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Electrospinning of chitosan nanofibers: Processing optimization

Homa Homayoni [a], Seyed Abdolkarim Hosseini Ravandi [a,*], Masoumeh Valizadeh [b]

[a] *Research Center of Fiber Science and Technology, Department of Textile Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran*
[b] *Faculty of Engineering, University of Guilan, Rasht 3756, Iran*

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ABSTRACT

In this study, the electrospinning of chitosan has been investigated. The problem of chitosan high viscosity, which limits its spinability, is resolved through the application of an alkali treatment which hydrolyzes chitosan chains and so decreases its their molecular weight. Solutions of the treated chitosan in aqueous 70–90% acetic acid produce nanofibers with appropriate quality and processing stability. Decreasing the acetic acid concentration in the solvent increases the mean diameter of the nanofibers. Optimum nanofibers are achieved with chitosan which is hydrolyzed for 48 h. Such nanofibers result in a moisture regain which is 74% greater than that of treated and untreated chitosan powder. The diameter of this nanofiber, 140 nm, is strongly affected by the electrospinning conditions as well as by the concentration of the solvent. FTIR investigations prove that neither the alkali treatment nor the electrospinning process change the chemical nature of the polymer.

1. Introduction

Electrospinning is a progressive method which produces fibers ranging from the submicron level to several nanometers in diameter in a high voltage electrostatic field. This is a relatively old approach to fiber spinning, but is currently one of the most advanced methods in manufacturing high performance nanofibers. These have been introduced into various technological fields because of their distinct specifications, such as high aspect ratio, porosity, and special chemical and physical properties which result from their unique structure (Frenot & Chronakis, 2003; Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005). Due to their characteristics, nanofibers have been studied in medical science; researchers have focused on the application of nanofibers to filtration, drug delivery, tissue engineering, and wound healing. However, in such studies, a significant problem arises in polymer selection (Ciechanshka, 2004; Prashanth & Tharanathan, 2007). Specifically, there is a wide range of spinnable polymers; however, only a few of them have the necessary parameters to make them suitable for use in such critical applications.

One polymer which does exhibit the necessary characteristics is chitin, along with its deacetylated derivative, chitosan. Chitin is the second most abundant polysaccharide after cellulose, and has a similar structure. Chitosan is insoluble in water, alkali, and most mineral acidic systems. However, though its solubility in inorganic acids is quite limited, chitosan is in fact soluble in organic acids, such as dilute aqueous acetic, formic, and lactic acids. In the presence of a limited amount of acid, chitosan is soluble in water–methanol, –ethanol, and –acetone mixtures. Chitosan also has free amino groups which make it a positively-charged polyelectrolyte in pH below 2–6 and which contribute to its higher solubility in comparison to chitin. However, this property makes chitosan solutions highly viscous and complicates its electrospinning (Muzzarelli, 1973, 1977). Furthermore, the formation of strong hydrogen bonds in a 3-D network prevents the movement of polymeric chains exposed to the electrical field (Geng, Kwon, & Jang, 2005; Neamnark, Rujiravaniti, & Supaphol, 2006; Whistler, 1993).

Although many researchers have made attempts to purify chitosan, few have succeeded. As one of the first to report on this process, Spasova, Manolova, Paneva, and Rashkov (2004) examined the electrospinning of chitosan/polyethylene oxide (PEO) blend. Their results showed that the electrospinning of this blend is possible with a chitosan/PEO mass ratio of <1. Similarly, Bhattaraia, Edmondson, Veiseh, Matsen, and Zhang (2005) reported the electrospinning of chitosan/PEO blend and stated that the formation of chitosan nanofibers strongly depends on the mass ratio of chitosan/PEO. Furthermore, Ignatova, Starbova, Markova, Manolova, and Rashkov (2006) claimed that preparing polyelectrolyte by electrospinning its aqueous solution is feasible only in the presence of a second polymer.

Additionally, Li and Hsieh (2006) used polyvinyl alcohol as a by-component and concluded that repulsive interactions between polycations prevent the degree of chain entanglement necessary for fiber formation. Moreover, Min et al. (2004) used the solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to produce pure chitosan nanofibers through deacetylation. In other attempts, Geng et al. (2005) electrospun chitosan with 106,000 g/mol molecular weight

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* Corresponding author. Tel.: +98 3113912425; fax: +98 3113912444.
E-mail address: hoseinir@cc.iut.ac.ir (S.A.H. Ravandi).

