Effects of Some Electrospinning Parameters on Morphology of Natural Silk-Based Nanofibers

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ABSTRACT: Natural silk, from *Bombyx mori* solutions were electrospun into nanofibers, with diameters ranged from 60 to 7000 nm. The effects of electrospinning temperature, solution concentration and electric field on the formation nanofibers were studied. Optical and scanning electron microscope were used to study the morphology and diameter of electrospun nanofibers. It was observed that the nanofibers became flattened with ribbon-like shape with increasing the electrospinning temperature. The nanofiber diameter increases with the increase in the concentration of silk solution at all electrospinning temperature. With increasing the voltage of electric field at 50°C, morphology of the nanofibers changes from ribbon-like structure to circular cross section. Referring to the literature the probable mechanism responsible for the change of morphology is pointed out. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 226–234, 2009

Key words: electrospinning; nanofiber; silk; morphology; ribbon; temperature; biopolymers

INTRODUCTION

The electrospinning process has gained much attention because it is an effective method to manufacture ultra fine fibers or fibrous structures of many polymers with diameter in the range from several micrometers down to a few nanometers. This method seem to be the most straightforward way to produce nanofibers by forcing a polymer melt or solution through a spinneret using of a high voltage electrostatic field. The electrostatic field is subjected to a droplet of polymer solution, held at the end of a capillary tube. With increasing the electrical field, electrostatic force overcomes the solution surface tension and a charged jet of the fluid is ejected from the hemi-spherical surface of the fluid at the tip of the capillary. The ejected polymer solution jet typically develops a bending instability and then solidifies to form fibers. Often the fibers produced in this way have a diameter in the range of nanometers. Several authors have explained the process; for the sake of brevity a few are mentioned in the list of references.[1–4]

Recently, many researchers in fiber and biomedical fields have been interested in fibrous protein based biopolymers, such as silk and collagen due to their unique biocompatible properties.[5,6] These types of proteins usually exhibit important mechanical properties in contrast to the globular proteins.

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Silks are generally defined as fibrous proteins that are spun into fibers by some Lepidoptera larvae such as silkworms, spiders, scorpions, mites, and flies.[7] Among the native silk proteins, the silkworm silk, mostly that of the domesticated *Bombyx mori* (*B. mori*) fibers, has been accepted as a high quality textile fiber and suture for a long time. *B. mori* silk fiber has been shown to be composed of two protein-monofilaments (named fibroin) embedded in a glue-like sericin coating. Silk has excellent properties such as lightweight (1.3 g/cm^3) and high tensile strength (up to 4.8 GPa as the strongest fiber known in nature).[8] Silk is thermally stable up to 250°C, allowing processing over a wide range of temperatures.[8] In addition to the outstanding mechanical properties, silk fibroin (SF) displays good biological compatibility that the sericin glue-like proteins are not biocompatible.[9] In practice, SF has been used in various fields, such as cosmetics, medical materials for human health, and food additives.

Number of researchers has investigated silk-based nanofibers as one of the candidate materials for biomedical applications, because it has several distinctive biological properties including good biocompatibility, good oxygen and water vapor permeability, biodegradability, and minimal inflammatory reaction.[10–12] Several researchers have also studied processing parameters and morphology of electrospun silk nanofibers[13–18] using Hexafluoroacetone, Hexafluoro-2-propanol and formic acid as solvents. In all these reports nanofibers with circular cross sections have been observed. Departures from

