

VŠB – Technical University of Ostrava

Nanotechnology Centre

University Study Programmes - Nanotechnology

Bachelor Thesis

**Electrostatic Spinning of Polyvinyl Alcohol and its Mixture with
Silver Nanoparticles Prepared by Biosynthesis**

**Elektrostatické zvlákňování polyvinylakoholu a jeho směsi se
stříbrnými nanočásticemi připravenými biosyntézou**

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Description:

Silver nanoparticles prepared by phytosynthesis using medicinal plants can be used in medical applications in the future due to their antimicrobial properties. Plant phytochemicals that stabilize the prepared nanoparticles can also enhance the final antimicrobial or healing effect. The anchoring of the biosynthetic silver nanoparticles into the biocompatible polymeric fiber matrix can then lead to the preparation of functional material usable in medicine.

- 1) to review available literature and summarize the current knowledge in the area of nanoparticles anchoring in polymer fibers,
- 2) to prepare silver nanoparticles according to the optimized and verified biosynthesis protocol,
- 3) verify the properties of the prepared nanoparticles by available analytical techniques,
- 4) familiarize yourself with the method of electrostatic spinning of the model polymer polyvinyl alcohol (PVA),
- 5) to conduct pilot experiments of PVA polymer spinning together with silver bionanoparticles and to characterize resulting composite by electron microscopy,
- 6) evaluate the experiments performed.

References:

- 1) Engineering Textiles: Research Methodologies, Concepts, and Modern Applications. Alexandr A. Berlin, Roman Joswik, Vatin Nikolai Ivanovich, Apple Academic Press 2015, 1. vydání, ISBN-10: 1771880783, ISBN-13: 978-1771880787, 386 s.
- 2) Metal Nanoparticles in Pharma. Mahendra Rai, Ranjita Shegokar (Eds.), Springer International Publishing 2017, ISBN 978-3-319-63789-1, 480 s.
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AUTHOR DECLARATION

The work submitted in this bachelor thesis is the result of my own investigation, except where otherwise stated.

It has not already been accepted for any degree, and is also not being concurrently submitted for any other degree.

Zuzana Vilamová

Declaration

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Abstract

This bachelor thesis focuses on the study of biosynthesized silver nanoparticles and electrospinning process poly(vinyl alcohol) and its mixture with prepared nanoparticles. The theoretical part describes partly the preparation and antibacterial properties of silver nanoparticles and partly the process of electrostatic spinning with focus on preparation of poly(vinyl alcohol) fibrous samples. The experimental part aims at characterization how silver nanoparticles are prepared via phytosynthesis and polymeric nanofibers as well as mixture of polymer with nanoparticles via electrospinning method. For characterization of prepared samples, several methods such as electron microscopy, infrared spectroscopy, ultraviolet visible spectroscopy, X-ray diffraction and dynamic light scattering were used. Silver nanoparticles were successfully synthesized via phytosynthesis. Then, electrostatic spinning of poly(vinyl alcohol) and its mixture with prepared silver nanoparticles was evaluated as successful and repeatable.

Keywords:

biosynthesis; phytosynthesis; *Tilia* sp.; nanoparticles; silver; nanofibers; electrostatic spinning; poly(vinyl alcohol)

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Abstrakt

Bakalářská práce se věnuje studiu biosyntetizovaných stříbrných nanočástic a elektrostatickému zvláknění čistého polyvinylalkoholu a jeho směsi s připravenými stříbrnými nanočásticemi. Teoretická část je z části věnována přípravě a antibakteriálním vlastnostem stříbrných nanočástic a z části elektrostatickému zvláknění polymerů se zaměřením na polyvinylalkohol. Experimentální část je zaměřena na charakterizaci připravených stříbrných nanočástic fotosyntézou, zvlákněného polyvinylalkoholu připraveného elektrostatickým zvlákněním i směsi polymeru a stříbrných nanočástic.

K charakterizaci bylo použito několik metod jako: skenovací a transmisní elektronová mikroskopie, infračervená mikroskopie, ultrafialovo-viditelná spektroskopie, rentgenová difrakční analýza a metoda založená na dynamickém rozptylu světla. Nanočástice stříbra byly za pomoci fyto syntézy úspěšně připraveny. Následně elektrostatické zvlákňování polyvinylalkoholu a jeho směsi s připravenými nanočásticemi bylo zhodnoceno jako úspěšné a opakovatelné.

Klíčová slova:

biosyntéza; fyto syntéza; *Tilia* sp.; nanočástice; stříbro; nanovlákna; elektrostatické zvlákňování; polyvinylalkohol

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List of abbreviations

CIF = ciprofloxacin

CS = chitosan

DLS = Dynamic light scattering

DNA = deoxyribonucleic acid

E. coli = *Escherichia coli*

FTIR = Fourier-transform infrared spectroscopy

ISO = The International Organization for Standardization

MRSA = Methicillin-resistant *Staphylococcus aureus*

NPs = nanoparticles

PCL = polycaprolactone

PGA = poly(glycolic acid)

PLA = poly(lactic acid)

PVA = poly(vinyl alcohol)

S. aureus = *Staphylococcus aureus*

SEM = Scanning Electron Microscopy

TCH = tetracycline hydrochloride

TEM = Transmission Electron Microscopy

UV/VIS = Ultraviolet Visible Spectroscopy

XRD = X-ray Diffraction

Introduction

Current research is confronted with various problems, and antibiotic resistance to microbes leads to a serious threat to worldwide public health. Convenient processing, high efficiency and stability of metallic nanoparticles seem to be a possible solution in medical field. Many studies describe beneficial properties of colloidal silver nanoparticles prepared by phytosynthesis in which plant phytochemicals in biomass reduce and stabilize the nanoparticles and can enhance final physical-chemical, antimicrobial or healing effects. Moreover, the incorporation of silver nanoparticles into the polymeric fibers can lead to the development of progressive materials with application in medicine [1].

This bachelor thesis devotes a preparation of silver nanoparticles and their incorporation to poly(vinyl alcohol) electrospun fibers. Poly(vinyl alcohol) is synthetic, biodegradable, water-soluble polymer and is biocompatible with human organism which has led to using it especially in medical field.

The bachelor thesis is divided into two parts – theoretical and experimental part. The theoretical part is based on the literature overview in the field of biosynthesis with respect to phytosynthesis of silver nanoparticles and electrospinning process of polymer. The electrospinning method is able to produce various nanofiber membranes in the wide range of materials and by easy changes of the processing parameters. Moreover, the antibacterial properties of silver nanoparticles in mixture with polymers are discussed too.

The experimental part represents the characterization of phytosynthesized silver nanoparticles as well as electrospun polymeric nanofibers. The experimental part also comprises the discussion of obtained results and interconnection of knowledge in the theoretical part.

Theoretical part

1. Metal nanoparticles and their preparation

Nanotechnology operates in nanoscale in the range from 1 and 100 nm and nanoscale structures can have a unique chemical, optical, electrical, mechanical or thermal properties which not appear for the same bulk materials and this can outline high application potential [2]. In 2008 the International Organization for Standardization (ISO) defined a NP as a discrete nano-object where all three Cartesian dimensions are in the size less than 100 nm [3].

According to the preparation methods, nanoparticles (NPs) and/or nanomaterial preparations can be classified into two main approaches such as "top-down" and "bottom-up". The "top-down" approach contains for example laser ablation or ball-milling and these methods start with a bulk material and lead to reduction to micro- or nano-scale sizes. On the other hand, the "bottom-up" approach includes organic synthesis or chemical reduction and these build structures from the elementary building blocks (atomic and molecular constituents) to an organized structure [2].

Another view on NPs preparation: silver (Ag), gold (Au), copper (Cu), zinc oxide (ZnO) or titanium dioxide (TiO₂) etc. - is classified as chemical, physical and mechanical methods [3] and can be combined with each other. The main disadvantage of the mechanical methods such as ball-milling is low purity and wide range of particle sizes. On the other hand, milling is applicable for obtaining high amount of metallic and ceramic NPs from bulk size. Benefits of physical methods are the narrow size distribution of NPs. However, they are usually financially demanding because expensive equipment (laser etc.) or special conditions such as pressure or temperature are needed. The use of chemical methods (for example chemical reduction) is more effective than the mechanical and physical approach as narrow size distribution is reached. Another benefit is gaining nanostructures by the mixing of solutions accompanied by supporting methods such as microwave irradiation. On the other hand, some chemical methods require a use of toxic chemicals, which are dangerous and harmful to the environment. Chemical methods also involve a use of organic molecules and organisms to produce metal NPs continuously. This approach is called biosynthesis [4, 5].

1.1. Biosynthesis of metallic nanoparticles

An alternative way to produce metallic NPs is biosynthesis, which is simple, rapid and inexpensive. Different microorganisms - bacteria, yeast, microscopic fungi and plant tissues - leaf, stem, root, peel, flower - are able to synthesize NPs in different shapes such as spherical, hexagonal or rectangular (Figure 1) [6–8].

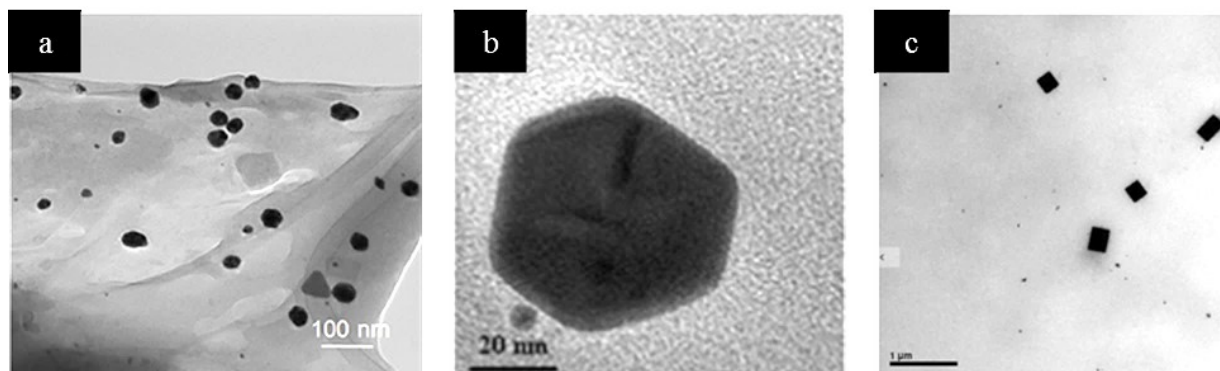


Figure 1: Different shapes of various metallic NPs prepared by using biosynthesis: (a) Au spherical NPs from *Elaeis guineensis* leaves extract; scale bar 100 nm [6], (b) TEM image of Ag hexagon produced by use of sunflower oil; scale bar 20 nm [7] and (c) rectangular Ag NPs using the marine red alga *Laurencia catarinensis*; scale bar 1 μm [8].

Microorganisms, parts of plants or their extracts/leachates are directly mixed with initial metal precursor. The precursor is a solution of metallic cations serving as basis for NPs nuclei growth. The most significant benefit is synthesis and stabilization of NPs in the same time by organic compounds. The final product can be in the form of colloid containing NPs of initial soluble salt and the precursor's transformation is usually accompanied by typical color change for every metal (Figure 2) [9, 10].

The Au NPs are mostly prepared by mixing the precursor tetrachloroauric acid (HAuCl_4) with biomass and silver nitrate (AgNO_3) is used for preparation of Ag NPs. Copper chloride (CuCl_2) is used for mixing with biomass to obtain Cu NPs and a lot of other NPs can be prepared by choosing the right initial salt. Precursors can be also mixed and bimetallic NPs can be prepared [6, 9, 11, 12].

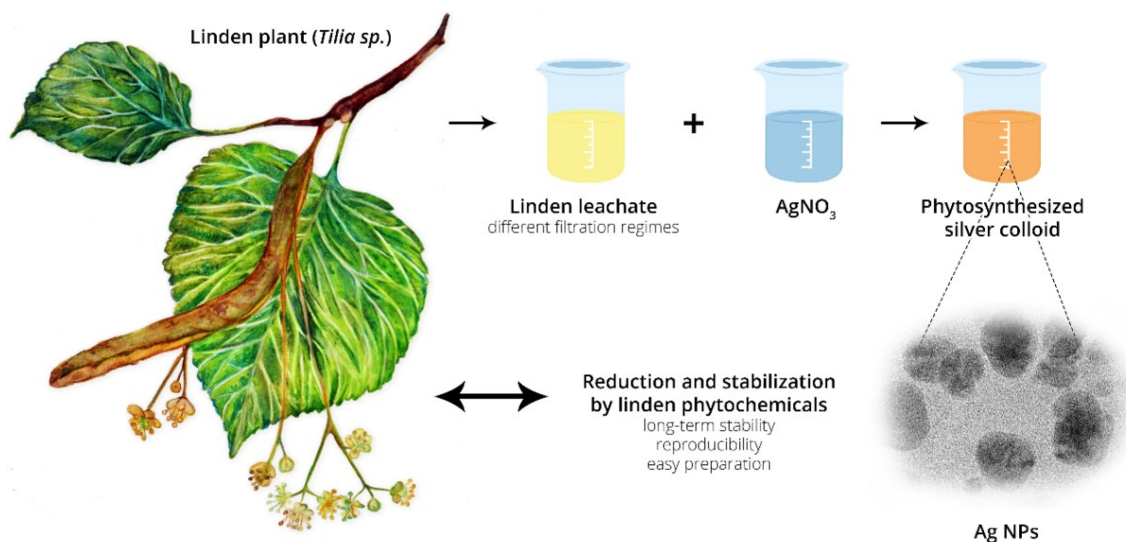


Figure 2: Schematic indication of NPs preparation process: collected biomass is mixed with initial salt precursor. This process is accompanied by visible color change of solution.