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using aqueous acetic acid 90% as a solvent, and Ohkawa, Cha, Kim, Nishida, and Yamamoto (2004) managed to produce pure electrospun chitosan nanofibers in tri-flouro acetic acid (TFA). Because TFA is a volatile, toxic, and expensive solvent, Ohkawa et al. (2004) used chitosan with 210,000 g/mol molecular weight, and in order to improve the quality of their electrospun chitosan nanofibers, they also added another toxic solvent, dichloromethane. However, Neamnark et al. (2006) cautioned that despite of this reported success of the preparation of electrospun chitosan nanofibers, the actual use of these fibers may be limited by the cost and toxicity of TFA and HFIP.

The main aim of this study is to electrospin pure chitosan nanofibers with a wide range of molecular weights from acetic acid 90% solution. Notably, we decrease the concentration of acetic acid in aqueous solvent and investigate the effect of this decrease on the structure of chitosan nanofibers.

2. Experimental

2.1. Materials and equipments

The materials used in this study include: (a) medium molecular weight chitosan powder with $\bar{M}_V = 1.095 \times 10^6$ g/mol and DD = 75–85% from ALDRICH (Goycoolea, Arguelles-Monal, Peniche, & Higuera-Ciapara, 2000; Maghami & Roberts, 1988; Tsaih & Chen, 1999), (b) glacial acetic acid (AcOH) from MERCK, and (c) deionized water. The equipment and their purposes include: (a) a 0.6-guage Ubbelohde viscometer for viscometry paired with the Mark–Howink equation for estimating the molecular weight of chitosan, (b) polymath bath (DataColor, UK) for keeping the temperature of the chitosan alkali treatment fixed, (c) an electrospinning unit for producing nanofibers from chitosan solution, (d) a Philips scanning electron microscope for observing the structure of chitosan nanofibers and calculating their mean diameters, and (e) an FTIR spectrophotometer for studying the functional molecular groups of untreated chitosan, hydrolyzed chitosan, and chitosan nanofibers.

2.2. Methods

2.2.1. Chitosan hydrolysis, decreasing molecular weight

To hydrolyze chitosan polymer chains, mixtures of 1/25 (w/v) chitosan/NaOH 50% were treated at 95 °C for 0.75, 2, 2.5, 3.5, 5, 8, 12, 16, 24, and 48 h. Each sample was strained and rinsed with deionized water, neutralized with acetic acid, rinsed, and dried at 60 °C for 16 h.

2.2.2. Measurement of viscosity

The molecular weight of the net chitosan and the hydrolyzed chitosan powders were measured using viscometry. Chitosan solutions in an NaCl 0.2 M + AcOH 0.1 M solvent system (Goycoolea et al., 2000; Maghami & Roberts, 1988) were prepared in four different concentrations: C = 0.01, 0.03, 0.07, and 0.1 g/l for each sample (Tsaih & Chen, 1999). The dropping times of solvent and chitosan solutions through an Ubbelohde capillary tube were considered to be t_0 and $t_{1,}$ respectively. The intrinsic viscosity was calculated using Eq. (1) extrapolated at C = 0. This equation gives the reduced viscosity. Plotting the measured reduced viscosity against concentration provides a linear graph, the intersection of which can be considered to be intrinsic viscosity: $([\eta] = \frac{1}{C}(\frac{t_1}{t_0} - 1)_{c=0})$.

$$\eta_{\mathrm{red}} = \frac{1}{C}\left(\frac{t_1}{t_0} - 1\right) \tag{1}$$

According to the Mark–Howink equation $[\eta] = k_m M_v^a$, where k_m and a are the constants for the selected solvent system and are equal to 1.8×10^{-3} cm^3/g and 0.93, respectively (Goycoolea et al., 2000; Maghami & Roberts, 1988).

2.2.3. Electrospinning

Chitosan solutions (untreated and hydrolyzed for different amounts of time) with different concentrations of polymer and solvent were prepared and inserted into syringes with 0.7 mm inner capillary diameter (gauge22). The filled syringes were then transferred to the electrospinning system. The syringe tips and collectors were connected to the electric generator's anode and cathode, respectively. It is important to note here that the preparation of chitosan nanofibers is strongly dependent upon the parameters of electrospinning, such as the fineness of a syringe tip, the gap between the tip and fiber collector, the feeding rate, and voltage. Fig. 1 shows a schematic of the electrospinning unit.

2.2.4. Measurement of nanofiber diameter

In order to measure fiber diameters for each sample, 100 fibers from each sample were randomly selected from SEM images and their diameters were measured using Motic software and microscopic observations of the SEM images.

