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# Optimization of Elecrospinning Process of Zein Using Central Composite Design

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**Abstract:** Biodegradable edible sub-micron electrospun zein fibers were prepared using acetic acid as solvent. The solution concentration at three levels: 22, 26 and 30 w/v %, the electrospinning voltage at three levels: 10, 20 and 30 kV, the solution flow rate at three levels: 4, 8 and 12 m*l*/h and the distance between needle tip and collector at three levels: 10, 15 and 20 cm were studied. Central composite design (CCD) was utilized to modeling the effect of electrospinning parameters of zein solution on average fiber diameters and the data were analyzed using response surface methodology (RSM). Coefficient of determination, $R^2$, of fitted regression model was higher than 0.9 for response. The analysis of variance table showed that the lack of fit was not significant for response surface model at 95 %. Therefore, the model for response variable was highly adequate. Results also indicated that the solution concentration had significant influence ($P<0.0001$) on morphology and diameter of fibers. By increasing the solution concentration, uniform and bead-free fibers were obtained. As the solution concentration was increased, the average fiber diameters were also increased. Furthermore, the electrospinning voltage had significant effect ($P<0.0001$) on average fiber diameters. By increasing the electrospinning voltage, the average fiber diameters increased. The solution flow rate and the distance between needle tip and collector had no significant influence on the average fiber diameters. According to model optimization, the minimum average fiber diameter of electrospun zein fiber is given by following conditions: 24 w/v % zein concentration, 10 kV of the applied voltage, 10 cm of needle tip to collector distance, and 4 m*l*/h of solution flow rate.

**Keywords:** Electrospinning, Zein, Optimization, Modelling, Central composite design

## Introduction

Zein is the major storage protein of corn and comprises about 45-50 % of the protein in corn. It was first identified in 1897, based on its solubility in aqueous alcohol solutions [1]. It is not a single protein but rather a mixture of protein of various molecular weights, classified as type $\alpha$, $\beta$, $\gamma$ or $\delta$ according to their solubility [2]. Zein is one of the most hydrophobic proteins as a consequence of the presence of nonpolar amino acids such as leucine, proline and alanine [3]. Furthermore, zein is thermal resistant and has a great oxygen barrier [2]. Apart from biodegradability and biocompatibility, zein has high elasticity, and film-forming capabilities [4]. These properties make it suitable for different applications like controlled release applications, coatings, fibers, biodegradable films and plastics [1].

Electrospinning, a spinning technique, is a unique approach using electrostatic forces to produce fine fibers from polymer solutions or melts and the fibers thus produced have a thinner diameter (from nanometer to micrometer) and a larger surface area than those obtained from conventional spinning processes [5]. A schematic diagram of a basic electrospinning process is shown in Figure 1. There are basically three components to fulfill the process: a high voltage supplier, a capillary tube with a pipette or needle of small diameter, and a metal collecting screen [6]. In the electrospinning, an electrically charged jet of polymer fluids is created by a high voltage power supply. The hemispherical shape of pendant droplet at the end of capillary tip changes into a conical shape with increasing voltage, which is known as the Taylor cone. By applying a critical voltage to the spinneret, the charge accumulated in the polymer droplet overcomes its surface tension and causes the polymer solution to eject from the spinneret tip to the collector. Once the jet flows away from the Taylor cone in a nearly straight line, the traveling liquid jet is subjected to a variety of forces, such as Coulomb force, an electrical force imposed by the external electrical field, a viscoelastic force, a surface tension force, a gravitational force, and an air drag force. The onset of bending instability can thus be observed. The

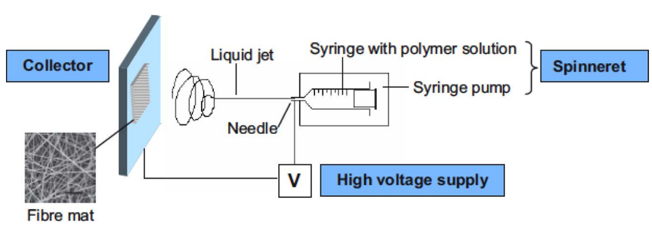


**Figure 1.** Schematic of the electrospinning unit.

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bending takes a complex path and other changes in the shape also occur. The electrical forces stretch and thin the jets by very large ratios, the solvent finally evaporates and the jet solidifies, resulting in a fibrous mat on the collector [7-9].

Because of their submicron diameter and large surface-to-volume ratio, electrospun fibers tend to exhibit enhanced interactions with their surrounding medium, making them an ideal material for sensors, controlled release systems, enzyme immobilization and filtration. Because heat is absent during electrospinning and the process is relatively simple, electrospun fibers may serve as innovative carriers to deliver bioactive components in foods [10,11].

