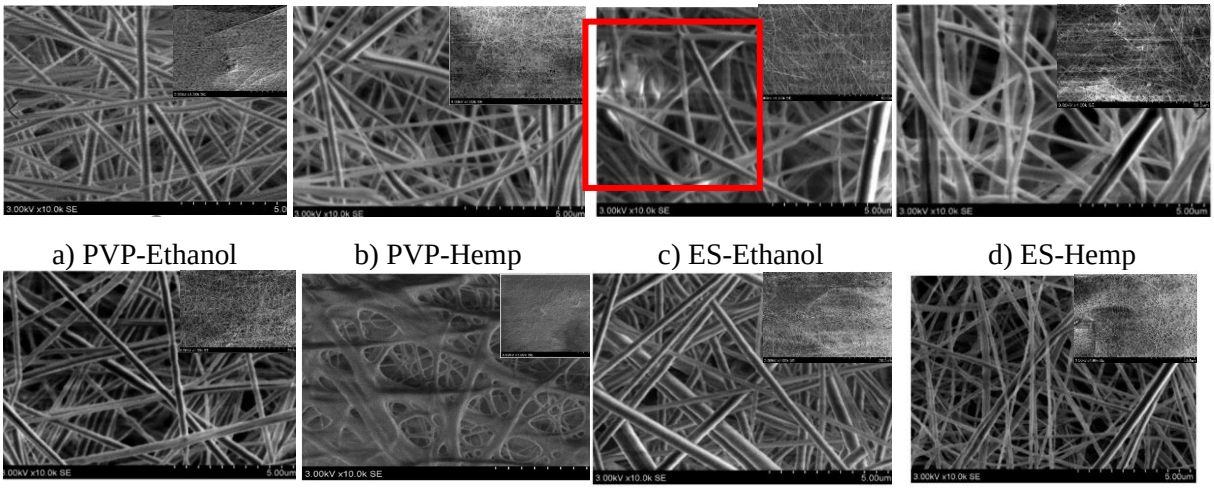
In multiple scientific research [27, 51, 96] for the analysis of nonwoven nano-microfiber morphology, nano-microstructure, diameter, particle size and defects SEM analysis were done. SEM is providing images in any region of the material and in a non-destructive way, also this method is low-cost and very efficient.

Only the samples fabricated at 50 kV applied voltage for 5 min were investigated further. From the SEM pictures (figure 3.3.2.1) it is seen that samples EE-Ethanol (c), EE-Hemp (f) were electrospraying, instead of electrospinning. Therefore, continuous nano-microfiber have not formed, it was impossible to measure the diameter of fiber and include these samples into the comparisons. It can be hypothesized, that by increasing the concentration of polymer, electrospinning process could be obtained. Alternatively, ES was used for making new polymeric compounds dissolved in ethanol

and hemp extract alone and EE was used in mixture with PVP dissolved in ethanol and in bioactive material hemp and comparisons of the nano-microfiber diameters were done.

Results of SEM investigation are divided into two parts in accordance to the targeted factor of influence: polymeric compound or hemp extract, that caused changes during the electrospinning process and morphology of the nano-microfiber nonwoven structure.

3.3.1. Hemp extract influence on the diameter of nano-microfibers



a) PVP-Ethanol | b) PVP-Hemp | c) ES-Ethanol | d) ES-Hemp

e) 50/50 PVP-Ethanol/EE-Ethanol | f) 50/50 PVP-Hemp/EE-Hemp | g) 75/25 PVP-Ethanol/EE-Ethanol | h) 75/25 PVP-Hemp/EE-Hemp

Figure 3.3.1.1 SEM pictures of the electrospun nonwoven materials in scale 5 μm, mag. 10 000x and 50 μm, mag. 1000x from: **a)** PVP-Ethanol; **b)** PVP-Hemp; **c)** ES-Ethanol; **d)** ES-Hemp; **e)** 50/50 PVP-Ethanol/EE-Ethanol; **f)** 50/50 PVP-Hemp/EE-Hemp; **g)** 75/25 PVP-Ethanol/EE-Ethanol; **h)** 75/25 PVP-Hemp/EE-Hemp

The effect of hemp extract inclusion into the polymeric solution was evaluated by the analyzing the morphology and determining the changes of structure by the SEM images (figure 3.3.1.1). According to SEM pictures that were taken to each sample in 5 different places in the scale of 5 µm (to see each nano-microfiber separately) and 50 µm (for the overall view of the structure) it is evident that PVP-Ethanol and PVP-Hemp compounds formed nano-microfibers with even diameter and surface as well as overall structure of the nonwoven. If compare 3.3.1.1 c) and 3.3.1.1 d) figures, nano-microfibers were formed during the electrospinning process, and it is seen that by adding hemp extract nano-microfibers formed more uniformly and was free of bundles that are formed in figure 3.3.1.1 c) (marked in red). This result is also presented in histogram (figure 3.3.1.2, B). It can be explained by the 3 times increased electrical conductivity of ES-Hemp sample, so electrospinning process was improved. However, EE polymer did not have enough viscosity and electrical conductivity was not enough to form nano-microfibers in the range of applied voltage and concentration that was chosen for this investigation with or without hemp extract. These results correlate with the results obtained in [27] research, where authors estimated minimum necessary concentrations for electrospinning of Eudragit S and Zein to avoid electrospraying. Bicomponent compounds of 50/50 concentrations were analyzed in the beginning and it was seen that PVP-Ethanol/EE-Ethanol nano-microfibers were not of even surface – some clusters can be seen on the nano-microfibers, and after incorporating hemp

extract into the bicomponent polymeric compound nano-microfibers formed sticked. It was impossible to measure the diameter of nano-microfibers of this sample (figure 3.3.1.1, f). This may have happened because adding hemp to this compound made the polymer jet instable. Hypothesis can be 50% concentration of EE in the polymer compound, when the weight concentration of this polymer was chosen too low to obtain electrospinning, as well as by adding hemp extract value of electrical conductivity increased 18 times, distortion of electrospinning process occured. 75/25 PVP-Ethanol/EE-Ethanol compound resulted in even nano-microfibers (figure 3.3.2.1, g) and addition of hemp resulted in nano-microfibers with small clusters and thinner in diameter (figure 3.3.2.1, h).

For each of the electrospun samples histograms were made to analyze the distribution of certain diameter of nano-microfibers to understand the percentage of mostly recorded nano-microfiber diameter sizes in the structure and compare the thickness of structures between each other. Analysis of hemp extracts' influence on the diameter of nano-microfibers is presented in figure 3.3.1.2. It was determined that 80,4 % of the nano-microfibers had a diameter in a range from 300 to 600 nm in PVP-Ethanol solution, while in PVP-Hemp diameter distribution patterns demonstrate that nano-microfibers were formed thinner, that is in the range from 100 to 400 nm of 81%. Addition of hemp extract into the PVP compound resulted in thinner nano-microfibers, because of 49 times increased electrical conductivity of the solution (figure 3.3.1.2, A).

