Electrospun Chitosan Based Nanofibers

Lenka Martinová and Daniela Lubasová
Technical University of Liberec, Department of Nonwovens, Liberec, Czech Republic.

ABSTRACT

The new electrospinning technology Nanospider™, offering a real potential for industrial production of nanofibers, is used for the preparation of nanofiber sheets from aqueous solutions of polymer blends. The nanofiber sheets are prepared from a mixture of chitosan and polyethylenoxide (PEO) and using the novel continual electrospinning process (Jirsak et al., 2005, www.nanospider.cz) affords a network with typical fiber diameters that are less than 500 nm. Effects of solvents, molecular weight of both polymers, monovalent salt, surfactant and composition of chitosan blend on electrospinning are also studied.

The optimal conditions for electrospinning by the Nanospider™ technology, including applied high voltage, distance between both electrodes, air temperature and air humidity, are also found. The crosslinking of the nanofiber sheet is achieved by heat treatment. The morphology of electrospun fibers is observed by using a scanning electron microscope (SEM). Chitosan in the nanofiber sheets format has a great potential to be widely used in various applications derived from its biocompatibility and biodegradability.

Keywords: chitosan, polyethylenoxide, polyelectrolyte, electrospinning, nanofibers, solvents, crosslinking

1. Introduction

Chitosan is a biosynthetic polysaccharide comprising of deacetylated chitin. Chitin is a naturally occurring β-1,4 linked polymer of 2-acetamido-2-deoxy-β-D-glucopyranose polysaccharide that can be extracted from crustacean exoskeletons or generated via a fungal fermentation process.

Chitosan is a β-1,4 linked polymer of 2-amino-2-deoxy-β-D-glucopyranose that is soluble in an aqueous solution of acids. The main parameters affecting the characteristic of chitosan are its molecular weight (Mₖ) and degree of deacetylation (DD) representing the proportion of deacetylated units.

Chitosan, known as biopolymer, is of interest to cartilage research. Past research has shown that chitosan is biocompatible and biodegradable and does not induce any strong immune response. Additional positive features are its low cost due to abundance and diverse methods that enable the chemical processing of this polymer (Chandy & Sharma, 1990).

Polyelectrolyte complexes are substances which are ionically crosslinked hydrogels formed by the co-reaction of highly and oppositely charged electrolytes. Our interest has been focused on polyelectrolyte complexes of biosynthetic and synthetic polymers; namely, chitosan and polyethylenoxide (PEO).

Chitosan molecules are neutral and form inter-molecular hydrogen bonds that make the dissolution of the polymer in water difficult. However, due to presence of amino groups in its chain, chitosan can be dissolved in an acidic aqueous solution and possesses properties of a cationic polyelectrolyte.

For example, chitosan forms strong intermolecular hydrogen bonds in hybrid polymer networks (HPN) with gelatin (Yin et al., 2000) or PEO (Khalid et al., 1999).
The conformation of any polymer is affected by a number of factors, such as polymer architecture and solvent affinity. Charge has also been accounted for in poly-electrolytes. Whereas an uncharged linear polymer chain usually remains in solution in a random conformation closely approximating a self-avoiding three-dimensional random walk, the charges on a linear polyelectrolyte chain repeal each other via Coulombic repulsion, which causes the chain to adopt a more expanded, rigid-rod-like conformation.

If the solution contains added salt, the charges are cancelled and consequently, the polyelectrolyte chain collapses to a more conventional conformation. The ability of a chitosan and poly-(vinyl alcohol) blend for continual electrospinning was studied previously (Růžičková et al., 2005), but the maximal contents of chitosan in the nanofiber sheets did not exceed 30wt%.

The mixing ratio of the two polymers and the presence of small molecules of an electrolyte as sodium chloride from the separation environment significantly affect the formation of nanofibers. These effects are due to the intermolecular interactions. The molecular weight of chitosan used in the electrospinning process needs to be very low since this polymer forms strong networks through the action of intermolecular and/or intramolecular hydrogen bonds.

Chitosan currently receives a great deal of interest in medical and pharmaceutical applications. The objective of this work is to prepare nanofiber sheets with a maximum content of chitosan that are applicable for cell proliferations, wood coverings or in dental practices for covering periodontal defects.