The advantages of electrospun fibrous membranes over conventional solution-cast membranes are the highly porous nature of the fibrous membranes; which exhibit a much greater surface area, thus allowing active molecules to diffuse out from the matrix much more freely [12-14]. Potential applications of nanofibrous membranes in food sectors are reported to act, for instances, as delivery vehicles for nutrients and other active compounds, protective entities for encapsulated active compounds during food processing, and also as bioseparation media to enhance food safety [11, 15,16]. WPC-based submicro and microcapsules were produced through electrospinning/electrospraying process for the protection of *bifidobacteria* [17]. It was demonstrated that electrosprayed WPC-based encapsulation structures prolonged *bifidobacterial* survival during storage at 4 and 20 °C and at various relative humidity conditions (0, 11, 53 and 75 % RH) [17]. Similarly, electrospun zein fibers were produced to encapsulate epigallocatechin-gallate (EGCG) and to evaluate the stability of encapsulated EGCG in water [11]. EGCG is a plant polyphenol found mainly in tea. It is not stable under alkaline conditions and may undergo oxidation, polymerization and epimerization during thermal processing [11]. Sodium alginate-pectin-poly(ethylene oxide) electrospun fibers were used as a carrier to stabilize folic acid [7]. It was concluded that ethanol-treated crosslinked alginate-pectin electrospun fibers could potentially be used as a folic carrier to protect the micronutrient in food products, especially acidic food products such as fruit juices and acidified beverage [7]. Wongsasulak *et al.* introduced edible egg albumen-based nanofibers by blending egg albumen with cellulose acetate which is an electrospinnable material. They concluded that electrospun fibers from edible biopolymers could be fabricated through regulation of solution composition as well as addition of surfactant to modulate the solution properties. Edible egg albumen-based nanofibers application was suggested in food packaging material due to their potential in increasing safety and quality of packed foods [18].

Although, there are many studies about electrospinning of zein, but ethanol was used as solvent. Whereas in this research, acetic acid was used as a solvent in electrospinning process of zein. There are only two papers in the literature reporting that this protein can be electrospun from acetic acid solutions to yield smooth fibers [19,20]. Unlike ethanol, acetic acid is legally approved to be used as food additive in many countries, including Canada, the EU, USA, Australia and New Zealand.

The objective of the present study was: 1- to produce sub-micron electrospun zein using acetic acid as solvent, 2- to optimize zein electrospinning process, 3- to study and to construct mathematical model for the effects of polymer concentration and electrospinning process parameters on morphology and diameter of zein elrctrospun fibers using Central Composite Design (CCD).

## Experimental

### Materials

Zein powder from corn (Z 3625) was obtained from Sigma-Aldrich (Madrid, Spain) and used without further purification. The acetic acid glacial of 99.7 % purity was purchased from molekula (England).

### Preparation of Electrospinning Solution

The solution for production of sub-micron zein was prepared by dissolving different amounts of zein powder in acetic acid glacial to obtain different concentrations of zein solution (22, 26 and 30 % w/v).

### Measurement of Viscosity

The viscosity of zein solutions was measured by Brookfield RV DVIII Ultra (Brookfield Co., USA) rotation viscometer. Viscosity were determined at ambient temperature (25 °C) using spindle type SC4-31 atconstant shear rate.

### Electrospinning Process

Zein protein solutions were electrospun using the electrospinning set equipped with a variable high voltage 0-35 kV power supplier (Fnm Co., Iran). Zein solutions were fed into a 5-m*l* plastic syringe and an18-gauge stainless steel needle spinneret was connected through Teflon tubing to syringe. A syringe pump was used to feed zein solutions into needle spinneret at 4, 8 and 12 m*l*/ h. Stainless steel needle spinneret attached to the positive electrode of a direct current (DC) power supply and the electrospinning voltage was applied at three levels: 10, 20 and 30 kV. A positively charged jet of the polymer solution was formed from the Taylor cone which traveled through the air gap and was deposited on an aluminum foil attached on the surface of the collector. The distance between the needle spinneret and collector varied at three levels: 10, 15 and 20 cm. All electrospinning experiments were carried out at 25 °C.

### Scanning Electron Microscopy (SEM)

The morphology of the sub-micron electrospun fibers, after coating with gold-palladium mixture (20 nm) with a sputter coater, was studied using a scanning electron microscope (Model VP-1450; LEO Co., Germany) at an accelerating voltage of 20 kV. Diameters of electrospun fibers were measured with image visualization software Image-J (National Institutes of Health, USA). Average fiber diameters for the samples were determined by measuring about 50 random fibers from the SEM images.

