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TECHNOLOGY OF MONOFILAMENT FIBERS BASED ON OXIDIZED HYALURONIC ACID

ZKRÁCENÁ VERZE DISERTAČNÍ PRÁCE

SUMMARY OF DOCTORAL THESIS

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ABSTRAKT

Předkládaná dizertační práce se zabývá vývojem technologie výroby nového typu biodegradabilních vláken na bázi oxidované kyseliny hyaluronové. V rámci práce je postupně představován vývoj jednotlivých jednotkových operací výroby, jejichž správné porozumnění a schopnost jejich řízení jsou klíčové pro žádaný chod celé vícestupňové technologie. V rámci práce je přestaven nezbytný vývoj technologického zařízení, průběžně konstruovaného pro účely laboratorního testování a následně až po samotnou linku pro finální výrobu vláken, která byla realizována v roce 2015. V rámci dizertační práce jsou dále navrhovány možnosti dodatečné chemické úpravy vláken s ohledem na zvyšování jejich stability ve vlhkém prostředí. S ohledem na cílené aplikace vláken pro vnitřní chirurgické implantace, jsou v práci vlákna též hodnocena z hlediska jejich materiálové biokompatibility (toxicity).

ABSTRACT

Following dissertation thesis discusses the technological development of novel type of fully biodegradable fibers based on oxidized hyaluronic acid. The thesis describes main aspects of the technological unit operations that are essential for the right understanding and management of the entire multistep fiber-forming production process. The thesis also mentions a design of main technological devices that were used within the laboratory development to gain the basic process knowledge and also the design of the further fiber-spinning production unit that was built in 2015. The thesis further discusses ways of potential chemical modification of fibers in order to increase their stability in wet environment. Regarding the targeted applications of discussed fibers within the internal surgical applications, the material biocompatibility or toxicity has been also evaluated.

KLÍČOVÁ SLOVA

Kyselina hyaluronová, oxidovaný hyaluronan, vlákno, mokré zvlákňování, síťování

KEYWORDS

Hyaluronic acid, hyaluronan, oxidized hyaluronan, fiber, wet-spinning, crosslink

CONTENT

1 THE OBJECTIVES OF THE THESIS

A) The study of processes related to the fiber formation by the coagulation technology

B) The realization of basic process apparates for the laboratory wet-spinning production method.

C) The design of a continual technological process of the oxHA fiber production

2 THEORETICAL PART

The problematics of fibers based on hyaluronic acid (HA) reflexes the recent effort of administration of HA into the human body in various forms of medical products others than the well-known gel form. The hyaluronan fiber formation opens the gate to a large field of textile-based products not only in a planar but also in 3D forms that excels in its mechanical flexibility, light-weight and the product shelf-life stability, allowing longer expiration periods in comparison to the liquid-based products. Novel applications of the HA-based fibers are appearing, such as biodegradable and active surgical sutures, meshes, stents or various textile patches, where the presence of HA can potentially support the wound healing post-surgical processes.

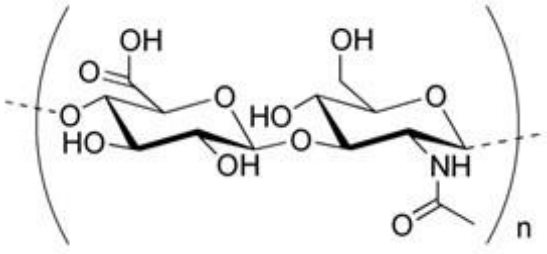
The industrial production of fibers based on HA has not been recently widespread yet therefore the recent literature mention rather simple laboratory experiments with limited proposals for the production-scale extensions. Regarding the actuality and the commercial potential of such fibrous products, the problematics is recently published rather in the patent literature than in scientific papers. The reason comes up from the necessity of the principal knowledge protection by the patent claiming. This concept was maintained also in the case of the data given within this dissertation thesis and the technological process has been patented [1]. The presented dissertation thesis represents a technological work for the 6-year period of work for the company Contipro, where the fiber spinning technology started to be explored from a scratch, following the base ideas of Assoc. Prof. Burgert. After the laboratory research realized within the years 2009-2013, the pilot-scale production was tested and finally in the year 2015 the production line has been built and started.

2.1 Hyaluronic acid (HA)

Hyaluronic acid (HA) belongs to the group of non-sulphated glycosaminoglycans consisting of disaccharidic units formed by *N*-acetyl-D-glucosamine and D-glucuronic acid *(Fig.1)*. The HA is commonly present in the human body, predominantly in the body fluids that manage the viscosupplementation or lubrication of the tissues. The related literature describes favorable effects of the HA within the wound healing processes since it supports the granulation of the regenerated tissue during the early stages of the healing process [2],[3]. For that reason, the HA belongs among the most sought-after active components within the wound-healing formulations.

One of the highlighted characteristics of HA is it´s affinity to the cellular receptors of the CD44 type. This special affinity of the HA is aimed to be used for a design of targeted delivery systems of biologically active molecules, that can be bonded to the HA and further accepted as a complex

drug delivery system by the cell. This special affinity of the HA to the CD44 cellular receptor is assumed to be given by the presence of a free carboxyl group in each disaccharide unit. This carboxylic group becomes therefore a key parameter wearing a “bioactive” function of the HA.[4],[5].



D-Glucuronic acid *N*-acetyl-D-Glucosamine

Fig.1: The structure of the hyaluronic acid

The HA is readily degradable in the human body by enzymes from a group of hyaluronidases, which are capable of a selective cleaving of glycosidic bonds, whereby the polymer molecular mass is gradually reduced to saccharidic mer-units which are subsequently metabolized in the human organism [6]. The turnover of HA in the human body is approximated to be 0,1-1 mg/min/kg of body weight [7].

Due to the lubricating and wound-healing properties of the HA, it is frequently used in a form of viscous hydrogel for a support of the bio-acceptation of implantable medical devices such as stents. However, for some surgical applications those hydrogel formulations have certain disadvantages, such as nonhomogeneous distribution of the gel and it´s migration from the place of application during the body movements. Therefore, the recent interest of numerous scientific groups is targeted to the expansion of the portfolio of various application forms of hyaluronan such as solid gels, foils, foams, pads based on nanofibers or textiles, within the medicine. [8]-[14]

2.2 Oxidized hyaluronic acid (oxHA)

The demand of the prolonged degradation stability of the products based on the HA is within the following thesis solved by the oxidation of the primar hydroxylic functions at *N*-acetyl-D-glucosamine part of the HA, followed by the potential further condensation with bifunctional hydrazides of organic acids. The oxidation is prefered to the carbonyle state in order to maintain it´s high reactivity. The common oxidation of polysaccharides (cellulose, starch or HA) is described in literature with a use of $NaIO_4$.[15],[16]

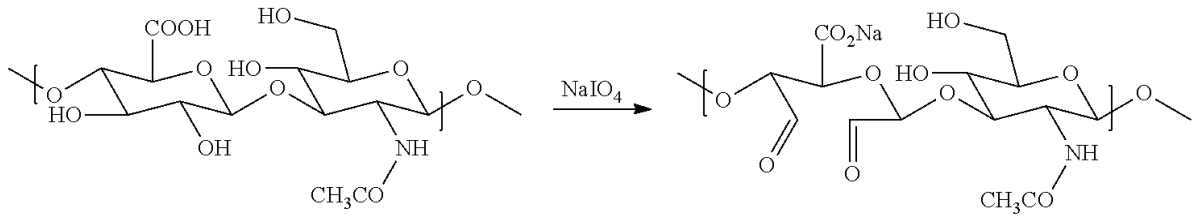


Fig.2: The oxidation of HA by $NaIO_4$. [15]

It is generally assumed that the oxidative reaction with use of the $NaIO_4$ prefferably proceeds on two vicinal hydroxyl groups of glucuronic part of HA yielding a dialdehydic product with opened pyranose ring (*Fig.2*). The supramolecular geometry of the oxidized HA (oxHA) is therefore significantly changed from a rather linear structure of HA to the more coil-like structures.

However, in the case of polymers used for the fiber production, the linearity of the base polymer chains is highly preferred. Such chains are better orientable along the fiber axis forming a regular and dense system stabilized by weak, non-covalent interactions, responsible for the macroscopic characteristics of the fiber (mechanical properties, swell ability or solubility).[17] Based on this point of view, much preferred strategy of HA-oxidation is showing to be by the mechanism designed by Dr. Buffa in Contipro a.s..[18],[19]

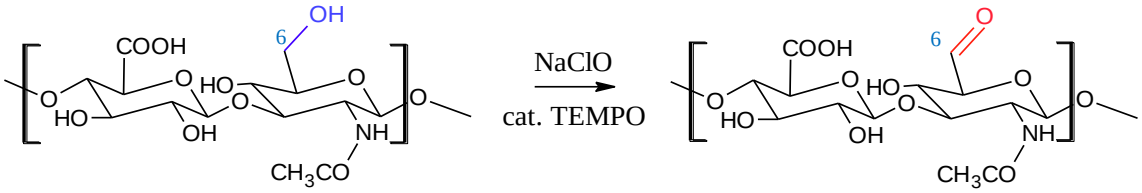


Fig.3: Strategy of regioselective oxidation oh hyaluronic acid at C6 of N-acetyl-D-glucosamine part.

The native HA is oxidized with use of NaClO under the presence of catalyst TEMPO (2,2,6,6-Tetramethylpiperidin-1-yloxy), that sterically favors the activation of the C6 carbon atom adjacent to the primary hydroxyl group of the *N*-acetyl-D-glucosamine part. Due to the presence of the catalyst, the oxidation runs rather regioselectively where the secondary hydroxyl groups remain mostly intact. The extent of the HA modification is rather mild, the author claims, that the degree of oxidation of the HA by the mechanism shown in (*Fig.3)* reaches up to 15%. The core polymer structure remains close to the native HA, therefore, similar behavior of the oxHA in terms of biocompatility and biodegradability in the human body can be expected. Simultaneously, as the oxidative process is rather mild, the polymer does not tend to be cleaved and the molecular weight does not fall dramatically.

The oxidation of the HA changes the hybrid state the C6 carbon on the *N*-acetyl glucosamine from a trigonal sp^3 to a planar sp^2, which becomes less sterically hindered. The presence of a multiple bond also causes a higher polarization of the carbonyl carbon atom which becomes more susceptible to the attack by general nucleophiles (e.g. OH-, NH_2-, SH-, NH_2NH-). Those functions can be used for further chemical modification of the oxHA.

2.3 Monofilament fibers based on the HA

The formation of textile processable fibers based on the HA represents a relatively cutting edge problematics. The technological basements of the problematics have been generally given by two patent applications Domard [20] and Burgert [21], where authors describe the possible methods of preparing fibers from HA-water solutions by extruding into concentrated (glacial) acetic acid, alternatively to binar solvent systems alcohol/organic acid (formic, acetic, propionic). The fibers produced by those technologies are claimed to have sufficient mechanical properties for further textile processing. However, the up-scalability of the proposed technology to larger production scales is slightly hindered by the fact, that the proposed process agents are problematical in terms of inadequately strong odors, volatility and elevated risk of toxic impacts on the operators. Those problems might be surely technically soluble, however from the economical point of view it might be beneficial to find more acceptable process agents allowing the technology to be scaled-up into the larger production. One of the main goals of the presented thesis is the overcoming of those technological problems and to design alternative process agents to be more acceptable for the technological scale-up.

However, fibers made of native hyaluronic acid represent material that is rapidly soluble when exposed to the wet environment. HA fibers turn into a viscous gel within seconds, which might cause problems with a sticking on the surgeon´s gloves during the surgical application of the HA-based textile. Therefore, it is desirable to enhance the fiber stability in the wet environment and thus decrease its water solubility by various chemical modifications. Interesting strategies have been shown by James [22] and Zhang [23] who have introduced a method for preparing fibers from hyaluronic acid modified by cetyltrimethylammonium, wherein the modification causes a carboxyl group on the glucuronic part of hyaluronan to be blocked. This conception leads to the water insoluble fibers, due to the increased hydrophobicity of the polymer. The blocking of the carboxylic functions and thus a loss of ability to form interchainal hydrogen bondings gives to the HA derivative an unique hydrophobic character - polymer becomes meltable by heat. This characteristics is generally extremely convenient for the fiber spinability, because the melt-

spinning techniques (described later) belong to the most efficient and economical fiber-forming processes. On the other hand it is assumed that the presence of free carboxylic functions on the hyaluronan backbone represents a key parameter for the polymer biodegradability by hyaluronidase enzymes.[6]

An another strategy has been chosen by Hadba and Ladet who described the preparation of HA fibers using the reactive spinning method based on the "Click chemistry"-crosslink. [24],[25] The process comprises extrusion of a couple of reactive polymers functionalized for crosslinking by the mechanism of click-chemistry. The process is then controlled by temperature, pressure and time. The crosslinking reaction is proposed among following groups: thiols, azides, alkynes, alkenes and carbonyls. The mechanism of the reaction is proposed to be a cyclo-addition (Huisgen 1,3-dipolar cycloaddition, producing a five-membered heterocyclic crosslinks. Other type of such reaction can include Diels-Alder diene-dienophyl conjugations. The click reaction within the patent is also described as Michael addition (maleinimide-thiol reaction), then metathesis or Staudinger type of reaction of phosphines with alkyl azides.

