Macromolecular Research, Vol. 21, No. 6, pp 649-659 (2013)
DOI 10.1007/s13233-013-1064-z

www.springer.com/13233
pISSN 1598-5032 eISSN 2092-7673

Preparation and Release Properties of Electrospun Poly(vinyl alcohol)/ Poly(ε-caprolactone) Hybrid Nanofibers: Optimization of Process Parameters *via* D-Optimal Design Method

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Received April 15, 2012; Revised July 8, 2012; Accepted July 11, 2012

Abstract: The main purpose of this work was to develop biomedical electrospun nanofibrous mats based on a poly(vinyl alcohol)/poly(ε-caprolactone) (80/20) hybrid with a defined drug release rate using tetracycline hydrochloride as a model drug. The electrospinning process parameters, such as polymer solution concentration, distance between injecting syringe tip/collector, voltage, injected flow rate and the polyvinyl alcohol crosslinking time were optimized *via* a D-optimal design method for a suitable nanofiber diameter with an optimal drug release rate. The morphology of nanofibers and their mean diameters were studied by a scanning electron microscopy technique. The results showed that the mean diameters of nanofibers were significantly reduced after drug loading. The swelling, weight loss and biodegradability of nanofibers samples investigated by FTIR were also determined. Two main mechanisms *via* penetration and erosion were evaluated. *In vitro* drug release in a phosphate buffer environment at pH=7.2 for the samples demonstrated that the polymer type and hydrophilic nature of the polymer/drug system is very effective in the kinetics and mechanism of drug release. Hybridization of poly(vinyl alcohol)/poly(ε-caprolactone) with a known ratio showed to be a suitable and useful method in the electrospinning of nanofibers samples for superior control of the drug release rate. Finally, nanofibrous mats of polyvinyl alcohol and polyvinyl alcohol/poly(ε-caprolactone) hybrid (80/20) had much better drug release rate characteristics for tetracycline hydrochloride as a model drug compared with cast film samples loaded with the same drug.

Keywords: nanofiber, poly(vinyl alcohol) (PVA), poly(ε-caprolactone) (PCL), hybrid, response surface methodology, drug release, tetracycline.

Introduction

The electrospun nanofibers show a great promise for developments of many new drug delivery systems (DDS) due to their unique properties. The current state of electrospun nanofibers with DDS is focused on drug-loaded nanofiber preparation from pharmaceutical and biodegradable polymers with different types of DDS. In recent years electrospinning has been an interesting process for the production of very fine fibers. The diameter of these fibers is in nanoscale or sub-micrometer. The ratio of surface area to volume or mass ratio of these fibers is high.[1] Electrospinning process is a simple method for production of nanofibers, especially for production of complex hybrid mats,[2,3] blending,[4-6] core-sheath[7-9] from biodegradable and immiscible polymers. As Figure 1 shows, in electrospinning a high electric potential is used for an electrically charged polymer melt or solution coming out of a syringe needle. The solvent is evaporated before reaching the collector and the rest is collected on a collector as an interconnected fibers and a web. In this work, the electrospinning system was equipped with two syringes in order to prepare hybrid nanofibers.

The morphology of the as spun nanofibers is governed by the following parameters: (1) the polymer material characteristics such as molecular weight, molecular weight distribution and structure (branched, linear, *etc*.) and the polymer solution properties (viscosity, conductivity, *etc*.), (2) electrospinning process parameters such as electric potential, flow rate, concentration, distance between the capillary and the collection screen, ambient parameters (temperature, humidity) and (3) the motion of target screen.[10]

One of the main advantages of electrospinning technique over the traditional film casting method is the high porous

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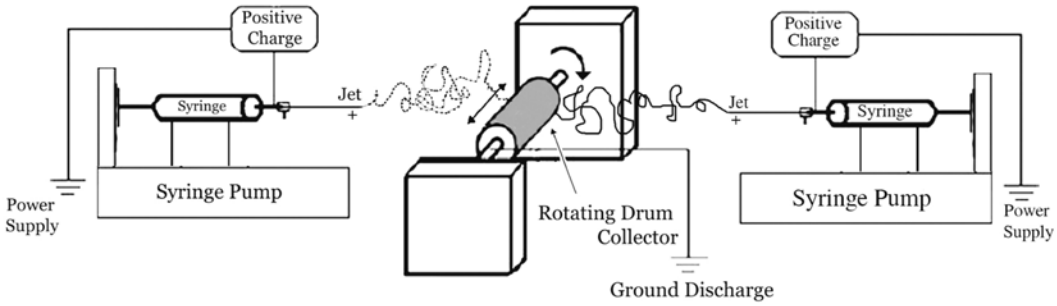