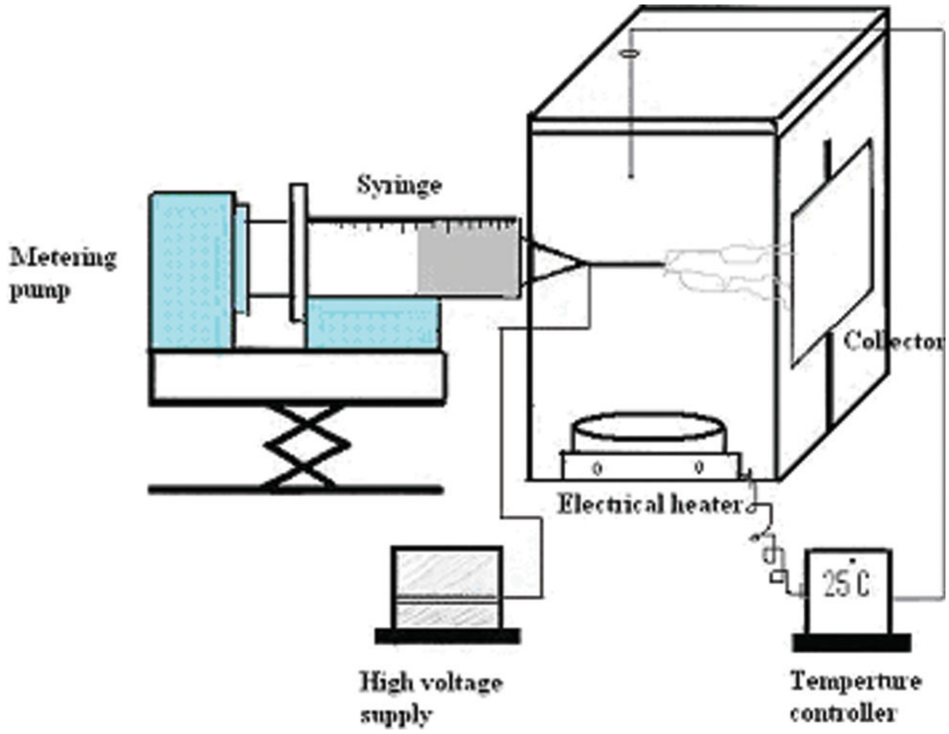

Figure 1 Schematic diagram of electrospinning apparatus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

circular symmetry around the axis of the jet have occasionally been reported.[19,20] Observation of ribbon-like jets of a synthetic polypeptide with elastin-like properties was described by Hung et al.[20]

In the present article, we report experimental observation of ribbon-like silk nanofibers. Effects of electrospinning parameters are studied and nanofibers dimensions and morphology are reported.

EXPERIMENTAL

Preparation of regenerated SF solution

Silks were obtained from domestic producer, Abrisham Guilan, Iran, and chemicals, Na_2CO_3 and formic acidwere from Merck, Germany and used as received.

The *B.mori* cocoons were degummed with 2 g/L Na_2CO_3 solution and 1 g/L commercial anionic detergent at 100°C for 1 h and then rinsed with warm distilled water to remove sericin from the surface of the silk fibers. Degummed SF was first dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (1 : 2 : 8 in molar ratio) at 70°C for 4 h. After dialysis with cellulose tubular membrane (Dialysis Tubing D9527, Sigma) in distilled water for 3 days, the SF solution was lyophilized to obtain the regenerated SF sponges. The regenerated SF sponge was dissolved in 98% formic acid for 30 min to prepare 8–14% (W/V) SF/formic acid solutions.

Electrospinning

In the electrospinning process, a high electric potential (Gamma High voltage Research) was applied to a droplet of SF solution at the tip (0.35 mm inner diameter) of a syringe needle. The electrospun nanofibers were collected on a target plate (aluminum foil), which was placed at a distance of 10 cm from the syringe tip. A syringe pump (New Eva Pump System) was used to form a constant amount of SF solution on the tip. The output of the injection pump was 20 μL/min. A charged jet is formed and ejected in the direction of the applied field. As the SF solution jet travels in the air, most of the solvent evaporates and the SF is collected on the grounded target as fine fibrous form. The syringe tip and the target plate were enclosed in a chamber for adjusting and controlling the temperature. Schematic diagram of the electrospinning apparatus is shown in Figure 1.

The processing temperature was adjusted at 25, 50, and 75°C. A high voltage in the range from 10 to 20 kV was applied to the droplet of SF solution.

Characterization

The fibers were examined by a Nikon Microphot-FXA optical microscope, Nikon, Japan. For better resolving power, morphology, surface texture and dimensions of the gold-sputtered electrospun nanofibers were determined using a XL-30 scanning electron microscope, Philips, Holland. A measurement

