# Polysaccharide based nanofibers with pH-sensitive function

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*The aim of the present study was to prepare a polysaccharide (cellulose acetate) based nanofibrous sensor for detection of pH change in the wound environment. In order to prepare cellulose acetate (CA) nanofibers, acetic acid was used as a solvent, and fabrication of fibers was performed on the needle-less electrospinning apparatus. Long uniform CA nanofibers, with diameters ranging from 250 to 300 nm, were electrospun from 15 wt% CA and 85% acetic acid, with addition of halochromic dye (Bromocrezol Green). The addition of Bromocrezol Green in the spinning formulation did not affect the fiber formation. Prepared nanofibrous sensors were characterized using CIE color space analysis in order to evaluate the color due to pH change. Nanofibrous sensors exhibit yellow color when exposed to pH4 and lower, simulating the wound environment beneficial to the wound healing, and blue color when exposed to pH 9 and higher, simulating the environment that hampers wound healing (chronic, infected wounds).*
**Key words:** *electrospinning, polysaccharide (CA) nanofibers, pH-sensitive dyes, sensors*

## 1. Introduction

Electrospinning is an environmental friendly method for production of nanofibers with unique properties that enables them to be used in versatile applications [1]. Due to their small diameter (from few tens to hundreds of nanometers), high porosity, small pore size and large active surface area [2], they became interesting material for biomedical applications, e.g. tissue engineering, vascular grafts, tissue repair, wound healing, and drug delivery [3]. All biochemical processes in the body, including wound healing, are influenced by pH. It is known that normal, healthy skin is slightly acidic (pH 4-6) and body's internal pH is 7.4. In case of a skin injury, the acidic environment of the skin is disturbed and body`s internal pH is exposed. However, when bacteria infect the wound, the pH is increased to alkaline region (pH 7-8) and the wound fails to heal [4]. The change in wound environments` pH is a very important information for the medical treatment of wounds; in these applications, electrospun nanofibers with high surface/volume ratio can be used as biosensors.

There have been few reports dealing with the preparation of electrospun pH-sensor nanofibers for different

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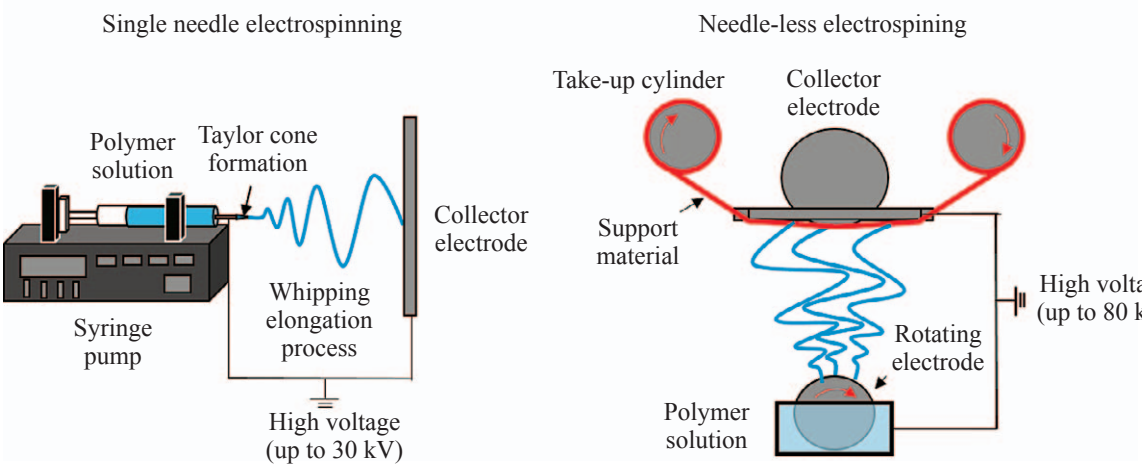



Fig.1 Schematic comparison of single needle electrospinning and needle-less electrospinning

applications e.g. smart packaging [5], textiles [6], tissue engineering and microbiological studies [7] by using indicator dyes, halochromic chemical compounds, which absorb different wavelengths of visible light depending on the pH. Nanofibers with pH-function can be prepared by subsequent dyeing procedure or by adding the pH-sensitive dye in the spinning solution prior to the fiber formation.