Figure 1. Schematic diagram of the electrospinning apparatus.

structure of the electrospun fibrous mats which allows a better drug penetration and release rate as compared to the cast films.[11] In order to have nanofibrous webs with high porosity, an effective control on diameter of nanofibers is needed. This can be realized by optimizing the electrospinning process parameters.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes.[12] This technique is an interesting method for optimizing several variable parameters. Yordom[13] investigated the effect of materials and process parameters on the diameters of polyacrylonitryl (PAN) electrospun fibers. The experimental design was carried out by RSM to set solution concentration, voltage, the distance between the syringe needle tip to the collector and solution flow rate. Sukigara *et al*.[14] used RSM for modeling and optimizing electrospinning parameters in order to get nano-silk fibers and used regeneration method from domestic silkworm, *Bombyx moni*. Electrical field and silk concentration were used as variables for the control of fibers diameter in the distance between the syringe needle tip and the collector. Gu and coworkers[15] used PAN/*N*,*N*-dimethylformamide (DMF) solution as a precursor of carbon nanofibers and obtained electrospun fibers with diameters ranging from 200 to 1,200 nm. They found that the concentration of solution played an important role in the average diameter of nanofibers.

Electrospun nanofibers were developed from a number of polymers as a matrix for drug release. Poly(lactic acid) (PLA) and poly(ethylene-*co*-vinylacetate) (PEVA) were successfully electrospun in the presence of tetracycline hydrochloride as a model drug in their first report by Kenawy *et al*..[16] The results of drug release profile showed that they had a better release rate for tetracycline hydrochloride compared with Actisite® commercial sample and also cast film sample. An early patent registered by Ignatious and Baldoni described electrospun polymer nanofibers for pharmaceutical compositions which can be designed to provide rapid, immediate, delay or modified dissolution such as sustained and/or pulsatile release characteristics.[17] Zong *et al*.[11] also used PLA as matrix and mefoxin was selected as a model drug. Their results were also similar to those reported by Kenawy *et al*. For poorly water soluble drugs such as intraconazole (an antifungal drug), ketanserin (as a drug for ischemic acute renal failure) and polyurethane was used as matrix. They concluded that the release rates of poorly water soluble drugs can be improved by hydrophilic polymers and the release rate of these drugs can be controlled by adjusting the polymer/drug ratio.

In recent decades, electrospun polymeric nanofibers for biomedical applications such as wound dressing,[18,19] tissue engineering[20] and controlled drug delivery[21-23] have been of much interest. In our recent work,[18] the effect of different concentration of tetracycline hydrochloride on the morphology and drug release rate from the electrospun nanofibrous matrices of PLA, poly(ε-caprolactone) (PCL) and PLA/PCL (50/50) blend were investigated. The results showed that PLA/PCL (50/50) blend and PCL with 500 μg/mL drug loading, after 48 h, have a drug release rate of about 65% and 70%, respectively. While, PLA nanofibrous mats due to their high crystallinity showed only about 30% release rate. Moreover, antibacterial *in vitro* tests on two bacteria, namely *E. coli* and *S. aureus* showed a better performance for nanofibers loaded with tetracycline on *S. aureus* bacteria. This is due to cellular nature of *S. aureus* which results in its Gram-positive.

