

Evaluation of Factors Affecting Size and Size Distribution of Chitosan-Electrosprayed Nanoparticles

Morteza Abyadeh¹, Ali Akbar Karimi Zarchi¹, Mohammad Ali Faramarzi², and Amir Amani^{1,3*}

1. Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

2. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran

3. Medical Biomaterials Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: Size and size distribution of polymeric nanoparticles have important effect on their properties for pharmaceutical application. In this study, Chitosan nanoparticles were prepared by electrospray method (electrohydrodynamic atomization) and parameters that simultaneously affect size and/or size distribution of chitosan nanoparticles were optimized.

Methods: Effect of formulation/processing three independent formulation/processing parameters, namely concentration, flow rate and applied voltage was investigated on particle size and size distribution of generated nanoparticles using a Box–Behnken experimental design.

Results: All the studied factors showed important effects on average size and size distribution of nanoparticles. A decrease in size and size distribution was obtainable with decreasing flow rate and concentration and increasing applied voltage. Eventually, a sample with minimum size and polydispersity was obtained with polymer concentration, flow rate and applied voltage values of 0.5 %w/v, 0.05 ml/hr and 15 kV, respectively. The experimentally prepared nanoparticles, expected having lowest size and size distribution values had a size of 105 nm, size distribution of 36 and Zeta potential of 59.3 mV.

Conclusion: Results showed that optimum condition for production of chitosan nanoparticles with the minimum size and narrow size distribution was a minimum value for flow rate and highest value for applied voltage along with an optimum chitosan concentration.

Avicenna J Med Biotech 2017; 9(3): 126-132

* **Corresponding author:**
Amir Amani, Ph.D., Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel/Fax: +98 21 88991118-20
E-mail: aamani@sina.tums.ac.ir
Received: 20 Jul 2016
Accepted: 10 Oct 2016

Keywords: Chitosan, Electrospray, Experimental design Nanoparticles, Particle size

Introduction

Biodegradable polymeric nanoparticles (NPs) have been used widely as drug carriers, especially for oral and pulmonary drug delivery purposes¹. Because of their small size, they can solubilize a concentrated payload of therapeutic agent, improve drug stability and bioavailability, and provide sustained delivery. Moreover, use of biodegradable and biocompatible materials decreases the risk of unwanted toxicities and adverse effects².

Natural polymers have been widely used as drug carrier in the literature. Chitosan (CS) is a natural polymer with properties such as biodegradability, biocompatibility, low toxicity³, efficacious delivery of therapeutic agent⁴, mucoadhesivity⁵ and ability to facilitate the macromolecules permeation through the epithelia

by opening tight junctions⁶. Chitosan nanoparticles (NPs) are being extensively investigated for delivery of drugs, proteins/peptides and genes⁷⁻⁹.

Recently, fabrication of polymeric NPs using electrospraying (electrohydrodynamic atomization) has received a great deal of attention for pharmaceutical purposes. Electrospray is based on the ability of electric field to create electrostatic forces within a liquid droplet to deform the droplet by repulsion between the cations. This results in an outwardly directed force when a charge is induced on the surface of the liquid¹⁰. Fabrication of polymeric particles by electrospray has the potential to overcome limitations of other techniques to provide reproducibly loaded nano- and microparticles¹¹⁻¹⁴. Compared with approaches such as solvent evapo-

ration and emulsification, electrospray has the advantages of narrower particle size distribution, higher drug-loading efficiency and lack of solvent residue^{15,16}. Additionally, this cost effective, simple and one-step technique does not require the use of template or surfactant and employs mild condition for sensitive therapeutic agents^{17,18}.

