

Optimization of Electrospinning Parameters for Chitosan Nanofibres

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Abstract

Electrospinning of chitosan, a naturally occurring polysaccharide biopolymer, has been investigated. In this paper, we report the optimization of electrospinning process and solution parameters using factorial design approach to obtain uniform chitosan nanofibres. The parameters studied were electric field strength, ratio of solvents - trifluoroacetic acid (TFA)/ dichloromethane (DCM), concentration of chitosan in the spinning solution, their individual and interaction effects on the diameter of nanofibres. The selected parameters were varied at three levels (-1, 0 and +1) using Box and Behnken factorial design. The interaction effect between electric field strength (FS) and concentration of chitosan (CC) as well as that between the ratio of solvents - TFA/DCM (SC) and electric field strength played the most significant role, followed by the concentration of chitosan and lastly by the electric field strength in obtaining uniform nanofibres.

Keywords: Chitosan, Electrospinning, Morphology, Nanofibres, Optimization.

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INTRODUCTION

In the past decade, research has seen resurgence in electrospinning of nanofibres from natural polymers owing to vast possibilities for their enhanced use in bio-based materials [1-5]. Many parameters like solution properties, processing and ambient conditions influence the transformation of polymer solution into nanofibres during the electrospinning process [1-12]. Owing to their distinctive properties, nanofibres have found applications in wound dressing, filtration, composites, tissue engineering and others [4, 13–17]. For most applications, it is always desirable to have smooth, ultrafine nanofibres with uniform diameter.

A number of research results on the optimization of electrospun chitosan nanofibres, by investigating one parameter at a time, have been reported in the literature. Torres-Geiner *et al.* 2008 studied the effect of electrospinning parameters (molecular weight, polymer concentration, ratio of solvents - TFA/DCM and distance between the nozzle tip and the collector) on the morphology and diameter of electrospun nanofibres from chitosan solutions [1]. They concluded that polymer concentration was the most influential parameter, followed by the tip-to-collector distance. Son *et al.* 2009 investigated viscosity and water content of chitosan /poly (vinyl alcohol) (PVA) nanofibres with antibacterial activity [18]. They discovered that the yield of low-viscosity chitosan/PVA nanofibres was higher than that of high-viscosity chitosan/PVA. Also, the study on the effect of solvent and the concentration of chitosan on the morphology of the resulting nonwoven fabrics was investigated by Ohkawa *et al.* 2004 [19]. They concluded that increased concentration of chitosan improved the morphology of electrospun fibres while the addition of dichloromethane to the chitosan-TFA solution improved the homogeneity of the electrospun nanofibres. In all the cases, none of the multiple variables were taken into account in establishing the relationship between the process parameters and fibre diameter. Neglecting the influence of other parameters associated with the process, sometimes leads to contradictory results as reported in some published literature [1–12]. In this regard, response surface methodology using Box and Behnken factorial design is a very useful tool in studying the influence of multiple variables acting simultaneously [20, 21].

The objective of this work is to study the interaction effect of the process and solution parameters in obtaining uniform and beadless nanofibres, aiming at obtaining nanofibres with smaller diameter. Box and Behnken factorial design with three variables (i.e. electric field strength, ratio of solvents - TFA/DCM and concentration of chitosan) varied at three levels (-1, 0 and +1) was selected. The response surface methodology was applied to predict fibre diameters from the processing parameters.