A large field of HA-based fibers has been introduced by the company Fidia Advanced Biopolymers S.R.L., who has developed fibers based on the esterified HA.[26] The esterification of HA is performed with use of aliphatic and aromatic alcohols.

2.3.1 Applications of the HA-based fibers

The HA textile is recently becoming an interesting form applied within the wound-healing management.[27] The textile sheet manages a regular coverage of the wounded tissue. In the wet conditions of the wound, the textile turns into a thin gel layer adhered on the surface of the wound. The HA applied in a thin layer increases the efficacy of the product in comparison to the hydrogel form, where larger HA-quantities need to be applied to cover the wound homogenously. Moreover, the HA in the form of a dry fabric provides a considerable advantage related to the increased product stability in terms of the shelf life. The applied textile can be sheared exactly and tailored in accordance to the size and geometry of the wound. The amount of the HA applied can be variably adjusted by the use of textile with various fiber density (mesh size) or the HA fibers can be blended with other material to decrease the product price.

The large field of use of the HA-based textile is also represented by the field of tissue engineering, where the HA-based fibers are used as scaffolding system to support the cell growths in the desired tissue.[28]

3 RESULTS AND DISCUSSIONS

3.1 The general HA-fiber spinning processes

The production of the HA-based fibers that were designed within this thesis used a modified wet-spinning technique. The fiber-forming processes have initially been tested in a laboratory scale, with use of simple laboratory techniques and devices. The technology was further scaled up into larger scales to a pilot-production and finally to a production line.

The fabrication process can be defined as a multi-step technology consisting of discrete unit operations that have been optimized within the technological research.*(Fig.4)*

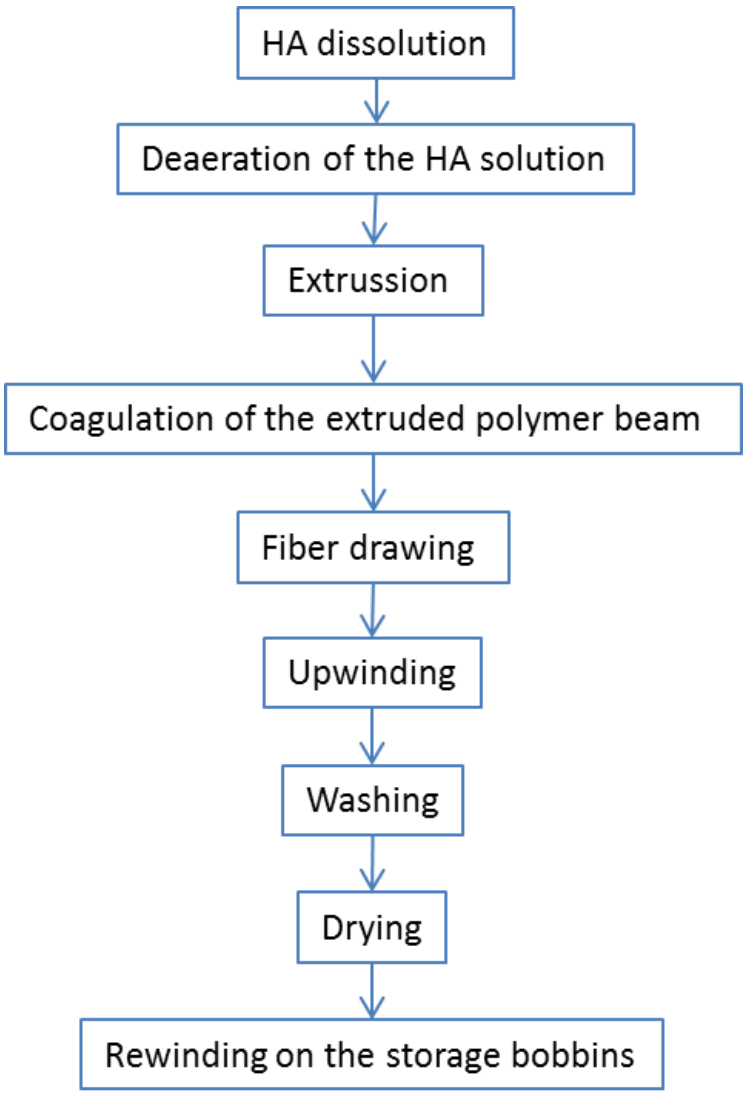


Fig.4: Process scheme of the HA-based-fiber production

3.2 The design of the fiber-spinning process

3.2.1 Laboratory-scale process conditions

The piston syringe with the deaerated polymer solution was inserted into a precise linear metering device and the extrusion rate was set at 200 µl/min. The solution was extruded through a spinning mono-nozzle having the outlet diameter of 500 µm into the coagulation bath of various contents (described later). Afterwards, the formed filament was continually up-winded on the process bobbin by various rates in a range of 0,5-2m/min.

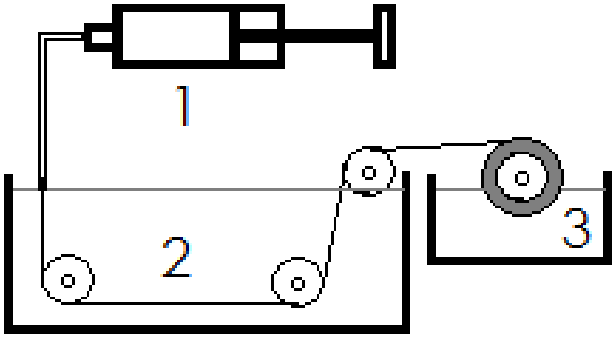
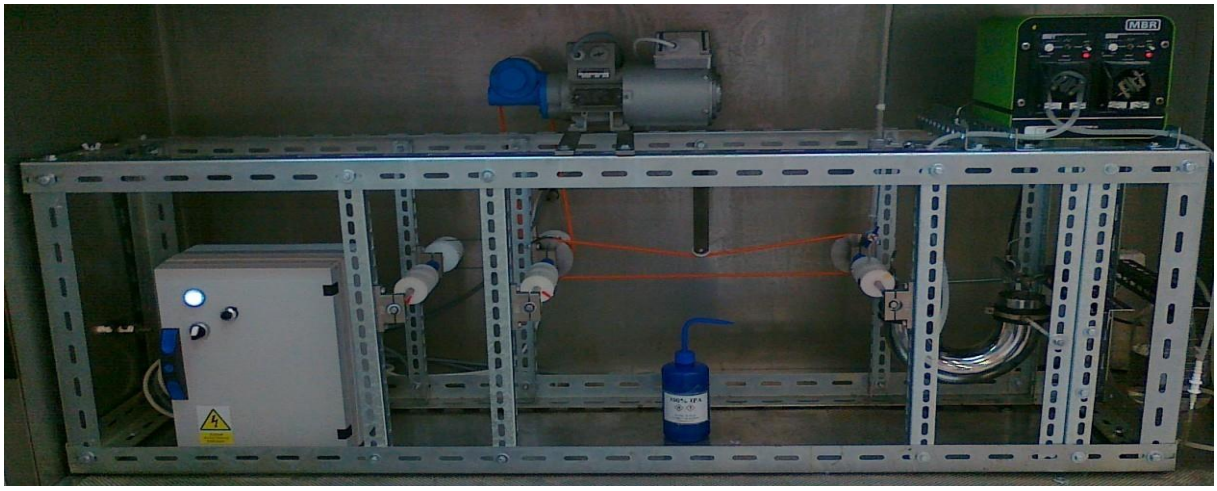


Fig.5: Scheme of the laboratory wet-spinning device: (1) linear syringe extruder, (2) coagulation bath, (3) stabilization bath

The maximal lenghts of the fiber prepared in the laboratory scale was 200m. The process bobbin was further immersed into the washing bath with pure ethanol, where the main content of coagulation baths has been washed out. The fiber was further rewinded on the storage bobbins. During the rewinding, the fiber-tension was controlled in order to maintain constant drawing conditions. The fiber was dried by the hot air during the rewinding. Finally the fibers were stored in a climatical chamber at 23±2°C and at the relative humidity of 50±3%.

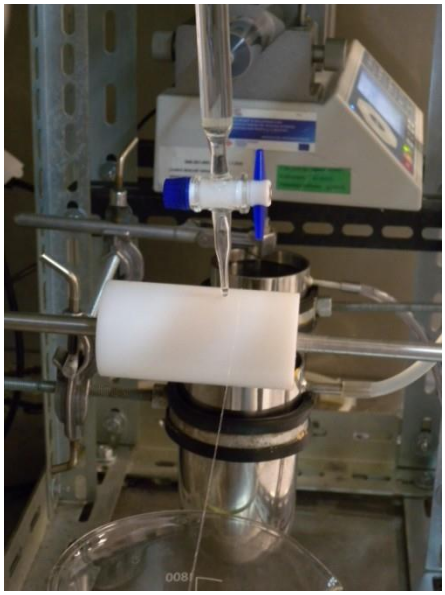
The laboratory spinning devices have been designed in 3 generations (*Fig.6, Fig.7* and *Fig.8*) and provided sufficient knowledge that might have been used for the further pilot and the production-spinning line building.



A

B

C



D

E

Fig. 6: Laboratory wet-spinning device 1st generation, a single fiber production (Běťák, J., 2010), A) An overview of the lab-spinning device, B) Piston syringe extruder, C) Coagulation bath (U-tube), D), E) Fiber up-winding, wetting of the surface in order to prevent fiber sticking

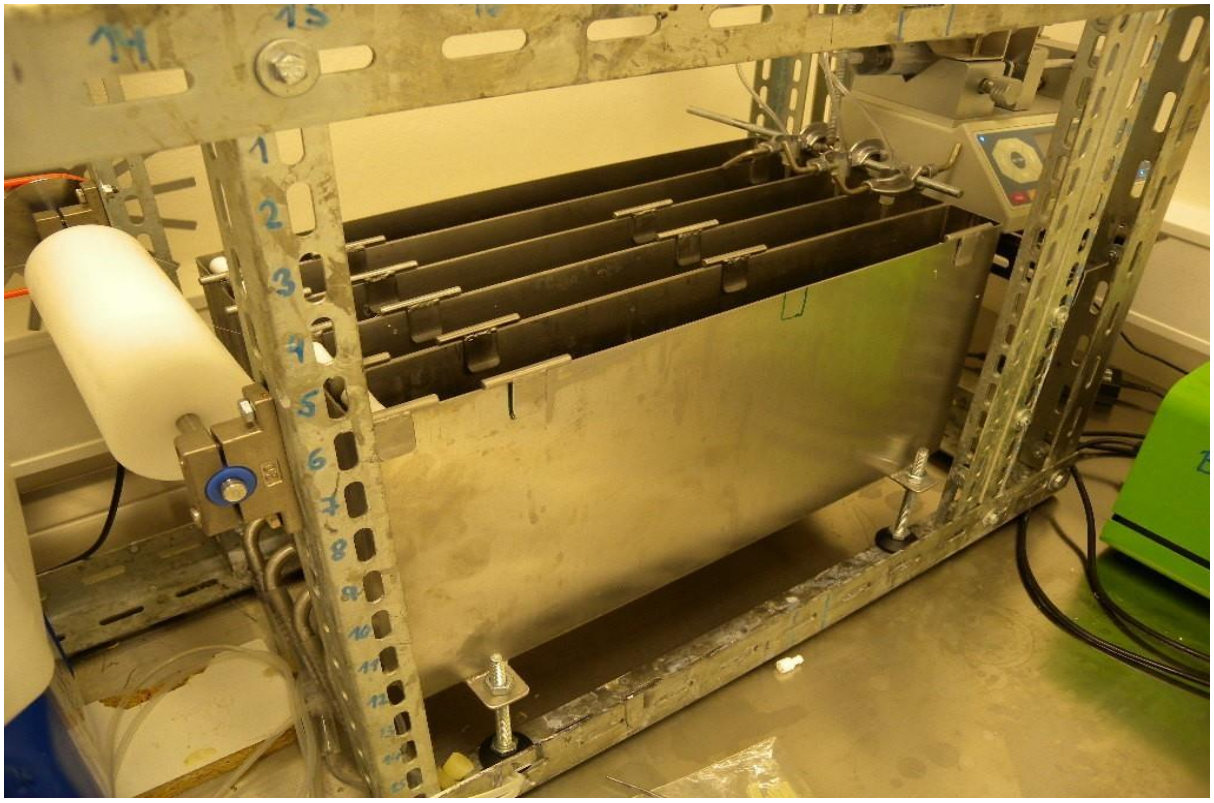


Fig. 7: Laboratory wet-spinning device 2nd generation, Extension to a triple-fiber production (Běťák, J., 2012)

Fig. 8: Laboratory wet-spinning device 3rd generation, (Běťák, J., 2015, the final design and construction by the company CERBET)

3.3 The computational design of the coagulation bath

3.3.1 The first generation of coagulation baths

The aim of the work was to design a suitable composition of coagulation bath that would eliminate technological problems of baths designed in the literature [20], [21] being based on formic or acetic acid. Although the mentioned coagulation baths work well for the fiber formation, their disadvantage is in their inadequate odor and elevated corrosivity that is a significant problem in a larger production scale. Those baths have been by the authors designed as a mixture of formic or acetic acid with an alcohol (methanol, ethanol or propan-2-ol) in ratios shown in tables *(Tab.2)* and *(Tab.3)* below.

