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# Regeneration of *Bombyx mori* silk by electrospinning—part 1: processing parameters and geometric properties

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**Abstract**

We studied the effect of electrospinning parameters on the morphology and fiber diameter of regenerated silk from *Bombyx mori*. Effects of electric field and tip-to-collection plate distances of various silk concentrations in formic acid on fiber uniformity, morphology and diameter were measured. Statistical analysis showed that the silk concentration was the most important parameter in producing uniform cylindrical fibers less than 100 nm in diameter.
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*Keywords:* Silk; *Bombyx mori*; Electrospinning

## 1. Introduction

### 1.1. Background

Polymer fibers of various diameters (μm to nm) have applications including use as reinforcing fibers in textile composites, nonwetting surface layer in textiles, high performance membrane filters [1], and tissue engineering applications like fabrication of cell-growth scaffolds, vascular grafts, wound dressings and drug delivery [2]. The large surface area to volume ratio allows cellular migration and proliferation in tissue engineering scaffolds. Several methods have been developed to fabricate highly porous biodegradable scaffolds including fiber bonding, solvent casting, particle leaching, phase separation, emulsion freeze drying, gas foaming and 3D- printing technique [3]. However, the simplicity of electrospinning is an attractive advantage for scaffolds construction. The small diameter structures produced by electrospinning process have a high surface area to volume ratio and morphology similar to natural tissues. In addition, the static charge of electrospun fibers incorporated into membrane filters enhances filtration efficiency [4].

Presently, biocompatible and biodegradable polymeric biomaterials are used in fabrication of scaffolds. These materials include collagen, poly-(lactide-*co*-glycolide), poly-(lactic acid), poly-(glycolic acid) and poly-(caprolactone). However, many of these scaffolding materials have insufficient mechanical integrity and often induce an inflammatory response [5]. The good mechanical properties of silk fibers (because of its secondary structure, β sheets) make it an unique candidate for scaffolds [6].

Renewed interest in silk fibers for biomedical applications is because of their favorable mechanical properties [6–9]. *Bombyx mori* silk has been of interest for over 5000 years not only for its textile properties of texture, tenacity and dyeing [10] but also its use in cosmetics creams, lotions, makeup, powders, bath preparations and pharmaceuticals [11]. Silkworm silk has also been extensively used commercially as biomedical sutures over the decades.

The electrospinning of *B. mori* cocoon silk and of *Nephila clavipes* dragline silks were first reported by Zarkoob et al. [12–14]. They used solution of 0.23–1.2 wt% in hexafluoro-2-propanol. Polymeric fibers with diameters in the range of 6.5–200 nm were produced with

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crystallographic order equivalent to that of the original natural fibers.

The electrospinning process as a technique for achieving highly porous silk scaffolds having nanoscale fiber diameter is employed by Jin et al. [2] and Ohgo et al. [10] as well. Initial research on electrospinning experiments on silk encountered problems with choosing a solvent [15] and controlling the conformational transitions of fibroin during electrospinning. The solvent for dissolving silk should not interfere with the biocompatibility of the processed material when exposed to cells in vitro or in vivo. Maintaining the secondary structure of silk (β sheets) in the electrospun fibers is necessary to attain optimal mechanical properties. Jin et al. [2] electrospun different blends of poly-(ethylene oxide) (PEO) and the silk using aqueous hexaflouro-2-propanol (HFIP). The uniform fibers (800 ± 100 nm) were obtained but this technique involves the use of PEO which might affect the mechanical properties and biocompatibility of fibers. Ohgo et al. [10] used hexafluoroacetone-hydrate (HFA) for preparation of the recombinant silk. The average diameter of the fibers obtained using this solvent was 300 nm. The use of HFA-hydrate might compromise the biocompatibility of the fibers and so methanol was used to remove HFA. This leads to undesirable effect of fiber shrinkage. The methanol treatment compromises the mechanical properties of the fibers. We are interested in studying the processing parameters in electrospinning. This study looks at the electrospinning process of protein-based biomaterials to fabricate scaffolds and membranes. Our goal is to obtain uniform nano scale silk fibers by electrospinning and to carry out detailed statistical analysis of the effects of the electrospinning parameters on fiber diameter.