MATERIALS AND METHODS

Chitosan from crab shells (minimum 85% of deacetylated) was obtained from Sigma- Aldrich. In the present work, various concentrations of chitosan were electrospun using different processing parameters. All laboratory grade chemicals and solvents were used without further purification, whereas all electrospinning experiments were carried out at room temperature. Based on our previous studies [11, 16-17], the electrospinning setup consisted of a pasteur pipette with 1 mm diameter, an electrically grounded metal screen and a high voltage power supply ranging from 0-30 kV. For all experiments, the flow rate of the polymer solution was determined by the angle at which the Pasteur pipette was tilted. The following parameters were varied at three levels from -1, 0 and +1 during electrospinning of chitosan nanofibres as per the factorial experimental design: electric field strength; 0.65, 0.70, 0.75 kV/cm, concentration of chitosan; 5, 5.5, 6 % w/v, ratio of solvents - TFA/DCM; 70/30, 75/25, 80/20 % v/v. Table 1 shows the coded levels of the parameters while the experimental design and average diameter of nanofibres for three factors at three levels is shown in Table 2. The structural morphology and diameter of the electrospun nanofibres were determined on Scanning Electron Microscope (SEM) FEI Quanta 200.

RESULTS AND DISCUSSION

Morphology of Nanofibres and Response Surface Function

By multiple regression analysis, coefficient of the parameters (electric field strength, ratio of solvents - TFA/DCM and concentration of chitosan), significance probability (*P*-value) and correlation coefficient were obtained (Table 3). The significance test at 95% confidence interval was conducted and *P*-values as a measure of statistical significance are shown in Table 3, if it is less than 0.05, then the variable has significant effect on the average fibre diameter and vice versa. The interactions between electric field strength and concentration of chitosan as well as interactions between ratio of solvents - TFA/DCM and electric field strength showed the most significant effect, followed by concentration of chitosan, and lastly by electric field strength as shown by calculated coefficients and *P*-values. In such a scenario, response surface plots could be used to further determine the coefficients that play a dominant role, which is discussed further. Equation (1) is a relationship between the dependent variable (diameter) and independent variable (process and solution parameters). This equation is established after a series of regression calculation done at 95% confidence interval.

The response surface equation for an average fibre diameter is given by:

$$y = C_0 + C_1x_1 + C_2x_2 + C_{12}x_1x_2 + C_{31}x_3x_1$$

The calculated equation is:

$$y = 198.8 - 9.750x_1 + 13.625x_2 + 14x_1x_2 + 14x_3x_1 \quad (i)$$

where, y is the average fibre diameter, x_1 , x_2 and x_3 are the coded values of electric field strength, concentration of chitosan and ratio of solvents - TFA/DCM, respectively. The co-efficient of determination (R^2) between the experimental and calculated values obtained from the response surface equation is 0.769. This value indicates a good correlation between the process and solution parameters in obtaining uniform fibre diameter.

The response surfaces of fibre diameter as a function of selected processing parameters are discussed along with their structural morphology. Three different cases have been considered as per the statistical significance and dominance of the selected parameters in obtaining uniform fibre diameters. Fig. (1) indicates some randomly selected SEM images from 15 sets of experiments showing the morphology of chitosan electrospun nanofibres.

Response Surface Plots of Fibre Diameter

(a) Function of Electric Field Strength and Ratio of solvents - TFA/DCM for Different Concentrations of Chitosan

The interaction effect of electric field strength and ratio of solvents - TFA/DCM on fibre diameters at 5.5% v/v concentration of chitosan is shown in Fig. (2a). The values of fibre diameter at -1, 0 and +1 levels of electric field strength are 222 nm, 208 nm and 194 nm, respectively. With the increase in electric field strength from -1 to 0 level, there is 6% decrease in fibre diameter and from 0 to +1 level, about 7% decrease in fibre diameter is observed. Overall, with the increase in electric field strength from -1 to +1 level, 13% decrease in fibre diameter results. This is attributed to the increased stretching of the jet at higher charge density as a result of increased electric field strength leading to formation of finer fibres [9, 11, 16]. Also, the fibre diameter at -1, 0 and +1 levels of ratio of solvents - TFA/DCM are 222 nm, 198 nm and 176 nm, respectively. With the increase in levels of ratio of solvents - TFA/DCM from -1 to 0 level, there is 11% decrease in fibre diameter and from 0 to +1 level, there is further 11% decrease in fibre diameter. Overall, with the increase in levels of ratio of solvents - TFA/DCM from -1 to +1 level, there is about 21% decrease in fibre diameter, thus yielding finer fibres. This may be due to the mixture of solvent (TFA/DCM, 70/30% v/v) reported as the best to electrospin chitosan [1]. Due to its lower dielectric constant and boiling point much lesser than that of water, the presence of DCM in the system increased the rate of evaporation of the solvent, which reduced the excessively strong charge density originated by TFA, thus, resulting in ultrafine fibres.

