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RESEARCH ARTICLE

Introducing humidity and temperature as important parameters determining the size of electrosprayed chitosan nanoparticles

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ABSTRACT

Particle size is an important parameter in determining the quality of drug delivery systems. In this study, the effect of four independent parameters on the size of nanoparticles, prepared via electrospray, was investigated using artificial neural networks (ANNs). The parameters included concentration of polymer, applied voltage, humidity and temperature, of which, the last two were investigated for the first time in this study.

The developed ANNs model showed that applied voltage and temperature had small and reverse effects on the size. However, the dominant factors determining the size of the nanoparticles were humidity and polymer concentration: an optimum value was required for obtaining the smallest size.

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INTRODUCTION

Electrohydrodynamic atomization (EHDA) or electrospray is being used to produce polymeric nanoparticles (NPs). In this method, liquid droplets are produced under the influence of a high electric field. The technique involves electromechanical and hydrodynamic forces, that control size and morphology of the NPs. Based on Fig. 1, electrospray apparatus consists of four parts: mechanical syringe pump, high voltage electric power supply, metal needle (nozzle) and grounded metal collector. During the electrospray process, the polymer solution is infused from the needle that is connected to the syringe pump and wired to the high voltage power supply.

Parameters that may affect size and size distribution of NPs in this method are solution parameters (e.g. type of polymer, concentration of polymer, molecular weight and type of solvent used), processing parameters (e.g. flow rate and applied voltage), as well as environmental parameters (e.g. humidity and temperature) (1, 2).

It is now well-known that size of NPs is an important parameter in determining their physicochemical properties. Many different biological properties have been reported to highly depend on size of particles. They include crossing through various biological barriers (3-5), rate of endocytosis (6) and lysosomal escape (7), response of immune system (8) as well as biodistribution in body and release profile (9, 10). Many factors have been reported to affect size of NPs, including concentration of ingredients (e.g. PLA-PEG-PLA copolymer (11), chitosan (CS) (12), CS and albumin (13), TPP (14), salt (15), surfactant (16)),

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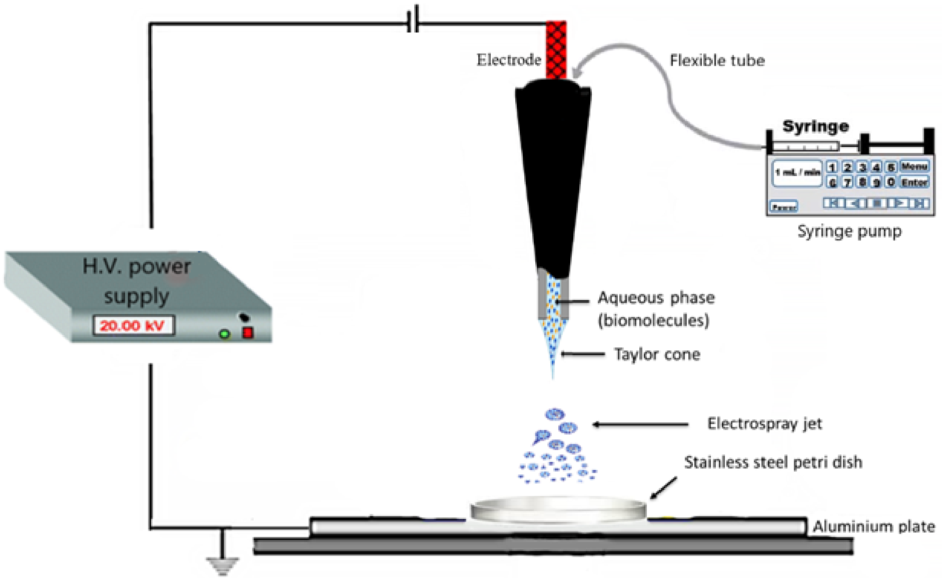

Fig. 1. Schematic of the experimental electrospray apparatus (setup).

polymer chain length (17), polymer molecular weight (18, 19), applied energy (20) and sonication time/ amplitude (21).

Artificial neural networks (ANNs) are computational models vaguely inspired by biological neural networks which plan to model patterns and learning capacity of the human brain (22). ANNs are usually used to find the patterns or relationships between inputs and output(s). ANNs are proper alternatives for situations where standard statistical analyses are not able to analyze complex, multi-dimensional and nonlinear patterns, or where the data are poorly organized (23). ANNs offer better validity and predictability compared with experimental designs (24). Examples of their uses include toxicity prediction (25, 26), pharmacokinetics and pharmacodynamics studies (27, 28).

