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Evaluation of process parameters for fiber alignment of electrospun tubular nanofibrous matrices: a DoE approach

Rossella Dorati[1,5], Enrica Chiesa[1], Silvia Pisani[2], Ida Genta[1,5], Tiziana Modena[1,5], Giovanna Bruni[3], Chiara R.M. Brambilla[1], Marco Benazzo[4], Bice Conti[1,5]

[1]Department of Drug Sciences and [3]Department of Chemistry, University pf Pavia, V.le Taramelli 12/14, 27100 Pavia, Italy.

[2] Department of Paediatric Oncoaematology IRCCS Policlinico S.Matteo, Pavia, P.zzle Golgi 1, 27100 Pavia, Italy

[4]Department of Otholaryngology Head Neck Surgery, University of Pavia and IRCCS Policlinico S.Matteo, P.zzle Golgi 1, 27100 Pavia, Italy.

[5]Polymerix srl, V.le Taramelli 20, 27100 Pavia

Abstract

Electrospinning is known to be an effective and straightforward technique to fabricate polymer non woven matrices made of nano and microfibers. Micro patterned morphology of electrospun matrices results to be outmost advantageous in the biomedical field, since it is able to mimic extracellular matrix (ECM), and favors cell adhesion and proliferation. Controlling electrospun fibers alignment is crucial for the regenerative purposes of certain tissues, such as neuronal and vascular. In this study we investigated the impact of electrospinning process parameters on fiber alignment in tubular nanofibrous matrices made of Poly (L-lactide-co-ε-caprolactone) (PLA-PCL); a Design of Experiments (DoE) approach is here proposed in order to statistically set up the process parameters. The DoE was studied keeping constants the previously set material and environmental parameters; voltage, flow rate and mandrel rotating speed were the process parameters here investigated as variables. Orientation analysis was based on ImageJ and plugin Orientation J analysis of SEM images. The results show that voltage combined with flow rate has significant impact on electrospun fiber orientation, and the greatest orientation is achieved when all the three input parameters (voltage, flow rate and mandrel rotation speed) are at their maximum value.

Keywords:
Nanomaterials; Electrospun nanofibers; biodegradable 3D scaffolds; Electrospinning;
Design of Experiments.

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1. Introduction

Electrospinning is known to be an effective and straightforward technique to fabricate polymer non woven matrices made of nano and microfibers. The technique is based on interfacial phenomena rising when an electric field is applied to a polymer solution. The electric field generates a charge in the polymer solution, and when the electric force overcomes the solution surface tension, a polymeric jet, making a typical Taylor cone, is generated from the surface tension and travels towards the collector [1, 2]. Viscosity of the polymer solution and its surface tension are interconnected since high viscosity stabilizes the forming fiber, while low surface tension promotes its stretching. Electrospun nanofibrous matrix forms due to deposition of entangled polymer fibres on the collector; polymer solvent evaporation from is provided in jet pathway between needle and collector.

Electrospinning technique has been applied to several fields such as filtration, electronic and photonic technology, and it has drawn particular attention in the biomedical area of tissue engineering because the electrospun matrices, made of suitable biodegradable and biocompatible polymers, can be seeded with cells and act as scaffold for tissue regeneration. An important advantage of the electrospun matrices is their micro patterned morphology that mimic extracellular matrix (ECM), thus favouring cell adhesion and proliferation. It has been demonstrated in the literature that aligned nanofibers are able to orient cells in a specific direction needed to provide the anisotropy encountered in certain organs including blood vessels, muscular tunic of esophagus, urethra, and in general of any hollow organ or tubular structure, and in tissues such as neuronal tissue [3-8]. Thus, the electrospinning parameters should be profitably adjusted in order to control diameter, alignment of fibers, meanwhile keeping suitable spinnable regimen of the polymer solution.

These parameters can be classified as follows: *i)* process parameters (electric field, polymer solution flow rate, syringe needle gauge, mandrel rotating speed), *ii)* material parameters (polymer solution viscosity, surface tension and ionic conductivity, polymer molecular weight) and *iii)* environmental parameters (relative humidity (RH) and temperature of electrospinnng chamber). Several authors account material parameters to be the most decisive factors for spinnability and uniform shape of the fibers, while environmental parameters are derived thereof. Eventually, once fixed the materials and environmental parameters, process parameters can play a significant role in affecting size and alignment of the fibers [9-11]. The topic drawn interest for several years, and it still is of interest for those scientists working in the area of tissue engineering, as demonstrate the papers found in the literature [12-17].

Aim of this work was study the electrospinning process parameters through a quality by design approach. A Design of Experiments (DoE) was set up in order to statistically evaluate which electrospinnig process parameters affect nanofiber alignment in electrospun tubular matrices made of Poly (L-lactide-co-ε-caprolactone) (PLA-PCL).