The combined influence of electric field strength, ratio of solvents - TFA/DCM and concentration of chitosan on the fibre diameter shows that +1 level of electric field strength (0.75kV/cm), +1 level of ratio of solvents - TFA/DCM (80/20% v/v) and 0 level of concentration of chitosan (5.5% w/v) give uniform nanofibre. The optimized region for the above selected parameters lies just below this region.

Similarly, the contour plots of effect of levels of electric field strength and ratio of solvents - TFA/DCM on fibre diameters at 6% concentration of chitosan is shown in Fig. (2b). With the increase in electric field strength from -1 to +1 level, there is 12% decrease in fibre diameter. This is following a similar trend observed in Fig. (2a), where a decrease in fibre diameter as a result of increased electric field strength is elucidated by increased electrostatic force that is encouraging the elongation of the jet, yielding thinner fibres. Also, an increase in ratio of solvents - TFA/DCM from -1 to +1 level indicates a 9% decrease in the diameter of fibres. This might be due to the reasons mentioned above, where a ratio of solvent (TFA/DCM, 70/30% v/v) is sufficient enough to balance the evaporation rate by DCM and reducing excessive charge density created by TFA, leading to finer fibres.

(b) Function of Electric Field Strength and Concentration of Chitosan for Different Values of Ratio of solvents - TFA/DCM

The interaction effect of levels of electric field strength and concentration of chitosan for different values of ratio of solvents - TFA/DCM is shown in Fig. (2c). The ratio of solvents - TFA/DCM was -1 level (70/30% v/v), 0 level (75/25% v/v) and +1 level (80/20% v/v), respectively. The average fibre diameter at -1 level, 0 level and +1 level of electric field strength are 220 nm, 184 nm and 151 nm, respectively. With an increase in levels of electric field strength from -1 to 0 levels, there is a 16% decrease in fibre diameter. Further increase in levels of electric field strength from 0 to +1 levels, there is a 18% decrease in fibre diameter. Overall, with the increase in levels of electric field strength from -1 to +1 level, there is a 31% decrease in fibre diameter, which indicates that the fibres are getting smaller. This might be due to the increasing charge density carried by the jet with the increasing electric field strength, leading to an increase in stretching and elongation of the jet and ultimately making the fibres thinner. With an increase in concentration of chitosan from -1 to +1 level, there is no change in fibre diameter (Fig. (2c)). This might be due to the influence by other two parameters namely, electric field strength and ratio of solvents - TFA/DCM.

Similar trend is observed in Fig. (2d), with the interaction effect of levels of electric field strength and concentration of chitosan for different values of ratio of solvents - TFA/DCM at -1 level (70/30% v/v), 0 level (75/25% v/v) and +1 level (80/20% v/v), respectively. The average fibre diameter at -1 level, 0 level and +1 level

of electric field strength are 210 nm, 185 nm and 163 nm, respectively. With an increase in levels of electric field strength from -1 to 0 levels, there is a 12% decrease in fibre diameter. Further increase in levels of electric field strength from 0 to +1 levels, there is a 12% decrease in fibre diameter. Overall, with the increase in levels of electric field strength from -1 to +1 level, there is a 22% decrease in fibre diameter. The results are concurrent with the results in Fig. (2a) and Fig. (2b) as discussed above. This is attributed to the coulombic repulsive forces in the jet, stretching the viscoelastic solution. Increase in electric field strength leads to an increase in charge density, thereby causing the jet to accelerate faster while encouraging more stretching and production of thinner fibres. Again, the increase in concentration of chitosan from -1 to +1 level, indicates no change in fibre diameter (Fig. (2d)). This might be due electric field strength and ratio of solvents - TFA/DCM.

