

Investigation of drug release from paclitaxel loaded polylactic acid nanofibers

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ABSTRACT

Objective(s): In this study, drug loaded electrospun nanofibrous mats were prepared and drug release and mechanism from prepared nanofibers were investigated.

Materials and Methods: Paclitaxel (PTX) loaded polylactic acid (PLA) nanofibers were prepared by electrospinning. The effects of process parameters, such as PTX concentration, tip to collector distance, voltage, temperature and flow rate on the mean diameter of electrospun PTX loaded PLA nanofibers were investigated. Scanning electron microscopy (SEM) was used to investigate the fiber morphology and mean fiber diameter of prepared nanofibers. Response surface methodology was used to model the average diameter of electrospun PLA/PTX nanofibers.

Results: The predicted fiber diameter was in good agreement with the experimental result. *In Vitro* drug release in phosphate buffer solution (PBS) and acetate buffer for the produced samples showed that diffusion is the dominant drug release mechanism for PTX loaded fibers.

Conclusion: Electrospinning was shown to be very promising approach to the formulation of Paclitaxel in order to enhance its release in a sustained and prolonged manner.

Keywords: Drug Release, Nanofiber, Paclitaxel

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INTRODUCTION

Electrospinning is a simple and efficient method that uses high voltage to form a liquid jet from polymer solutions. Fibers in the nano-scale range are formed as the charged polymer solution is stretched continuously due to electrostatic repulsions of the surface charges and the solvent [1]. Electrospun nanofibers have been used in various fields which include ultrasonication, electrical, and biomedical applications (scaffolds for tissue engineering, wound dressing and drug delivery) [2].

The morphology of the fibers was found to be dependent upon the process parameters; including solution concentration, applied electric field strength, tip to collector distance and flow rate [3].

Fiber diameter of electrospun nanofibers could affect physical, mechanical and biological properties. So, optimizing the mean fiber diameter which is a function of process parameters is crucial. Response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical modeling and has been widely used to optimize and design operating conditions. RSM has been used successfully for material and process optimization in numerous studies [4-8]. Gu *et al.* [5], who employed RSM, reported no significant effect of voltage on the processing of PAN nanofibers. They found that the concentration of solution played an important role in the average diameter of nanofibers. Several local delivery systems such as drug loaded fibers, hydrogel and nanoparticles, have been studied for biomedical applications in recent years [9-11].

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Among these systems, biodegradable polymer electrospun nanofibers have attracted much attention because of their appealing features such as high surface to area ratio, high loading capacity and encapsulation efficiency [12, 13]. Xie *et al.* [13] fabricated cisplatin-loaded PLA/PLGA (30/70) fibers for long-term sustained delivery of cisplatin to treat C6 glioma *in vitro*.

The drug encapsulation efficiency was more than 90% and the cisplatin-loaded fibers showed sustained release for more than 75 days without initial burst release. Poly (lactic acid) (PLA) polymer solutions with different amounts of paclitaxel (PTX) were used for fabrication of electrospun nanofibrous mats. In this contribution, the simultaneous effects of five electrospinning parameters, such as PTX concentration in polymer solution, voltage, tip to collector distance, flow rate and temperature on the mean fiber diameter (MFD) of PLA-PTX nanofibers were systematically investigated. Also, *in vitro* drug release characteristics, release mechanism and kinetics analysis were investigated in this study.

MATERIALS AND METHODS

Materials

PLA with molecular weight of 75 kDa was supplied by Sigma Aldrich. PTX (>99.0%) was purchased from Sigma Aldrich. Chloroform was bought from Merck (Germany). All other chemicals were used without further purification.

Preparation of electrospun nanofibrous scaffolds

PTX (0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 w/w %) was added into 10 mL chloroform with stirring until a clear solution was obtained.

Then, 1.0 g of PLA was added to the PTX/Chloroform solution. This was followed by magnetic stirring until the polymer dissolved completely. An electrospinning machine (ANSTCO-RN/1, Asian Nanostructures Technology Co., Iran) was used to produce PLA/PTX nanofiber mats.

The syringe containing PLA solution with different concentrations of PTX was placed on a syringe pump (New Era NE-100, USA) used to dispense the solution at a controlled rate (0.3-1.5 (ml/h)).

A high-voltage DC power supply (Nano spinner TM, Iran) was used to generate the electric field needed for fiber production (9-23 kV). The collector was a

rotating cylindrical drum which was placed at different distances from the needle (10-30 cm).