2. Experimental

2.1 Materials

Three grades of commercial chitosan were used in the electrospinning experiments. The first, designated as Chitosan A, was chitosan-10 from Wako Pure Chemical Industries Ltd. in Japan, with viscosity of a 0.5% solution in 1% acetic acid being 5-20 cP and DD 0.78. The second polymer, designated as Chitosan B, was chitosan with midsize molecular weight from Aldrich characterized by viscosity of 1% solution in 1% acetic acid in the range 200-800cP and DD 75-85%. The third Chitosan C, was a high molecular weight product from Aldrich, featuring viscosity of a 1% solution in 1% acetic acid in the range 800-2000cP and DD over 75%.

Chitosan was dissolved in 10, 30 or 60wt% acetic acid and 30wt% lactic acid to achieve a polymer concentration of 10wt% (Chitosan A), 2wt% (Chitosan B), and 1wt% (Chitosan C).

Alternatively, 10wt% citric acid was used to obtain a Chitosan A concentration of 8wt%. The citric acid solution was used not only as a solvent for chitosan, but also enabled crosslinking through esterification reaction between hydroxyl groups of chitosan and carboxylic groups of the acid.

PEO with a molecular weight of 400kD and 900kD, and surfactant Triton® X-100 were obtained from Aldrich.

2.2 Preparation of Solution for Electrospinning

Deionized water was used to prepare all solutions. Sodium chloride was used by adding salt together with a water solution of 5wt% PEO in concentrations ranging from 0.24 to 0.85mol/L.

Chitosan solutions and a 5wt% PEO solution were prepared separately by dissolving chitosan in different solutions of acids and PEO in water with sodium chloride under magnetic stirring at room temperature overnight. Both solutions were then mixed and stirred again at room temperature overnight. The chitosan and PEO solution were combined to obtain mixtures with a weight ratio of chitosan to PEO ranging from 70/30 to 95/5.
Surface tension of chitosan/PEO solutions measured using tensomether Krüss K-12 was adjusted to 51.0–32.0 mN/m using non-ionogenic surfactant Triton X-100. The surface tension is strongly influenced by the concentration of acetic acid used for dissolution of chitosan.

2.3 Electrospinning of Nanofibers

The modified electrospinning setup was used in all experiments (Jirsák et al., 2003).

The electrospinning originates from the surface of the polymer solution and the process does not need any nozzles. High-voltage source generates voltages of up to 55 kV and the polymer solution was connected with the high-voltage source. Electrospun nanofibers were deposited on the grounded collector electrode.

The scheme of this system is shown in Figure 2 and was described in detail elsewhere (Jirsák et al., 2003).

This original method affords fibers with a diameter ranging from tens of nanometers to tens of micrometers. The metal roller (1), which serves as the positive electrode, raises a thin film of polymer solution. The so-called Taylor cones (4) are then formed due to the effect of applied high voltage (20–70 kV). The method is based on the possibility of creating Taylor cones from a thin layer of polymer solution. The cones cleave into fibers, which are carried onto the negative electrode (8) and retained at a polypropylene non-woven fabric (6). During this process, the solvent evaporates and the fibers become stretched at either ambient or elevated temperatures.

The reservoir (3) is filled with 20 ml of polymer solution and rotation of the metal roller (1) ensures its perfect coating with the solution. The high-voltage power supply (Glassman High Voltage, Inc., USA) allows control of voltage in the range of 0–55 kV. The electric field is switched on and slowly increased until the desired electrospinning is obtained. The optimum value of the electrostatic field differs for each polymer solution. Once the optimum value of voltage is known, it can be already set next time at the beginning of the process. In the process that is described further, formation of optimum Taylor cones and hence, achieving optimal electrospinning process is tuned by adjusting the rotational speed of the metal roller. The Taylor streams are formed next to each other, throughout the entire length of the metal roller, resulting in the high production capacity of Nanospider’s™ spinning head.

Electrospinning of mixture of chitosan/PEO solutions in this study is carried out at a voltage of 50–55 kV, distance of electrodes 10 cm, air temperature 22°C and air humidity 38%. Nanofiber sheets were subsequently crosslinked by treatment at 135-140°C for 30 min.

3. Results and Discussion

3.1 Interaction between PEO and Chitosan Chains

It is well known that polyelectrolyte behavior changes after adding salt. This effect is confirmed since sodium chloride added at low concentration affects the interaction of chitosan and PEO macromolecules. This effect is attenuated at increased salt concentration. This rule is valid for
all chitosan/PEO blends containing chitosan or PEO with different molecule weights. This finding can be explained by the following mechanism. At low salt concentration, the chitosan chains are much more stretched and dispersed by the electro-static repulsion, thus enabling flexible PEO chains to easily form molecular complex through hydrogen bonding between the chitosan and PEO.