(c) Fibre Diameter as a Function of Concentration of Chitosan and Ratio of solvents - TFA/DCM for Different Electric Field Strengths

The interaction effect of concentration of chitosan and ratio of solvents - TFA/DCM on fibre diameters at different electric field strength is shown in Fig. (3a). From the regression analysis, there is no significant interactions between concentration of chitosan and ratio of solvents - TFA/DCM as shown in Table 3. The interaction effect of concentration of chitosan and ratio of solvents - TFA/DCM on fibre diameters for electric field strength of 0.65kV/cm (-1 level) is shown in Fig. (3a). With an increase in concentration of chitosan from -1 to +1 level, there is no change in fibre diameter. However, with an increase in levels of ratio of solvents - TFA/DCM from -1 to +1 level, there is a decrease in fibre diameter. In (Fig. (3b)), the contour plots of interaction effect of concentration of chitosan and ratio of solvents - TFA/DCM on fibre diameters show a completely different trend. With an increase in levels of concentration of chitosan and ratio of solvents - TFA/DCM from -1 to +1 level, there is an increase in fibre diameter (Fig. (3b)). This is attributed to a lack of significance in the interaction between the variables involved (Table 3). Such responses were not observed in the previous scenarios (Fig. (2a) and Fig. (2b)), where significant interactions between the variables were observed (Table 3). Both these plots (Fig. (3a) and Fig. (3b)) indicate contrast in situations where there is no interaction between the selected set of variables, similar contradiction is generally found in electrospinning process for a number of variables as reported in published literatures [1-10]. Clearly, it shows that it is crucial to optimize the process by simultaneously studying interaction effect of variables rather than drawing conclusions on the basis of investigating the effects of individual parameters only.

CONCLUSIONS

The interactions between electric field strength and ratio of solvents - TFA/DCM as well as between electric field strength and concentration of chitosan showed the most significant effect, followed by concentration of chitosan, and lastly by electric field strength alone to obtain nanofibres with uniform diameters. The combined influence of electric field strength, ratio of solvents - TFA/DCM and concentration of chitosan on fibre diameter shows that +1 level of electric field strength (0.75kV/cm), +1 level of ratio of solvents - TFA/DCM (80/20% v/v) and 0 level of concentration of chitosan (5.5% w/v) provided uniform nanofibres with the lowest diameter of 176 nm. This study has proven that the interaction between the different variables played a significant role compared to a singular parameter in obtaining uniform nanofibres.