Characterization of nanofibers morphology

The fiber morphology and diameter of the electrospun PLA/PTX fibers were determined using scanning electron microscopy (SEM; Vega II XMU instrument Tescan, Czech Republic).

A small section of the nonwoven mat was placed on the SEM sample holder and sputter-coated with gold. For each experiment, MFD was determined from about 100 measurements of random fibers in four SEM micrographs taken from different areas of the mat.

In vitro PTX release

PTX loaded electrospun PLA nanofibrous mats were immersed in 20 mL of PBS at pH=7.4 and acetate buffer (pH=4.8) under gentle shaking at 37 °C. At predetermined time intervals, 4 mL of each extracted solution was analyzed by UV-vis spectroscopy at a wavelength of 480 nm.

This amount of the solutions was immediately replaced with an equal volume of the dissolution medium to keep the volume constant.

Kinetics and mechanism of drug release

Drug release mechanism from prepared nanofibers was investigated using Korsmeyer-peppas equation (Eq. (1)) [14,15]. In Eq. (1), M_t/M_∞ is a fraction of drug released at time t , k is the release rate constant and n is the release exponent. The n value is used to characterize different release kinetics.

$$M_t/M_\infty = kt^n \quad (1)$$

In this model, the value of n characterizes the release mechanism of the drug. In the case of cylindrical tablets, $0.45 e^{-n}$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to the non-Fickian transport, $n = 0.89$ to the Case II (relaxational) transport, and $n > 0.89$ to the super case II transport.

Experimental design

Central composite design (CCD) was employed to study the effects of electrospinning parameters on the MFD of nanofibrous mats.

The independent variables X_1, X_2, X_3, X_4, X_5 were as follows (low/high value): PTX/PLA concentration ((w/w)%) 0/30; distance (cm) 10/30; voltage (kV) 9/23, flow rate (ml/h) 0.3/1.5 and temperature (°C) 25/45.

The ranges of the variables were selected from trial experiments and represented the attainable limits for nanofiber formation and/or equipment operation. Each variable was coded at five levels: -2, -1, 0, +1, and +2.

The selected factors and their levels are shown in Table 1. The coded values of these factors were obtained according to equation 2.

$$X_i = (x_i - x_0) / \Delta x_i \quad (2)$$

Where X_i is the coded value of the factor, x_i is the real value of the factor, x_0 is the real value of the factor at the center point, and Δx_i is the step change value of the factor. The design matrix is shown in Table 2.

RESULTS AND DISCUSSION

Construction of model equations

Thirty two experiments were designed using CCD methodology. The experimental process conditions

Table 1. Selected factors and their levels

Variable	Low axial (-2)	Low factorial (-1)	Center (0)	High factorial (+1)	High axial (+2)
X ₁ (A)	0.0	7.5	15.0	22.5	30.0
X ₂ (B)	10.0	15.0	20.0	25.0	30.0
X ₃ (C)	9.0	12.5	16.0	19.5	23.0
X ₄ (D)	0.3	0.6	0.9	1.2	1.5
X ₅ (E)	25.0	30.0	35.0	40.0	45.0

X₁ (A) = Concentration (w/w) %; X₂ (B) = Distance (cm); X₃ (C) = Voltage (kV); X₄ (D) = Flow rate (ml/h); X₅ (E) = Temperature (°C)

Table 2. Experimental conditions and their responses

Run No.	Coded value of X ₁ (A)	Coded value of X ₂ (B)	Coded value of X ₃ (C)	Coded value of X ₄ (D)	Coded value of X ₅ (E)	Response MFD (nm)
1	1	-1	-1	-1	-1	237
2	0	0	2	0	0	393
3	0	0	0	0	0	305
4	0	0	0	0	-2	356
5	1	1	-1	-1	1	281
6	0	0	0	0	0	290
7	2	0	0	0	0	276
8	0	0	0	-2	0	320
9	-2	0	0	0	0	310
10	1	1	1	-1	-1	345
11	1	1	1	1	1	445
12	0	0	-2	0	0	170
13	1	-1	1	1	-1	434
14	-1	-1	1	-1	-1	227
15	-1	1	-1	1	1	265
16	-1	-1	-1	-1	1	210
17	-1	1	-1	-1	-1	312
18	0	0	0	0	0	290
19	0	2	0	0	0	285
20	0	0	0	2	0	325
21	-1	-1	-1	1	-1	235
22	0	0	0	0	2	315
23	-1	1	1	-1	1	345
24	0	0	0	0	0	301
25	0	0	0	0	0	315
26	0	0	0	0	0	308
27	0	-2	0	0	0	245
28	-1	1	1	1	-1	345
29	1	-1	-1	1	1	265
30	-1	-1	1	1	1	367
31	1	1	-1	1	-1	278
32	1	-1	1	-1	1	248

and their responses are presented in Table 2. Design Expert 7 Software (trial version, Stat-Ease Inc., USA) applied to analyze the results. A polynomial model for the average variations of PTX/PLA nanofibers was chosen and fitted to the results.