PLA-PCL was selected because it is a biodegradable and biocompatible polymer whose properties, such as long biodegradation time and elasticity, make it suitable for application in regeneration of blood vessel and other hollow tubular human organs. The copolymer can also be used in combination with other biodegradable polymers such as Polylactide or Polylactide-co-glycolide. However, a lot of studies in the literature characterize these last polymers, while less investigation has been performed on PLA-PCL copolymer, highlighting there is room for studies on PLA-PCL addressed to regeneration purposes [17-20].

Quality by design approach is quite common in the pharmaceutical industry and it was applied to electrospinning process with interesting results towards different variables and diverse polymers [21][22]

Data processing: ImageJ software was used in this work to measure the orientation distribution function of the fibers in a plane environment. This post processing technique is based on digital imaging and takes advantages of using algorithm method to measure fiber orientation. The technique finds consolidated application more in general with quality target product design and more specifically for fiber alignment evaluation in particular in electrospinning [23][24, 25]

The investigated electrospun matrices are intended for tissue engineering applications, namely vascular grafts or esophagus reconstruction, for this reason in this work attention was focused on a biodegradable biocompatible polymer already approved by FDA for use in the human body.

2. Materials and methods

2.1. Materials

Poly (L-lactide-co-ε-caprolactone) 70:30, Mw 160241 Da (PLA-PCL, RESOMER LC 703 S) was from Evonik Industries (Evonik Nutrition & Care GmbH, 64275 Damstadt, Germany).

Methylen chloride (MC) 99,9% purity and N,N-Dimethylformammide (DMF) 99,8% purity were from Carlo Erba (Carlo Erba SpA, Milano, Italia).

2.2. Methods

2.2.1. Choice of polymer solvent and polymers ratio/concentration (material parameters)

The solvents used in electrospinning process should have low boiling points, in order to evaporate on the way to the electrospinning collector, and high dielectric constant in order to promote fiber stretching. PLA-PCL is soluble in several organic solvents such as methylene chloride, chloroform, carbon tetrachloride, dimethylformamide, tetrahydrofuran. The solvents selected to solubilize PLA-PCL were Methylene chloride (MC) and Dimethylformamide (DMF). MC was chosen because, is the one showing the lowest boiling point, i.e. 40 °C, among the polymer solvents. However, its dielectric constant is 8.93, very low, and mixing with DMF, whose dielectric constant is 36.7, although its high boiling point is 153 °C, permits to rise the dielectric constant of polymer solution, improving nanofiber formation process. The properties of the selected polymer solvents, to be considered for electrospinning process, are summarized in **Table 1**.

Table 1: Properties of the polymer solvents (Boiling point, Viscosity, Electric conductivity, Dielectric constant and Surface tension) selected for electrospinning process

SOLVENTS	Boiling point (°C)	Viscosity (mPa·s)	Electric conductivity (mho)	Dielectric constant	Surface tension (Dyne/cm)
Methylene chloride (MC)*	39.7	0.410	4.3×10^{-11}	10.7	28.20
(N,N) - Dimethylformamide (DMF)*	153	0.802	6.0×10^{-2}	36.70	36.42

*Data from PubChem and Dow Chemical Company product information

Polymer concentration in the solvent mixture, and the solvents ratio was determined by evaluating the crossover point between surface tension and viscosity curves of the copolymer solutions at fixed concentration and different solvent ratios. According to the literature [16] this corresponds to the best condition for electrospinning, since it is the point of lowest surface tension and highest viscosity (namely high viscosity stabilizes the forming fiber and low surface tension favors its stretching).

Surface tension of PLA-PCL 10 % and 15 % w/v solutions in the solvent mixtures of MC:DMF 60:40, 70:30, 75:25, 80:20 v/v ratios, were measured by Du Nouy's tensiometer with a manual force tensiometer K6 (KRÜSS GmbH – Hamburg, Germany). The method involves slowly lifting a platinum ring from the surface of a liquid. The force required to raise the ring from the liquid's surface is directly proportional to its surface tension (γ) according to the following Equation (1):

$$\gamma = \frac{F \times k}{2 \times \Phi} \qquad \text{Equation} \quad (1)$$

where F is the force, k is the correction factor and Φ is the ring's circonference.

Viscosity values of the PLA-PCL solutions at 10% and 15 % w/v in solvent mixtures of MC:DMF 60:40, 70:30, 75:25, 80:20 v/v ratios were measured by Rheometer Kinexus Pro Malvern (Alfa Test, Cinisello Balsamo, Italy) with cone-plate geometry, constant share rate 100 s^{-1}, 25 °C and solvent trap. The share rate value was chosen because it simulates the one that develops inside electrospinning apparatus during ejection process.