### 1.2. The Electrospinning process

Electrospinning is a simple and efficient technique for the production of nano to micro scale fibers. The use of electrospinning in the fabrication of various nonwoven materials was first reported in 1934 in a patent by Formhals A [16]. The electrospinning technique involves the generation of a strong electric field between a polymer solution contained in a reservoir such as a glass syringe with a capillary tip or needle, and a metallic collection plate (Fig. 1). When the voltage reaches a critical value, the charge overcomes the surface tension of the deformed drop of suspended polymer solution formed on the capillary tip or needle, and a jet is produced. The diameter of electrically charged jet decreases under electro-hydrodynamic forces, and under certain operating conditions this jet undergoes a series of electrically induced bending instabilities during passage to the collection plate, which results in extensive stretching [2]. The stretching process is accompanied by a rapid evaporation of the solvent, which leads to a reduction in the diameter of the jet. The dried fibers are deposited randomly or in aligned manner on the surface of the collection plate.

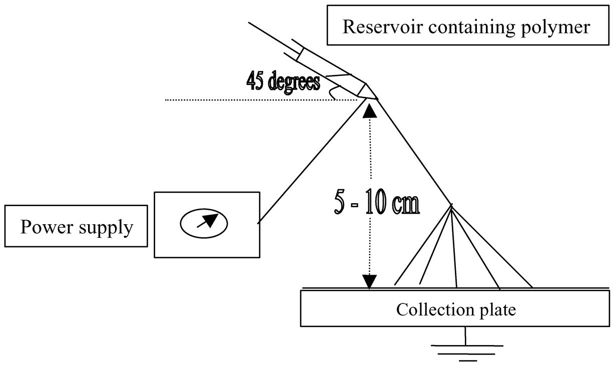



Fig. 1. Schematic diagram describing electrospinning technique. The polymer solution is filled in a reservoir like syringe and high voltage is passed through it. The fibers are collected at the collection plate which is grounded.

The fiber diameter can be controlled by varying the processing parameters such as polymer solution concentration, viscosity and electric field; type of solvent employed, distance from tip-to-collection plate, flow rate [4], diameter and angle of the spinneret [17].

## 2. Experimental

### 2.1. Spinning dope preparation

All concentration measurements were done in weight by weight (w/w). All basic chemicals were obtained from Sigma–Aldrich unless mentioned. The fibroin silk fibers were purchased from Pfaltz and Bauer Inc. The fibers were heated (100 °C for 30 min) in an aqueous $Na_2CO_3$ (0.02 M) and rinsed with water to extract sericin. The extracted fibers (1.3 g) were readily dissolved in 50% aqueous $CaCl_2$ (100 °C, then cooled) to obtain silk concentration of 6% solution. The solution (22 g) was poured into regenerated cellulose dialysis tubing (Fisher Scientific: T3 membrane, pore size 25 Å) to carry out dialysis against 1000 ml of deionized water (for 48 h at 23 °C). The regenerated silk fibroin sponge was obtained by lyophilization. The silk sponge solution (5–20%) was electrospun in formic acid (98–100%).

### 2.2. Electrospinning

The silk–formic acid solution was placed in a 3-ml syringe (18-G and spinning angle 45°). The tip-to-collection plate (covered with aluminum foil) distance varied from 5 to 10 cm vertically under the needle tip (Fig. 1). The electric field expressed in terms of voltage/distance between the collection plate (cathode) and the needle tip (anode) ranged from 2 to 5 kV/cm. A factorial experiment was designed to investigate and identify the relative significance of the processing parameters on fiber diameter (Table 1).

Table 1
Factorial design of experiment

| Factor | Factor level |
|---|---|
| Concentration (%) | 5, 8, 10, 12, 15, 19.5 |
| Electric field (kV/cm) | 2, 3, 4, 5 |
| Spinning distance (cm) | 5, 7, 10 |

### 2.3. Characterization

The morphology of the gold sputtered electrospun fibers was examined by field emission environmental scanning electron microscope (Phillips XL-30 ESEM). The average fiber diameter and its distribution were determined from 100 random fibers obtained from each spinning conditions.

