

Investigation of the electrospinning parameters on the diameter of the polyvinylpyrrolidone nanofibers containing silver nanoparticles using Box-Behnken design

H. Çavuşoğlu Vatansever, and A. Ersoy Mericboyu,

Department of Chemical Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Istanbul, 34469, Turkey

Nanofibers with antibacterial properties have gained great attention especially in biomedical applications where bacterial contamination should be prevented. In this study, it is aimed to produce antibacterial polyvinylpyrrolidone (PVP) nanofibers containing silver nanoparticles with electrospinning technique. In order to investigate the effects of electrospinning parameters such as, applied voltage, flow rate and tip to collector distance on fiber diameter, Box Behnken design technique was employed. SEM images were used to determine average fiber diameter. Based on the results of the statistical analysis, a model equation was developed that establishes a relationship between the electrospinning parameters and the fiber diameter. The adequacy and accuracy of the model equation were verified by residual graphs and validation experiments. The occurrence of the minimum and maximum fiber diameter areas were determined by the aid of contour and surface plots.

Keywords: Electrospinning, Polyvinylpyrrolidone, Silver nanoparticles, Fiber diameter, Box-Behnken Design

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Corresponding authors: cavusogluha@itu.edu.tr

1. Introduction

Nanofibers mostly expressing the fibers with the diameter less than several hundred nanometers but also broadly considering as less than 1 micrometer are of great interest in different areas including energy, environmental, and biomedical applications due to highly increased specific surface area, high porosity and flexibility [1]. Interconnected porous network of nanofibers resembles the extracellular matrix, supports cellular attachment moreover, high surface to volume ratio and porosity enables biomolecules and active species loading efficiently. Nanofibrous materials have particular importance in healthcare and biomedical engineering containing tissue engineering, wound dressings, drug delivery, biological sensing applications [2]. Functionalization of nanofibrous materials with antibacterial property is necessary for these applications [1, 2]. In this regard, silver nanoparticles represent a widely addressed as an antimicrobial agent with their broad-spectrum antimicrobial effects toward various type of bacteria, fungi and virus [3, 4].

Electrospinning is a largely used technique whose principles based on electrostatic interaction to fabricate polymeric or composite nanofibers from various synthetic or natural polymer solutions, polymer blends or melts in a simple, cost effective and reproducible way [5-7]. Technically, high electrical potential is applied to the solution droplet that extruded from the needle, and then polymer jet emerges out when the electrostatic force overcomes the surface tension. During acceleration of the charged polymer jet toward to the oppositely charged collector, the jet is elongated and solvent is evaporated and ultimately, a nonwoven fibrous mat composed from randomly-oriented continuous fibers is obtained [2, 5, 8, 9]. In this technique, the microstructure and diameter of fibers are a function of a number of parameters that are combined under three groups as solution properties, electrospinning parameters and ambient conditions and they have a determining role in physical, biological and chemical characteristics of electrospun mats. Therefore, controlling the fiber diameter to obtain electrospun fibers with the tuned morphology and diameter is crucial. [10, 11].

Response surface methodology (or RSM) based on mathematical and statistical techniques aims to modeling, analysis and optimization of an interested response which is effected by different variables [12]. While one-factor-at-a-time approaches are not sufficient in the case of possible factor interaction, RSM allows investigating both individual factors and their binary interaction simultaneously. Box-Behnken design is extensively used an experimental design technique based on RSM together with the Central Composite Design [10, 13]. In literature, there are several studies using Box-Behnken Design to investigate the relationship between fiber diameter and electrospinning parameters with different polymers, such as cellulose acetate [11], polypropylene [13], chitosan/hydroxyapatite [14], chitosan/bacterial cellulose [15], polyacrylonitrile/polymethylmethacrylate [16].

In this work, antibacterial polyvinylpyrrolidone nanofibers containing silver nanoparticles were prepared by electrospinning method. The effects of electrospinning parameters on average nanofiber diameter were investigated by response surface methodology based on three level, three variable Box-Behnken design. A model equation was obtained and individual and interactive effects of the parameters on the average fiber diameter were investigated.