2.2.2 Electrospinning of PLA-PCL

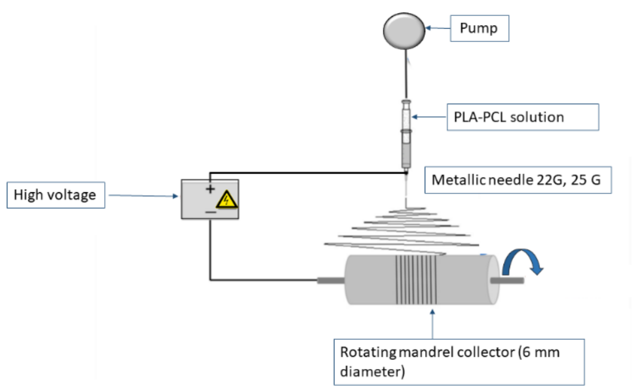

Figure 1 – Scheme of basic electrospinning set up equipped with pump, high voltage supply and rotating mandrel collector.

Electrospinning apparatus Nanon-01A (MECC Instruments, Ltd, Ogo-shi, Fukuoka, Japan) equipped with a dehumidifier system (196-1 Fukudo Ogori-Shi, Japan) was used. Electrospinning process schematized in **Figure 1** works as follows. Polymer solution is loaded in a 5 mLLuer lock plastic syringe (Luer lock syringe, DB) and it is pumped through the syringe needle, across an electric field generated by application of high voltage between the syringe needle (primary electrode) and the collector (counter electrode). The electrospinning spinneret set is vertical and moves with 50 mm/s fixed speed, a rotating mandrel was chosen as collector, whose diameter was 6.0 mm, and 46.5 cm^2 surface area. The gap between nozzle and the rotating mandrel collector was maintained at 15 cm. Fiber deposition is driven by both electric field and mandrel rotation, this latter developing a centripetal force on fibers. Electrospinning time was 15 minutes for all samples. This time was chosen because it allows to form entangled matrices completely coating the rotating mandrel surface independently of rotating mandrel speed. The electrospinning time was fixed and it was not considered a variable, as long as electrospinning temperature and relative humidity (RH%) that were 25 °C and 40% respectively.

2.2.3 Design of experiment (DoE)

DoE is a systematic method to determine the relationship between variables affecting a process and the output of that process. It is a statistical driven powerful data collection and analysis tool that has been recognized useful in order to set up and optimize process parameters [20].

A DoE full factorial experimental design was applied, applied voltage (kV), mandrel rotating speed (rpm) and polymer solution flow rate (mL/h) were the input variables (process parameters), and fiber orientation was the output parameter analyzed as summarized in **Table 2**. The design matrix for the evaluated variables took into account all the possible combinations of maximum and minimum values for each input variable, as reported in **Table 3**. Maximum value was defined +1 and minimum value -1, intermediate value 0. Number of possible combinations depends on number of input variables considered and was 2^n, where n corresponded to the number of input variables evaluated. **Table 4** reports the design matrix of the 9 possible combinations of the 3 input variables considered in this work.

The experiments were executed in random order as protection against dormant variables; two degree of freedom were available to evaluate the experimental error. Response surface analysis was applied as optimization technique, where the response of a series of full factorial experiments was mapped to generate mathematical equations that describe how factors affect the response. Materials parameters, i.e. PLA-PCL solution concentration and solvent mixture composition were set following the results of preliminary evaluation on surface tension and rheology measurements, and they were not considered input variables of DoE.

Table 2: DoE matrix set up

Number of input variables	**3**
Number of output parameters	1
Number of runs	9, including one central point per block
Degree of freedom	2
Randomized	Yes

Table 3: Minimum, maximum and intermediate values of set input parameters

	Minimum value (-1)	**Intermediate value (0)**	**Maximum value (+1)**
Voltage (kV)	22	26	30
Flow rate (mL/h)	1	3	5
Mandrel rotation speed (rpm)	1000	1750	2500

Table 4: Screening design matrix of the 9 possible combinations of the 3 input variables considered.