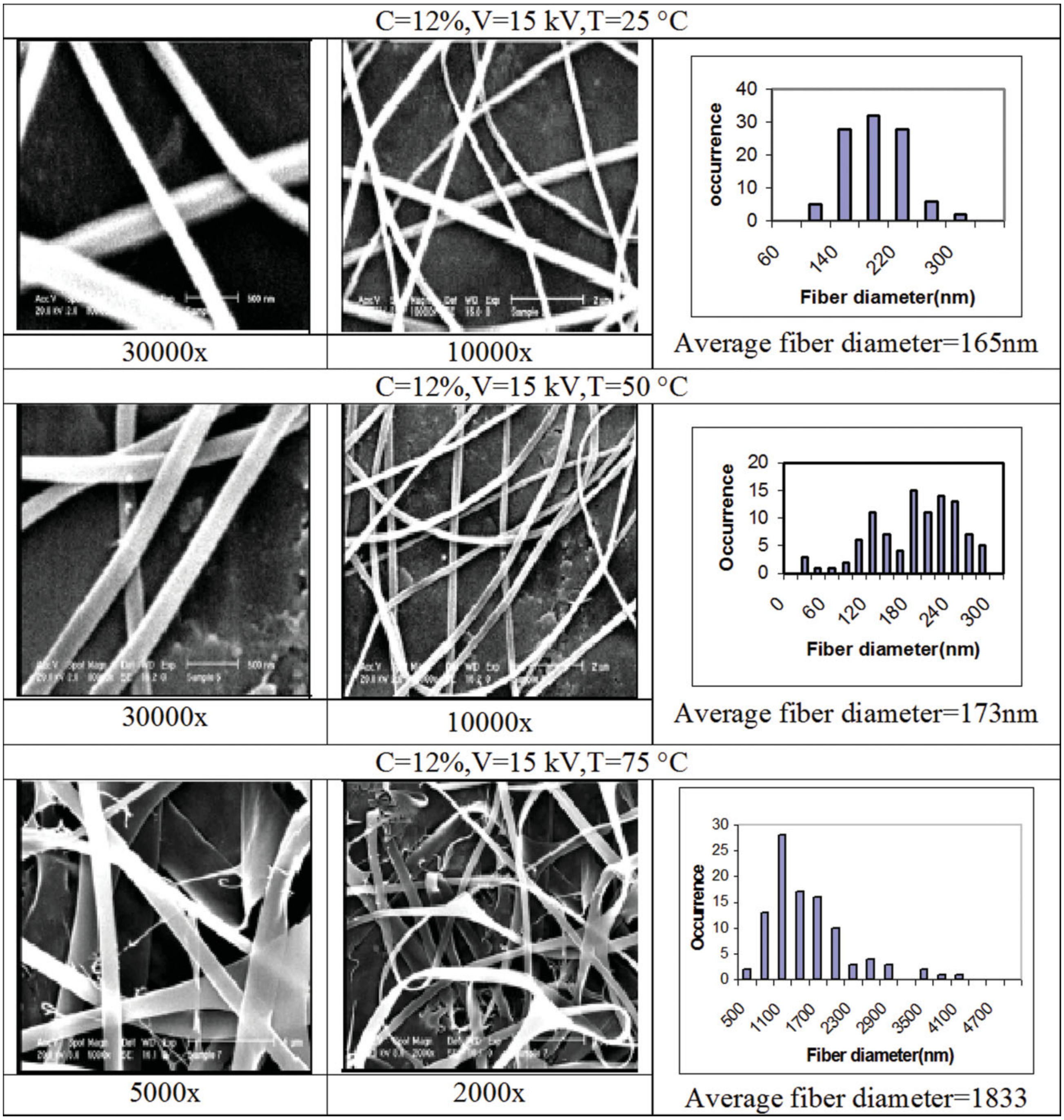

Figure 2 SEM micrograph and fiber distribution of electrospun SF at 25, 50, and 75°C , fibroin concentration $C = 12\%$, applied voltage $V = 15$ kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of about 100 random fibers was used to determine average fiber diameter and their distribution.

RESULTS AND DISCUSSION

Effect of electrospinning temperature

Studies on the electrospinning show[1–4] that many parameters may influence the transformation of polymer solution into nanofibers. Some of these parameters include (1) the solution related properties such as viscosity and surface tension, (2) process variables such as electric potential at the capillary tip, and (3) ambient parameters such as air temperature in the electrospinning chamber. To study the effect of electrospinning temperature on the morphology and texture of electrospun silk nanofibers, 12% silk solution was electrospun at various temperatures of 25, 50, and 75°C. Results are shown in Figure 2. Interestingly, the electrospinning of silk solution showed flat fiber morphology at 50 and