2. Experimental details

2.1 Materials

Silver nitrate (AgNO_3 , Merck), ethanol (Merck), polyvinylpyrrolidone (PVP, Sigma-Aldrich) with the average molecular weights of 40000 (L-PVP) and 360000 (H-PVP) were used in the experiments.

2.2 Preparation of Solution

Firstly, %7.5 (w/v) L-PVP/ethanol and %7.5 (w/v) H-PVP/ethanol solutions were prepared separately by stirring for 24 h at room temperature. Then, AgNO_3 salt was added to the L-PVP/ethanol solution with an amount based on the polymer/salt weight ratio of 50. After complete dissolution with further stirring, the colour of the solution changed from colourless to brown which indicates reduction of silver ions to silver nanoparticles. Finally, electrospinning solution was prepared by stirring silver nanoparticles containing L-PVP/ethanol solution with the same volume of H-PVP/ethanol solution for 1 h.

2.3 Electrospinning Process and Experimental Design

Electrospinning was carried out in Nanospinner24-XP, Inovenso electrospinning device that using bottom-up electrospinning method. During the electrospinning experiments, temperature and relative humidity were kept at the range of 25-28°C and 61-63%, respectively to minimize the possible effects of ambient conditions on the fibers.

Box-Behnken experimental design was employed to understand the relationship between the fiber diameter (dependent variable) and electrospinning parameters

(independent variables), namely, applied voltage, flow rate and tip to the collector distance. These variables were used at three equally spaced levels coded with -1, 0 and 1 to identify low, medium and high levels, respectively, as shown in Table 1. For, Box-Behnken design, a quadratic model developed to predict the fiber diameter in terms of electrospinning parameters is given as follows:

$$y = C_0 + \sum_{i=1}^3 C_i X_i + \sum_{i=1}^3 C_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 C_{ij} X_i X_j \quad (1)$$

where y is the measured response of dependent variable, X_i , X_{ii} , and X_{ij} are linear, quadratic and interaction terms of related independent variables, C_0 , C_i , C_{ii} and C_{ij} are model coefficients. The results of the model were statistically analyzed by using analysis of variance (ANOVA) and the adequacy of the model verified by the coefficient of determination (R^2). Minitab 18.0 software (Minitab Inc., State Collage, PA, USA) was used for all statistical calculations.

Table 1: Independent variables and their levels.

Independent variables	Levels		
	-1	0	1
X_1 : Applied voltage (kV)	15	18	21
X_2 : Flow rate (ml/h)	1	3	5
X_3 : Tip to collector distance (mm)	170	200	230

2.4 Characterization

Scanning electron microscope (SEM, JEOL JSM-6510LV) was used to investigate the morphology and diameter of the electrospun nanofibers. The average fiber diameter and the standard deviation were determined from 40 randomly selected fibers from each SEM images with by the aid of Image-J software program (<https://fiji.sc/>).

3. Results and discussion:

Box-Behnken design technique for 3 variables at 3 levels offer a design matrix comprising of 15 experimental runs (12 factorial points and 3 replicates at the center point). Electrospinning experiments were performed according to this design matrix and corresponding average fiber diameter and standard deviation values are listed in Table 2. In addition, SEM images of the obtained electrospun nanofibers are shown in Fig. 1. According to the Table 2 and Fig. 1, the electrospun nanofibers having good morphology with the average diameter ranging from 335 nm (run 2) to 566 nm (run 1) were produced.

The experimental average fiber diameters were used to obtain a second order response surface model. The results of the response surface model with uncoded units were given in Table 3. Among these results, R^2 value of 0.9049 represents that the 90.49 % of total variability in the fiber diameter can be explained by the regression model. However, adjusted R^2 was calculated as 0.7336 and should be improved in order to obtain a well-fitted model. The significance probability (p-value) of the model was found as 0.041 which indicates that

the investigated model is statistically significant at the confidence interval of 95 %. Additionally, 2-way interaction terms have significant effect on the average fiber diameter having p-value as 0.011. However, p value of square terms was calculated as 0.920 meaning that they were insignificant, therefore they must be eliminated from the model. The results of the refined response surface model with uncoded units were given in Table 4.