Voltage (kV)	**Flow rate (mL/h)**	**Mandrel rotating speed (rpm)**
+1	+1	+1
+1	+1	-1
-1	+1	+1
+1	-1	+1
-1	+1	-1
+1	-1	-1
-1	-1	+1
-1	-1	-1
0	0	0

Starting from the matrix reported in **Table 4**, preliminary electrospinning tests were carried out in order to select which could be the highest and the lowest value of each parameter achieving a stable polymer jet in the electrospinning process, i.e. able to generate a Taylor cone. Please note that the maximum voltage value corresponded to the maximum value allowed by the apparatus specifications. The values of set input parameters derived from these preliminary tests are listed in **Table 3**, and the consistently built design matrix is reported in **Table 5.**

Table 5: DoE design matrix derived from the values in **Tables 3 and 4**

Batch n. (run number)	**Voltage (kV)**	**Flow rate (mL/h)**	**Mandrel rotation speed (rpm)**
1	30 (+1)	5 (+1)	2500 (+1)
2	30 (+1)	1(-1)	2500 (+1)
3	22 (-1)	5 (+1)	2500 (+1)
4	30 (+1)	5 (+1)	1000 (-1)
5	22 (-1)	1 (-1)	2500 (+1)
6	30 (+1)	1(-1)	1000 (-1)
7	22 (-1)	5 (+1)	1000 (-1)
8	22 (-1)	1 (-1)	1000 (-1)
9	26 (0)	3 (0)	1750 (0)

Input and output factors were statistically processed by Statgraphics XVIII software (ANOVA, regression coefficient R^2), in order to avoid false positive and to get statistically significant results. Voltage was set up as Factor A, flow rate was Factor B and mandrel rotation speed was Factor C. The effect of the 3 factors was evaluate in 9 runs according to the matrix reported in **Table 5**. Experiments execution was completely randomized in order to protect from effect of dormant variables. Only 2 degree of freedom were involved in the evaluation of experimental error.

2.2.4 Characterization of the electrospun nanofibrous matrices

All electrospun samples were analyzed by Scanning Electron Microscopy (SEM) with a Zeiss EVO MA10 apparatus (Carl Zeiss, Oberkochen, Germany). All samples were dried and gold sputtered before undergo SEM analysis.

Morphometric analysis of the electrospun nanofibers was quantitatively perfomed processing SEM images by ImageJ software. This is an image processing program designed for scientific multidimensional images and based on plugin series Diameter J Segment e Diameter J 1-018.

Diameter J Segment plugin algorithm works by segmenting SEM image and allows selection of images. Selected segmented image/or images to best represent a sample should have the following characteristics: absence of partial fibers, fiber intersection shouldn't contains black spots/hole. When two or more images of the sample are very similar, best choice should be the one containing highest number of fibers. Super Pixel is the plugin that calculate mean fibers' diameter, using a super pixel determination (the scale length set was 202 pixels for 1 cm). Mean fibers' diameter was determined by Histogram Mean, fitting a Gaussian Curve to the radius data and finding the curves mean value. Histogram SD, delineates the standard deviation of the Gaussian fit of the radius histogram. Histogram Mode and Histogram Median determine respectively the most occurring fiber diameter in the histogram and the middle fiber diameter. Histogram Min. Diam. and Histogram Max. Diam defines the smallest and the largest diameter measured. Histogram Integrated Density calculates the product of length of the fibers and the average radius, then Histogram Raw Integrated Density determines the sum of the radii at all pixels in the image or selection. Diameter Skewness measures the third order moment about the mean; Diameter Kurtosis the fourth. All analyzed data were then plotted by using Microsoft Excel.

The values of orientation were obtained analyzing SEM images with ImageJ and plugin Orientation J software , that analysed the degree of fiber orientation distribution from −90° to +90°, of each SEM image using Fast Fourier transform (0° angle corresponded to the horizontal orientation parallel to mandrel axis and increased counter-clockwise). Analysis was performed on 6 SEM images for each electrospun matrix sample. Fiber alignment is expressed as frequency %, calculated by taking how many times same + 90 ° fiber orientation repeats during analysis of the same area of sample divided by the total number of total counted fibers in the area and multiplied by 100. The greater frequency % value the more oriented are the electrospun fibers.

Yield of process was determined for each sample from the following Equation (2)

$$Yield\ of\ process = \frac{Ws}{Wi} \times 100 \qquad \text{Equation (2)}$$

Where W_s is the sample weight and W_i is the amount of polymer in the starting polymer solution, and it is calculated following Equation 3:

$$W_i = \frac{15g}{100\ mL} \times \text{mL of electrospun solution making a sample} \qquad \text{Equation (3)}$$

Samples were prepared in triplicate and yield process variability evaluated in terms of mean ± standard deviation (sd).

