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DEVELOPMENT OF COAXIAL ELECTROSPINNING TECHNOLOGY

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SUMMARY OF THE THESIS

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Abstract

There is a growing interest in nanofibers for biomedical applications in the last years. Core-shell nanofibers have a high potential of use in tissue engineering to replace damaged tissue or as materials for a drug delivery system due to their structure mimic the extracellular matrix and the possibility of incorporation of the active substance as their core part.

Generally, the needle is the most commonly used electrode for the electrospinning. This technology was investigated in this work and different needle coaxial spinning electrodes were developed and tested. A disadvantage of the needle technology is its very low productivity. Needleless coaxial spinning electrodes developed within this work allow the increase of productivity of core-shell nanofibers. The needleless coaxial electrospinning is a relatively new technology for production of core-shell nanofibers from a free surface of polymeric two-layer. This method was developed at Technical University of Liberec and patented in 2009 (Pokorný). The apparatus called the Weir spinner and the cylindrical coaxial spinning electrode were developed and tested within this work. There are just few companies offering equipment for production of core-shell nanofibers in the world. Unique equipment presupposing the industrial production of nanofibers and core-shell were developed at Department of Nonwovens and Nanofibrous materials and Department of Textile Machine Design at TUL in 2012 within this work and the cluster Nanoprogres.

Theoretical relations of hydrodynamics for needleless electrospinning from a free liquid surface were derived in this work. They allow a determination of a relaxation time of electrospinning. This is the time of Taylor cone formation after HV switching. Dispersion relations were verified with experiment of needleless electrospinning of polyvinyl alcohol solutions. The dependence of the time delay T on the electrospinning number Γ was found.

The analysis of the core-shell structure of nanofibers is not easy. This work describes various methods leads to a detection of the core-shell nanofibrous structure. The phase contrast method developed within this work is relatively inexpensive and easy method allows proving the core-shell structure using elements with higher atomic number in the core.

Keywords: coaxial electrospinning, core-shell nanofibers, needleless coaxial electrospinning, spinning electrodes

Anotace

V posledních letech vzrůstá zájem o nanovlákna v biomedicínských aplikacích. Koaxiální nanovlákna, připomínající svou strukturou extracelulární matrici a umožňující zapouzdření aktivních látek do své jádrové části, mají vysoký potenciál použití v oblasti tkáňového inženýrství, kde mohou nalézt uplatnění např. jako náhrada poškozené tkáně nebo jako materiál vhodný pro cílenou dopravu léčiv.

Jehla je obecně nejpoužívanější elektrodou pro výrobu nanovláken. V rámci této práce byla tato technologie detailně zkoumána a bylo vyvinuto několik druhů jehlových elektrod. Limitujícím prvkem je jejich velmi nízká produktivita. Vyšší výrobnosti lze docílit bez-jehlovou technologií, což je poměrně nová metoda pro výrobu koaxiálních nanovláken z volného povrchu polymerní dvojvrstvy vyvinutá a patentovaná roku 2009 na Technické univerzitě v Liberci (Pokorný, 2009). V rámci této práce byly vyvinuty bez-jehlové koaxiální zvlákňovací elektrody zvyšující výrobnost koaxiálních nanovláken - tzv. přeplavovací spinner a kruhová elektroda. V současné době je známo pouze malé množství společností nabízejících zařízení pro výrobu koaxiálních nanovláken. Ve spolupráci s Katedrou netkaných textilií a nanovlákenných materiálů a Katedrou textilních a jednoúčelových strojů Technické univerzity v Liberci byla v rámci klastru Nanoprogres a této předložené práce vyvinuta zvlákňovací zařízení s předpokládaným použitím v průmyslovém měřítku.

Teoretické vztahy hydrodynamiky popisující vznik bez-jehlového elektrostatického zvlákňování vedoucí k odvození tzv. relaxačního času byly odvozeny v teoretické části této práce. Relaxační čas je čas nezbytný k formování Taylorova kužele po sepnutí VN zdroje. Odvozené vztahy pro relaxační čas byly ověřeny experimentálně bez-jehlovou technologií elektrostatického zvlákňování polyvinyl alkoholu, přičemž byla nalezena závislost časového zpoždění T na elektrospinningovém čísle Γ.

Analýza vnitřní struktury koaxiálních nanovláken není jednoduchou záležitostí. Tato práce popisuje řadu metod vedoucích k detekci jádro-plášť struktury vyrobených nanovláken. Jednou s možných metod je metoda tzv. fázového kontrastu - poměrně snadná a finančně nenáročná metoda založená na látce vyššího atomového čísla v jádře vedoucí k prokázání koaxiální struktury nanovláken.

Klíčová slova: koaxiální elektrostatické zvlákňování, koaxiální nanovlákna, hladinové koaxiální elektrostatické zvlákňování, zvlákňovací elektrody

Content

1 Introduction

The thesis is focused on the coaxial electrospinning. This work examines and describes the process of electrospinning itself, the development of special coaxial spinning electrodes and the analysis of the core-shell structure of formed nanofibers. Parameters of the process for coaxial electrospinning are investigated as a fundamental basis for a design and development of the new coaxial spinning electrodes. The coaxial electrospinning is less widespread in industry. Currently this is primary laboratory method for the development of sophisticated nanofibrous systems for special applications such as tissue engineering, drug delivery system or special probes for example. Significant part of this work is aimed at an optimization of needleless coaxial spinning electrodes for productivity enhancement of core-shell nanofibers. Detailed analysis and investigation of the coaxial electrospinning process are one of the main aims of this work. New knowledges obtained within this work are necessary to ensure the optimal formation of core-shell nanofibers. Onset of the electrospinning process is observed with the focus on formation of a bi-component droplet or a polymeric two-layer at a needle or a needleless case, respectively. Morphology analysis of the core-shell structure of produced nanofibers realized using various methods is the next part of this thesis.

2 Purpose and the aims of the thesis

The development and experimental testing of the needle and needleless coaxial electrospinning electrodes and an optimization of process and materials parameters of the coaxial electrospinning leading to the core-shell nanofibers formation are main goals of this work. The next part of this work is focused on a description of the dispersion laws for non-viscose and viscose liquids and derivation of a relaxation time of the electrospinning process. The last part deals with the characterization of produced nanofibers and an experimental proof of core-shell nanofibrous structure. An optimization of process and materials parameters of the coaxial electrospinning leading to the core-shell nanofibers formation and a determination of the suitable and easy methods for an experimental proof of core-shell nanofibrous structure are main goals of this thesis.

3 Overview of the current state of the problem

Electrospinning is a relatively simple nanofibers producing technique known since the beginning of the former century (Formhals, 1934; Zeleny, 1914). This is a process that employs electrostatic forces to produce ultra-fine fibers with diameters ranging from micrometers down to hundreds of nanometers.

The coaxial electrospinning is less widespread in industry. Currently this is primary laboratory method for the development of sophisticated nanofibrous systems for special applications such as tissue engineering, drug delivery system or special probes for example. Core-shell nanofibers have a high potential of use in tissue engineering to replace damaged tissue or as materials for a *drug delivery system* (Dzenis, 2004; Moghe, 2008). The structure of nanofibers is reminiscent of the extracellular matrix. Due to these specific properties and the possibility of incorporation of the active substance as their core part, core-shell nanofibers have an excellent usage in a medical field as a replacement of a damaged tissue (e.g. a skin, muscle tissue, or neural tissue), wound dressing or as systems for drug delivery. Materials with incorporated drugs, antibiotics, disinfection, enzymes, liposomes, spheres or even with

DNA can be created and used in this field. They can be used as nanofibrous scaffolds enabling a support of cell proliferation and a creation of the new tissue.

The coaxial electrospinning also eliminates any damage caused by direct contact of the incorporated drug with organic solvents or harsh conditions during emulsification (Jiang, 2014). The high interest in core-shell nanofibers for medicine is given by the possibility of spinning of completely or hardly spinnable materials. The coaxial electrospinning can produce nanofibers with incorporated antibiotics (Huang, 2003), drug (Su, 2012), growth factors (Liao, 2006), enzymes (Reznik, 2006) or cells (Klein, 2009). Great advantage of this technology is a core-shell formation with core and shell components represented by materials with different degradation times. The medicine is not the only one possible field of use of the core-shell nanofibers. They can be also used in optics, filtration, as composites, electrically conductive fibers or as special probes for detection systems (A.G. MacDiarmid, 2001; Song, 2006; Dumas, 2007; Greiner, 2007). The technology of coaxial electrospinning also allows incorporation of solid nanoparticles into nanofibers. Iron particles, silver particles, magnetic particles or nanodiamonds for example can be used as core part of the core-shell nanofibers. These systems can be suitable as special magnetic filters, sensors, special probes or they find them application in electronics (Dumas, 2007; Song, 2006).

The first equipment for coaxial electrospinning has already been patented in the early 20th century (Cooley, 1902). Two liquid materials are independently delivered through the coaxial capillary, i.e. spinneret, to its orifice. A composite polymeric droplet is created in an orifice of this spinneret. *Taylor cone* (Taylor, 1964) is created on the top of the composite droplet and both liquids in common are drawn and elongated by electric forces and collected on the grounded collector as nanofibers with a core-shell structure (Reznik, 2006). This technology is known as the needle coaxial electrospinning.

Research teams began to examine coaxial electrospinning in more detail at the turn of the 20th and 21st centuries. Yarin and Zussman dealt with the combination of organic and inorganic materials (Sun, 2003). Hollow nanofibers can be also produced using this technology (Dror, 2009; Li, 2004). Two materials with different solvents are formatted into nanofibers using the coaxial electrospinning and follow the removal of the core part by a treatment of its solvent system.

The coaxial electrospinning is not just the only option to form core-shell nanofibers. An emulsion electrospinning is another way to production of nanofibers with the core-shell structure (Bazilevsky, 2007). His single-nozzle technique allows production of the core-shell nanofibers from a polymeric mixture of immiscible liquids.

Generally, the needle is the most commonly used electrode for the electrospinning. A disadvantage of this technology is a very low production of core-shell nanofibers. As described by Haitao (2009), a needle can mostly create only one polymer jet and the productivity of nanofibers is less than 300 mg/h per needle. Higher productivity can be reached by increasing the number of needles (Ding, 2004). However, this so-called multiple-jet setup has a problem with non-uniform electric field, a large operating space is necessary and the cleaning of a spinning electrode is more demanding (Vysloužilová, 2010). A new idea of needleless electrospinning to increase productivity of nanofibers was introduced by Yarin and Zussman in 2004 (Yarin, 2004). The main aim of their work was a realization of multiple upwards jets from a free surface of polymeric solution without any needles. Technology of mass production of nanofibers and commercialization of the needleless electrospinning was developed in 2003 at TUL in cooperation with Elmarco Ltd. and patented under the brand name Nanospider^{TM} in 2005 (Jirsak, 2005).

First apparatus for the needleless coaxial electrospinning was developed at TUL and patented in 2009 under the name "Weir spinner" (Pokorný, 2009). This technology is based on the electrospinning from a very thin two-layer of polymer solutions flowing over the electrode as is shown in Figure 1. This technology was called the weir spinner due to its similarity to the weir on a river (Vysloužilová, 2010).

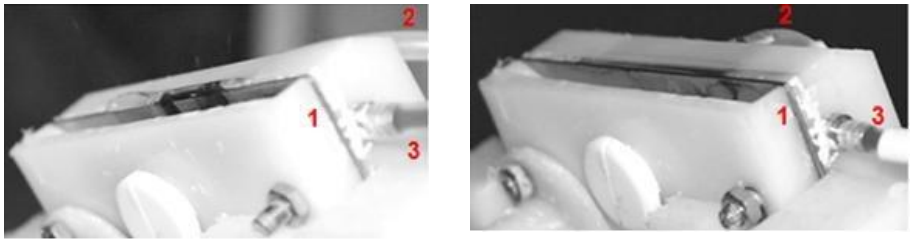


Figure 1 *The weir spinner: polymer two-layer flowing over the spinning electrode (1), feed of the shell polymer solution (2), feed of the core polymer solution (3), (Vyslouzilova, 2010).*

Unique equipment for the industrial production of nanofibers and core-shell nanofibers (Spinner 1 and Spinner 2) were developed at Department of Nonwovens and Nanofibrous materials and Department of Textile Machine Design at TUL in 2012 within this work and the cluster Nanoprogres, see (Figure 2), (Vysloužilová, 2012). Spinner 2 (Figure 2 b, c) is unique equipment allowing the production of nanofibers and core-shell nanofibers in cleanroom in grade A (European Standard). This is certificated inside the electrospinning chamber of this equipment. An integral part of the Spinner 2 is a laminar box ensuring the cleanroom in grade B (European Standard). The uniqueness of this highly productive equipment can be assessed by the fact that only a few other institutions are currently deals with the development of the needleless coaxial electrospinning.

