

## Increasing mechanical strength of electrospun gelatin nanofibers by the addition of aluminum potassium sulfate

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**ABSTRACT:** Increasing mechanical strength of gelatin-based materials is required to expand the range of their applications, which is desirable because of biocompatibility, biodegradability, and low cost of gelatin. The effect of aluminum potassium sulfate on preparation and properties of nanofibrous gelatin were investigated. Samples were electrospun from 10M aqueous acetic acid and analyzed by scanning electron microscopy (SEM), Fourier transform infrared microscopy (FTIR), energy-dispersive x-ray analysis (EDX), and tensile test. The addition of  $\text{AlK}(\text{SO}_4)_2$  considerably increases the elastic modulus of the material up to about 10% salt content. The elastic modulus of electrospun gelatin meshes prepared as described in the present work increased from 20 MPa to 70 MPa and the elastic modulus of the fiber material increased from 150 MPa to 620 MPa as the salt content in the fibers increased from 0% to 9.6%. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 42431.

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### INTRODUCTION

Gelatin-based materials are promising bioplastics because of their biocompatibility, biodegradability, and cost-effectiveness. In its nanofibrous form, gelatin is suitable for various applications including bio- and regenerative medicine, filtration, and protective clothing. However, low mechanical strength of gelatin has been limiting the use of fibrous gelatin-based materials. It has been shown previously that electrospun gelatin fabrics can be cross-linked by glucose, resulting in an excellent material for tissue engineering and cell culture applications.<sup>1</sup> Nevertheless, even higher mechanical strength is required for several other applications.

Aluminum potassium sulfate has long been used in leather tanning to enhance its mechanical properties and make it more durable.<sup>2</sup> This suggests that the addition of  $\text{AlK}(\text{SO}_4)_2$  could also increase the strength of fibrous gelatin meshes, since gelatin is derived by hydrolysis from collagen, a naturally occurring protein acting as the main structural component of soft tissue such as skin.<sup>3,4</sup> However, in case of leather tanning the additive is used to treat existing bundled fibers, whereas in case of artificially prepared fibers the additive can be placed directly inside the fibers. Therefore, the effect of the additive can be expected to be stronger and last longer than in tanning applications.

Nanofibrous materials can be used in various applications in biomedical field (wound dressing, tissue engineering, drug

delivery, and release control), energy storage, filtration, protective clothing, and sensors.<sup>5–7</sup> The usability of a material in these applications is closely connected to its mechanical properties. Several methods like phase separation, self-assembly, surface patterning, wet spinning, biospinning, interfacial complexation, microfluidic spinning, melt spinning, and electrospinning can be used to prepare nanofibrous materials.<sup>8–11</sup> The advantage of the latter method is that it is straightforward and easy to upscale.

Untreated electrospun gelatin is water-soluble and mechanically weak. In order to make it stronger and insoluble, gelatin must be cross-linked. Cross-linking decreases solubility by creating chemical bonds between polymer chains. This has been shown to increase mechanical strength of fibrous gelatin-based materials.<sup>12,13</sup>

Gelatin can be cross-linked thermally, but it results in a very low degree of cross-linking.<sup>14</sup> Therefore, the improvement in strength of the material is also little and the use of chemical cross-linking agents is necessary to achieve considerable increase in strength. In the current work, glucose was used as a cross-linking agent. This enables achieving high extent of cross-linking and significant increase in mechanical strength of the fibers.<sup>15</sup> Reducing sugars like glucose have an effect on the cross-linking process by enabling the Maillard reaction.<sup>16,17</sup> The reaction involves mainly certain amino groups of gelatin ( $\epsilon$ -amino groups of lysine and  $\alpha$ -amino groups of terminal

amino acids).<sup>18</sup> In order to further increase mechanical strength of the material, other functional groups must be involved in the cross-linking process. It is known that aluminum forms cationic basic ions, capable of reacting with collagen and the reaction sites for Al(III) are the carboxyl groups of collagen.<sup>19</sup> This suggests that the addition of  $\text{AlK}(\text{SO}_4)_2$  could also lead to an increase in mechanical strength of gelatin-based materials by initiating additional cross-linking mechanism involving carboxyl groups of gelatin.