**Table 1.** Actual and coded values of the variables based on central composite design for electrospun zein fibers

| Coded values | Actual values | | | |
|---|---|---|---|---|
| | Polymer concentration (% w/v) | Applied voltage (kV) | Distance between needle tip and collector (cm) | Flow rate (m*l*/h) |
| -1 | 22 | 10 | 10 | 4 |
| 0 | 26 | 20 | 15 | 8 |
| +1 | 30 | 30 | 20 | 12 |

### Atomic Force Microscopy (AFM)

AFM measurements were performed using AFM device (Ara Research Co., Tehran, Iran) to investigate the morphology of the fibers surface. The images were scanned in non contact mode in air using commercial Si cantilevers (Ara Research Co.) with a resonance frequency of 180 kHz.

### Statistical Analysis

In this study, the effects of four electrospinning parameters namely zein solution concentration (% w/v), applied voltage

**Table 2.** Central composite design for the variables and experimental response

| Run | Polymer concentration ($X_1$) | Applied voltage ($X_2$) | Distance between needle tip and collector ($X_3$) | Flow rate ($X_4$) | Response average fiber diameter ($\mu$m) |
|---|---|---|---|---|---|
| 1 | +1 | -1 | -1 | -1 | 0.8 |
| 2 | +1 | +1 | +1 | -1 | 1.211 |
| 3 | -1 | +1 | +1 | +1 | 0.356 |
| 4 | +1 | -1 | +1 | -1 | 0.922 |
| 5 | +1 | -1 | +1 | +1 | 0.738 |
| 6 | 0 | -1 | 0 | 0 | 0.541 |
| 7 | 0 | 0 | 0 | 0 | 0.56 |
| 8 | -1 | +1 | -1 | -1 | 0.343 |
| 9 | 0 | +1 | 0 | 0 | 0.614 |
| 10 | 0 | 0 | 0 | 0 | 0.69 |
| 11 | -1 | -1 | -1 | +1 | 0.149 |
| 12 | +1 | -1 | -1 | +1 | 0.824 |
| 13 | 0 | 0 | -1 | 0 | 0.549 |
| 14 | 0 | 0 | 0 | +1 | 0.646 |
| 15 | +1 | 0 | 0 | 0 | 1.258 |
| 16 | 0 | 0 | 0 | 0 | 0.715 |
| 17 | 0 | 0 | 0 | 0 | 0.7 |
| 18 | +1 | +1 | -1 | +1 | 1.142 |
| 19 | 0 | 0 | +1 | 0 | 0.639 |
| 20 | -1 | -1 | -1 | -1 | 0.252 |
| 21 | -1 | +1 | +1 | -1 | 0.306 |
| 22 | -1 | 0 | 0 | 0 | 0.393 |
| 23 | 0 | 0 | 0 | 0 | 0.71 |
| 24 | 0 | 0 | 0 | -1 | 0.621 |
| 25 | +1 | +1 | -1 | -1 | 1.322 |
| 26 | -1 | -1 | +1 | -1 | 0.179 |
| 27 | -1 | +1 | -1 | +1 | 0.421 |
| 28 | +1 | +1 | +1 | +1 | 1.6 |
| 29 | -1 | -1 | +1 | +1 | 0.174 |
| 30 | 0 | 0 | 0 | 0 | 0.589 |

(kV), distance between needle tip and collector (cm) and flow rate (m*l*/h) on sub-micron fiber diameter ($\mu$m) were studied using central composite design (CCD). The experiment was performed at least three levels for each factor to fit the quadratic model. The actual values and the coded design experiments for each parameter are given in Tables 1 and 2, respectively.

**Table 3.** Viscosity, average fiber diameter, and electrospun fiber morphology of zein at various concentrations at a constant applied voltage of 20 kV, flow rate of 8 m*l*/h and distance between needle tip and collector of 15 cm

| Concentration (w/v %) | Viscosity (m pa·s) | Average fiber diameter ($\mu$m) | Morphology |
|---|---|---|---|
| 10 | 18.39 | - | Beads |
| 15 | 50.65 | - | Beads |
| 20 | 117.27 | - | Beads |
| 22 | 247 | 0.393 | Fibers and small beads |
| 26 | 492.28 | 0.660 | Fibers free of beads |
| 30 | 888.28 | 1.258 | Fibers free of beads |
| Viscosity of solvent | 1.8 | - | - |

## Results and Discussion

### Effect of Zein Concentration on Diameter of Electrospun Fibers

As shown in Table 3 and Figure 2 with increasing zein concentration, the viscosity of zein solution was increased and electrosprying changed to electrospinning process. Such trend was also reported by other workers [19,21,22]. At a low viscosity, it is common to find beads along the fibers deposited on the collector. When the viscosity increases, there is a gradual change in the shape of the beads from spherical to spindle-like until a smooth fiber is obtained [23]. At lower viscosity, the higher amount of solvent molecules and fewer chain entanglements will form beads along the fiber.