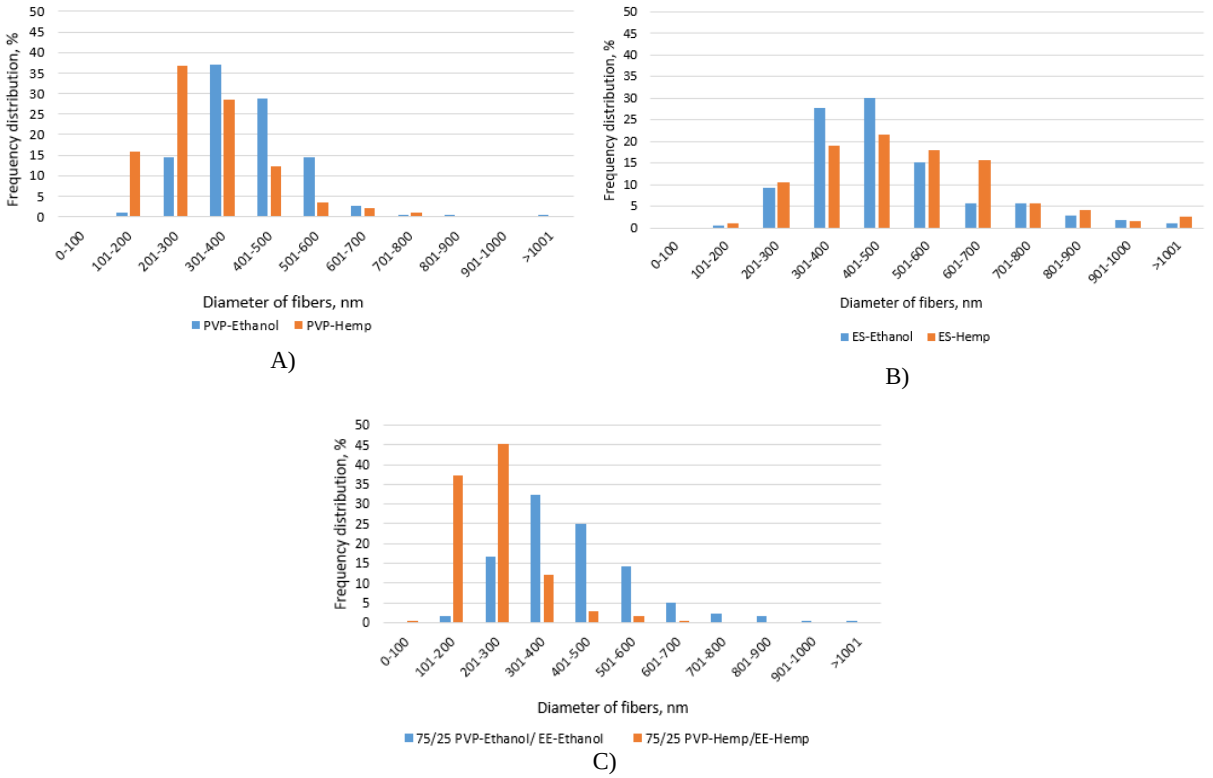


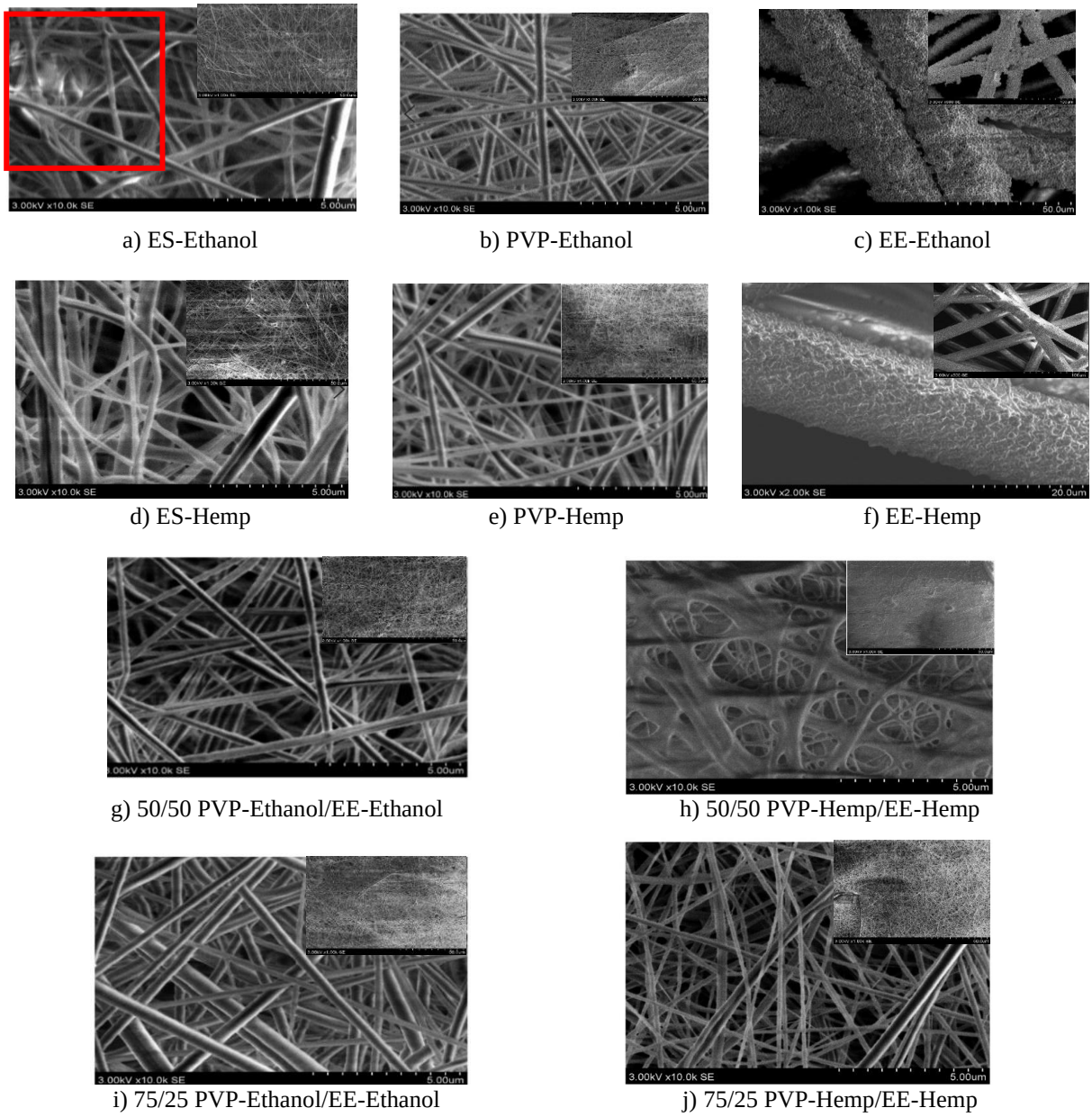
Figure 3.3.1.2. A) Distribution of diameter of PVP nano-microfibers electrospun from PVP-Ethanol and PVP-Hemp; **B)** Distribution of diameter of ES nano-microfibers electrospun from ES-Ethanol and ES-Hemp; **C)** Distribution of diameter of PVP and EE from 75/25 PVP-Ethanol/ EE-Ethanol and 75/25 PVP-Hemp/ EE-Hemp

Diameter of ES-Ethanol compounds' nano-microfibers were 82,2% in the range of 300-600 nm, while ES-Hemp range of diameter of fibers was more broad – 85% of nano-microfibers were in a range of diameter from 200 to 700 nm (or 58,6% in the range of 300 to 600 nm). Adding hemp extract

into the ES polymer compound made the range of diameter of nano-microfibers broader, that means that structure is less even with more nano-microfibers that are different in their diameter (figure 3.3.1.2, B).

In concentration of 75/25 PVP-Ethanol/EE-Ethanol 74% of nano-microfibers diameter was in the range of 200 to 500 nm, while adding hemp extract to this compound resulted in 94% of nano-microfibers of diameter with 100 to 400 nm (figure 3.3.1.2, C). This result is similar to the PVP-Ethanol and PVP-Hemp compounds results, when addition of hemp extract resulted in thinner nano-microfibers.

3.3.2. The influence of electrospun polymer solution content on diameter of nano-microfibers



a) ES-Ethanol b) PVP-Ethanol c) EE-Ethanol

d) ES-Hemp e) PVP-Hemp f) EE-Hemp

g) 50/50 PVP-Ethanol/EE-Ethanol h) 50/50 PVP-Hemp/EE-Hemp

i) 75/25 PVP-Ethanol/EE-Ethanol j) 75/25 PVP-Hemp/EE-Hemp

Figure 3.3.2.1. SEM pictures of the electrospun nonwoven materials in scale 5 μm, mag. 10 000x and 50 μm, mag. 1000x **a)** ES-Ethanol; **b)** PVP-Ethanol; **c)** Electrosprayed EE-Ethanol in scale 50 μm, mag. 1000x and 100 μm, mag. 500x; **d)** ES-Hemp; **e)** PVP-Hemp; **f)** Electrosprayed EE-Hemp in scale 20.0 μm, mag. 2000x and 100 μm, mag. 500x; **g)** 50/50 PVP-Ethanol/EE-Ethanol; **h)** 50/50 PVP-Hemp/EE-Hemp; **i)** 75/25 PVP-Ethanol/EE-Ethanol; **j)** 75/25 PVP-Hemp/EE-Hemp

Three polymers were used to electrospin nano-microfibers in ethanol and in hemp extract solutions: PVP, ES and EE. From the SEM pictures it is seen depending on the polymer type, nano-microfiber structure nonwoven were formed the most even of PVP polymer (figure 3.3.2.1, b). ES polymer dissolved in ethanol solution have the highest electrical conductivity from all other samples, however, fibers are glued together with some small bundles (figure 3.3.2.1, a) marked in red). EE polymer electrosprayed and nano-microfibers were not formed (figure 3.3.2.1, c). Hypothesis could be because of very low viscosity of 3.1 ± 0,25 mPa*s of EE-Ethanol compound and 3,6 ± 0,36 mPa*s of EE-Hemp compound.

As it was mentioned before, bicomponent solution of PVP and ES did not form because of their chemical structure, so instead of ES, EE polymer was used. In 50/50 concentration of PVP and EE polymers dissolved in ethanol it is seen that nano-microfibers are formed (figure 3.3.2.1, g), although not of even diameter, while after incorporating hemp extract, electrospinning process was distorted and nano-microfibers were formed sticked (figure 3.3.2.1, h). By changing the concentration of the solution and adding 75% of PVP and 25% of EE, value of electrical conductivity increased 28 times, but as there was higher percentage of PVP polymer in the structure and nano-microfibers were formed of even surface and even overall structure of nonwoven was obtained (figure 3.3.2.1, i). As well as after incorporation hemp extract into the polymeric solution, nano-microfibers were formed, although it is seen that their surface is changed and is not so sharp anymore (figure 3.3.2.1, j).

In concentration of 75/25 PVP-Ethanol/EE-Ethanol high viscosity of PVP (85,9 ± 0,35 mPa*s) overpowered the low viscosity of EE (3.1 ± 0,25 mPa*s) and nano-microfibers with diameter of 425,09 ± 13,08 nm were formed. Adding hemp to this concentration of polymers reduced the diameter of electrospun nano-microfibers to the 235,50 ± 7,49 nm.

In conclusion, PVP polymer formed the most appropriate nano-microfiber structure and had the lowest impact of the hemp extract incorporated into the structure. For the bicomponent compound, increased percentage of PVP resulted in a better-quality nano-microfiber nonwoven structure.

Analysis of the polymeric solutions' influence on the diameter of nano-microfibers is presented in figure 3.3.2.2. It was determined that the biggest amount of nano-microfibers in ES-Ethanol compound was 30% in the range of 401 to 500 nm, while PVP-Ethanol nano-microfibers were thinner, because 37% of nano-microfibers were distributed in the range of 301 to 400 nm (figure 3.3.2.2, A). It is well known that from solution of the same polymer type, but of higher viscosity, thicker nano-microfibers are formed [88, 27]. Viscosity of PVP solution is 5 times higher than ES solution, but from PVP is possible to form thinner nano-microfibers. Such results show that type of polymer has significant influence on the structure of electrospun material and to compare viscosity of different polymers types is not correct.

Adding hemp extract into the ES polymer compound made the distribution of different diameter of fibers more even and 21,6 % was maximum amount of nano-microfibers ranged in 401 to 500 nm, while 36,8% of nano-microfibers formed of PVP-Hemp compound were ranged in the 201-300 nm (figure 3.3.2.2, B).

Different concentrations of polymer in the ethanol solution compounds resulted that 48% of 50/50 concentration fibers were in the range of 301 to 400 nm, while in the concentration ratio of 75/25 the percentage in the same range of fiber diameter was 32,2% (figure 3.3.2.2, C).