In this work, the effect of  $\text{AlK}(\text{SO}_4)_2$  on preparation and properties of nanofibrous electrospun gelatin meshes cross-linked by glucose was investigated. It was found that  $\text{AlK}(\text{SO}_4)_2$  increases mechanical strength of electrospun gelatin thermally cross-linked by glucose up to about 10% salt content.

## EXPERIMENTAL

### Preparation of Fibrous Gelatin Meshes

Gelatin meshes containing glucose as a cross-linking agent and aluminum potassium sulfate as an additive were prepared by electrospinning from aqueous acetic acid. Gelatin type A from porcine skin, glucose, and glacial acetic acid were obtained from Sigma-Aldrich. Aluminum potassium sulfate dodecahydrate was purchased from Lach-Ner. Gelatin and glucose were mixed so that the mixtures contained 10% w/w glucose. Aluminum potassium sulfate dodecahydrate was added so that the mixtures contained up to 17% w/w of  $\text{AlK}(\text{SO}_4)_2$ . The mixtures were dissolved in 10M aqueous acetic acid to obtain 20–25% w/w solutions, which were electrospun into fibrous meshes. A 5 mL syringe containing the solution was mounted on a New Era Pump Systems NE-511 pump operating at 10  $\mu\text{L}/\text{min}$ . High voltage (17.5 kV) was applied to a blunted metallic 22 gauge (0.7 mm outer diameter and 0.4 mm inner diameter) syringe needle using Heinzinger LNC 30,000 high voltage power supply. A grounded target (plate collector) was placed at 14–15 cm distance from the needle tip. Fibrous meshes were collected from the target after electrospinning for 25–30 min and cross-linked by placing them in a preheated oven for 3 h at 170°C. The meshes were analyzed by energy-dispersive x-ray analysis (EDX) using Helios NanoLab 600 to confirm the presence of Al, K, and S in the fibers.

### Scanning Electron Microscopy (SEM)

Samples were dried using Leica EM CPD300 critical point drier, covered with 7 nm layer of gold using Polaron SC7640 sputter coater and analyzed by SEM (Tescan Vega) to confirm the fibrous structure and analyze the morphology of the meshes. Fiber diameters were measured from 21,000 times magnified images. In order to obtain smooth and representative histograms, fiber diameter distribution graphs were constructed by measuring the number of fibers with diameters  $\pm 30$  nm from a certain point on the graph.

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra between 400 and 4000  $\text{cm}^{-1}$  were recorded using Bruker Vertex 70 spectrometer equipped with an attenuated total reflection (ATR) accessory. The ATR spectra were converted to absorbance spectra after baseline correction and normalized to a constant penetration depth using OPUS software.

### Tensile Test and Evaluation of Mechanical Properties

Rectangular 3 cm  $\times$  1.5 cm pieces were cut out from electrospun meshes and their thickness was measured using a calliper. The samples were fixed between clamps and tested using a self-built tensile testing station (10  $\mu\text{m}$  distance measurement accuracy) equipped with Sauter FH100 force sensor. The samples were pulled at the rate of 10 mm/h, elongation and force were recorded. Tensile test results were used to determine elastic moduli of the meshes.

Tensile test results and numerical simulations were used to study mechanical properties of the fiber material as described in a previously published model,<sup>15</sup> which allows calculating the elastic modulus of cross-linked polymeric materials using tensile test results (sample dimensions, force, and elongation) and fiber diameters measured from SEM images. It was assumed that the addition of  $\text{AlK}(\text{SO}_4)_2$  does not change porosity of the meshes. The model simulates tensile test of fibrous materials, varying the hypothetical elastic and shear moduli of the fiber material. Elastic modulus of the fiber material is determined by comparing the elastic regions of the simulated and experimental stress–strain curves – when they overlap, then the hypothetical elastic modulus is equal to the elastic modulus of the fiber material. Thus, the measured data were used in the calculations to determine the elastic moduli of fibers containing various amounts of  $\text{AlK}(\text{SO}_4)_2$ .