Higher viscosity means that there is greater interaction between the solvent and polymer molecules thus when the solution is stretched under the influence of electric field, the solvent molecules will tend to spread over the entangled polymer molecules thus reducing the tendency for the solvent molecules to come together and finally smooth fibers will be created. When the viscosity is increased, the diameter of the fiber also increases [11,23]. Figure 4 shows

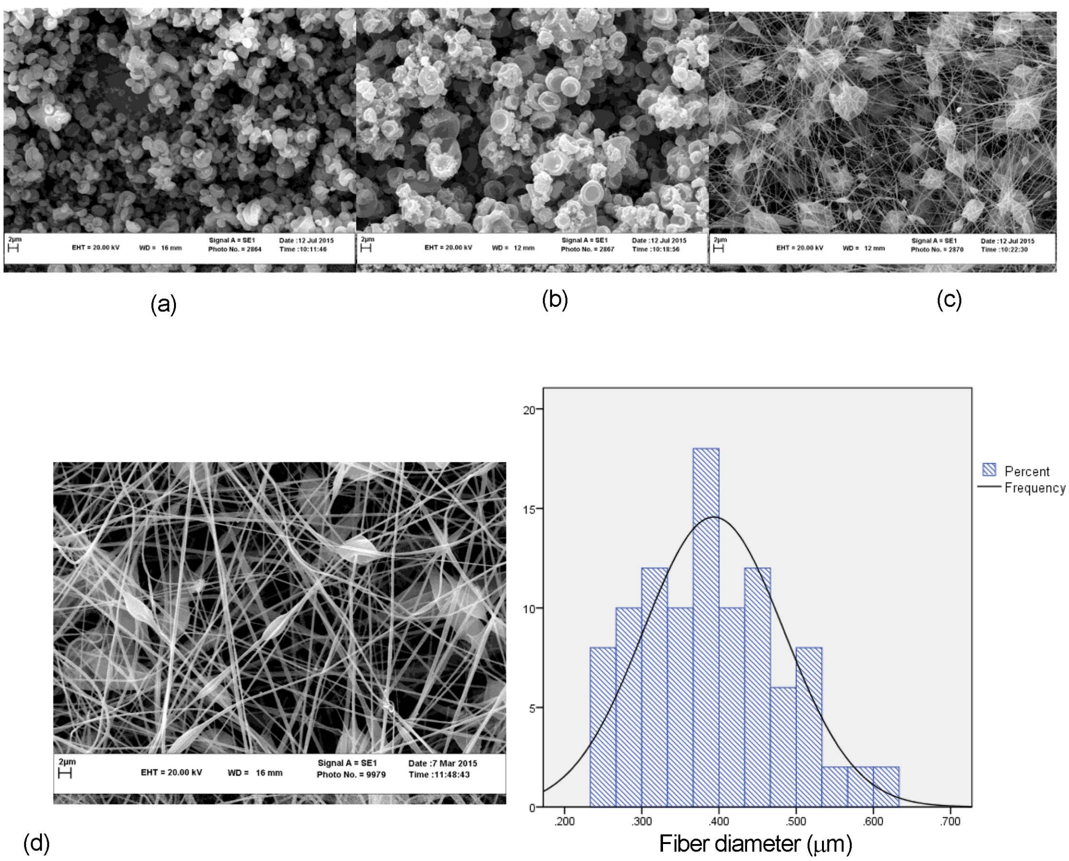


**Figure 2.** SEM images of zein fibers electrospun at a constant applied voltage of 20 kV, flow rate of 8 m*l*/h, and distance between needle tip and collector of 15 cm and at various zein concentrations; (a) 10 w/v %, (b) 15 w/v %, (c) 20 w/v %, (d) 22 w/v %, (e) 26 w/v %, and (f) 30 w/v %.

(e)

(f)