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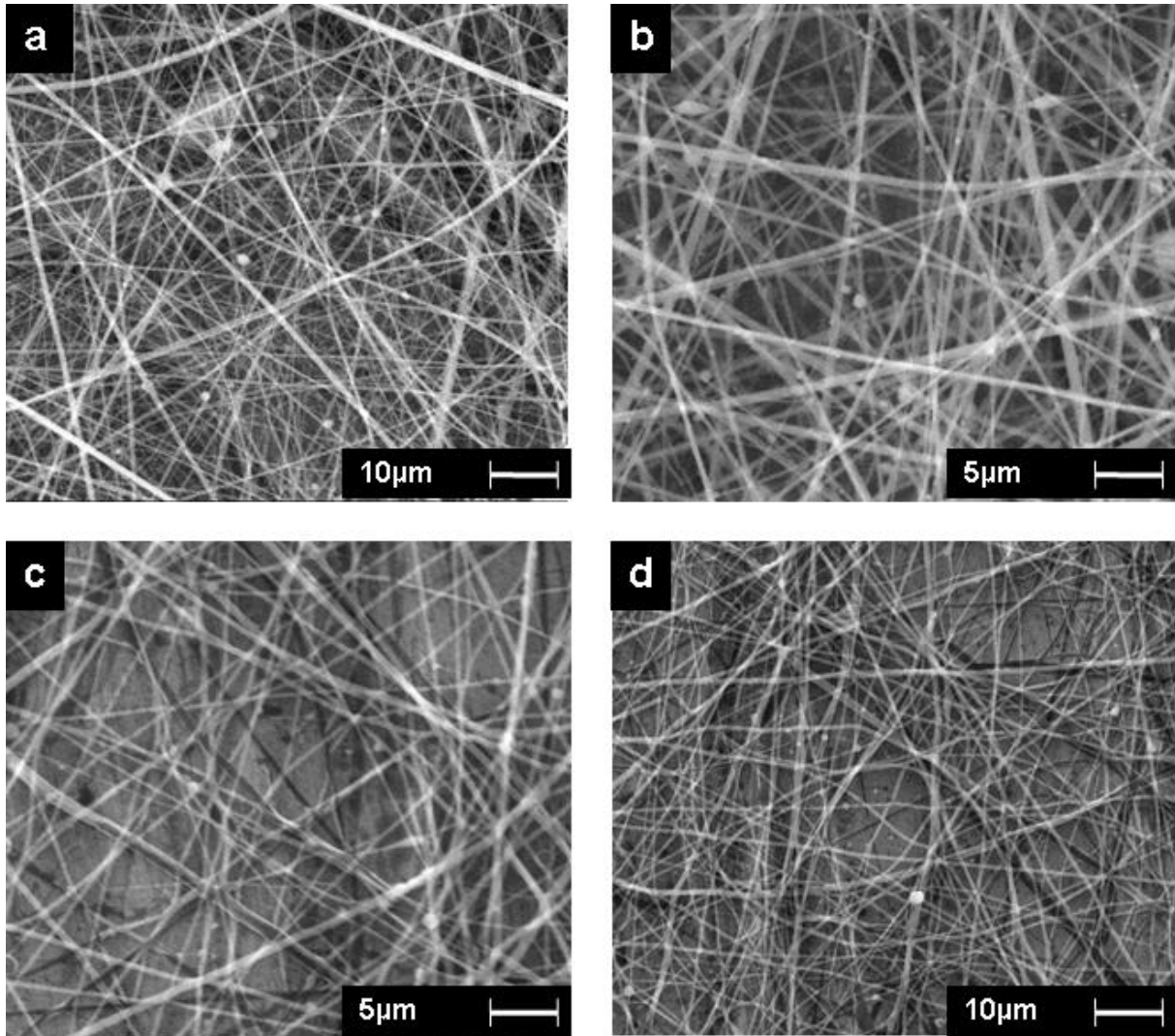


Fig. (1). SEM images showing the morphology of chitosan electrospun nanofibres for experimental combination numbers: (a) 9 (b) 15 (c) 1 (d) 6.