As 50/50 PVP-Hemp/EE-Hemp nano-microfibers were formed sticked, only data of 75/25 PVP-Hemp/EE-Hemp was obtained, and results showed that 94,6% of nano-microfibers were distributed in the diameter range from 100 to 400 nm. (figure 3.3.2.2, D).

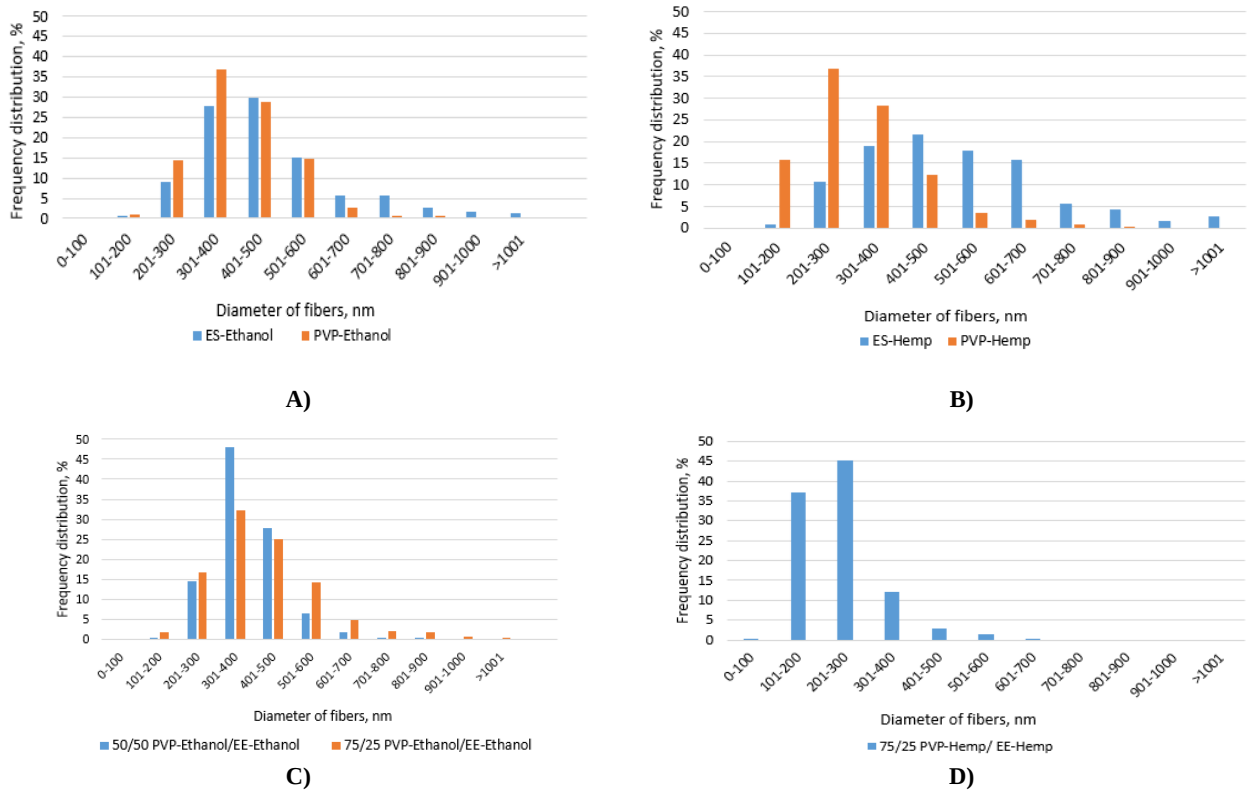


Figure 3.3.2.2. Distributions of diameter of **A)** ES and PVP nano-microfibers electrospun from ES-Ethanol and PVP-Ethanol; **B)** ES and PVP nano-microfibers electrospun from ES-Hemp and PVP-Hemp; **C)** PVP and EE nano-microfibers electrospun from 50/50 PVP-Ethanol/ EE-Ethanol and 75/25 PVP-Ethanol/ EE-Ethanol; **D)** PVP and EE nano-microfibers electrospun from 75/25 PVP-Hemp/EE-Hemp

In conclusion, diameter of nano-microfibers was influenced by the polymer itself as well as by the chose of the solvent. Hemp extract significantly increased electrical conductivity of solutions, this is why it had a great influence on the diameter of formed nano-microfibers when compared with the samples where polymers were dissolved in ethanol.

3.4. Analysis of Fourier Transform Infrared Spectroscopy of electrospun material

Analysis of the nonwoven material from nano-microfibers by FTIR equipment was done in a research [51] where PLA with PEE and AgNPs were electrospun for wound dressing material with antiseptic and antimicrobial activity. FTIR was done in accordance to confirm the presence of functional groups of PEE compounds in PLA microfibers and it was verified.

FTIR was done for pure polymers EE, ES and PVP, as well as for compounds dissolved in ethanol and hemp and mixtures of them.

Figure 3.3.5 shows that characteristic peaks of EE are quite intensive and narrow. At 1146 cm^{-1}, 1239 cm^{-1} and 1273 cm^{-1} C-O stretching's of ester are seen, at 1383 cm^{-1}, 1457 cm^{-1} and 2954 cm^{-1} C-H

vibrations and at 1726 cm^{-1} C=O (carbonyl) stretching of ester is visible. At 2767 cm^{-1} and 2820 cm^{-1} peaks are because of the vibration of dimethylamino groups [76].

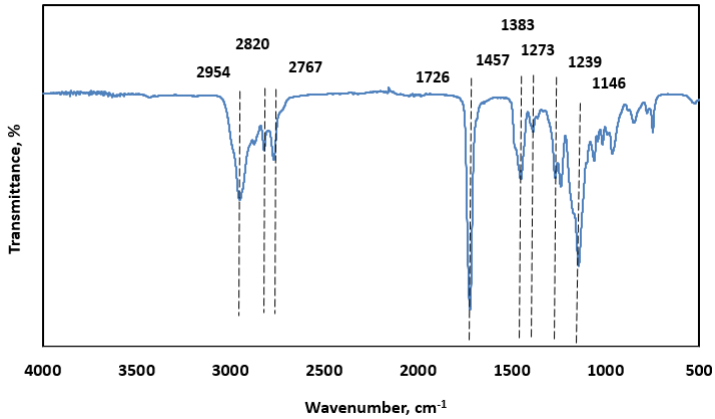


Figure 3.3.5. FTIR spectra and peaks of pure EE

In figure 3.3.6 peaks of ES, ES-Ethanol and ES-Hemp are presented. The strongest peaks are at: OH stretch at 3230 cm^{-1}, aliphatic CH stretching at 2990 cm^{-1}, aromatic C-H alkene stretching at 2950 cm^{-1}, C=O stretching at 1720 cm^{-1}, at 1390 cm^{-1} CH3 bending, C-O stretching (aromatic ester) at 1250 cm^{-1}, C-O stretching at 1140 cm^{-1}, C=C bending at 961 cm^{-1}. Similar results of the Eudragit S-100 FT-IR were obtained in [77] research. ES and ES-Ethanol samples' values of the peaks did not have any sharp changes in values, only in OH stretch place at 3230 cm^{-1} the peak shows lower transmittance because of OH functional groups in ethanol chemical structure.

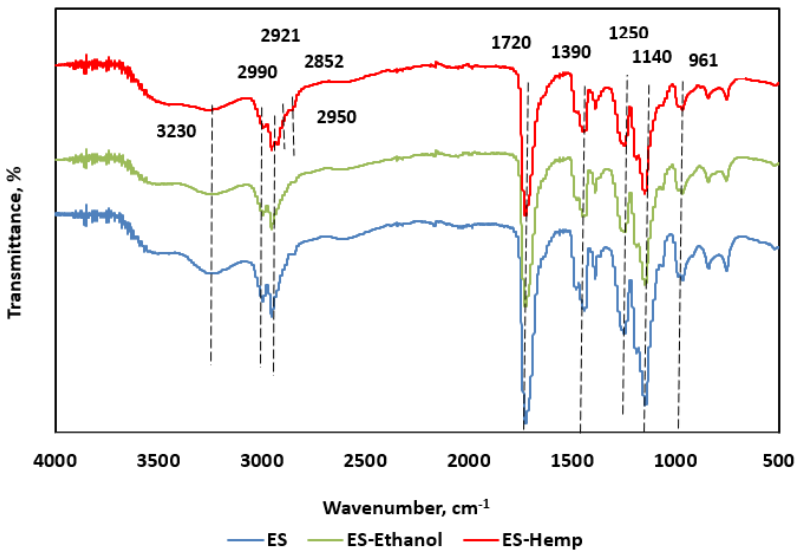


Figure 3.3.6. FTIR spectra of pure ES, ES-Ethanol and ES-Hemp

On the spectra of hemp extract all the functional groups that were seen in pure ES and ES-Ethanol samples were seen as well in this sample. However, small peaks can be noticed at 2921 cm^{-1} and 2852 cm^{-1}.

In figure 3.3.7 peaks of the PVP, PVP-Ethanol and PVP-Hemp FT-IR spectra are presented. OH stretching at 3468 cm^{-1}, CH2 bending at 2955 cm^{-1} and 2915 cm^{-1}, C-H stretching at 2876 cm^{-1}, C=O stretching at 1657 cm^{-1}, C-H stretching at 1422 cm^{-1}, C-N stretching at 1279 cm^{-1} were seen

for both PVP and PVP-Ethanol samples. Researchers Safo et al. [78] did FT-IR for PVP and obtained similar spectra.