(a) (b) (c)

Figure 2 *Equipment for industrial production of nanofibers and core-shell nanofibers developed within this work and cluster Nanoprogres: (a) Spinner 1, (b, c) Spinner 2 for clean rooms: The coaxial equipment (1), the coaxial spinner in the holder (2), the collector (3), a nonwoven spun-bond material to collect nanofibers (4), a camera (5), a hydraulic dosing system (6), dosing syringes (7), a control panel (8), a laminar flow box (9).*

In 2012, American Society Arsenal Medical, Inc. introduced a high-performance slit electrode for core-shell nanofibers formation (Yan, 2012). In 2014, a Contipro Company introduced 4SPIN device for the production of composite nanomaterials and core-shell nanofibers (Contipro, 2014). In 2013, Forward *et al.* described a coaxial electrospinning technology using a wire electrode. The principle of their technology is a passing of a wire

through two immiscible solutions (Forward, 2013). In 2014, Jiang and Qin introduced a high throughput one stepped pyramid-shaped spinneret with productivity 4 g/h, which is several hundred times higher than that of conventional sigle-needle electrospinning (Jiang G., 2014). In recent years, an interest in the highly productive coaxial spinning electrodes is greatly higher. An increasing number of both local and foreign institutions deal with the idea of core-shell nanofibers formation.

4 Materials and Methods

4.1 Materials used

Water soluble polymer polyvinyl alcohol (PVA) for initial testing of spinning electrodes was used. Polyvinyl butyral (PVB), polyethylene oxide (PEO), polycaprolactone (PCL), polyvinyliden fluoride (PVDF) and polyvinyl pyrolidon (PVP) were used for next experiments with development spinning electrodes. Solutions of PVA (Sloviol, Chemicke zavody Novaky, SK) were prepared at concentration ranging from 5 to 15 % (w/v) dissolving in distilled water for investigation of relaxation time of electrospinning.

In the last step, nature polymers for the anticipated use in medicine were selected. Nanofibers from natural biopolymers (e.g. silk fibroin, chitosan, collagen and hyaluronic acid) have a high potential to be utilized in biomedicine, because these are biodegradable, biocompatible and non-toxic materials. Chitosan, Hyaluronic acid (HA) and its sodium salt were used. Carboxymethyl cellulose (CMC) was used as inexpensive variant of HA for initial experiments.

4.2 Electrospinning equipment

Initial experiments were realized using laboratory setup for coaxial electrospinning, see (Figure 3). This apparatus was designed and manufactured exclusively for research activity within this work. In the next step, new unique equipment for core-shell electrospinning was developed in framework of this work within cluster Nanoprogres (Spinner 1 and Spinner 2), see (Figure 2).

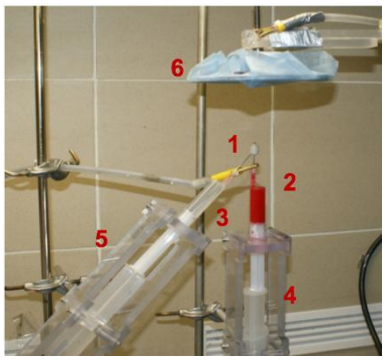


Figure 3 *Laboratory set-up for the needle coaxial: the coaxial needle spinning electrode connected to the HV source with a positive charge (1), the syringe with the core polymer solution (2), the syringe with the shell polymer solution (3), the hydraulic system for the core solution (4) and for the shell solution (5), the collector with the Spunbond nonwovens connected to the HV source with a negative charge (6).*

4.3 Coaxial spinning electrodes

Needle and needleless spinning electrodes were designed, constructed and tested in this work. Nanofibers were collected on different kinds of 2D and 3D collectors in dependence of the desired structure of nanofibrous products.

4.4 Relaxation time of electrospinning

Determination of the relaxation time of electrospinning

The dispersion law for the non-wiscose liquids is given as

$$\omega^2 = gk + \frac{p}{\rho}k^2 - \frac{\varepsilon_0 E^2}{\rho}k^2, \tag{1}$$

where ω denotes the angular frequency, $\varepsilon_0$is permittivity of vacuum and E is field strength. The relaxation time of electrospinning T can be determined on the bases of the dispersion law for viscose fluids:

$$(-i\omega + 2\nu k^2)^2 + \omega_0^2 = 4\nu^2 k^4 \sqrt{\frac{-i\omega}{\nu k^2} + 1}, \tag{2}$$

where ω denotes the angular frequency, ν is velocity and k is the wave vector. Relaxation time is the time necessary to Taylor cones formation and start of electrospinning after applying of the high voltage on the electrospun liquid. The electric forces deformed the free liquid surface and Larmor-Tonks-Frenkel instabilities are formed (Frenkel, 1955; Tonks, 1935; Larmor, 1890). The electrospinning originates from the fastest growing instability when the critical value of the electric strength E_c is overcoming. Levich (1962) introduced the solution of the dispersion law (2) in the case of the low viscosity liquids and long wavelengths, $\frac{-i\omega}{\nu k^2} \gg 1$ as:

$$-i\omega \cong -i\omega_0 - 2\nu k^2. \tag{3}$$

The solution of the dispersion law (2) for highly viscous liquids and short wavelengths, $\frac{-i\omega}{\nu k^2} \ll 1$, is given as (Levich, 1962):

$$-i\omega \cong -\nu k^2 \pm \sqrt{\nu^2 k^4 - \omega_0^2} = -\frac{\omega_0^2}{2\nu k^2}\,. \tag{4}$$

Electrospinning originates from the fasters growing instability on the free surface of the liquid. This process is managed by the dispersion law (2). The instability occurs for a root of this equation with $-i\omega > 0$. The reciprocal value of this growth rate $\tau = \frac{1}{-i\omega}$ can be taken as a characteristic time of the Taylor cone formation.

It is advantageous to introduce dimensionless variables and relations for further adjust:

$$\Omega_0^2 = \omega_0^2 \cdot \frac{a}{g}\,,\ A = -i\omega\sqrt{\frac{a_c}{g}}\,,$$

$$K = a_c k,\ O_h = \sqrt{\frac{\nu^2}{g a_c^3}}\,, \tag{5}$$

where A is the dimensionless growth rate, K denotes the dimensionless wave number, a_c denotes capillary number $a_c = \sqrt{\gamma/\rho g}$ and $O_h$is the *Ohnesorge number*[1]. The dispersion relations (4) and (1) can now be rewritten respectively as:

[1] The Ohnesorge number is a dimensionless constant that describes liquid cohesion. This number relates the viscous forces to inertial and surface tension forces.

$$(A + 2O_h K^2)^2 + \Omega_0^2 = 4O_h^2 K^4 \sqrt{\frac{A}{O_h K^2} + 1}\,, \tag{6}$$

$$\Omega_0^2 = K^3 - 2\Gamma K^2 + K \tag{7}$$

We want to gain dimensionless growth rate A according K. The equation (6) can be rewritten using a new dimensionless variable $x = A/(O_h K^2)$ as:

$$(x+2)^2 - 4\sqrt{x+1} + \Omega_0^2/O_h^2 K^4 = 0. \tag{8}$$

The first term is dominant for large values of x and the second one can be linearized for $x \to 0$ using $\sqrt{x+1} \cong 1 + x/2$. In this approximation, the dimensionless dispersion relation (8) can become a quadratic function in x, $x^2 + 2x + \Omega_0^2/(O_h^2 K^4) = 0$. The dimensionless growth rate A can be then obtained as its positive root:

$$A = -O_h K^2 + \sqrt{O_h^2 K^4 - \Omega_0^2}\,. \tag{9}$$

Now, we need to obtain the relations between the dimensionless wave number K and the electrospinning number $\Gamma = \frac{a\varepsilon E^2}{2\gamma}$ (Lukas, 2008). The condition for maximization of the growth rate A follows from the (9) rewritten in the form of an implicit function. Then, the conditions for extremes of this implicit function *f(K,A)* are:

$$f(K,A) = A^2 + 2O_h K^2 A + K^3 - 2\Gamma K^2 + K = 0, \tag{10a}$$

$$\frac{\partial f(K,A)}{\partial K} = 4O_h AK + 3K^2 - 4\Gamma K + 1 = 0. \tag{10b}$$

By analyzing the roots of (10) we can obtain the relations between the electrospinning number Γ and the dimensionless wave number K. This one is valid in case of the maximal growth rate A. In the first step, the (10b) can be rewritten as $\Gamma = O_h A + 3K/4 + 1/(4K)$ and $= O_h AK + 3K^2/4 + 1/4$. The equation for ΓK, together with that for $f(K,A)$, (10a), gives $A = \sqrt{(K^3 - K)/2}$. Thus, substituting A from here back into (10b) we obtain the resultant Γ-K relationship (11) and maximal A value for medium viscosity liquids. This relationship is compared to the one for non-viscous liquids (12):

$$\Gamma = \frac{3}{4}K + \frac{1}{4K} + O_h\sqrt{\frac{K^3 - K}{2}}\,, \tag{11}$$

$$\Gamma = \frac{3}{4}K + \frac{1}{4K}\,. \tag{12}$$

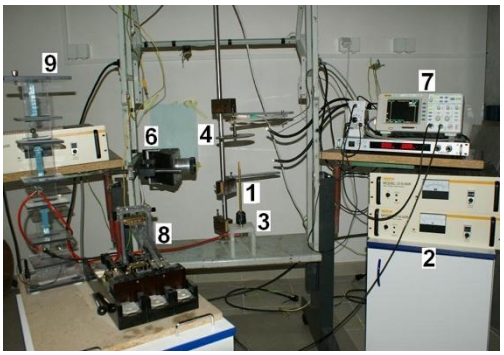
The equation (9), together with the inversion of the equation (11), i.e., $K = K(\Gamma)$, gives the dependence of the maximal value of A on the electrospinning number Γ for a given O_h. The relation $A = A(\Gamma)$ means that the theoretical results can be compared with experimental data on the dimensionless time of Taylor cone formation, $T = 1/A$:

$$T = \frac{1}{-O_h K^2 + \sqrt{O_h^2 K^4 - K^3 + 2\Gamma K^2 - K}} \tag{13}$$

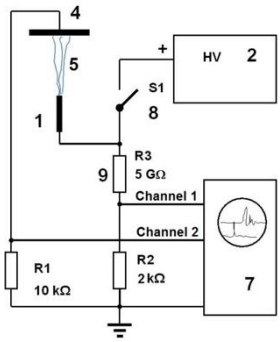
Experimental examination of the relaxation time of electrospinning

The equipment for investigation the relaxation time of the electrospinning was manufactured at the KNT, TUL within this work. Rod spinning electrode with diameter 8.85 mm was used for this experiment and located 100 mm under the metal disc collector with diameter 150 mm. A 300 Watt High Voltage DC Power Supply (AU-60PO.5-L, Matsusada Precision Inc.) was

used. The rod was connected to the positive pole of the high voltage source, while the collector was grounded. Photographic snapshots of electrospun polymeric droplets were taken using stereopticon Meopta, see in Figure 5a, and Nikon camera Coolpix 4500. Surface tension measurements were done using the digital tensiometer (Krűss K9) and viscosity of polymer solutions was obtained using viscometer Haake Roto Visco 1.