## RESULTS AND DISCUSSION

### Preparation of Fibrous Meshes

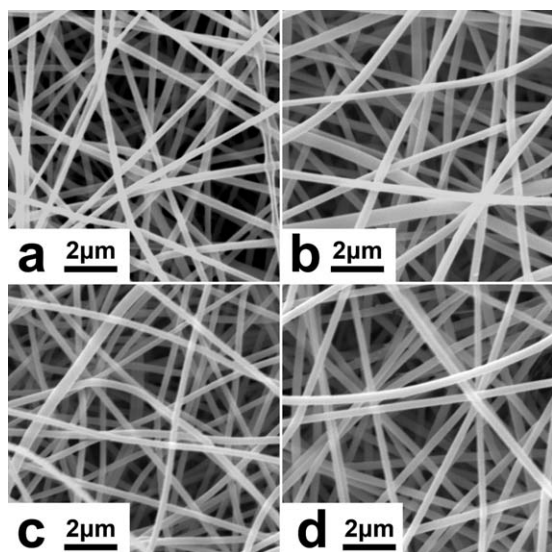
Fibrous gelatin meshes containing glucose and  $\text{AlK}(\text{SO}_4)_2$  were prepared by electrospinning. As a result, 40–110  $\mu\text{m}$  thick, insoluble, yellowish-white meshes were prepared.  $\text{AlK}(\text{SO}_4)_2$  did not have a significant effect on the fiber preparation process even at higher concentrations.

EDX results confirmed the presence of Al, K, and S in the fibers and that the additive was quite evenly distributed in the fibers. Samples without additives also contained small amounts of sulfur, originating from gelatin powder (gelatin type A from porcine skin was used).

### SEM

Morphology of the meshes was examined by SEM. Fibers were smooth and without beads (Figure 1). Average fiber diameters varied between 170–300 nm.  $\text{AlK}(\text{SO}_4)_2$  did not have a significant effect on the morphology of the meshes, except for on fiber diameters. The addition of  $\text{AlK}(\text{SO}_4)_2$  brought about two changes. Firstly, average fiber diameters increased with increasing salt content. This was caused mainly by the fact that the same amount of solvent was used to prepare all the electrospinning solutions, but the concentration of the solution increased as the salt was added. The effect of  $\text{AlK}(\text{SO}_4)_2$  on average fiber diameters was not detected, probably because it is small compared to the effect of the concentration of the electrospinning solution.

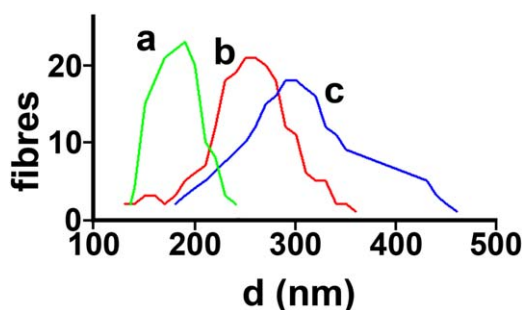
Secondly, fiber diameter distribution graphs (Figure 2) revealed that the measured fiber diameters were more dispersed in case of samples containing more salt. This is probably caused by the