Each of those coagulation baths has been initially evaluated by a calculation of the Hansen solubility (HSP) parameters of the mixtures from their pure components shown in the *(Tab.1)*, and based on the numerical data, an alternative coagulation bath having similar HSP characteristics has been designed.

Tab.1: The HSP of pure components used in coagulation baths [29]

	Acetic acid	**Formic acid**	**Lactic acid**	**Propan-2-ol**	**Water**
δ_D (MPa)	14,5	14,3	17	15,8	15,5
δ_P (MPa)	8	11,9	8,3	6,1	16
δ_H (MPa)	13,5	16,6	28,4	16,4	42,3

Tab.2: The coagulation baths based on Formic acid.[21] Calculated HSP parameters of the mixture

1. Formic acid/Methanol bath	**% content**	δ_D (MPa)	δ_P (MPa)	δ_H (MPa)
Formic acid	30	14,86	11,1	20,59
Methanol	70			

2. Formic acid/Ethanol bath	**% content**	δ_D (MPa)	δ_P (MPa)	δ_H (MPa)
Formic acid	30	15,35	9,73	18,56
Ethanol	70			

Tab.3: The coagulation bath based on Acetic acid. [21] Calculated HSP parameters of the mixture

3. Acetic acid/propan-2-ol bath	**% content**	δ_D (MPa)	δ_P (MPa)	δ_H (MPa)
Acetic acid	80	14,76	7,62	14,08
Propan-2-ol	20			

Based on the above computations of the HSP of the basic coagulation baths an alternative composition with use of more friendly components could have been designed.

3.3.2 The alternative coagulation bath based on the Lactic acid

The main target of the novel coagulation bath design was the decrease of odor and price. Simultaneously the non-toxicity and biodegradability must have been maintained. The acetic or formic acid has been therefore replaced by the less-volatile lactic acid that is a compound naturally occurred in the human body. The decreased volatility of the lactic acid is caused by the fact that it is an α-hydroxyl acid, forming strong hydrogen intermolecular interactions. However, pure lactic acid is in a solid state, in a liquid form usually its 80-90% water solutions are available on the market. Due to the role of hydrogen interactions, the solution of the lactic acid is much more viscous liquid then the acetic or formic acid. The elevated viscosity and also a lower density of the coagulation bath based on the lactic acid, led to the need of its dilution by isopropyl alcohol (IPA) in order to maintain similar behavior of the coagulated fiber in the bath. (Defined in the following table)

Tab.4: The flow behavior of coagulated fibers in coagulation bath of various content of lactic acid

Lactic acid content (%)	IPA content (%)	Fiber behavior	Fiber up-winding
90	0	Flow on surface	Impossible – not coagulated
80	20	Flow on surface	Impossible – not coagulated
60	40	Slow sedimentation	Hardly possible – not coagulated
40	60	Sedimentation	Possible – flexible transparent fibers
20	80	Sedimentation	Possible – flexible transparent fibers
10	90	Sedimentation	Possible – flexible white fibers
0	100	Sedimentation	Hardly possible – brittle white fibers

The flow behavior of the coagulated fibers was tested in various dilution ratios of the lactic acid/IPA coagulation baths. In cases of higher content of lactic acid the fibers were floating on the level and were also hardly coagulated to a mechanically resistant fibrous form to be possible to wind it up. On the other hand, too high concentrations of IPA over 90% led to a formation of brittle white fibers that were hardly up-windable. A suitable ratio of the Lactic acid/IPA has been therefore determined to be in a range between 20-40% of lactic acid. Regarding the higher price of the lactic acid in a comparison to the acetic or formic acids the lowest and still working composition was desirable, therefore the final composition was set in a ratio of 20% lactic acid and 80% IPA.

The HSP computations of the designed coagulation bath have been done to evaluate the differences among the baths designed in the previous studies containing the acetic and formic

acids shown in *(Tab.5)*. As the lactic acid is usually distributed in a liquid form as a 80% water solution, the 4% of water must have been considered.

Tab.5: The content of the coagulation bath based on the Lactic acid, calculated HSP parameters of the mixture

Lactic acid bath with 4% water	**% content**	δ_D (MPa)	δ_P (MPa)	δ_H (MPa)
Propan-2-ol	80	15,98	6,84	19,31
Lactic acid	16			
Water	4			

3.3.3 The evaluation of baths by the HSP differences

The similarity-evaluation of coagulation baths has been calculated as the differences of each of the HSP parameters $\Delta\delta_i$. The calculated values have been compared with the reference coagulation bath based on the Formic acid/methanol that was evaluated as well-working composition.[21]

Tab.6: The HSP differences $\Delta\delta_i$ of the designed coagulation baths

Compared coagulation baths	**$\Delta\delta_D$ (MPa)**	**$\Delta\delta_P$ (MPa)**	**$\Delta\delta_H$ (MPa)**
1. Formic acid/Methanol bath - REFERENCE	0	0	0
2. Formic acid/Ethanol bath	0,49	1,37	2,03
3. Acetic acid/Propan-2-ol bath	0,10	3,48	6,51
4. Lactic acid/Propan-2-ol bath	1,18	4,56	1,79
5. Lactic acid/Propan-2-ol bath with 4% water	1,12	4,25	1,23

By the theory the acceptable HSP differences for two closely similar compounds are up to 2MPa in each parameter.[29] It can be therefore stated, that the designed coagulation bath based on the lactic acid acts similarly to the reference bath composition in terms of dispersion and hydrogen interaction parameter where the $\Delta\delta_D$ and $\Delta\delta_H < 2$ MPa. In terms of the polar parameter $\Delta\delta_P$, the difference is more significant stating that the alternative coagulation bath is slightly less polar then the reference-bath composition. (The polar parameter of the lactic-acid bath is lower than in the case of the referenced bath).

3.4 The influence of the concentration of the spinning solution on fiber mechanical properties.

Molecular weights of the spun polymers were selected from the highest that were accessible in a range of 600-700 kDa. The longer molecular chains allow higher interchainal entanglements and the final fiber is further intensively stabilized by the interchainal non-covalent interactions leading to the increased mechanical properties of the final fiber. During the spinning of solutions of different polymer concentrations it has been observed that the minimal polymer content in the solutions leading to a continual fiber-spinning process is 3,5% w/w. (Measured on the lab-spinning device at the up-winding rate of 1,5m/min)

The fibers formed from solutions with lower polymer content tend to break during the fiber formation in the coagulation bath. As shown in the *(Fig.9)*, with the increasing polymer content in the spinning solution, the mechanical strengths measured as the „load at break“ has been increased. The important complementary information to the fiber mechanical measurements is shown in the red–marked record, showing the changes of the linear mass density of the measured fibers. The higher polymer concentration in the polymer solution, the higher linear mass density, Therefore, the increased concentration of the polymer solution leads to a formation of fibers with increased mass which is followed by the increased break force.

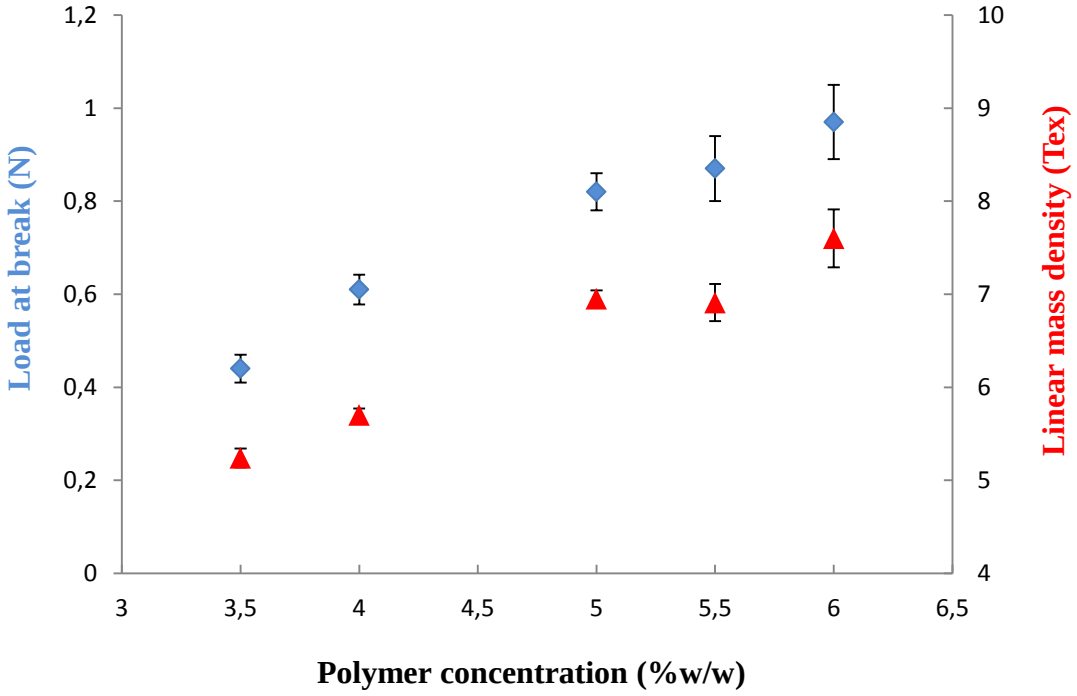


Fig.9: The influence of the polymer concentration in the spinning solution on the fiber mechanical strenghts and linear mass density.

However, by the highest tested concentrations the measurements showed increased standard deviations in the measured characteristics. This observation might correlate with the increased viscosity of the polymer solution that is in the case of 6% solution a rather highly vicous non-homogenous gel. The viscosity profiles are shown in the *(Fig.10)* bellow.

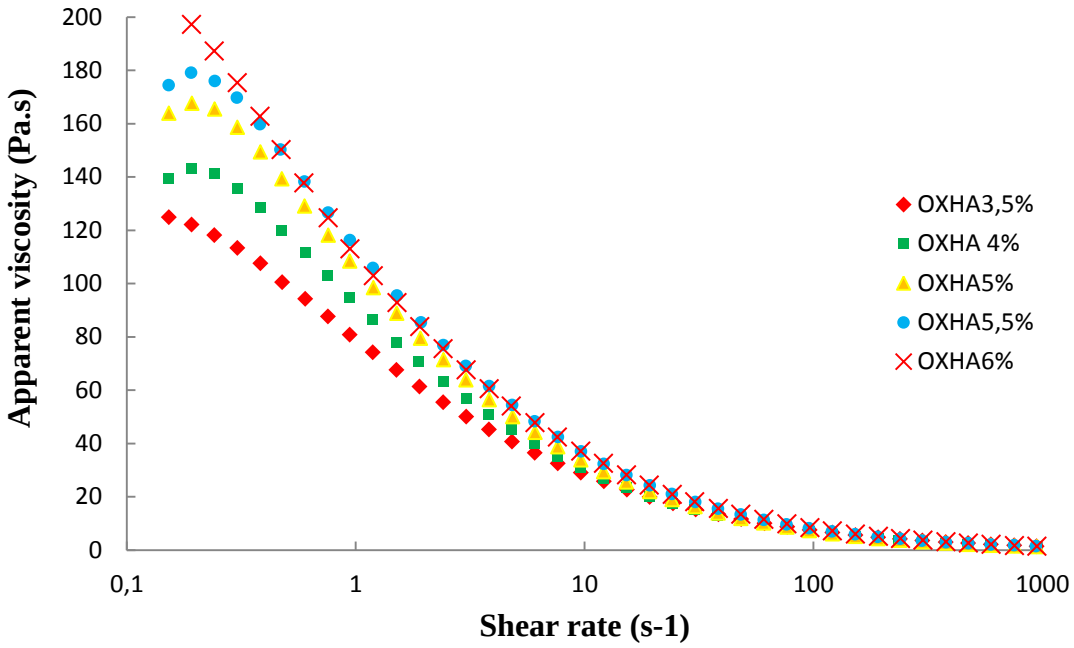


Fig.10: The viscosity profiles of the oxHA spinning solutions. A range of polymer concentrations (oxHA MW 650kDa, measured at 25°C)

This observation can be explained by the enhanced entanglement-formation leading to a more dense systems. However, by the highest tested concentrations the measurements showed increased standard deviations in the measured characteristics. The homogenity of the extruded polymer solution of the higher concentrations is therefore lower, which further affects the homogenity of the fiber characteristics. Based on the observations, a suitable polymer concentration in the spinning solution was defined to be 5% to form homogenous fibers with sufficient mechanical properties for the further textile processing.