Size and size distribution of generated nanoparticles in electrospray method may be affected by many variables. By optimizing solution, process and environmental parameters, one may obtain a desired size and size distribution. No need to mention that size and size distribution of polymeric nanoparticles notably influence properties such as blood circulation time, bioavailability and cellular uptake¹⁹⁻²¹. Few researches have studied parameters that affect size and/or size distribution of nanoparticles so far which are produced by electrospray. Size and size distribution of nanoparticles is greatly influenced by solution properties including polymer molecular weight²², surface tension, conductivity, polymer concentration and acid concentration^{1,23} as well as process parameters such as flow rate, needle gauge, applied voltage and distance between the electrodes²⁴. For instance, Enayati *et al* reported that lowest PLGA particle size and size distribution was obtained at concentration of 5 (%wt) in range of study (2% wt-10% wt)²⁵. In a study by our group, mean particle diameter decreased as both flow rate and polymer concentration were reduced²⁶. However, majority of such studies are PLGA-based reports. Very limited reports on chitosan are found which include chitosan/ampicillin (520 nm)²⁷, doxorubicin/chitosan/tripolyphosphate (200 nm)²⁸, chitosan/indomethacin (340 nm)²² as well as chitosan alone, (167 nm)²³ and (124 nm)¹. However, the result of investigation of parameters that simultaneously affect size and/or size distribution of chitosan nanoparticles produced with electrospray process has not been reported yet. More importantly, there is no detailed report focusing on size distribution of polymeric nanoparticles with this method.

Our investigation focused on the concurrent effect of three variables including polymer concentration, applied voltage and flow rate on nanoparticles size and size distribution to obtain the optimum conditions [(i.e. smaller size and narrowed Size Distribution (SD))].

Materials and Methods

Materials

High molecular weight chitosan (CS) (MW=500 kDa, DD=85%) was purchased from Zhengzhou Sigma Chemical Co. (China). Acetic acid was purchased from Merck Chemicals (Germany).

Solution properties

17 solutions of chitosan in aqueous acetic acid were prepared with a different chitosan concentration. Acetic acid concentration was fixed at 50% (v/v) in all samples.

Preparation of chitosan nanoparticles

To prepare chitosan nanoparticles, solutions with concentrations of 0.1, 0.4 and 0.7 (%w/v) were prepared by dissolving chitosan polymer into aqueous acetic acid solvent. The polymer solutions were stirred magnetically for 1 hr at room temperature before electrospraying. Then, the polymer solutions were transferred into a 2 ml plastic syringe and continuously forced through the spraying nozzle which was wired to the high voltage power supply (13, 14 and 15 kV) using a programmed pump (flow rate: 0.05, 0.2 and 0.35 ml/hr). For all samples, the needle gauge and the distance between nozzle and collector were kept at 27 g and 10 cm, respectively. Droplets were formed at the nozzle tip, in form of a cone called Taylor cone, when the electrical field overcame the surface tension of the polymer solution. The solvent was evaporated before reaching the collector to deposit the polymer in form of nanoparticles on the collector (i.e. alumina foil).

Particle size and zeta potential

Size and morphology of nanoparticles were determined by scanning electron microscopy (SEM) (ZEISS DSM 960A Oberkochen, Germany). Samples were sputter coated with gold (20 kV for 3 min). Zeta potential of optimized nanoparticle was measured using a Zetasizer (Nano-ZS, Malvern Instruments Ltd., UK).

Experimental design

Determination of the most important parameter and optimum level of each parameter with trial-and-error experiments is a time-consuming process. Therefore, Box-Behnken was employed as a response surface methodology (RSM) to optimize the three independent parameter levels. RSM is a statistical method for fitting the experimental data to a model for optimization²⁹.

Design-Expert (version 7.0.0, Stat-Ease, USA) was used to define the values of three independent parameters including applied voltage, chitosan concentration and flow rate in three levels as low (-1), basal (0) and high (+1), as given in table 1. Total number of experiments was 17, including 12 factorial points and 5 replicates at the center point for estimation of pure error sum of squares (Table 2).