Table 2: Experimental design matrix and the results.

Run	X ₁	X ₂	X ₃	Average Fiber Diameter (nm)	
				Experimental	Calculated
1	-1	-1	0	566±110	581
2	1	-1	0	335±55	371
3	-1	1	0	427±99	406
4	1	1	0	500±216	501
5	-1	0	-1	486±68	504
6	1	0	-1	382±154	379
7	-1	0	1	489±86	483
8	1	0	1	521±124	494
9	0	-1	-1	451±152	416
10	0	1	-1	466±178	467
11	0	-1	1	547±197	536
12	0	1	1	416±158	441
13	0	0	0	457±160	465
14	0	0	0	474±108	465
15	0	0	0	457±147	465

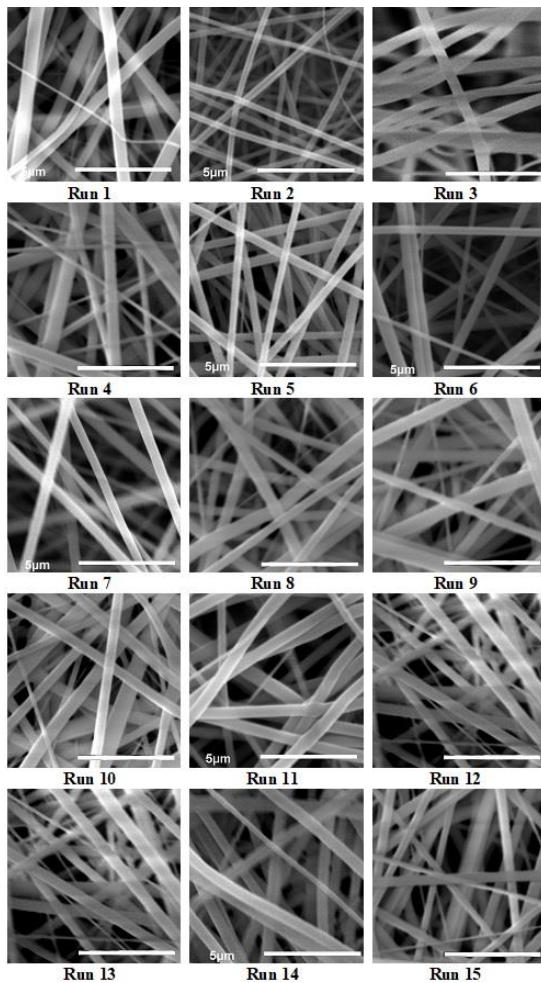


Fig.1: SEM images of the obtained electrospun nanofibers at a magnification of ×5000.

Table 3: The results of the response surface model.

Term	Coefficient	P-value
Constant	C ₀	2498.000 0.000
Linear		0.078
X ₁	C ₁	-110.800 0.047
X ₂	C ₂	-108.100 0.351
X ₃	C ₃	-8.600 0.085
Square		0.920
X ₁ ²	C ₁₁	-0.34 0.856
X ₂ ²	C ₂₂	-0.650 0.879
X ₃ ²	C ₃₃	0.011 0.565
2-way Interaction		0.011
X ₁ .X ₂	C ₁₂	12.670 0.004
X ₁ .X ₃	C ₁₃	0.378 0.079
X ₂ .X ₃	C ₂₃	-0.608 0.065
R ²	90.49 %	F-value p-value
R ² (adj)	73.36 %	Model 5.28 0.041
	Lack of fit	15.91 0.060