2.2.5. FTIR spectroscopy

FTIR spectroscopy of untreated chitosan, hydrolyzed chitosan, and chitosan nanofibers was performed after finely grinding a portion of each sample with KBr, a specially purified salt used to remove the scattering effects from large crystals (the mass ratio of the sample/KBr was 1/100). The powder mixture was then crushed in a mechanical die press to form a translucent pellet through which the beam of the spectrometer could pass.

3. Results and discussion

3.1. Primary examination

Chitosan solution in AcOH 90% with a molecular weight of 1.095×10^6 g/mol results in a very high viscosity, which prevents the solution from elongating as a jet in the electrical field of the electrospinning unit. However, varying the concentration of chitosan in AcOH 90% (from as little as 1 wt%, up to as much as 2.75 wt%, which is the most accessible concentration in this situation) and changing the electrospinning parameters did not solve this problem, nor improve the quality of the products depositing on the collector as droplets. Furthermore, while decreasing the concentration of AcOH from 90% to 50% results in a lower viscosity, it also increases surface tension (Geng et al., 2005).

As such, because surface tension and viscosity are two of the most crucial parameters in the electrospinning of polymer solutions there is a need for an efficient method which either decreases solution viscosity, or optimizes both surface tension and viscosity. In this study, in order to achieve these improvements, decreasing the molecular weight of chitosan was investigated.

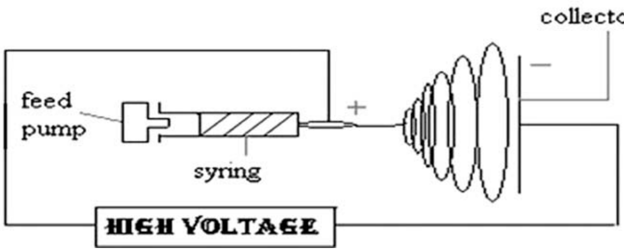

Fig. 1. Schematic of the electrospinning unit.

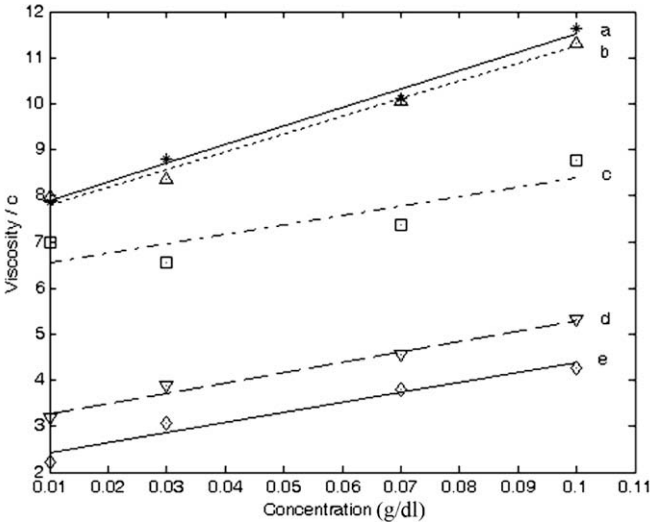

Fig. 2. Intrinsic viscosity versus the concentrations for different times of hydrolyzed chitosan: 0 h (a), 3.5 h (b), 8 h (c), 24 h (d), and 48 h (e).

3.2. Chitosan hydrolysis

Chitosan powder was hydrolyzed for 0.75, 1.5, 2, 3.5, 8, 12, 16, 24, and 48 h. Fig. 2 shows the regression lines of reduced viscosity against the concentrations. The intercepts present the mean value of intrinsic viscosity.

Table 1 reveals the relationship between the duration of alkali treatment, the molecular weight of the hydrolyzed chitosan, and the intrinsic viscosity of chitosan in the NaCl 0.2 M + AcOH 0.1 M solvent system.

As these data illustrate, the reduction of molecular weight and viscosity versus time is both nonlinear and less sensitive during the early stages. This tendency may be explained by the role NaOH plays in the further deacetylation of chitosan and its depolymerization, which probably leads to an additional increase in the positive charge of the polymer chains, thus compensating for the decrease in molecular weight.

Table 1
The relations of hydrolysis time, intrinsic viscosity, and molecular weight.