Furthermore, NPs can obtain high catalytic activity, and/or biocompatibility. They usually do not contain high contents of residual chemicals. However, the biomolecules or their residues are present in the final solution and often have stabilization function. Biosynthesis can be easily optimized by many factors: pH, type of biomass, precursor, temperature, stirring and these parameters can influence final NPs sizes and shapes [4].

1.1.1. Bacteria used for biosynthesis

The bacteria are able to reduce metal ions to the form of NPs. Enzymes, proteins and other organic compounds participate on metal ion reduction or transformation [10]. Aforementioned proteins and other metabolic residues can act as stabilizers to prevent aggregation and agglomeration of particles. Both phenomena present the creation of larger cluster of particles. Agglomeration means that cluster of particles can be damaged for example by shaking. Aggregation of the particles means that cluster of particles is an irreversible process [10].

Bacteria have been extensively studied because they can be simply cultivated and harvested. Au NPs can be synthesized by *Stenotrophomonas maltophilia*, *Rhodopseudomonas capsulate* or the thermophilic bacterium *Geobacillus species*. Au NPs ranged between 10 and 20 nm [9].

1.1.2. Fungi and algae used for biosynthesis

Majeed et al. (2017) used fungi *Penicillium italicum* for the extracellular biosynthesis of Ag NPs. They evaluated antibacterial and anticancer effects. Antibacterial studies against *Staphylococcus aureus* (*S. aureus*), *Salmonella enterica*, *Bacillus cereus*, and *Escherichia coli* (*E. coli*) showed successful bacteria inhibition [11]. Chan and Don (2012) studied five fungi species able to synthesize Ag NPs. *Pycnoporous sanguineus* was chosen as a potential candidate for synthesis Ag NPs with a yield at 98.9%. Obtaining NPs exhibited spherical shapes with average diameter from 50 to 100 nm [13].

Lakhia et al. (2017) used algae called *diatoms* naturally present in salt water and fresh water bodies. Micrographs from scanning electron microscope (SEM) (Figure 3) revealed that Ag NPs had spherical shape with different sizes from 30 to 70 nm. Electrocatalytic activity of as-prepared Ag NPs has been demonstrated towards the hydrazine [14].

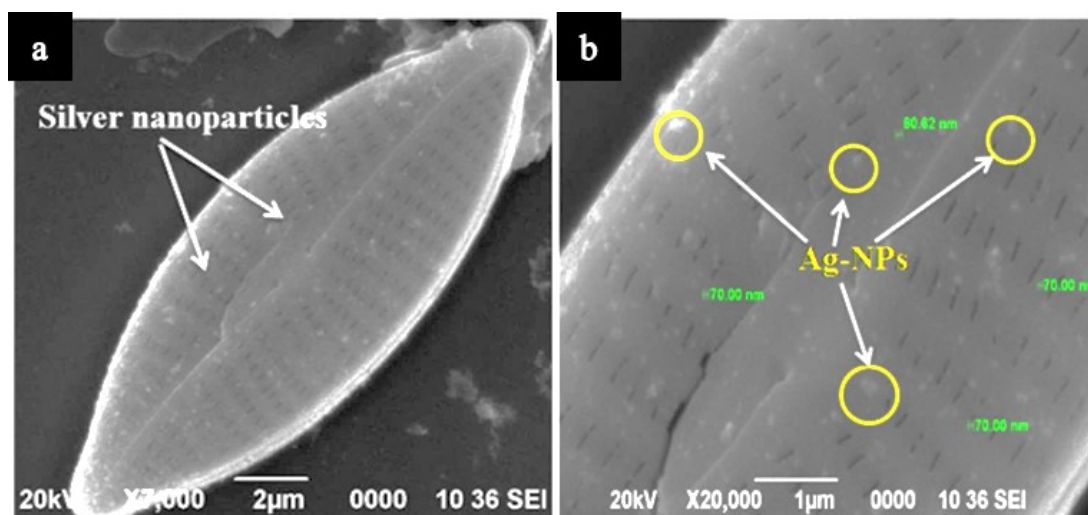


Figure 3: SEM micrographs show Ag NPs: (a) diatoms frustule made of biosilica serves as a bedding for an attachment of Ag NPs reduced and stabilized by biomolecules released from diatom cell; (b) formation of Ag NPs is obvious with diameters 70, 70, 70 and 86 nm [14].

1.1.3. Plants, their parts and extracts used for biosynthesis

Generally, different parts of plants such as leaves, bark, flowers etc. have the main role as synthesis agent for metallic NPs and they can be harvested everywhere and provide easy handling [10]. Plant parts can be treated into small pieces to release profitable compounds to distilled water [15], ethanol [8] etc. and then extract, leachate or essential oil are purified by centrifugation or filtration. Final extract/leachate is mixed with salt

solution at laboratory temperature or can be heated. Phytosynthesis of NPs are predominantly an extracellular process. Amino acids, polysaccharides, flavonoids and vitamins contained in plants play an important role in reducing, transforming and stabilization of final NPs [15].

Au NPs were obtained by an aqueous extract from *Juglans regia* plant. Synthesized NPs exhibited an average size of 19 nm at room temperature and 14 nm at 45 °C. The samples were dried and the yield of NPs was enhanced as better for the sample at 45 °C. Both NPs had spherical shape and proved non-cytotoxic effect on cell line in high concentration, which makes them suitable for application in various biological and medicinal field [16].

Bimetallic Ag/Au NPs were successfully synthesized by *Aloysia triphylla* plant extract at room temperature with diameter size of around 20-35 nm [17] and essential oil *Coleus aromaticus* with diameter size of around 28 nm [18]. The shape of obtained NPs by both plants was spherical [17, 18].

1.2. Ag NPs as effective antibacterial agent

Ag compounds have been known for their antibacterial activity for many years and this activity can be detected at low concentrations. The principle of antibacterial action is the mechanism of inhibitory action by Ag ions (Ag^+) and Ag NPs < 10 nm through the cell plasma membrane. Ag NPs ($d > 10$ nm) aggregate and adsorb on bacteria surface, serve as reservoir of Ag^+ and can provide further antibacterial activity for several days. Cellular functions such as permeability and respiration are disturbed. Moreover, NPs can interact with bacterial DNA, which causes the loss of their replication ability [19, 20]. The mechanism of penetration and destruction of bacterial cell is briefly indicated in Figure 4.

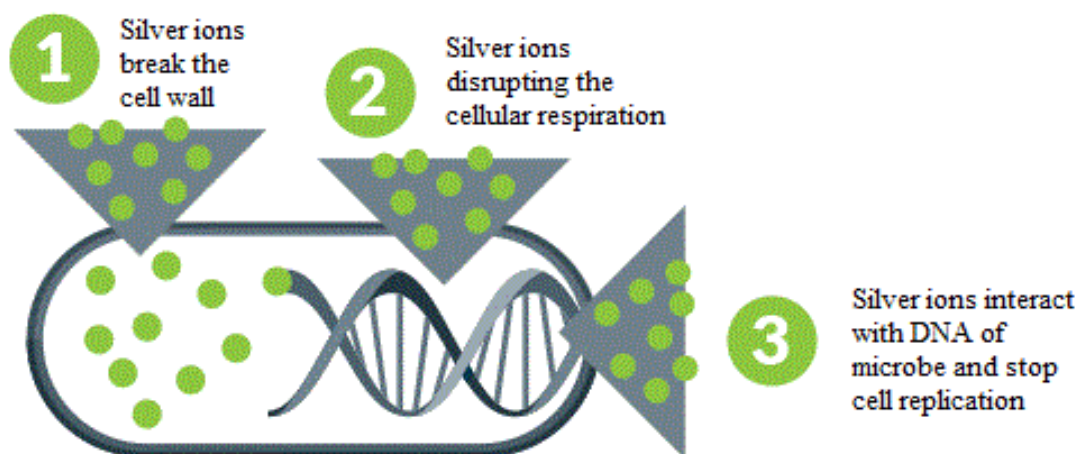


Figure 4: Schematic image of basic principal interaction of Ag ions/Ag NPs < 10 nm with the bacteri: (1) Ag ions break cell membrane, than (2) disrupt respiration process and (3) interact with bacterial DNA.

Usually antimicrobial properties of metallic NPs are studied with human pathogenic gram-positive and gram-negative bacteria for example *E. coli* and *S. aureus* [21]. In the study of Benakashani et al. (2016) [22] describes phytosynthesized Ag NPs gained from medicinal plant *Capparis spinosa*. Pure leaf extract did not exhibit any microbial activity. However, the addition of initial precursor and subsequent Ag NPs synthesis exhibited antimicrobial properties against *E. coli* and *Salmonella typhimurium* [22]. Organic compounds of *Cerasus serrulata* leaves served as reduction and stabilization agent for Ag NPs synthesis with spherical shape and 15–24 nm size. The prepared Ag NPs exhibit antibacterial effect on *S. aureus* and *Streptococcus mutants* [23]. Ag NPs were also obtained by synthesis of *Anacardium occidentale* [24], *Tamarix gallica* [25] or *Cichorium intybus* [26] and their antibacterial activity has been tested successfully as well.

On the other hand, in a recent study a resistance of Ag NPs to bacteria *E. coli* was proved, but by adding pomegranate rind extract the resistance was reduced [27]. In other study [28] Graves et al. (2015) confirmed rapid resistance in *E. coli*.

2. Electrospinning: an effective method for membrane preparation

Nanofibers are fibers with diameter in nanometer scale from 50 to 1000 nm and they have been a significant class of nanomaterials in the last 20 years [19]. Electrospinning or electrostatic spinning is a simple and efficient technique for the fabrication of polymer nanofibers [29, 30].

2.1. Brief history of electrospinning

The first mention of the process of spinning in electrostatic field was in 16th century. At the beginning of 20th century J. F. Cooley and W. J. Morton got a patent for improving methods for electrostatic spinning. Unfortunately, high-tech equipment such as electron microscopy was not available and they could not detect presence of nanofibers. In the 80's an industrial potential of fibers was developed and over the last 20 years electrospinning has been intensively investigated [30, 31 pp. 34–39].

Czech scientist prof. Oldřich Jirsák from Technical University of Liberec worked on important development in mass production of nanofibers and he constructed and patented a unique needle-less device called NanospiderTM. It can produce fibrous samples larger than 1 m with a uniform web, which can be commercially used [32].

Since 1990 a Czech private company CONTIPRO a. s. has been the producer of compounds for cosmetic and pharmaceutical industries. One of the important contributions in the field of nanotechnology of this company is the development of the needle device called 4SPIN. The main advantages of 4SPIN are: spinning of wide range of polymers, an enormous variety of applied parameters during the process or easy maintenance of the device and its components too. Components, such as collectors or needles, can be easily replaced by another, which led to production of various fibers [33].

For comparison, another technique for fiber preparation is a centrifugal spinning. This technique is based on centrifugal force; due to it, fibers are pulled out of the spinning unit. It has advantages such as the possibility to set up high temperature near the spinning unit and solid material can be melted and spinned without a previous chemical preparation. Furthermore, centrifugal spinning can be utilized for the preparation of fibers from solutions of higher weight concentrations than electrostatic spinning [34].

2.2. Technical setup of 4SPIN

The device is constructed of three major components (Figure 5): a high voltage power supply, a spinneret and a grounded collector (usually metal screen, plate or rotating mandrel also called as drum collector) [29, 35].

High voltage electrostatic field charge is applied between the surface of liquid solution, usually polymers or melts, and the collector. Electrical repulsive forces overcome forces of surface tension in polymer and it generates Taylor cone between the spinneret and the collector, which has opposite polarity than polymer liquid solution [35, 36].