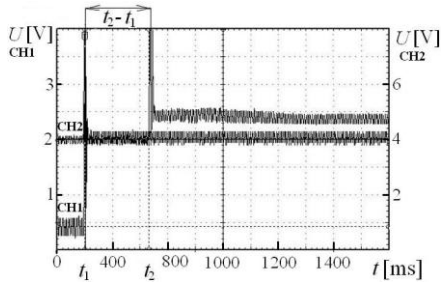
(a)



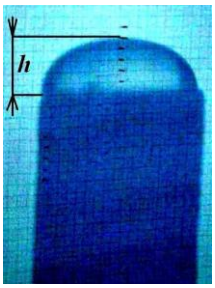
(b)

Figure 4 *(a) The electrospinning setup: rod spinning electrode (1), HV source (2), chuck (3), disk collector (4), polymer jet (5), (b) The oscilloscope (7), switcher (8) and voltage divider (9)*

The experimental setup allows read two voltaic signals at channels 1 and 2 of the oscilloscope. The channel 1 records the switch-on state of the rod that is triggered by the switch S1. This entry is represented by a lower voltage signal on oscilloscope records, see (Figure 5). Voltage waveforms are recorded at channel 1 of the oscilloscope using voltage divider formed by resistors R3 and R2. The switch point appears as a peak at time t_1. The oscilloscope channel 1 records the voltage on the rod, and hence, on a droplet. The electric current caused by the charges reaching the collector is recorded at channel 2 of the oscilloscope and appears as a next delayed signal having its peak at time t_2. These charges are formed in the vicinity of the Taylor cone and are accelerated by electric field pushing them toward the collector. The time of ion transport to the collector is measured in milliseconds, hence can be safely neglected here (Pokorny, 2010). Therefore the time difference $\Delta = t_2 - t_1$ is defined as the characteristic time of Taylor cone formation.



(a)



(b)

Figure 5 *(a) Oscilloscope records from channels 1 (CH1) and 2 (CH2) provide the time delay* $\Delta = \boldsymbol{t_2 - t_1}$ *between the moments of switching on the field and the onset of jet formation. (b) The droplet of height h on the top of the electrospinning rod electrode.*

4.5 Visualization of the Electrospinning proces

Visualization methods were used for record and investigation of electrospinning process. The broad range of methods was used to observed nanofibrous morphology and to analysis of core-shell structure (Vysloužilová, 2015). A web camera Microsoft LifeCam Studio with a 1080p sensor and the camera Nikon Coolpix S8100 (Nikon, Jap.) were used for general view over the electrospinning process, observation of the polymer droplet/layer creation at the spinning electrode orifice and state of electrospinning. The figure 6 shows the formation of polymeric two-layer at the orifice of the cylindrical coaxial spinning electrode of the 2nd generation and DC and AC electrospinning realized by this one.

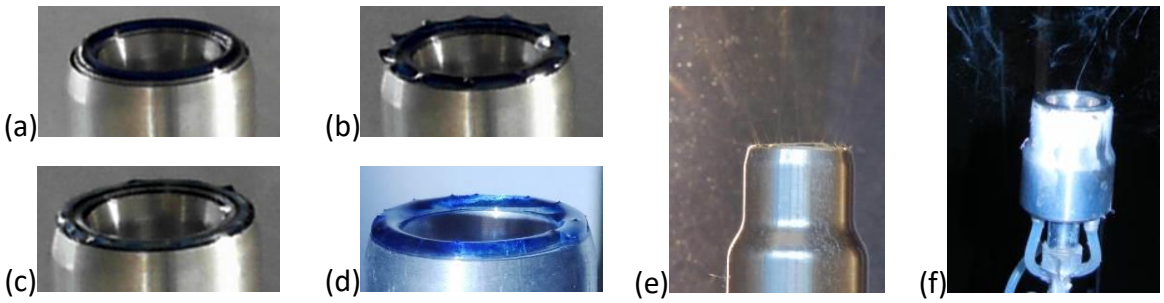


Figure 6 *The record of needleless electrospinning realized by cylindrical coaxial spinning electrode: (a) the core polymer layer formation, (b) the emerging instability on the polymer layer before reaching value of Ec, (c) Taylor cones, (d, e) DC electrospinning, (f) AC electrospinning (Vysloužilová, 2015).*

The formation of bi-component Taylor cone during needle coaxial electrospinning was observed by optical microscope within Clemson University, NC, USA. Process was recorded by a camera (Dalsa - Falcon-14M100, Stemmer). Taylor cone created from the shell polymer solution pulled up the core one, see (Figure 7) and both materials were drawn and elongated together by electric forces to produced core-shell nanofibers (Vysloužilová, L., 2014; Vysloužilová, 2015).

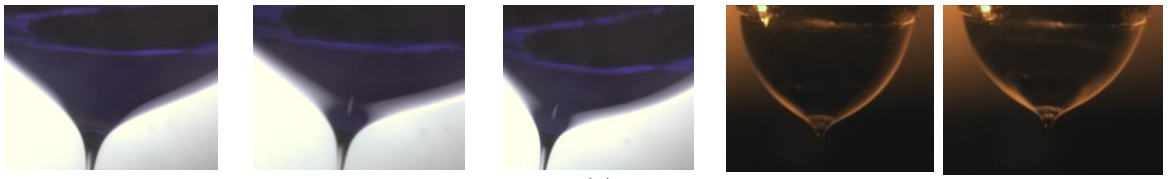


Figure 7 *The bi-component polymeric droplet during coaxial electrospinning (a, b, c) and the non-coaxial electrospinning without the core solution (d, e) recorded by the camera Dalsa - Falcon-14M100, Stemmer (Clemson, NC, USA): The shell Taylor cone (the light transparent part of the bi-component droplet) pulls up the core solution (the dark part of the bi-component droplet). The different displaying of coaxial and non-coaxial polymer droplet is caused by different light during experiments. This was necessary to clear observed of the core solution inside the shell one in case of coaxial variant of electrospinning (Vysloužilová, L., 2014; Vyslouzilova, 2015).*

The HD camera Panasonic HC-W850 with 120 fps (Panasonic, UK) and the high speed camera iSPEED 3 with up to 150 000 fps (Olympus, USA) were used to observation of the formation of the polymer bi-component droplet at the orifice of the needle coaxial spinning electrode, see (Figure 8a) and the layer/two-layer, respectively in case of needleless coaxial electrospinning, see (Figure 8b-d). The emerging instabilities, the formation of Taylor cones and polymeric jets as well as their shape, number, stability, density (characteristic wavelength λ) and their behavior throughout electrospinning process were investigated (Valtera, 2014; Vysloužilová 2015).

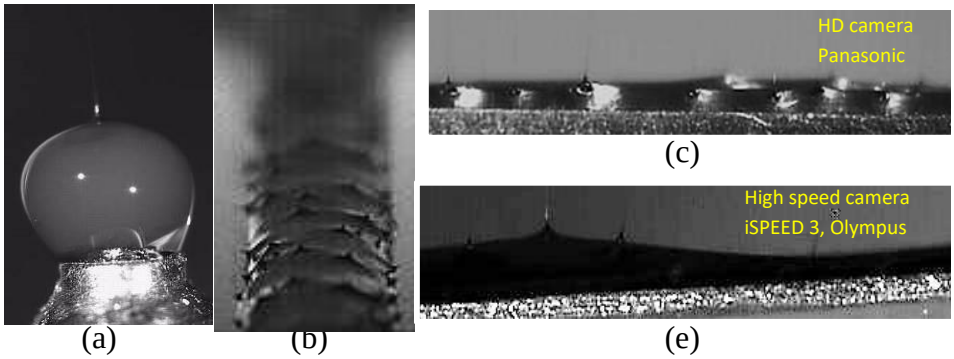


Figure 8 *Polymer jets during electrospinning realized by (a) the coaxial needle spinning electrode, (b) the cleft electrode, (c, d) the weir spinner of the 2nd generation (Valtera 2014; Vysloužilová 2015)*

The UV Corona camera CoroCam 1 (Uvirco Technologies, SA) was used for investigation of the distribution of the Taylor cones and polymeric jets (characteristic wavelength λ) as well as for observation of corona discharges occurrence as can be seen in Figure 9 b-d and Figure 18 (Vysloužilová, 2015). Characteristic wavelength λ and its critical value could be identified and behavior of polymeric jets throughout electrospinning process could be observed as is shown in Figure 9. This method can also detect an unsuitably chosen dosage of polymer solutions (low speed), resulting in uncovering the surface of the spinning electrode and the emergence of spark discharges.

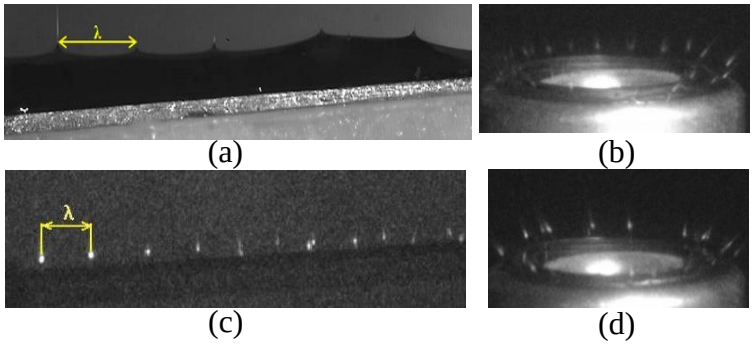


Figure 9 *The characteristic wavelength λ in case of needleless electrospinning recorded by (a) the high speed camera and (b-d) the UV camera (Vyslouzilova, 2015)*

4.6 Analysis of core-shell structure

The morphology of nanofibrous layers was observed using scanning electron microscopy (SEM) Phenom G2 (FEI, USA), Tescan Vega (Tescan, CZ) and Carl Zeiss ULTRA plus (Zeiss, DE). The analysis of core-shell structure was investigated using confocal laser scanning microscope Zeiss LSM 5 DUO (Zeiss, DE), transmission electron microscope High-resolution TEM – H9500 (Hitachi, Jap.) and SEM Carl Zeiss ULTRA plus (Zeiss, DE). Cuts of nanofibers was done using CryoMill, (Retsch GmbH, DE) and Jeol JSM 7600F (Jeol Ltd., DE). The spectrofotemeter Avatar 320 FT – IR (Elsichrom, SE) and Energy Dispersive X-ray Spectrometer JED 2300 (Jeol Ltd., DE) were used for detection of incorporated materials.

5 Summary of the results achieved

5.1 Needle coaxial spinning electrodes

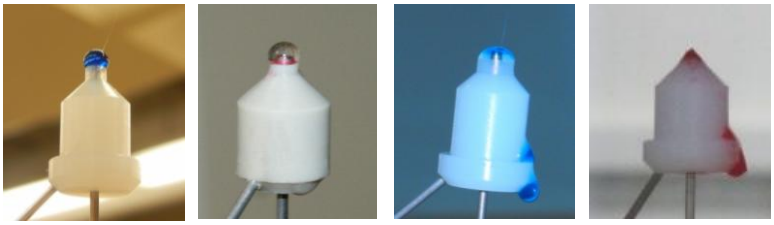
Many variants of needle spinning electrodes were developed within this work and in collaboration with Audacio, Ltd. Company. We designed electrodes from duraluminium, teflon, polypropylene (PP), polyoxymethylene (POM) and high density polyethylene TIVAR 1000 (HDPE). The reason for choosing of a wide range of construction materials was a finding of optimal spinning electrodes allows electrospinning of broad range of materials. Spinning electrodes from conductive and non-conductive construction materials were designed a developed for the purpose of easy manipulation, maintenance and with respect to chemical and mechanical resistance by intended use. In the next step, the investigation was focused on the shape of construction of a spinneret orifice. Spinnerets with a different shape of the orifice and an angle were developed. The HDPE coaxial needle spinning electrode was selected as the best material for the manufacture of coaxial needle spinning electrodes. This material is suitable for electrospinning, easily to handle and there were not any problems with core-shell nanofibers formation during electrospinning process (Vysloužilová, L., 2014).

Based on the knowledge gained from the testing of developed coaxial spinning electrodes were designed and constructed another series of spinning electrodes from HDPE with different designs. The next work was focused on the shape of the orifice of the spinning electrodes. New spinning electrodes with and without so-called “neck”, with different shapes, bevel angle and diameter of the neck were designed within this work. The new developed needle coaxial spinning electrodes are shown in Table 1.

Table 1 *Developed needle coaxial spinning electrodes with different shapes of their orifice (Vysloužilová, L., 2014)*

				
Material: POM	Material: TIVAR 1000	Material: TIVAR 1000	Material: TIVAR 1000	Material: TIVAR 1000
With the neck	With the neck	With the neck	With the neck	Without the neck
diameter of the neck: 3 mm	diameter of the neck: 3 mm	diameter of the neck: 2 mm	diameter of the neck: 6 mm	diameter of the neck: 3 mm
Suitable electrospinning materials: universal for a wide range of materials.	Suitable electrospinning materials: universal for a wide range of materials.	Suitable electrospinning materials: containing of volatile substances.	Suitable for needle non-coaxial electrospinning of water soluble liquids.	Unsuitable for electrospinning, slumping of the polymeric droplet

It was found, that the neck allows keeping of polymeric droplet at the orifice of the spinner throughout the electrospinning process. Electrodes with a tiny orifice are appropriate for electrospinning of volatile solvents. Electrodes with a large orifice and without a neck, see (Figure 11 c, d) are unsuitable for using. Optimal bi-component droplet can not to be created, this is slumping from the electrode and core-shell nanofibers are not produced.