Hydrolyzation time (h)	Intrinsic viscosity (dl/g)	Molecular weight: $\bar{M}_V$ (g/mol $\times 10^6$)
0	7.49	1.095
3.5	7.429	1.084
8	6.329	0.914
24	3.043	0.416
48	2.202	0.294

3.3. Electrospinning of hydrolyzed chitosan

Hydrolyzing chitosan powder in alkali treatment for up to 5 h decreases the viscosity of its solution in AcOH 90%, but has no considerable effect on the formation of nanofibers. Furthermore, increasing treatment time to up to 8 h leads to uneven nanofiber formation. However, increasing the duration of alkali treatment to up to 24 h improves the continuity of the electrospinning process and the quality of electrospun fibers. Additionally, electrospinning chitosan which has been treated for 48 h eventually leads to a steady processing condition, as well as high-quality nanofibers, as shown in Fig. 3. Here, the proper concentration of treated chitosan in AcOH 90% is 5 wt%. The molecular weight of chitosan which was been hydrolyzed for 48 h is 2.94×10^5 g/mol, which represents an almost 73% decrease in comparison to the net sample (1.09×10^6 g/mol), and a 30% decrease in comparison to the chitosan which was hydrolyzed for 24 h (4.16×10^5 g/mol). This suggests that the polymeric chains of the chitosan hydrolyzed for 48 h to a low molecular weight align more effectively in the electromagnetic field of the electrospinning unit. One possible explanation of this is that the average length of polymer chains is below the threshold required for entanglement coupling formation. However, this hypothesis requires more investigation in respect to solution viscosity and the observation of the Newtonian or non-Newtonian behavior of the solution, since a Newtonian behavior would be expected for a solution which does not entangle.

The average diameter of this product is 140 nm and the standard deviation is 51. Fig. 4 shows a histogram of the measured diameters.

3.4. Effect of electrospinning parameters

The electrospinning of chitosan and other natural polymers is a complex process due to the special behavior of the solution of this polymer, its polycationic specification, its high molecular weight, and its wide molecular weight distribution. Therefore, electrospinning control parameters such as needle inner diameter, tip-collector gap, electric field voltage, and feedrate all significantly affect the spinability and the quality of the product.

The electrospinning process is initiated at the point at which the electrostatic force in a solution overcomes the surface tension of the solution. This requires high voltage. Specifically, a strong electrical charge accelerates jet extending and causes a greater volume of solution to be drawn from the needle. However, a higher voltage also leads to greater stretch of the solution. This directly affects the

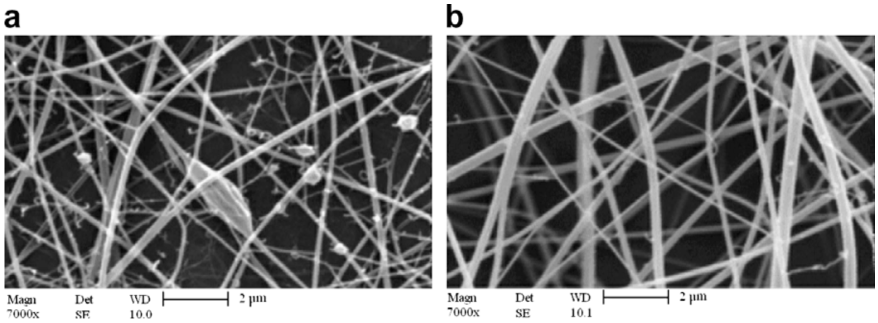

Fig. 3. SEM photographs (magnification: 7000) of nanofibers of chitosan hydrolyzed for 48 h 4 wt% (a), 5 wt% (b).

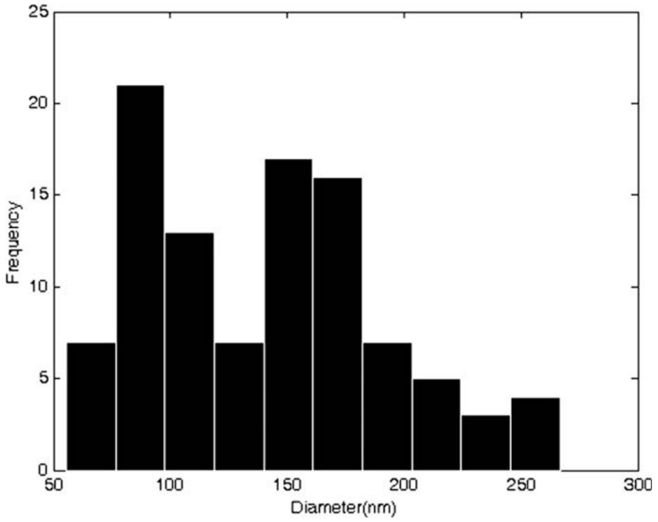

Fig. 4. Diameter histogram of the nanofibers, prepared from 48 h hydrolyzed chitosan.

Table 2
Electrospinning parameters of 48 h hydrolyzed chitosan.