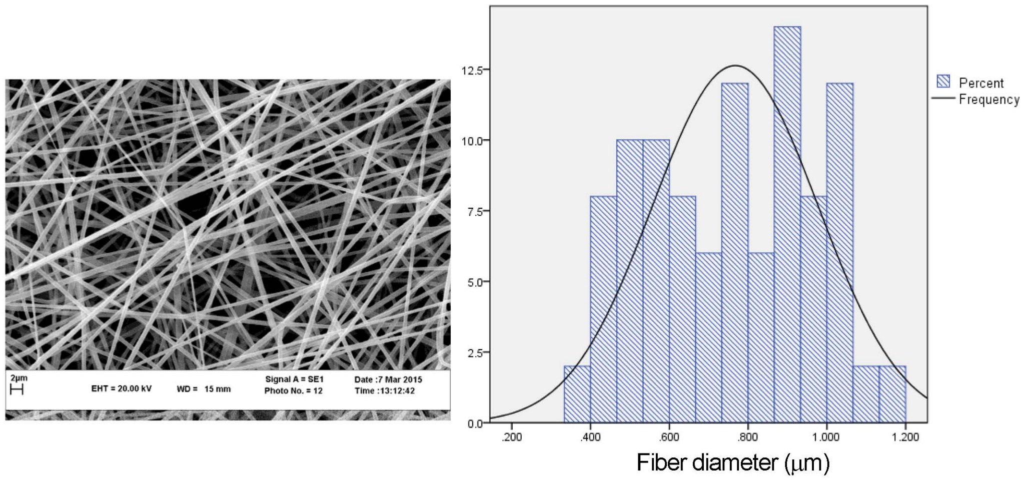


**Figure 2.** Continued.

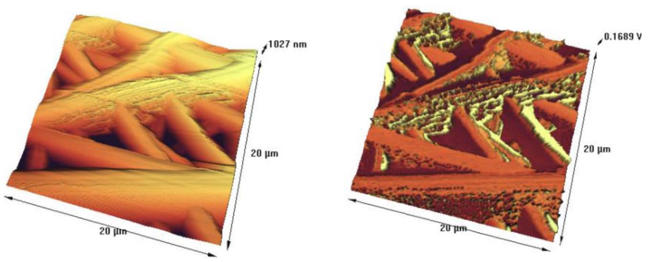


**Figure 3.** AFM 3D-topograph (left) and amplitude (right) images of zein fibers electrospunat a constant concentration of 26 w/v %, applied voltage of 20 kV, flow rate of 8 m*l*/h, and distance between needle tip and collector of 15 cm.

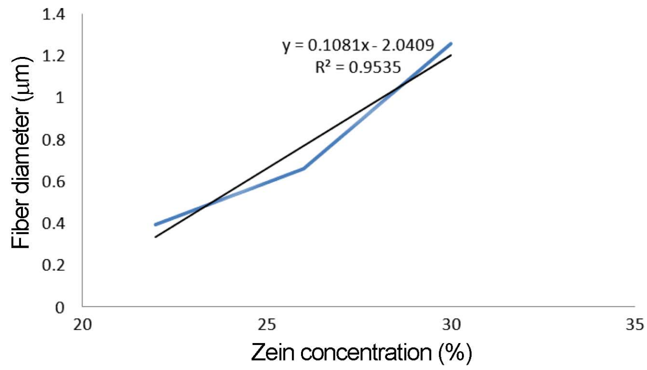


**Figure 4.** Effect of zein concentration (w/v %) on diameter ($\mu$m) of zein fibers electrospun at a constant applied voltage of 20 kV, flow rate of 8 m*l*/h and distance between needle tip and collector of 15 cm.

that zein concentration was proved to be the most significant factor controlling the fiber diameter in the electrospinning process [3,22]. With increasing concentration, the viscosity increases and thus, the diameter of the fiber also increases; this is probably due to the greater resistance of the solution to be stretched by the charges on the jet [23].

Other than beaded and non-beaded fibers, electrospinning is able to produce other types of fibers such as tubular structures, branched fibers, flat ribbons, ribbons with other shapes and fibers split longitudinally from larger fibers [3,24]. Atomic force microscopy (AFM) was used to better describe the surface topography of fibers. Figure 3 shows an AFM image of zein fibers electrospun at a constant concentration of 26 w/v %, applied voltage of 20 kV, flow rate of 8 m*l*/h, and distance between needle tip and collector of 15 cm. From Figure 3, it is observed that the morphology of the fibers is entirely tubular that it is in accordance with the SEM image of this sample. Round or tubular fibers are thought to be formed when the skin collapses with the formation of longitudinal wrinkles in the skin [3].

**Effect of Processing Parameters on Diameter of Electrospun Fibers**

The effects of processing parameters (applied voltage, flow rate and distance between needle tip and collector) on electrospun zein fiber diameter are shown in Figures 5 to 7.

Figure 5 shows the influence of various applied voltages (10-30 kV) on the diameter of zein fibers. The zein solution concentration was kept constant at 26 w/v %, and the electrospinning was done at flow rate of 8 m*l*/h. As shown in this figure, fiber diameter increased with increasing applied voltage. Similar phenomenon was reported by [2,3,11,20,25]. With increased voltage, the acceleration of the fibers also increases. This reduces the flight time of the electrospinning jet which may results in the formation of thicker diameter fibers [23,26].