Mathematical relationship of the response (Yi, particle size and size distribution) with the independent variables (Xi, concentration, applied voltage and flow rate) can be modeled by a second-order polynomial

Table 1. Variables used in Box–Behnken design

Independent variable	Levels		
	-1	0	1
Applied voltage (kV)	13	14	15
Flow rate (ml/hr)	0.05	0.2	0.35
Concentration (%w/v)	0.1	0.4	0.7
Dependent variables	Constraints		
Y ₁ = particle size (nm)	Minimize		
Y ₂ = size distribution	Minimize		

Table 2. Box-Behnken experimental design in 17 runs and the correspondent responses

Run no.	Independent variables			Dependent variables	
	A	B	C	Y ₁ (size)	Y ₂ (size distribution)
1	0	0	0	122	57
2	1	-1	0	122	53
3	1	1	0	171	68
4	0	0	0	119	48
5	0	0	0	121	51
6	-1	-1	0	121	43
7	0	0	0	122	67
8	0	-1	1	105	36
9	0	1	-1	170	78
10	1	0	1	131	53
11	-1	1	0	132	56
12	1	1	-1	170	84
13	-1	0	-1	137	59
14	0	-1	-1	139	63
15	0	1	1	142	71
16	-1	0	1	128	51
17	0	0	0	125	50

A) Concentration; B) Flow rate; C) Voltage.

function model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where Y is the predicted response, β_0 , intercept, β_1 , β_2 and β_3 , linear coefficients, β_{11} , β_{22} and β_{33} , squared coefficients and β_{12} , β_{13} and β_{23} , the interaction coefficients of the equation and X_1 , X_2 and X_3 are the independent variables.

Contour plots and 3D graphs were used to show the relationship and interaction of independent variables with the dependent response. Software-proposed optimized samples to prepare nanoparticles, considering minimum value for particle size and size distribution were used to experimentally prepare the samples. The results were then compared with the predicted values by the software to investigate the ability of the model to estimate the optimum conditions.

Results

Compared with conventional methods of generating aerosol droplets, electrospray is a straightforward technique which commonly produces less hetero-dispersed nanoparticles. In this approach, an electrical potential is applied on the nozzle. Above a certain critical charge, the electrical force overcame the liquid surface tension and broke the solution down to small electrically charged droplets that repel each other and produce a shower of fine polymer droplets³⁰. Nanoparticles with nearly monodispersed size distribution and spherical shapes can be produced by controlling the electro-spraying parameters².

Different jet types may be obtained in an electrosprary process; dripping, spindle, ramified-meniscus modes in low applied voltage and single cone-jet at an

optimum voltage value as well as multi-jet at higher values are commonly observed. A stable single cone-jet mode is essential for production of nearly monodispersed micro- and nanoparticles³¹.

Our experiments were performed at atmospheric pressure and room temperature. The simultaneous effect of three variable parameters on nanoparticles size and size distribution was investigated. Chitosan nanoparticles were prepared with size and size distribution ranging from 105 to 171 nm and 27 to 84 (Table 2), respectively. To obtain the best lack of fit and model F-values, quadratic second-order polynomial equation was used.

The equations fitted to the data were as follows:

$$Y_1 = +2386.92 + 263.00X_1 - 226.22X_2 - 316.55X_3 + 211.11X_1 X_2 - 25.00 X_1 X_3 + 10.00 X_2 X_3 + 95.56 X_1^2 + 271.11X_2^2 + 11.10 X_3^2 \quad (2)$$

$$Y_2 = +1430.08 + 288.75 X_1 - 401.67 X_2 - 191.29 X_3 - 19.17 X_1 X_3 + 33.33 X_2 X_3 + 6.54 X_3^2 \quad (3)$$

where Y_1 and Y_2 code size and size distribution and X_1 , X_2 and X_3 are concentration, flow rate and voltage, respectively. The coefficients of determination (R^2) of the model for size and size distribution were 0.99 and 0.88, respectively, with adjusted R^2 of 0.98 and 0.81, respectively, implying capability of the model to predict the two responses.