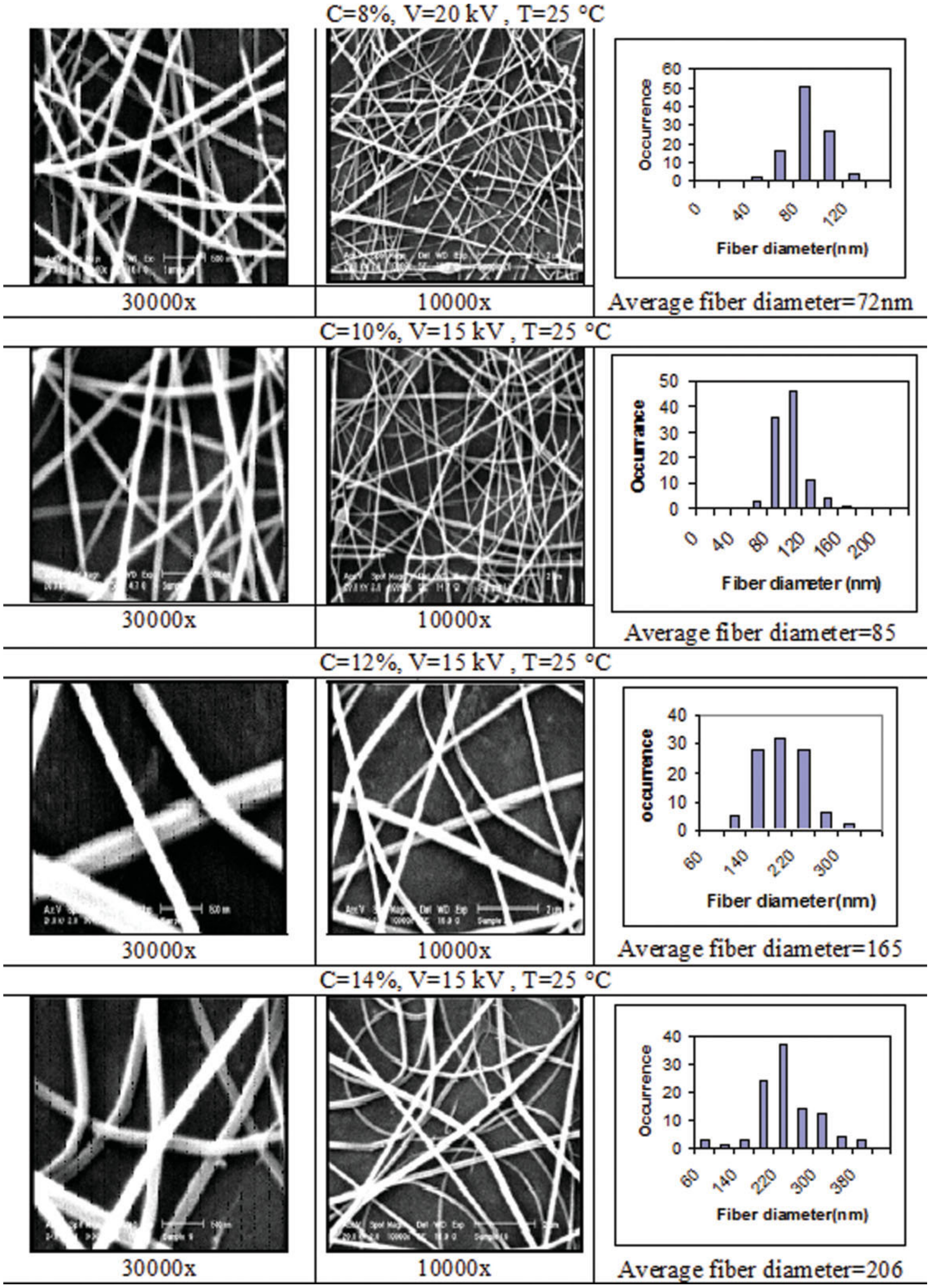

Figure 3 SEM micrograph and fiber distribution of electrospun SF at 25°C, fibroin concentration C = 8, 10, 12, and 14%, applied voltage V = 15 kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

75°C, whereas circular structure was observed at 25°C. At 25°C, the nanofibers with a rounded cross section and a smooth surface were collected on the target. Their diameter showed a size range of ~ 100 to 300 nm with 180 nm being the most frequently occurring. They are within the same range of reported size for electrospun silk nanofibers.[14,16] With increasing the electrospinning temperature to