1.1 The influence of the relative humidity on fiber-mechanical properties

The oxidized hyaluronic acid represents a highly hydrophilic material, therefore it could have been expected, that even the mechanical properties of the dry fibers will be highly dependent on the environmental air humidity. Therefore, series of experiments have been processed when the air

humidity in the measuring room has been adjusted in range from 30-62 %Rh. The results are shown in the following graph.

As shown in the *(Fig.11)* the elevated environmental humidity leads to a loss of the fiber mechanical strengths and on the other hand to the increase of its deformability. The reason of the observation might be in the process of the fiber swelling where the water molecules penetrate to the interchainal polymer regions and increases the mutual distances. The impact of the low-range cohesive forces is therefore lowered and the mechanical strengths (load at break) of the fiber decreases. On the other hand, as the interchainal distances are increased, the polymer chains become more relaxed as the penetrating water molecules act as interchainal spacers and the mobility of the polymer chains rises.

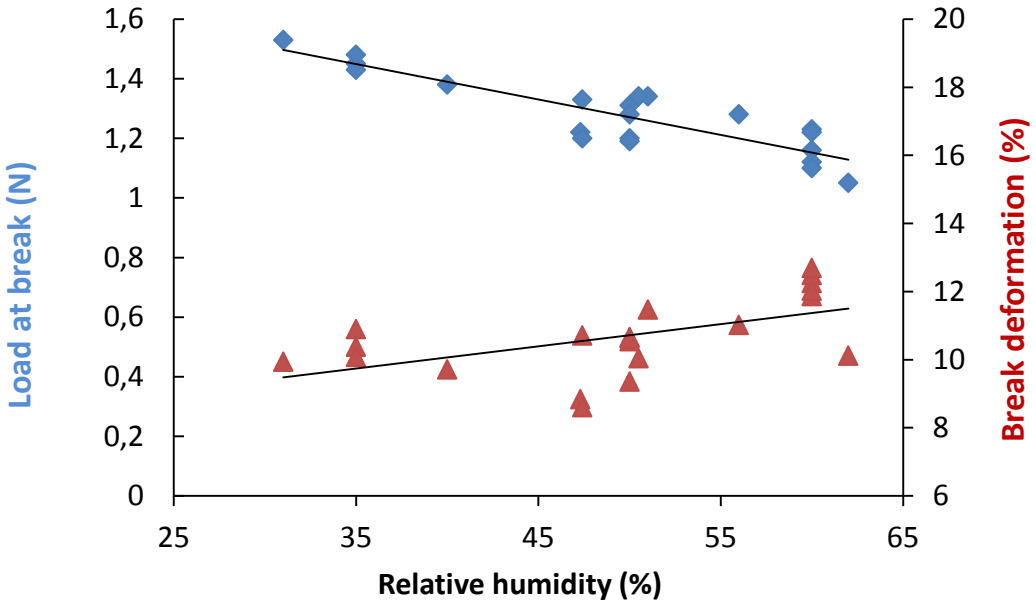


Fig.11: The influence of relative humidity on the mechanical properties of oxHA fibers

The increased environmental humidity therefore leads to the increased fiber flexibility that becomes one of the most critical parameter for the further textile processing of the fiber. Based on the further testing it has been stated, that the suitable conditions for the textile processing seems to be at humidity of 50-60%. At that range the fibers are still mechanically strong and sufficiently flexible enough to overcome the mechanical loading by the knitting textile technology that follows.

1.2 The design of the washing process

The further process that has been studied within the complex technological design was the fiber-washing from the residual coagulation agents, particularly from the lactic acid. The

requirements on the washing agent are firstly a good affinity and mixability with the washed compound and simultaneously an insolubility of the washed fiber. Based on those assumptions, the alcoholic washing baths from a group of methanol, ethanol, isopropyl alcohol and their mixtures with water have been selected. The washing process was determined as a decrease of concentration of the lactic acid in the fiber measured at distinct time periods. The (*Fig.12)* shows the kinetical profiles of the extraction processes in a range of washing agents. The washing experiment was set as static, where the fibers were statically immersed into the washing solutions.

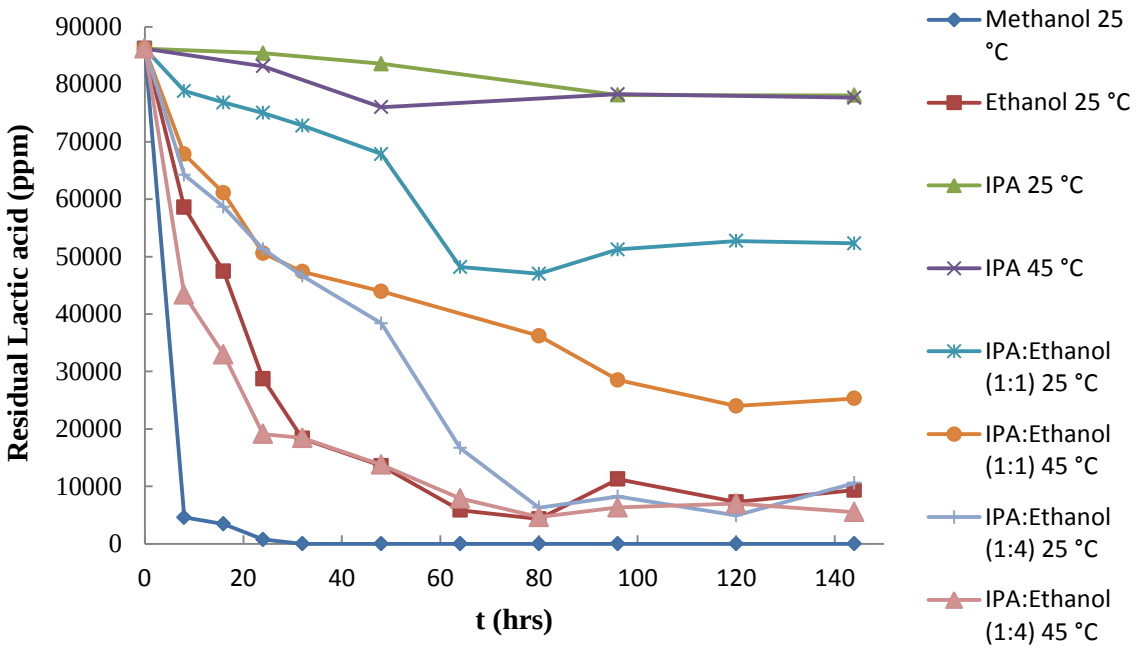


Fig. 12: The kinetical profiles of the lactic acid washing from the fiber in different alcoholic baths. Measured by ing. Jaromír Kulhánek, Contipro a.s.

The residual concentration of the lactic acid was evaluated by the ion-exchange HPLC measurement. Based on the measurement the best washing results have been observed in methanol where the lactic acid was washed out within the 24 hrs. below the detectable limits of the HPLC. On the other hand the low washing efficacy showed the isopropyl alcohol even at elevated temperatures. The reason might be in the decreased polarity of isopropyl alcohol in comparison to the methanol. The use of methanol in a larger production scale is however connected with potential health and safety risks as the methanol. The further suitable washing agent seemed to be ethanol at 25°C or a mixture of ethanol/IPA in a ratio of 1:4 at elevated temperature of 45°C. For the further process, the ethanol was therefore chosen as the washing agent. However, if there is a request of further lowering of the production costs, the pure ethanol can be potentially replaced by its mixture with IPA in the previously discussed ratio of 1:4.

1.2.1 The evaluation of the fiber shelf-stability

The 5 bobbins with dry fibers in the final form have been placed into the environmental chamber, where a constant temperature and humidity was maintained. The fiber stability was tested for a period of the 3 months and has been tested in terms of the loss of their mechanical properties (Load at break).

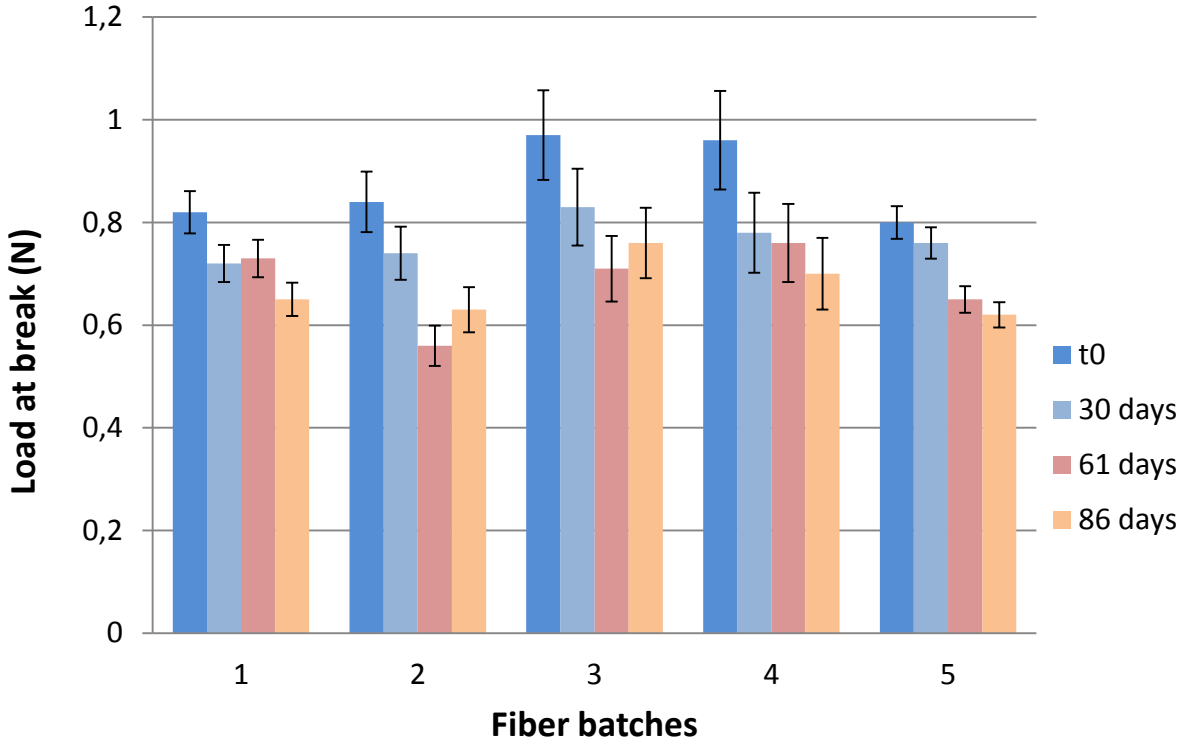


Fig.13: The shelf-life fiber stability, tested on 5 independent fibers, 3 months stability study, fibers left in a environmental chamber at T=23±0,5°C, Rh = 50±2%

The (*Fig.13)* shows that the mechanical properties of fibers decrease slowly within the tested time-frame of the 3 months. The cause of the observation might be in the fact that the oxHA is a biodegradable material that is an excellent substrate for the bacteria. As the conditions of the test were not set to be sterile, the decrease of the fiber mechanical properties is probably caused by the bacterial decomposition of fibers. The results therefore might support the assumption of the material biodegradability.