3. Results and discussion

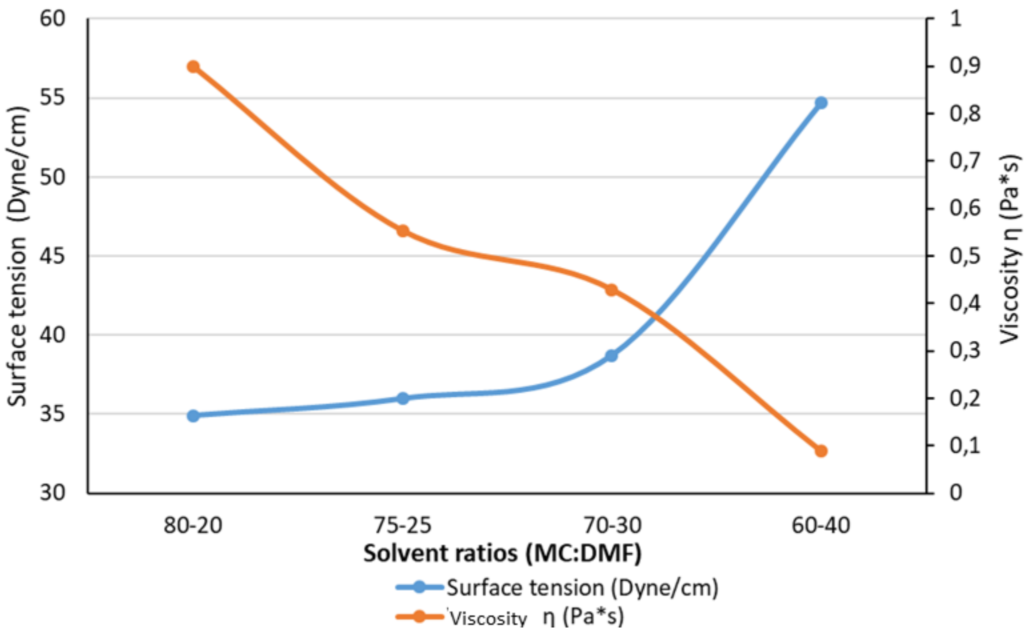

Figure 2 – Surface tension and viscosity curves of PLA-PCL 15 % w/v as a function of different MC:DMF ratios **.**

The results of preliminary evaluation carried out to set up the material parameters are reported in **Figure 2** and show that PLA-PCL 15 % w/v solution increases its surface tension as long as DMF content increases, while viscosity decreases in the same conditions. The crossover point of the two curves resides between 70:30 and 60:40 solvent ratios. Similar result was obtained for PLA-PCL 10 % w/v solution (curves not reported). Considering this result, two intermediate solvent ratios 65:35 and 68:32 were selected and the PLA-PCL solutions were electrospun at 2500 rpm mandrel rotation speed. The preliminary set of tests were carried out in order to select and fix material parameters i.e. PLA-PCL concentration in the starting solution and one solvent ratio between those that could be suitable. The results of SEM analyses (data not reported) show that electrospinning of PLA-PCL 10% w/v solutions did not reach satisfactory results independently of MC-DMF ratios tested; the fibers did not form regularly and some of them were melted together originating irregular cluster inside the matrix. Electrospinning of PLA-PCL 15 % w/v solutions gave better results in terms of regular fibers formation originating a network with homogeneous structure. Starting from these results only electrospun matrices obtained from PLA-PCL 15 % were processed further.

Table 6: Results in terms of fiber diameter and porosity obtained by software ImageJ, plugin Diameter J and plugin Diameter J 1-018.

Sample composition	Mean fibre diameter (µm)	Range of fiber size (µm)	Porosity percentage (%)	Mean pore area ± sd (μm^2)
PLA-PCL 15%w/v 65:35 MC:DMF	0.315	0.039 – 0.630	38.08	0.402 ± 0.484
PLA-PCL 15%w/v 68:32 MC:DMF	0.551	0.039 – 1.102	49.56	0.949 ± 2.124

SEM images referred to electrospun matrix obtained starting from 15 % w/v PLA-PCL solutions in 65:35 and 68:32 MC-DMF blends, were processed by software ImageJ, and mean fiber diameter, rank of fiber diameter porosity percentage, mean and standard deviation of pore area were determined. The results reported in **Table 6** highlight that mean diameter of electrospun fibers is significantly smaller (0.314 µm with respect to 0.551 µm), and with narrower range of fiber diameter (0.0394-0.6299 µm with respect to 0.0394 – 1.1024 µm), when the solvent mixture MC:DMF 65:35 % v/v was used. However, the samples obtained from 65:35 MC:DMF solution show lower porosity percentage and mean pore area with respect to samples obtained from 68:32 v/v MC:DMF solution (38.08 % with respect to 49.56 %, and 0.4020 ± 0.484 μm^2 with respect to 0.9492 ± 2.124 μm^2 respectively). Moreover, the matrices obtained from 65:35 MC:DMF solution are more reproducible in these parameters, with lower standard deviation.

These results led to select 65:35 v/v MC:DMF solvent ratio as the composition giving the most consistent results.

All SEM images show random orientation of the electrospun fibers. Starting from the set material parameters, following DoE was addressed to achieve the best conditions to get fiber alignment.

The results of input and output variables processed by Statgraphics XVIII ANOVA in order to analyze which variable or couple of variables mostly affect fiber orientation, are reported in **Table 7**.

