Electrospinning and stabilization of chitosan nanofiber mats

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Abstract. Chitosan is of special interest for biotechnological and medical applications due to its antibacterial, antifungal and other intrinsic physical and chemical properties. The biopolymer can, e.g., be used for biotechnological purposes, as a filter medium, in medical products, etc. In all these applications, the inner surface should be maximized to increase the contact area with the filtered medium etc. and thus the chitosan’s efficacy. Chitosan dissolves in acidic solutions, opposite to neutral water. Electrospinning is possible, e.g., by co-spinning with PEO (poly(ethylene oxide)). Tests with different chitosan:PEO ratios revealed that higher PEO fractions resulted in better spinnability and more regular fibre mats, but make stabilization of the fibre structure more challenging.

1. Introduction
Chitosan was investigated with respect to its electrospinning properties by several groups. Varying morphologies were found, depending on the chitosan:PEO ratio \cite{1,2}, including the existence of membranes, beads or pure nanofibers. In former studies, we could point out the possibility to tailor the electrospun nonwoven between nanofibers and a thin layer membrane by modifying the chitosan:PEO ratio \cite{3,4}. This raised the question how stabilizing processes, performed by different chemical post-treatments \cite{2}, would change the surface structure of the nonwovens and thus their mechanical, optical, and other physical properties – which are directly linked to possible applications.

Such applications can, e.g., aim at medical purposes due to the hemostatic properties of chitosan \cite{5}. Being bioactive, chitosan can be used as a filter material for organo-chemicals or heavy metals from aqueous solutions \cite{6}. Its fungicidal and bactericidal properties can be used in preserving agents \cite{7} or agriculture \cite{8}.

To increase the inner surface of the chitosan material and thus the interaction with its environment, chitosan is often spun to nanofibers. Here, the material properties can additionally be found in a quite pure form. Pure chitosan nanofibers with a high molecular weight could be synthesized, e.g., from \textit{Drosophila melanogaster} \cite{9}. However, chitosan is usually blended with diverse other polymers to increase spinnability. Chitosan/poly(lactic acid) nanofibers, e.g., were investigated with respect to their antibacterial properties against \textit{E. coli} \cite{10}. Antimicrobial activities against \textit{E. coli} and \textit{S. aureus} were tested using chitosan/poly(\epsilon-caprolactone) blends \cite{11} or chitosan/PEO blends \cite{12}.

On the other hand, chitosan blended fibers can be used in the area of tissue engineering. 3D scaffolds from polyamide-6,6/chitosan or from poly(epsilon-caprolactone)/chitosan nanofibers were produced for this application \cite{13,14}, while chitosan nanofibers were used for capturing tumor cells from blood \cite{15} or for transdermal drug delivery \cite{16}.

In spite of these broad variety of possible applications, investigations of needleless nanospinning of chitosan and chitosan blends are scarce \cite{3,4}. This article concentrates on different approaches of electrospinning and crosslinking chitosan/PEO blends.
2. Methods and Materials
Nanofiber mats were produced with the needleless electrospinning machine “Nanospider Lab” from Elmarco (Czech Republic). Spinning was performed at a voltage of 80 kV, carriage speed 250 mm/s, nozzle diameter 0.9 mm, electrode-substrate distance 160 mm, relative humidity in the chamber 38 %, and temperature 22.5 °C.

A chitosan solution was prepared from 2 wt% chitosan (190-310 kDa; 200-800 mPa·s; Sigma Aldrich) with 1 % acetic acid. The chitosan was solved in aqueous solution with sodium bicarbonate (NaHCO₃), using a concentration of 20.16 g/l. PEO with a molecular weight of 600,000 daltons (concentration 8 %, S3 Chemicals) was added as a spinning agent in different chitosan:PEO ratios. Water was added to modify the viscosity of the polymer solution.

For stabilization, different procedures were performed, based on Ref. 2. The dried nanofiber mats were subjected to neutral water, to H₂O with 1 mol/L K₂CO₃, ethanol/H₂O (70/30) with 1 mol/L K₂CO₃, 0.01 mol/L NaOH, etc.