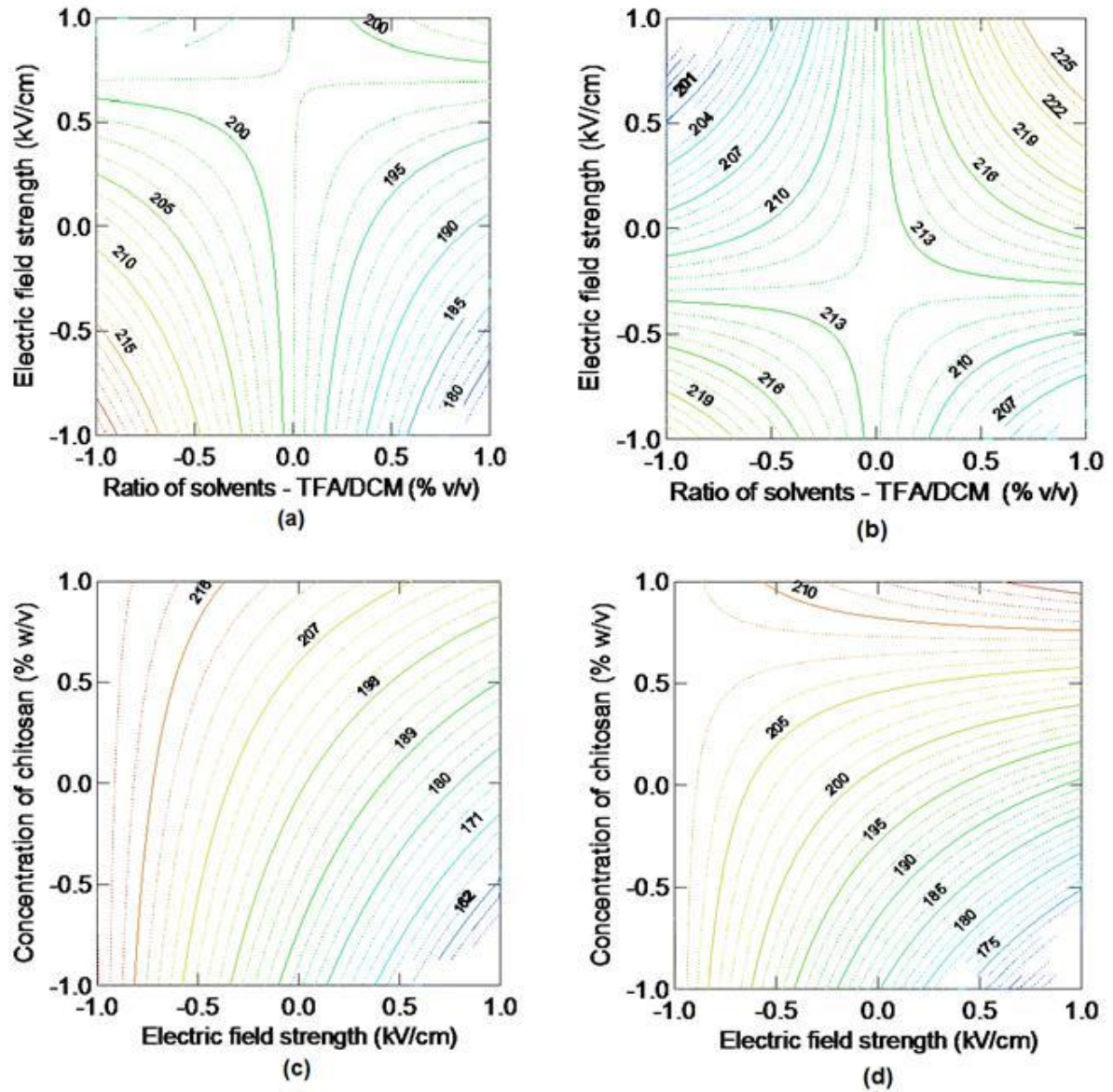


Fig. (2). Contour plots of interaction effect of levels of (a) and (b) Electric field strength and ratio of solvents - TFA/DCM on nanofibre diameters for variable concentration of chitosan: (c) and (d) Electric field strength and concentration of chitosan on nanofibre diameters for variable ratio of solvents - TFA/DCM.