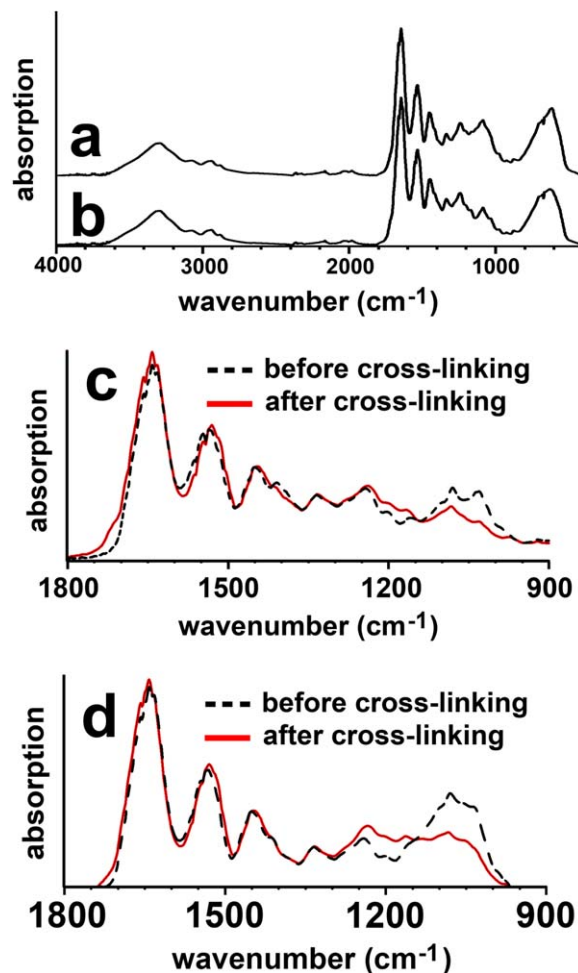
**Figure 1.** SEM images of gelatin meshes cross-linked by glucose (21,000 times magnification); (a) 0%, (b) 4.8%, (c) 11.3%, (d) 17.1% AlK(SO<sub>4</sub>)<sub>2</sub>.

higher electrical conductivity of the polymer solution, which in return makes the charged polymer solution less stable.

Fiber diameters are an interesting factor when considering mechanical properties of biopolymer nanofibers, and rarely considered. In order to be able to compare mechanical properties of fibers with different composition, it is vital to keep the average fiber diameters of the samples as close as possible due to various surface effects, which cause fibers with larger diameters to be mechanically stronger. Firstly, gelatin molecule only has limited number of possible cross-linking sites mentioned in the introduction (certain amino groups of gelatin in case of glucose-initiated cross-linking and possibly carboxyl groups of gelatin in case of AlK(SO<sub>4</sub>)<sub>2</sub>-initiated cross-linking). As the fiber diameter decreases, surface effects become more dominant and the properties of the bulk material become more distorted. The percentage of possible cross-linking sites located on the surface of the fiber increases with decreasing fiber diameter. On the surface of the fiber cross-links can form only between individual fibers. However, the probability for this is low due to the high porosity of electrospun materials. Therefore, fibers with smaller diameters have fewer cross-links inside the fiber and are mechanically weaker than fibers with larger diameters. Hydro-



**Figure 2.** Fiber diameter distributions of samples containing 0% (a), 4.8% (b), 13.4% (c) AlK(SO<sub>4</sub>)<sub>2</sub>. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



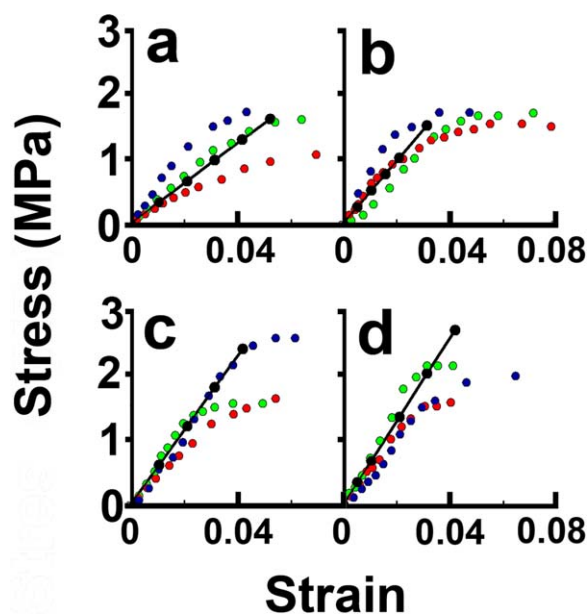
**Figure 3.** FTIR spectra of the meshes: (a) 8.8% AlK(SO<sub>4</sub>)<sub>2</sub>; (b,c) without additives; (d) 8.2% AlK(SO<sub>4</sub>)<sub>2</sub>. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

gen bonding and other non-covalent forces present in protein fibers also have a similar effect on the mechanical strength of the fibers.