Reviewing the literature, some reports may be found on the effect of different independent parameters on properties of electrosprayed particles, especially their particle size. Such information would provide insight into how to control the electrospray parameters to obtain the desired particle. Effect of polymer concentration on electrosprayed nanoparticles has been reported at concentration range of 0.1-0.7 (%w/v) (29), while in this study, a wider range of concentration was investigated on CS NPs. Additionally, little to no research was found on the effect of temperature/ humidity on the size of electrosprayed CS NPs. This investigation focused on identifying the influence of four independent parameters, namely, applied electric voltage, concentration of polymer used (i.e. CS), temperature and humidity, on particle size of CS NPs prepared by electrospray.

MATERIALS AND METHODS

Materials

CS (MW=7 kDa, DD~72%) was purchased from Zhengzhou Sigma Chemical Co. (China). Tripolyphosphate (TPP) was obtained from Sigma-Aldrich (USA).

Preparation of CS NPs

To prepare CS NPs, electrospray method was employed in an environmental chamber being able to control the temperature. During the electrospray process, the CS solution was first injected through a nozzle. The injected solution was pulled (sprayed) immediately towards a collector using a high voltage generator (Fanavaran Nano Meghyas ltd., Tehran, Iran) to form a Taylor cone-jet. During the spray process, the solvent was evaporated to form NPs on the collector (see Fig. 1) (30). Different values were used for the four factors (i.e. applied voltage: 7.5-13.9 kV, CS concentration: 1-10 %W/V, temperature: 20-35 °C and humidity: 13-49 %). Remaining parameters were fixed during the process included flow rate (0.7 ml/h), distance between nozzle and collector (12.75 cm), inner needle diameter (0.260 mm), and concentration of TPP (0.0001 %w/v) in collector. CS polymers were dissolved in distilled water using a magnetic stirrer at 50 °C, followed by probe sonication (30 s,

Table 1. Training parameters set with the software

Back propagation type	Angle Driven Learning	
Transfer function	Output	Linear
	Hidden layer	Asymmetric Sigmoid

Table 2. The validation data set

Sample No.	Concentration of polymer (%W/V)	Humidity (%)	Temperature (°C)	Applied Voltage (kV)	Real particle size (nm)	Predicted particle size (nm)
1	5	17	30	9.7	173	227
2	2	35	20	9.3	340	318
3	10	30	24	13.5	227	265
4	7	41	26	13.9	165	176
5	6	31	27	11.9	213	202
6	5	37	20	12.7	213	185
7	5	17	33	9.4	192	207

power 70, Fapan Ultrasound Probe-400R, Fanavari Iranian Pajouhesh Nasir Co., Iran). A regular visual inspection was performed to check maintenance of a stable cone-jet during the electrospray process.

Characterization of NPs

Total weight of 0.01 g chitosan was electrosprayed onto 10 ml TPP solution. Then, 300 µl of the CS/TPP solution was diluted with distilled water to final volume of 3 ml. Particle size was investigated using dynamic light scattering (DLS) using Scatteroscope I (K-ONE, Korea).

ANNs studies

In this study, modeling the parameters affecting particle size was performed using INForm v4.02 (Intelligensys, UK). The input variables were concentration of polymer, humidity, temperature and applied voltage and the output was size of NPs.

Forty-seven CS NPs samples were experimentally prepared having different values for the four factors, while the remaining factors were fixed as mentioned in section 2.2. Their particle size was then measured using DLS. Afterwards, the data sets were randomly divided into three sets (categories) to perform the ANNs modeling. The sets included test, validation and training data. Thirty-six data were used to train network, using learning parameters/ algorithms listed in Table 1. The network structure included one hidden layer with 5 nodes. Remaining parameters were given previously (31). The software selected 10% percent of the training data as test data (four points) to check fit of the model and avoid overtraining of the chosen model (32). The remaining data sets (7 samples (were excluded from training the model and taken as validation (unseen) data (Table 2) to validate the generated model. After developing the model and training the network, predictive ability and quality of the model were evaluated using the determination coefficient (R^2) for the data sets.

$$R^2=1-\frac{\sum_{i=0}^{n}(yi-\hat{y})^{2}}{\sum_{i=0}^{n}(yi-\bar{y})^{2}}$$

Where n was number of samples, $\bar{y}$ is the mean obtained values, and ŷ represented the predicted values by the model for the dependent variable. High R^2 value (close to 1) is preferred for the unseen, test and training data sets.

Afterwards, response surfaces produced by the software were used to investigate the effects and relationships between the input data and the output parameter.