The lack of fit F-values model for the size and size distribution were 0.20 and 0.95, respectively, indicating insignificance relative to the pure error and significant models, respectively³². ANOVA results for two responses are shown in tables 3 and 4. Three-dimensional response surface plots, generated by the software which indicates the relation of the independent variables with the responses, are demonstrated in figures 1 and 2. In each plot, the interaction of two independent variables is investigated simultaneously while the third variable is at its middle-level value.

Figure 1A shows the effect of applied voltage and concentration on the size of nanoparticles produced by electrospray. The findings show that particles size decreases with increase in applied voltage and decrease the concentration that is against the other report focusing on the role of applied voltage in spray mode and after forming stable cone jet, increase in applied voltage makes slight decrease in size of particles^{2,25}.

As shown, by increasing the concentration, the size increases. Literature shows that by manipulating chitosan concentration, some properties of the solution such as viscosity and conductivity¹ are influenced which in turn affects the droplets diameter. The relation is explained by an equation suggested by Hartman *et al*³²:

$$d = \alpha \left(\frac{\rho \epsilon_0 Q^4}{I^2} \right)^{1/6}$$

$$I \propto (\gamma K Q)^{1/2}$$

where d is the droplet diameter, α is a constant, ρ is the solution density, ϵ_0 is the permittivity of vacuum, Q is the liquid flow rate, I is the current, γ is the surface

Table 3. ANOVA results for size as the responses

Source	Sum of squares	df	Mean square	F value	p-value probe>F
Model	5971.64	9	663.52	87.14	<0.0001
A-Concentration	722.00	1	722.00	94.82	significant
B-Flow rate	2048.00	1	2048.00	268.97	<0.0001
C-Voltage	1512.50	1	1512.50	198.64	<0.0001
AB	361.00	1	47.41	0.0002	<0.0001
AC	225.00	1	29.55	0.0010	
BC	9.00	1	1.18	0.3130	
A ²	311.41	1	40.90	0.0004	
B ²	156.67	1	20.58	0.0027	
C ²	518.78	1	68.13	< 0.0001	
Residual	53.30	7	7.61		
Lack of Fit	34.50	3	11.50	2.45	0.2037
Pure Error	18.80	4	4.70		not significant
Cor Total	6024.94	16			

R-Squared 0.9912, Adj R-Squared 0.9798, Pred R-Squared 0.9035.

Table 4. ANOVA results for size distribution as the responses

Source	Sum of squares	df	Mean square	F value	p-value probe>F
Model	2140.24	6	356.71	12.75	0.0004
A-Concentration	300.12	1	300.12	10.72	significant
B-Flow rate	760.50	1	760.50	27.17	0.0084
C-Voltage	666.13	1	666.13	23.80	0.0004
AC	132.25	1	4.73	0.0548	0.0006
BC	100.00	1	3.57	0.0880	
C ²	181.24	1	6.48	0.0291	
Residual	279.88	10	27.99		
Lack of Fit	68.68	6	11.45	0.22	0.9518
Pure Error	211.20	4	52.80		not significant
Cor Total	2420.12	16			

R-Squared 0.8844, Adj R-Squared 0.8150, Pred R-Squared 0.7527

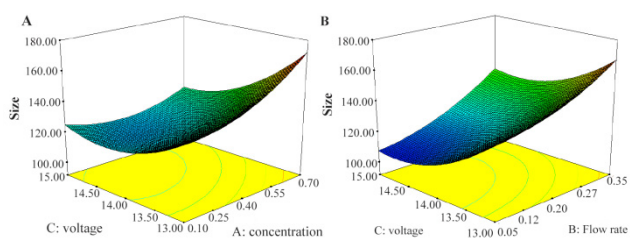


Figure 1. Effects of voltage and concentration; A) as well as voltage and flow rate; B) on size of nanoparticles.