In the present study, we report about the preparation of polysaccharide nanofiber mats with incorporated pH-sensitive dye, Bromcresol Green that has a pH range from 3.8 to 5.4, with color change from yellow to blue. For fiber formation cellulose acetate (CA) was used, which is extensively used in different electrospinning applications [8,9], since it can be easily converted to cellulose. Different solvents for electrospinning of CA have been used, e.g. chloroform, methanol, N,N-dimethylformamid, dichloromethane, pyridine, and their mixtures [10]. In our study, acetic acid was employed as solvent, which was first reported by Han [11]. For formation of nanofibers they used 17 wt% concentration of CA dissolved in 70% or higher concentration of acetic acid, by using single needle electrospinning setup. In our research, we used needle-less electrospinning apparatus ElMarco Nanospider, which is, in comparison to electrospinning single needle setup, slightly modified; a high voltage power supply (up to 80 kV), feeding unit (a bathtub with rotating electrode – cylinder or wire) and a grounded collector (cylinder or wire electrode), seen in Fig.1. When an external electric field is applied, the polymer solution, covering the rotating electrode, is charged, causing the formation of conical droplets, due to the equilibrium of polymer surface tension and applied electric field. With increasing voltage, the electric field overcomes the polymer solutions` surface tension and jets start to form spontaneously on the free liquid surface. The polymer jets solidify on their way towards the collecting electrode and are collected as nonwoven fabric [12,13]. The production rate of Nanospider setup is up to several 10g of fibres per hour, making it suitable for large-scale production [14,15].

## 2. Materials and methods

### 2.1. Materials

Cellulose acetate (CA), with Mn= 30.000 and acetic acid (≥ 99.8 %) were kindly supplied by Sigma Aldrich. Pegatex® S non-woven, supplied by PEGAS NONWOVENS s.r.o. (Znojmo, Czech Republic) was used as a mat for nanofiber deposition. The mat is a non-woven fabric manufactured by means of spun bond technology made from 100 % polypropylene fibers.

pH-sensitive dye Bromocrezol Green was purchased from Kemika.

### 2.2. Preparation of electrospinning solutions

12, 15 and 17 wt% cellulose acetate (CA) solutions were prepared by dissolving appropriate amount of CA in different concentrations of acetic acid (75, 80 and 85 %). All the solutions were stirred until a homogenous mixture was obtained. For characterization of viscosity, surface tension and conductivity of prepared solutions, Fungilab Viscometer, Goniometer OCA35 Dataphysics and Mettler Toledo Conductivity Meter, were used, respectively.

Electrospun solutions with pH sensitive dyes were prepared from 15wt% CA solution in 85 % acetic acid, by adding pH-sensitive dye and stirred for 2 hours. Concentration of pH-sensitive dyes was 0,5 wt% relative to the CA mass in the solution.

### 2.3. Electrospinning

Electrospinning was performed on the pilot scale apparatus Nanospider NS Lab 500 from ElMarco Co. Fiber formation was performed by introducing the polymer solution in the Nanospider bathtub, where the wired electrode was placed. Process parameters were; voltage 75 kV, electrode distance: 160 mm, wired electrode rotation speed: 3,8 rpm and collecting time: 40 min.

### 2.4. Fiber characterization

Fiber morphology was analyzed by using scanning electron microscopy

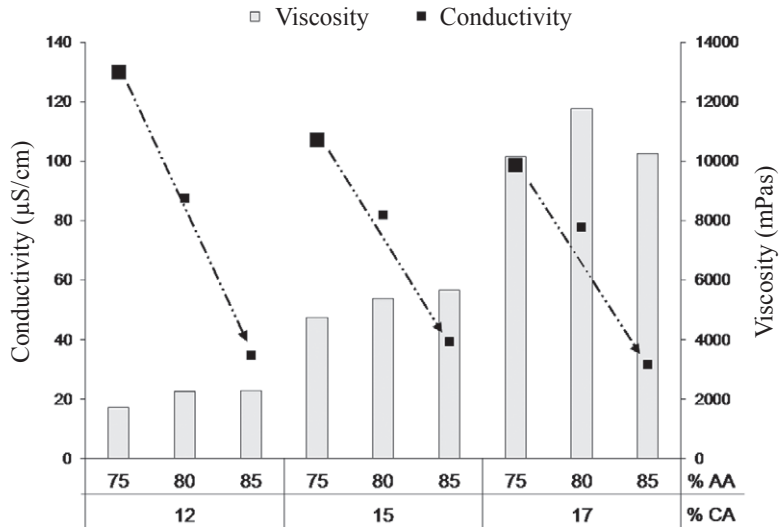



Fig.2 Viscosity and conductivity of prepared electrospinning solutions with different concentrations of acetic acid (AA) and cellulose acetate (CA)

(FE-SEM SUPRA 35 VP, Carl Zeiss). Fibrous samples were placed on a sample holder by using conductive carbon tape. The samples were analyzed with 1 kV accelerating voltage and working distance of 4,5 mm.