RESULTS

Electrospray is an effective and one-stage method to produce polymeric NPs. In this method, some processing and formulation parameters may be tuned to optimize the properties of produced NPs, including their size. In our work, the influence of applied voltage, temperature, polymer concentration and humidity on size was investigated using an ANNs model. Subsequent to the modeling with ANNs, R^2 value was calculated as 0.70, 0.63 and 0.74 for the unseen (validation), test and training data, respectively. The obtained values demonstrated an appreciable degree of validation for the model.

Effect of temperature and applied voltage

Effect of temperature and applied voltage on particle size was studied when polymer concentration and humidity were fixed. The effects were then graphed 3-dimensionally using the software (see Fig. 2). From Figure 2, increasing the temperature generally made the size slightly smaller. Furthermore, a minor decrease in particle size may be observed when applied voltage increases.

Effect of applied voltage and polymer concentration

In Fig. 3, the effect of polymer concentration and applied voltage on the size of NPs was investigated when humidity and temperature were fixed. From Figure 3, in general, increasing the applied voltage may decrease the particle size slightly. However, the effect of concentration on particle size is complicated and depends on temperature and humidity: a certain level of polymer concentration is required to provide minimum particle size. Higher or lower values produce larger particles. This value depends on humidity and temperature and tends to increase by increasing either of humidity or temperature. For instance, when humidity was low, medium and high, the optimum concentation values were ~2 %, ~3.5 % and ~ 5.5 %, respectively.

Effect of humidity and polymer concentration

In Fig. 4, the effect of humidity and polymer concentration on the size of NPs is shown when temperature and applied voltage were fixed at 22.5, 27.5, and 32.5 °C and 8.6, 10.7, and 12.8 kV, respectively.

From Figure 4, an optimum value for both concentration and humidity is required to obtain the smallest size. Values above or below the optimum point increase the size. The value of this point is not fixed for either of humidity and concentration and depends on the other factors. When humidity is low, the solution concentration must be also low