Some of the most relevant and more commonly used mathematical models describing the dissolution curves for drug transport inside pharmaceutical systems are shown in Table I. These models better describe the drug release from pharmaceutical systems when it results from a simple phenomenon or when that phenomenon by the fact of being the rate limiting step, conditions all the other processes. In these models, Q_t is the amount of drug dissolved at time t, Q_0 is

Table I. Mathematical Models Used to Describe Drug Dissolution Curves

Zero Order	$Q_t=Q_0+K_0t$
First Order	$\ln Q_t=\ln Q_0+K_1t$
Higuchi	$Q_t=K_H\sqrt{t}$
Hixon-Crowell	$Q_0^{1/3}-Q_t^{1/3}=K_st$
Korsmeyer-Peppas	$M_t/M_\infty=K_kt^n$

the initial amount of drug in the solution (often Q_0=0) and K is the release constant. In Korsmeyer-Peppas equation, M_t is the accumulative amount of drug released at time t, M_∞ is the initial drug loading and n is the diffusion exponent suggesting the nature of the drug release mechanism.[22]

The aim of this work is to optimize the electrospinning process parameters *via* introduction of a D-optimal design approach for production of electrospun nanofibrous mats based on poly(vinyl alcohol) (PVA), PCL and (80/20) PVA/PCL hybrids. The morphology, *in vitro* drug release characteristics, release mechanism and kinetics analysis using appropriate mathematical models as well as weight loss and swelling behavior of the mats are studied and compared to that of cast films.

Experimental

Materials. Poly(vinyl alcohol) (PVA) (trade name, GH-17R-GOHSENOL, a white-yellowish powder, viscosity of 4% solution=30 mPa.s, melting point 180 °C) was purchased from NIPPON GOHSEI Co., Japan. Poly(ε-caprolactone) (PCL) (biodegradable, weight average molecular weight 90 kg/mol) was obtained from Sigma-Aldrich Co., Pilsburg, the Netherlands. Tetracycline hydrochloride, an antibiotic (99.99% purity, yellow powder) was supplied by Merck, Germany. All the other chemicals were analytical reagent grades and were used without further purification.

Preparation of Neat and Drug-Loaded Electrospun PVA, PCL and PVA/PCL Mats, Cast Films from PVA and PCL. Weighed PVA powder was dissolved in distilled water at 80 °C, stirred for about 2 h and finally a 8% (w/v) solution of PVA in distilled water was obtained. After cooling the PVA solution to room temperature, weighed tetracycline hydrochloride dissolved in 3 mL methanol was added to PVA solution to get a 500 μg/mL drug concentration. The mixture was stirred for about 30 min. Similarly, weighed granules of PCL was dissolved in 7/3 (v/v) solution of chloroform/DMF in order to get a 9.5% (w/v) concentration and stirred for about one hour. In the next stage, the PCL solution containing tetracycline hydrochloride with 500 μg/mL concentration was prepared. First, drug was weighed and dissolved in 3 mL DMF; and then, it was added to polymer solution samples with known concentration containing 7 mL of chloroform. Then, the mixture was stirred for about 30 min. Since PVA and PCL are immiscible with different characteristics they should be processed under different electrospinning conditions. An electrospinning device model (eSpinner NF-COEN/II) Asian Nanostructures Technology Co. Iran, used for preparation of the samples. The electrospinning conditions for PVA sample were: solution flow rate=0.8 mL/h, voltage=19.5 kV, gauge needle=0.7 mm and distance between the needle tip to the rotational collector=10 cm. The electrospinning conditions for PCL sample were as follows: solution flow rate=0.45 mL/h, voltage=19.5 kV, gauge needle=0.7 mm and distance between the needle tip to the rotational collector=14.5 cm. (80/20) PVA/PCL nanofibers hybrid mats were prepared by using a syringe containing PVA/drug and the other syringe containing PCL/drug. According to the above conditions, a simultaneous injection from both syringes was carried out. The spun nanofibrous mats were collected on a sterile aluminum foil.

Crosslinking of both neat and tetracycline hydrochloride containing PVA and also PVA/PCL (80/20) fiber mats was carried out by clamping each of the fibrous mat between a pair of supporting stainless steel frames (4.5 cm×10 cm) with adhesive tapes. The clamped fiber mat samples were then placed into a sealed chamber saturated with the vapor from 20 mL of the glutaraldehyde (GTA) 25% (v/v) aqueous solution. The temperature of the chamber was maintained at 32 °C and each fiber mat sample was exposed to the moist GTA vapor for 12 h. After exposure, the samples were heat treated in an oven at 45 °C for 24 h to enhance the crosslinking reaction and to remove excess moist, of unsaturated GTA. To achieve fully dried electrospun nanofiber samples and to ensure evaporation of the organic solvents from the spun fibers, the samples were then dried at room temperature for about 12 h at a relative humidity of 32% until a constant weight was attained.