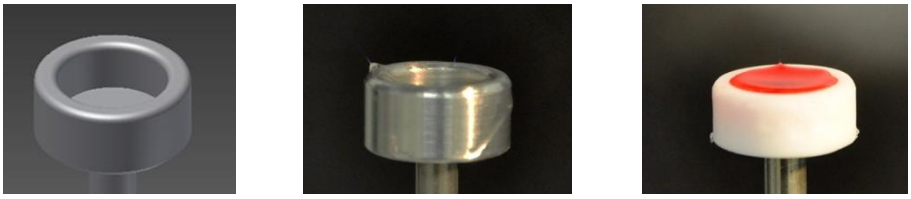
(a) (b) (c) (d)

Figure 11 *Developed coaxial needle spinning electrodes with the neck and optimal bi-component polymeric droplet (a, b), the too large of diameter of the spinning electrode orifice caused the droplet slumping (c), the spinning electrode without the neck causes the droplet slumping (d).*

In the next step, new coaxial spinning electrodes with different bevel angle of the orifice and of the upper part of the spinning electrode was designed and tested within this work. There was found, these structural modifications have no influence on the coaxial electrospinning.

5.2 Needleless coaxial spinning electrodes

Dis-continual and continual needleless coaxial spinning electrodes were developed within this work to increase of productivity of core-shell nanofibers. Dis-continual spinning electrodes were intended for a laboratory use to investigate of the coaxial electrospinning process with focus on the behavior of Taylor cones on the free surface of the polymer solution, see (Figure 12). The produced nanofibrous layers are shown in Figure 13. Continual needleless spinning electrodes were developed in cooperation with KTS TUL under the project Nanoprogres.



(a) (b) (c)

Figure 12 *Pool needleless spinning electrodes: The model of the pool spinning electrode (a), the pool spinning electrode from the electrically conductive (b) and electrically non-conductive (c) material.*

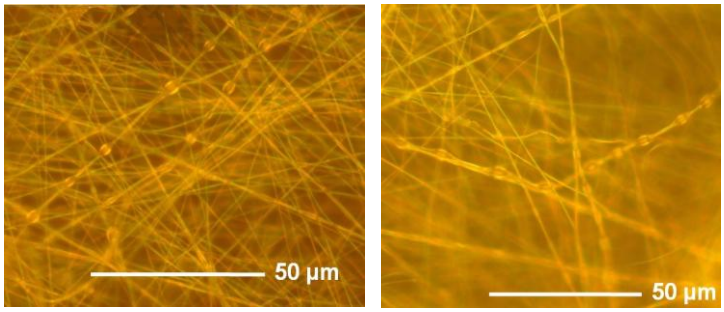


Figure 13 *Core-shell nanofibers with incorporated vegetable oil realized using electrically non-conductive material observed using optical microscope Olympus.*

The weir spinner

The weir spinner is the needleless coaxial spinning electrode for electrospinning from the free surface of the polymeric two-layer. This one was developed at TUL and patented in 2009 (Pokorný). This technology gained its name from the similarity to the weir on the race (Vysloužilová, 2010). Two polymers are spun together by the action of electrostatic field. Core-shell nanofibers are collected on the oppositely charged collector as is shown in Figure 14. A great number of Taylor cones and polymer jets are created on the top of free liquid surface of the polymeric two-layer.

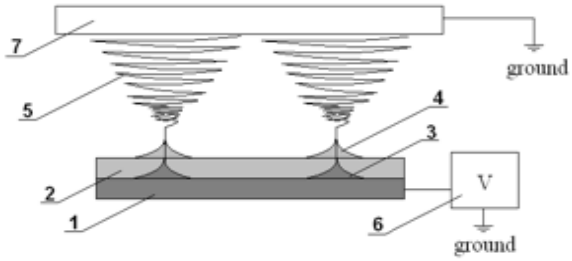


Figure 14 *The schema of the needleless coaxial electrospinning from the free surface of the polymeric two-layer: Layer of the core polymer (1), layer of the shell polymer (2), Taylor cones (3, 4), the polymer jet (5), HV supply (6), grounded collector (7), (Vysloužilová, 2010).*

The weir spinner with parameters 80 x 70 x 28 mm (weight x high x depth) consists of two feeding chambers with volume 8 ml (the shell chamber) and 7 ml (the core chamber) for dispensing of shell and core liquids and of one outflow chamber for the waste material as is shown in Figure 1. The middle part represents the plate electrode with thickness 1 mm that is placed in between two side parts. The work length of the weir spinner is 47 mm.

There were found some disadvantages of this spinning electrode. First of all, there was too large volume of feeding chambers. A lot of the spinning material has been lost due to large capacity of chambers. This is a big problem in a case of electrospinning of expensive materials such as drugs, enzymes, liposomes, special liquids with nano-diamonds and others. The next disadvantage was a uneven polymer solution feed to the orifice of the weir spinner. To ensure the uniform supply of polymer solutions is crucial for production of bi-component nanofibers with core-shell structure. Based on these knowledges from electrospinning realized using this apparatus, the new optimized *weir spinner of the 2nd generation* was designed and tested within this work, see (Figure 16). Volumes of feed chambers were reduced to 1 ml. This is great advantage for electrospinning of expensive and special polymers and liquids. The next advantages of this type of spinning electrode are thin feed chambers ensuring a laminar flow of liquids. The result is the homogeneous polymeric two-layer at the spinning electrode orifice. The work length of the weir spinner of the second generation is 42 mm (Vysloužilová, 2011).

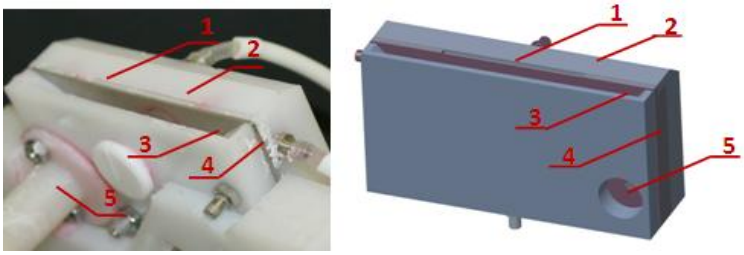
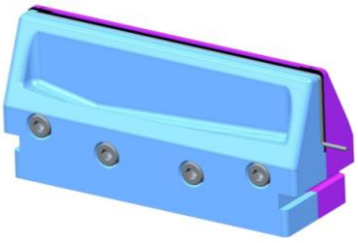
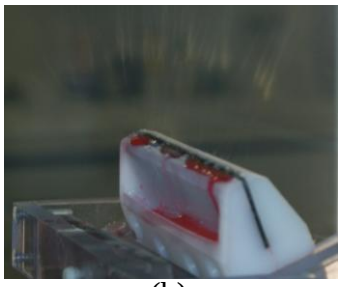


Figure 16 *The Weir spinner of the 2nd generation: the chamber for the core polymer solution (1), the chamber for the shell polymer solution (2), the outflow chamber (3), the core polymer feeding tube (4), the shell polymer feeding tube (5), the electrode (6), the holder for HV cord (7), (Vysloužilová, 2011).*

There was founded, that this electrode has some disadvantage. The new *weir spinner of the 4th generation* with parameters 35 x 155 mm (width of the base x length) was designed within this work, project SGS 28012 and Nanoprogres cluster, see (Figure 17). Its orifice was significantly narrowed and raised from the base of the spinning electrode to the maximal exposure of the electrospinning liquid to the electric field. The remaining part of the apparatus (the massive base of the apparatus) was inserted as deeply as possible from the action of the electric field. The aim was the maximum exposure of the electrospinning liquid to the electric field. The plate electrode was replaced by the wire electrode placed to the orifice of the core chamber. A separator plate was inserted between core and shell chamber.



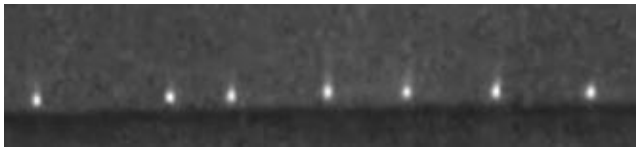
(a)



(b)

Figure 17 *3D model of the weir spinner of the 4th generation (a), coaxial electrospinning realized by the weir spinner of the 4th generation (b)*

Experiments have shown, the weir spinner is suitable spinning electrode to increase of productivity of core-shell nanofibers. The large numbers of Taylor cones and polymeric jets were created as is shown in Figure 18. The productivity of this spinning electrode is 3.45±0.83 g/h. It is approximately 5 times more than needle coaxial technology, see in Table 2. The disadvantage of this spinning electrode is susceptibility to creation of spark discharges caused by its edges as is shown in Figure 18b. Electrospinning is accompanied by corona discharges (Figure 18). Corona discharges are generated by highly charged polymeric jet roots, so-called Taylor's cones. Sometimes, the corona discharge converts into the spark discharge. This is unsuitable for electrospinning process, since the discharge drains away energy from the technological process (Vyslouzilova, 2014).



(a)

(b)

Figure 18 *Corona discharges (a) and spark discharges (b) during electrospinning realized using the weir spinner of the 4th generation. UV spots in the figure determine the position of Taylor cones. Photographs are obtained using CoroCam I, Uvirco Technologies, SA (Vysloužilová, 2014).*

Cleft electrode

Cleft electrode with the rounded edges, see (Figure 19a) was developed at KTS TUL within this work, project SGS 21012 and project Nanoprogres as a new modification of the weir spinner. The modification increases productivity of needleless coaxial electrospinning due to the suppression of spark discharges. The idea of the cleft electrode is the elimination of a loss of electric energy caused by sharp edges of the weir spinner. This spinning electrode was designed from the duraluminium. The reason for this material was the possibility to use cleft electrode in environment of the cleanroom as in Spinner 2 (Figure 2). The next advantage of this construction material is its easy workability and accuracy machining (Valtera, 2014).

Cylindrical coaxial spinning electrode

Based on the results of the spinning electrode with the cleft geometry, a new design of the electrode was developed at KTS TUL within this work and project SGS 21012 (Valtera, 2014). The aim of the new concept was to replace the linear shaped electrode by the circular one, which would eliminate problems at the ends of the cleft electrode and with spark discharges, see (Figure 9 and 18). The cylindrical coaxial spinning electrode, see (Figure 19b) has a smooth surface and it consists of three cylindrical feeding chambers. The outer chambers are intended for the supply of the shell polymer solution, the middle one is for supply of the core liquid. The diameter of the spinning electrode orifice was 31 mm. The core chamber was designed with the protrusion 0,5 above the shell one. The assumption was that the core liquid overlaps the shell one. This spinning electrode used a special tubes system and feeding chambers with auxiliary distribution ring located horizontally at the bottom of the core chamber. This system supports the uniform dosing of polymer solutions into feed chambers and the formation of the homogeneously polymer two-layer at the orifice of the spinning electrode.

(a) (b) (c) (d)

Figure 19 *needleless coaxial electrospinning realized by the cleft electrode (a, c) and by the cylindrical coaxial spinning electrode (b, d).*

The cylindrical coaxial spinning electrode with a smooth, nearly constant surface curvature of its orifice allows running electrospinning without loss of energy caused by spark discharges from sharp edges. This new concept of the spinning electrode allows the minimal polymer waste consisting in elimination of polymer solution slumping. This electrode is

suitable for DC and AC electrospinning. The productivity of developed spinning electrodes is listed in Table 2.