Table 7: Analysis of variance (ANOVA) on the three input variables (A, B, C) *vs* orientation (output variable)

Nomenclature	**Square sum**	**Degree of freedom**	**Square mean**	**F ratio**	**P-value**
Input variable, A: voltage	1.9345×10^6	1	1.9345×10^6	3.29	0.2113
Input variable, B: flow rate	2.34943×10^6	1	2.34943×10^6	4.00	0.1836
Input variable, C: mandrel rotation speed	8.95266×10^6	1	8.95266×10^6	15.23	0.0598
Effect AB	3.37049×10^7	1	3.37049×10^7	57.33	**0.0170**
Effect AC	7.04504×10^6	1	7.04504×10^6	11.98	0.0743
Effect BC	9.28908×10^6	1	9.28908×10^6	15.80	0.0578
Total error	1.17575×10^6	2			
Total (corrected)	6.44513×10^7	8			

ANOVA Table (**Table 7**) splits out orientation variability for each effect (made of couple of variables) and verifies the statistic significance of each effect by comparing the square mean with an evaluation of experimental error.

R-squared (R^2) analysis indicated that the adjusted model fits 98.1758 % of variability in orientation. R^2 adjusted to degree of freedom, that better compares models with independent variables and different numbers, resulted to be 92.703 %. R^2 adjusted and R^2 adjusted to degree of freedom resulted of same rank order and confirmed the dependent variable is well explained by the independent variables and that the model was statistically significant. The standard error of the estimated (residual standard deviation) was 766.729; mean absolute error (MAE) was 284.55 and corresponded to residues mean value. Based on the regression coefficients derived, the following second degree equation (4) was applied to evaluate how each input variable or couple of input variables (**Table 7**) affected electrospun fiber orientation.

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$$\text{Fiber Orientation} = 42263.2 - 1440.07 \times A - 7657.09 \times B - 8.8776 \times C + 256.573 \times A \times B + 0.312806 \times A \times C + 0.718373 \times B \times C \qquad \text{Equation (4)}$$

When the result of equation (4) is a positive value it indicates orientation of electrospun fibre, whereas a negative result indicates lack of fibre orientation. Each number multiplied for the value of each variable or couple of variables, indicates how each variable, or variables interaction, affect fiber orientation. A greater value corresponds to stronger effect, and a lower value to a weaker one.

The results in **Table 7** and **Figure 3** show that only AB effect (voltage and flow rate) significantly affected electrospun fibers orientation with P-value lower than 0.05 indicating that this effect significantly differs from 0 with a level of confidence of 95.0 %.

Moreover, Pareto chart in **Figure 3** clearly show that each single input variable is not able to significantly affect fiber orientation; voltage is the input variable with lower effect on electrospun fiber orientation; only the combination of input variables voltage (A) and flow rate (B) significantly affects electrospun fiber orientation.

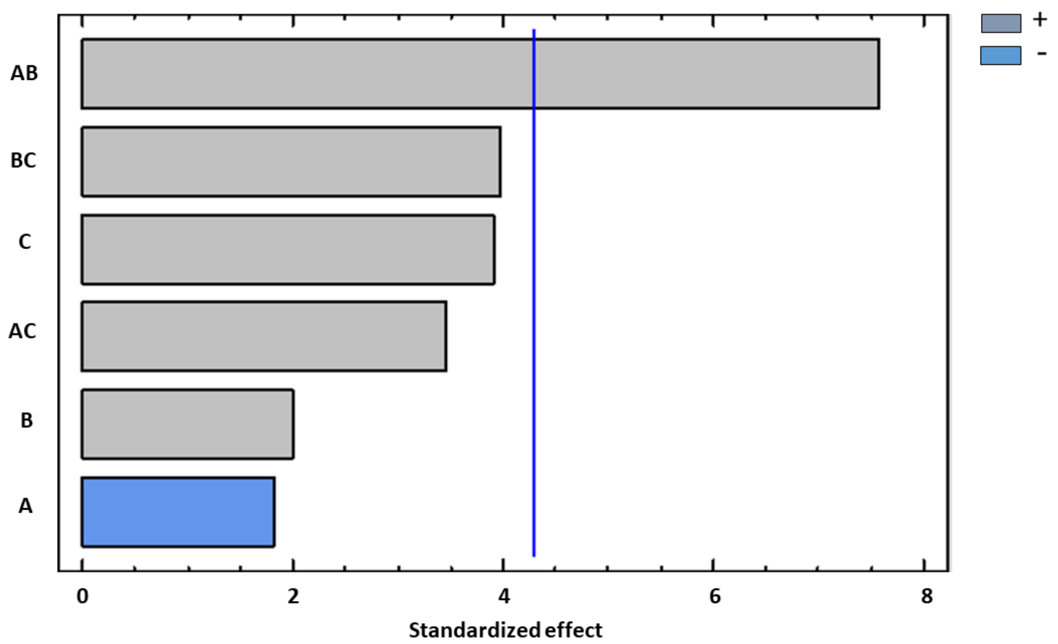

Figure 3 – Pareto chart: influence of the input variables and combination of input variables on electrospun fiber orientation; A voltage, B flow rate, C mandrel rotation speed.