For testing of nanofibers' color change the following solutions were used: for pH 0-2 different molarities of HCl, for pH 4, citrate buffer, for pH 7, phosphate buffer, for pH 9, carbonate-bicarbonate buffer, for pH 10 - 14 different molarities of NaOH. Nanofibrous samples were soaked in prepared buffer solution for 1 min. In order to evaluate the color change, CIE color measurements were performed within a spectral range of 400-700 nm wavelengths by means of a two-ray Spectraflash SF600 Plus spectrophotometer (Datacolor) under a standard illuminant D56 (LAV/Spec.Incl.), and a measuring geometry of s/8°.

## 3. Results

Electrospinning is a fiber forming method, which strongly depends of the solution properties, especially in the needle-less electrospinning process. Therefore, prior to the electrospinning procedure, all electrospinning solutions were characterized according to their viscosity, conductivity and surface tension, which are the solution parameters that have biggest influence on the formation of nanofibers. The results, from solutions surface tension measurements, show values 34 mN/m, irrespectively to the composition of the solvent and CA concentration. However, in the case of the viscosity measurements (Fig.2) an expected increase is observed with increasing concentration of CA. While, on the other hand, a decrease in conductivity of solutions with increasing concentration of ace-

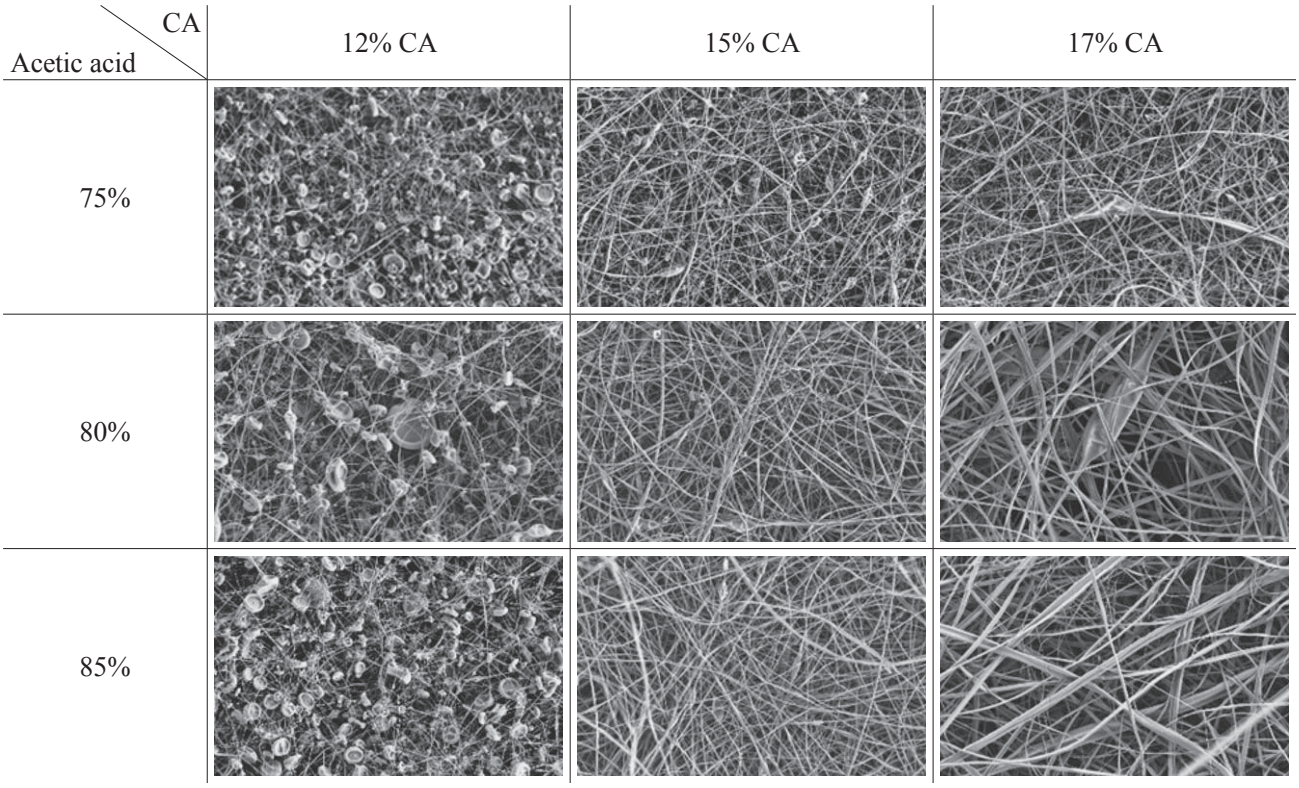
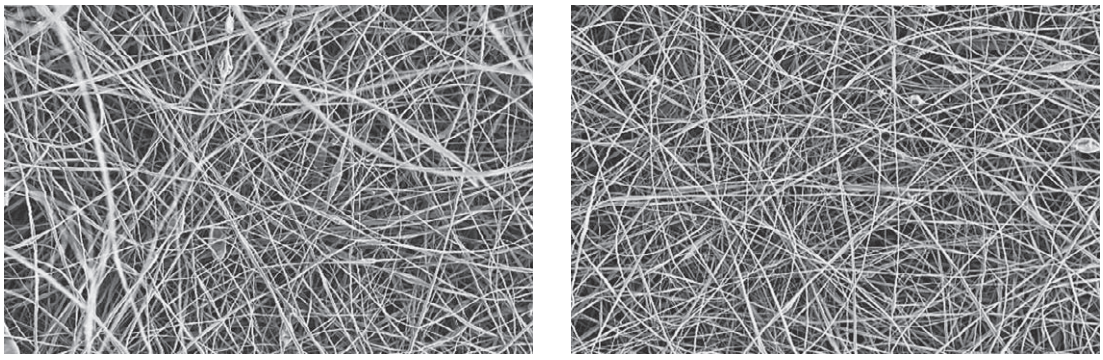