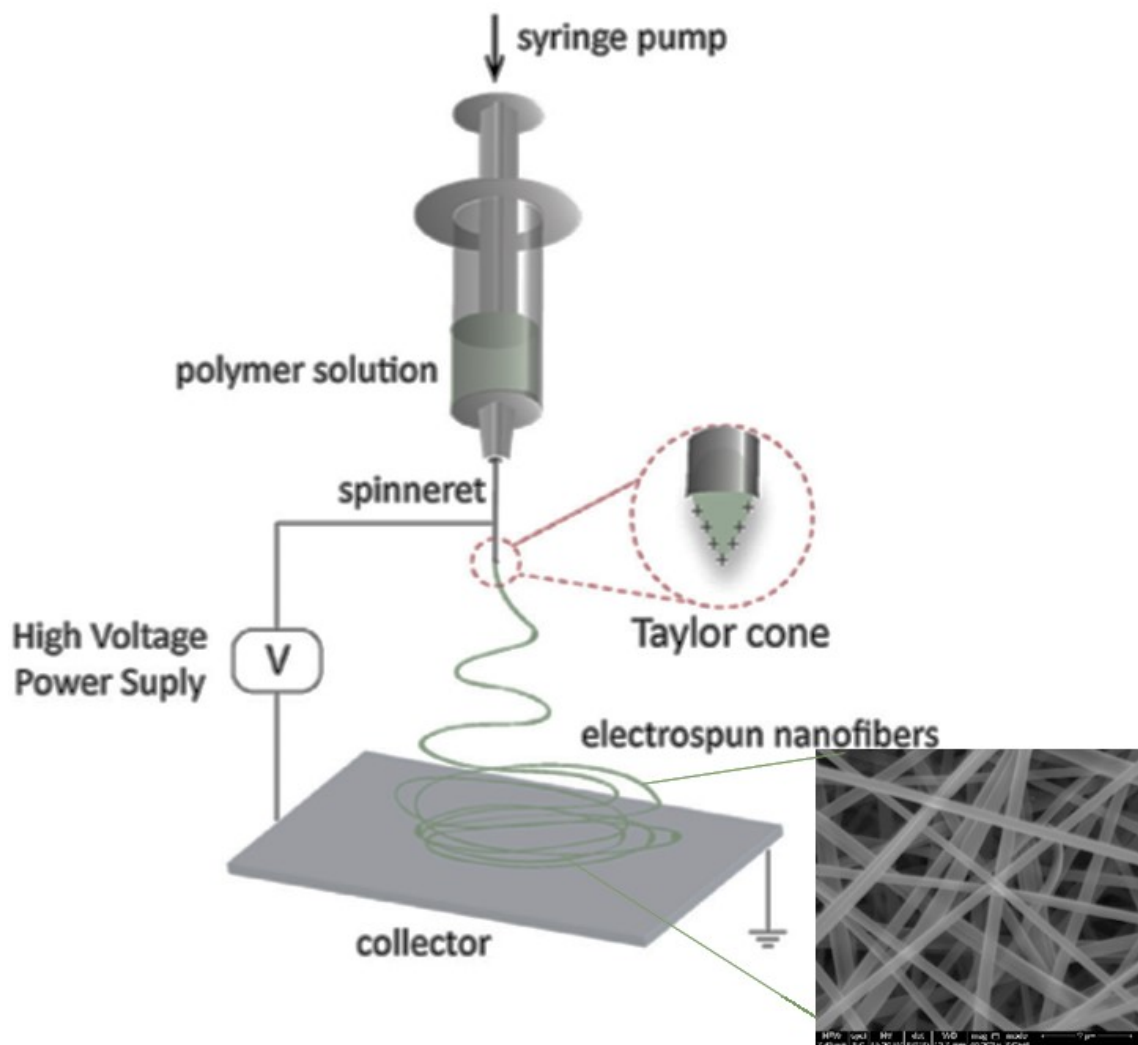


Figure 5: Schematic diagram of electrospinning device [36].

The process of electrospinning can be easily influenced by many parameters. We can divide them into three groups:

(1) technical parameters of the process: applied voltage, flow rate, needle/collector type and distance between the needle and collector;

(2) parameters of solution: viscosity, molecular weight and concentration of the polymer, conductivity, surface tension; and

(3) environmental factors such as humidity, temperature [21, 35].

The main factors to control the diameter of the fiber are molecular weight concentration of polymer in solution and the type of solvent used, applied voltage and flow rate [29].

2.2.1. Technical parameters

The different distance between the needle and collector is related to the changing morphology of fibers caused by different deposition time and evaporation rate. It was shown that the fiber diameter decreased as the distance increased [29, 35]. An increasing applied voltage can result in the formation of numerous defects such as beads. High applied voltage causes decreasing of Taylor cone and increasing jet velocity for the same feed rate and these effects lead to reduction in the fiber diameter and also faster evaporation of solvent [29, 37].

2.2.2. Solution characteristics

Low weight concentration of the polymeric solution can cause breaking of polymer chains into fragments, caused by applied high voltage and surface tension of solution, which leads to the formation of beads or fibers of chain type. On the other hand, high increasing of the weight concentration of the polymeric solution leads to an increase in the viscosity, which also increases the chain structure of fibers. For every type of solution there should be optimum solution weight concentration for the process [29]. Increasing the conductivity of the solution increases the charge on the surface of the droplet, which forms Taylor cone but also causes decrease in the fiber diameter [35].

Also, the choice of polymer leads to different webs of nanofibers. The selection of solvents for electrospinning process is important too for obtaining of smooth and beadless electrospun nanofiber [37]. Polymer should be completely soluble in solution which have a moderate boiling point for easier evaporating such as distilled water [24, 35].

Molecular weight of the chosen polymer has remarkable effect on properties such as viscosity, conductivity or surface tension. Too low polymer molecular weight led to formation of beads rather than fibers and increasing value of molecular weight of polymer give fibers with larger average diameters (Figure 6) [37].

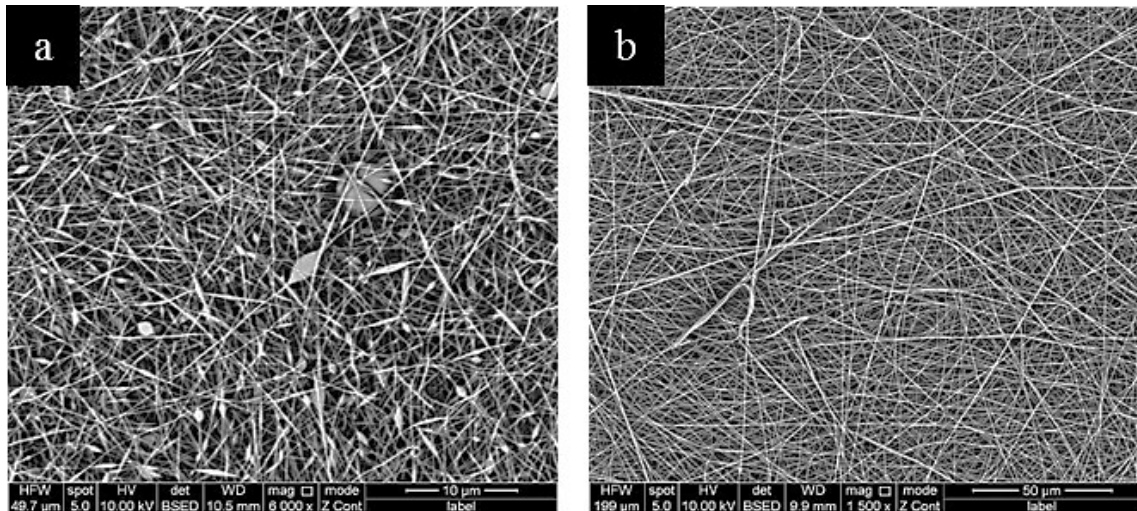


Figure 6: Influence of different molecular weight to final structure of polymer's fibers electrospun at same condition: (a) $M_w = 61\,000\text{ g}\cdot\text{mol}^{-1}$; scale bar $10\ \mu\text{m}$ and (b) $M_w = 130\,000\text{ g}\cdot\text{mol}^{-1}$; scale bar $50\ \mu\text{m}$.

2.2.3. Environmental parameters

Increasing temperature results in decrease in fiber diameter and high humidity causes pores on the surface of the fibers. Unfortunately, these factors cannot be easily changed [29].

By controlling these parameters, not even the mentioned ones, it is possible to easily fabricate electrospun nanofiber scaffolds for the various application such as drug delivery [38], tissue engineering [38], wound healing applications [21] high porous filters for example for removal of dye from water [39], ultrafine phase change fibers for thermal energy storage [40], (bio)sensors for food and agricultural application [36] and a lot of others.

3. Polymer nanofibers used for medical application

3.1. Historical progress in dressing materials

Dressing materials which are used for wounds or burns covering should accelerate the recovery of damaged areas of skin [41]. Historically, dressing materials were classified into three main groups: (1) traditional, (2) biological and (3) artificial dressings [41, 42].

(1) Traditional materials such as gauze or gauze-woven cotton composite dressing is a frequently used material, which has been used since the beginning of 1970. It is cheap, easy to handle and it has large absorbent capacity. On the other hand, it causes a rapid dehydration of wound and promote growth number of bacteria and wound contamination. Furthermore, at the end of healing, removing of gauze can cause bleeding or damage to renewed epithelial flora [41].

(2) Biological dressings are established to heal deep, chronic wound and burns by covering normal skin from animals or human dead body. The basic drawback of these materials is, in some cases, inappropriateness of donor to donate part of skin to a patient [41].

Recently, scientists have focused on (3) artificial dressings. They are fabricated from natural polymeric materials such as collagen, gelatin or synthetic polymeric materials such as poly(vinyl alcohol) (PVA) or poly(lactic acid) (PLA), which are not found in skin. Structure of artificial dressing should be a harmless for human organism, mechanically stable, biodegradable, and provide a proper environment for the tissue repair. Also, they must fulfill two essential requirements: rapid hemostasis and good antibacterial property [41].

3.2. Currently used polymers in medical applications

There is a large number of natural and synthetic polymers that can be used in electrospinning process forming fine nanofibers for various applications such as tissue engineering [38], filtration membrane [39] and a lot of various biomedical application [29]. Polymers for preparation of dressing can be prepared alone or combined with each other [41].

Generally, natural polymers such as collagen, gelatin or silk fibroin are widely used for biomedical application due to their biocompatibility and without negative effects to organism compared with synthetic polymers so far. On the other hand, during spinning process natural polymer fibers may transform their structure due to denaturation and it leads

to fact that electrospinning of natural polymers is financially demanding because of the need for the use of additives which improve the stability [29].

Cellulose nanofibers prepared by electrospinning can be interesting material in the field of wound dressing. Derivative of cellulose, acetyl cellulose could have application as semi-permeable membranes in dialysis, ultrafiltration and reverse osmosis. Collagen is characterized by excellent biological and physical-chemical properties for wound dressing or tissue engineering applications. On the other hand, for obtaining uniform fibers webs, collagen and/or cellulose must be dissolved in costly and not environmental-friendly solvents. Choosing other solvents leads to destruction of the collagen structure and loss of unique properties [29, 35].

Synthetic polymers such as PLA, poly(ϵ -caprolactone) (PCL), polyglycolic acid (PGA) or PVA are appropriate for electrostatic spinning as well. They are used for their convenient characteristics such as biocompatibility with human organism, biodegradability and appropriate mechanical properties [29].

PGA and PCL are used for nonwoven tissue engineering scaffolds. However, hydrophobic and uncontrolled biological interactions of PCL with material are disadvantageous. One solution to correct this issue is mixing PCL with other polymers such as PGA. This mixture displays better biocompatibility, hydrophilicity as well as mechanical properties than using the polymers separately especially for tissue engineering (Figure 7) [35, 43].

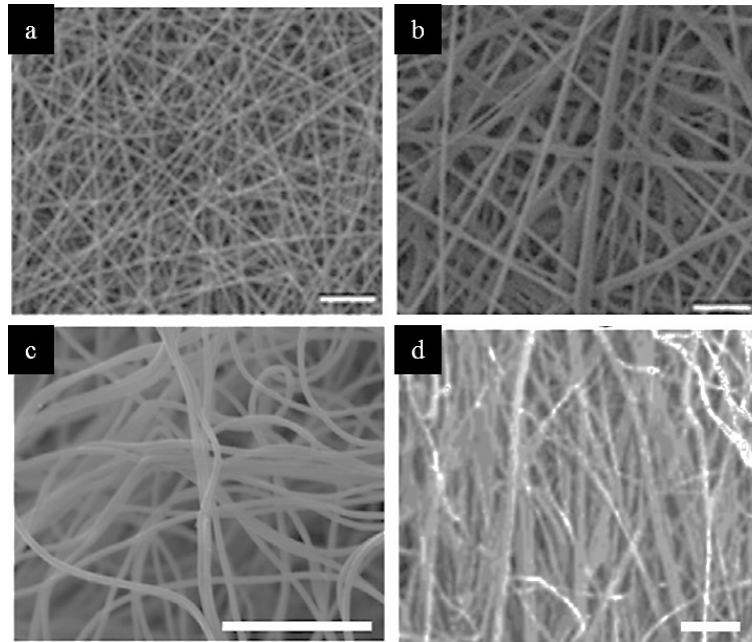


Figure 7: Different typical webs of fibers of different synthetic polymers (a) PGA, (b) PCL and (c) PVA, and natural polymer (d) collagen; scale bar 10 μ m.