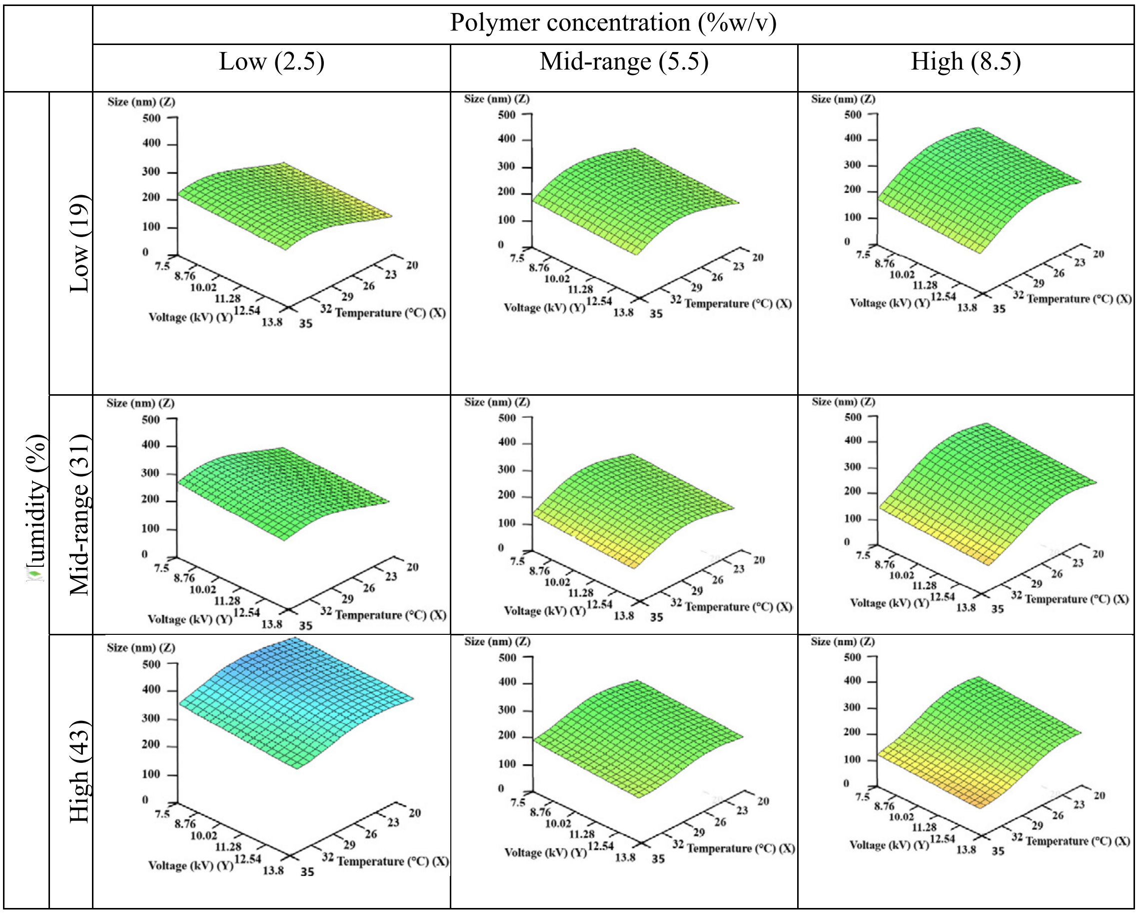

Fig. 2. Particle size (nm) as a function of temperature (°C) and applied voltage (kV), predicted by the ANNs model. The effect of applied voltage and temperature on the particle size is shown at low, medium or high values of other input factors (polymer concentration and humidity).

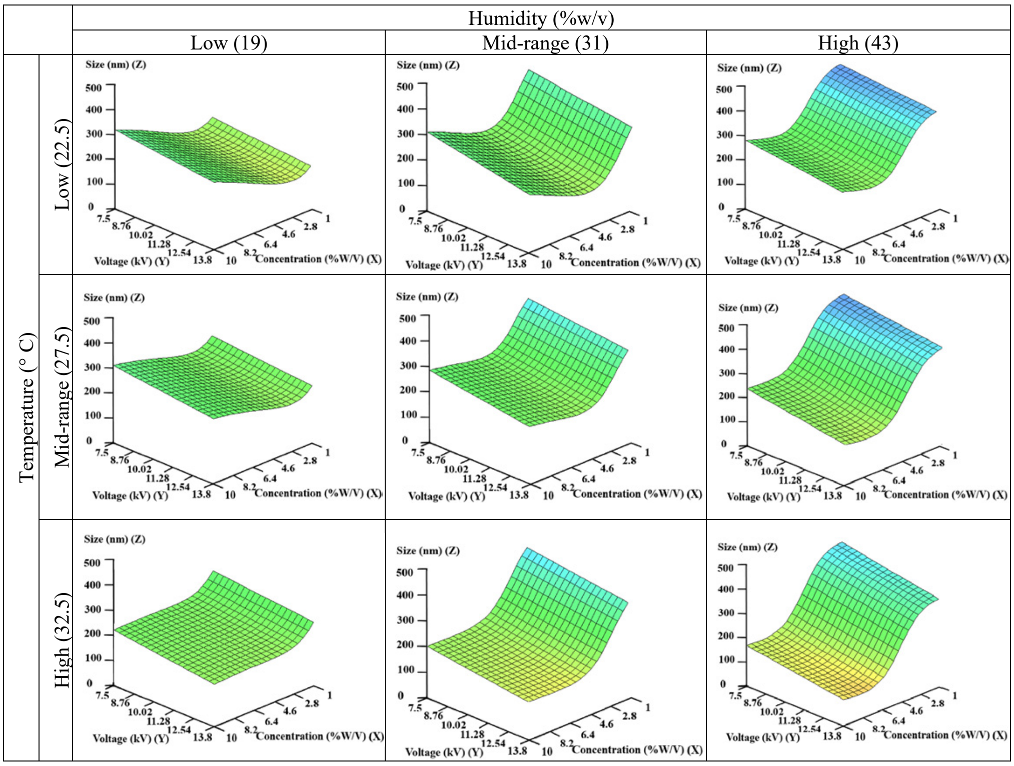

Fig. 3. Particle size (nm) as a function of amount of polymer concentration (%w/v) and applied voltage (kV), predicted by the ANNs model. The effect of polymer concentration and applied voltage on particle size is shown at low, medium or high values of other input factors (humidity and temperature).