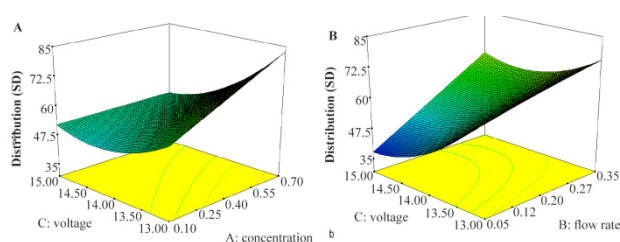


Figure 2. Effects of voltage and concentration: A) as well as voltage and flow rate; B) on nanoparticles size distribution.

tension in ambient air, and K is the liquid conductivity. From the equation, viscosity and conductivity have contrary impacts on the size. For instance, Zhang *et al* reported that increase in concentration of chitosan solution, increased the nanoparticles size as the increase in viscosity was more effective than increase in conductivity of solution¹.

Looking more closely to the diagram, in our work, decrease in chitosan concentration from 0.7 to 0.4 (%w/w) shows substantial decrease in nanoparticles size but decrease from 0.4 to 0.1 (%w/w) made no fur-

ther decrease and even small increase in nanoparticles size. It is already reported that when chitosan concentration is low, changing the polymer concentration makes substantially more variation in viscosity compared with conductivity¹. So, at such situations, the decrease in viscosity as a function of decrease in chitosan concentration is more than that of conductivity. Thus, decrease in size is expected. However, further decrease in viscosity makes the electrospray process unstable, which could be an explanation to the observed size increase³³.

The second most important parameter after solution parameters in electro spraying process is probably flow rate²⁵, which together with solution parameters can control polymer entanglements and Coulomb fission; consequently, affect particles size and size distribution². As shown in figure 1B, in our study, reduction in flow rate makes decrease in particles size, as documented previously³⁴. When flow rate increases, the droplet size increases which makes faster movement of droplets towards the collector. Thus, solvent may not evaporate completely when reaches the collector. This makes agglomeration of wet nanoparticles which in turn leads to formation of larger particles^{22,24}.

From figure 1B, increasing the voltage from 13 to 15 kV makes a decrease in the size. This was described above and is in agreement with the previous results²⁴.

Figure 2 details the effect of independent variables on the size distribution. From figure 2A, voltage shows a reverse effect of size distribution. It seems applied voltage has important effect on size distribution of nanoparticles. Forming the stable single cone-jet mode is essential for near monodispersed nanoparticles production and can be obtained with optimized applied voltage. As other researchers showed, after forming stable cone jet, more increase in applied voltage makes multi-jet²⁴ appear in an irregular shape²⁷ and leads to increase in size distribution.

Also, as other researchers reported, size distribution of nanoparticles increases with increasing polymer concentration^{26,27}. Increase in concentration makes an increase in viscosity and decrease in conductivity as described above, makes cone jet unstable and leads to increase in size distribution.

Figure 2B indicates that decrease in flow rate makes an important decrease in size distribution. As other researchers show, the jet mode also depends on flow rate and increased flow rate needs increase in applied voltage to make cone jet mode stable that is essential for monodisperse particles formation²².

Optimization

By solving equations 2 and 3 using the software, optimum values of 0.52 (%w/w), 0.05 (ml/hr) and 15 (kV) were given for chitosan concentration, flow rate and applied voltage, respectively. Using these values, 110 nm for size and 30 for size distribution were predicted by the software. Values were then used for experimental preparation of the samples. The results for three replicates were 110.6 (4.7) nm, 32 (5.8) and 59.3 mV for size, size distribution and zeta potential, respectively (Figure 3). These results show a good agreement between predicted value and obtained value.