In the biomedical field, PVA or PLA is investigated for main application in wound dressing and healing. Usually PVA is used as matrix for incorporation of inorganic NPs or antibiotics, because it is one of the biodegradable, water-soluble and biocompatible synthetic polymer [21].

3.3. PVA-based wound dressings for tissue engineering

Nanofibrous dressing materials have found a wide application in wound dressing. Nanofibers are characterized by their high surface area, high porosity, high capacity for incorporating of biological substances and active materials such as drugs or NPs [42]. Bacteria commonly contaminate wound and cause problem during wound healing, so the efficacious antimicrobial dressing must be used [44]. The antibacterial nanofibers were usually fabricated by incorporating antibacterial materials such as antibiotics, for example ofloxacin and his derivate ciprofloxacin [45, 46], Ag NPs [47] or another metallic and metal oxide [19] NPs.

Five methods of active agent incorporation into the fibers are shown in Figure 8. Antibacterial wound dressing materials can be prepared by the active agent blended with polymer solution before electrospinning (Figure 8a) or by fabricating core/shell

(also called core/sheath) structures through coaxial electrospinning (Figure 8b). For example, Kaleemullah et al. (2016) prepared core/sheath fibers by coaxial electrospinning using PCL and chitosan (CS) by the same method as is shown on Figure 8b. Incorporation of Ag NPs has been done by soaking fibers in AgNO₃ solution and reducing by NaBH₄. CS improved hydrophilicity of PCL and Ag NPs released active Ag⁺ as shell [48].

Another method is encapsulating the active agent before blending it with electrospinning solution (Figure 8c) or post-treatment of the fiber (Figure 8d) based on converting a precursor to its active form after electrospinning process. The last method is called post-immobilization where drugs are conjugated onto fabricated nanofiber matrices through physical or chemical methods, (Figure 8e) for example by spraying or soaking fiber into solution [42, 49].

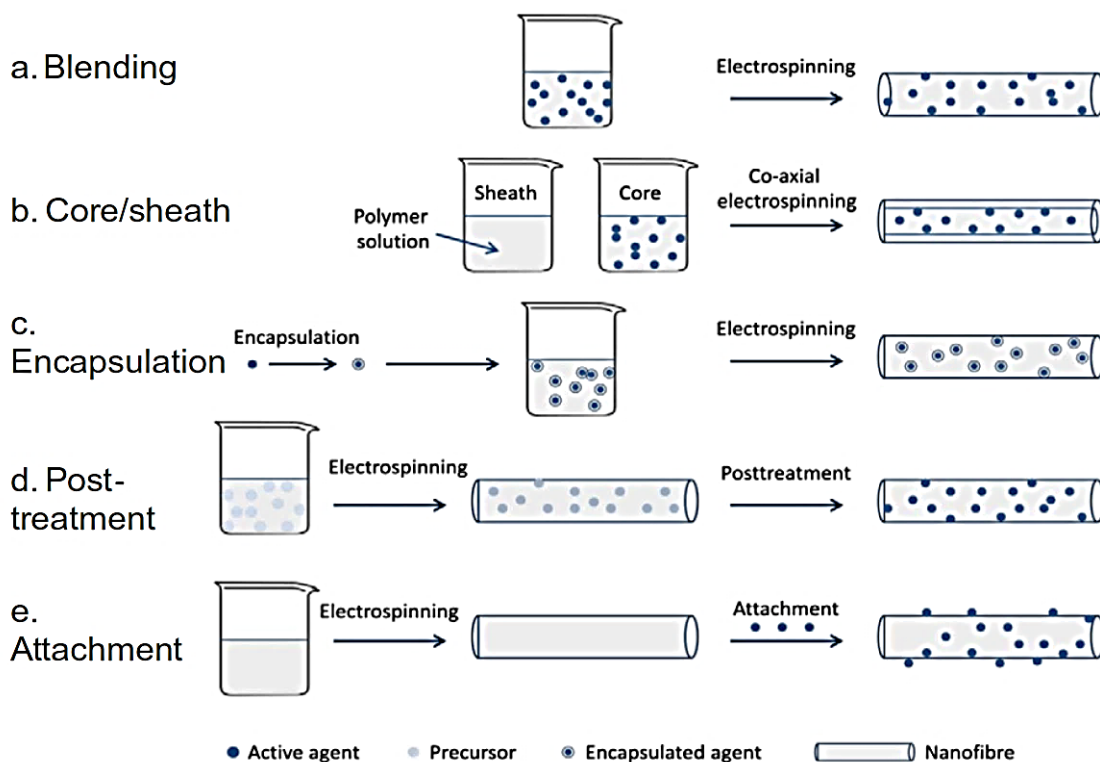


Figure 8: Processes of incorporating active agents in the fibers: (a) the active agent mixed with polymer solution before electrospinning, (b) fabricating core/shell structure through coaxial electrospinning, (c) encapsulating the active agent before mixing them with electrospinning solution, (d) post-treatment of the fiber after electrospinning and (e) post-immobilization of active agent [42].

Incorporation of drugs or inorganic NPs seems to be the best option for better antibacterial properties. Interaction with bacteria in wound can be caused by multiple different ways: (1) by fibers chemistry (characteristics and structure of polymer) and physical properties (fiber diameter, porosity etc.) or (2) by molecules attached to or incorporated into nanofibers [42, 49].

3.3.1. Polymer conjugates with antibiotics as more active agents

In the study of the research group of Vashisth et al. (2017) ofloxacin and is known as an antibiotic, was incorporated into the gellan / PVA nanofibers prepared by electrospinning. They stirred pre-pared gellan / PVA and ofloxacin, which created an amorphous dispersive solution. The research demonstrated that gellan / PVA nanofibers matrix acted as a preservative agent for ofloxacin. The stability of ofloxacin was improved and led to the better efficacy of the drug against microorganisms such as *E. coli* or *S. aureus* [46].

Another group loaded antibiotic ciprofloxacin (CIF) to PVA nanofiber by blending the solution before electrospinning. In this study, they investigated the influence of differently used ratios of solution on nanofibers morphology, size distribution and antibacterial activity. During electrospinning process, they applied voltage of 16 kV between the needle and collector. The distance needle-collector was fixed at 15 cm. The composite fibers also inhibited the growth of *E. coli* and *S. aureus*. The prepared fibers loaded with CIF exhibit better wound healing process compared with commercial gauze. These composite material shows huge potential for wound dressing application [45].

Antibacterial and cytotoxic properties of PVA/Chitosan/Tetracycline hydrochloride (TCH) mats against *E. coli*, as well as *S. epidermis* and *S. aureus* were successfully proved by Alavarse et al. (2017). Moreover, TCH loaded nanofibers exhibit a uniform distribution along the nanostructures [50].

3.3.2. Polymer conjugates with Ag NPs as effective antimicrobial agents

Polymer fibers/mats show an excellent result when conjugated with NPs. Also, the polymer which is used as matrix can protect the NPs from oxidation or corrosion. It has been reported that the presence of Ag NPs, which is added for example to PVA

solutions, not only enhance the antibacterial activity, but also the electrospinning ability [41].

Spraying of NPs for incorporation to natural polymer microfibrillar, nanofibrillar cellulose and filtrate paper was used in the study of Yan et al. (2016). Ag NPs were well dispersed with a size range from 10 to 30 nm and antibacterial activity was tested against *E. coli* by using inhibition zone test and viable cell counting. The best result was reached by using microfibrillar cellulose as the matrix [51].

Spherical Ag NPs prepared by biosynthesis with *Momordica charantia* fruits were used as filler into polymeric nanofiber as it is described in the article of Alippilakkotte et al. (2017). This research group electrospun different solutions of polymer PLA and Ag NPs in different concentration of PLA (1, 2 and 3 percentage by volume of Ag NPs). Antimicrobial activity tests showed the best efficiency for the PLA/Ag NPs conjugates with already the lowest (1%) Ag concentration.[52].

Another study [53] describes preparation of nanofibers from solution sulfanilamide and PVA and mixing them with Ag NPs. The obtained composite nanofibers had a narrow size distribution, which depended on the use of ration substances. The presence of Ag NPs was proven by transmission electron microscope (TEM) see on Figure 9. The antimicrobial and *in vivo* wound healing evaluation exhibit superior results because of successful incorporation of Ag NPs to solution of PVA and sulfanilamide synergism against pathogen cells.

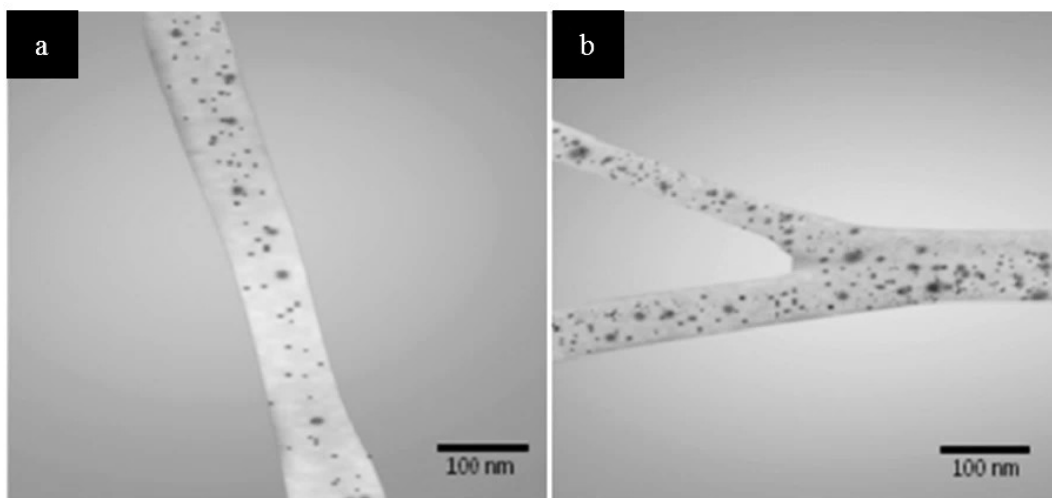


Figure 9: TEM images of incorporation Ag NPs into polymer fiber of PVA/sulfanilamide [53].

An object of another study was the preparation of composite made of nanofibers of natural natural polymer chitosan as a matrix and Ag NPs as a filler. Both components were successfully electrospun and exhibited high degree of antibacterial activity against *Methicillin-resistant S. aureus (MRSA)* and *Pseudomonas aeruginosa* [44].

Another case of nanofibers of PVA combined with chemically prepared Ag NPs was reported in the review written by Z. Zhijie et al. (2016). PVA and Ag NPs were mixed before spinning process in different ratios. The result of this study was successful fabrication of the Ag/PVA composite mats with well-dispersed Ag NPs, which exhibited antibacterial ability mainly attributed to the attached Ag NPs. The electrospun materials showed much better bacterial inhibition against *S. aureus* than *E. coli* [21].

Another researcher group prepared Ag NPs by a chemical bottom up method. Silver particles were added to the prepared PVA water solution, and intentionally different concentration of composite have been obtained (1, 2, 3, 4 and 5 percentage by volume of Ag NPs). Subject of interest was the influence of different concentration of solution on morphology of fibers. Fiber size exhibits a trend: thinner fiber (around 20 nm) at higher Ag NPs concentration. It was found that antibacterial activity is mainly attributed to the attached Ag NPs. Nanofibrous material show better bacterial inhibition against gram-positive *S. aureus* than that of gram-negative *E. coli* [54].

Experimental part

4. Materials and methods

4.1. Phytosynthesis of Ag NPs

Ag NPs were synthesized from parts of deciduous tree *Tilia* (*Tilia* sp.) which were collected in the Czech Republic. Leaves and blooms were dried at room temperature. For synthesis of Ag NPs, leachate of *Tilia* sp. was prepared as a reducing and stabilizing agent from 4 g of dried plant leached for 5 minutes in 200 ml of distilled water which was warmed to 80 °C. After that, the leachate was filtrated via 0.2 µm syringe filter. For synthesis of Ag aqueous solution of salt AgNO₃ with concentration of 0.01 mol·dm⁻³ NPs was used. Plant water extract and solution of AgNO₃ were mixed in the ratio 1:1 and after 24 hours the colour change from yellow to dark red/brown due to reduction of Ag NPs (Figure 10) was observed. Then final silver colloid was put into fridge (T = 4 °C).



Figure 10: An illustration photo (a) leachate of *Tilia* sp. and (b) colloid of Ag NPs 24 hours after mixing the plant leachate with aqueous solution of AgNO₃.