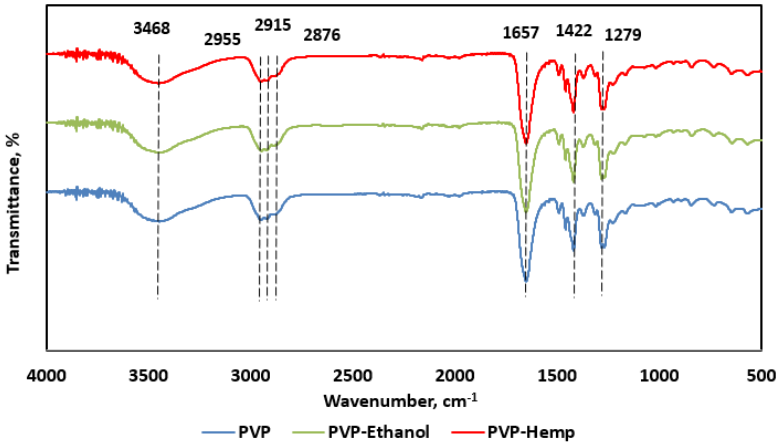


Figure 3.3.7. FTIR spectra of PVP, PVP-Ethanol and PVP-Hemp sample

In figure 3.3.7 also spectra of PVP-Hemp nano-microfiber nonwoven sample is presented. Despite the incorporation of hemp extract, there is no evidence of CBD of CBG. Hemp functional groups (Benzene ring vibrations from 2000 to 1700 cm-1 and OH group stretch at 3200 to 3500 cm-1)) were not seen in the spectra. Hypothesis could be done that by higher concentration of hemp, functional groups of hemp may be detected.

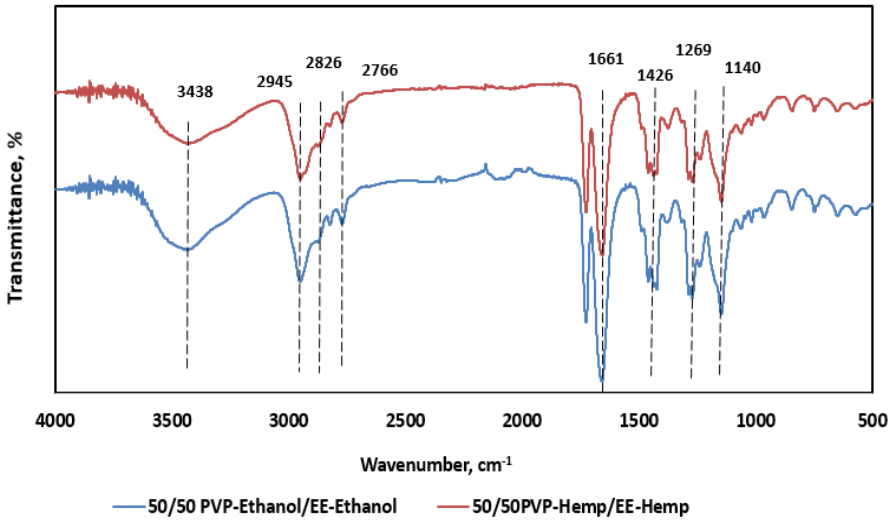


Figure 3.3.8. FTIR spectra of 50/50 PVP-Ethanol/EE-Ethanol and 50/50 PVP-Hemp/EE-Hemp

In figure 3.3.8 spectra of 50/50 PVP-Ethanol/EE-Ethanol and 50/50 PVP-Hemp/EE-Hemp are presented. The peaks are visible at around: OH stretching at 3438 cm^{-1}, CH2 bending at 2945 cm^{-1} and 2826 cm^{-1}, C-H stretching at 2766 cm^{-1}, C=O stretching at 1661 cm^{-1}, C-H stretching at 1426 cm^{-1}, C-N stretching at 1269 cm^{-1} , C-O stretching at 1140 cm^{-1} for both samples with and without incorporated hemp extract.

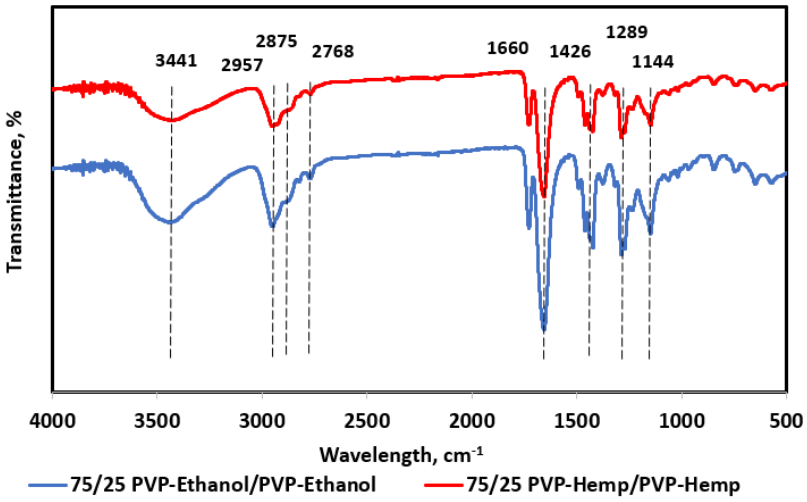


Figure 3.3.9. FTIR spectra and peaks of 75/25 PVP-Ethanol/EE-Ethanol and 75/25 PVP-Hemp/EE-Hemp

In figure 3.3.9 spectra of 75/25 PVP-Ethanol/EE-Ethanol and 75/25 PVP-Hemp/EE-Hemp are presented. The peaks are visible at around: OH stretching at 3441 cm^{-1}, CH2 bending at 2957 cm^{-1} and 2875 cm^{-1}, C-H stretching at 2768 cm^{-1}, C=O stretching at 1660 cm^{-1}, C-H stretching at 1426 cm^{-1}, C-N stretching at 1289 cm^{-1} , C-O stretching at 1144 cm^{-1} for both samples with and without incorporated hemp extract.

From the obtained FTIR spectra it can be concluded that hemp extract cannot be seen in the chemical structure of the electrospun nano-microfiber material. Hypothesis can be that currently the concentration of hemp extract in the structure is too low, so by increasing the concentration, functional groups of CBD and CBG could be noticed in the spectra.

3.5. Analysis of Differential Scanning Calorimetry of electrospun material

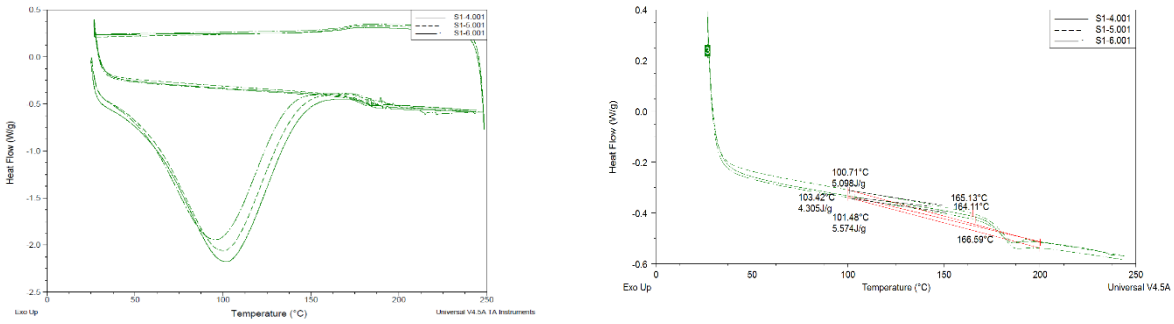
DSC is a technique used to investigate the response of polymers exposed to heat. DSC shows the results when polymer starts to crystallize, melt T_m or its glass transition temperature T_g. However, not all polymers undergo all three transitions during heating. The crystallization and melting peaks are observed only for polymers that can form crystals. Purely amorphous polymers undergo a glass transition peak.

The set-up of DSC equipment is composed of a measurement chamber where number of pans are placed and a computer to monitor the temperature and regulate the rate at which the temperature of the pans changes. Two pans are heated in the measurement chamber during one measurement. The sample pan contains material, that has to be investigated, while second pan is typically empty as it is used as a reference pan. The rate of temperature change for a given amount of heat will be different

between the pan with sample and reference pan, because of the composition of the content that is placed in the pan as well as physical changes, such as phase changes.

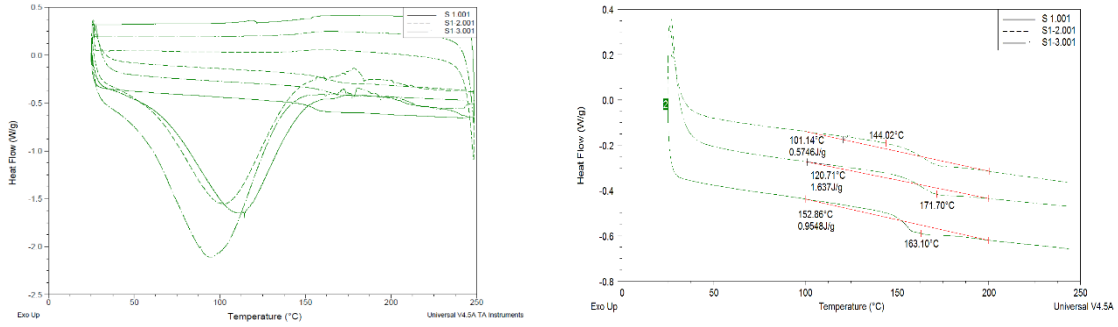
Both PVP and PMMA polymers are of an amorphous structure and can be characterized by their glass transition temperature. The value of Tg of an amorphous polymer determines its physical as well as chemical properties and is a crucial parameter in the understanding of material performance.