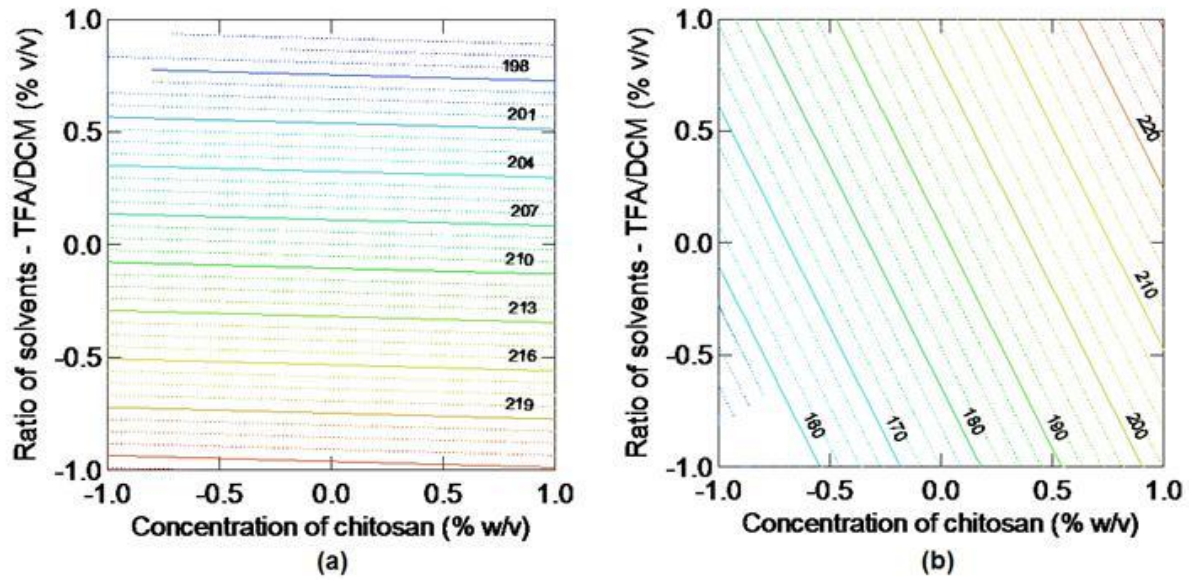


Fig. (3). (a) and (b) Contour plots of interaction effect of levels of ratio of solvents - TFA/DCM and concentration of chitosan on nanofibre diameters for variable electric field strength

Tables
Table 1. Levels of variables for design of experiment

Variables (or parameters)	Coded levels		
	-1	0	+1
Electric field strength, x_1 (kV/cm)	0.65	0.70	0.75
Concentration of chitosan, x_2 , (% w/v)	5	5.5	6
Ratio of solvents - TFA/DCM x_3 , (% v/v)	70/30	75/25	80/20

Table 2. Coded levels and actual values of variables for different experimental combinations along with average fibre diameter

Experimental combination number	Coded level of variables			Actual values			Average fibre diameter (nm)
	x_1	x_2	x_3	x_1	x_2	x_3	
1	-1	-1	0	14.95/23	5	75/25	211
2	+1	-1	0	20.25/27	5	75/25	169
3	-1	+1	0	14.95/23	6	75/25	193
4	+1	+1	0	20.25/27	6	75/25	207
5	-1	0	-1	14.95/23	5.5	70/30	229
6	+1	0	-1	20.25/27	5.5	70/30	176
7	-1	0	+1	14.95/23	5.5	80/20	199
8	+1	0	+1	20.25/27	5.5	80/20	202
9	0	-1	-1	17.50/25	5	70/30	189
10	0	+1	-1	17.50/25	6	70/30	233
11	0	-1	+1	17.50/25	5	80/20	174
12	0	+1	+1	17.50/25	6	80/20	219
13	0	0	0	17.50/25	5.5	75/25	191
14	0	0	0	17.50/25	5.5	75/25	194
15	0	0	0	17.50/25	5.5	75/25	196

Table 3. Analysis of variance for three variables (electric field strength, concentration of chitosan, ratio of solvents - TFA/DCM), significance probability (P -value) and correlation coefficient

Term	Coefficient	P -value*
Constant	$C_0 = 198.000$	0.000
Electric field strength: x_1	$C_1 = -9.750$	0.028
Concentration of chitosan: x_2	$C_2 = 13.625$	0.005
Electric field strength \times Concentration of chitosan: $x_1.x_2$	$C_{12} = 14.000$	0.026
Ratio of solvents - TFA/DCM \times Electric field strength: $x_3.x_1$	$C_{31} = 14.000$	0.026

* $R^2 = 0.769$