Fig.3 SEM micrographs of CA nanofibers electrospun from different CA concentration dissolved in different concentrations of acetic acid



a) b)

Fig.4 SEM micrographs of electrospun CA nanofibers without - a) and with incorporated Bromocrezol Green - b)

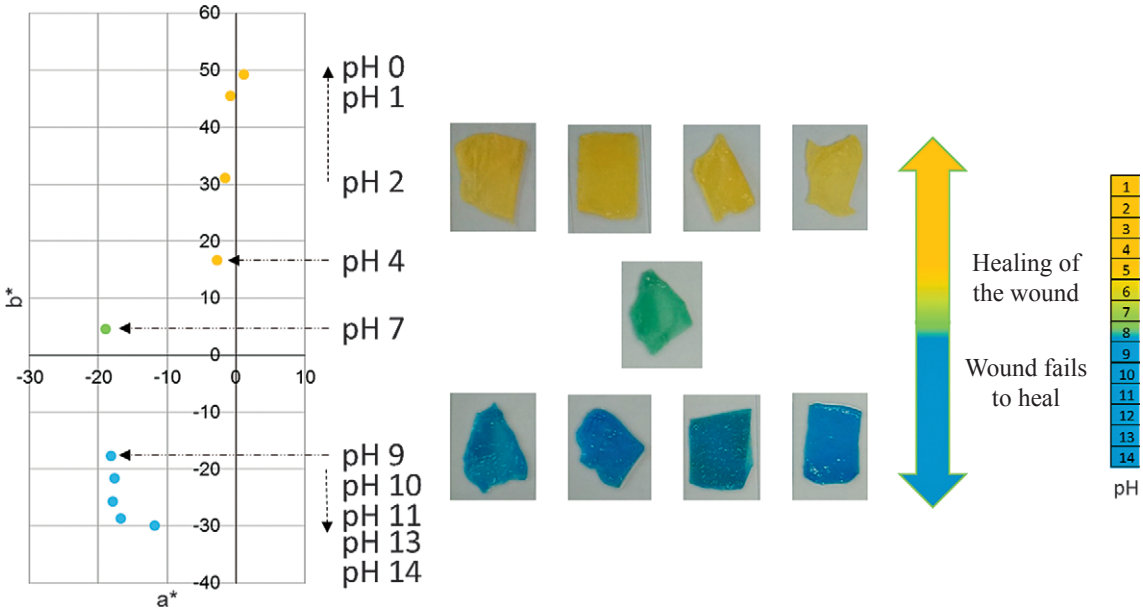



Fig.5 CIE a*b* values of colored nanofibers depending on the pH

tic acid was observed, which could be attributed to dissociation of solvent - acetic acid. As the content of water in acetic acid solution is decreasing, the dissociation of ions becomes more difficult, which results in lower charge densities in CA solutions [11]. In order to determine an optimal solution formulation, which is required to obtain smooth and uniform nanofibers, SEM microscopy was used. In Fig.3, SEM micrographs of nanofibers are shown. We can observe different morphologies depending on the CA concentration as well as concentration of solvent. SEM micrographs of 12 wt % CA in all solvent concentrations show hollow spheres or particles due to inadequate solution formulation leading to emergence of electrospraying. When the solution viscosity is to low, the polymer jets are disintegrating in droplets, before they reach the collecting material, due to the effect of surface tension. However, with an increase in CA concentration to 15 wt%, the beaded morphology of nanofibers is reduced and by increasing the concentration of acetic acid (80 and 85%) the beads are completely absent and uniform fibers with diameter from 300-400 nm are formed. Nanofibers, electrospun from 17wt% CA, show uneven size and shape with diameter ranging from 500 – 1000nm, independent on the solvent concentration. Increase in fiber diameter with thicker parts alongside the fibers' axis is due to the high viscosity of the polymer solution, causing uneven movement of the polymer jet from the electrode surface to the collecting material. According to these results, we can conclude, that uniform nanofibers are formed with the solution formulation of 15wt% CA dissolved in 85% acetic acid, which was used in further research.

In order to prepare pH-sensitive nanofibers, halochromic dye Bromocrezol Green was added to defined optimal spinning solution. In Fig.4, SEM micrographs of electrospun nanofibers without and with Bromocrezol Green are shown in order to evaluate the effect of dye on the electrospinning. We observed that the addition of pH-sensitive dye did not influence the fiber morphology, fibers are uniform with diameter ranging from 250 – 300 nm.

Prepared nanofibrous mats with incorporated pH-sensitive dye were ex-