Figure 6 shows the influence of solution flow rate (4-12 m*l*/h) on the diameter of zein fibers. The zein solution concentration was kept constant at 26 w/v %, and the electrospinning was done at distance between needle tip and collector of 15 cm. As shown in this figure, the diameter of the electrospun fibers was not significantly changed with the varied flow rate as suggested by [2,27,28].

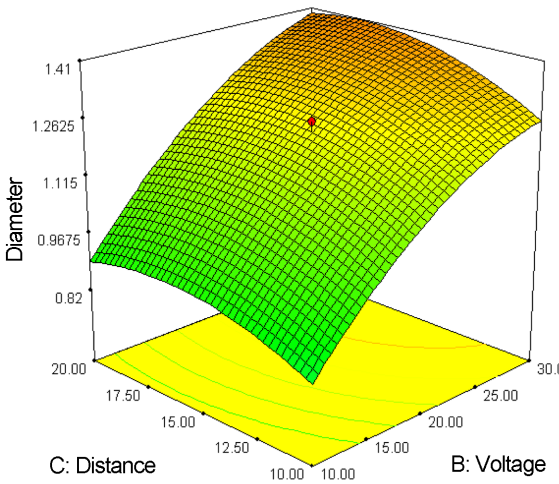


**Figure 5.** Influence of applied voltage (kV) on fiber diameter ($\mu$m) in electrospinning at a constant zein solution concentration of 26 w/v %, flow rate of 8 m*l*/h.

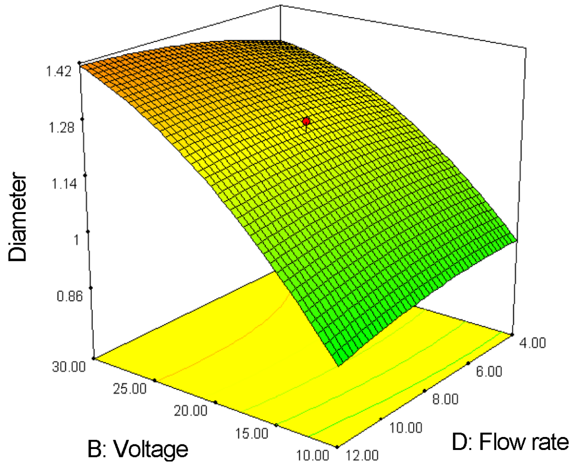


**Figure 6.** Influence of solution flow rate (m*l*/h) on fiber diameter ($\mu$m) in electrospinning at a constant zein solution concentration of 26 w/v %, and distance between needle tip and collector of 15 cm.

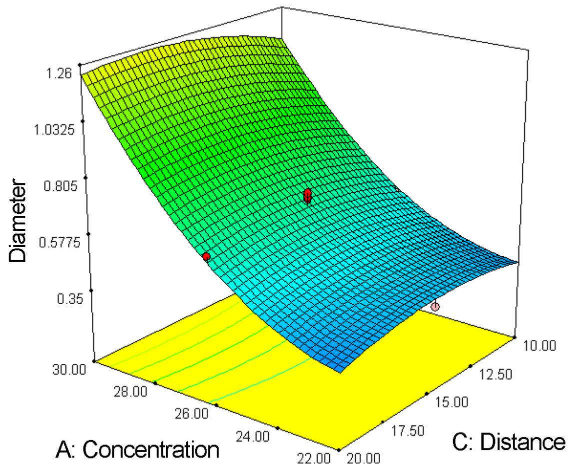


**Figure 7.** Influence of distance between needle tip and collector (cm) on fiber diameter ($\mu$m) in electrospinning at applied voltage of 20 kV and flow rate of 8 m*l*/h.

Figure 7 shows the influence of varying distance between needle tip and collector (10-20 cm) on the diameter of zein fibers. The electrospinning was done at applied voltage of 20 kV and flow rate of 8 m*l*/h. As shown in this figure, the diameter of the electrospun fibers was not significantly changed with the varied distance. But, fiber diameter gradually increased with increasing distance. This is probably due to the decrease in the electrostatic field strength resulting in less stretching of the fibers [23,29].

**Modelling**

Analysis of variance results has been summarized in Table 4. The values of *P*-value less than 0.05 indicates that the model terms are significant, whereas, the values greater than