The drug loaded PVA and PCL films were prepared by solution casting technique, from PVA and PCL solution having concentrations of 8 and 9.5% (w/v), respectively. The thickness of all the spun mats (for the mats that were electrospun for about 4 h) and the cast films were controlled between 700-900 μm.

Morphological Studies. The electrospun nanofibers on aluminum foil were coated with a thin layer of gold by a Bio-Rad E5200 auto sputter coater (England). For the morphological observations of the samples, a scanning electron microscope (CamScan MV2300 model, Oxford) with 5000X magnification was used. The mean values of the nanofiber diameters from five different sections were recorded.

Degree of Swelling and Weight Loss. The degree of swelling and weight loss for nanofiber mats and films were calculated by eqs. (1) and (2), respectively. Both tests were carried out in the release medium (phosphate buffer solution, PBS, pH=7.2) at 37°C for 1, 6, and 24 h.

$$\text{Degree of swelling}=\frac{M-M_d}{M_i}\times 100 \quad (1)$$

$$\text{Weight loss (\%)}=\frac{M_i-M_d}{M_i}\times 100 \quad (2)$$

where M is the weight of swollen nanofibers and films samples which were wiped dry with filter paper, M_d is the dried mass of immersed sample in PBS, measured by drying the swollen nanofiber mats and films in an oven at 45 °C until a constant weight was reached, and M_i is the initial dry mass of the sample.

Biodegradability of the Samples. Biodegradability of nanofibrous PVA, PCL, and (80/20) PVA/PCL hybrid samples and also their cast films after 24 h immersion in PBS environment was studied by Fourier transformed infrared (FTIR) spectroscopy. A FTIR model EQUINOX 55, Bruker Co., with an attenuated total reflection technique at room temperature was used. The wave number range used in FTIR spectroscopy was 1000-4000 cm^{-1}.

***In vitro* Drug Release Studies.** Drug release from electrospun nanofibrous mats and films were measured by placing a known mass with an approximate dimensions (2.5 cm×2.5 cm) of material into 20 mL of PBS at pH=7.2 under constant stirring at 37 °C.

An ultraviolet-visible spectrometer (UNICAM Series 8700 model, Philips Co., Amsterdam, the Netherlands) was used for the determination of the antibiotic (tetracycline hydrochloride) release rate. The maximum wavelength for tetracycline hydrochloride solution was 364.8 nm. The calibration curve, with r^2 value of 0.9966, plotted with Beer-Lambert law according to the following linear equation:

$$A = \varepsilon \times l \times c = 0.037 \times c \tag{3}$$

where "A" is the absorption percentage, "c" is the concentration of the antibiotic, "l" is the distance the light travels through the material and "ε" is the extinction coefficient of the antibiotic (Figure 2).

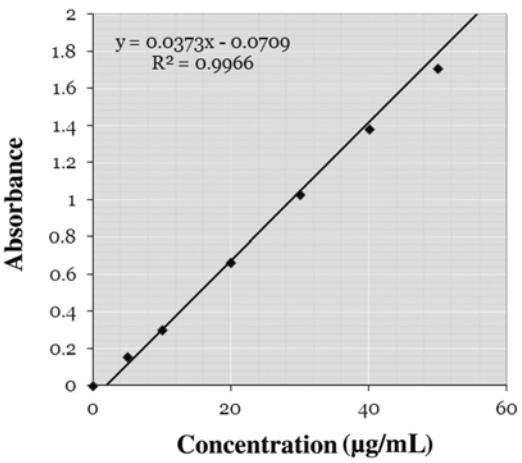

Figure 2. Calibration curve according to Beer-Lambert equation for tetracycline hydrochloride by UV-visible spectroscopy.