Coaxial spinning electrode	L[(a)] [mm]	U_c[(b)] [kV]	U_{opt}[(c)] [kV]	v_c[(d)] [ml/h]	v_s[(e)] [ml/h]	n[(f)]	P[(g)] [g/h]	d[(h)] [nm]
Needle spinner	130	36	40	2.5	4.8	1	0.64±0.03	330±60
Conductive pool spinner	130	34	36	-		6±3	0.82±0.99	290±62
Nonconductive pool spinner	130	30	34	-		10±5	0.9±0.96	280±50
Weir spinner (50 mm)	130	40	48	8	12	40±8	2.67±0.99	270±60
Weir spinner (100 mm)	140	66	60	15	35	50±10	3.45±0.83	270±52
Cylindrical spinner (95 mm)	190	56	66	6	25	50±10	2.18±0.66	280±97
Cleft electrode	150	60	65	3	9	20±8	2.67±0.99	270±60

Table 2 *Process parameters and productivity of the developed coaxial spinning-electrodes*

(a) L is the distance between electrodes
(b) U_c is the critical value of the voltage
(c) U_{opt} is the optimal value of the voltage
(d) v_c is the feed rate of the core polymer solution
(e) v_s is the feed rate of the shell polymer solution
(f) n is the number of polymer jets
(g) P is the productivity of the spinning electrode
(h) d is the average value of fiber diameters

5.3 Relaxation time of electrospinning

The dimensionless electrospinning number $\Gamma = a\varepsilon E^2/2\gamma$ and capillary number $a_c = \sqrt{\gamma/\rho g}$ (Lukas, 2008) are necessary to characterize the process of Taylor cone and polymeric jet formations for excitation of the electrospinning process. The polymeric jet is expected to emanate from the Taylor cone when the electrospinning number exceeds the critical value. This criticality condition defines the critical field at the liquid surface $E_c = \sqrt[4]{4\gamma\rho g/\varepsilon^2}$, which depends only on the physico-chemical parameters of the liquid (Lukas, 2008).

In these experiments, the voltage U as a global characteristic of the electric field was defined. In the next step, the local field strength values E was assigned to the applied voltages U. The process started with the experimental determination of the so-called critical voltage U_C as the lowest voltage for which the liquid surface was destabilized and a polymeric jet appeared. The relationship between U and E is assumed to be linear for a fixed geometry of the spinner, $E = \beta U/L$, where β is a dimensionless enhancement factor and L is the distance between the spinning electrode and the collector. The enhancement factor β was determined from the measured critical voltage U_C and the theoretically predicted critical field E_C as $\beta = E_C L / U_C$. The experimental values of U_C were found inside the interval 12 - 30 kV.

The time delay obtained from experiment was about 24 ms for low viscosity PVA solutions (5% (w/v)) with high electrospinning numbers ($\Gamma = 6$). For highly viscous solutions (15% (w/v)) and the critical electrospinning number ($\Gamma \approx 1$), the time delay increased up to 1.269 s. These time delays Δt were measured ten times for each concentration and for each

value of the electrospinning number. The relationship between the experimentally determined dimensionless time delay $T_{EXP} = \Delta t\sqrt{g/a}$ and the dimensionless electrospinning number Γ is plotted in Figure 20. One polymeric jet was observed in case of low electrospinning numbers ($\Gamma \cong \Gamma_c = 1$), while for greater electrospinning numbers ($\Gamma = 6$) five polymeric jets were observed. The ability to generate multiple jets with the existing setup can be used as evidence for the formation of nanofibers in the so-called needleless electrospinning mode (Yarin, 2004).

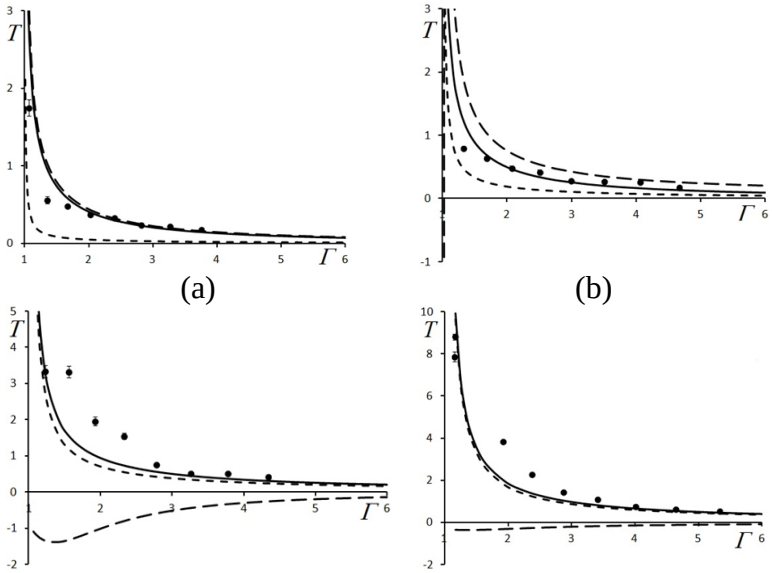


Figure 20 *The time delay T as a function of electrospinning number Γ. Experimental data points correspond to the mean values averaged over 10 measurements each. (a) PVA solution at concentration of 5 % (w/v), Oh = 0.033, (b) PVA solution at concentration of 8 % (w/v), Oh=0.132, (c) PVA solution at concentration of 12 % (w/v), Oh=0.628 (d) PVA solution at concentration of 15 % (w/v), Oh=1.640.*

Values of the time delay were obtained from the experimental measurement for different concentration of PVA solution. This time delay denotes the time required to the build of Taylor cone. There were founded that experimental data corresponds to the theoretical results.

5.4 Analysis of core-shell structure

Polymer nanofibers are very fine fibers and an analysis of the core-shell structure is not easy. The cut of micro fibrous layer and the observation of a cross-section on using microscope is a relatively simple matter for the cross-section analysis. This method does not easily apply in case of polymer nanofibers. These very fine fibers tend to pull inwards the structure and this is hard to find the cross-section of nanofibers, see (Figure 21).

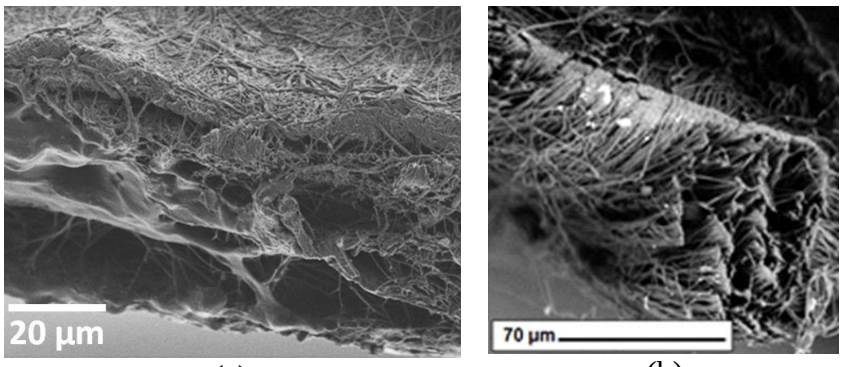


Figure 21 *The cross-sections of the core-shell nanofibers observed using SEM (Zeiss, Germany) (a) and the mechanical cross-section of nanofibrous yarn observed using SEM Phenom (G2, FEI)*

The method of ion beam cutting, see (Figure 22 c) and cryogenic mill using CryoMill (Retsch, Germany), see (Figure 22 a, b) were used for analysis of CMC incorporated in core-part nanofibers as their core part. These methods allow observation of core-shell nanofibers. However, finding of the cross-section of nanofiber with clear core-shell structure in case of cryogenic mill is not easy, because there are many cross-sections with deformed structure, as can be seen in Figure 22 a, b (Vysloužilová 2011).

(a) (b) (c)

Figure 22 *Core-shell nanofibers PVA (shell) - CMC/PEO (core) analyzed by cryogenic mill by Retsch (Germany) and observed by SEM Phenom (FEI) (a, b) and core-shell nanofibers PCL (shell) and CMC (core) observed by Jeol (Germany). The core part has white color and the shell one has grey color (c), (Vysloužilová, L., 2011).*

Vegetable oil incorporated into nanofibers as their core part using pool electrode was analyzed by optical microscopy. The analysis was composed of two parts. First, the core-shell nanofibers were observed by the optical microscope. In the second part, this nanofibrous layer was placed at an angle of 45° under the optical microscope and this was left in this position for 48 hours. This experiment led to the verification of the incorporation of the vegetable oil into core-shell nanofibers and excluded its location on the surface of the nanofibers. In the last case, the beads with incorporated vegetable oil would slump down from nanofibers and the oil foil would be created. The result of this experiment is shown in Figure 23.

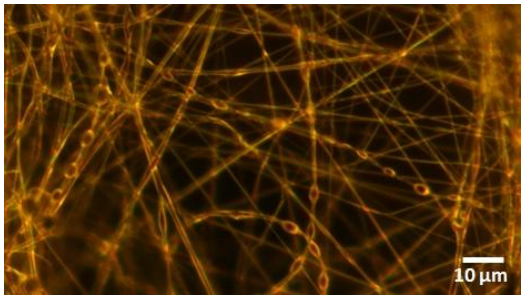
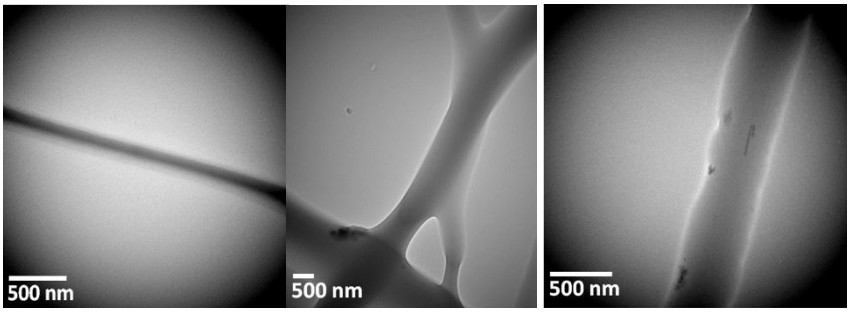


Figure 23 *Core-shell nanofibers with incorporated vegetable oil observed by optical microscopy after 48 hours.*

Transmission electron microscopy (TEM) is the next possible method to proof of core-shell structure of coaxial nanofibers. Core-shell nanofibers of PVA (shell) and PVA with Prussian blue (core) were prepared for TEM analysis. Samples were observed by TEM Hitachi H7650 in The Electron Microscopy Center at the University of South Carolina, USA. Images show that nanofibers were produced by coaxial electrospinning technology, but fuzzy boundaries of nanofibrous “edges” are there, see (Figure 24 a). Core-shell nanofibers of PVA (shell) and PVDF with incorporated magnetic nanoparticles (core) are shown in Figure 24 b, c. Figures shown lines of core and shell part of nanofibers, but these nanofibrous “edges” are not focused.



(a) (b) (c)

Figure 24 *TEM images of PVA/PVA core-shell nanofibers with Prussian blue (a) and PVA/PVDF core-shell nanofibers with incorporated magnetic nanoparticles (b, c).*

A pitfall of TEM analyses of core-shell nanofibers is a difficult choice of the suitable investigated materials and demanding preparation of suitable specimens. Water-soluble polymeric materials are difficult to analysis for that reason the impossibility of observing the sharp boundary between core and shell material as can be seen in Figure 24 a, b.

FT-IR analysis was used for characterization of core-shell structure of produced core-shell PVA nanofibers with incorporated disinfection in the core part. PVA powder, disinfection and core-shell nanofibers with incorporated disinfection in the core were analyzed. Results from this analysis are shown in Figure 25.

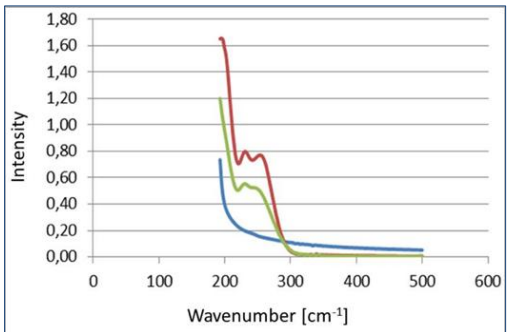


Figure 25 *FTIR spectroscopy of PVA (blue line), disinfection (red line) and core-shell nanofibers PVA/disinfection (green line).*

The course of individual curves in the graph shows a presence of the disinfection in core-shell nanofibers. The intensity of itself disinfection is higher than intensity of the disinfection incorporated into core-shell nanofibers enveloped by the PVA shell.

The phase contrast method is relatively simply analysis of core-shell structure of nanofibers. This one uses elements with higher atomic number incorporated in core part of core-shell nanofibers. Due to these elements, the core part appears significantly brighter than the shell one during observed by SEM. This indicates their presence in the core part of nanofibers. The contrast increases with increasing atomic weight of elements. Phase contrast method could be relatively easy, fast and inexpensive way to receive proof of core-shell structure of coaxial nanofibers, because expensive material as well as expensive analyses are not needed. PVA nanofibers with incorporated Prussian Blue as the element with higher atomic number was prepared for this analyzis. The Prussian blue $[Fe_4[Fe(CN)_6]_3]$ is a dark blue pigment containing iron. The morphology of produced nanofibrous layers was observed using SEM by Zeiss (Germany). SEM images of these samples are shown in Figure 26. There are obvious brightly illuminated areas in core-shell nanofibers. This is the core material with the Prussian blue containing iron (element with higher atomic number), (Vysloužilová, 2013).