4.2. Polymer solutions to electrospinning

Two solutions 1) clear and homogenous PVA in distilled water (molecular weight $M_w = 130\,000\text{ g}\cdot\text{mol}^{-1}$) and 2) PVA with colloidal Ag NPs without visible aggregation of Ag NPs were prepared for electrospinning:

- PVA,
- PVA + Ag NPs.

Both solutions were stirred for 24 hours at 50 °C to obtain liquid solution and were prepared as 10 % weight concentration of solution.

4.3. Electrospinning process

Samples of fibers were prepared per needle device 4SPIN (Contipro a.s., Czech Republic) by needle with internal diameter 0.8 mm and collected by static continual collector C1. Distance between collector and needle was set at 18 cm and flow rate was fixed at $20\text{ }\mu\text{l}\cdot\text{min}^{-1}$. Furthermore, time (20 minutes) of spinning was the same for every sample. The process of electrospinning is showed on Figure 11.

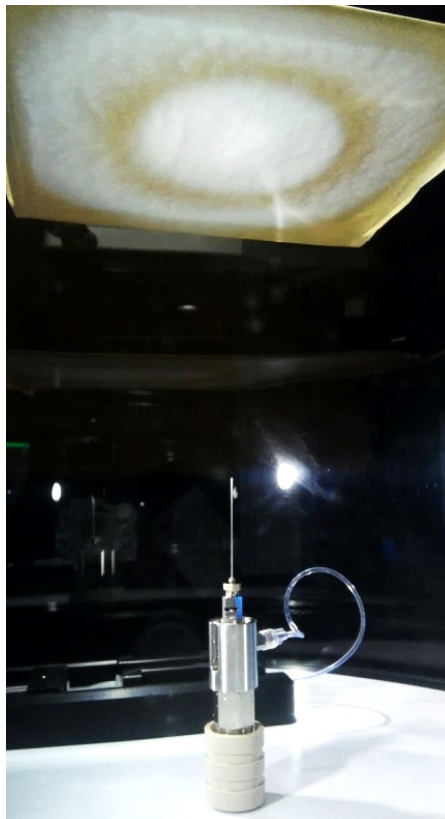


Figure 11: An illustration image of sample electrospinning by 4SPIN device.

The Table 1 shows applied voltage and environmental parameters: temperature and humidity values, observed during spinning process. Two sets were prepared to find out if process could be repeated with similar results.

Table 1: Summary of changed parameters during electrospinning process of fibrous samples PVA and PVA with loaded Ag NPs.

SET	PVA				PVA + Ag NPs			
	Sample number	U [kV]	T [°C]	ϕ [%]	Sample number	U [kV]	T [°C]	ϕ [%]
1	1	40	20	28	13	40	21	27
	2	35	20	27	14	35	22	28
	3	30	20	29	15	30	22	29
	4	25	21	28	16	25	22	29
	5	20	21	28	17	20	22	27
	6	15	21	28	18	15	22	28
2	7	40	22	27	19	40	24	25
	8	35	22	26	20	35	24	26
	9	30	23	25	21	30	24	24
	10	25	23	25	22	25	24	24
	11	20	23	25	23	20	24	24
	12	15	23	25	24	15	24	25

4.4. Methods for characterization

4.4.1. Ultraviolet visible spectroscopy (UV/VIS)

The colloid of Ag NPs was characterized by two-beam UV/VIS spectrophotometer LAMBDA 11 (Perkin Elmer, USA). UV/VIS is based on absorption of light (visible light in range 800 - 400 nm and ultraviolet in range 400 - 200 nm) through the liquid sample.

4.4.2. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns were recorded under $\text{CoK}\alpha$ irradiation ($\lambda=1.789\text{\AA}$) by Bruker D8 Advance diffractometer (Bruker AXS, Billerica, USA) equipped with a fast position sensitive VANTEC 1 detector. Measurements were performed in reflection mode and sample was pressed in a rotational holder; a goniometer with Bragg-Brentano geometry in 2θ range from 3 to 75° and 0.03° step, and X-Ray-pattern was evaluated by EVA software.

The sample of silver colloid was repeatedly applied on microscopic slide and dried at 36°C for 45 minutes to obtain a thin layer. In this way the prepared sample was analyzed by XRD.

4.4.3. Chemical-physical methods of characterization

Size distribution of NPs in colloid was also studied by dynamic light scattering (DLS) utilization ZetaSizer Nano-ZS (dynamic light scattering DLS; type ZEN 3600; Malvern Instruments Ltd.). The DLS analysis was conducted in a plastic cuvette cell with 2 mL of NPs solution with respect to 1 % deviation.

The ζ -potential was controlled utilization ZetaSizer Nano-ZS especially by laser doppler velocimetry. This system applied an electric field into the disposable ζ -potential cell approximately of 2 ml of sample and measured particle motion by electrophoretic light scattering. ζ -potential values show electrostatic stability of tested solutions.

Changes of pH in solution with NPs were measured using EcoScan Series pH 5+ (Eutech Instruments) immediately after mixing leachate with solution of AgNO_3 and also 24 hours later.

Viscosity of homogenous polymer solutions were measured by Visco Basic Plus (FungiLab). The dynamic viscosity was determined from the magnitude of the torque measured in the steady-state rotation, which were set to 50, 60 and 100 RPM/SP

of the appropriate spindle (R4) in the sample. An average value of viscosity was counted in the study.

Conductivity of polymer solutions were measured by COND 730 WTW (InoLab) at laboratory temperature (22.8 °C). Potassium chloride ($\text{KCl } c = 0.1 \text{ mol} \cdot \text{l}^{-1}$) was used as standard for re-counted to final value of measured polymeric solutions.

4.4.4. Transmission electron microscopy (TEM)

Morphology and size distribution of Ag NPs in colloid and incorporated as well, were characterized by JEOL 1011 at Faculty of Science in Charles University in Prague.

2 μl of colloid was applied on carbon/formvar coated grids and after 15 minutes of drying the sample was characterized. Size distribution of biosynthesized NPs was evaluated by JMicroVision program on the base of image analysis. At least 650 NPs from TEM images underwent the size distribution analysis per sample. Histograms were created by MATLAB software. Grids with samples of nanofibers were prepared by direct electrospinning polymer solution to them for 1 minute and 30 seconds.

4.4.5. Scanning electron microscopy (SEM)

Samples of fibers were characterized by SEM FEI Quanta FEG 450 at Regional materials science and technology centre in VŠB - Technical University of Ostrava. Two detectors: Secondary Electron Detector (SED) and Back-Scattered Electron Detector (BSED) were used along with energy dispersive analysis spectroscopy (EDX).

Size distribution of electrospun fibers was evaluated by JMicroVision program (image analysis). At least 150 average diameters of fibers from SEM micrographs underwent the image analysis per sample. Histograms were created by MATLAB software.

4.4.6. Fourier transformation infrared spectroscopy (FTIR)

FTIR analysis was realized in VŠB – Technical University of Ostrava. FTIR spectrometer Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, USA) is an analytic spectroscopic system for many applications preferably for identification and structural characterization of organic and inorganic materials. The operating unit is fully automatic and takes place via computer software called Omnic. The spectra were measured in the range of $400 - 4\,000 \text{ cm}^{-1}$. Spectra were modified by using the ATR correction with automatically modified baseline; also presence of CO_2 bands was removed.

5. Results and discussion

5.1. Properties of Ag NPs in colloid

5.1.1. Morphology and size distribution of particles

The DLS size distribution of Ag particles was measured in a range 25 – 615 nm and average diameter of size was determined as 164 nm (with respect to 1 % deviation) (Figure 12). Measurement was repeated after one week with the same values of size. It is a known fact that measurement by this method includes the thickness of biocompounds present on the surface of NPs originally called “core diameters” of metallic NPs [6]. Afterwards, this fact was confirmed by TEM analysis.

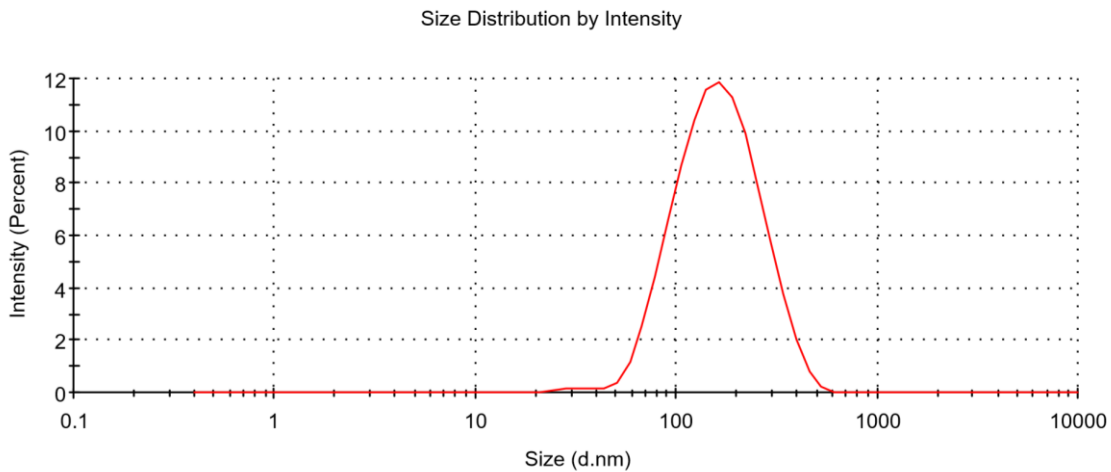


Figure 12: First evaluation of size distribution measured by DLS with size of NPs with coating around 164 nm.

The TEM analysis of the sample confirmed presence of Ag particles in colloid as well. Mostly spherical shapes were observed as it can be seen on Figure 13. By assumption of spherical particles an average size of particles from TEM images was achieved around 14 ± 9 nm as it could be seen in histogram (Figure 13). Mostly represented sizes were in the range 5 - 10 nm. Large particles were noticed too and detected in the range of 50 - 55 nm.

The Ag NPs in colloid formatted clusters of NPs because organic components from leachate of *Tilia* sp. were presented and coated them as it is seen on TEM photos (Figure 13). The visual agglomeration of Ag NPs in TEM photograph can be caused during sample

preparation (drying of colloid sample). However, in colloidal form aggregation/agglomeration of Ag NPs was not apparent.

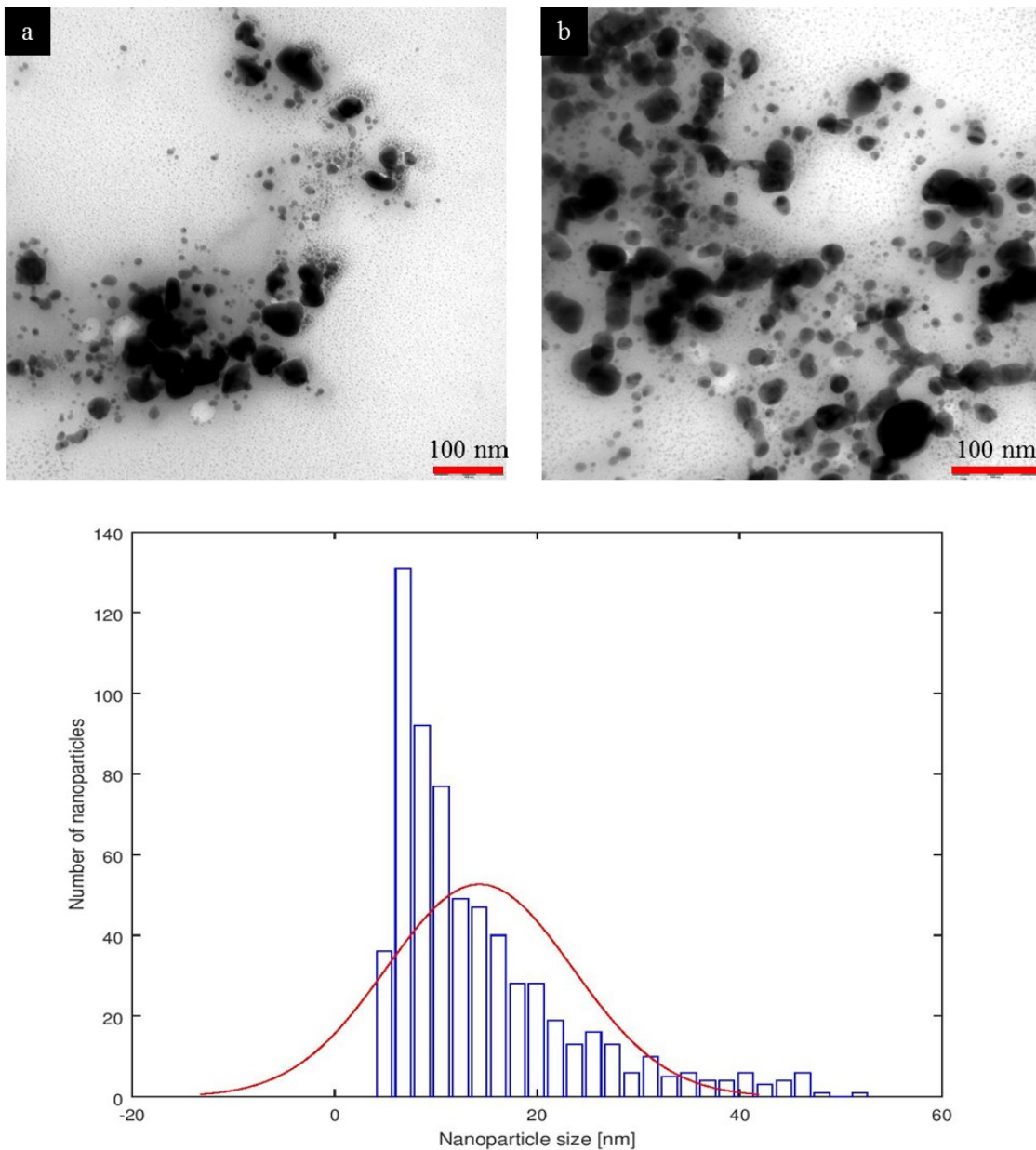


Figure 13: TEM micrographs of Ag NPs present in colloid; scale bar 100 nm and histogram of size distribution prepared Ag NPs. The average size was 14 ± 9 nm, where small and even big NPs were include.