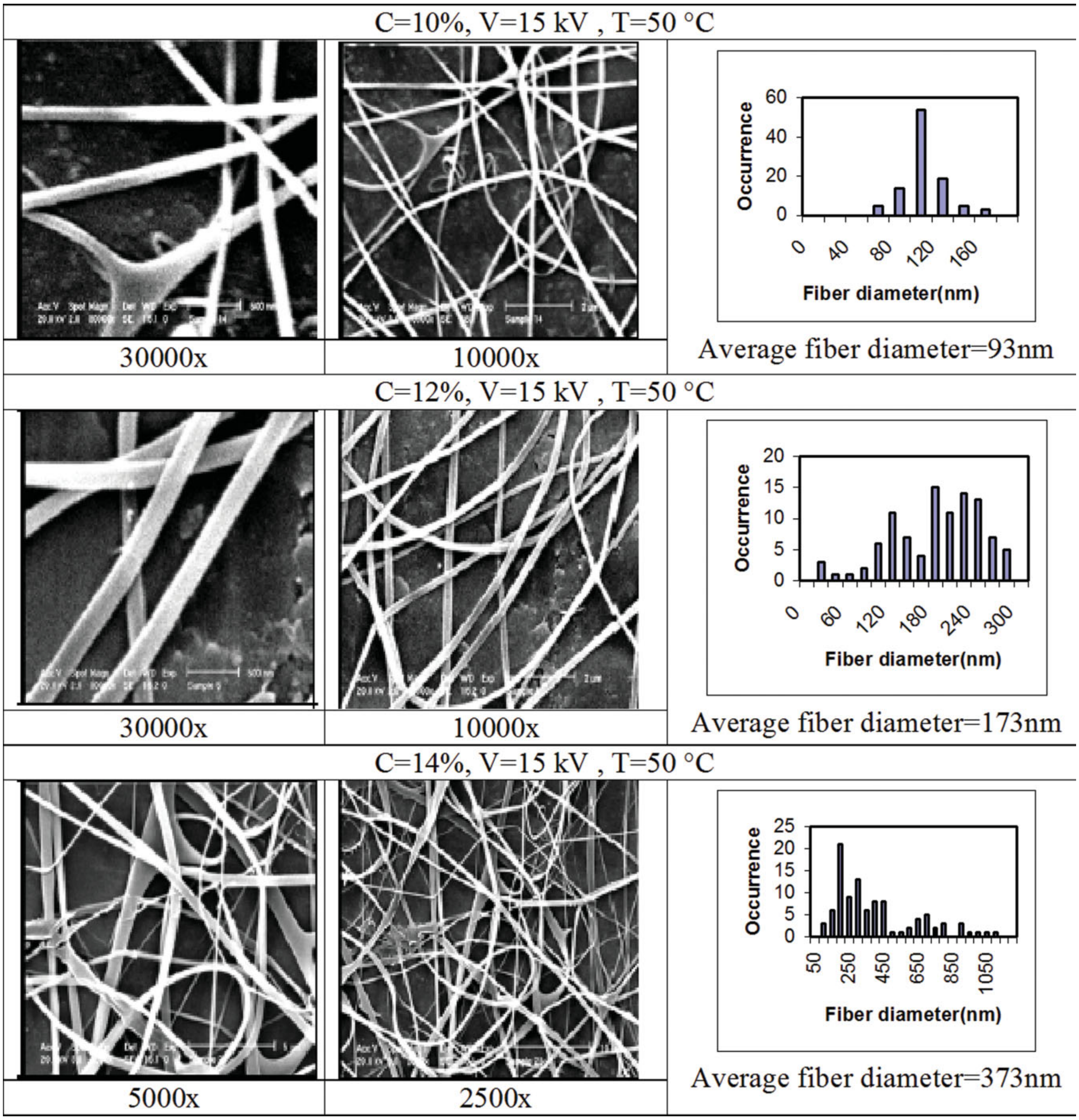

Figure 4 SEM micrograph and fiber distribution of electrospun SF at 50°C, fibroin concentration $C = 10$, 12, and 14%, applied voltage $V = 15$ kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

50°C, The morphology of the fibers was slightly changed from circular cross section to ribbon-like fibers. Fiber diameter was also increased to a range of ~ 20 to 320 nm with 180 nm the most occurring frequency. At 75°C, The morphology of the fibers was completely changed to ribbon-like structure. Furthermore, fibers dimensions were increased significantly to the range of 500 to 4100 nm with 1100 nm the most occurring frequency.

One of the most important quantities related to electrospun nanofibers, which affect their end use, is their diameter. Since nanofibers are resulted from evaporation of solvent from polymer solution jets, the fiber diameters will depend on the jet sizes, elongation of the jet, and evaporation rate of the solvent.[1] The main factors determining the nanofiber morphology are the elasticity of the fluid, the electrical charge carries by the jet and skin formation at the jet surface in conjunction with the evaporation of the solvent and elongation of the jet.[2,19] The elongation of the jet and evaporation rate of the solvent both changes the shape and the charge per unit area