Possibly, two kinds of hydrogen bonds can be formed; the first between the hydrogen atoms of hydroxyl groups of chitosan and ether oxygen of PEO, while the second type occurs between hydrogen atom of the quarternary ammonium group and the ether oxygen of PEO (Figure 3) (Khalid, 1999).

![Fig. 3. Chitosan/PEO interactions](image)

With an increase in the concentration of the added salt, the effect of charges residing on chains diminishes and the chitosan chains become coiled, resulting in a decrease in contribution of the second type of hydrogen bonds mentioned above. This leads to the formation of intramolecular hydrogen bonds. Hence, the strength of interaction between PEO and chitosan chains decreases.

### 3.2 FTIR Spectroscopy

FTIR spectroscopy is used to monitor changes in the chemical structure of nanofiber sheets from blending chitosan/PEO in 10wt% aqueous solution of acetic acid and 10wt% aqueous solution of citric acid after crosslinking upon heat treatment at 145°C for 15 min. This is followed by water treatment at 25°C for 24h and subsequent drying (Figures 4, 5 and 6).

The FTIR spectra of sheets prepared from a mixture of chitosan/PEO dissolved in acetic acid are shown in Figure 4.

They demonstrate that acetic acid is completely extracted from sheets after heat and water treatment. The FTIR spectra confirm formation of chemical bonds between acetic acid.

![Fig. 4. FTIR spectrum of nanofiber sheets prepared from a mixture of chitosan A/PEO dissolved in acetic acid](image)

The FTIR spectra of sheets prepared from chitosan that are dissolved in citric acid are shown in Figure 5. The band at 1716cm⁻¹ representing valency vibrations of ester C=O groups of citric acid confirms the crosslinking reaction between citric acid and chitosan.

The FTIR spectra show changes of nanofiber sheets processed from a blend of chitosan/PEO (Mₙ 400 and 900kD) as a function of molecular weight of PEO (Figure 6). First, PEO with Mₙ 900kD is not extracted even after 24 h from nanofiber sheets as demonstrated by a peak at 1260cm⁻¹, while PEO with Mₙ 400kD is completely extracted under the same condition.
3.3 Effect of Molecular Weight of PEO

The possibility of formation of covalent bonds between chitosan and PEO chains were tested using FTIR spectroscopy before and after heat treatments. We found that no chemical bonds appeared between hydroxyl groups of chitosan A that was dissolved in citric acid and hydroxyl groups of PEO with molecular weight 400kD. The water soluble PEO could then be extracted with water for 24h from nanofiber sheets formed from a chitosan/PEO blend, while the chitosan was not soluble in water.

SEM micrographs of the nanofibers prepared from chitosan A/PEO blends shown in Figures 7 and 8, demonstrate better stability of morphology after 15min water treatment for fibers prepared from PEO with molecular weight 900kD.

(a) Chitosan A in citric acid with PEO 400kD (ratio 8/2),
(b) Chitosan A in citric acid with PEO 900kD (ratio 8/2)
3.4 Effect of Sodium Chloride on Electro-Spinning of Polymer Blend Chitosan/PEO

The concentration of NaCl in PEO solution was varied from 0 to 2.4 mol/L. Optimum for the desired process is found in the range 0.24-0.66 mol/L (Figure 9). Effect of electric conductivity of the polymer blends on electrospinning was determined for a range of 6.2 - 32.3 mS/cm.

Conductivity controlled by the added salt is almost always an important feature that affects the start of the electrospinning. However, in the case of polyelectrolytes, the salt also affects conformation of the makromolecules and therefore, their interactions with the environment. Chitosan is dissolved in aqueous acid and behaves as a cationic polyelectrolyte.

Chitosan is also rigid as a result of its intramolecular hydrogen bonds. Solution properties and in particular, its viscosity, change during the shrinkage of the macromolecule as a consequence of interaction of dissociated NaCl and chitosan polycation (Jing & Han, 1999).

3.5 Effect of Heat Treatment for Stabilization of Chitosan/PEO Nanofibers Sheets

No crosslinking agents, except citric acid, were used for the preparation of chitosan/PEO nano fiber sheets. Water-resistant nano fiber sheets are obtained by thermal crosslinking at 145°C for 15 min (Figure 10).