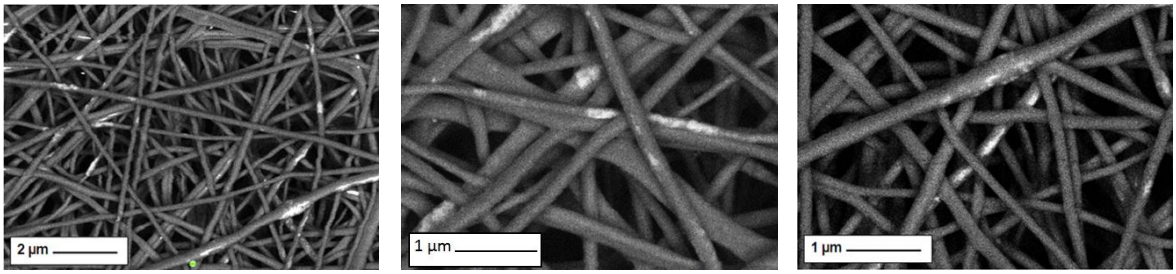


Figure 26 *SEM images of the core-shell nanofibrous structures with incorporated Prussian Blue observed by Pavel Kejzlar, KMT, FS, TUL(Zeiss, GE), (Vysloužilová, 2013).*

The new method to proof of the coaxial structure of core-shell nanofibers was designed and verified in this work. Bi-component nanofibers with core-shell structure were produced by the needle coaxial electrospinning and analyzed by the SEM. Due to the use of elements with higher atomic number in core material (Prussian blue), the core-shell structure could be detected. An advantage of the phase contrast method is a low price and easy and relatively fast analysis (Vysloužilová 2013).

Confocal laser scanning microscopy was used for investigation of core-shell structure using fluorescent substances. In the first step, fluorochrome Rhodamine B was added to the core solution and FITC Dextran to the shell one. Rhodamine B excites at wavelength 540 nm and emits at 625 nm with red color signal. Excitation maximum of fluorophore FITC Dextran is 490 nm and the emission maximum is 520 nm. FITC Dextran emits with green color signal. In the next experiment, carried out in collaboration with Matej Buzgo (2nd Faculty of Medicine, Charles University in Prague), the core solution was prepared with a protein conjugated with phycoerythrin and Alexa Fluor 700. Phycoerythrin absorbs blue light (420–700 nm) and with combination of the red-absorbing dye Alexa Fluor 700, a red shift in the emission spectrum occurs as a result of Förster resonance energy transfer. These properties of the selected fluorophores allowed single-wavelength excitation of both fluorophores (i.e., excitation at 488 nm for both FITC-dextran and IgG-PE-AF700) but difference between the emission spectra of FITC-dextran and IgG-PE-AF700.

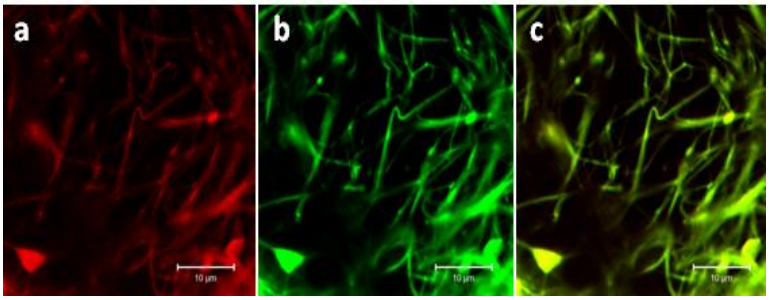


Figure 27 *Core-shell nanofibers with incorporated FITC-dextran in the shell (green signal) and Rhodamine B with IgG-PE-AF700 (red signal) in the core observed using confocal fluorescent microscopy Zeiss LSM 5 DUO: (a) the core, (b) the shell, (c) observing realized with two lasers to analysis of core-shell structure. Collaboration with Matej Buzgo (2nd Faculty of Medicine, Charles University in Prague)*

The fluorescent analysis was supplemented by the investigation of signal intensity profiles of fluorophores of the core-shell nanofibers. This analysis was done within Matej Buzgo (2nd Faculty of Medicine, Charles University in Prague) and Jiří Kula (The Department of Textiles Evaluation, TUL). The micrograph shows the signal of the shell-incorporated

fluorophore (FITC-dextran, green signal) and the signal of the core-incorporated fluorophore (IgG-PE-AF700, red signal), as well as the combination of both signals from core-shell nanofiber. There is recorded a tracking of imaginary nanofiber cross-section from one side of the nanofiber to the other, see in (Figure 28). The signal intensity of the IgG-PE-AF700 in the core is lower than FITC-dextran in the shell. The energy loss during resonance transfer, its lower quantum yield, and signal loss is caused by crossing through the boundary between the core and the shell part of the core-shell nanofiber. The signal intensity profile of the core-shell nanofiber (Figure 28a) shows that the signals are increasing near the periphery of the nanofiber and peaked in the central part of the nanofiber. The signal from the core (red line) has a delayed increase in the fluorescence intensity compared to the shell one (green line). The full width at half-maximum (FWHM) was determined for both the core and the shell intensity profiles to characterization of the peaks. The ratio of the shell FWHM to the core FWHM of the core-shell nanofibers produced by the needle spinning electrode was 1.64 ± 0.35 and 1.35 ± 0.13 in case of the weir spinner of the second generation, see (Figure 28b). This means, the shell part is thicker than the core one and this leads to the next proof of the core-shell morphology of the examined nanofibers. These results indicate that the both the needle and needleless coaxial spinning electrodes enables the production of the nanofibers with the core-shell structure.

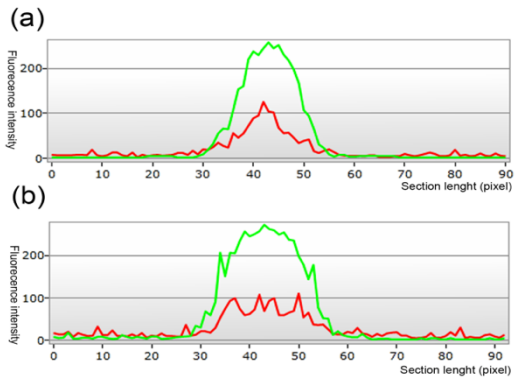
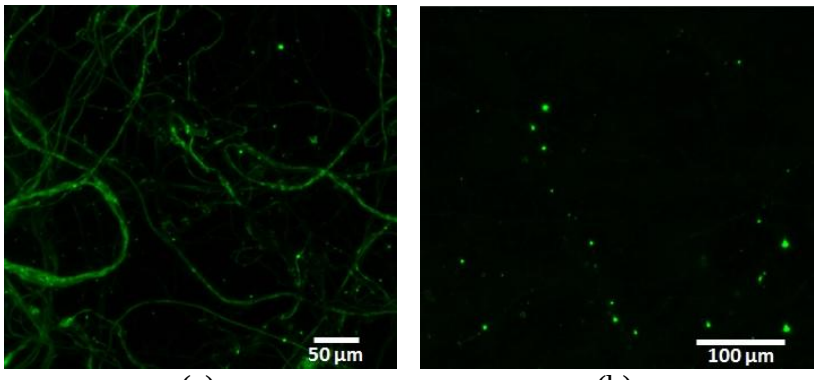


Figure 28 *Signal intensity profiles of fluorophores for core-shell nanofibers realized by (a) the needle coaxial spinning electrode and (b) the weir spinner of the second generation. Fluorescence intensity profiles of FITC-dextran incorporated in the shell (green line) and IgG-PE-AF700 incorporated in the core (red line). Collaboration with Jiří Kula and Matej Buzgo.*

Confocal laser scanning microscopy was also used to proof the core-shell structure of nanofibers with liposomes, see (Figure 29). FITC-dextran was encapsulated into liposomes and these were electrospun as the core part of the PCL(shell)/PVA(core) nanofibers using cylindrical coaxial spinning electrode in cooperation with Andrea Míčková (2nd Faculty of Medicine, Charles University in Prague). Liposomes need to located in aqueous environment and protected against ambient environment – the last one was managed by the PCL shell.



(a) (b)

Figure 29 *Core-shell nanofibers of PCL/PVA with FITC-Dextran (a) and core-shell nanofibers of PCL/PVA with FITC-Dextran incorporated in liposomes (b). Collaboration with Andrea Míčková).*

Figure 29a shows core-shell nanofibers of PCL/PVA with incorporated FITC-Dextran in the core. Green signal of the FITC-Dextran can be observed across the length of fibers. Liposomes with incorporated FITC-Dextran are shown in Figure 29b. In this case can be observed significant change due to fluorophore encapsulated in liposomes. This also means that the liposomes in the core were not damaged and the fluorophore remained safely incorporated inside of the liposomes.

Many different analysis leads to proof the core-shell structure of core-shell nanofibers were done within this work. This is not easy to provide a clear proof of core-shell structure in case of very fine fibers. Therefore, different ways allowing achievement this proof of core-shell structure of nanofibers or combination of these methods were designed and investigated within this work.

6 Evaluation of results and new finding

Technology of coaxial electrospinning was investigated in this work. Different kinds of needle and needleless coaxial spinning electrodes were designed, developed and tested. Equipment for production of core-shell nanofibers (Spinner 1 and Spinner 2) were developed within this work and project Nanoprogres in 2012. This work focused on the development of sophisticated spinning coaxial electrodes from different construction materials and various shape for the purpose of the finding of the optimal spinning electrodes for a wide range of electrospun materials. Duraluminium, Teflon, PP, POM and HDPE TIVAR 1000 were selected as possible construction materials of needle coaxial spinning electrodes. Needle spinning electrodes from these materials were designed and their functionality was verified. In the next step, optimal design of spinning electrodes focusing on the shape of their orifice was investigated and designed within this work. Coaxial needle spinning electrodes allowing the electrospinning of polymer solutions with water-based solvents as well as volatile solvents and various chemical-based liquids and biomedical liquids were developed and tested.

Needleless coaxial electrospinning electrodes were developed and tested in the next part of this work to increase the productivity of core-shell nanofibers. This method was developed at TUL and patented in 2009 (Pokorný). The developing of this needleless spinning electrode took place in conjunction with this work. The apparatus called the Weir spinner and the cylindrical coaxial spinning electrode were designed, developed, investigated and tested within this work. The main advantage of the cylindrical construction of the last one is electrospinning process without a loss of electric energy caused by spark discharges. Another its advantage is a reduction of polymer solution slumping from this spinning electrode.

Experiments with needleless electrospinning lead to determination of the characteristic wavelength λ. This one is a distance between neighboring polymeric jets during needleless electrospinning. Electrospinning process starts with a certain critical value of electric field strength E_c and critical value of the characteristic wavelength λ_c. These parameters were examined during electrospinning process and their effect on the process was investigated in detail.

Dispersion laws for a free surface of non-viscous and viscous liquids were described in this work. They allow a determination of a relaxation time of electrospinning. This is time of Taylor cone formation after HV switching. Dispersion relations were verified with experiment of electrospinning of PVA solutions with concentration from 5 – 15 % (w/v). The dependence of the time delay T on the electrospinning number Γ was found.

The analysis of the core-shell structure of nanofibers was investigated with this work. This analysis is not easy. Transmission electron microscopy (TEM), confocal laser scanning microscopy or cutting by an ion beam are commonly used methods. The problem is that these methods are both difficult and expensive. Only some centers and universities have these special equipments. This thesis described the special new method leads to easy detection of core-shell nanofibrous structure. The phase contrast method allows proving the core-shell structure using elements with higher atomic number in the core part of core-shell nanofibers. Scanning electron microscopy (SEM) was used for this investigation. Brightly illuminated areas of core with these heavy elements can be observed in comparison with grey areas without these heavy elements. The advantage of the phase contrast method is a low price and easy and relatively fast analysis. Other different analysis leads to proof the core-shell structure of produced core-shell nanofibers were done within this work. TEM analysis, FT-IR analysis, fluorescent analysis and special method combining the last one with investigation of the signal intensity profiles of fluorophores in core and shell part of the nanofibers were used and verified.