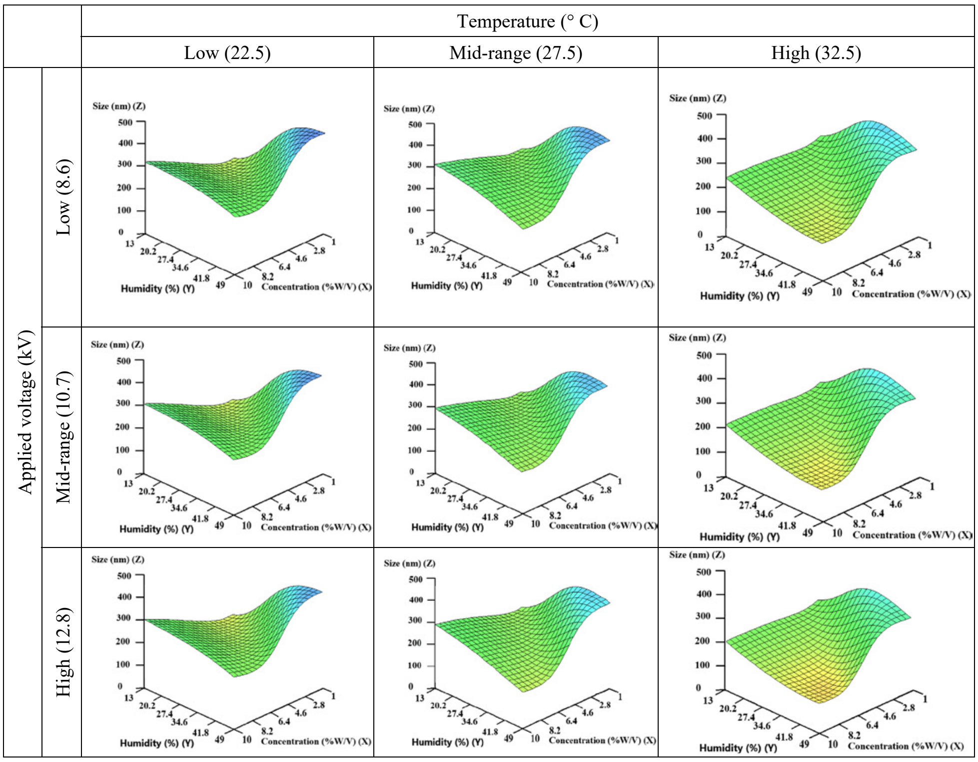

Fig. 4. Particle size (nm) as a function of amount of polymer concentration (%w/v) and humidity (%), predicted by the ANNs model. The effect of polymer concentration and humidity on particle size is shown at low, medium or high values of other input factors (temperature and applied voltage).

too to yield the smallest size. Conversely, when the humidity is high, the concentration must increase to obtain the smallest size.

Effect of humidity and temperature

In Fig. 5, the effect of humidity and temperature on the size of NPs is shown when both the polymer concentration and applied voltage were fixed at 22.5, 27.5, and 32.5 °C and 8.6, 10.7, or 12.8 kV, respectively. The data confirm the complicated effect of humidity on size as reported above. A small decrease and increase may be observed by increasing the humidity. Furthermore, temperature makes a small effect on size.

Effect of polymer concentration and temperature

In Fig.6, the effect of temperature and polymer concentration on the size of NPs is shown when humidity and applied voltage are fixed.

From Figure 6, temperature has a reverse effect on size: usually, by increasing temperature the size tends to become smaller. The effect on concentration on size has also been described previously: at a critical point, the size becomes minimum while values above or below this point make the size larger. The value of this certain point depends on the other parameters, including humidity and applied voltage.

Effect of applied voltage and humidity

In Fig. 7, the effect of humidity and applied voltage on the size of NPs is shown when polymer concentration and temperature are fixed at 2.5, 5.5, and 8.5 % W/V and 22.5, 27.5, and 32.5 °C, respectively. From the details, the previous findings about the influence of applied voltage/ humidity on the size are confirmed.

In total, the effects of the dependent variables on size could be summarized as:

-with increasing voltage, the particle size becomes slightly smaller.

- humidity and polymer concentration are dominant parameters determining the size. An optimum value of each parameter is required for