In the refined model equation, a small decrease in R² value and an increase in adjusted R² was observed. Such a decrease in R² value is expected because the term number in the refined model decreased after elimination of insignificant square terms. On the contrary, the adjusted R² depends on significant terms that improves the model rather than the term number in the case of R² [17]. Since the new model includes statistically significant terms after elimination of unnecessary terms, adjusted R² improved. A more reliable model was developed with a reasonable agreement with R² and adjusted R². Also, it is seen that the significance of the refined model increased by considering associated p-value of the model as 0.001. According to these results the model equation was expressed in the following form for actual value of each parameters;

$$y = 2177 - 123.1X_1 - 112X_2 - 4.19X_3 + 12.67X_1X_2 + 0.378X_1X_3 - 0.608X_2X_3 \quad (2)$$

The model equation (Eq. 2) was used to calculate fiber diameters and the results were given in Table 2. It is clearly seen from Table 2 that the calculated fiber diameters were consistent with the experimental values.

To test model associated errors, lack of fit was investigated. Lack of fit refers to the comparison between the residual error from model error and the pure error from the replicated runs. Insignificant lack of fit with the p-value higher than 0.05 is desired to confirm the model fits the response surface properly [18]. Thus, the computing lack of fit as 0.106 was improved in the refined model.

Table 4: The results of the refined response surface model.

Term	Coefficient	P-value
Constant	C_0	2177.000 0.000
Linear		0.018
X_1	C_1	-123.100 0.013
X_2	C_2	-112.000 0.249
X_3	C_3	-4.190 0.032
2-way Interaction		0.001
$X_1.X_2$	C_{12}	12.670 0.000
$X_1.X_3$	C_{13}	0.378 0.029
$X_2.X_3$	C_{23}	-0.608 0.021
R^2	89.59 %	F-value p-value
$R^2(\text{adj})$	81.77 %	Model 11.47 0.001
	Lack of fit	8.74 0.106

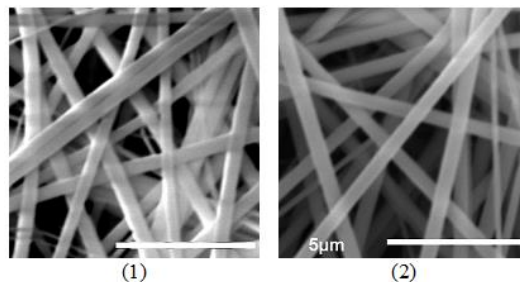


Fig.3: SEM images of the validation experiments (1) and (2) at a magnification of $\times 5000$.

For the further testing of the adequacy of the model, normal probability plot (Fig. 2 (a)) and residual graph (Fig. 2 (b)) were used. In the normal probability plot, residuals scattered around a linear line demonstrating that the errors (difference between predicted values from model and the experimental outcomes) are normally distributed without a systematic trend. On the other hand, the residual graph shows that residuals were independent from the observation order since they were randomly distributed without a pattern.

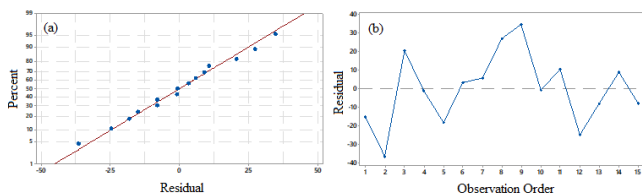


Fig.2: Normal probability plot (a) and residual graph (b).

In order to test the prediction accuracy of the obtained model, two additional experiment which were not involved in the design matrix but remained in the limits of design space were performed. Average fiber diameters that calculated from SEM images of the validation experiments (Fig. 3) and predicted from the model equation were compared in Table 5. 95% confidence intervals of the validation experiments (1) and (2) were found as 468 nm – 520 nm and 463 nm – 514 nm, respectively. Since the experimental findings stayed between these limits, it can be said that the model is adequate in predicting the fiber diameter in terms of electrospinning parameters.

Table 5: The results of validation experiments.