In figure 3.5.1 DSC thermogram of PVP-Ethanol is presented. It is seen that the glass transition temperature for PVP is in the range of 149 to 156 ˚C, and then it degrades at 175 to 200 ˚C. Furthermore, a broad endothermic event is visible from 35 ˚C to 100 ˚C due to the evaporation of adsorbed moisture by the hydrophilic PVP polymer. This result is similar with the result obtained in [79].



a) b)

Figure 3.5.1. 3 repetitions of PVP-Ethanol sample test during a) 3 full cycles; b) during the second cycle at the temperature range from 100 to 200 ˚C

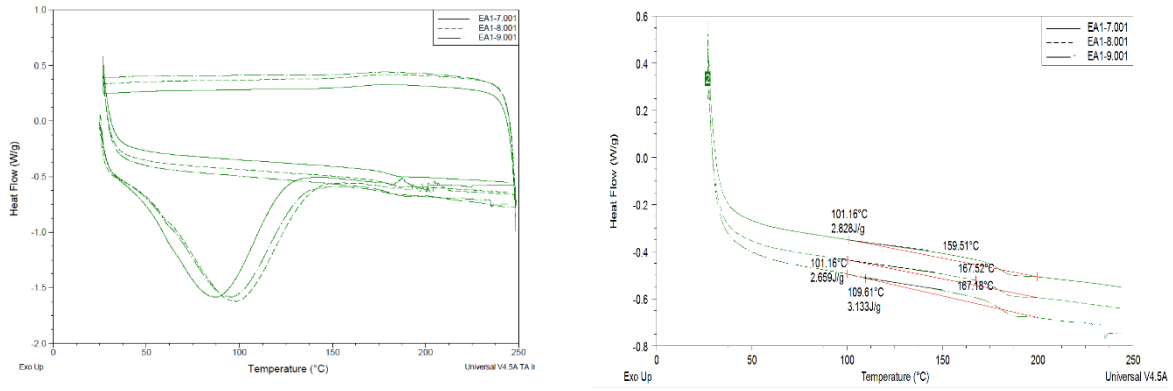


a) b)

Figure 3.5.2. 3 repetitions of PVP-Hemp sample test during a) 3 full cycles; b) during the second cycle at the temperature range from 100 to 200 ˚C

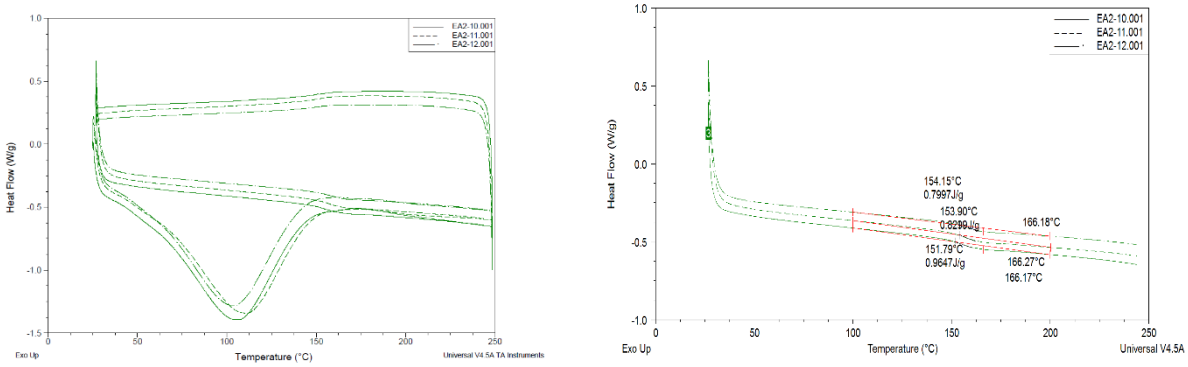
In the figure 3.5.2 endothermic event as well as glass transition and degradation temperatures are seen, so it can be concluded that PVP-Ethanol and PVP-Hemp samples contain the PVP, although it is not possible to see the effect of incorporated hemp extract in the composition.

In accordance to the scientific research [80], glass transition temperature of EE is at 45 ˚C, however, from the following graphs (figure 3.5.3 and 3.4.4) it is not possible to verify that EE is present in the samples.



a) b)

Figure 3.5.3. 3 repetitions of 75/25 PVP-Ethanol/EE-Ethanol sample test during a) 3 full cycles; b) during the second cycle at the temperature range from 100 to 200 ˚C



a) b)

Figure 3.5.4. 3 repetitions of 75/25 PVP-Hemp/EE-Hemp sample test during a) 3 full cycles; b) during the second cycle at the temperature range from 100 to 200 ˚C

To conclude, from the DSC thermograms PVP glass transition temperature and broad endothermic event is visible, however, it was impossible to verify the presence of EE polymer in the structure, as well as there was no evidence on changes of thermograms because of the hemp extract influence.

3.6. Analysis of hemp extract kinetic release from electrospun material

Drug delivery technique has a very important requirement for the quality of the function – release of dosage of the drug has to be controlled. The purpose controlling the release system is to obtain wanted drug concentration in the blood or in the target tissues at a desired value as long as possible. This means it is possible to control drug release amount and continuation. There is a big amount of formulations, devoted to oral controlled drug release as well as varied physical properties that has influence on the release of the drug from these formulations. Release can vary in its patterns – slow rate or initial rapid dose rate, followed by slow order release of sustained component. In general, for rapidly attaining the effective therapeutic concentration of the drug, controlled release system initially releases part of the dose contained in the medicine. After this, kinetics of the drug release follows a distinct behavior in order to supply the maintenance dose enabling the fulfillment of the chosen drug concentration.

Worth to mention, for receiving more effective products, engineers and pharmacists work together and mathematical modeling turns out to be very useful approach to predict kinetic release before the

release systems are realized. Usually it allows the measurement of the drug diffusion coefficient and resorting to model fitting on the release data obtained from experiments. In this case, mathematical modeling is very important in the process of optimization of the awareness of all the phenomena that can affect kinetics of drug release [81].

In the research of E. Adomavičiūtė et al. [51] kinetic release of propolis phenolic acid test was done for the electrospun nonwoven from PLA, PEE and AgNPs. Results showed that only trace quantities of propolis phenolic acids were released from PLA based electrospun material. This could be explained by *in vitro* testing as propolis ability to preserve the viability of keratinocytes, which constitute 90% of the cells of epidermis, the outermost layer of skin [51].

Kinetic release determines the release of the bioactive material from the nonwoven nano-microfiber material. In this way bioactive material with some special properties are released to the inflammation place and cure. Kinetic release was done in scientific research [51] when PEE and AgNPs were incorporated into PLA, but only low amount of PEE was released from PLA based nano-microfiber materials.

During the in vitro release study in Lithuanian University of Health Sciences of CBG and CBD from electrospun nonwoven mats experiments were performed by testing the CBG and CBD dissolution of containing nano-microfibers.

Kinetic release investigation was done for the samples:

- PVP-Hemp;
- ES-Hemp;
- 75/25 PVP-Hemp/EE-Hemp.

Results are presented in the following tables:

Table 3.6.1. PVP-Hemp kinetic release test results

Actives	Time, h					
	0.25	0.5	1	2	3	4
CBD	60 ± 3.5	64 ±2.4	64 ±1.4	65 ± 5.1	65 ± 4.5	63 ± 2.4
CBG	75 ± 1.2	76 ±3.8	76 ±4.9	76 ± 2.5	75 ± 7.9	75 ± 2.3

Table 3.6.2. ES-Hemp kinetic release test results

Actives	Time, h					
	0.25	0.5	1	2	3	4
CBD	75 ± 1.3	78 ± 10.8	79 ± 7.8	78 ± 4.9	78 ± 9.1	79 ± 5.2
CBG	80 ± 4.6	83 ± 12.3	80 ± 9.8	81 ± 7.6	82 ± 4.8	80 ± 2.3

Analysis of the release of CBD and CBG from hemp extract from electrospun PVP and ES nano-microfibers showed that the release was immediate and constant during the investigation. Value of the release when the test was beginning (after 15 min) and when it finished (after 4 hours) were constant for CBG cannabinoid for both PVP-Hemp and ES-Hemp. However, CBD cannabinoid was released in a 5% bigger amount for both PVP-Hemp and ES-Hemp sample amount after 4 hours of

investigation. Important to note that there was no kinetic release of cannabinoids from the bicomponent sample 75/25 PVP-Hemp/EE-Hemp.

To conclude, there was a significant release of both CBD and CBG cannabinoids from one-component polymer compound electrospun into nano-microfiber structure. So, the presence of hemp extract inside the electrospun material can be confirmed. Thus, from bicomponent sample no kinetic release of cannabinoids was detected.

3.7. Analysis of the UV-VIS of electrospun material

The method of functioning of the Ultraviolet-Visible (UV-VIS) instrument is easy and straightforward. First, a beam of visible and/or UV light is separated into its component wavelengths by a prism or diffraction grating. Each single wavelength beam in turn is split into two equal intensity beams by a half-mirrored device. The sample beam passes through a small transparent container (cuvette) with a solution of the compound which is studied in a transparent solvent. The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent. Over a short period of time, the spectrometer automatically scans all the component wavelengths. The intensities of these two light beams are measured by electronic detectors and compared with each other. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm [82].

In the research of Matysiak W. et al [60] authors produced composite nano-microfibers from PVP as a polymer matrix doped with the iron oxide (Fe_2O_3) (30% wt.) and analyzed its potential of photocatalytic activity due to visible-light responsive compound. After UV-VIS spectroscopy there was indicated a slight increase in absorption level in the range of visible light wavelengths from 310 nm to 560 nm in a composite nano-microfibers with a doping with Fe_2O_3. This result leaded to conclusion that this composite nano-microfiber can be used in photocatalytic processes in this range of visible light and can be used in UV light protection shields.

The effect of hemp extract that is incorporated in the electrospun samples influence on the changing of color of the nonwoven nano-microfiber structure is analyzed by the UV-VIS irradiation. With the naked eye it was already seen that samples with incorporated hemp extract are slightly greenish in comparison with pure polymeric nonwoven structure, but further investigations must be done.

In order for a compound to have a color, it must absorb the visible light, that is in a range of the 400 nm to 800 nm of electromagnetic spectrum wavelengths. Compounds that are of a certain color absorb the light in the visible region, but human eyes see the complementary color that is reflected most completely.