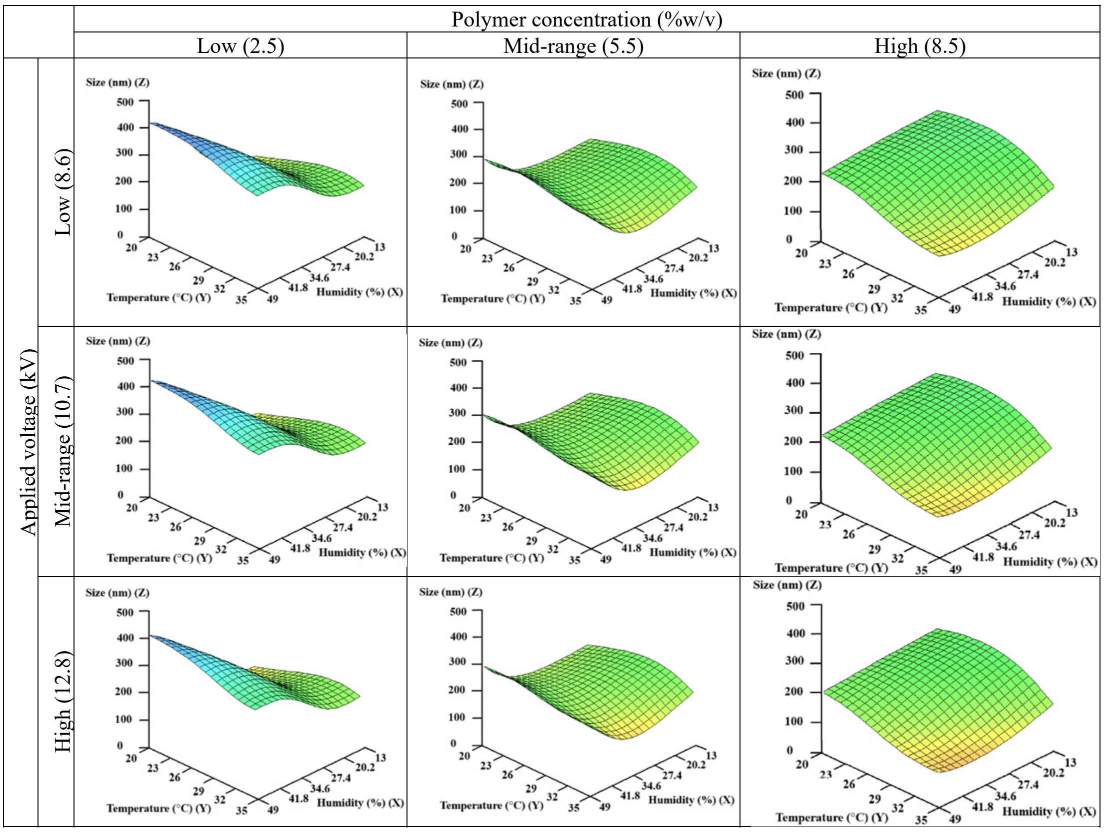

Fig. 5. Particle size (nm) as a function of humidity (%) and temperature (°C), predicted by the ANNs model. The effect of humidity and temperature on particle size is shown at low, medium or high values of the other factors (polymer concentration and applied voltage).

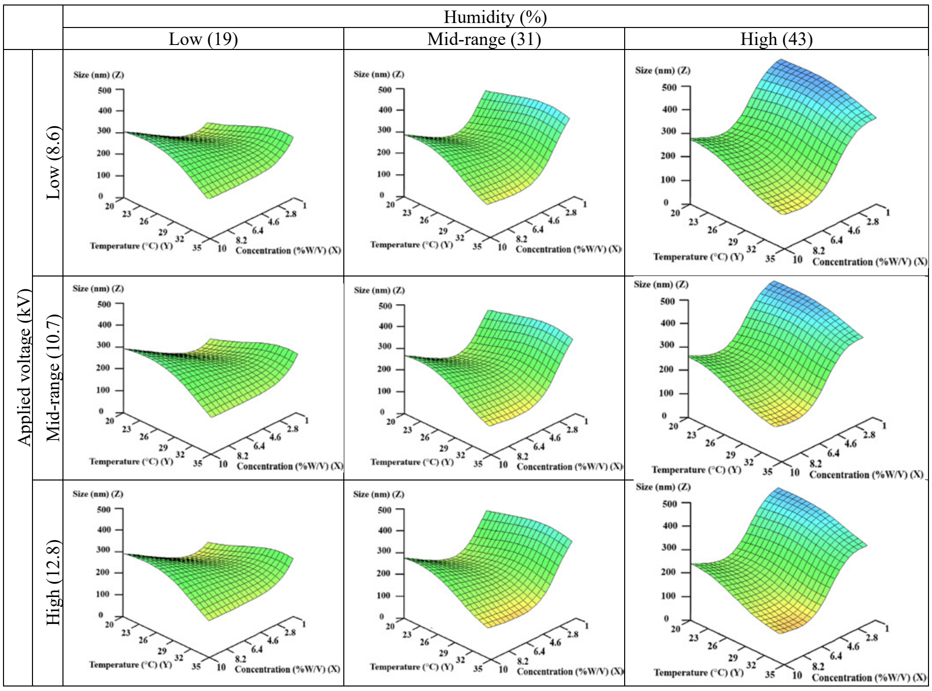

Fig. 6. Particle size (nm) as a function of amount of polymer concentration (%w/v) and temperature (° C) predicted by the ANNs model. The effect of polymer concentration and temperature on particle size is shown at low, medium or high values of the other factors (humidity and applied voltage).