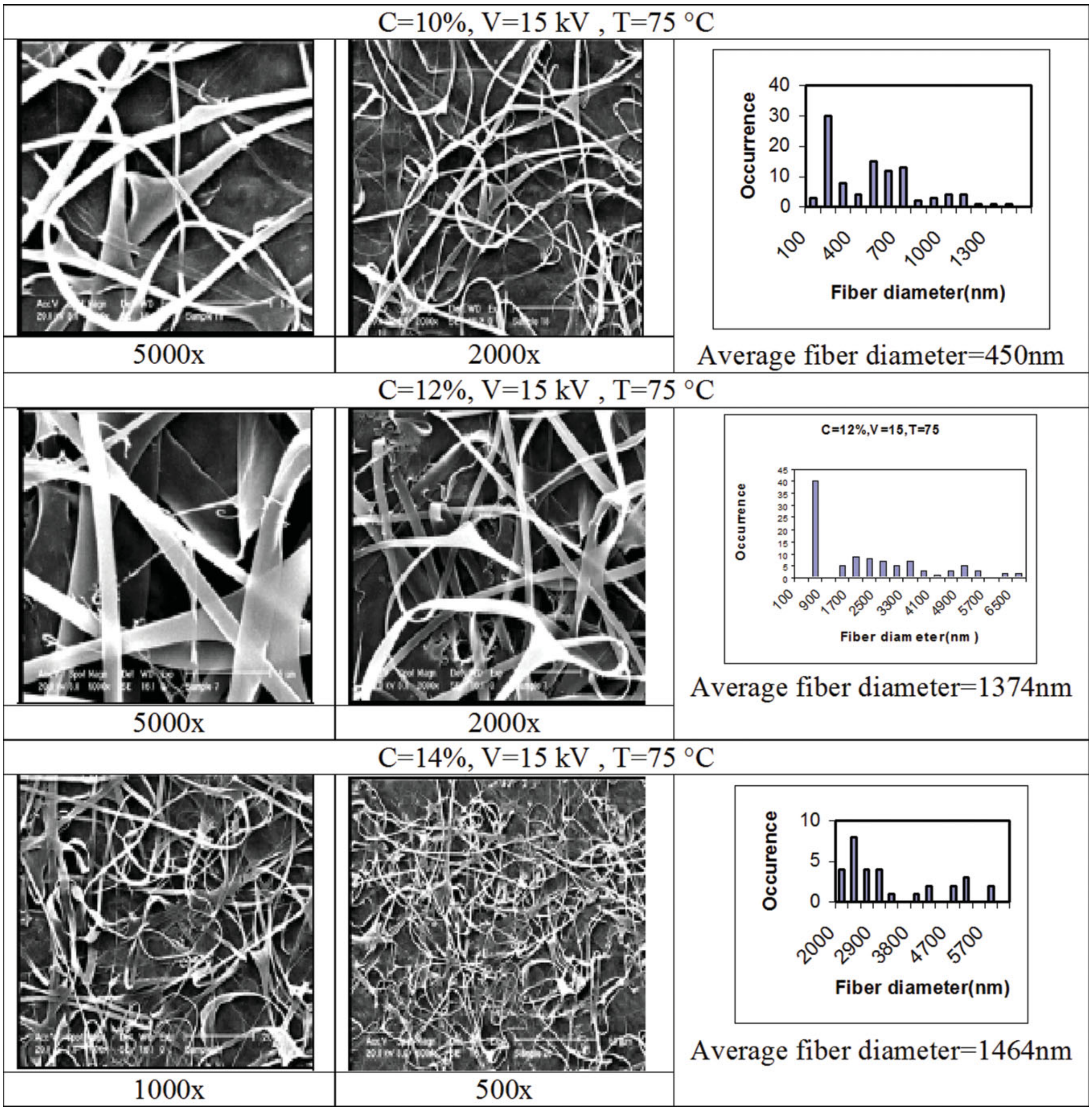

Figure 5 SEM micrograph and fiber distribution of electrospun SF at 75°C, fibroin concentration $C = 10$, 12, and 14%, applied voltage $V = 15$ kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

carried by the jet. Koombhongse et al.[19] explained that a thin and mechanically distinct polymer skin forms on the liquid jet during the electrospinning of some polymer solutions. According to Koombhongse et al.[19] after the skin is formed, the solvent inside the jet escapes and the atmospheric pressure tends to collapse the tube like jet. The circular cross section becomes elliptical and then flat, forming a ribbon-like structure. In this work we believe that ribbon-like structure in the electrospinning of SF at higher temperature thought to be related with skin formation at the jets. With increasing the electrospinning temperature, solvent evaporation rate increases, which results in the formation of skin at the jet surface. Nonuniform lateral stresses around the fiber due to the uneven evaporation of solvent and/or striking the target make the nanofibers with circular cross section to collapse into ribbon shape.

Bending of the electrospun ribbons were observed on the SEM micrographs as a result of the electrically driven bending instability or forces that occurred when the ribbon was stopped on the collector.[19] Another problem that may be occurring in the electrospinning of SF at high temperature is the branching of jets. With increasing the temperature of electrospinning process, the balance between the surface tension and electrical forces can shift so that the shape of a jet becomes unstable. Such an unstable jet can reduce its local charge per unit surface area by ejecting a smaller jet from the surface of the primary jet or by splitting apart into two smaller jets. Branched jets, resulting from the ejection of the smaller jet on the surface of the primary jet were observed in electrospun fibers of SF. The axes of the cones from which the secondary jets originated were at an angle near 90° with respect to the axis of the primary jet. Similar results were obtained for some polymers by Koombhongse et al.[19]

Effect of silk concentration

A series of experiments were carried out when the SF concentration was varied from 8 to 14% at the 15 kV constant electric field and 25°C constant temperature. Below the silk concentration of 8% as well as at low electric filed in the case of 8% solution, droplets were formed instead of fibers. The breakage of the jet and formation of droplets in the electrospinning of low concentration solutions have been reported by previous investigators.[1,15] However, at 8%, when the electric field was increased to 20 kV fibrous structure was formed. Figure 3 shows morphology of the fibers from 8% silk solution at 20 kV. The fibers are not uniform in diameter that could be due to the low viscosity of the solution. The average fiber diameter is 72 nm and a narrow distribution of fiber diameters is observed. It was found that continues nanofibers were formed above silk concentration of 8% at the applied electric field of 10 to 20 kV. The morphology of these electrospun nanofibers at 15 kV were shown in Figure 3. Similar sets of experiments were performed when the electrospinning temperature was 50 and 75°C. Figures 4 and 5 show morphology and diameter distribution of the resulted fibers at 50 and 75°C, respectively. At 50°C using 10% SF concentration, fibers with circular cross section and a narrow distribution of fiber diameter were formed. As the solution concentration increases, the fiber diameter increases and there is gradual shift from circular cross section to ribbon-like fibers. When the electrospinning temperature was 75°C, this shift from circular to ribbon-like fibers occurs at 10% SF concentration. Furthermore, the average size of the fibers increases and thick fibers with broader distribution of the size was collected on the target at this temperature.