Discussion

Compared with conventional methods of generating aerosol droplets, electro spray is a straightforward technique which commonly produces less hetero-dispersed nanoparticles. In this approach, an electrical potential

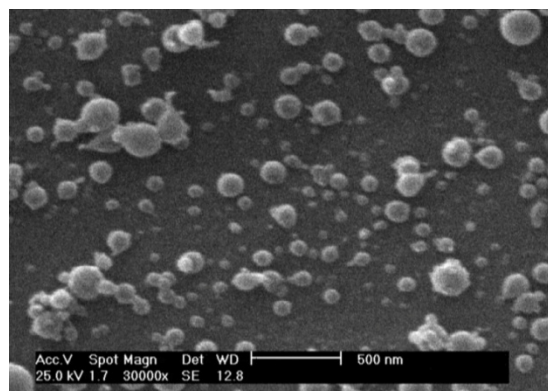


Figure 3. SEM image of chitosan nanoparticles

is applied on the nozzle. Above a certain critical charge the electrical force overcomes the liquid surface tension and breaks the solution down to small electrically charged droplets that repel each other and produce a shower of fine polymer droplets³¹. Nanoparticles with nearly monodispersed size distribution and spherical shapes can be produced by controlling the electro-spraying parameters².

Different jet types may be obtained in an electro-spraying process; dripping, spindle, ramified-meniscus modes in low applied voltage and single cone-jet at an optimum voltage value as well as multi-jet at higher values are commonly observed. A stable single cone-jet mode is essential for production of nearly monodispersed micro- and nanoparticles³². Our experiments were performed at atmospheric pressure and room temperature. Reviewing the graphs indicate that decreased size and narrow size distribution could be obtained with decreasing flow rate and concentration and increasing applied voltage.

As shown in figure 1A and figure 1B by increase applied voltage and decrease the concentration and flow rate, size decrease, that is against other report that most role of applied voltage is on spray mode and after forming stable cone jet increase in applied voltage make slight decrease in size of particles^{2,24}.

Literature shows that by manipulating chitosan concentration, some properties of the solution such as viscosity and conductivity¹ is influenced which in turn affects the droplets diameter. The relation is explained by an equation suggested by Hartman *et al*³²:

$$d = \alpha \left(\frac{\rho \epsilon_0 Q^4}{I^2} \right)^{1/6}$$

$$I \propto (\gamma K Q)^{1/2}$$

where d is the droplet diameter, α is a constant, ρ is the solution density, ϵ_0 is the permittivity of vacuum, Q is the liquid flow rate, I is the current, γ is the surface tension in ambient air, and K is the liquid conductivity. From the equation, viscosity and conductivity have contrary impacts on the size. For instance, Zhang *et al* reported that increase in concentration of chitosan solu-

tion, increased the nanoparticles size as the increase in viscosity was more effective than increase in conductivity of solution¹.

Looking more closely to the diagram, in our work, decreases in chitosan concentration from 0.7 to 0.4 (%w/w) shows substantial decrease in nanoparticles size but decrease from 0.4 to 0.1 (%w/w) made no further decrease and even small increase in nanoparticles size. It is already reported that when chitosan concentration is low, changing the polymer concentration makes substantially more variation in viscosity compared with conductivity¹. So, at such situations, the decrease in viscosity as a function of decrease in chitosan concentration is more than that of conductivity. Thus, decrease in size is expected. However, further decrease in viscosity makes the electrospray process unstable, which could be an explain to the observed size increase³³.

The second most important parameter after solution parameters in electrospraying process is probably flow rate²⁴, which together with solution parameters can control polymer entanglements and Coulomb fission; consequently, affect particles size and size distribution². As shown in figure 1B, in our study reduction in flow rate makes decrease in particles size, as documented previously³⁴. When flow rate increases, the droplet size increases which makes faster movement of droplets towards the collector. Thus, solvent may not evaporate completely when reaches the collector. This makes agglomeration of wet nanoparticles which in turn leads to formation of larger particles^{22,24}.

As shown in figure 2A and 2B increase in applied voltage and decrease the concentration and flow rate makes decrease in size distribution.