#### FTIR

FTIR was used to study the changes brought about by thermal treatment of the meshes and to confirm cross-linking. In case of all analyzed spectra, peaks were detected at 3300 and 3073 cm<sup>-1</sup> (mainly OH and NH vibrations), 2938 and 2878 cm<sup>-1</sup> (CH<sub>2</sub> asymmetric and symmetric vibrations, respectively), 1657, 1641, and 1632 cm<sup>-1</sup> (amide I band, mainly C=O vibrations), 1546 and 1530 cm<sup>-1</sup> (amide II band, mainly NH bending), 1444 and 1333 cm<sup>-1</sup> (different in plane vibrations), 1239 cm<sup>-1</sup> (amide III band, NH bending, and CN stretching), and 1082 and 1031 cm<sup>-1</sup> (mainly CO vibrations overlapping with over vibrations in glucose) [Figure 3(a,b)].<sup>20–25</sup>

Notable differences in FTIR spectra between cross-linked and non-cross-linked meshes were observed between 1300 cm<sup>-1</sup> and 900 cm<sup>-1</sup> [Figure 3(c,d)]. The comparison between spectra is made harder by the fact that perfect contact between a sample and ATR crystal is hard to achieve due to the nanofibrous structure of the meshes. The intensity of peaks at 1082 and



**Figure 4.** Tensile test results: samples without additives (a) and samples containing 3.0% (b), 5.6% (c), 8.8% (d)  $\text{AIK}(\text{SO}_4)_2$  and the simulated elastic region of the stress–strain curve (lines on a–d). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

$1031\text{ cm}^{-1}$  decreased after thermal treatment of the meshes in case of all samples. This was accompanied by a slight increase in the intensity of the amide I band, confirming the assumption that one of the main reactions occurring during thermal treatment of the meshes is the Maillard reaction, the reaction between amino group of gelatin and carbonyl group of glucose. The increase in the intensity of the amide I band is small compared to the decrease in the intensity of peaks at  $1082$  and  $1031\text{ cm}^{-1}$ , because mainly the  $\epsilon$ -amino groups of lysine are involved in the Maillard reaction,<sup>18</sup> but lysine content in gelatin is low<sup>26</sup> (4–5% of dry ash-free protein<sup>27</sup>). Notably,  $\text{AIK}(\text{SO}_4)_2$  brought about significant changes in spectra between  $950$ – $1300\text{ cm}^{-1}$ , which is probably connected to the mechanism of  $\text{AIK}(\text{SO}_4)_2$ -initiated cross-linking, although the cross-linking chemistry is beyond the scope of the current work and needs to be studied further.

### Mechanical Properties

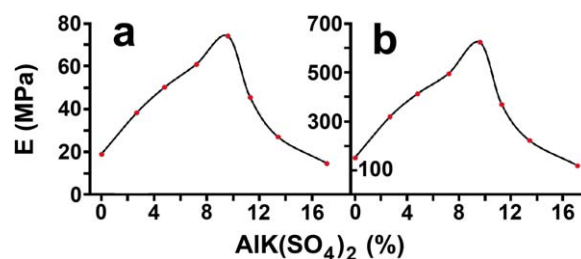
Mechanical behavior of the meshes was studied by tensile test. Tensile test results and stress–strain curves are demonstrated in Figure 4. The simulation results shown in the figure correspond to the best fit of the elastic moduli of the meshes. The main problem causing uncertainties and slightly different results between samples with the same composition is the fact that the thickness of the samples is not uniform. Due to the nature of electrospinning process, the thickness of the samples varied up to 20%. This is somewhat compensated in the model used to evaluate the elastic modulus of the fibers by taking into account the total length of the fiber per volume, but is nevertheless a possible cause of uncertainty.