Fig.14: The HA-based fibers from the production-scale line. A paralell production of 9 HA fibers

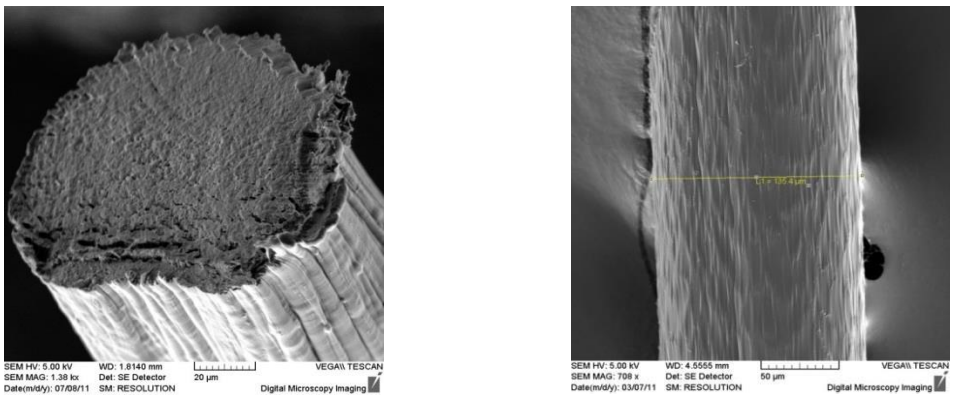


Fig.15 The oxHA monofilaments

3.4.1 Statistical evaluation of the process-reproducibility

The fiber-spinning processes designed within the thesis have been finally tested in terms of reproducibility and repeatability with use of statistical tests (F-Test and the Student´s T- Test). The technological process has been evaluated on 2 different polymer batches, from each polymer batch have been prepared 5 individual fiber windings. Each winding was analyzed by 5 individual measurements. The total data points in each statistical group were minimally 25. The zero hypothesis that was statistically tested was defined as following: "*The mean value of the fiber-tested characteristics is not dependent on the polymer batch used for the fiber spinning*". The polymer batches were selected having similar molecular weights and polydispersity.

Based on the large number of statistical results shown in the full version of the dissertation thesis, it has been stated that the defined zero hypothesis cannot be declained on the tested signifficancy level α=0,05. Therefore, regarding the signifficancy level, fiber-forming process can be considered independent on the used polymer batch (having comparable characteristics).

3.5 The technological scale-up of the fiber spinning production

The demand of the production scale-up led to the necessity of a technological change of distinct process operations that have been used within the laboratory spinning system. The main changes must have been designed in the process of the spinning-solution preparation and it´s dosing to the coagulation bath. Instead of the discontinual piston-syringe dosing used in laboratory, the continual pumping system has been designed. Simultaneously the technology of the polymer-solution deaeration must have been changed. The production line has been designed as described on the general scheme *(Fig.16)*.

The fiber-spinning process starts with the polymer dissolution and deaeration in the stirred reactor vessel. The homogenous solution is further transported into the precise gear-pump system by the elevated pressure in the reactor vessel. The solution is pumped to the 3-line beam separator, where the polymer solution is split and further extruded and coagulated in a form of 3 parallel fibers. Finally, the fibers are up-winded to the process bobbins by the cross-winding mechanism.

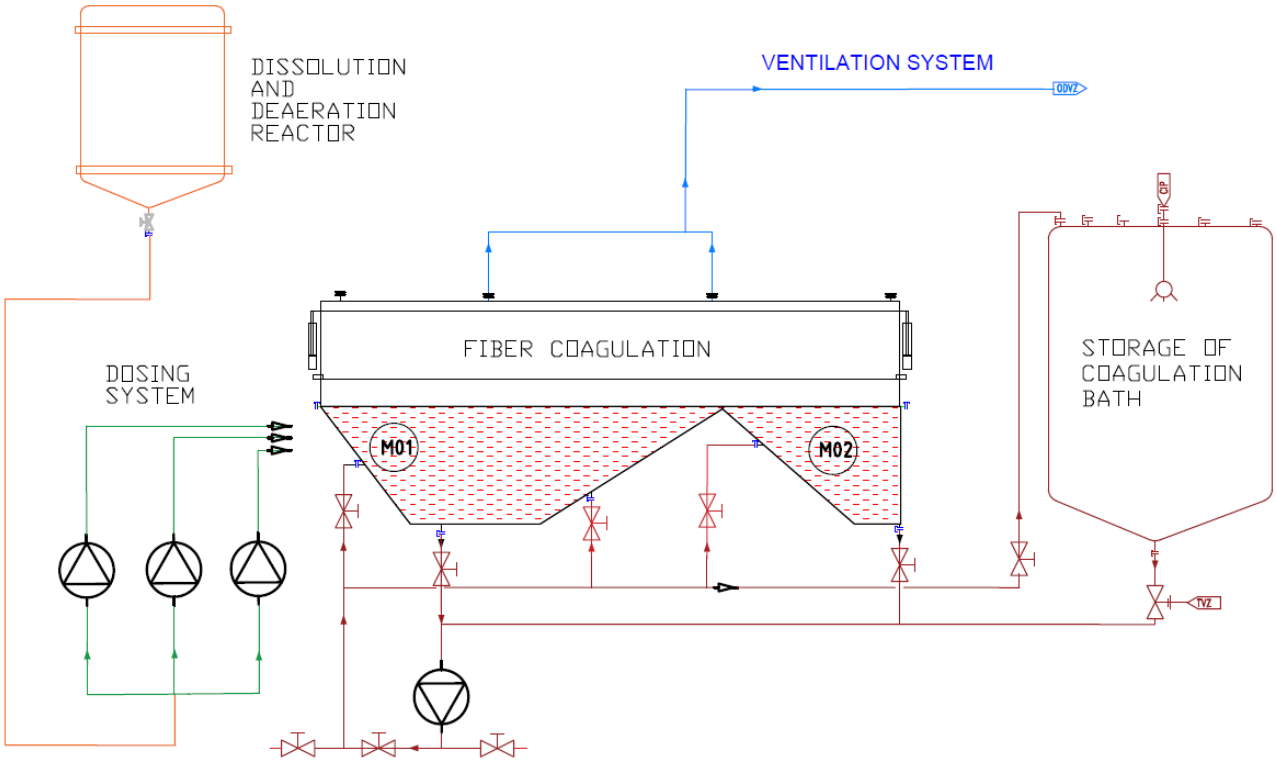


Fig.16: The general scheme of the fiber-spinning process device

3.5.1 The production- process conditions

The major technological modifications in comparison to the laboratory production must have been done in the system of polymer-solution formation, deaeration and its further extrusion. The pilot-scale technologies were designed with a stress to their further up scalability and a possible automatization.

The oxHA (600-700kDa, Contipro a.s.) was added to a stirred reactor filled with 5l of water to form 5% solution. The dissolution was processed under a lower pressure of ~70mBar and temperature 25°C for minimally 8 hrs (the system was maintained in a non-boiling state). The stirring rate was set at 100 rpm, an anchor stirrer was used. Afterwards, the stirrer was stopped and the solution was left still under the vacuum for 4hrs to obtain homogenous and clear deaerated solution.

The reactor vessel was connected to the dosing system (gear pump with extrusion tips at the end). The extrusion tips were connected to a vacuum pump and the polymer solution was sucked to fill the pipeline without any further aeration. The extrusion tips were immersed into the coagulation bath containing IPA/lactic acid in a ratio of 4:1, and the pumping rate was set to the desired value. The coagulated fibers (3-9 parallel lines) were guided to the up-winding device and the desired value of the winding rate was adjusted.

The raw fibers on the process bobbins were further washed by 96% ethanol (25°C) for 1 hour to wash out the residuals of the coagulation bath. Fibers were further dried under a low pressure of 50mBar at 50°C for 2 hrs. Afterwards, the fibers were left to regenerate in a controlled environment (T= 23 ±2°C and Rh=50±3%) for minimally 12 hrs (usually overnight). Dry fibers were further rewinded to storage bobbins and the qualitative measurement was performed.

3.5.2 The deaeration of the spinning solution

The process device consists of the dissolution reactor vessel where the process of polymer dissolution takes place. The dissolution run under a lowered pressure in order to get rid of air bubbles dissolved in the solvent (water). Further the polymer solution is left under the deeper vacuum to complete the deaeration.

The efficacy of the vacuum-deaeration process has been further tested in a 2-liter stirred reactor.

Fig.17: The trials of the vacuum deaeration process in the 2l-stirred reactor

Based on the theoretical assumptions, the intensification of the deaeration process is highly dependent on the depth of the vacuum and the viscosity of the deaerated solution. The depth of the vacuum was set constant and was given by the maximal power output of the vacuum pump (Vacuubrand MD1) of 50mBar (measured in the reactor vessel). The target was to decrease the viscosity of the polymer solution in order to decrease the resistance to the bubble motion. The recepture of the spinning solution must have been maintained constant, (the parameters influencing the viscosity such as polymer molecular weight, concentration, polydispersity has to be kept constant), a possible way was the increase of the temperature and the shear rate. The viscosity changes of the spinning solutions at different temperatures have been measured.

As shown in the *(Fig.18),* the flow curves show the non-Newtonian "shear-thinning" behavior of the spinning solutions where the apparent viscosity η decreases with the shear rate. This phenomenon can be explained by the organization of the polymer chains in the flow direction. The apparent viscosity also decreases with the temperature which can be explained by the increased molecular motion of the polymer chains.

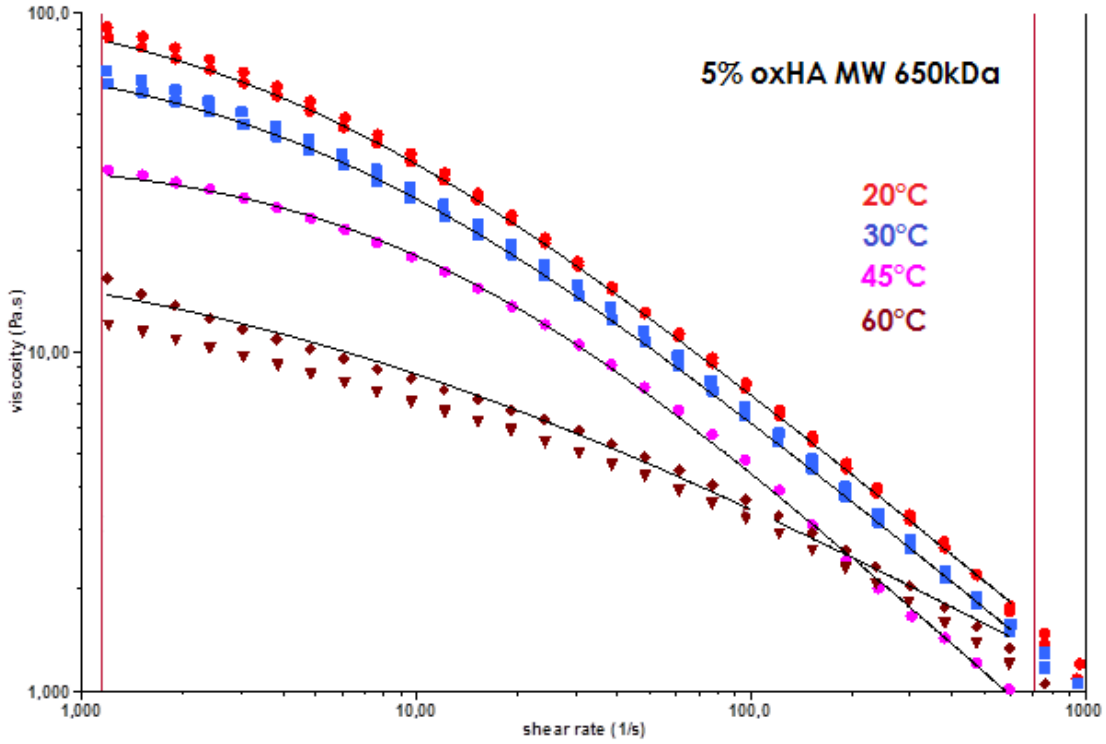


Fig.18: The flow (viscosity) curves of 5% oxHA (650kDa) spinning solution at temperature range (20-60°C).

The flow curves shown in the (*Fig.18)* were best-fitted by the Williamson rheological model and the particular flow parameters for each curve were calculated.

$$\eta = \eta_0 - K\left[\frac{d\gamma}{dt}\right]^{n-1} \tag{16}$$

Tab.7: The parameters of rheological model, the Williamson type

5% oxHA 650 kDa in water - parameters of Williamson model				
Temperature (°C)	**η_0 (Pa.s) (zero-rate viscosity)**	***K* (s) (consistency)**	***n* (-) (rate index)**	**Standard error**
20	113,8	0,2589	0,8162	3,11
30	79,71	0,2075	0,8175	3,805
45	37,95	0,09584	0,8969	9,241
60	22,04	0,228	0,5403	18,01

The flow curve measured at 60°C shows a higher variation that might be caused by the polymer cleavage. By the literature the maximal acceptable value of the flow-curve error for a sufficient fit

is up to 20.[30][30] Based on the findings it has been stated that the suitable temperature-loading during the process of deaeration should not exceed 60°C.