By comparison DLS and TEM analysis, the mentioned influence of organic compounds was confirmed as well. The average size of Ag NPs was determined by DLS analysis as 164 nm and by analysis based on TEM photos was 14 ± 9 nm.

Based on the results, the influence of the thickness of biocompounds in DLS analysis was significant.

5.1.2. Analysis of form Ag NPs by X-ray diffraction (XRD)

The XRD analysis was included to confirm crystallic character of Ag NPs in colloid sample and the XRD diffractogram (Figure 14) was adjusted in a range $25^\circ - 80^\circ$ of 2θ angle.

The diffractogram shows one intense peak around 44° of 2θ angle which presents cubic form of Ag (111). Other intense peak around 52° of 2θ angle was present in cubic form (200), but because of huge noise it was hard to identify. The peak around 32° of 2θ angle could be AgCl. The presence of Cl was confirmed by EDX analysis as well and could be caused by pollution [55].

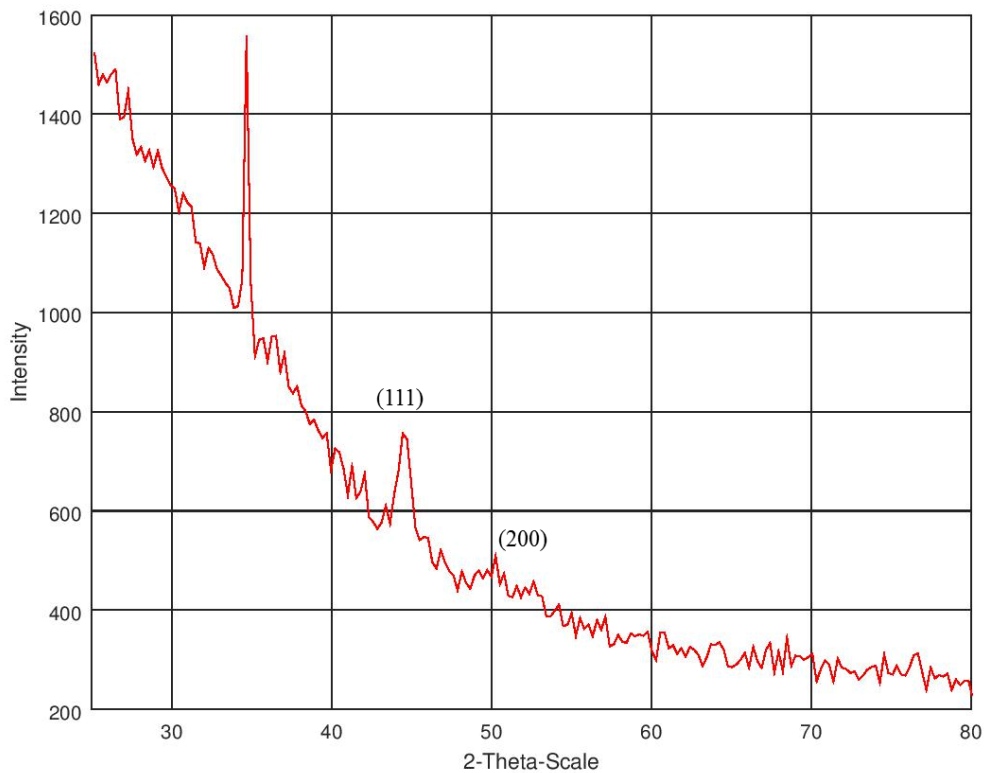


Figure 14: The diffractogram of Ag NPs colloid with one intense peak around 44° of 2θ angle.

There are several facts influencing our XRD diffractogram. Primarily, experimental setup is set on Bragg-Brentano configuration with high measurement angles. The Small Angle X-RAY dispersion (SAX) should be used because setting probably could produce

lower noise, but this could not be guaranteed in every case. Secondary, an ambiguity of final diffractogram was caused by choosing a method of preparation of the sample (thin layer of colloid). The XRD analysis is primary focused on powdered samples. On the other hand, confirming of cubic character of prepared Ag NPs was sufficient.

5.1.3. UV-VIS measurement results

After mixing the leachate of *Tilia* sp. with the aqueous solution of the AgNO_3 , the color started change to dark brown. The presence of NPs was confirmed by obtaining a spectrum in visible range of 380 – 600 nm using UV-VIS spectrophotometer (Figure 15). From this analysis, absorbance peak was found around 460 nm. Presence of Ag NPs was confirmed in agreement with reported results in the literature [52, 54, 56].

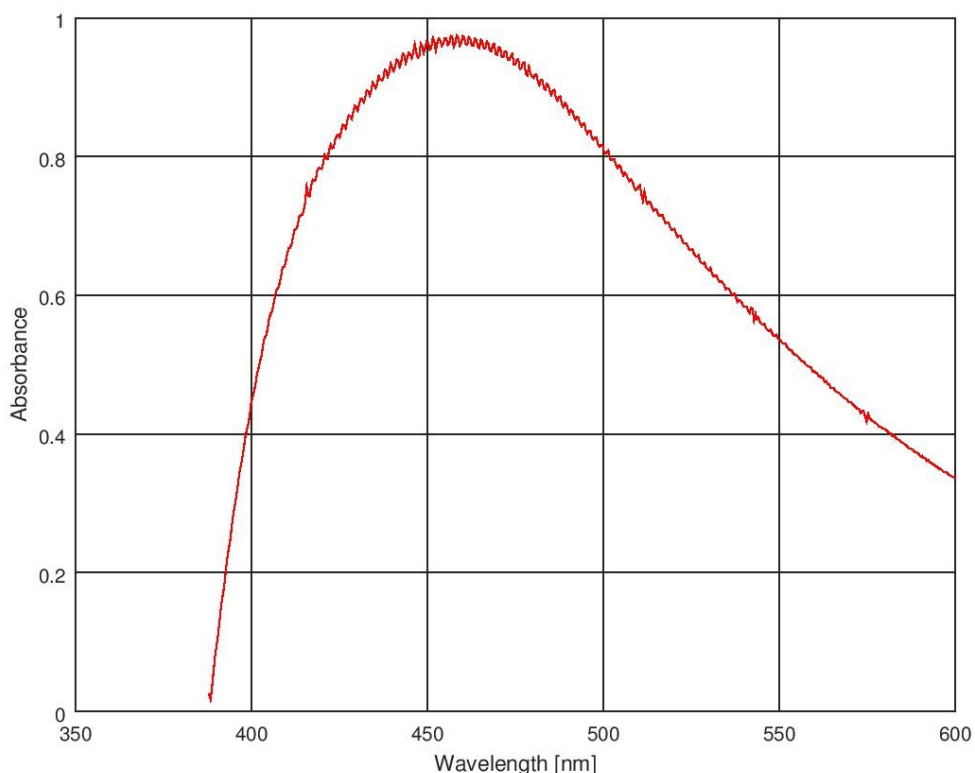


Figure 15: UV-VIS absorption spectrum of Ag NPs colloid with characteris peak around 460 nm.

The wide peak showed us that in colloid there were presented Ag NPs of different sizes with most of them around 10 nm which is in agreement with TEM analysis. The presence of organic stabilizers, which were included in leachate of *Tilia* sp, were confirmed as well by widely peak in UV-VIS absorption spectrum.

5.1.4. Stability of prepared colloid

Generally, the value of ζ -potential above ± 30 mV describes a stable system, but stability of the system depends on several parameters, mostly on pH providing sufficient/insufficient charge to confirm stability of colloid [57].

The negative value of ζ -potential of colloidal Ag NPs was determined to -26 mV (Figure 16). Despite the fact that the colloidal system did not achieve theoretical boundary value, TEM and visual analysis did not confirm aggregation, and stability of the colloidal system can be predicted.

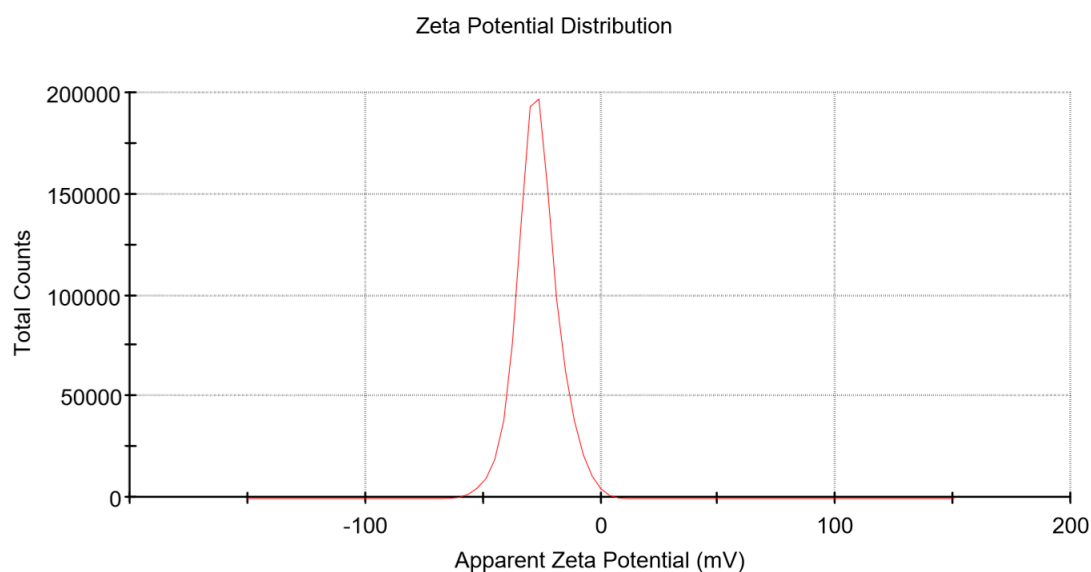


Figure 16: Value of the ζ -potential of Ag NPs colloidal system was determined to -26 mV.

Based on the previous analysis and theoretical knowledge about the ζ -potential, changes of pH during phytosynthesis were measured as another subject of the study. pH value of the plant leachate was 5.4 ± 0.1 . Immediately after mixing the leachate with Ag-precursor measured pH was 4.9 ± 0.1 . After 24 hours colloid was measured again and value of pH decreased to 4.3 ± 0.1 . The decreasing of pH value after 24 hours was observed during the process of NPs reduction. The pH value of colloid after 24 hours was considered constant, and based on this assumption colloid was evaluated as stable.

5.2. Characteristics of fibers

5.2.1. Properties of PVA and its mixture with Ag NPs

Conductivity and viscosity of prepared polymer solutions were included, because influence of these parameters led to different properties of final fibrous samples as was mention in theoretical part as well. Summary of viscosity (η) and conductivity (κ) of prepared polymers was given in Table 2.

Table 2: Value of conductivity and viscosity of PVA and PVA + Ag NPs.

	PVA	PVA + Ag NPs
$\kappa [\mu S. cm^{-1}]$	322	733
$\eta [cP]$	672	629

The PVA + Ag NPs solution had increased conductivity due to presence of conductive Ag NPs. Presence of Ag NPs had influence on viscosity of polymer which decreased as expected. It was impossible to compare it with other values of viscosity and conductivity of PVA in other studies because both parameters were dependent on molecular weight and, of course, on mass concentration (especially viscosity).