Forming the stable single cone-jet mode is essential for near monodisperse nanoparticles production and can be obtained with optimize applied voltage. As other researcher showed after forming stable cone jet, more increase in applied voltage makes multi-jet²⁴, appear irregular shape²⁷ and leads to increase in size distribution. Also as other researcher reported size distribution of nanoparticles increase with increasing in polymer concentration^{26,27}. Increase in concentration make increase in viscosity and decrease in conductivity as above described, that make unstable cone jet and lead to increase in size distribution and as other researcher shows, the jet mode also depends on flow rate and increased flow rate needs increase in applied voltage to making stable cone jet mode that is essential for monodisperse particles formation²².

Conclusion

The aim of this study was to present optimized condition for production of solid chitosan nanoparticles using an electrospray device. The optimum condition for obtaining the minimum size and narrow size distribution was a minimum value for flow rate and highest value for applied voltage along with an optimum chi-

tosan concentration. Afterwards, the optimum conditions were evaluated and solid chitosan nanoparticles were successfully prepared with size of 110.6 nm, size distribution of 32 and zeta potential of 59.3 by electrospray method which can be used for pharmaceutical applications.

Acknowledgement

This research has been supported by Tehran University of Medical Sciences and Health Services grant No. 93-01-87-25230.

References

1. Zhang S, Kawakami K. One-step preparation of chitosan solid nanoparticles by electrospray deposition. *Int J Pharm* 2010;397(1-2):211-217.
2. Almeria B, Deng W, Fahmy TM, Gomez A. Controlling the morphology of electrospray-generated PLGA microparticles for drug delivery. *J Colloid Interface Sci* 2010; 343(1):125-133.
3. Lee KY, Ha WS, Park WH. Blood compatibility and biodegradability of partially N-acylated chitosan derivatives. *Biomaterials* 1995;16(16):1211-1216.
4. Chen MC, Mi FL, Liao ZX, Hsiao CW, Sonaje K, Chung MF, et al. Recent advances in chitosan-based nanoparticles for oral delivery of macromolecules. *Adv Drug Deliv Rev* 2013;65(6):865-879.
5. Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharm* 1992;78(1-3):43-48.
6. Grenha A, Grainger CI, Dailey LA, Seijo B, Martin GP, Remuñán-López C, et al. Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro. *Eur J Pharm Sci* 2007;31(2):73-84.
7. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. *J Control Release* 2006;115(2):216-225.
8. Krauland AH, Alonso MJ. Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system. *Int J Pharm* 2007;340(1-2):134-142.
9. Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. *Int J Biol Macromol* 2014;64:353-367.
10. Hayati I, Bailey AI, Tadros TF. Mechanism of stable jet formation in electrohydrodynamic atomization. *Nature* 1986;319(6048):41-43.
11. Reyderman L, Stavchansky S. Electrostatic spraying and its use in drug delivery-cholesterol microspheres. *Int J Pharm* 1995;124(1):75-85.
12. Valo H, Peltonen L, Vehviläinen S, Karjalainen M, Kostiaainen R, Laaksonen T, et al. Electrospray encapsulation of hydrophilic and hydrophobic drugs in poly(LG-lactic acid) nanoparticles. *Small* 2009;5(15):1791-1798.
13. Ding L, Lee T, Wang CH. Fabrication of monodispersed Taxol-loaded particles using electrohydrodynamic atomization. *J Control Release* 2005;102(2):395-413.