Electrospinning parameters	Amount
Needle inner diameter	0.7 mm
Tip-collector gap	16 cm
Voltage	17 kv
Feedrate	8×10^{-2} mg/h (1.6 mm^3/h)

morphology of the electrospun fibers and usually decreases the diameter of the fibers and increases the possibility of bead formation.

Table 3
Regain moisture%[*].

Sample	Original powder	Chitosan hydrolyzed for 48 h	48 h hydrolyzed chitosan nanofibers
Regain moisture%	7.97	8.008	13.95

[*] Regain moisture% $= 100 \times \frac{\text{Sample weight}-\text{Dried sample weight}}{\text{Sample weight}}$.

Feedrate, another important parameter, determines the amount of solution available. An increase in feedrate in turn increases fiber diameter and bead size. Additionally, the internal diameter of the tip has a certain effect on electrospinning: a smaller internal diameter reduces clogging, as well as the number of beads, and leads to less exposure of the solution to the atmosphere during the process.

Tip-collector distance has a direct influence on jet flight time and electric field strength. A decrease in this distance shortens flight times and solvent evaporation time, and increases the electric field strength, which results in more bead formation. The effect of decreasing tip-collector distance is almost the same as for increasing voltage (Frenot & Chronakis, 2003; Ramakrishna et al., 2005). These parameters changed during the electrospinning process. The optimum conditions which resulted in these stable, high-quality chitosan nanofibers are featured in Table 2.

SEM images of Figs. 5 and 6 show the effect of tip-collector distance and electrical field voltage, respectively, on the structure of electrospun nanofibers. As shown in these figures, increasing of gap distance and voltage not only decreases and refines nanofibers diameters, but also improves the quality of electrospun nanofibers.

3.5. Properties

3.5.1. FTIR spectroscopy

The FTIR spectrum of untreated chitosan shows resonance bands at 1154 and 893 cm^{-1} identifying its saccharide groups.

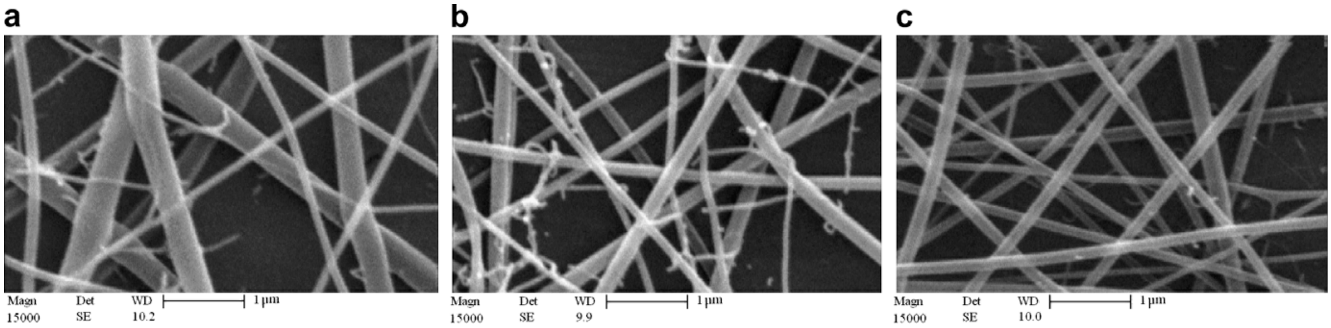

Fig. 5. SEM images of 5 wt% chitosan hydrolyzed 48 h nanofibers in aqueous acetic acid 90%, tip needle – collector distance: 14 cm (a), 15 cm (b), 16 cm (c).

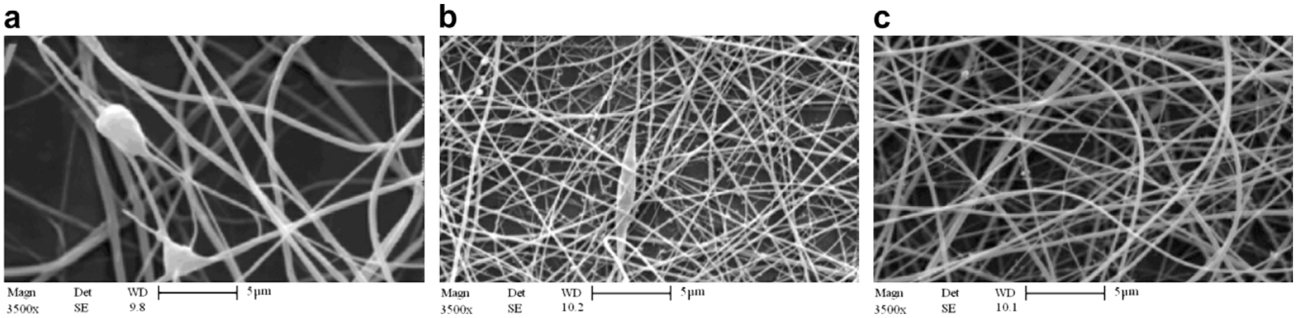

Fig. 6. SEM images of 5 wt% chitosan hydrolyzed 48 h nanofibers in aqueous acetic acid 90%, electric field voltage: 14 kv (a), 16 kv (b), 17 kv (c).