Equation 3, found to be adequate for the MFD prediction of PTX/PLA electrospun nanofibers.

$$MFD = 298.45 + 25.74B + 51.24 D + 21.64 E - 22.65 AB + 32.24 CE \quad (3)$$

A, B, C, D and E are the coded values for PTX concentration, tip to collector distance, applied voltage, temperature and flow rate, respectively. ANOVA analysis implies that the fitted model is significant in 95% confidence level (p-value < 0.05). Values of R² and adjusted R² for the model are 0.95 and 0.89, respectively. The high value of R² indicates that the polynomial equation is capable of representing the system under the given experimental domain.

Optimization

An optimization technique was used in this research for optimization of PTX/PLA nanofibers diameter using design expert software. The aim of this study was to find the conditions of producing fibers with minimum diameter. The amounts of process parameters at optimum conditions are as follows: 12 kV voltage, 20.5 (w/w) % concentration, 17 cm distance, 35 °C temperature and 1.0 ml/h flow rate.

According to the above conditions, the minimum average diameter of PTX/PLA nanofibrous mats was

about 138 nm. In order to investigate the reliability of the fibers produced from the electrospinning process, a test was conducted to measure the fiber diameter from the given set of process parameters. SEM image of optimum conditions is shown in Fig. 1.

The mean fiber diameter was about 129 nm. Comparing the experimental result with the response provided by the model shows that they are close to each other.

In Vitro drug release from the optimized nanofibrous mats

Fig. 2 shows accumulative release of PTX from nanofibrous mats under optimized conditions at different pH.

As shown, the cumulative release amount was only about 15% in PBS 7.4 in 10 h. For the lower pH, a faster PTX release from the fibers was observed with about 40% in 10 h. It is reasonable that the solubility of PTX increases under pH 1.2, leading to a faster PTX diffusion from the fibers into medium. It is well known that diffusion is the typical drug release mechanism for drug loaded fibers in the early stage owing to the slow degradation of PLA, thus the mobility of polymer chains seems play the dominant role [16].

In order to investigate the release mechanism of BSP molecules from drug-loaded samples the resultant data were fitted to the Korsmeyer–Peppas model.

The data obtained from in vitro drug release studies were plotted as log cumulative percentage of drug release versus log time, to study the release kinetics. The results are presented in Table 3.