Investigation started with 2% hemp extract dissolving in ethanol and measuring the spectra of liquid solution with the Perkin Elmer Lamba 25 UV-VIS spectrophotometer. Visible light spectra is presented in figure 3.7.1. Strong, narrow absorbance peak with an absorbance of 0,71 is seen at around 670 nm. It is known that materials which appear green, absorb in the red (about 650 nm) and the blue (about 425 nm) band shape and color. This spectrum indicates that hemp extract is greenish because it absorbs in the red spectra.

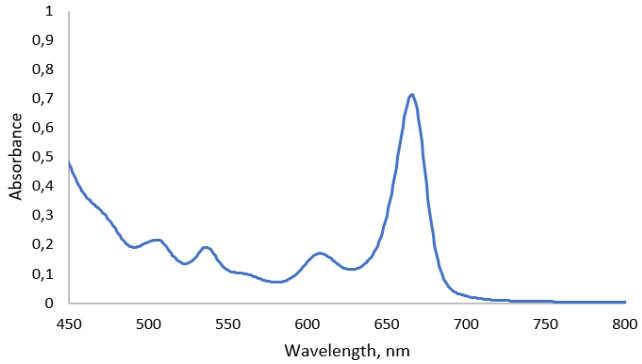


Figure 3.7.1. UV-VIS spectrum of liquid hemp extract solution

Further, investigation for the solid samples with Perkin Elmer Lambda 35 UV-VIS spectrophotometer has been done. In figure 3.7.2 the spectrums of solid samples of PVP-Ethanol and PVP-Hemp are presented. PVP-Ethanol shows a dull and broad peak at around 700 nm to 750 nm with absorbance of 0,1. While PVP-Hemp sample shows a narrow, but weak absorbance peak of 0,2 at around 660 nm. The similar results are obtained for ES polymer (figure 3.7.3), just the peak of absorbance at 660 nm is of lower intensity. For the bicomponent electrospun nano-microfiber material the peak of absorbance in red band shape and color is the most intense and absorbance reaches the 0,25 value at 660 nm.

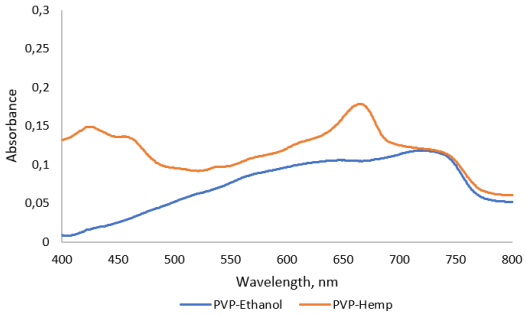


Figure 3.7.2. UV-VIS spectrum of electrospun material PVP-Ethanol and PVP-Hemp

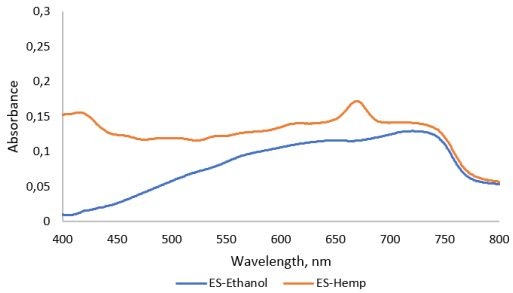


Figure 3.7.3. UV-VIS spectrum of electrospun material ES-Ethanol and ES-Hemp

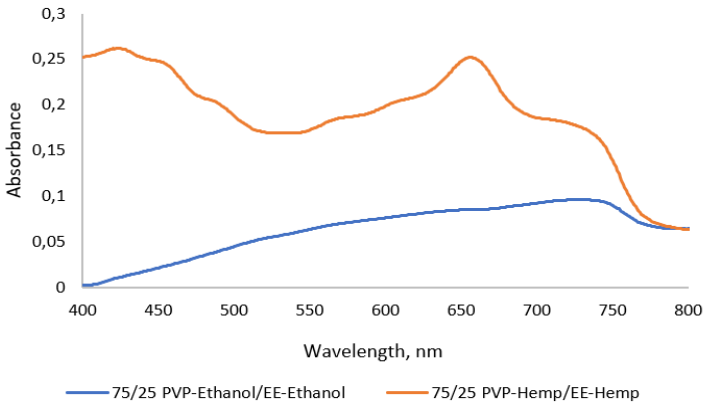


Figure 3.7.4. UV-VIS spectrum of electrospun material 75/25 PVP-Ethanol/EE-Ethanol and 75/25 PVP-Hemp/EE-Hemp

To conclude, it is evident that after incorporation of hemp extract into the polymeric compound, the nonwoven sample changes its' color and narrow peak at 660 nm is visible with the intensity of

absorbance for one-component compound at 0,2 and for bicomponent compound at 0,25, so the presence of hemp extract in the nonwoven nano-microfiber structure is confirmed.

Conclusions

1. In this work, electrospun material from three pharmaceutical polymers and mixtures of them dissolved in ethanol were fabricated. Hemp extract was successfully incorporated into 8 wt.% ES, EE, PVP polymers and liquid solutions for electrospinning were prepared. Furthermore, mixture of 50/50 and 75/25 concentrations of PVP and EE polymers were prepared, and 8 samples were successfully electrospun into nano-microfiber nonwoven.
2. PVP/ES mixtures of polymers coagulated, due to strong electrostatic interaction between anionic groups of ES polymer and cationic groups of PVP, the molecules of these both ionized polymers tend to form polyelectrolytic complex compounds and coagulation occur.
3. After analysing the influence of viscosity on the liquid samples, it was estimated, that viscosity of polymeric compound strongly depends on the polymer type (when concentration is the same of 8 wt.% EE-Ethanol viscosity was 3,1±0,25 mPa•s and it was too low to form nano-microfibers, ES-Ethanol – 15,2±0,30 mPa•s , PVP-Ethanol - 85,9±0,35 mPa•s). After analysing hemp extract influence on polymeric compounds viscosity, it was estimated that there are no significant changes after adding hemp extract to the compound on viscosity value, except for PVP polymer, that average viscosity value reached 100,0±0,43 mPa•s. No significant changes of viscosity value were demonstrated after incorporating hemp extract into the polymeric mixture solutions.
4. Hemp extract possess 156 times higher electrical conductivity than pure ethanol and after adding it to ES, EE and PVP polymers, rapid increase in electrical conductivity of polymeric compound was seen: 2,57 times, 18 times and 49 times respectively, while using compounds dissolved in ethanol as a reference. Bicomponent polymeric solution of 50/50 concentration electrical conductivity value increased 18 times, while 75/25 concentration - 26 times.
5. Electrospun material of uniform nano-microfibers is possible to form from PVP and ES polymers, as well as from bicomponent PVP/ EE solutions (50/50 and 75/25). After analyzing the diameter of electrospun nano-microfibers it was estimated that from PVP nor from ES and from 50/50 PVP/ EE nor from 75/25 PVP/ EE solution is possible to form nonwoven materials with thinner nano-microfibers.
6. From the obtained FTIR analysis for pure polymers and electrospun nano-microfiber nonwoven it can be concluded that hemp extract cannot be seen in the chemical structure of the electrospun nano-microfiber material. Hypothesis can be that by increasing the concentration in the structure, functional groups of CBD and CBG could be noticed in the spectra.
7. DSC thermograms show broad endothermic event at the 100°C and it can be concluded that polymers are hydrophilic due to absorbed moisture. From DSC thermograms was not possible to confirm the presence of EE and hemp extract.
8. Analysis of the release of CBD and CBG demonstrated that release was immediate and constant during the investigation. Values of the release after 15 min and after 4 hours of test were constant for CBG cannabinoid for both PVP-Hemp and ES-Hemp samples. However, CBD cannabinoid was released in a 5% bigger amount for both PVP-Hemp and ES-Hemp sample amount after 4 hours of investigation. So, the presence of hemp extract inside the electrospun material can be confirmed. There was no kinetic release of both cannabinoids from the bicomponent sample 75/25 PVP-Hemp/EE-Hemp.
9. From UV-VIS spectra analysis it is seen that after incorporation of hemp extract into the polymeric compound, narrow peak at 660 nm is visible with the intensity of absorbance for one-component compound at 0,2 and for bicomponent compound at 0,25, so the presence of hemp extract in the nonwoven nano-microfiber structure is confirmed.

Acknowledgement

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Recommendations

- It is possible to form PVP, ES and 75/25 PVP/EE electrospun materials with incorporated hemp extract and such materials may be used for biomedical application;
- In order to use such materials in biomedical application additional evaluation of particular medical properties such as analgesic or anti-inflammation must be done.

During the 2018 – 2019 academic years of Master's degree I, the author of the Master's Final Degree Project Sandra Goroškaitė, studied in École nationale supérieure des arts et industries textiles (ENSAIT) (France), Lodz University of Technology (Poland), University of Boras (Sweden) as a member of European Textile Engineering Advanced Master programme (E-TEAM) organized by the Association of Universities for Textiles (AUTEX) and coordinated by the Ghent University Departament of Materials, Textiles and Chemical Engineering.

Publications:

- S.Goroškaitė, E. Adomavičiūtė, J. Baranauskaitė – „The Influence of Pharmaceutics Polymers Type on Structure of Electrospun Material“ (Industrial Engineering 2020)
- It is planning to prepare a scientific paper S.Goroškaitė, J. Baranauskaitė, E. Griškonis, M. Skrifvars, E. Adomavičiūtė with preliminary title "Investigation of Structure and Properties of Electrospun Materials with Hemp Extract".

List of references

[1] - DEITZEL, Joseph M., et al. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, 2001, 42.1: 261-272. DOI: https://doi.org/10.1016/S0032-3861(00)00250-0.