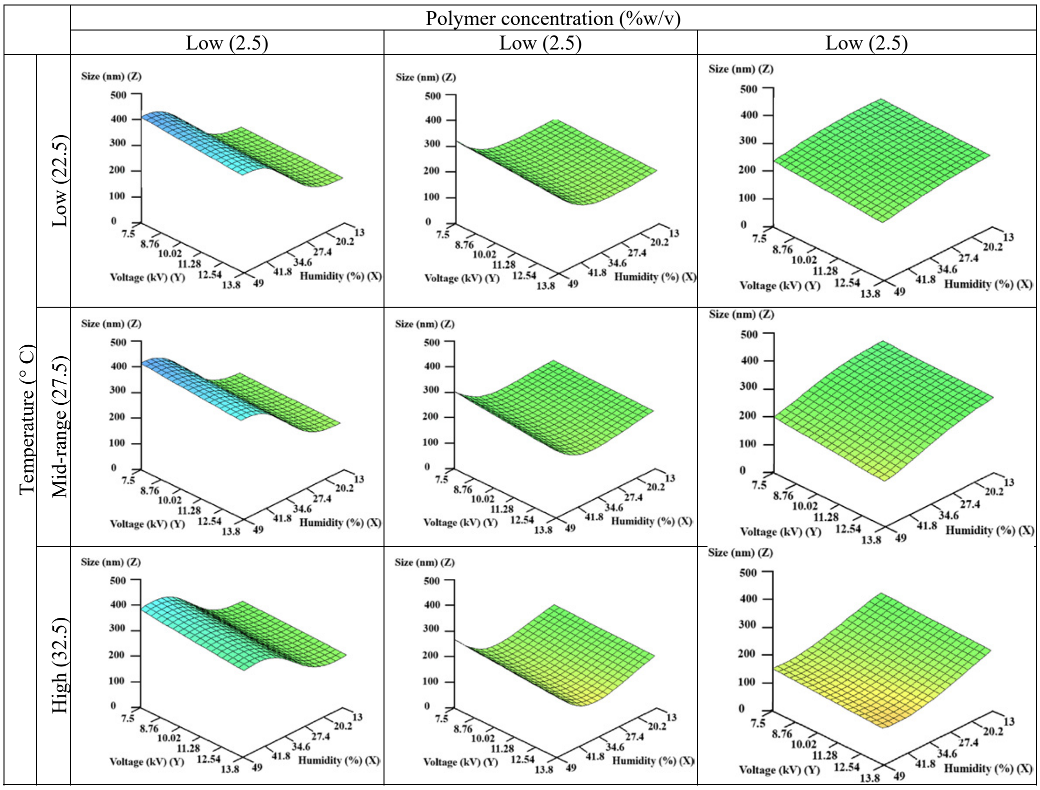

Fig. 7. 3D plots of particle size (nm) predicted by the ANNs model versus the amount of applied voltage and humidity, predicted by the ANNs model. In each chart, the effect of two input factors (applied voltage and humidity) on the output factor (particle size) is shown at low, medium or high values of the other factors (temperature and polymer concentration).

obtaining smallest size.

- increasing the temperature leads to a small decrease in size.

DISCUSSION

In electrospray, a conductive liquid should be injected through the nozzle. The applied voltage then renders a charge at the surface of the liquid. This will lead to breaking the liquid into fine droplets. The droplets are then sprayed and evaporated to form smaller particles with higher surface charge values due to Rayleigh disintegration (33). This creates strong repulsive forces within droplets, leading to dissipating and dispersing finer smaller droplets. Ultimately, micro/nano-particles are formed on the collector (34).

In this study, we measured the size of the produced particles was using DLS when dispersed in the solution of TPP. Four independent variables were chosen which could be easily tuned during the electrospray process (i.e. polymer concentration, humidity, temperature, applied voltage). These variables appear to affect the electrospray process indirectly. For instance, temperature or polymer concentration render their effects through changing viscosity, conductivity or surface tension of the solution. However, tuning these "secondary" variables is not straightforward during the experiment.

Findings of this study showed that applied voltage made a small decrease in size. Partovinia et al. worked on production of alginate beads by means of the electrospray. According to their results, a minimum bead size of 130 µm was obtained at applied voltage of 11 kV and alginate concentration of 1.5%. In their report, an important factor affecting the size of particles was applied voltage. Depending on working distance, by increasing the value of applied voltage from 7.5 to 12.5 kV, particle size decreased (35). In another work, using CS concentrations of 0.1, 0.4 and 0.7 (% w/v) and applied voltage of 13, 14, and 15 kV, the minimum particle size of electrosprayed nanoparticles was obtained when the applied voltage was highest (29). During the electrospraying process, the solution that comes out of the nozzle is exposed to electrostatic forces to overcome the surface tension (36). When the solution leaves the nozzle, droplets are formed in three different modes: Dripping mode, single cone-jet mode and multiple cone-jet mode. These modes affect size/ size distribution of electrosprayed particles (37). The single jet mode is the preferred regime which is able to produce the most stable jet and forms small and monodispersed particles (38). Single jet is formed at optimum applied voltage (36, 39). Selection of appropriate applied voltage is necessary for formation of a stable and continuous spray: by changing the applied voltage unstable spray is expected (40).