## 3. Results

### 3.1. Effect of silk polymer concentration on fiber diameter

We observed that silk concentration plays a major role in fiber diameter. No fibers were formed at less than 5% silk concentration for any electric field and spinning distances. Figs. 2 and 3 show the morphology of fibers obtained at the electric fields of 3 and 4 kV/cm, respectively, at silk/formic acid concentrations of 5, 8, 10, 12, 15, and 19.5% with a constant tip-to-collection plate distance of 7 cm. At 8% concentration less than 30 nm diameter fibers were formed with beads (drops of polymer over the woven mesh) and they were not uniform and were branched off (Fig. 3). At 10% concentration with 5 cm spinning distance and 2, 3 and 4 kV/cm electric fields, drops were formed instead of fibers. Continuous fibers were obtained above 12% regardless of electric field and distance (Figs. 2 and 3). At 19.5%, the average fiber diameter was much larger than that of fibers spun at lower concentrations. The distribution of fiber diameters at 12, 15 and 19.5% concentration is shown in Fig. 4. Fiber diameters less than 100 nm were successfully electrospun at each concentration and the distribution of the fiber diameter was plotted. The fiber diameter distribution is skewed to the left but the values of skewness were under acceptable limits and thus the distribution was assumed as normal to carry out further statistical analysis on the data.

### 3.2. Effect of voltage and spinning distance on morphology and diameter

We optimized the process parameters by studying the influence of electric field and distance on fiber diameter. Fig. 5 shows the relationship between mean fiber diameter and electric field with concentration of 15% at spinning distances of 5, 7 and 10 cm. The mean fiber diameter obtained at 2 kV/cm is larger than other electric fields. The relationship between the fiber diameter and concentration at 2, 3 and 4 kV/cm is shown in Fig. 6 along with the regression line. The results suggest an exponential relationship between fiber diameter and silk concentration. The effect of two factors, concentration and electric field on fiber diameter was investigated by two-way analysis of variance. The interaction effect between two factors is also obtained from this analysis. Fig. 6 shows that the concentration apparently has more effect on the fiber diameter than electric field. The multiple regression analysis was carried out to evaluate the contribution of concentration and electric field on the fiber diameter. The fiber diameters were transferred to the natural logarithmic values and then linear model was applied to our data as seen in Table 2. Table 2 shows the analysis of variance and coefficients for this model. The coefficient of determination ($R^2$) was found to be 0.742. However, difference between the observed and predicted values was within one standard deviation. The standardized coefficient of the concentration was much larger than that of the electric field.

## 4. Discussions

In our study, solution concentration was found to be the most significant factor controlling the fiber diameter in the electrospinning process.

### 4.1. Polymer concentration

We were not able to obtain the fibers below the silk concentration of 5% because a stable drop at the end of spinneret was not maintained. In the short distance as well as low concentration (10%), the solution reaches the collection plate before the solvent fully evaporates. This explains the formation of droplets and beads at the low concentration and distance. Fewer beads were observed in electrospun fibers at higher concentration. Increase in the regenerated silk concentration in the formic acid increases the solution viscosity. Fong et al. [18] showed that the viscosity plays a major role in the formation of beads. They indicated that higher viscosity tends to facilitate the formation of fibers without beads. Our results are consistent with their study. Dietzel et al. [17] demonstrated that solution surface tension and viscosity play important roles in determining the range of concentrations from which continuous fibers can be obtained in electrospinning. At low concentrations beads are form instead of fibers and at high concentrations the formation of continuous fibers are prohibited because of inability to maintain the flow of the solution at the tip of the needle resulting in the formation of larger fibers. In our study continuous nanofibers were obtained above 12% regardless of electric field and distance and at higher concentration of 19.5% the average fiber diameter was larger than at lower concentrations.