	X_1	X_2	X_3	Average fiber diameter (nm)	
				Experimental	Predicted
1	15	3	200	506 \pm 118	494
2	18	3	230	473 \pm 160	488

Contour and surface plots depict the relationship between the dependent and independent variables within the design space in 2D and 3D display, respectively. In these graphs, dependent variable is presented with respect to two independent variables. Contour and surface plots of the investigated model were given in Fig 4 and 5. Two electrospinning parameters were investigated in each figure, while the other parameter was hold constant at the medium level (coded as 0). Increasing voltage led to decrease in fiber diameter until a threshold value of flow rate and after that point, the trend reversed. Similarly, fiber diameter decreased when voltage increased in low and medium levels of distance, but in the high value of distance, it was not affected from voltage change. On the other hand, increasing distance until the medium level of flow rate caused an increase in fiber diameter, above this limit, fiber diameter remained constant regardless of distance.

Moreover, minimum and maximum fiber diameter formation regions can be observed from the contour and surface plots. Minimum fiber diameter was seen in the region of high voltage, low flow rate and low distance where the electrospinning parameters are 21 kV, 1 ml/h and 170 mm. Nevertheless, maximum fiber formation was seen in the region of low voltage, low flow rate and high distance where the electrospinning parameters are 15 kV, 5 ml/h and 230 mm.

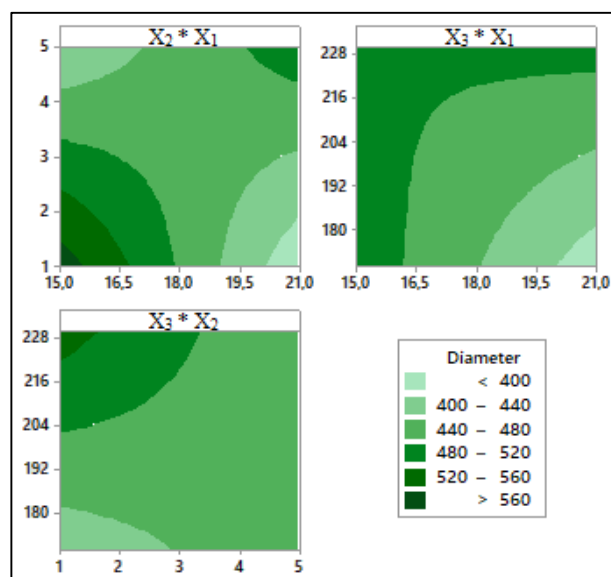


Fig.4: Contour plots of the model.

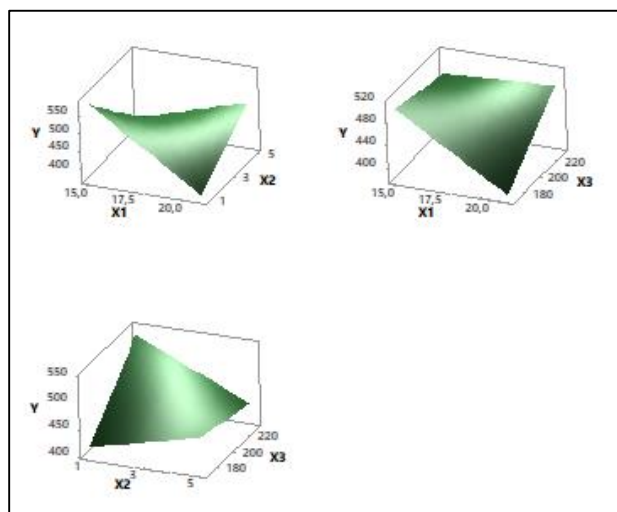


Fig.5: Surface plots of the model.

Conclusions

PVP nanofibers containing silver nanoparticles for the potentially usage in biomedical applications were successfully produced by electrospinning method. In order to understand the effects of the electrospinning parameters on fiber diameter and eventually control it, a response surface model based was developed by employing three level-three factor Box Behnken design technique. By evaluating the ANOVA results, statistically significant model equation consisting of linear and interaction effects of the electrospinning parameters was obtained with 89.59% R^2 at 95% confidence level. Validation experiments proved the accuracy of the model in predicting the fiber diameter in altered electrospinning parameters. By the aid of contour and surface plots, the response of the fiber diameter on electrospinning parameters were observed. As a result, targeted fiber diameter according to the requirements of the different applications can be provided by carrying out the experiments on certain response surface regions.

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