Benefits of this work can be seen in the expansion of knowledge about the coaxial electrospinning and in the possibility of application of this technology in industrial production. Needleless coaxial spinning electrodes increase the productivity of core-shell nanofibers manufacturing. Equipment presupposing the use of the coaxial electrospinning on an industrial scale were developed within this work. The process of the electrospinning and its coaxial variant was investigated in detail and visualization methods were established and verified. Numerous analyzes providing the proof of the core-shell structure of the produced nanofibers were designed and tested.

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Kejzlar, P., Vysloužilová, L., Voleský, L., Andršová, Z., and Kroisová, D., Bionics: Egg shell. In: *PANMS 2012 Conference Proceedings*. Hejnice, 2012. P. 55-58. ISBN 978-80-7372-890-8

Vysloužilová L., Mohrová J., Lukáš D., Electrospinning of biopolymers, In: *Strutex*, (2011), Liberec, Czech Republic, ISBN 978-80-7372-786-4

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Pokorny, P., Mikes, P., Vyslouzilova, L., Hazuchova, A., Lukas, D.: Relaxation Time of Electrospinning, in Proceedings of the International Conference of the Czech Society of Biomechanics, Human Biomechanics 2010, Sychrov, Czech Republic, October 4-6, 2010, Number Publication: 55-090-10, ISBN 978-80-7372-648-5

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Daňková J., Buzgo M., Vejpravová J., Kubíčková S., Sovková V., Vysloužilová L., Mantlíková A., Nečas A., Amler E., Highly efficient mesenchymal stem cell proliferation on poly-ε-caprolactone nanofibers with embedded magnetic nanoparticles, *International Journal of Nanomedicine* (2015), 10 7307–7317, pp. 7307—7317, doi: 10.2147/IJN.S93670

Batory, M., Grabicka, A-A., Guzenda A-S., Bartoszek, N., Komorowski, P., Vyslouzilova, L., Rozek, Z., Budzisz, E., The use of liposomes in the modification of polycaprolactone fibers, *Applied Polymer Science*, (2015), doi: 10.1002/app.43299

Plencner M, Prosecká E, Rampichová M, East B, Buzgo M, Vysloužilová L, Hoch J, Amler E., Significant improvement of biocompatibility of polypropylene mesh for incisional hernia repair by using poly-ε-caprolactone nanofibers functionalized with thrombocyte-rich solution, I*nternational Journal of Nanomedicine* (2015), 10:2635-46

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Cudlinova, M., Vyslouzilova, L., Novak, P., Coaxial electrodes for Electrospinning, In: *Nanocon 2015*, October, 14 - 16. 2015, Brno, Czech Republic, pp. 457-462, ISBN:978-80-87294-63-5

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Vysloužilová L., Chvojka J., Lukáš D., Oriented nanofibrous Layers, In: *TEXCOMP 10*, Lille, France (2010), pp. 187-193, ISBN 978-1-60595-026-6

Curriculum Vitae

Personal details

Name: Lucie Vysloužilová
Possition: Production Manager at Nanopharma, a.s.
Adress: Vrahovická 169, 798 11 Prostějov
Date of birth: 1.7.1985
Nationality: Czech
E-mail: Lucie.Vyslouzilova@email.cz,
Tel: +420 776 656 032

Education

Since 2009 **Technical University of Liberec, Czech Republic**

Department of Nonwovens and Nanofibrous materials

Ph.D. Student, program: Textile and material Engineering, Faculty of Textile Engineering

2007 – 2009 **Technical University of Liberec, Faculty of Textile Engineering**

Textile and Material Engineering (Master Degree, Ing.)

Thesis title: Production of yarn nanofibers

2004 – 2007 **Technical University of Liberec, Faculty of Textile Engineering,**

Technology and Control of Clothing Production (Bachelor Degree, Bc.)

Thesis title: The program module formation in MATLAB for processing of frequency analysis in the context of size assortment

Professional experience

Since 2015 The Production Manager at Nanopharma, a.s.

2014 – 2015 The Researcher at the University Centre for Energy Efficient Buildings, Buštěhrad, Czech Republic (January 1, 2014 – January 31, 2015)

2013 – 2015 Completion of the **"Course of University education"**

2014 Presentation at an International Webinar on the topic "Electrospinning", Nanoprogres, April 25, 2014, Liberec

2011- 2014 The Senior Researcher in a Czech nanotechnology cluster Nanoprogres.

2013 Clemson University, South Carolina, USA, Department of Materials Science and Engineering, three weeks (October 28 – November 15, 2013)

2012 Technical University of Koszalin, Poland, Department of Biomedical Engineering, A presentation in a seminar (March 5, 2012)

2011 Technical University of Lodz, Department of Material and Commodity Sciences and Textiles Metrology, Lodz, Poland, Training agreement, 2,5 months (10.10.2011 – 20.12.2011)

Attend courses

2015 Course of Matlab at TUL

2014 Course of function and structure of cell membranes, Institute of Physiology ASCR, v.v.i., Prague, one week (October 20 – 24, 2014)

2013 The course of Statistic data processing in program MS Excel, The training center VIRIDIS, Ltd. (November 19, 2013)

2013 Course of tissue engineering, Institute of Physiology ASCR, v.v.i., Prague, one week (April 22 – 26, 2013)

2009 2nd Faculty of Medicine Charles University, Department of Biophysics, Prague, Internship, two weeks (October 2009)

Evaluation

6st place in the Prix International Théophile Legrand de l'Innovation Textile (2015)

1st place in the Fiber Society Poster Competition (2014)

3rd place in the SVOČ competition

Additional Professional Activities

MATLAB
MS Excel – course of statistical data processing in program MS Excel
Image analysator LUCIA™G and NIS Elements 3.0.
Scanning electron microscopy TESCAN VEGA
Scanning electron microscopy Phenom, FEI

Language skills

English – pre-intermediate B1
German – fundamental
Polish – pre-intermediate
Russian - fundamental

Brief description of the current expertise, research and scientific activities

Doctoral studies

Studies	Textile Engineering Textile and material engineering Part time
Exams	Thermal and chemical technology of nonwoven, 5.2.2010 Mathematical statistics and data analysis, 15. 3. 2010 Tissue Engineering (The 2nd Faculty of Medicine, Prague), 7.9.2012 Macromolecular Chemistry, 17.9.2012
SDE	State doctoral Exam completed on 3.12.2014 with the overall result passed.

Teaching activities

Teaching	Production of Nonwovens, 2009-2013 Polymer physics, 2009 – 2015 Textile Nanomaterials, 2009 - 2013
Leading Bachelors/Master Students	Martin Štryncl, *Koaxiální zvlákňování biologických materiálů*, 2012 - supervisor Ladislav Baláž, *Jehlové koaxiální elektrostatické zvlákňování*, 2012 – consultant. Kateřina Matulová, *Vývoj nosičů pro tkáňové inženýrství pomocí koaxiálního elektrostatického zvlákňování*, 2011 - consultant
Research projects	„Research and Development of Devices for Production of Nanofibers" no. 21012, *Ministry of Education, Youth and Sports of the Czech Republic*, (Student's Grant Competition „Mentor for university students," project Open university, CZ.1.07/2.3.00/35.0036 Development of the institute for nanomaterials, advanced technologies and innovations" no. LO1201, National Programme for Sustainability I – LO (NPU I) "Research and development of equipment for producing of nanofibers" no. 28012, *Ministry of Education, Youth and Sports of the Czech*

Republic, (Student's Grant Competition)

NANOPROGRES, The Czech nanotech cluster, *Czech Invest.*

"Applied research of new generation protective masks with nanofilters to increase men protection from design, technological and material point of view," no. MV VG20122014078, *Ministry of Interior of the Czech Republic*

"Electrospinning of Biopolymers using coaxial electrospinning" no. 4849, *Ministry of Education, Youth and Sports of the Czech Republic*, (Student's Grant Competition)

"Nanomaterials to persons protection against CBRN substances", program BV II/2-VS, grant No. 1656, *The Ministry of Interior of the Czech Republic*

"Development of scaffolds for tissue engineering using coaxial needleless electrospinning" no. 4844, *Ministry of Education, Youth and Sports of the Czech Republic*, (Student's Grant Competition)

„Modification of Nanofibrous Materials by plasmatic technology for biological application", *the Grant Agency of the Academy of Sciences*, project: no. ME10145.

"Tissue scaffolds from nanofibrous materials with incorporated Liposome" no. IAA500390702, *the Grant Agency of the Academy of Sciences of the Czech Republic*

"Non-standard application of physical fields – analogy, modeling, verification and simulation," no. 102/08/H081, *the Grant Agency of the Czech Republic*.

Record of the state doctoral exam

TECHNICKÁ UNIVERZITA V LIBERCI
Fakulta textilní

ZÁPIS O VYKONÁNÍ STÁTNÍ DOKTORSKÉ ZKOUŠKY (SDZ)

Jméno a příjmení doktorandky:	**Ing. Lucie Vysloužilová**
Datum narození:	**1. 7. 1985**
Doktorský studijní program:	**Textilní inženýrství**
Studijní obor:	**Textilní materiálové inženýrství**
Termín konání SDZ:	**3. 12. 2014**

prospěla **neprospěla**

Komise pro SDZ:		*Podpis*
Předseda:	prof. RNDr. Oldřich Jirsák, CSc.	
Místopředseda:	doc. RNDr. Miroslav Šulc, Ph.D.	
Členové:	prof. Mgr. Jiří Erhart, Ph.D.	
	doc. Ing. Antonín Kuta, CSc.	
	doc. Ing. Eva Košťáková, Ph.D.	
	prof. Ing. Ivan Stibor, CSc.	
	prof. Ing. Jaroslav Šesták, DrSc., dr.h.c.	

V Liberci dne 3. 12. 2014

O průběhu SDZ je veden protokol.

Recommendation of the supervisor

TECHNICKÁ UNIVERZITA V LIBERCI
Fakulta textilní

Katedra netkaných textilií a nanovlákenných materiálů | Studentská 1402/2 | 461 17 Liberec 1

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Disertační práce:

DEVELOPMENT OF COAXIAL ELECTROSPINNING TECHNOLOGY

Autorka: Ing. Lucie Vysloužilová

Hodnocení školitele

Disertační práce Ing. Lucie Vysloužilové se zabývá koaxiálním elektrickým zvlákňováním. Doktorandka v práci zkoumá a popisuje vybrané fyzikální charakteristiky procesu, vývoj speciálních koaxiálních zvlákňovacích elektrod a analyzuje vnitřní strukturu vytvořených koaxiálních nanovláken. Významná část práce je zaměřena na optimalizaci prostorového uspořádání kapilár zvlákňovacích elektrod za účelem zvýšení produktivity. Doktorandka prokázala schopnost pracovat ve výzkumných týmech. Řada vyvinutých zvlákňovacích elektrod uvedených v této práci byla testována ve spolupráci s dalšími odborníky z katedry netkaných textilií a nanovlákenných materiálů, katedry textilních a jednoúčelových strojů Technické univerzity v Liberci a ve spolupráci s firmou AUDACIO a.s. v rámci řešení projektů klastru Nanoprogress. Práce se neomezuje pouze na klasické jehlové metody tvorby koaxiálních nanovláken, ale zkoumá i možnosti koaxiálního zvlákňování z volného povrchu dvojité vrstvy dvou nemísících se polymerních roztoků. Tuto část její práce považuji za průkopnickou. Pro důkaz koaxiality a studium vnitřní struktury vyrobených koaxiálních vláken použila L. Vysloužilová řadu přímých i nepřímých metod. Téma práce je vysoce aktuální díky rostoucímu zájmu o aplikace nanovláken například pro biomedicínské účely.

Lucie Vysloužilová se své doktorské práce chopila s velkou intenzitou ihned po jejím zadání. Je schopna samostatně provádět vybrané kroky teoretické analýzy zvlákňovacích procesů, ale především je silná v plánování, provádění a vyhodnocování experimentů. Zvláště si cením její schopnosti připravit technologický experiment, pečlivě jej provést, s citem jej vyhodnotit a navrhnout cestu k dalšímu zdokonalení přístrojového provedení nebo materiálového složení. Závěry jejích experimentů byly na KNT FT TUL základem pro rozvoj technologie koaxiálního zvlákňování. Svůj inženýrský talent po dobu dvou let uplatnila v rámci řešení projektu klastru Nanoprogres I a II, který se tematicky kryl se zadáním její disertační práce.