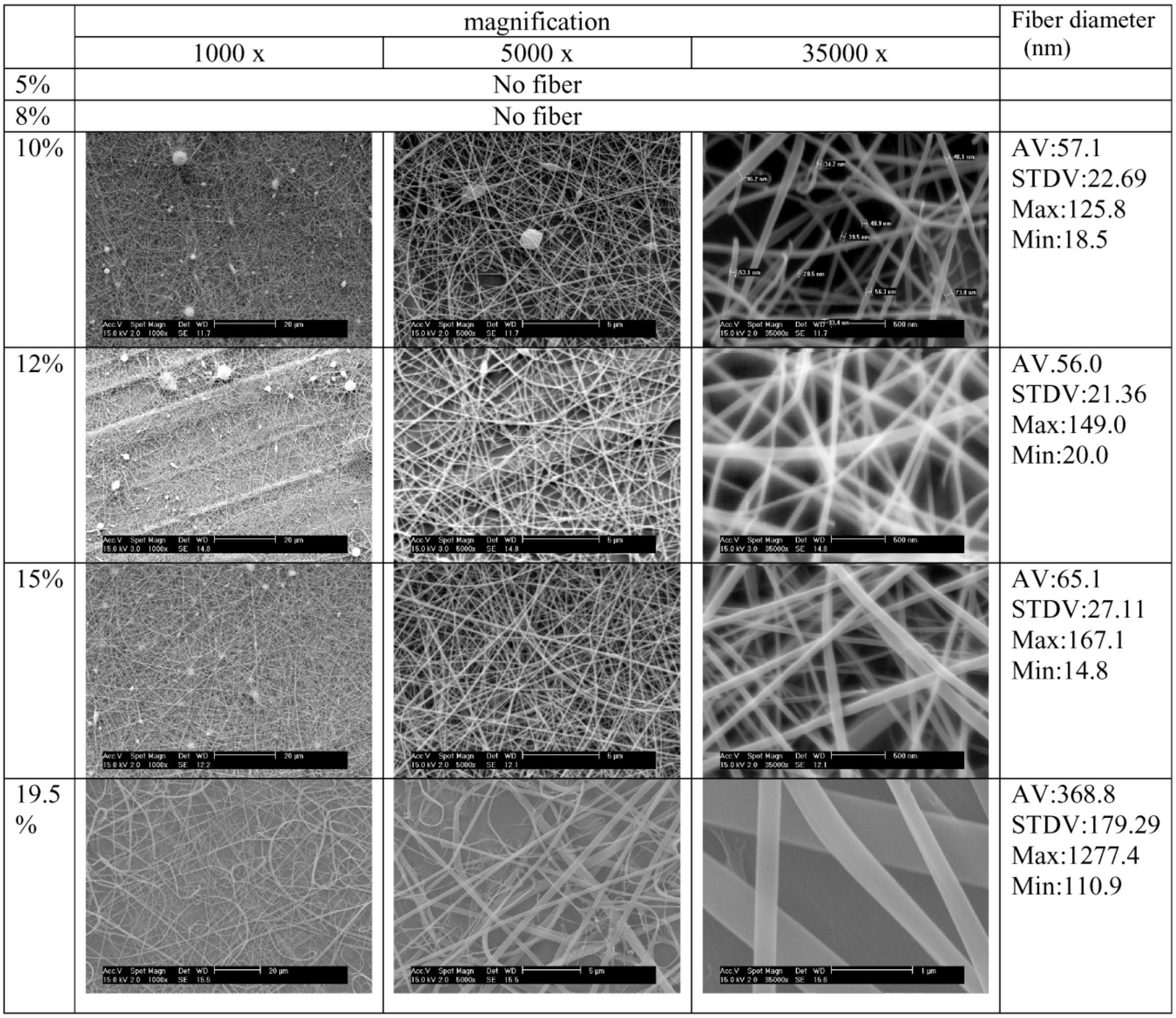



Fig. 2. The morphology of fibers at electric field of 3 kV/cm at concentrations from 5 to 19.5% with a constant spinning distance of 7 cm. The figure also shows the average, standard deviation, maximum and minimum values of the fiber diameter.

Table 2
Analysis of variance for the two factors (electric field, concentration) and coefficients of the model

| Variable | Unstandardized coefficients | | Standardized coefficients:[a] β | |
|---|---|---|---|---|
| | $C_0$ | 7.653 | | |
| Electric field: $x_1$ | $C_1$ | −0.611 | −0.668 | |
| Concentration: $x_2$ | $C_2$ | −0.497 | −2.027 | |
| $x_1^2$ | $C_{11}$ | 0.04556 | 0.346 | |
| $x_2^2$ | $C_{22}$ | 0.02189 | 2.668 | |
| $x_1x_2$ | $C_{12}$ | 0.01373 | 0.325 | |
| | *F* | Sig. | *R* | *R* square |
| Model[b] | 778.171 | 0.000 | 0.862 | 0.742 |

[a] Standardized coefficients explain the contribution of each parameter to the dependent variable (fiber diameter).
[b] Model: $\ln y = C_0 + C_1x_1 + C_2x_2 + C_{11}x_1^2 + C_{22}x_2^2 + C_{12}x_1x_2$, where $y$ is fiber diameter.