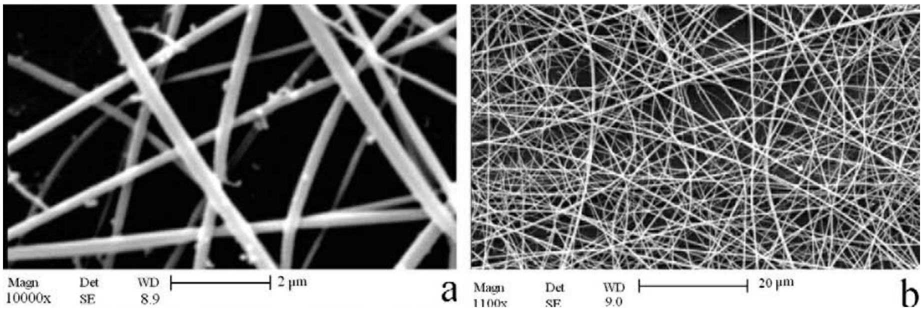

Fig. 7. SEM photographs of nanofibers of chitosan hydrolyzed for 48 h in aqueous 80% acetic acid 7 wt% (a, magnification: *10,000, b, *1100).

Other bands at 1600, 1651 and 3425 cm^{-1} depict N–H bending of the primary amino groups, the carbonyl stretching of the amide bands, and the N–H stretching of the primary amino groups, respectively.

FTIR spectroscopy of chitosan treated with alkali for 48 h and the nanofibers of this chitosan indicate that these processes do not affect the chemical nature of the polymer. However, slight shifts in the location of N–H stretching bands reveal the changes in secondary bonds, i.e., hydrogen bonds.

3.5.2. Regain moisture

Untreated chitosan powders, chitosan hydrolyzed for 48 h, and chitosan nanofibers were placed in standard conditions (relative humidity 65% and temperature 20 °C) for 48 h and were then weighed.

Next the samples were transferred to an oven at a temperature of 70 °C. The dried samples were removed from the oven and weighed every 15 min (using desiccators), until constant weight (I0.0001 g) was achieved. This process was carried out for electrospun nanofibers on a slide. Each test was repeated three times and the average regain moistures were calculated. Table 3 shows the regain moisture of both untreated chitosan and chitosan treated for 48 h to be approximately 8%. The most interesting result is in respect to the nanofibers electrospun from chitosan hydrolyzed for 48 h, which has a regain moisture of about 13.95% and presents a 74% increase in regain moisture relative to the treated and untreated chitosan.

The standard error of the nanofibers regain moisture is 0.87, and its CV is 6.24%. These characteristics seem to be the result of high porosity and high aspect ratio, which produce high specific surface, enhancing the wicking property, and increasing the number of suitable sites for hydrogen bond formation.

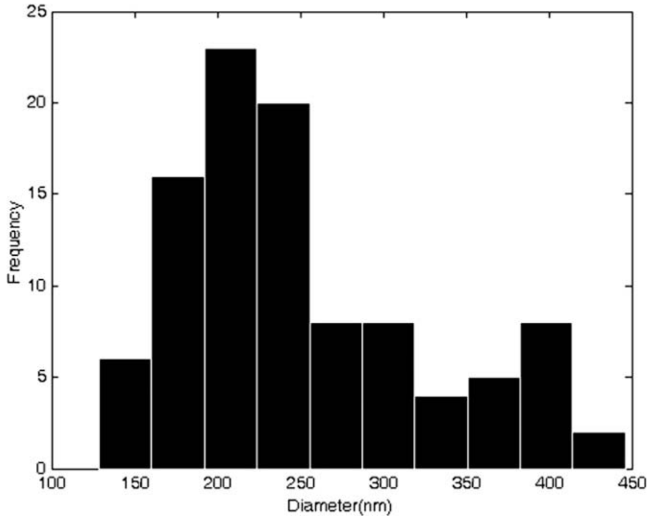

Fig. 8. Diameter histogram of the nanofibers, prepared from 48 h hydrolyzed chitosan in AcOH 80%.