**Table 4.** Analysis of variance results for fiber diameter

| Source | Sum of squares (SS) | Degree of freedom (DF) | Mean of squares (MS) | F-value | *P*-value Prob>F | Remarks |
|---|---|---|---|---|---|---|
| | 3.51 | 5 | 0.70 | 74.6 | 0.0001 | Significant |
| $X_1$-Concentration | 0.38 | 1 | 0.38 | 40.06 | 0.0001 | Significant |
| $X_2$-Voltage | 0.47 | 1 | 0.47 | 49.54 | 0.0001 | Significant |
| $X_1X_2$ | 0.11 | 1 | 0.11 | 11.57 | 0.0023 | Significant |
| $X_1^2$ | 0.065 | 1 | 0.065 | 6.90 | 0.0148 | Significant |
| $X_2^2$ | 0.042 | 1 | 0.042 | 4.50 | 0.0444 | Significant |
| Residual | 0.23 | 24 | 9.398 | - | - | |
| Lack of fit | 0.20 | 19 | 0.011 | 2.31 | 0.179 | |
| Pure error | 0.023 | 5 | 4.613 | - | - | |
| Total | 3.73 | 29 | - | - | - | |

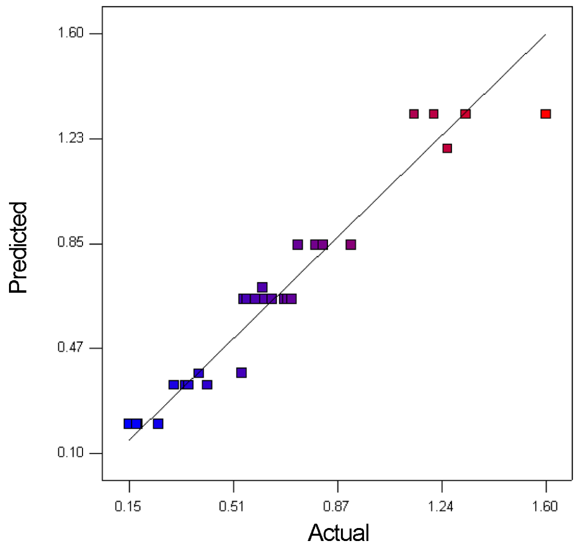


**Figure 8.** The predicted versus actual plot for average fiber diameter of electrospun zein fiber.

0.05 are not significant. Table 4 shows that the model terms, $X_1$, $X_2$, $X_1^2$, $X_2^2$ and $X_1X_2$ were significant ($P$<0.05), while the other model terms were not significant. The response surface model equation for the average fiber diameter can be proposed as followed:

$$Y = 1.19 + 0.85X_1 + 0.23X_2 + 0.1X_1X_2 + 0.21X_1^2 - 0.11X_2^2$$

where $Y$ is the average fiber diameter ($\mu$m), $X_1$ is the zein concentration (w/v %), $X_2$ is the applied voltage (kV).

The prediction model suggests that the average fiber diameter of electrospun zein fibers was directly affected by zein concentration and applied voltage. Therefore, with increasing of zein concentration and applied voltage, the average fiber diameter increases. According to the model equation, zein concentration is the most significant factor controlling the fiber diameter in the electrospinning process.

The model *P*-values (<0.0001) and lack of fit value (0.1799) suggested that the obtained experimental data has a good agreement with the model. Coefficient of determination, $R^2$, of fitted regression model were higher than 0.9 for response

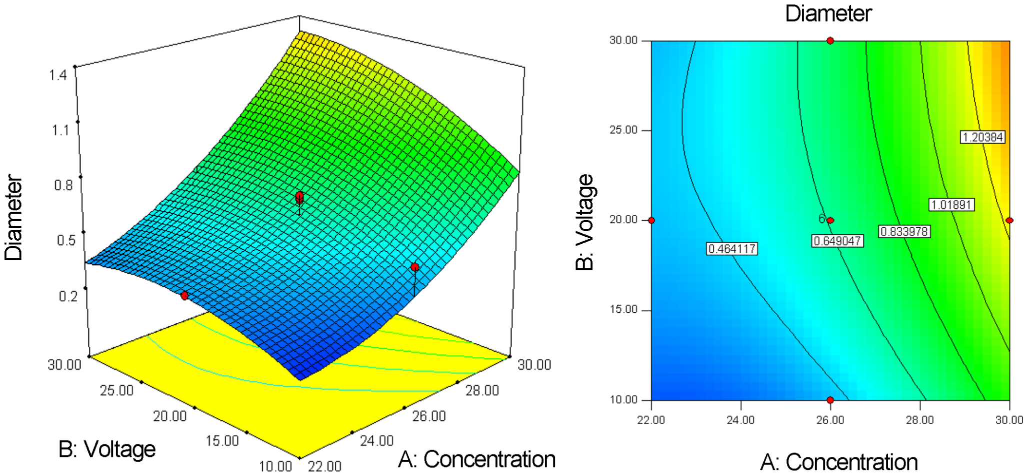


**Figure 9.** Response surface and contour plots of fiber diameter ($\mu$m) showing the effect of solution concentration (w/v %) and applied voltage (kV).

(0.9395).