As was shown from the rheological measurements, the viscosity of the spinning solution can be lowered by the temperature increase. The measured data proved that the vacuum deaeration of the spinning solution can be therefore supported by the temperature elevation. However, in the situation when the increased temperature is combined with a decreased pressure in the spinning reactor, the polymer solution will start boiling at some distinct point. During the boiling novel bubbles appear, therefore the boiling of the spinning solution represents a negative process that needs to be avoided. In order to determine the boiling characteristics, the pressure/temperature dependences of the spinning solution have been measured. As the spinning solution contains 95-97% of water, the boiling curve was expected to be close to the pure water. Initially, the boiling points at various vacuum/temperature sets were determined in pure water. Afterwards the measurement was processed with the 3% HA solution (97% water content).

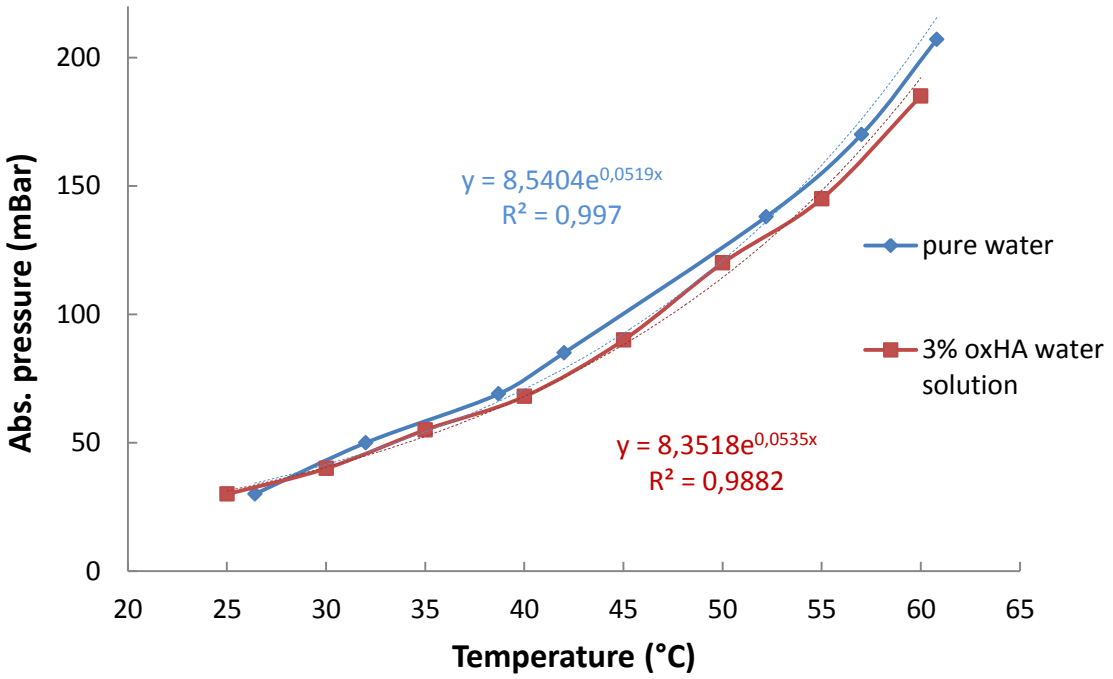


Fig.19: The boiling point determination at various pressures for a) pure water – a blue curve, b) 3% HA/water solution – a red curve

The (*Fig.19)* shows, that the addition of the polymer led to a moderate shift of the boiling curve to higher boiling temperatures. The explanation might be in the potential fact that the hydrophilic polymer chains, having intensive affinity to the water molecules, tend to keep the water molecules

in the solution and prevent them to move to the gaseous phase. A more energy (heat) is therefore needed to force the water molecules to the phase transition.

Based on the experiments shown above, the condition of the deaeration process has been set to moderately elevated temperature of the polymer solution not exceeding ~35°C and a maximal vacuum given by the pump ~60mBar. The elevated temperature was maintained by the moderate stirring of the polymer solution (~10rpm). At that condition the solution was not boiling and the bubble motion was enhanced by the temperature-induced decrease of viscosity.

3.5.3 The testing of the up-winding rate on the fiber mechanical properties

The properties of the fibers were studied in a relation to the up-winding rate. The results shown in the following graph *(Fig.20)* show the decreasing tendency of the fiber fineness that is given by the rising fiber-tension during the spinning process.

The produced fibers were further evaluated in terms of their tension break loads. The measurements shown in the graph *(Fig.21)* bellow show the decreasing tendency in the relation to the rising up winding rates. The observation can be explained by the gradually decreasing fiber diameter and the polymer mass - shown in the previous graph *(Fig.20)*.

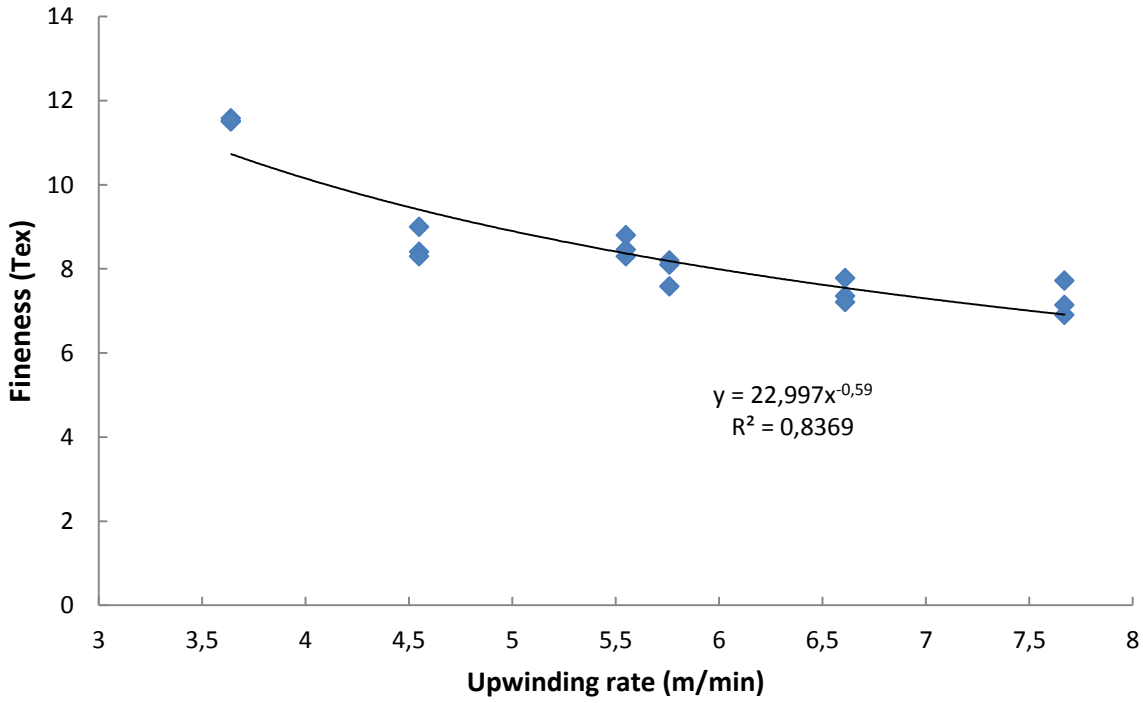


Fig.20: The impact of the up-winding rate on the fiber fineness

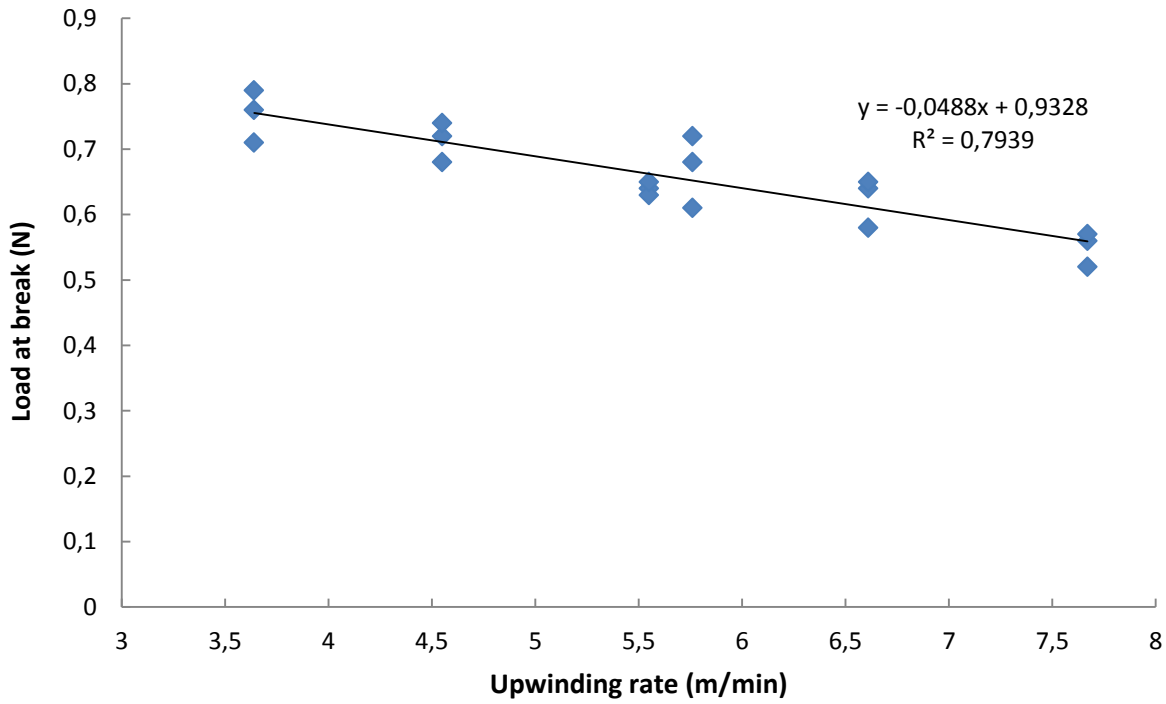


Fig.21: The impact of the up-winding rate on the fiber break load

Based on the demands of the textile processability, the fibers were further prepared to maintain the fineness values of 9±0,5Tex which is approximately related to the up-winding rates of 5-5,5 m/min.

3.6 The crosslinking of the oxHA fibers

The pure oxHA fibers are naturally cross-linked by the acetal-bond formation that is occurring among the aldehydic and hydroxyl functions. The reaction proceeds spontaneously as the auto-crosslink during the water elimination. Due to this spontaneous crosslink the aldehydic fibers are naturally much more stable in water then the fibers from the native hyaluronan. This thought has been proved by numerous experiments, where the aldehydic fiber was inserted into the water or phosphate-buffer solution. The water resistancy of the diacetal-crosslinked aldehydic fibers varies in relation to the fiber diameter, however all fibers prepared within this research proved a minimal water-stability of 30 min at room temperature. In a comparison to the fibers from the native hyaluronan, which are usually completely dissolved within a minute, the aldehydic fibers represent much more useful material for the practical handling with fibers within the wet environment (e.g. wet gloves of the surgeon.)

Moreover, the aldehydic group attached to hyaluronan has an exceptionally high reactive variability. Therefore, the fibers formed from this material can be further modified by a various reactive species containing amines, hydrazides, thiols, hydroxyl groups, etc. This reactivity was

therefore used for the further fiber stabilization with the dihydrazidic crosslinking agents. The dihydrazides are readily used within the food and drug industry, especially the adipic acid dihydrazide that is considered to be metabolically non-toxic. The crosslinking reaction was alternatively performed with the succinic acid dihydrazide to prove the evaluated reaction mechanism.

3.6.1 The study of the crosslinking reaction

The crosslinking process was tested with a use of a couple of different dihydrazides of organic acids (succinic C4 and adipic C6 acids), that differ in the number of carbon atoms. The results have been summarized into the reaction maps, illustrating the solubility/swellability behavior of the modified fiber in a relation to the reaction conditions. This summary of the thesis shows only the tested case of the adipic acid dihydrazide.

Each reaction map, represented by a separate table in a table set *(Tab.8)* represents a reaction system where one concentration of the dihydrazide was used. The fiber stability/swellability in water was taken as the resulting parameter of the reaction evaluation. The reaction maps (tables) were arranged in a direction of a decreasing concentration of the used dihydrazide in the reaction bath. The rows in the tables represent reaction results in different reaction times, the column represent the time of the fiber decay when exposed to water. The values in the reaction maps represent the solubility/swelling state of the modified fiber in a scale (0-5), where 0 equals to fully dissolved state, 5 to fully insoluble and even a non-swelling state. The increasing water stability of the fibers in the 0-5 scale was also marked by the darkness of the color-tone, the darker the more stable fiber in water. The values were further averaged for each concentration resulting in the parameter ***R*** that was taken as a measure of the crosslink efficacy of each reaction bath.

The reproducibility of the reaction procedure has been proved with use of 4 different fiber batches. As shown in the *(Fig.22)* below, in all cases the parabolical shape of the reaction curve have been proved and the crosslinking maximum has been found nearby the concentration region of 5.10^{-3} mol/l.