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The effect of each single input variable and of their interactions was decomposed and analysed in the charts reported in **Figure 4** .

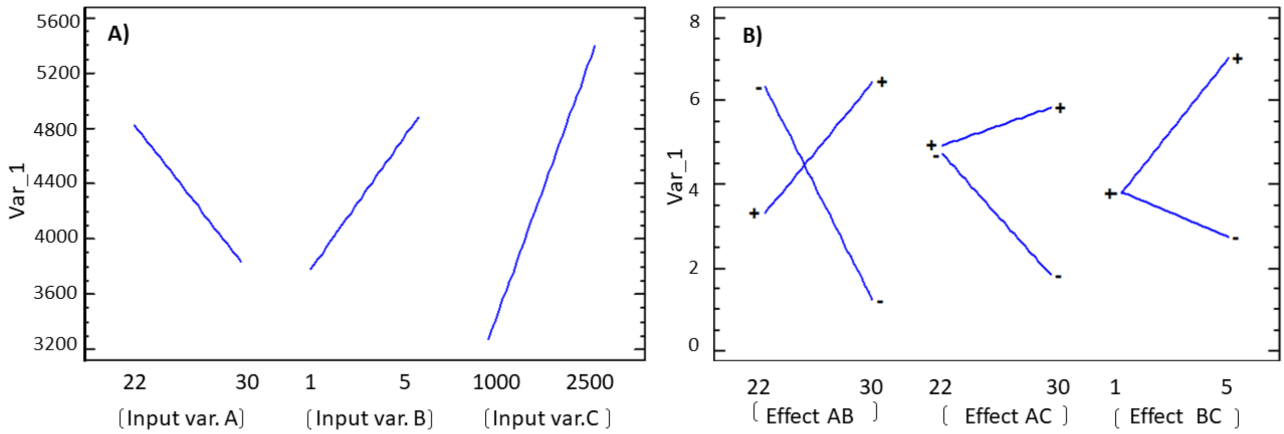

Figure 4 – Graphical representation of DoE results: A) effect of the single input variable on electrospun fiber orientation (output variable); B) Effect of input variables interactions on output variable.

Figure 4A shows that electrospun fiber orientation is promoted by low voltage (20 kV), high flow rates (up to 5 mL/h) and high mandrel rotation speeds (2500 rpm).

As long as the interaction between input variables is concerned, three input variables combination (AB, AC, BC) were investigated and the results in **Figure 4B** confirm that main interaction is voltage/flow rate (AB) that significantly affects fiber orientation degree (**Figure 3**). High voltage should be combined with low flow rate and vice versa, in order to achieve high orientation degree. AC interaction show that high mandrel rotation speed always favors high orientation degree, independently from the voltage applied. BC interaction indicates that either low or high mandrel rotation speed can be combined to low flow rates in order to get high fiber orientation, whereas in case of high flow rates, high mandrel rotation speed should be applied for better fiber orientation.

The values of electrospun fiber orientation obtained from ImageJ and plugin Orientation J analysis of SEM images are reported in **Table 8,** and they confirm that the highest degree of the electrospun fiber orientation (98.29 % frequency) is achieved for batch n. 1, i.e. when all the three input parameters (Voltage (A), flow rate (B) and mandrel rotation speed (C)) are at their maximum value. The results confirm those obtained by DoE statistical analysis. Electrospun fiber orientation is very good also for batch n. 8 (71.82% frequency), where all the three input variables are at minimum value, as confirmed by **Figure 5** showing SEM images of batches 1 and 8. Mandrel rotation speed demonstrated to be a crucial factor to fibre alignment, but the combination of the three input variables also plays a significant role in fiber alignment when electrospinning PLA-PCL copolymer. Most of the results of other authors reported in the literature are in keeping with the influence of mandrel rotating speed on fibre alignment Anyway, when comparing results found in the literature it should be taken into account that, set up of mandrel rotation speed depends on type of electrospinning apparatus, namely horizontal or vertical electrospinning, its brand, and the rotating mandrel diameter in addition to the type of polymer and solvents used. [26] [27].