[2] - KARIDURAGANAVAR, Mahadevappa Y.; KITTUR, Arjumand A.; KAMBLE, Ravindra R. Polymer synthesis and processing. In: *Natural and Synthetic Biomedical Polymers*. Elsevier, 2014. p. 1-31. ISBN: 9780123969835.

[3] – BURGESS, Kieran, et al. The Effect of Molecular Properties on Active Ingredient Release from Electrospun Eudragit Fibers. *Pharmaceutics*, 2018, 10.3: 103. DOI: 10.3390/pharmaceutics10030103.

[4] - ARAKAWA, H., et al. Endovascular embolization of the swine rete mirabile with Eudragit-E 100 polymer. American journal of neuroradiology, 2007, 28.6: 1191-1196. DOI: 10.3174/ajnr.A0536

[5] - CASIAN, Tibor, et al. Electrospun amorphous solid dispersions of meloxicam: Influence of polymer type and downstream processing to orodispersible dosage forms. International journal of pharmaceutics, 2019, 569: 118593. ISSN: 0378-5173

[6] - DEIANA, S. Potential Medical Uses of Cannabigerol: A Brief Overview. In: *Handbook of Cannabis and Related Pathologies*. Academic Press, 2017. p. 958-967. ISBN: 9780128008270.

[7]- HU, Xiuli, et al. Electrospinning of polymeric nanofibers for drug delivery applications. *Journal of controlled release*, 2014, 185: 12-21. ISSN: 0168-3659.

[8]- THERON, S. A.; ZUSSMAN, E.; YARIN, A. L. Experimental investigation of the governing parameters in the electrospinning of polymer solutions. Polymer, 2004, 45.6: 2017-2030. ISSN: 0032-3861

[9] - AGARWAL, Seema, et al. *Electrospinning: A Practical guide to nanofibers*. Walter de Gruyter GmbH & Co KG, 2016. ISBN: 9783110331806.

[10] - HUANG, Zheng-Ming, et al. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites science and technology*, 2003, 63.15: 2223-2253. ISSN: 0266-3538

[11] - STEPANYAN, R., et al. Nanofiber diameter in electrospinning of polymer solutions: Model and experiment. Polymer, 2016, 97: 428-439. ISSN: 0032-3861

[12] - ZAAROUR, Bilal; ZHU, Lei; JIN, Xiangyu. A Review on the Secondary Surface Morphology of Electrospun Nanofibers: Formation Mechanisms, Characterizations, and Applications. ChemistrySelect, 2020, 5.4: 1335-1348. DOI: 10.1002/slct.201903981.

[13] - HAIDER, Adnan; HAIDER, Sajjad; KANG, Inn-Kyu. A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. Arabian Journal of Chemistry, 2018, 11.8: 1165-1188. DOI: 10.1016/j.arabjc.2015.11.015.

[14] - DERSCH, R., et al. Nanoprocessing of polymers: applications in medicine, sensors, catalysis, photonics. *Polymers for Advanced Technologies*, 2005, 16.2-3: 276-282. ISSN: 1042-7147

[15] - RYU, Young Jun, et al. Transport properties of electrospun nylon 6 nonwoven mats. European Polymer Journal, 2003, 39.9: 1883-1889. ISSN: 0014-3057

[16] - LIU, Xiaoli, et al. Electrospinnability of poly lactic-co-glycolic acid (PLGA): the role of solvent type and solvent composition. *Pharmaceutical research*, 2017, 34.4: 738-749. ISSN: 0724-8741

[17] - NÚÑEZ, Carla Nathaly Villacís, et al. Effect of organic solvents in morphology and mechanical properties of electrospun polyvinylpyrrolidone fibers. In: Congreso de Ciencia y Tecnología ESPE. 2018. DOI: 10.24133/cctespe.v13i1.790.

[18] - SILL, Travis J.; VON RECUM, Horst A. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials, 2008, 29.13: 1989-2006. DOI: 10.1016/j.biomaterials.2008.01.011.

[19] - MATABOLA, K. P.; MOUTLOALI, R. M. The influence of electrospinning parameters on the morphology and diameter of poly (vinyledene fluoride) nanofibers-effect of sodium chloride. Journal of Materials Science, 2013, 48.16: 5475-5482. ISSN: 0022-2461

[20] - BHARDWAJ, Nandana; KUNDU, Subhas C. Electrospinning: a fascinating fiber fabrication technique. Biotechnology advances, 2010, 28.3: 325-347. ISSN: 0734-9750

[21] - WENG, Lin; XIE, Jingwei. Smart electrospun nanofibers for controlled drug release: recent advances and new perspectives. Current pharmaceutical design, 2015, 21.15: 1944-1959. DOI: 10.1002/slct.201903981.

[22]- ZAFAR, Muhammad, et al. Potential of electrospun nanofibers for biomedical and dental applications. *Materials*, 2016, 9.2: 73. DOI: 10.3390/ma9020073.

[23] - MO, X. M., et al. Electrospun P (LLA-CL) nanofiber: a biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation. Biomaterials, 2004, 25.10: 1883-1890. ISSN: 0142-9612.

[24] - CHEN, Shixuan, et al. Electrospinning: An enabling nanotechnology platform for drug delivery and regenerative medicine. Advanced drug delivery reviews, 2018, 132: 188-213. ISSN: 0169-409X.

[25] - MA, Junyu; HE, Xuezhong; JABBARI, Esmaiel. Osteogenic differentiation of marrow stromal cells on random and aligned electrospun poly (L-lactide) nanofibers. Annals of biomedical engineering, 2011, 39.1: 14-25. ISSN: 0090-6964.

[26] - MCCANN, Jesse T.; LI, Dan; XIA, Younan. Electrospinning of nanofibers with core-sheath, hollow, or porous structures. Journal of Materials Chemistry, 2005, 15.7: 735-738. DOI: 10.1039/B415094E.

[27] - KWON, Il Keun; KIDOAKI, Satoru; MATSUDA, Takehisa. Electrospun nano-to microfiber fabrics made of biodegradable copolyesters: structural characteristics, mechanical properties and cell adhesion potential. Biomaterials, 2005, 26.18: 3929-3939. ISSN: 0142-9612.

[28] - CARLBERG, Björn, et al. Electrospun polyurethane scaffolds for proliferation and neuronal differentiation of human embryonic stem cells. Biomedical Materials, 2009, 4.4: 045004. ISSN: 1748-6041

[29] - GHOLIPOUR-KANANI, Adeleh, et al. Tissue engineered poly (caprolactone)-chitosan-poly (vinyl alcohol) nanofibrous scaffolds for burn and cutting wound healing. *IET nanobiotechnology*, 2014, 8.2: 123-131. ISSN: 1751-8741.

[30] – THAKKAR, Shreya; MISRA, Manju. Electrospun polymeric nanofibers: New horizons in drug delivery. European Journal of Pharmaceutical Sciences, 2017, 107: 148-167. ISSN: 0928-0987.

[31] - AGUILAR, Ludwig Erik, et al. Electrospun polyurethane/Eudragit® L100-55 composite mats for the pH dependent release of paclitaxel on duodenal stent cover application. International journal of pharmaceutics, 2015, 478.1: 1-8. ISSN: 0378-5173.

[32] – CHOU, Shih-Feng; WOODROW, Kim A. Relationships between mechanical properties and drug release from electrospun fibers of PCL and PLGA blends. Journal of the mechanical behavior of biomedical materials, 2017, 65: 724-733. ISSN: 1751-6161.

[33] - KENAWY, El-Refaie, et al. Release of tetracycline hydrochloride from electrospun poly (ethylene-co-vinylacetate), poly (lactic acid), and a blend. Journal of controlled release, 2002, 81.1-2: 57-64. ISSN: 0168-3659.

[34] - NATU, Mădălina V.; DE SOUSA, Hermínio C.; GIL, M. H. Effects of drug solubility, state and loading on controlled release in bicomponent electrospun fibers. International journal of pharmaceutics, 2010, 397.1-2: 50-58. ISSN: 0378-5173.

[35] - ZAHEDI, Payam, et al. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. Polymers for Advanced Technologies, 2010, 21.2: 77-95. ISSN: 1042-7147.

[36] - CHARERNSRIWILAIWAT, Natthan, et al. Lysozyme-loaded, electrospun chitosan-based nanofiber mats for wound healing. International Journal of Pharmaceutics, 2012, 427.2: 379-384. ISSN: 0378-5173.

[37] - LI, Heyu, et al. Thermosensitive nanofibers loaded with ciprofloxacin as antibacterial wound dressing materials. International journal of pharmaceutics, 2017, 517.1-2: 135-147. ISSN: 0378-5173.

[38] - FRONTERA, Patrizia, et al. Manufacturing and Assessment of Electrospun PVP/TEOS Microfibres for Adsorptive Heat Transformers. *Coatings*, 2019, 9.7: 443. DOI: 10.3390/coatings9070443.

[39] - POONGUZHALI, R.; BASHA, S. Khaleel; KUMARI, V. Sugantha. Synthesis and characterization of chitosan/poly (vinylpyrrolidone) biocomposite for biomedical application. Polymer Bulletin, 2017, 74.6: 2185-2201. ISSN: 0170-0839.

[40] – LIU, Xinhua, et al. Ultrasound-mediated preparation and evaluation of a collagen/PVP-PCL micro-and nanofiber scaffold electrospun from chloroform/ethanol mixture. Fibers and Polymers, 2016, 17.8: 1186-1197. ISSN: 1229-9197.

[41] - MATYSIAK, W.; TAŃSKI, T.; ZABOROWSKA, M. Electrospinning process and characterization of PVP/hematite nanofibers. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing, 2018. p. 012050. DOI: 10.1088/1757-899X/461/1/012050.

[42] - KARAYEĞEN, Gökay, et al. Aligned polyvinylpyrrolidone nanofibers with advanced electrospinning for biomedical applications. Bio-medical materials and engineering, 2018, 29.5: 685-697. DOI: 10.3233/BME-181017.