Our findings also indicated that the effect of polymer concentration is not linear and appears to be dependent on other parameters, mainly humidity: generally, a critical concentration value to obtain minimum size is required. Other reports show that by increasing the concentration, the size increases. In a study, various concentrations of alginate (1–5 %) were investigated to prepare alginate micro-beads using electrospray. By analyzing the data, they showed that when the concentration increased from 1 to 5%, the particle size increased from ~ 800 to 1120 µm, depending on the other independent parameters (35). In our previous report, N-Acetylcysteine (NAC) was loaded on PLGA nanoparticles via electrospray. PLGA concentration was 0.5 to 5 (%w/v), and the smallest particle size was obtained when concentration was 0.5 (%w/w) (39). Another paper used electrospray to produce estradiol coated with PLGA. In this paper, 2, 5, and 10 % of the polymer were used, and it was shown that the smallest nanoparticles were obtained at concentrations of 2 and 5 %, with formation of polymer agglomerates at 10 % polymer (41). It is believed that by increasing polymer concentration, polymer entanglements increases which, along with increasing viscosity, prevents breaking aerosol droplets to smaller droplets during the electrospray process, thus, make the particles larger (42, 43). Concerning the reverse effect of concentration on size at very low values (i.e. below the critical point), it is believed that when the concentration drops below a certain level, the interactions between the polymer chains will be reduced. This reduces condensation of the polymer chains and deformation of the particle during evaporation, resulting in a larger particle size (44). Furthermore, as the polymer concentration increases, the viscosity and conductivity of the solution increase. As particle size is expected to decrease by increasing conductivity or decreasing viscosity (45), the fluctuation in particle size as a function of concentration variation may be explained through these two parameters. A third reason explaining the reason of reverse effect of concentration on the size could be the fact that when the concentration is too low, the formed jet from the nozzle is unstable, thus, cannot produce optimum nanoparticles, thus, the size becomes larger.

Our results showed that with increasing humidity, the particle size decreases temporarily, followed by an increase in size above an optimum value. Although little research has been performed on effect of humidity and temperature in electrospray, the effect of humidity and temperature on electrospun nanofibers has been investigated in the literature. In a study on PVA solution, by increasing humidity from 4 to 60 %, diameter of nanofibers reduced from 667 ± 83 nm to 161 ± 42 nm (46). In another electrospinning report, using cellulose acetate (CA) and poly(vinylpyrrolidone) (PVP), it was shown that when the humidity increased, diameter of the CA nanofibers increased, while PVP nanofibers indicated decrease in diameter (47). In electrospinning of Poly (lactic acid) (PLA), it was concluded that increasing the humidity from 40 to 80 %, increased the diameter from 0.73 ± 0.08 µm to 1.78 ± 0.16 µm (48). The effect of humidity can be explained by two different factors: the rate of evaporation of the polymer solution and the length of the polymer jet. According to other studies, it can be said that when the humidity is reduced, the rate of evaporation of the polymer solution becomes faster, so, polymer jet solidifies much earlier. By reducing the solidification time of the polymer jet, the jet is less affected by the voltage-induced stretching, thus, the particle size increases. A second mechanism is the formation of larger particles as a function of a longer jet. When the moisture content exceeds a certain value, the jet becomes larger, causing the polymer chains to entangle with one another before the start of the solidification process, resulting in formation of larger particles (De Vrieze, Van Camp et al. 2009, Pelipenko, Kristl et al. 2013, Liu, Zhao et al. 2019).

Findings of this study also show that as the temperature increases, the particle size slightly decreases. When producing nanofibers from cellulose acetate (CA)/ Dimethylacetamide (DMAc) and poly(vinylpyrrolidone) (PVP) / ethanol solutions, temperatures of 10, 20, and 30 °C were used for electrospinning. It was found that changing the temperature from 10 to 20 °C the nanofiber diameter of both polymers increased, but increasing the temperature from 20 to 30 °C reduced the diameter of the nanofibers (47). It is believed that as the temperature increases, viscosity of the polymer solution decreases and molecular mobility increases, which facilitates the effect of voltage on the solution. Thus, a smaller size is expected (47, 49).

CONCLUSION

Effect of CS concentration, temperature, applied voltage, and humidity was studied using ANNs in CS NPs. Of the different parameters, humidity and temperature showed dominant. Maximum temperature and optimum humidity values were necessary to obtain minimum particle size.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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