Figure 6 shows the relationship between mean fiber diameter and SF concentration at different electrospinning temperature. There is a significant

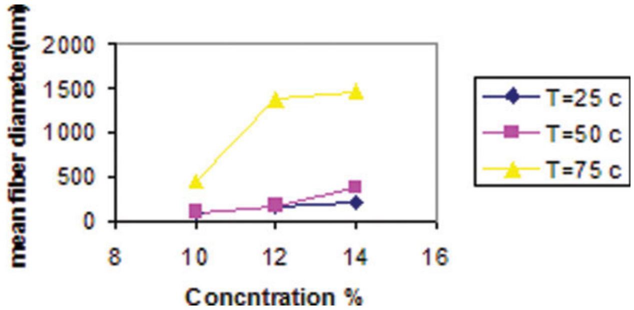

Figure 6 Mean fiber diameter of electrospun silk fibers at 25, 50, and 75°C at 15 kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

increase in mean fiber diameter with increasing of the silk concentration, which shows the important role of silk concentration in fiber formation during electrospinning process. It is well known that the viscosity of polymer solutions is proportional to concentration and polymer molecular weight.[21] For concentrated polymer solution, concentration of the polymer solution reflects the number of entanglements of polymer chains, thus have considerable effects on the solution viscosity. At fixed polymer molecular weight, the higher polymer concentration resulting higher solution viscosity. Ditzel et al.[1] showed that solution viscosity plays an important role in determination the range of concentration from which continues fiber can be obtained in electrospinning. The jet from low viscosity liquids breaks up into droplets more readily and few fibers are formed, while at high viscosity, electrospinning is prohibit because of the instability flow causes by the high cohesiveness of the solution. Experimental observations in electrospinning confirm that for fiber formation to occur, a minimum polymer concentration is required. Below this critical concentration, application of electric field to a polymer solution results electro spraying and formation of droplets to the instability of the ejected jet.[22] As the polymer concentration increased, a mixture of beads and fibers is formed. Further increase in concentration results in formation of continuous fibers as reported in this article. It seems that the critical concentration of the silk solution in formic acid for the formation of continuous silk fibers is 10% when the applied electric field was in the range of 10 to 20 kV.

Effect of electric field

Sukigara et al.[16] have studied the effects of electric field on fiber diameter of SF at ambient temperature. They reported that for a 10% concentration, increasing the electric field from 1 to 5 kV/cm leads to decrease in the fiber diameter. They also reported that at higher concentration, the effect of the electric field becomes