The most important part of the stress–strain curve for practical applications is the linear region. Therefore, plastic region and failure of the meshes were not studied. The elastic moduli of

the meshes with different composition were calculated using the linear region of the stress–strain curve. Next, tensile test was simulated by varying the hypothetical elastic modulus of the fiber material, until the elastic region of experimental stress–strain curves and simulated stress–strain curves overlapped. In this case the elastic modulus of a fibrous material calculated from the simulation is equal to the experimentally determined elastic modulus of the mesh. The elastic modulus of the fiber material was found in this way for all samples.

$\text{AIK}(\text{SO}_4)_2$  increased the elastic modulus of glucose-cross-linked gelatin fibers up to about 10% (Figure 5) salt content. However, it should be kept in mind that mechanical anisotropy resulting from lengthwise orientation of polymer chains in the fibers, the orientation of macromolecules, fiber size, and humidity has an influence on the properties of electrospun fibers.<sup>28–30</sup> In the present case the elastic moduli are also greatly dependent on the molecular mass of gelatin, extent of cross-linking determined by the cross-linking method (the amount of cross-linking agent, period of thermal treatment, temperature), denaturation (10M aqueous acetic acid causes denaturation of gelatin, which in turn causes a decrease in the elastic modulus of the fiber material due to lowering of molecular mass of gelatin), surface effects (which are dependent on fiber diameters). In this work, these parameters were kept as constant as possible in order to distinguish the effect of the additive from other parameters. Thereby, the effect of  $\text{AIK}(\text{SO}_4)_2$  was easy to detect. However, varying the abovementioned parameters can cause fibrous gelatin-based materials to be either mechanically stronger or weaker than presented in this work.

The elastic modulus of the fiber material is roughly eight times higher than the elastic modulus of the mesh. This difference is caused firstly by the high porosity of electrospun materials and secondly by random fiber orientation.<sup>15</sup> The fact that the addition of  $\text{AIK}(\text{SO}_4)_2$  increased the elastic modulus of the material indicates that the presence of the salt also induces cross-linking, which in return determines mechanical strength of the material. At about 10%  $\text{AIK}(\text{SO}_4)_2$  content maximum extent of cross-linking that can be reached by the addition of  $\text{AIK}(\text{SO}_4)_2$  is achieved and the elastic modulus of the material drops at higher salt concentrations. It is not certain whether the addition of  $\text{AIK}(\text{SO}_4)_2$  brings about covalent or ionic cross-linking, but significant changes in FTIR spectra between  $950$ – $1300\text{ cm}^{-1}$  brought about by the presence of the salt, in addition to considerable increase in strength of the material indicate that thermal



**Figure 5.** Dependence of the elastic modulus of gelatin meshes (a) and the fiber material (b) on  $\text{AIK}(\text{SO}_4)_2$  concentration. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

treatment initiates covalent cross-linking (probably involving carboxyl groups of gelatin), although the exact chemistry of it requires further studies.

## CONCLUSIONS

Increasing mechanical strength of gelatin-based materials is required to expand the range of their applications beyond food and medical fields, which is desirable because of biocompatibility, biodegradability, and low cost of gelatin. The addition of  $\text{AlK}(\text{SO}_4)_2$  caused an increase in mechanical strength of gelatin fibers cross-linked by glucose up to a bit less than 10% salt content. The elastic modulus of electrospun gelatin meshes increased from 20 MPa to 70 MPa and the elastic modulus of the fiber material from 150 MPa to 620 MPa as the  $\text{AlK}(\text{SO}_4)_2$  content in the fibers increased from 0% to 9.6%. The addition of  $\text{AlK}(\text{SO}_4)_2$  most likely causes the involvement of certain functional groups (possibly carboxyl groups) of gelatin in the cross-linking process in addition to amino groups involved in glucose-mediated cross-linking, leading to a higher extent of cross-linking and increased mechanical strength of the material.

## ACKNOWLEDGMENTS

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