Further, the goodness-of-fit of central composite design was evaluated by the predicted fiber diameter versus actual fiber diameter plot as shown in Figure 8. From Figure 8 it can be observed that experimental values are in good agreement with the predicted values.

**Effects of Significant Parameters on Average Fiber Diameter**

The response surface and contour plots in Figure 9 indicated that there was a considerable interaction between zein solution concentration and applied voltage at the middle of spinning distance (15 cm) and flow rate (8 m*l*/h). An increase in average fiber diameter can be seen with increase in solution concentration at any given voltage which is in agreement with previous observations [3,30,31]. The Response surface indicates that by increasing polymer concentration, fiber diameter increases which is probably due to the greater resistance of the solution to be stretched by the charges on the jet [23]. With increasing applied voltage, diameter increases, with increased voltage, the acceleration of the fibers also increases. This reduces the flight time of the electrospinning jet which may results in the formation of thicker diameter fibers [23,26].

**Determination of Optimal Conditions**

The conditions of the electrospinning parameters were established from the quadratic form of the RSM. Independent variables namely, the solution concentrations from 24 to 26 w/v %, the applied voltage, the distance between needle tip and collector, and the solution flow rate were set in range and dependent variable (the average fiber diameter) was fixed at minimum. The optimal conditions in the tested range for minimum average fiber diameter of electrospun zein fiber are shown in Table 5. This optimum condition was a predicted value, thus to confirm the predictive ability of the RSM model for response, a further electrospinning was carried out according to the optimized conditions and the agreement between predicted and measured responses was verified. The experimentally measured average fiber diameter was 0.168 $\mu$m was very close to the predicted value estimated to 0.158 $\mu$m. Figure 10 shows the SEM image and the average fiber distribution of electrospun fibers prepared at optimized conditions.

**Table 5.** Optimum values of electrospinning parameters for minimum average fiber diameter of electrospun zein fiber

| Parameter | Optimun value |
|---|---|
| Solution concentration (w/v %) | 24 |
| Applied voltage (kV) | 10 |
| Solution flow rate (m*l*/h) | 10 |
| Distance between needle tip and collector (cm) | 12 |

## Conclusion

This study used acetic acid as solvent to produce electrospun zein fibers. Central composite design (CCD) was used to modeling the electrospun zein fiber diameters. The response surface and contour plots of the predicted electrospun fiber diameters indicated that the zein concentration and applied voltage were factors affecting the average fiber diameters. It was concluded that the zein concentration was the most significant factor controlling the average fiber diameters of electrospun fiber mat. With increasing of zein concentration and applied voltage, the average fiber diameter increases. The $R^2$ value was 0.9395 for average fiber diameter, which indicates a good fit of the models with experimental data. The optimum value of the solution concentration, applied voltage, spinning distance, and flow rate were found to be 24 w/v %, 10 kV, 10 cm and 4 m*l*/h, respectively, for minimum average fiber diameter of electrospun zein fiber.

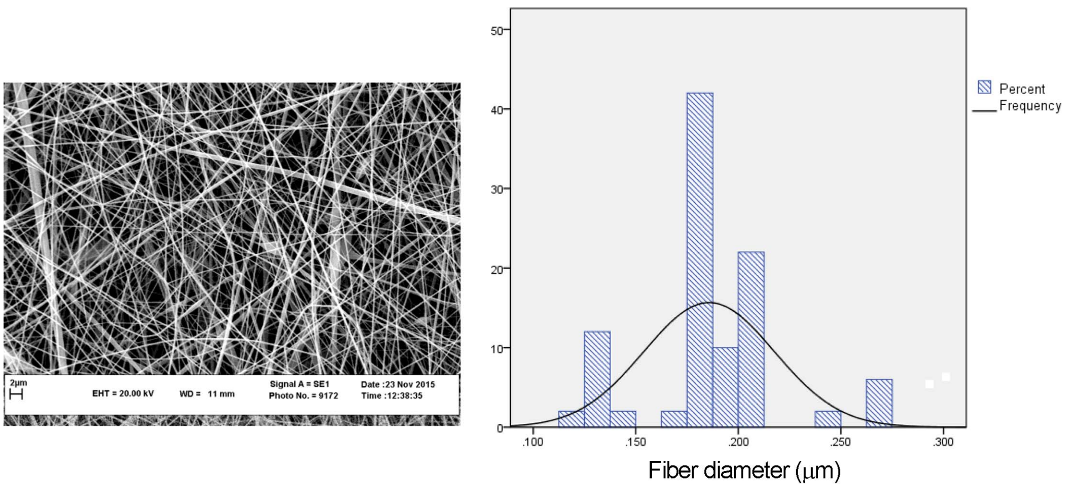


**Figure 10.** SEM image and fiber diameter distribution of zein fibers electrospun at optimized condition.

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