3. Results and Discussion
Fig. 1 shows, as an example, a nanofiber mat with thin-film parts, electrospun from a chitosan:PEO 1:2 solution (left panel). After 30 s in H₂O (middle panel), the nanofiber mat is visibly thinner, and its structure has changed. Especially on top of the thicker fibres from the substrate, the fibres are apparently dissolved.

For stabilization of the nanofiber mats, it is necessary to wash out the PEO completely and before to neutralize the chitosan fibers [2]. It should be emphasized that the chitosan:PEO ratio of 1:2 is lower than the ratios tested in Ref. 2 due to the different electrospinning processes – here, we investigate needleless electrospinning, while in Ref. 2, a needle-based process is examined. This results in a higher ratio of PEO being necessary to enable fiber formation.

Fig. 2 depicts the morphology of the chitosan/PEO nanofiber mat after stabilization for 30 s in H₂O with 1 mol/L K₂CO₃ (left panel). Apparently, the original nanofiber structure is not retained. While in Ref. 2, the resulting morphology after stabilization exhibits relatively thick interconnected fibers, here a membrane-like structure with only some fibers being superimposed is visible. After washing the stabilized structure in water (Fig. 2, right panel), the original fiber structure is completely lost.
Next, stabilization was performed for 30 s in Ethanol/H₂O (70/30) with 1 mol/L K₂CO₃. The result is depicted in Fig. 3 (left panel). While in Ref. 2, stabilization with ethanol results in the finest and least connected fibers, here again the fiber structure is mostly lost after stabilization and completely vanished after washing in water (Fig. 3, right panel).

This is why in the next test, 0.01 mol/L NaOH was used for stabilization. The resulting membrane is very fine, as can be recognized from the typical interference colors (Fig. 4, left panel), and thus more promising than the thicker mats observed before. However, after washing with water, the structure is again completely vanished (Fig. 4, right panel).

Finally, to test whether the aforementioned methods stabilize the chitosan/PEO nanofiber mat at all, a contrasting test with immersing the nanofiber mat in acidic solution was performed. Fig. 5 (left panel) depicts the effect of immersing the mat for 30 s in 0.01 mol/L HCl, leading to large open areas where the mat is vanished. After washing, all remains of the nanofiber mat are disappeared, leaving back the thick fibers of the spinning substrate.

Apparently, the routes given in literature for stabilization of chitosan/PEO nanofiber mats do not provide satisfying results when transferred from needle-based to needleless electrospinning. This
problem can be attributed to the necessity to increase the ratio of PEO:chitosan to enable fiber formation, resulting in lower amounts of chitosan which can be stabilized with these approaches. While the changes in fiber mat morphology due to the stabilization process are known from the literature, this effect is strongly increased for needleless spun fiber mats with necessarily higher PEO concentration, resulting in a complete loss of the fiber structure. Interestingly, immersing chitosan/PEO nanofiber mats without stabilization in pure water has the smallest effect on the fiber morphology. This finding may be used for developing a new approach, using low-concentrated basic solutions in a first stabilization step and increasing the concentration in further steps.

**Figure 4.** Chitosan:PEO nanofiber mat after stabilization for 30 s in 0.01 mol/L NaOH and after washing in water for 30 s.

**Figure 5.** Chitosan:PEO nanofiber mat after immersing for 30 s in 0.01 mol/L HCl and after washing in water for 30 s.

### 4. Conclusion

Chitosan belongs to the “active” biopolymers with intrinsic antibacterial, antifungal and other properties. It can be co-spun with PEO from an acidic solution, resulting in different morphologies of the respective nonwoven, depending on the chitosan:PEO ratio and other spinning and solution parameters. Stabilizing such electrospun chitosan/PEO mats by different chemical routes changes the surface structure again. Depending on the stabilizing agent, membranes of different thickness and morphology
are formed with increased water resistance, but without the original nanofiber surface structure. Apparently, needleless electrospinning of chitosan/PEO blends necessitates new or modified stabilization strategies and/or an optimized spinning process to decrease the necessary amount of PEO.

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