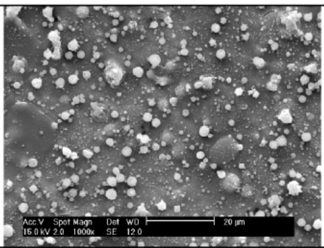
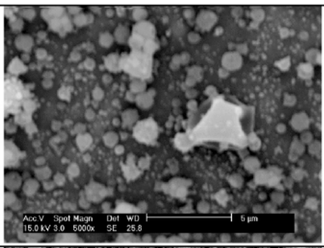
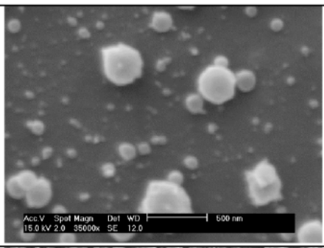
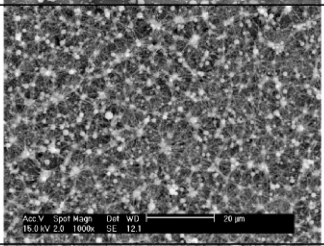
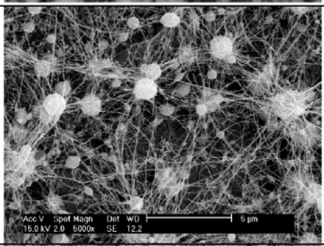
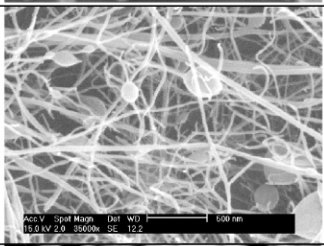
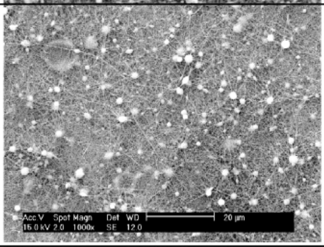
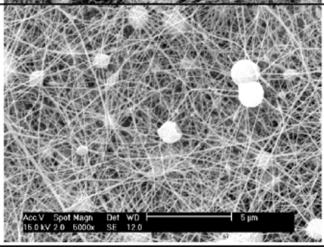
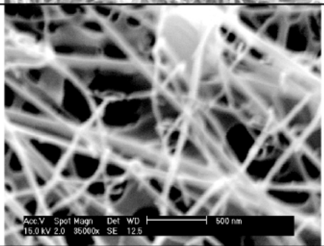
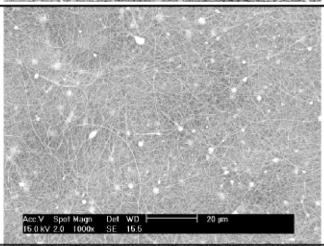
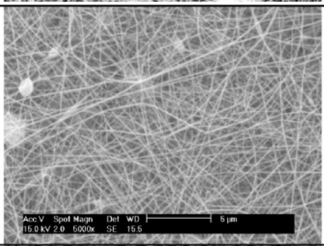
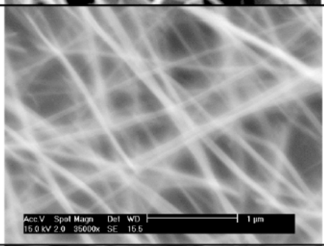
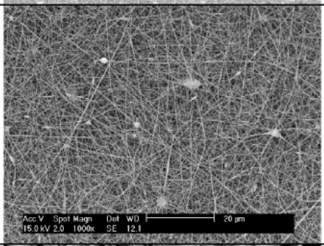
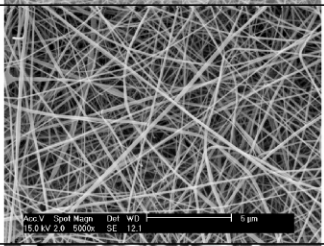
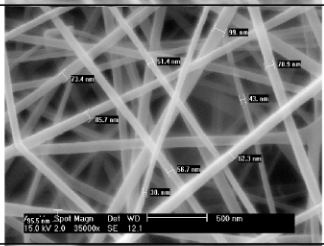
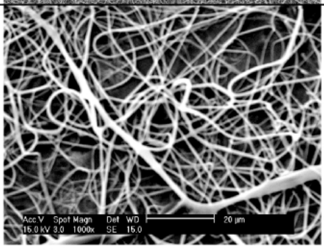
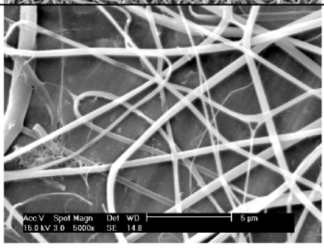
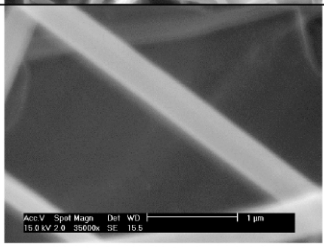
| | magnification | | | Fiber diameter (nm) |
|---|---|---|---|---|
| | 1000x | 5000x | 35000x | |
| 5% |  |  |  | |
| 8% |  |  |  | AV:26.3<br>STDV:12.58<br>Max:79.1<br>Min:11.9 |
| 10% |  |  |  | AV:40.5<br>STDV:14.23<br>Max:91.0<br>Min:15.38 |
| 12% |  |  |  | AV:41<br>STDV:17.0<br>Max:118.6<br>Min:15.5 |
| 15% |  |  |  | AV:71.0<br>STDV:29.29<br>Max:188.0<br>Min:19 |
| 19.5 % |  |  |  | AV:379.3<br>STDV:201.36<br>Max:1472.9<br>Min:109.66 |