Jako velmi uspokojivou hodnotím publikační činnost Ing. Lucie Vysloužilové, která podle záznamu v databázi WoS ISI čítá 12 položek. Z toho je 8 konferenčních příspěvků ve sbornících a 4 časopisecké publikace. Celkový ohlas na její časopisecké práce čítá 33 položek bez auto-citací. Doktorandčin H-index je 2.

Práce L. Vysloužilové přispěla k rozvoji technologií výroby nanovlákenných materiálů, které mohou díky různorodé vnitřní struktuře nést celou řadu technických nebo biologických funkcí.

Navrhuji, aby práce Ing. Lucie Vysloužilové byla přijata k obhajobě.

Prof. RNDr. David Lukáš, CSc.

Opponents' reviews

Prof. DSc. PhD Stanislaw F. Mitura, dr hc TUL
Professor of the Koszalin University of Technology
e-mail: stanislaw.mitura@gmail.com

Koszalin, 31 December 2016

Review of the PhD thesis

Eng. Lucie Vysloužilová:

„Development of Coaxial Electrospinning Technology"

(Vývoj technologie koaxiálního elektrostatického zvlákňování)

Supervisor: Prof. RNDr. David Lukáš, CSc.

Re: TUL – 16/4814/041973, 16 November 2016

I made this review based on the letter of the **Dean of the Textile Engineering Faculty of the Technical University of Liberec Eng. Jana Drašarová, Ph.D.**

1. Evaluation of importance of the PhD. Thesis for the field of science

The PhD thesis of Eng. Lucie Vysloužilová: *„Development of Coaxial Electrospinning Technology"* concerns the problems connected with the **Textile Techniques and Materials Engineering (Textilní materiálové inženýrství)** field. From one side textile materials, had been known in various forms for thousands of years. The history of modern composites for textile industry probably began in 90 decades of the twentieth century and it is associated with new technologies, especially of nanotechnology.

The PhD thesis of Eng. Lucie Vysloužilová is focused on the coaxial electrospinning; work examines and describes the process of electrospinning itself, the development of special coaxial spinning electrodes and the analysis of the core-shell structure of formed nanofibers. Parameters of the process for coaxial electrospinning are investigated as a fundamental basis for a design and development of the new coaxial spinning electrodes. Significant part of this work is aimed at an optimization of needleless coaxial spinning electrodes for productivity enhancement of coreshell nanofibers.

There is a growing interest in nanofibers, especially as a material for biomedical applications in the last years. Nanofibers are really unique materials with a low area weight and a high specific surface area. It means, they are composed of very fine fibers with diameters ranging from 100 nm to 1 μm and with high porosity and very small

- Where is relation 2.25?.
- The dissertation contains small editor's faults.

Despite of this and other mistakes and misinterprets in my opinion the dissertation presents adequate level and deserves for positive opinion.

2. Evaluation of the Student's publications

I analyzed the publication of Eng. Lucie Vysloužilová in the database Web of Science:

Web of Science [v.5.23] Export
Transfer Service Web of Science™
AUTHOR: (Vysloužilová L*)
Timespan=2004-2017.
Indexes=SCIEXPANDED, SSCI, A&HCI, CPCIS, CPCISSH, BKCIS, BKCISSH, ESCI, CCREXPANDED, IC.

Results found:	**14**
Sum of the Times Cited:	**37**
Average Citations per Item:	**3.19**
h-index:	**2**

Especially interesting are papers:

Rampichová M., Chvojka J., Buzgo M., Prosecká E., Mikeš P., Vysloužilová L., Tvrdík L., Kochová P., Gregor T., Lukáš D., Amler E., Elastic three-dimensional poly (ε-caprolactone) nanofibre scaffold enhances migration, proliferation and osteogenic differentiation of mesenchymal stem cells, Cell Proliferation, Vol. 46, Issue 1, (2013), pp 23–37. (Citations 22)

Prosecká, E., Buzgo, M., Rampichová, M., Kocourek, T., Kochová, P., Vysloužilová, L., Tvrdík, D., Jelínek, M., Lukáš, D. and Amler, E., Thin-Layer Hydroxyapatite Deposition on a Nanofiber Surface Stimulates Mesenchymal Stem Cell Proliferation and Their Differentiation into Osteoblasts, Journal of Biomedicine and Biotechnology, Article ID 428503, (2012), doi:10.1155/2012/428503. (Citations 8)

Valtera J., Vysloužilová L., Komárek, J., Skřivánek, J., Žabka, P., Beran J., Lukáš D., Protrusion of the Rod Electrode in the Electrospinning Process, Journal of Nanotechnology, (2015), Article ID 301636, doi:10.1155/2015/301636

Vysloužilová L., Valtera J., Pejchar K., Beran J., Lukáš D., Design of coaxial needleless electrospinning electrode with respect to the distribution of electric field, Applied Mechanics and Materials, Vol. 693 (2014), pp 394-399, ISSN: 1660-9336, doi:10.4028

Rampichová M., Chvojka J., Buzgo M., Prosecká E., Mikeš P., Vysloužilová L., Tvrdík L., Kochová P., Gregor T., Lukáš D., Amler E., Elastic three-dimensional poly (ε-caprolactone) nanofibre scaffold enhances migration, proliferation and osteogenic differentiation of mesenchymal stem cells, Cell Proliferation, Vol. 46, Issue 1, (2013), pp 23–37

inter-fiber pores. Due to these unique properties and their structure nanofibers can be used in medicine as scaffolds, wound dressing, materials for drug delivery system or as a replacement of a damaged tissue. Nanofibers can also be used in electronics, optics, filtration, as composite materials. Number of published articles and their citations on the coaxial electrospinning and core-shell nanofibers topic by Web of Knowledge (Figure 1 in PhD. Thesis) are very significant for importance of the dissertation in the field of science.

2. Evaluation of content-related of the work

The work contains 115 pages of typescript, 93 figures and 20 tables, includes a literature review (44 pages, 25 figures, 4 tables, 40 equations) with very important Chapter 2 "The theoretical part" and experimental researches. In the literature review candidate for a doctor's degree introduced the notions applied in the field of coaxial electrospinning, the core-shell nanofibers, thermodynamics of polymer solutions, needle and needleless coaxial spinning electrodes of different shapes and design. The literature review was worked out on the basis of 95 references, including few papers where Lucie Vysloužilová is co-author.

Interdisciplinary considerations in the textile engineering and material science field are conducted with high dose of cognition and criticism which proves the ability to knowledge synthetization and scientific maturity of the candidate for a doctor's degree.

In reviewer's opinion, the most important parts of the dissertation are as follows:

- The research of technology of coaxial electrospinning.
- The works focused on the development of sophisticated spinning coaxial electrodes from different construction materials and various shape for the purpose of the finding of the optimal spinning electrodes for a wide range of electrospun materials.
- Theoretical study.
- The very good conformity of a mathematical model, based on the parameters of the process with the measured results; it means the possibility of prediction of behavior during PECVD process. though it wasn't specially emphasized by the candidate for a doctor's degree.
- The analysis of the core-shell structure of nanofibers.
- It should be also underlined the utilitarian aspect of realized work. There is a possibility of utilization of obtained research results in practice.

The scientific level of the elaboration is high, although the candidate for a doctor's degree made few mistakes. I will quote some of them in presented review.

- *„It means, they are composed of very fine fibers with diameters ranging from 100 nm to 1 μm."* What about possibility to manufacture fibers with diameter smaller then 100 nm?
- The chemical mechanism of the procedures is not explained.
- It is lack of chemical analysis of the material (comparison *before* and *after* manufacturing of fibers).

Prosecká, E., Buzgo, M., Rampichová, M., Kocourek, T., Kochová, P., Vysloužilová, L., Tvrdík, D., Jelínek, M., Lukáš, D. and Amler, E., Thin-Layer Hydroxyapatite Deposition on a Nanofiber Surface Stimulates Mesenchymal Stem Cell Proliferation and Their Differentiation into Osteoblasts, Journal of Biomedicine and Biotechnology, Article ID 428503, (2012), doi:10.1155/2012/428503

Vysloužilová L., Váša P., Soukupová J., Pokorný P., Chvojka J., Pejchar K., Žabka P., Lukáš D., Beran J., Předávací dokument ke spineru 1, Nanoprogres – The Czech-based nanotechnology cluster, Technická Univerzita v Liberci, Liberec (2012), Czech Republic.

I had the pleasure watch the invited presentation at the conference NANOMED in Warsaw in November 2016.

In particular, I appreciate the achievements of publishing Lucie Vysloužilová. This demonstrates not only the scientific level, which represents PhD Student, but also of the ability to work in a research team.

3. Final evaluation of the dissertation

The candidate for a doctor's degree demonstrated very good knowledge of the subject and proper formulation of the scientific thesis. I am fascinated by the extraordinarily high level of dissertations, PhD Student erudition. In particular, I appreciate the achievements of publishing Lucie Vysloužilová.

The theoretical and experimental researches realized by her doesn't arouse any doubts and content-related stipulates. Its interpretation proves the scientific maturity of the candidate for a doctor's degree.

The dissertation is written in comprehensible way, it possesses the attributes of originality and brings in the new cognitive values with comparison to the past state of the art in **Textile Techniques and Materials Engineering** field **(Textilní materiálové inženýrství)**.

Taking into consideration remarks mentioned above I voice an opinion that the dissertation of Eng. Lucie Vysloužilová: „*Development of Coaxial Electrospinning Technology*" corresponds with the terms established in the titles and degrees law.

On this ground, I move to Textile Engineering Faculty Scientific Council of the Technical University of Liberec to allow the PhD thesis Eng. Lucie Vysloužilová to its public defense.

Oponentský posudek disertační práce Ing. Lucie Vysloužilové

Vývoj technologie koaxiálního elektrostatického zvlákňování

a) Předložená práce řeší vysoce aktuální problematiku technologie koaxiálního elektrostatického zvláknění a její výsledky přináší významný posun v inovaci této technologie.

b) Postup řešení i použitá metodika jsou originální a technické řešení má aplikační potenciál. Stanovené cíle jsou jasně definované v kap.1.1 a splněné. Pouze splnění dílčího cíle 5 není v teoretické části textu dostatečně zvýrazněno jako vlastní přínos autorky, což chápu spíše jako formální nedostatek, protože autorka projevila dostatečnou erudici v dané problematice v teoretické části práce.

c) Práce je výsledkové velmi bohatá a svědčí o tom, že kromě teoretického popisu procesů autorka zvládla i jejich experimentální charakterizaci. Práce obsahuje originální řešení zvlákňujících elektrod a velké množství experimentálních dat a to jak z oblasti charakterizace samotného procesu elektrospinningu, tak i charakterizace produktů elektrospinningu - nanovlákenných materiálů. Závěry shrnují výsledky práce a jejich přínos v širším kontextu a dokazují autorčin přehled v dané problematice. Pro odbornou rozpravu při obhajobě mám dva doplňující náměty k diskuzi, které by autorka měla rozebrat podrobněji:

- Jaké parametry jsou klíčové pro nastavení tloušťky core a shell vrstvy a jejich homogenitu???
- Jak budou různá aditiva ovlivňovat proces koaxiálního zvláknění a výsledný produkt????

d) Formální úprava práce je přehledná, obrázkům a grafické úpravě nelze nic podstatného vytknout. Pár drobných překlepů v textu doporučuji ještě opravit, než se práce vystaví na webu. Musím ale upozornit na překlepy v tabulce 15 pro „weir spinner" je uvedeno kritické napětí vyšší než optimální. Popis tabulky 15 postrádá informaci, pro jaký polymer jsou ta naměřená data. V tabulce 14 chybí jednotky pro vysoké napětí a obrázku č. 26 by velmi prospělo, kdyby se čísla popisovaných částí objevila i na obrázku samotném.

e) Publikační aktivita studentky zahrnuje autorství a spoluautorství odborných článků, monografie, výzkumné zprávy, konferenčních příspěvků a také spoluautorství jednoho patentu. Z pěti odborných publikací v zahraničních časopisech jsou dva excelentní výsledky s IF > 3. Tato publikační aktivita je mimo jiné také zárukou úspěšné obhajoby.

f) Závěrem konstatuji, že předložená práce Ing. Lucie Vysloužilové: *„Vývoj technologie koaxiálního elektrostatického zvlákňování"* splňuje po všech stránkách podmínky, kladené na disertační práci a **doporučuji ji proto k obhajobě.**

Prof. RNDr. Pavla Čapková, DrSc

Oponent