Although chitosan powder does not dissolve in water at near to neutral pH, nano fiber sheets from chitosan/PEO without heat treatment dissolve when in contact with water. This is due to their high specific surface area and local residual acids molecules originally used as a solvent. Water resistance of the nanofibers depends on the composition of the spinning solution and increases with the increased PEO contents and concentration of acid used as a solvent. Negative effects of solvents can be reduced by an initial dipping of the sheets into a solution of 0.01 mol/L NaOH.
Monofunctional PEO moieties, such as alkoxyderivates, can be attached to chitosan using a variety of methods (WO01/00246, PCT/US00/40163). We assume that terminal hydroxyl groups of PEO react with amino groups of chitosan provided the reaction conditions are suitable. Moreover, we cannot exclude the intermolecular reaction of chitosan after extraction of PEO that was confirmed by FTIR. The nanofiber sheets were swollen, but stable in water.

3.6 Effect of Citric Acid on Electrospinning Process

A variety of solvents were tested in the electrospinning of chitosan blends using the Nanospider technology. Some of them were not able to make this process possible. Higher concentration of aqueous acetic acid affects the surface tension of chitosan solutions (Geng, et al., 2005) and allows for a complete elimination of the non-ionogenic surfactant Triton X-100.

Aqueous solution of citric acid improves the recovery factor of electrospinning the Nanospider process compared to chitosan solutions in acetic acid. Nanofiber sheets that were prepared from a chitosan/PEO solution in acetic acid and subsequently subjected to heat treatment, dissolved completely in water in 10 days. In contrast, sheets prepared from chitosan/PEO solution in citric acid after an identical heat treatment, were stable in water for a period of 2 months. Stability of these nanofiber sheets after heat treatment (145°C/15min) and water treatment (15min) is demonstrated in Figures 10C and D.

3.7 Optimal Condition Heat Treatment

Optimal conditions for heat treatment of nanofiber sheets prepared from chitosan dissolved in citric acid after electrospinning were also investigated. Temperatures of 120°C, 130°C, 140°C and 145°C were used for 30 and 60min followed by water treatment for 24h. We found that a temperature of 120°C was not adequate because the sheets dissolved in water. In contrast, a temperature of 145°C was too high and the sheets turned yellow after heat treatment due to the onset of degradation of the polymer. A temperature of 135-140°C applied for 30min is found to be optimal (see Figure 11).

4. Conclusions

The nanofiber sheets from chitosan/PEO blends containing 90-95% chitosan are successfully electrospun by using the Nanospider continual electrospinning process. Effect of surface tension defined by concentration of acetic acid as a solvent of chitosan and addition of non-ionogenic surfactant Triton X-100 is found to be important. Mono-valent salt ion strongly affects the electrospinning process throughout the formation of molecular complexes.

Heat treatment of nanofiber sheets prepared from a chitosan/PEO solution in acetic acid at different temperatures does not lead to chemical crosslinking as confirmed by FTIR. However, the stability of nanofiber sheets in water strongly increases.

Water-resistant nanofiber sheets are obtained by thermal crosslinking when aqueous citric acid is used as a solvent for chitosan. The FTIR spectra confirm the crosslinking reaction between citric acid and chitosan.

Optimal compositions of a chitosan/PEO polymer blend with or without the non-ionogenic surfactant Triton X-100 and sodium chloride are found for the Nanospider technology.

An improvement in nanofiber stability in deionized water related to small changes in their morphology is achieved by using PEO with molecular weight 900kD instead of 400kD. PEO with M_w 900kD is not extracted even after 24h.

Fig. 11. SEM micrographs of nanofiber sheets; chitosan in citric acid with PEO 900kD: (A) After heat treatment 140°C for 30min and water treatment for 24h, (B) After heat treatment 130°C for 30min and water treatment for 24h.
from the nanofiber sheets, while PEO with $M_w$ 400kD is completely extracted under the same condition. We assume that this effect results from formation of semi-interpenetrating polymer network (IPN) enabled by attractive forces between chitosan hydroxyl groups and electron pairs on oxygen in PEO chains.

We conclude that the optimal condition for heat treatment of chitosan/PEO sheets comprise of heating to a temperature of 135-140°C for 30min.

FTIR experiments show that the crosslinking of nanofibers sheets from chitosan/PEO is not based only on Coulomb interactions, but also on chemical interactions between functional groups of chitosan and citric acid molecules that is primarily used as a solvent. The stability of sheet in water is excellent in a period of two months. Crosslinked chitosan/PEO sheets are generally well tolerated, biocompatible and promising for use in medical and pharmaceutical applications. Chitosan/PEO nanofiber sheets are successfully tested as supports for the proliferation of chondrocytes.

Acknowledgements

This work was supported by research Centrum, Project 1M 0554 of the Czech Republic.

REFERENCES