Fig. 3. The morphology of fibers at electric field of 4 kV/cm at concentrations from 5 to 19.5% with a constant spinning distance of 7 cm.

*4.2. Electric field and spinning distance*

Two-way analysis of variance was carried out at the significance level of 0.05 to study the influence of electric field and distance on fiber diameter. For 12 samples (four levels of electric field × three levels of distance), multiple comparison analysis was carried out to find out the significant differences in mean fiber diameter among samples. The results suggest that there is no significant difference (at 0.05 level) in fiber diameter between 7- and

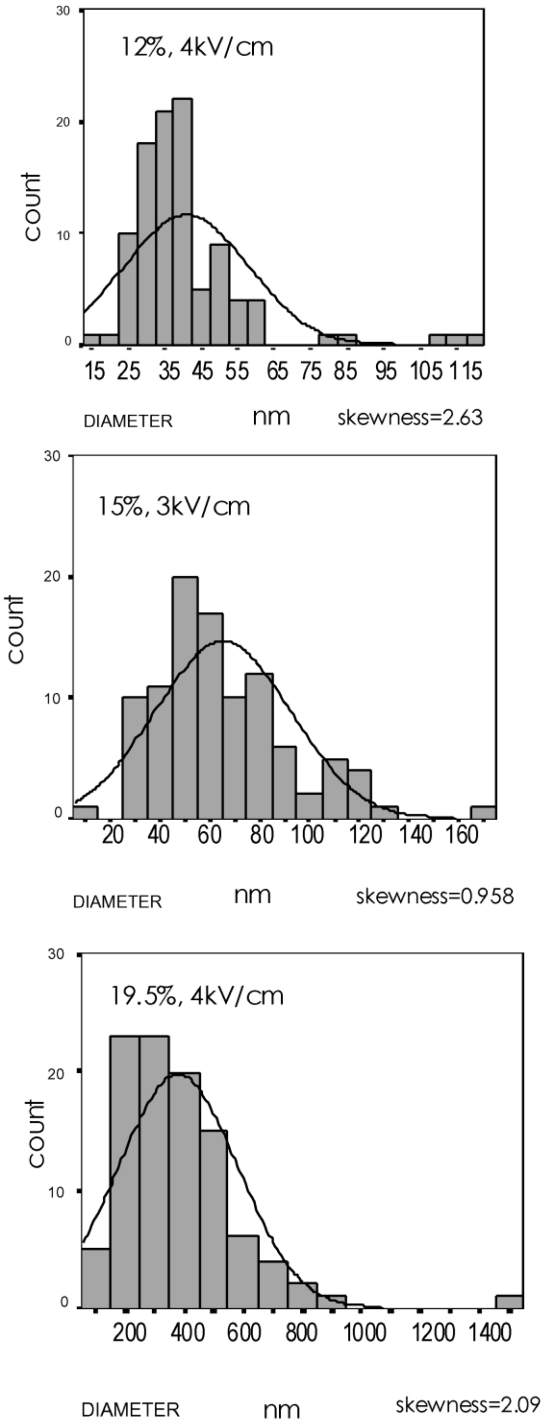



Fig. 4. The distribution of fiber diameter at with concentration of 12, 15 and 19.5% with a constant spinning distance of 7 cm. The distribution of the fiber diameter can also be seen from the values of skewness.