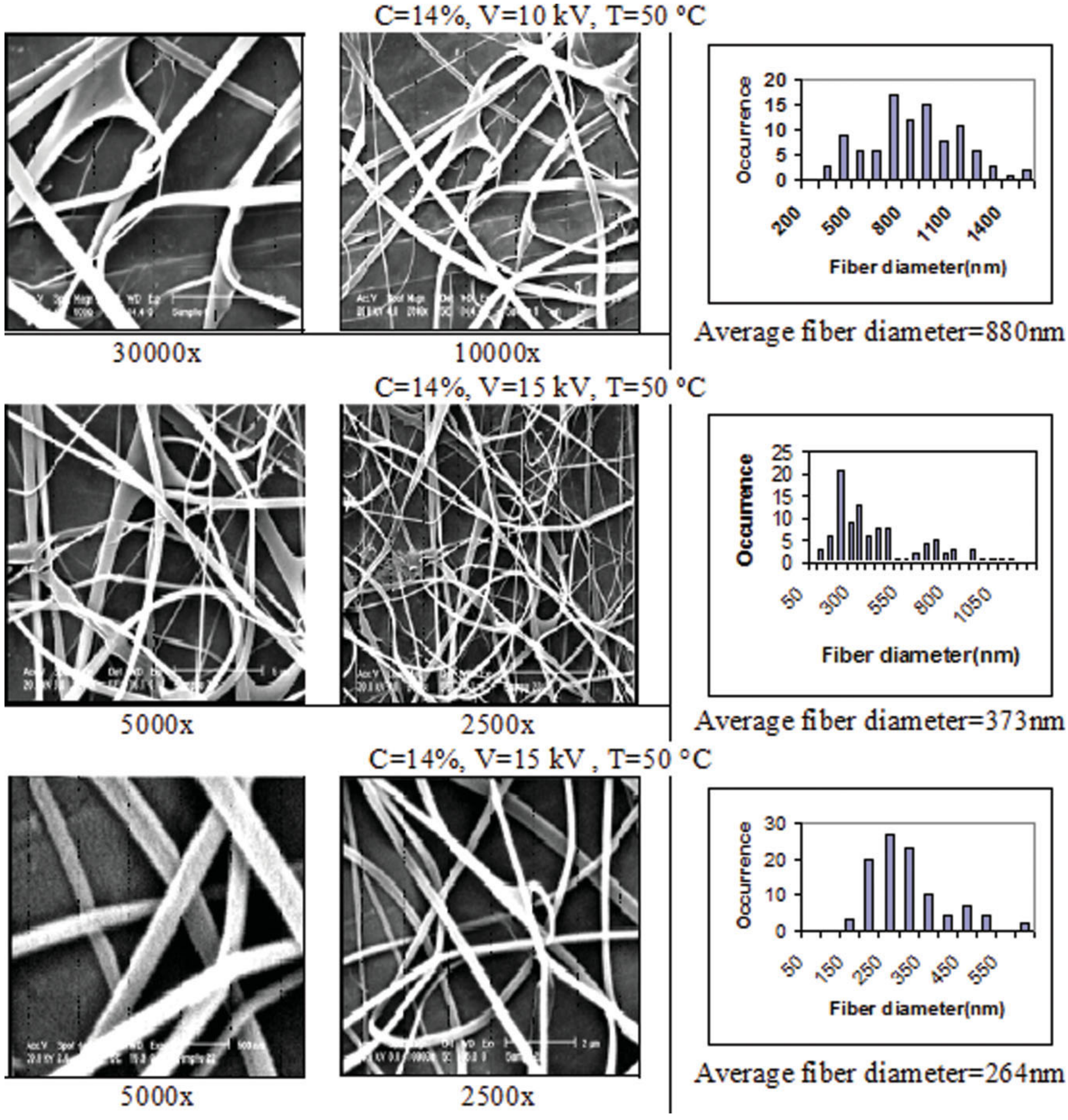

Figure 7 SEM micrograph and fiber distribution of electrospun SF at 50°C, fibroin concentration C = 14%, V = 10, 15, and 20 kV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

negligible. However, it was reported that the effect of the electric field strength on the diameter of electrospun nanofibers is much lower than the effect of the solution concentration. In this study, to examine the effect of the electric field at higher electrospinning temperature, SF solution with concentration of 14% was electrospun at 10, 15, and 20 kV. The electrospinning temperature was 50°C. Morphology of the obtained nanofibers, variation of the mean fiber diameter and their distributions at different applied voltage were shown in Figure 7. SEM micrographs showed a gradual shift in nanofiber morphology from ribbon-like structure to circular cross section. There is also a significant decrease in the nanofiber diameter, which may be due to changing of the flattened fiber structure to the circular cross section.

Increasing the electric field strength will increase the electrostatic force on the fluid jet, which favors the more elongation of the jet and the formation of thinner fibers. On the other hand, at higher electric field, a larger amount of solution would be removed from the capillary tip, which results in the increase of the fiber diameter. As the results of this finding it seems that electric field shows different effects on the nanofiber morphology. This effect depends on the polymer solution concentration and electrospinning conditions.

Based on the observations of Koombhongse et al.[19] it can be imagined that the noncircular and flat ribbons fibers form in two distinct processes. First, the polymer molecules leave the solvent and form the skin of the fibers. Then the solvent evaporates and makes the fibers to collapse into flattened form. It is expected that temperature have effects on the ribbon formation in these two distinct process involved. It has effect on molecular interactions between formic acid and polar groups in SF molecules that make the skin and it has effects on the rate of evaporation of solvent that causes the skin to collapse into flattened forms. Then increasing the temperature would increase the possibility of the formation of flattened fibers. Nevertheless, increasing the electric field strength will increase the elongation rate and traveling speed of the jet in the distance between nozzle and collecting plate. Therefore, the fluid jet may reach the collector before the formation of any skin on the surface of the jet and the fibers would have circular cross section.

The same trend was observed at the silk concentration of 10 and 12%. (The results are not shown here). Flattening of the electrospun nanofibers increases fiber dimensions. Hence, thicker nanofibers are resulted at lower applied electric field.

CONCLUSIONS

The solutions of SF in formic acid were electrospun into nanofibers and the effect of some parameters on the morphology of fibers were examined. The effects of electrospinning temperature, solution concentration and electric field on the formation nanofibers were studied. The electrospinning temperature, the solution concentration and the electric field have a significant effect on the morphology of the electrospun silk nanofibers. There effects were explained to be due to the change in the rate of skin formation and the evaporation rate of solvents. To determine the exact mechanism of the conversion of polymer into nanofibers require further theoretical and experimental work.

From the practical view the results of the present work can be condensed. Concentration of regenerated silk solution was the most dominant parameter to produce uniform and continuous fibers. The jet with a low concentration breaks into droplets readily and a mixture of fibers and droplets as a result of low viscosity is generated. On the other hand jets with high concentration do not break up but traveled to the grounded target and tend to facilitate the formation of fibers without beads and droplets. In this case, fibers become more uniform with regular morphology. In the electrospinning of SF, when the silk concentration is more than 10%, thin and rod-like fibers with diameters range from 60 to 450 nm were obtained. In the electrospinning of SF, when the process temperature is more than 25°C, flat, ribbon-like and branched fibers with diameters range from 60 to 7000 nm were obtained.

The results of this article can be used in the mathematical modeling of electrospinning silk nanofibers or for the designing of the specific type of silk nanofibers.

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