3.6. Decreasing acetic acid concentration

Regarding environmental and cost issues, decreasing solvent concentration is generally desirable. Therefore, the electrospinning of chitosan nanofibers in aqueous AcOH with concentrations less than 90% is detailed below.

3.6.1. AcOH 80% as a solvent

In this step, the concentration of AcOH in the solvent was lowered to 80%. Fig. 7 shows the nanofibers which were prepared from the chitosan hydrolyzed for 48 h in a 7 wt% of its solution in two different magnifications presenting detailed descriptions of the prepared nanofibers and the total scheme from the larger area. Here, the quality of nanofibers and the speed and continuity of the electrospinning process are satisfactory. The electrospinning parameters are the same as outlined in Table 2. The average diam-

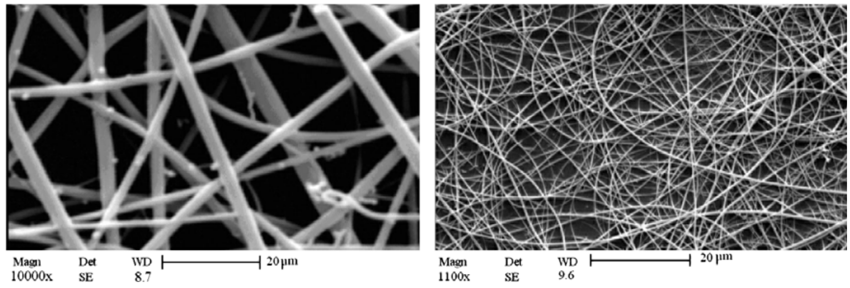

Fig. 9. SEM photographs of nanofibers of chitosan hydrolyzed for 48 h in aqueous 70% acetic acid 7.5 wt% (a, magnification: *10,000, b, *1100).

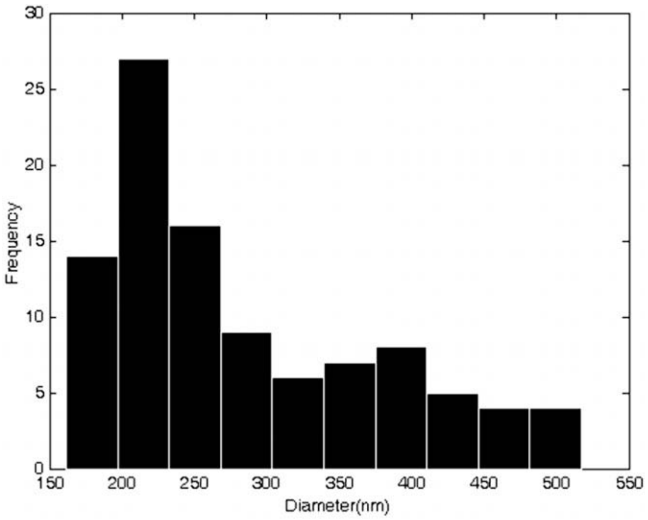

Fig. 10. Diameter histogram of the nanofibers, prepared from 48 h hydrolyzed chitosan in AcOH 70%.

eter of these electrospun nanofibers is 250 nm and the standard deviation is 76. Fig. 8 is a histogram of the diameter distribution of chitosan nanofibers electrospun from AcOH 80% solution.

3.6.2. AcOH 70% as a solvent

The concentration of AcOH in solvent was lowered even more, to 70%. Fig. 9 shows the nanofibers which were prepared from the chitosan hydrolyzed for 48 h in this solution at 7.5 wt%. In such a situation, the quality of nanofibers and the speed and continuity of the electrospinning process are satisfactory, but not as ideal as with AcOH 80% and 90%.

The electrospinning parameters were set as outlined in Table 2. The average diameter of these electrospun nanofibers is 284 nm and the standard deviation is 93.5. Fig. 10 is a histogram of the diameter distribution of chitosan nanofibers electrospun from AcOH 70% solution.

It should also be mentioned that the solution of chitosan hydrolyzed for 48 h in AcOH 60% was prepared and examined in the electrospinning unit, but the results were poor and unreliable.

4. Conclusion

In this study, the electrospinning of chitosan was investigated using different molecular weights of chitosan and different concentrations of AcOH as a solvent, and the optimum conditions leading to high-quality chitosan nanofibers in a steady electrospinning process were introduced. The diameter of the optimum product is 140 nm, which corresponds to chitosan hydrolyzed for 48 with the molecular weight of 2.94×10^5 g/mol. These nanofibers present 13.95% moisture uptake, which is 74% higher than that of the polymer powder.