Based on the results it was proved that the crosslinking reaction has a parabolic shape of the cross linker concentration. This observation supports the initial assumption that the crosslinking process has a distinct concentration optimum where the crosslinking is efficient. Bellow this optimum the crosslink density is low which leads a poor stabilization of polymer chains in the fiber and it´s poor hydrolytic stability.

Tab.8: The map of crosslinking reactions of oxHA fibers by the adipic acid at elevated temperature of 30±1°C(Reaction was set in a furnace)

Adipic-acid dihydrazide (C6)																													
	c = 5.10-2 M						c = 1.10-2 M						c = 5.10-3 M						c = 5.10-4 M						c = 5.10-5 M					
	Time of reaction (min)						Time of reaction (min)						Time of reaction (min)						Time of reaction (min)						Time of reaction (min)					
Water-stability of fiber (min)		1	3	5	10	15		1	3	5	10	15		1	3	5	10	15		1	3	5	10	15		1	3	5	10	15
	10	3	3	3	3	3	10	5	5	5	4	4	10	5	5	5	5	5	10	5	5	5	5	5	10	3	3	4	4	4
	30	3	3	3	2	2	30	5	5	5	4	4	30	5	5	5	5	5	30	2	2	5	5	5	30	2	2	2	2	2
	50	3	3	3	2	2	50	5	5	5	4	3	50	5	5	5	5	5	50	2	2	5	5	5	50	2	2	2	2	2
	70	3	3	2	2	2	70	5	5	5	4	3	70	5	5	5	5	5	70	2	2	5	5	5	70	2	2	2	2	2
	90	3	3	2	2	2	90	5	5	5	3	3	90	5	5	5	5	5	90	2	2	5	5	5	90	2	2	2	2	2
	110	3	3	2	2	2	110	5	5	5	3	3	110	5	5	5	5	5	110	2	2	4	5	5	110	2	2	2	2	2
	130	3	2	2	2	2	130	5	5	5	3	3	130	5	5	5	5	5	130	2	2	4	5	5	130	2	2	2	2	2
	150	3	2	2	2	2	150	5	5	5	3	3	150	5	5	5	5	5	150	2	2	4	5	5	150	2	2	2	2	2
	170	3	2	2	2	2	170	5	5	5	3	3	170	5	5	5	5	5	170	2	2	4	5	5	170	2	2	2	2	2
	190	3	2	2	2	0	190	5	5	5	3	3	190	5	5	5	5	5	190	2	2	4	5	5	190	2	2	2	2	2
	210	3	2	2	2	0	210	5	5	5	3	3	210	5	5	5	5	5	210	2	2	4	5	5	210	2	2	2	2	2
	230	3	2	2	2	0	230	5	5	5	3	3	230	5	5	5	5	5	230	2	2	4	5	5	230	2	2	2	2	2
	250	3	2	2	2	0	250	5	5	5	3	3	250	5	5	5	5	5	250	2	2	4	5	5	250	2	2	2	2	2
	270	3	2	0	0	0	270	5	5	5	3	3	270	5	5	5	5	5	270	2	2	4	5	5	270	2	2	2	2	2
	∞	3	0	0	0	0	∞	5	5	4	3	3	∞	5	5	5	5	5	∞	2	2	4	5	5	∞	0	2	2	2	2
	$\bar{R}$	2,1						4,3						5,0						3,7						2,1				

On the other hand, above the concentration optimum, the too high concentration of the crosslinking agent leads to the saturation of reactive aldehydic functions of the polymer by individual molecules of the crosslinker and thus the crosslink efficiency is lowered as well. Based on the experiments the following reaction scheme was proposed.*(Fig.60)*

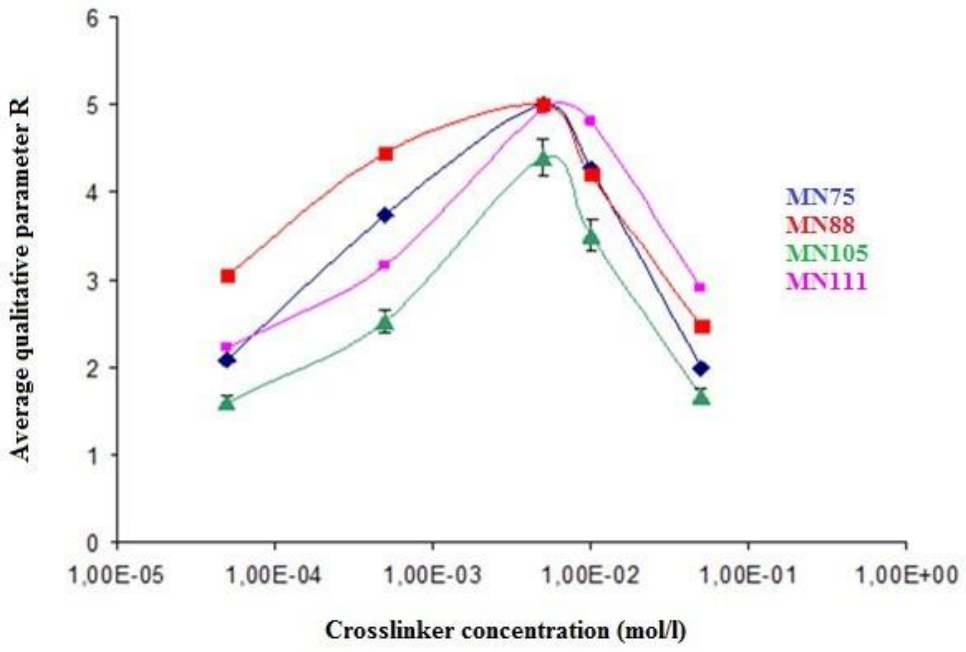


Fig.22: The evaluation of the reaction repeatability, the optimal crosslinking concentration was proved by four independent repetitions showing similar concentration reaction maximum

Based on the observations and measured data the reaction mechanism shown in the *(Fig.23)* has been proposed.

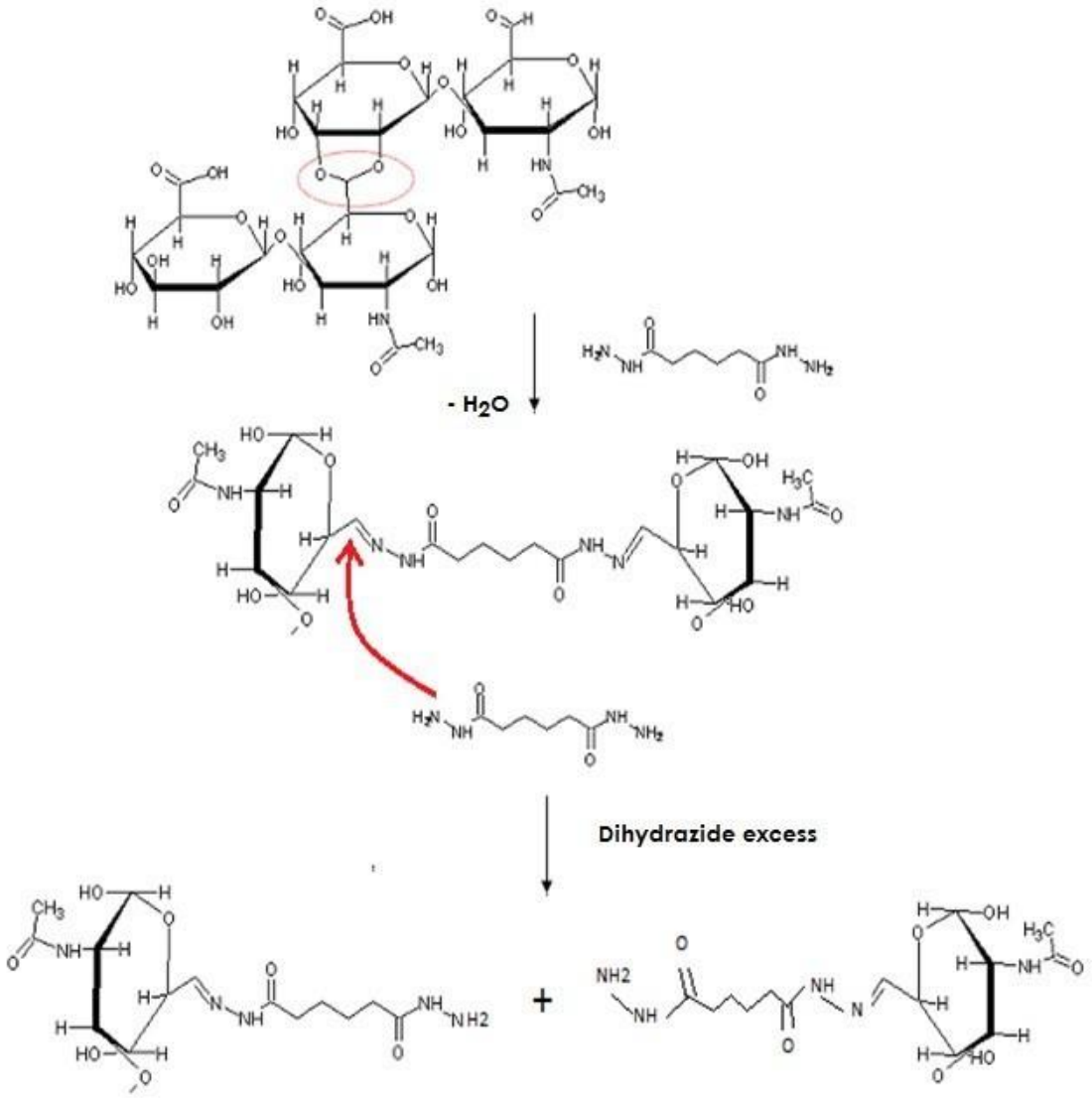


Fig.23: The reaction scheme of the hydrazone crosslink and it´s unfavorable cleavage at higher concentration of the dihydrazide

3.7 The evaluation of material biocompatibility

The fibers developed within this work have been targeted to the possible use within the human-body-internal implantations. Therefore the evaluation of biological and toxicological characteristics must have been determined in order to prove the material applicability within this field.

3.7.1 The evaluation of the endotoxine content

The endotoxins are represented by the lipopolysaccharidic particles coming from the decomposed cellular shells. The monitoring of the endotoxin content is highly important in case of materials and medical devices that are dedicated to be implanted into the body. The endotoxin content in the material gives the complementary information to the material sterility and cytotoxicity. The increased endotoxin content causes an elevated activation of the immune system leading to the inflammatory side reaction that might be a cause of the undesirable rejection of the implanted material. The endotoxin content informs about the material pyrogene-contamination and gives partial information about the material purity.

The measurement of the endotoxin content is usually processed by the standard assay kits, in this case The PyroGene™ recombinant Factor C (rFC), that has been accepted by the regulatory authority FDA and is generally accepted as a standard endotoxin measurement test.

Tab.9: An evaluation of endotoxin content in the fiber-spun materials (measured by the Laboratory of Microbiology, Contipro a.s.)

rFC assay	MN116	MN112	MN113	HA 2MDa
Endotoxin content (IU/mg)	0,19	0,12	0,28	0,22

The endotoxin content was evaluated in 4 different fiber-spinning materials (oxHA: MN112, MN113 and MN116 and a control material pure hyaluronic acid HA 2MDa (pharma grade quality, Contipro Pharma a.s.). In all cases the endotoxin content was detected to be below 0,5 IU/mg, that is by the Pharmacopoeia [31] an acceptable limit for the further *In-Vivo* experiments.

3.7.2 The evaluation of material biocompatibility/cytotoxicity

The biocompatibility was evaluated with a use of human dermal fibroblasts that are usually taken as an accessible and suitable screening cell type. The biocompatibility/cytotoxicity test is based on the presumption that the potential cytotoxic material hinders the natural cellular growth and the number of viable cells is decreasing within time. During the experiment the cellular viability of 3T3 cells treated by oxHA fibers was determined by MTT test (Microculture tetrazolium test) at 24, 48 and 72 hrs. time points. The biocompatibility test evaluated two batches of oxHA (MN112 and MN113) and two batches of native hyaluronic acid (Contipro, Pharma-grade) as standards. The results have been evaluated by the statistical T-test, by a comparison of the tested materials with untreated controls (ctrl). The significancy level was stated at $p < 0,05$.