The same 9 runs data were analysed and processed by ImageJ software plugins Diameter J 1-018 and the results in **Table 8** compared to those of fiber orientation highlighted no correlation among fibers orientation, mean fiber diameter, range of fiber diameter and porosity. These results are keeping with those of other authors found in the literature such as H.Wang ad coll [5] who performed wet electrospinning process parameters evaluation on High amylose maize starch (HAMS), and Choi and coll. [28] who evaluated electrospinning parameters on poly(ε-caprolactone)/ collagen. In agreement with the literature diameter of electrospun fibers is mainly governed viscous forces surface tension and electric field that determine jet elongation and Taylor con stability.

Process yield was always lower than 64% (**Table 8**), and it did not result to be related to electrospun fiber orientation. The result represent a drawback of the electrospinning process that should be further studied and optimized.

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Table 8: Results of Electrospun fibers characterization by ImageJ, plugin Diameter J 1-018 and plugin Orientation J software on the 9 batches obtained in the conditions selected from DoE design matrix

Batch n.	Process yield (%)	Fiber orientation +90° (Frequency %)	Mean fiber diameter (µm)	Range of fiber size (µm)	Porosity %	Mean pore area (µm²) ± sd
1	55.68	98.29	0.590	0.039 – 1.181	47.56	1.8738 ± 3.508
2	57.33	20.54	0.413	0.039 – 0.826	36.04	0.7651 ± 2.774
3	55.52	43.99	0.511	0.039 – 1.023	40.20	1.7254 ± 3.756
4	58.61	32.50	0.492	0.039 – 0.984	43.81	2.4967 ± 8.146
5	47.73	56.96	0.374	0.039 – 0.748	41.60	0.9931 ± 1.640
6	63.47	6.47	0.315	0.039- 0.630	32.64	0.4764 ± 0.530
7	52.64	24.36	0.374	0.039 – 0.784	46.60	1.3462 ± 2.270
8	62.67	71.82	0.433	0.039 – 0.866	36.12	0.9047 ± 1.025
9	56.62	34.85	0.492	0.039 – 0.984	45.95	1.6908 ± 2.624

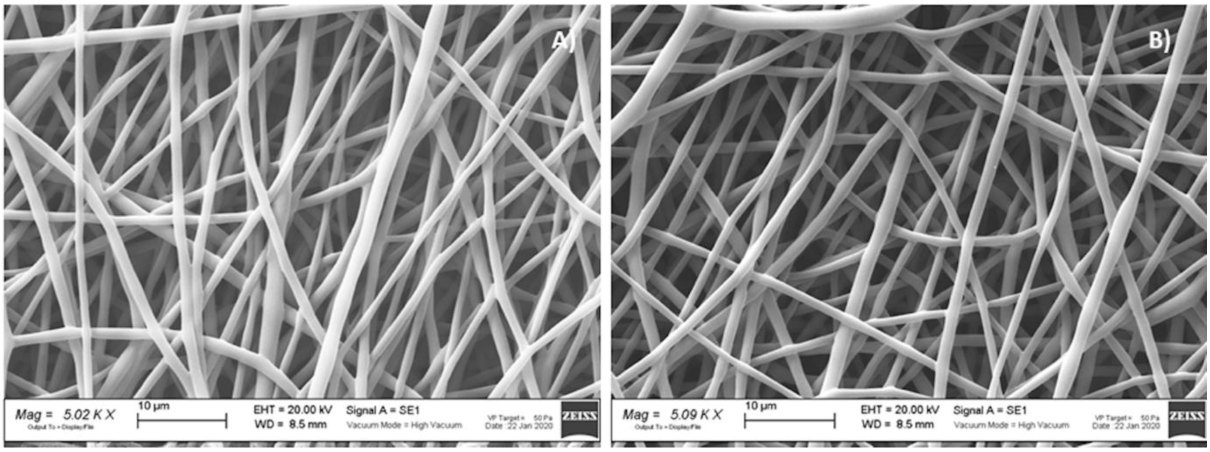

Figure 5 - SEM images of: A) batch 1 and B) batch 8.

5. Conclusions

The results of this study permit to identify from a statistical stand point the values of some process parameters affecting electrospun fiber orientation, when the polymer is PLA-PCL solubilized in the solvent mixture MC plus DMF. Namely, high voltage combined to high flow rate and mandrel rotation speed demonstrated to promote fiber orientation on a rotating mandrel collector and with a standard electrospinning apparatus. These set process parameters are strictly dependent on the type of polymer and polymer solvents processed.

Author Contributions

Rossella Dorati supervised experimental plan, Enrica Chiesa carried out DoE, Silvia Pisani performed data processing, Ida Genta supervised the manuscript, Tiziana Modena and Marco Benazzo contributed to funding acquisition, Giovanna Bruni carried out SEM analysis, Chiara R.M. Brambilla carried out electrospinning and ImageJ analysis, Bice Conti supervised the research and wrote the final version of the manuscript

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Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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