5.2.2. Fourier transformation infrared spectroscopy (FTIR) results

FTIR spectroscopy was used to study and analyze the organic functional groups of PVA molecules. Figure 17 shows FTIR spectra of PVA (red line) and of PVA + Ag NPs (blue line). The major absorbance peaks presented in the spectrum of PVA are at 3336, 2941, 2914, 1735, 1715, 1430, 1375 and the last one 1092 cm^{-1} . The first intensive peak around 3336 cm^{-1} represents bonds from the intermolecular and intramolecular hydrogen bonds O-H. The peaks at 2941 and 2914 cm^{-1} attributed to bond C-H from alkyl group $-CH_2$. The peaks at 1735 and 1715 cm^{-1} responded to C=O bond. The peak at 1430 cm^{-1} corresponded to $-CH_2$ twisting vibration bond. The peak 1375 cm^{-1} attributed C-H and O-H bonds. The last peak at 1092 cm^{-1} corresponded to C-O-C structure of bonds.

Comparing with FTIR spectra of PVA and mixture PVA + Ag NPs there was not shown any visible and significant difference. There was only one shift of the peak from 3336 to 3315 which could be caused by Ag NPs or moisture absorption of PVA [54].

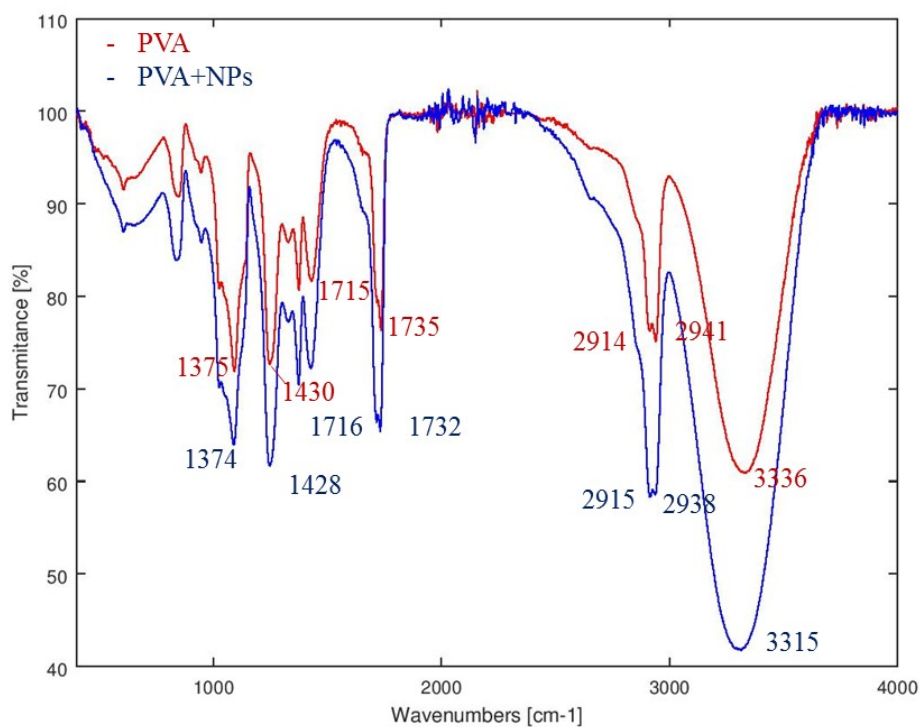


Figure 17: FTIR spectrum of PVA and mixture PVA + Ag NPs which are very similar.

5.2.3. The morphology and size distribution of fibers

The porosity of fibers was not found out for any PVA or PVA + Ag NPs sample. Random defects were presented in each sample such as on Figure 18a have been seen drops (points 1) and beads (points 2) or on Figure 18b was obvious some drops (point 1).

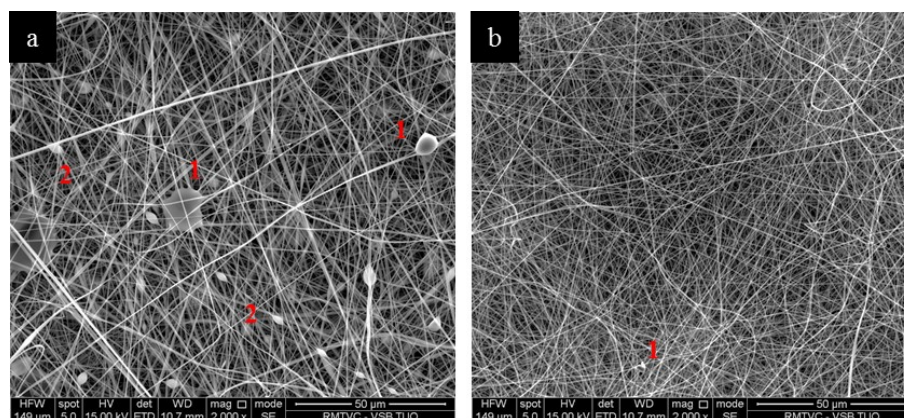


Figure 18: SEM images (scale bar 50 µm) of defects present in nanofibrous webs: (a) sample number 1 at 40 kV with drops and beads and (b) sample number 5 at 20 kV with drops

All of fibrous PVA samples showed this trend: with increasing applied voltage an amount of beads and defects increased (Figure 19a-c). On the other hand, the samples of PVA + Ag NPs (Figure 19d-f) did not show this trend, which was caused probably by presence of Ag NPs and led to improving of electrospinning ability as was mentioned in the theoretical part.

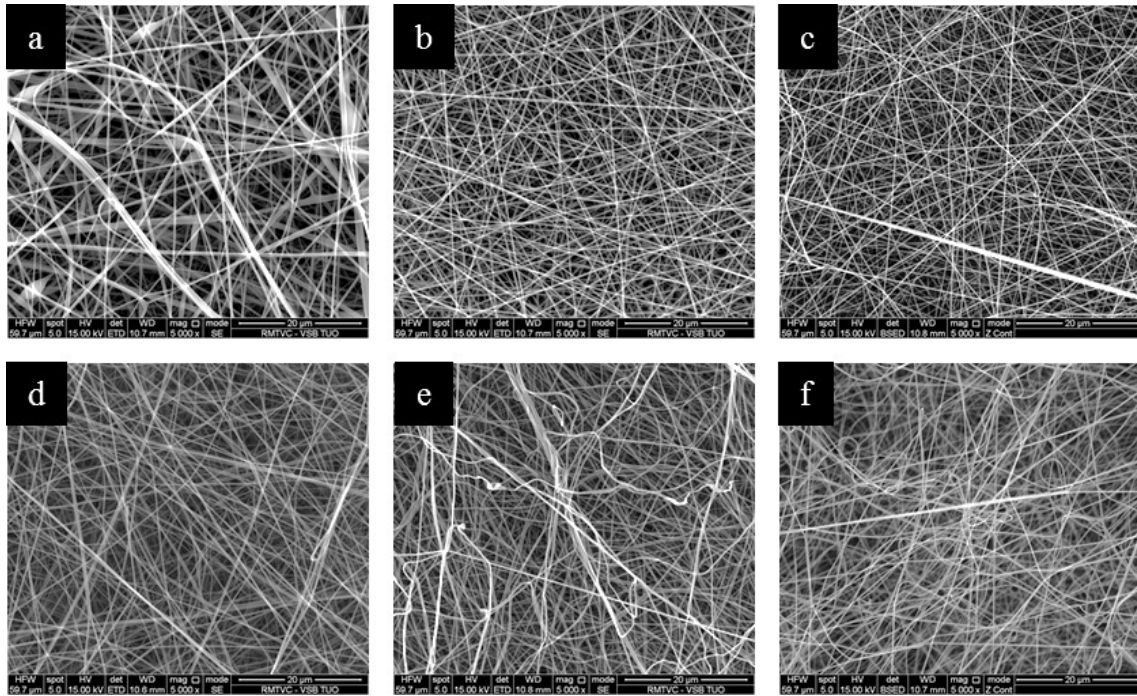


Figure 19: SEM photos (scale bar 20 μm) of PVA and PVA + Ag NPs nanofibrous webs from set 1 to demonstrate influence of increasing applied voltage to sturcture PVA solution: (a) sample number 2 at 35 kV, (b) sample number 4 at 25 kV, (c) sample number 6 at 15 kV, and PVA + Ag NPs solution: (d) sample number 14 at 35 kV, (e) sample number 16 at 25 kV, (f) sample number 18 at 15 kV.

The size distribution of each fibrous samples was analyzed using the program JMicroVision. Average diameters are presented in Table 3.

Table 3: Summarization of average diameters of fibers and divergence of fibrous samples. Samples prepared at 25 kV are highlighted and choosed as representative samples

SET	PVA			PVA + Ag NPs		
	Sample number	\varnothing [nm]	$\bar{\varnothing}$ [nm]	Sample number	\varnothing [nm]	$\bar{\varnothing}$ [nm]
1	1	275	51	13	307	48
	2	278	48	14	303	45
	3	286	47	15	305	50
	4	291	44	16	301	41
	5	279	41	17	305	45
	6	275	43	18	304	46
2	7	217	36	19	231	38
	8	221	31	20	228	35
	9	233	33	21	227	37
	10	242	33	22	221	24
	11	235	27	23	224	29
	12	220	24	24	231	34

The presence of Ag NPs led to unified of average diameters despite different applied voltages. In the set 1 (temperature around 21 °C, humidity around 28 %) the size of fibers (diameters) was evaluated in the range of 250 - 300 nm and in set 2 (temperature around 23 °C, humidity around 26 %) it was 210 – 250 nm. This difference was caused by laboratory conditions (temperature, humidity) which cannot be easily regulated.

Due to the fact that it was impossible to analyze and characterize all the samples prepared for time and capacity (see Table 3), two representative samples of spun fibers with applied voltage 25 kV were chosen samples (number 4 and number 16). The webs of PVA fibers (sample number 4, Figure 20a) were without beads with average diameter 291 ± 44 nm, but webs were not homogenous as webs of PVA + Ag NPs (sample number 16, Figure 20b) with average diameter 301 ± 41 nm (Table 3). With respect to the theoretical part, presence of Ag NPs in polymer solution improved ability of electrospinning and more homogenous webs were observed. This may be caused by increasing conductivity of polymer solution PVA + Ag NPs.

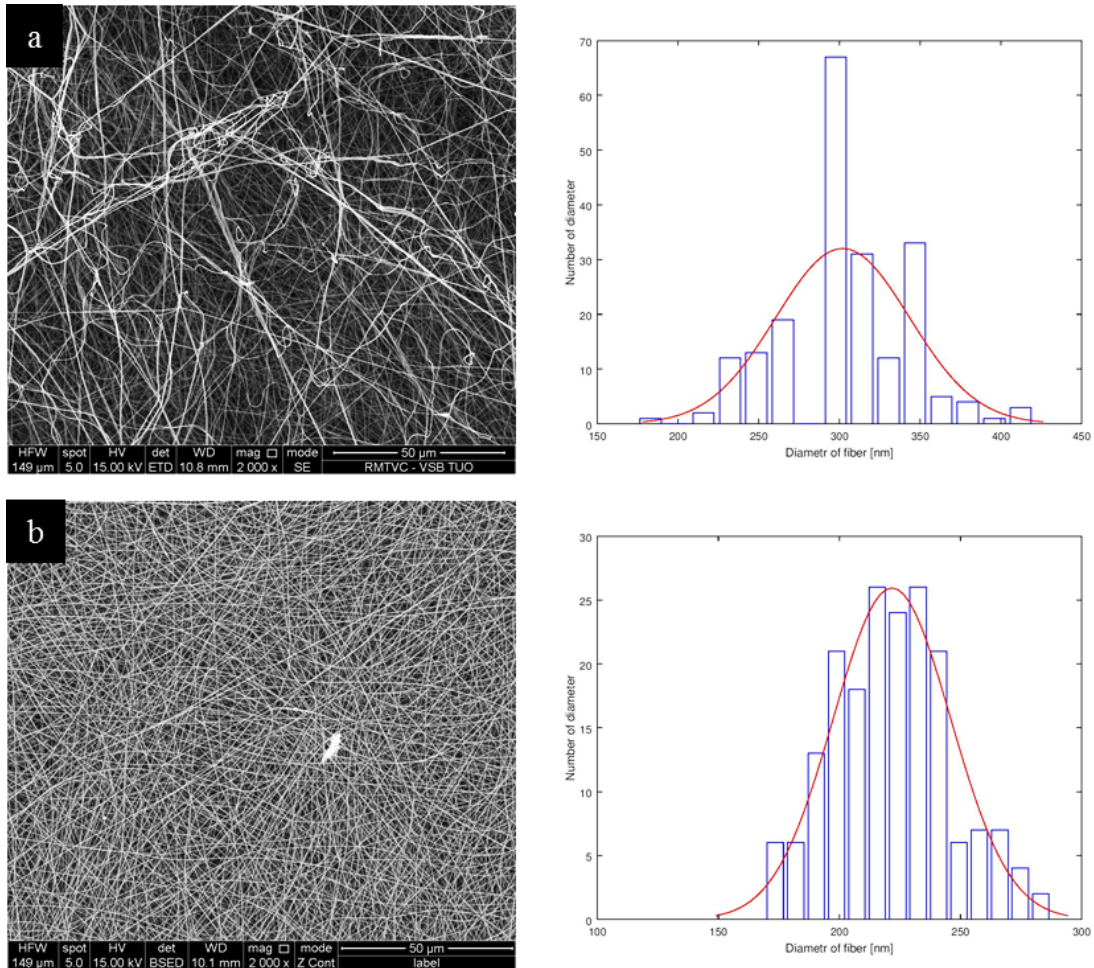


Figure 20: SEM images of representative nanofibrous samples with scale bar 50 μm and their histograms of nonafibers: (a) PVA sample number 4 with $291 \pm 44 \text{ nm}$ and (b) PVA + Ag NPs sample number 16 with $301 \pm 41 \text{ nm}$.