posed to solutions with different pH values and the color change was analyzed using spectrophotometer. Color change took place in approximately 10 seconds upon exposure. For comparing the samples` color, the CIE (International Commission of Illumination) color coordinates have been determined and the results are shown in Fig.5. A color is defined by a* and b* color values, where a* represents the position on green/red axis, negative value indicates green, and positive value red region. b* represents the position of sample on yellow and blue axis, negative value represents blue and positive value represents yellowness. Fig.5 clearly shows that exposure of dye-functionalized nanofibers to low pH values (high acidic region) turns the color to yellow, while neutral environment (pH 7), changes the color to green with an only slight yellowish tint. When electrospun mats are exposed to alkaline pH, they turn blue with slightly green tint. Since pH values in wound environment change from pH4 to pH9, developed nanofibrous sensors should give immediately observed color change in this region that patients can quickly assess. Therefore, prepared nanofibrous mats show excellent results since they show yellow color in environment that is desirable for successful wound healing and blue color when the wound environment does not allow the wound to heal. By using these kinds of sensors in wound dressings, the patients would be able to establish the status of wound healing on their own. Benefits in sensing the wound environment include reduction of hospitalization time, prevention of amputations and better understanding of the processes, which impair healing [4].

## 4. Conclusions

Presented research deals with the preparation of halocromic or pH-sensitive nanofibrous material for detecting changes in wound environments` pH values. Nanofibrous material, as a potential sensor platform, was successfully prepared by needle-less electrospinning using cellulose acetate (CA) dissolved in acetic acid. Long and uniform nanofibers, with diameter of 250 to 300 nm, were obtained from polymer solutions` formulation of 15 wt% CA and 85% acetic acid. Prior to electrospinning, halocromic dye Bromocrezol Green was added to the spinning solution. The addition of pH-sensitive dye did not affect the electrospinning process and morphology of formed nanofibers. Developed halocromic nanofibrous sensor shows distinct change in color upon exposure to different pH environments. In acidic pH, the sensor is colored yellow, indicating a good environment for fast and effective healing, while in the alkaline region the sensor is colored blue, indicating the infection and failing of the wound healing. Since humans are naturally visual and more effectively process information in visual format, developed sensor materials show high potential in wound healing treatments.

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