14. Jaworek A. Micro-and nanoparticle production by electro-spraying. *Powder Technol* 2007;176(1):18-35.
15. Enayati M, Chang MW, Bragman F, Edirisinghe M, Stride E. Electrohydrodynamic preparation of particles, capsules and bubbles for biomedical engineering applications. *Colloids Surf A Physicochem Eng Asp* 2011;382(1-3):154-164.
16. Bohr A, Yang M, Baldursdóttir S, Kristensen J, Dyas M, Stride E, et al. Particle formation and characteristics of Celecoxib-loaded poly (lactic-co-glycolic acid) micro-particles prepared in different solvents using electro-spraying. *Polymer* 2012;53(15):3220-3229.
17. Rezvanpour A, Wang CH. Computational and experimental studies of electro-spray deposition process in pharmaceutical micro-pattern formation. *Chem Eng Sci* 2011;66(17):3836-3849.
18. Lee YH, Mei F, Bai MY, Zhao S, Chen DR. Release profile characteristics of biodegradable-polymer-coated drug particles fabricated by dual-capillary electro-spray. *J Control Release* 2010;145(1):58-65.
19. Singh R, Lillard JW Jr. Nanoparticle-based targeted drug delivery. *Exp Mol Pathol* 2009;86(3):215-223.
20. Xie Y, Castracane J. High-voltage, electric field-driven micro/nanofabrication for polymeric drug delivery systems. *IEEE Eng Med Biol Mag* 2009;28(1):23-30.
21. Gomez A, Bingham D, De Juan L, Tang K. Production of protein nanoparticles by electro-spray drying. *J Aerosol Sci* 1998;29(5-6):561-574.
22. Thien DVH, Hsiao SW, Ho MH. Synthesis of electro-sprayed chitosan nanoparticles for drug sustained release. *Nano Life* 2012;2(1):1250003.
23. Hu JF, Li SF, Nair GR, Wu WT. Predicting chitosan particle size produced by electrohydrodynamic atomization. *Chem Eng Sci* 2012;82(12):159-165.
24. Bock N, Dargaville TR, Woodruff MA. Electro-spraying of polymers with therapeutic molecules: state of the art. *Prog Polym Sci* 2012;37(11):1510-1551.
25. Enayati M, Ahmad Z, Stride E, Edirisinghe M. One-step electrohydrodynamic production of drug-loaded micro-and nanoparticles. *J R Soc Interface* 2010;7(45):667-675.
26. Karimi Zarchi AA, Abbasi S, Faramarzi MA, Gilani K, Ghazi-Khansari M, Amani A. Development and optimization of N-Acetylcysteine-loaded poly (lactic-co-glycolic acid) nanoparticles by electro-spray. *Int J Biol Macromol* 2015;72:764-770.
27. Arya N, Chakraborty S, Dube N, Katti DS. Electro-spraying: a facile technique for synthesis of chitosan-based micro/nanospheres for drug delivery applications. *J Biomed Mater Res B Appl Biomater* 2009;88(1):17-31.
28. Songsurang K, Praphairaksit N, Siraleartmukul K, Muangsinsin N. Electro-spray fabrication of doxorubicin-chitosan-tripolyphosphate nanoparticles for delivery of doxorubicin. *Arch Pharm Res* 2011;34(4):583-592.
29. Gunst RF. Response surface methodology: process and product optimization using designed experiments. *Technometrics* 1996;38(3):284-286.
30. Jaworek A. Electrostatic micro-and nanoencapsulation and electroemulsification: a brief review. *J Microencapsul* 2008;25(7):443-468.
31. Hartman RPA, Brunner DJ, Camelot DMA, Marijnissen JCM, Scarlett B. Jet break-up in electrohydrodynamic atomization in the cone-jet mode. *J Aerosol Sci* 2000;31(1):65-95.
32. Garg UK, Kaur MP, Garg VK, Sud D. Removal of nickel (II) from aqueous solution by adsorption on agricultural waste biomass using a response surface methodological approach. *Bioresour Technol* 2008;99(5):1325-1331.
33. Smith DP. The electrohydrodynamic atomization of liquids. *IEEE Trans Ind Appl* 1986;(3):527-535.
34. Yao J, Lim LK, Xie J, Hua J, Wang CH. Characterization of electro-spraying process for polymeric particle fabrication. *J Aerosol Sci* 2008;39(11):987-1002.