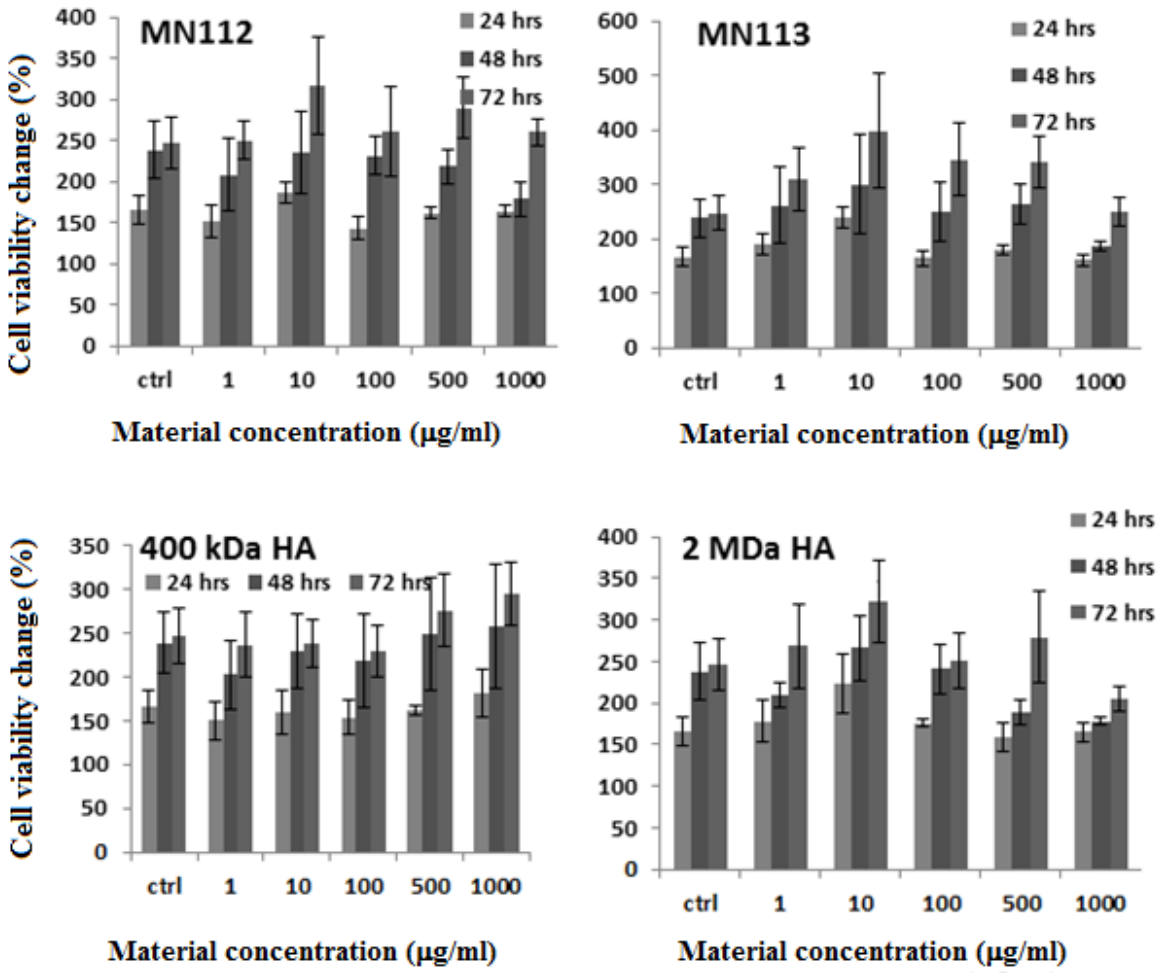


Fig.24: The evaluation of the material cytotoxicity in concentrations 1, 10, 100, 500 and 1000 µg/ml. The viability of 3T3 cells treated by oxHA materials (~700kDa) NM112 (A) and NM113 (B) and by standard native hyaluronic acid C) HA 400kDa and D) HA 2MDa was measured in 24, 48 and 72 time points by MTT test.

The cell-viability tests shown in the *(Fig.24)* showed that the both tested oxHA materials represented by the materials MN112 and MN113 show a normal, positive cell proliferation behavior in all tested concentrations (1-1000µg/ml) and therefore the number of cell is increasing in time. The tested materials were simultaneously compared with the two samples of native hyaluronan (MW400 and 2000kDa). The results confirmed that also in a presence of these native materials the cell-viability-response is similar with a positive proliferation cell-behavior. Based on the measurement it can be stated that the biocompatibility of ox-HA material tested in terms of the cell viability shows to be equivalent as in the case of the native hyaluronan.

3.7.3 The evaluation of potential inflammatory side-reactions

Samples of microfibers were tested for their proinflammatory behavior in the complex system of full human blood followed by the detection of proinflammatory cytokine tumor necrotic factor alpha (TNF-alpha) and reactive oxygen species (ROS). (The results from the ROS not shown in this shortened version of the thesis).

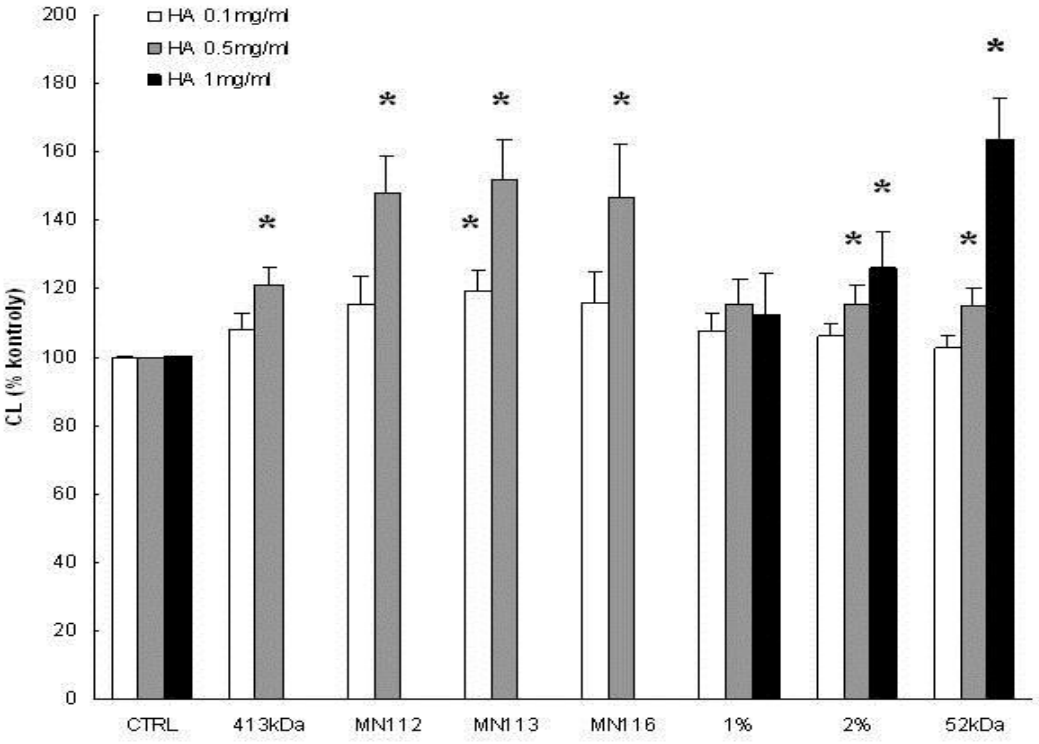


Fig.25: The evaluation of the pro-inflammatory reaction of immune cells on oxHA materials by the ROS chemiluminiscence, oxHA materials (MN112-116) were compared to the 1 and 2% native hyaluronic acid MW 413 and 52 KDa and a clear control.

Results showed that the fibrous samples (MN112 and MN116) as well as the native HA of an adequate molecular weight did not significantly activate the immune system. The moderate increase of the TNF-α *(Fig. 25) and ROS (not shown)* concentrations was observed, however, these levels are not clinically significant.

4 CONCLUSION

The goals of this research have been defined as the complex study of the technological aspects of formation of endless fibers based on the oxidized hyaluronic acid. The motivation for the oxHA material selection was in its enhanced reactivity potential (compared to the native HA) that might have been used for the further fiber stabilization in order to get hydrolytically stable fibers with an enhanced stability in the environment of the human body. The raw aldehydic fibers can survive in the water environment for approx. 30-40mins and after the chemical crosslink the fiber becomes totally insoluble.

Initially the suitable process-device portfolio must have been designed in order to be able collect relevant process data. The laboratory-fiber spinning devices have been developed in three generations *(Fig. 18- Fig.20)* supporting an adequate process-data accuracy.

The oxHA-fiber forming process has been based on the coagulation technology of the extruded polymer solution therefore a high attention was paid to the design of a suitable coagulation bath. The design was supported by the calculations based on the Hansen solubility theory *(Chapter 3.1)*. A novel type of a coagulation bath based on lactic acid has been designed having similar fiber-spinning efficacy as the baths designed in the cited literature. The advantage of the newly designed coagulation bath is in its lower odor and lowered health & safety risks in comparison to the acetic or formic acid-based baths used previously. Due to the demand of the further process automatization, the suitable methods of the coagulation-bath quality monitoring was designed being based on the conductivity measurement.*(Chapter 3.2)*

The process of the fiber formation has been studied by a range of impacts (influence of the temperature of the coagulation bath, concentration of the spinning solution, dosing and up-winding rates) *(Chapter 3.3-3.6)*. Further the process of the fiber washing was evaluated and relevant washing agents have been proposed. Based on the gained information the standard process parameters have been set and the reproducibility of the process was further statistically evaluated *(Chapter 3.7)*, resulting in the statement that the fiber-forming process is reproducible and the technology lead to the formation of fibers with homogenous and reproducible properties.

The fiber-forming technology was further extended into a larger production scales. This step demanded some major technological changes in the way of the spinning-solution formation, especially the technology of the deaeration *(Chapter 3.8.1)* and the change of the discontinual piston-dosing system into a continual gear-pumping system. *(Chapter 3.8.2)*

Based on the pilot-scale process data, the qualitative norms of the oxHA fiber have been set and the production line was tested in a mode of a parallel-fiber production, where the 9 fibers

could have been produced simultaneously. The results from the initial trials *(Chapter 3.8.4)* shown that the parallel production can yield fibers with sufficiently homogenous parameters with a need of minor adjustments.

The presented thesis further discusses the possibilities of the further chemical stabilization of oxHA fibers based on the hydrazone-bond crosslink.*(Chapter 3.9.)* The crosslinking mechanism was evaluated with a use of two different crosslinking agents (dihydrazides of succinic and adipic acids). Based on the large sets of the experiments shown in reaction maps *(Tab.19 and Tab.20)* it has been proved that the crosslinking reactions have a parabolic dependency of the crosslink-efficacy on the cross linker concentration. The optimal concentration of the cross linker yielding hydrolytically stable fibers has been found.

Finally, the fibers were evaluated from the point of view of their biocompatibility and potential toxicity-risks.*(Chapter 3.10.2)* The biocompatibility was evaluated on the standard 3T3-mice-fibroblast-cells showing that the material in all tested concentrations (up to 1000μg/ml) does not lower the cell proliferation (natural reproduction) and can be therefore considered as biocompatible. Further the potential inflammatory side-effects of the oxHA material have been evaluated by the standard TNF-α and ROS (Reactive oxygen species) tests. The results shown that the pro-inflammatory reaction in the presence of the oxHA material is clinically insignificant and the material can be therefore considered as biologically inert. The fibers based on the cross-linked oxHA therefore represent a potentially promising platform within the field of tissue engineering.

The discussed problematics has been covered by an international patent.

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6 CURRICULUM VITAE

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Technical University Brno, Faculty of Chemistry

2006 – 2007 **Materials chemistry** (6 months Erasmus stay)

Technical University Vienna, Faculty of Chemistry, Austria

2002 – 2005 **Bc. in Technical chemistry**

Technical University Brno, Faculty of Chemistry

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WORK EXPERIENCE:

2016 – now **Adfors Saint-Gobain a.s.**

Research engineer, Technology of glass fiber

2013 – 2016 **Contipro Pharma a.s.**

Research engineer, technological design of hyaluronan fiber production

2009 – 2015 **Contipro Biotech sr.o.**

Researcher and chemical technologist, fiber development

2008 – 2009 **CPN spol. s r.o.**

Research assistant – laboratory of chemical synthesis

2007 – 2008 **Fischers services Ltd.**, Perth, Scotland (Production operative)

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PROJECTS:

2015 - 2016	„**Vlatex_Prostory**“ Building of a production hall for instalation of fiber-forming processes
2013 – 2015	**„Zavedení výroby plně biodegradabilních vláken a textilií“** Responsibility for the fiber-production plant design with respect to ATEX and future GMP, Preparation of device tenders and technological runs.
2013 – 2015	„**AMCARE – CARDIOPATCH**“ - European research project Responsibility for the design of multi-step technological processes leading to a degradable cell-adhessive textile.
2013	**Fibers based on oxidized hyaluronic acid** (project ZPŘ2)
2012	**Fibers based on native hyaluronic acid** (project ZPŘ2

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6.1.1 Patents

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