10-cm spinning distance at the same electric field and the concentration of 15%. Our study indicated that the fiber diameter increases exponentially with increasing concentration. Similar relationship between fiber diameter and solution concentration was observed for PEO/water solution [17]. Jin et al. [2] observed that electric field of more than 2 kV/cm was necessary for fiber formation in silk/HFA and silk/PEO solutions. Our results were consistent with Jin et al. as we obtained the fibers of less than 100 nm at 3 and 4 kV/cm electric fields.

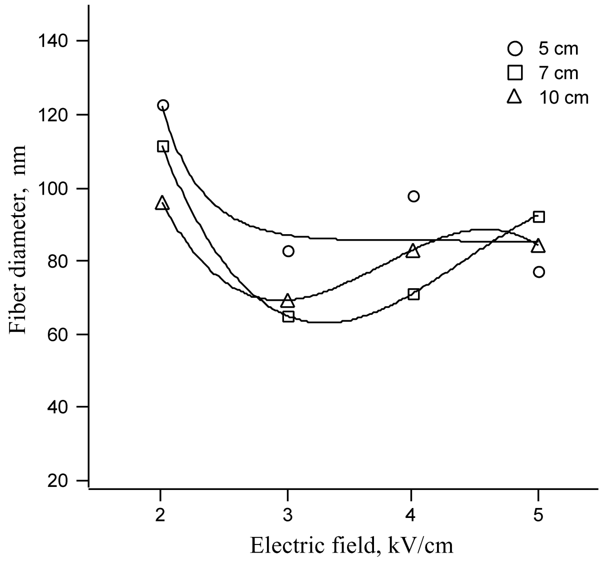



Fig. 5. The relationship between mean fiber diameter and electric field with concentration of 15% at spinning distances of 5, 7 and 10 cm. (Please refer to text for explanation).

## 5. Conclusions

The electrospinning of *B. mori* silk fibroin in formic acid was processed and fiber diameters ranging from 12 to 1500 nm were obtained depending on the electrospinning conditions. Morphology of fibers and distribution of fiber

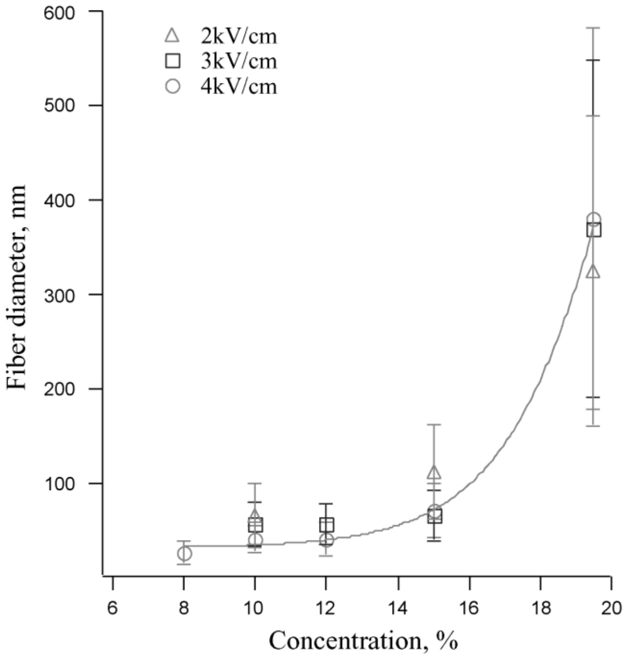



Fig. 6. The relationship between the fiber diameter and the concentration at three electric fields (2, 3, 4 kV/cm).

diameter were investigated varying electric field, concentration of regenerated silk solution and distance between tip and collection plate. The concentration of regenerated silk solution was the most dominant parameter to produce uniform and continuous fibers. The uniform fibers with diameter less than 100 nm were produced in the spinning conditions of 12–15% concentration and electric field of 3 and 4 kV/cm. The mechanical properties of an electrospun nonwoven mesh from silk fibroin, and further optimization analysis in electrospinning will be carried out in our subsequent studies.

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