[43] - HUANG, Siwei, et al. Preparation and properties of electrospun poly (vinyl pyrrolidone)/cellulose nanocrystal/silver nanoparticle composite fibers. Materials, 2016, 9.7: 523. DOI: 10.3390/ma9070523.

[44] - KARTHIKEYAN, K., et al. Electrospun zein/eudragit nanofibers based dual drug delivery system for the simultaneous delivery of aceclofenac and pantoprazole. *International journal of pharmaceutics*, 2012, 438.1-2: 117-122. ISSN: 0378-5173.

[45] - KARTHIKEYAN, K., et al. Development and characterization of zein-based micro carrier system for sustained delivery of aceclofenac sodium. *AAPS PharmSciTech*, 2012, 13.1: 143-149. DOI: 10.1208/s12249-011-9731-x.

[46] - KUMARI, Avnesh; YADAV, Sudesh Kumar; YADAV, Subhash C. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and surfaces B: Biointerfaces*, 2010, 75.1: 1-18. ISSN: 0927-7765.

[47] - LINARES, Valentina, et al. Relationship between Degree of Polymeric Ionisation and Hydrolytic Degradation of Eudragit® E Polymers under Extreme Acid Conditions. Polymers, 2019, 11.6: 1010. DOI: 10.3390/polym11061010.

[48] - RIEGER, Katrina A.; BIRCH, Nathan P.; SCHIFFMAN, Jessica D. Designing electrospun nanofiber mats to promote wound healing–a review. *Journal of Materials Chemistry B*, 2013, 1.36: 4531-4541. DOI: 10.1039/c3tb20795a.

[49] - ANDREU, Vanesa, et al. Smart dressings based on nanostructured fibers containing natural origin antimicrobial, anti-inflammatory, and regenerative compounds. *Materials*, 2015, 8.8: 5154-5193. DOI: 10.3390/ma8085154.

[50] - RAD, Zahra Pedram; MOKHTARI, Javad; ABBASI, Marjan. Calendula officinalis extract/PCL/Zein/Gum arabic nanofibrous bio-composite scaffolds via suspension, two-nozzle and multilayer electrospinning for skin tissue engineering. *International journal of biological macromolecules*, 2019, 135: 530-543. ISSN: 0141-8130.

[51] - ADOMAVIČIŪTĖ, Erika, et al. Formation and investigation of electrospun PLA materials with propolis extracts and silver nanoparticles for biomedical applications. *Journal of nanomaterials*, 2017, 2017. ISSN: 1687-4110.

[52] - DASGUPTA, Nandita, et al. Extraction-based blood coagulation activity of marigold leaf: a comparative study. *Comparative Clinical Pathology*, 2014, 23.6: 1715-1718. ISSN: 1618-5641.

[53] - UN, Rabia Nur, et al. Phyto-niosomes: in vitro assessment of the novel nanovesicles containing marigold extract. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 2015, 64.17: 927-937. ISSN: 0091-4037.

[54] - AHMED, Safwat A., et al. Cannabinoid ester constituents from high-potency Cannabis sativa. Journal of natural products, 2008, 71.4: 536-542. ISSN: 0163-3864B.

[55] - KHAN, Belas Ahmed; WARNER, Philip; WANG, Hao. Antibacterial properties of hemp and other natural fibre plants: a review. *BioResources*, 2014, 9.2: 3642-3659. ISSN: 1930-2126.

[56] - LOPATRIELLO, Annalisa, et al. Iodine-mediated cyclization of cannabigerol (CBG) expands the cannabinoid biological and chemical space. Bioorganic & medicinal chemistry, 2018, 26.15: 4532-4536. ISSN: 0968-0896.

[57] - ATALAY, Sinemyiz; JAROCKA-KARPOWICZ, Iwona; SKRZYDLEWSKA, Elzbieta. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants, 2020, 9.1: 21. ISSN: 2076-3921.

[58] - LONE, Tariq Ahmad; LONE, Reyaz Ahmad. Extraction of cannabinoids from Cannabis sativa L. plant and its potential antimicrobial activity. Universal Journal of Medicine and Dentistry, 2012, 1.4: 51-55. ISSN: 0022-1147.

[59] - LIGRESTI, Alessia, et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. Journal of Pharmacology and Experimental Therapeutics, 2006, 318.3: 1375-1387. DOI: 10.1124/jpet.106.105247.

[60] - RIEDER, Sadiye Amcaoglu, et al. Cannabinoid-induced apoptosis in immune cells as a pathway to immunosuppression. Immunobiology, 2010, 215.8: 598-605. ISSN: 0171-2985.

[61] - O'SULLIVAN, Saoirse Elizabeth. Cannabinoid activation of peroxisome proliferator-activated receptors: an update and review of the physiological relevance. Wiley Interdisciplinary Reviews: Membrane Transport and Signaling, 2013, 2.1: 17-25. DOI: 10.1002/wmts.73.

[62] - LEE, Chi-Ya, et al. A comparative study on cannabidiol-induced apoptosis in murine thymocytes and EL-4 thymoma cells. International immunopharmacology, 2008, 8.5: 732-740. ISSN: 1567-5769.

[63] - APPENDINO, Giovanni, et al. Antibacterial cannabinoids from Cannabis sativa: a structure activity study. Journal of natural products, 2008, 71.8: 1427-1430. DOI: 10.1021/np8002673.

[64] - AHN, Yongjun, et al. Electrospinning of lignocellulosic biomass using ionic liquid. Carbohydrate polymers, 2012, 88.1: 395-398. ISSN: 0144-8617.

[65] - BARANAUSKAITE, Juste, et al. Development of extraction technique and GC/FID method for the analysis of cannabinoids in Cannabis sativa L. spp. santicha (hemp). Phytochemical Analysis. DOI: 10.1002/pca.2915.

[66] – MAGNETIC STIRRER WITH STAINLESS STEEL HEATING PLATE MSH BASIC [online]. [Accessed 15 July, 2019]. Availabile from Internet: https://www.imlab.com/yel/YL%20ENGL/yl%20page.htm

[67] – SINE-WAVE VIBRO VISCOMETER SV-10 [online]. [Accessed 20 July, 2019]. Availabile from Internet: http://www.aandd.jp/products/test_measuring/sv10/sv10.html

[68] – HQ40D PORTABLE MULTI METER [online]. [Accessed 20 June, 2019]. Availabile from Internet: https://www.hach.com/hq40d-portable-multi-meter-ph-conductivity-salinity-tds-dissolved-oxygen-do-orp-and-ise-for-water/product?id=7640501639

[69] - PUPKEVIČIŪTĖ, Solveiga; ADOMAVIČIŪTĖ, Erika; STANYS, Sigitas. Analysis of Structure of Electrospun Nonwoven Mats from Pure PCL Nano/Micro Fibres. *Materials Science/Medziagotyra*, 2013, 19.3. ISSN: 1392-1320.

[70] – SCANNING ELECTRON MICROSCOPE S-3400N [online]. [Accessed 20 June, 2019]. Availabile from Internet: http://apc.lei.lt/en/equipment-and-services/energy-accumulation/hydrogen-energy/1/scanning-electron-microscope-229

[71]- INTRODUCTION TO FTIR SPECTROSCOPY [online]. [Accessed 20 November, 2019]. Availabile from Internet: https://www.thermofisher.com/lt/en/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental-isotope-analysis-learning-center/molecular-spectroscopy-information/ftir-information/ftir-basics.html.

[72] - NICOLET IS 10 FTIR SPECTROMETER [online]. [Accessed 20 November, 2019]. Availabile from Internet: https://www.thermofisher.com/order/catalog/product/IQLAADGAAGFAHDMAPC#/IQLAADGAAGFAHDMAPC

[73] – DIFFERENTIAL SCANNING CALORIMETER Q2000 [online]. [Accessed 20 November, 2019]. Availabile from Internet: https://www.mcgill.ca/mc2/instrumentation/thermal-analysis-and-spectroscopy/dsc-q2000

[74] – PERKIN ELMER LAMBA 25/35/45 UV-VIS SPECTROPHOTOMETERS [online]. [Accessed 20 March, 2020]. Availabile from Internet: https://www.perkinelmer.com/cmsresources/images/44-74448bro_lambda.pdf

[75] – MILAŠIUS, Rimvydas. *Tekstilės eksperimento atlikimas : metodiniai nurodymai studentams : mokomoji knyga;* Kaunas: Technologija, 2000. UDK: 677.017(075.8).

[76] - LIN, Shan-Yang, et al. DSC-FTIR microspectroscopy used to investigate the heat-induced intramolecular cyclic anhydride formation between Eudragit E and PVA copolymer. Polymer journal, 2011, 43.6: 577-580. ISSN: 0032-3896.

[77] - KUMARA, Manoj; AWASTHIA, Rajendra. Development of Metronidazole-Loaded Colon-Targeted Microparticulate Drug Delivery System. Polim. Med, 2015, 45.2: 57-65. ISSN: 0370-0747.

[78] - SAFO, I. A., et al. The role of polyvinylpyrrolidone (PVP) as a capping and structure-directing agent in the formation of Pt nanocubes. Nanoscale Advances, 2019, 1.8: 3095-3106. DOI: 10.1039/c9na00186g.

[79] - SRIYANTI, Ida, et al. Mangosteen pericarp extract embedded in electrospun PVP nanofiber mats: physicochemical properties and release mechanism of α-mangostin. International journal of nanomedicine, 2018, 13: 4927. DOI: 10.2147/IJN.S167670.

[80] - RAJABI-SIAHBOOMI, Ali R. (ed.). Multiparticulate drug delivery: formulation, processing and manufacturing. Springer, 2017. ISBN: 9781493970124.

[81] - DASH, Suvakanta, et al. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm*, 2010, 67.3: 217-23. ISSN: 0001-6837.

[82] - UV-VIS VISIBLE SPECTROSCOPY [online]. [Accessed 20 March, 2020]. Availabile from Internet: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/uv-vis/uvspec.htm