5.2.4. Incorporation of Ag NPs into PVA fibers

SEM and EDX analysis were applied to confirm the presence of Ag NPs in PVA nanofibers and were successful as it is seen in Figure 21 were analysis of sample number 22 is shown. The Ag NPs were found to be incorporated in PVA nanofibers as it is proved by analysis in points 1 and 2. Furthermore, the creation of random located cluster was probably caused by applying of whole electrospinning process.

Regarding the distribution of clusters in samples, it was observed that the locations and amount of Ag was easier to find out with respect to decreasing value of applying voltage. Other chemical elements, as C or O, which were detected, belong to the structure of PVA. Presence of Au was caused by necessity for sputtering of sample for SEM analysis.

The presence of Cl was not clear and should be subject for further analysis, but probably it was caused by leachate of *Tilia* sp respectively by pollutants in the biomass [55].

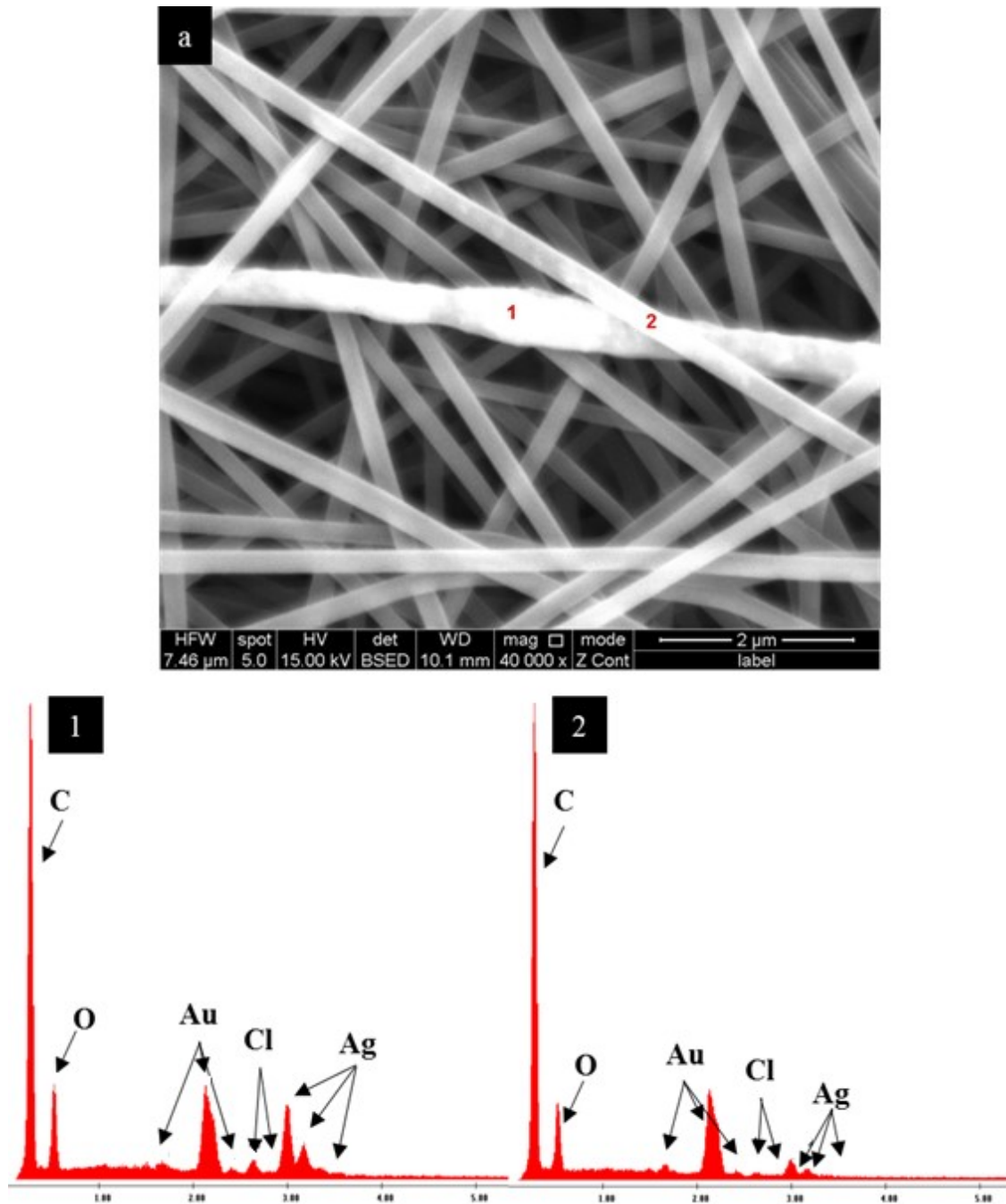


Figure 21: SEM images of fibers PVA + Ag NPs (scale bar 2 μm) sample number 22 with two location of aggregate Ag NPs and their EDX analysis.

For detailed detection of Ag NPs in PVA fibers TEM analysis (Figure 22) was used as well. The detected Ag NPs (point 1) had 40 nm which corresponds with previous results.

The point 2 was evaluated as a defect caused by degradation of PVA due to high applied voltage (80 kV) during TEM analysis. The diameters of PVA fibers were around 190 nm, which was in the same range determined by SEM analysis. PVA + Ag NPs nanofibers had diameters around 180 nm corresponding with SEM analysis as well.

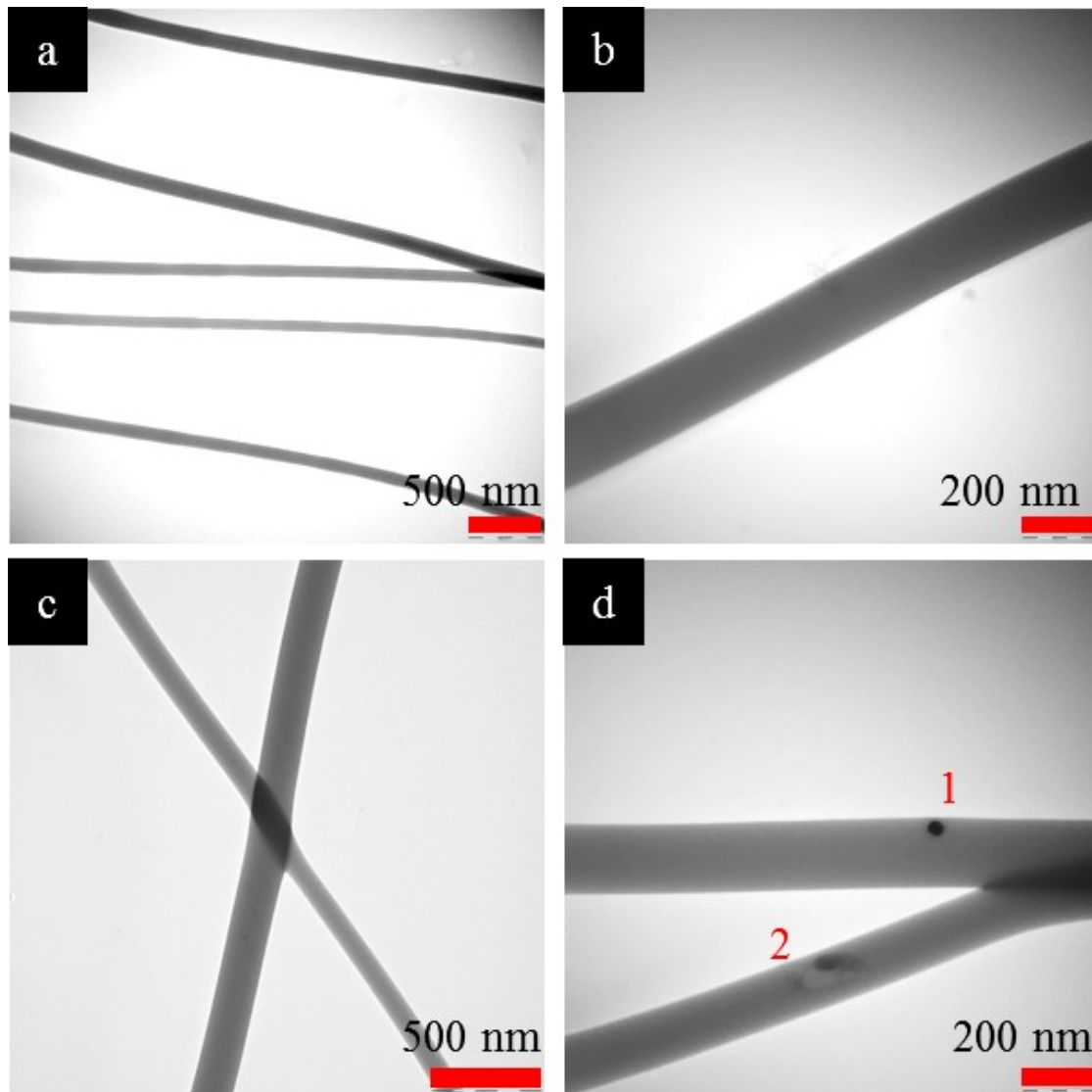


Figure 22: TEM micrographs of fibers from SET 2 prepared by applied voltage 25 kV: (a) + (b) sample of PVA fibers; scale bar 500 nm and 200 nm, and (c) + (d) sample of PVA + Ag NPs fibers; scale bar 500 nm and 200 nm; point 1 - incorporated Ag NPs and point 2 – defect caused by degradation of PVA due to high applied voltage during TEM analysis.

The lesser quantity of nanofibers on TEM micrographs is caused probably due to preparation of polymers fibers by direct electrospinning to grid for 1 minute and 30 second, which guaranteed a thin layer of polymer nanofibers for successful TEM

observation. Extending the spinning time should lead to the preparation of a sample with a higher frequency of fibers in subsequent tests and thus to better observation of distribution of nanoparticles.

In the conclusion, the crystalline Ag NPs were successfully prepared and they showed a stabilized system as well. The final Ag NPs were successfully incorporated into PVA nanofibers without obvious technical complications. The presence of Ag NPs in solution led to improvement of electrospinning ability, which can have been seen in SEM images.

6. Conclusion

This bachelor thesis was divided into two main parts – theoretical and experimental. The theoretical part contains three main topics: (1) a brief overview of basic preparation methods of metal nanoparticles with more detailed focus on phytosynthesis of silver nanoparticles and its antibacterial activity, (2) methods of nanofibers preparation by electrospinning and influence of spinning parameters on fiber properties and (3) progressive materials based on polymer nanofibers used in medical field.

In the experimental part, spherical and crystalline form of silver nanoparticles via phytosynthesis using leachate of *Tilia* sp. were successfully synthesized with average size 14 ± 9 nm. The presence of organic phytochemicals from leachate led to reduction, creation and stabilization of nanoparticles which was confirmed by ζ -potential (-26 mV) and with respect to pH.

Both the prepared polymers poly(vinyl alcohol) and mixture of poly(vinyl alcohol) and silver nanoparticles were homogenous and successfully spun with reproducible results. The average diameter poly(vinyl alcohol) and mixture of poly(vinyl alcohol) with silver nanoparticles fibers was repeatedly in the range 200 – 300 nm. Silver nanoparticles obviously influenced the ability of electrospinning process leading to homogenous webs without a large number of defects. The presence and incorporation of silver nanoparticles into poly(vinyl alcohol)nanofibers was proved and silver nanoparticles did not significantly disrupt the structure of poly(vinyl alcohol) fibers. Unfortunately, silver nanoparticles probably aggregated during the electrospinning process, which was mostly observed by SEM analysis. However, using TEM detailed analysis, separate nanoparticles were observed as well.

For the above reasons, this bachelor thesis determinates the following aims for the future experiments:

- to prove/disprove influence of inorganic element such as Cl on silver nanoparticles morphology and structure;
- optimization of electrospinning process to avoid creation of nanoparticles clusters and to improve properties of final material;
- to confirm antibacterial activity of promising fibrous material as well as of silver nanoparticles probably used in medicine.

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