FTIR spectroscopy also revealed that alkali treatment has no effect on the chemical nature of chitosan and its nanofibers, though it may affect secondary bonds.

Chitosan hydrolyzed for 48 h is spinnable in lower concentrations of AcOH, and the solution of this polymer at 7 and 7.5 wt% in AcOH 80% and 70% leads to nanofibers with mean diameters of 250 and 284 nm, respectively. The advantage of this work in comparison to other attempts at the electrospinning of pure chitosan (Geng et al., 2005; Ohkawa et al., 2004) include, first, the omission of toxic solvents and the decrease in the concentration of acetic acid from 90% to 70%, and second, the molecular weight of electrospun chitosan is increased.

References

Bhattaraia, B., Edmondson, D., Veiseh, O., Matsen, F. A., & Zhang, M. (2005). Electrospun chitosan-based nanofibers and their cellular compatibility. *Biomaterials, 26*, 6176–6184.

Ciechanshka, D. (2004). Multifunctional bacterial cellulose/chitosan composite materials for medical application. *Fibers & Textiles in Eastern Europe, 12*, 69–72.

Frenot, A., & Chronakis, I. (2003). Polymer nanofibers assembled by electrospinning. *Current Opinion in Colloid and Interface Science, 8*, 64–75.

Geng, X., Kwon, O. H., & Jang, J. (2005). Electrospinning of chitosan dissolved in concentrated acetic acid solution. *Biomaterials, 26*, 5427–5432.

Goycoolea, F. M., Arguelles-Monal, W., Peniche, C., & Higuera-Ciapara, L. (2000). Chitin and chitosan. In G. Doxastakis & V. Kiosseoglou (Eds.). *Navel macromolecules in food systems* (Vol. 41, pp. 265–308). Amsterdam: Elsevier.

Ignatova, M., Starbova, K., Markova, N., Manolova, N., & Rashkov, L. (2006). Electrospun nano-fibre mats with antibacterial properties from quaternised chitosan and poly(vinyl alcohol). *Carbohydrate Research, 341*, 2098–2107.

Li, L., & Hsieh, Y. L. (2006). Chitosan bicomponent nanofibers and nanoporous fibers. *Carbohydrate Research, 341*, 374–381.

Maghami, G. G., & Roberts, G. A. F. (1988). Evaluation of the viscometric constants for chitosan. *Makromolekulare Chemie, 189*, 195–200.

Min, B. M., Lee, S. W., Lim, J. N., You, Y., Lee, T. S., Kang, P. H., et al. (2004). Chitin and chitosan nanofibers: Electrospinning of chitin and deacetylation of chitin nanofibers. *Polymer, 45*, 7137–7142.

Muzzarelli, R. A. A. (1977). *Chitin* (pp. 50–85). Oxford: Pergamon Press.

Muzzarelli, R. A. A. (1973). In R. Belcher & H. Freisers (Eds.), *Natural chelating polymer* (pp. 144–176). Oxford: Pergamon Press.

Neamnark, A., Rujiravaniti, R., & Supaphol, P. (2006). Electrospinning of hexanoyl chitosan. *Carbohydrate Polymers, 66*, 298–305.

Ohkawa, K., Cha, D., Kim, H., Nishida, A., & Yamamoto, H. (2004). Electrospinning of chitosan. *Macromolecular Rapid Communications, 25*, 1600–1605.

Prashanth, K. V. H., & Tharanathan, R. N. (2007). Chitin/chitosan: Modifications and their unlimited application potential. *Trends in Food Science & Technology, 18*, 117–131.

Ramakrishna, S., Fujihara, K., Teo, W. E., Lim, T. C., & Ma, Z. (2005). *Introduction to electrospinning and nanofibers* (pp. 90–152). Singapore: Word Scientific Publishing.

Spasova, M., Manolova, N., Paneva, D., & Rashkov, L. (2004). Preparation of chitosan-containing nanofibres by electrospinning of chitosan/poly(ethylene oxide) blend solutions. *e-Polymers, 056*, 1–12.

Tsaih, M. L., & Chen, R. H. (1999). Molecular weight determination of 83% degree of deacetylation chitosan with non-Gaussian and wide range distribution by high-performance size exclusion chromatography and capillary viscometry. *Journal of Applied Polymer Science, 71*, 1905–1913.

Whistler, R. L. (1993). Introduction to industrial gums. In R. L. Whistler & J. N. Bemiller (Eds.), *Industrial gums polysaccharides and their derivatives* (pp. 9–19). San Diego: Academic Press.