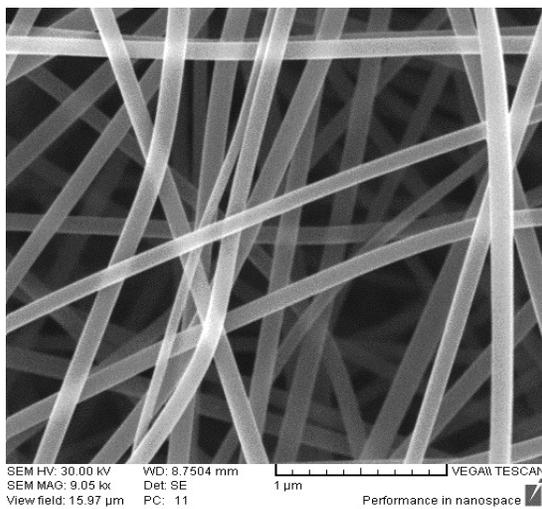


Fig. 1. SEM image of nanofibers at optimum condition

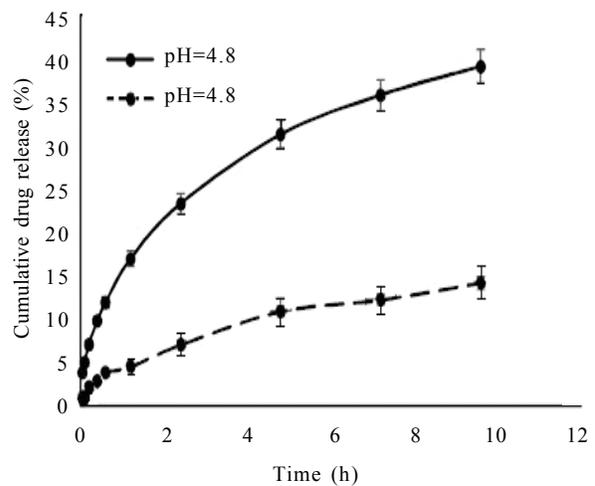


Fig. 2. Cumulative release of PTX from nanofibrous mats in different buffers

Table 3. PTX release kinetic parameters for nanofibrous mats in different media

Model	R ²	Mechanism	n	k	Release medium
Korsmeyer–Peppas	0.94	Non-Fickian	0.65	0.065	PBS
	0.98	Fickian	0.42	0.243	Buffer 4.8

It shows that in PBS media the drug was released by a non-Fickian diffusion mechanism, but in buffer with pH 4.8, the drug was released by a Fickian diffusion kinetics.

CONCLUSION

In this study, PLA electrospun nanofibrous mats loaded with PTX were prepared. PTX/PLA concentration, spinning distance, applied voltage, temperature and flow rate were optimized using RSM with central composite design.

In Vitro drug release from prepared nanofibrous mats and the release mechanism were investigated.

Obtained results showed that diffusion mechanism is dominant.

The prepared PLA/PTX nanofibers are highly promising as local implantable scaffolds for the treatment of a tissue defect after tumor resection. PTX loaded PLA nanofibers could be an excellent substrate for biomedical applications.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

- Zeng J, Xu X, Chen X, et al. Biodegradable electrospun fibers for drug delivery. *J Control Release*. 2003; 92: 227–231.
- Altman GH, Diaz F, Jakuba C, et al. Silk-based biomaterials. *Biomaterials*. 2003; 24: 401–416.
- Chen JP, Ho KH, Chiang YP, et al. Fabrication of electrospun poly(methyl methacrylate) nanofibrous membranes by statistical approach for application in enzyme immobilization. *J Mem Sci*. 2009; 340: 9–15.
- Heikkilä P, Harlin A. Parameter study of electrospinning of polyamide-6. *Eur Polym J*. 2008; 44: 3067–3079.
- Gu SY, Ren J and Vancso GJ. Process optimization and empirical modeling for electrospun polyacrylonitrile (PAN) nanofiber precursor of carbon nanofibers. *Eur Polym J*. 2005; 41: 2559–2568.
- Yordem OS, Papila M and Menciloglu YZ. Effects of electrospinning parameters on polyacrylonitrile nanofiber diameter: An investigation by response surface methodology. *Mater Des*. 2008; 29: 34–44.
- Sukigara S, Gandhi M, Ayutsede J, et al. Regeneration of Bombyx mori silk by electrospinning. Part 2. Process optimization and empirical modeling using response surface methodology. *Polymer*. 2004; 45: 3701–3708.
- Zahedi P, Rezaeian I, Jafari SH, et al. Preparation and release properties of electrospun poly(vinyl alcohol)/poly(L-caprolactone) hybrid nanofibers: Optimization of process parameters via D-optimal design method. *Macromol Res*. 2013; 21: 649–659.
- Sill TJ and Von Recum HA. Electro spinning: Applications in drug delivery and tissue engineering. *Biomaterials*. 2008; 29: 1989–2006.
- Liang D and Chu B. Functional electrospun nanofibrous scaffolds for biomedical applications. *Adv Drug Deliv Rev*. 2007; 59: 1392–1412.
- Jeong B, Bae YH, Lee DS, et al. Biodegradable block copolymers as injectable drug delivery systems. *Nature*. 1997; 388: 860–862.
- Jin HJ, Fridrikh SV, Rutledge GC, et al. Electrospinning Bombyx mori silk with poly(ethylene oxide). *Biomacromolecules*. 2002; 3: 1233–1239.
- Okuda T, Tominaga K and Kidoaki S. Time-programmed dual release formulation by multilayered drug-loaded nanofiber meshes. *J Control Release*. 2010; 143: 258–264.
- Korsmeyer RW, Gurny R, Doelker E, et al. Mechanisms of solute release from porous hydraulic polymers. *Int J Pharm*. 1983; 15: 25–35.
- Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm Acta Helv*. 1985; 60: 110–111.
- Xu X, Chen X, Wang Z, et al. Ultrafine PEG-PLA fibers loaded with both paclitaxel and doxorubicin hydrochloride and their in vitro cytotoxicity. *Eur J Pharm Biopharm*. 2009; 72: 18–25.