Analysis of Drug Release Kinetics and Modeling. According to Table I, the release kinetics of tetracycline hydrochloride from the all samples can be described using the Korsmeyer-Peppas equation:

$$\frac{M_t}{M_\infty} = Kt^n \tag{4}$$

where M_t is the accumulative amount of drug released at time t, M_∞ is the initial drug loading, K is a constant characteristic of the drug-polymer system, and n is the diffusion exponent suggesting the nature of release mechanism. In addition to Fickian theory, four more models were used to further analyze the profile of drug release including zero order, first order, Higuchi and Hixon-Crowell models.[24]

D-Optimal Design Method. The experimental plan was carried out by using Design Expert V.6 (Stat-Easy Inc., Minneapolis, MN). Table II shows the six main effective independent parameters PVA concentration, flow rate, distance between the needle tip to the collector, applied voltage, crosslinking time and PCL concentration which are represented respectively by x_1, x_2, x_3, x_4, x_5, and x_6. Factors x_1-x_6 are shown as coded and numeric formats in two actual surfaces. Tables III and IV give the experimental design results, which were obtained by D-optimal in 28 and 26 runs with the analytical data of the nanofibers diameters for PVA and PCL mats, respectively. On this basis, the effects of all the parameters (x_1-x_6) for the answers (y_1 and y_2) were investigated. Finally for each response, a polynomial model on the basis of effective parameters and their interactions are fixed and represented as a generalized scheme.

Results and Discussion

Optimization of PCL, PVA and Their Hybrid Nanofibrous Mats. Figure 3(a)-(i) shows 3-dimensional graphs of optimized effective parameters on the diameter of PVA nanofibers. General polynomial equation for the average variations of PVA nanofibers diameters without drug with (x_1-x_5) factors and especially their interactions is as follows:

Table II. The Range of Independent Variables and Coded Level for Experimental Design

Factor	Name	Low Actual	High Actual	Low Coded	High Coded
x_1	PVA Concentration	6	10	-1	1
x_2	Flow Rate	0.4	1	-1	1
x_3	Distance between Needle Tip/Collector	10	18	-1	1
x_4	Applied Voltage	10	25	-1	1
x_5	Crosslinking Time	2	12	-1	1
x_6	PCL Concentration	8	12	-1	1

Table III. D-Optimal Design to Study the Effects of PVA Concentration (x_1), Flow Rate (x_2), Distance between Needle Tip to Collector (x_3), Applied Voltage (x_4), and Crosslinking Time (x_5) on the Nanofiber Diameter (y_1) of Electrospun PVA

Run	x_1: PVA %(w/v)	x_2: Flow Rate (mL/h)	x_3: Distance (cm)	x_4: Voltage (kV)	x_5: Cross linking Time (h)	y_1: Diameter (nm)
1	10	1	10	10	12	618
2	10	0.4	18	25	2	524.5
3	10	0.4	18	10	12	558.9
4	6	1	18	10	12	316.0
5	10	1	10	25	2	532.4
6	10	0.4	10	10	2	635.5
7	6	1	18	25	2	541.9
8	8	0.7	14	17.5	7	426.7
9	8	0.7	14	17.5	7	421.5
10	10	1	18	10	2	440.6
11	6	0.4	18	10	2	338.3
12	6	0.4	10	25	2	288.0
13	6	1	10	25	12	420.7
14	6	0.4	10	10	12	239.7
15	6	0.4	18	25	12	528.8
16	6	1	10	10	2	618.5
17	10	0.4	10	25	12	520.2
18	10	1	18	25	12	543.0
19	10	1	18	17.5	12	424.7
20	6	0.7	18	25	2	422.9
21	8	1	10	10	2	521.1
22	10	0.4	10	17.5	2	499.3
23	8	1	10	25	12	263.2
24	10	1	14	25	2	426.4
25	10	0.7	18	10	2	536.0
26	6	0.4	10	10	7	528.2
27	6	0.4	14	25	12	722.4
28	6	1	18	10	7	472.8

$$y_1 = 2522.9 - 678.9x_1 + 1091x_2 + 54.3x_3 - 51.3x_4 + 57.2x_5 + 51.1x_1^2 - 1.8x_3^2 + x_4^2 - 7.1x_5^2 - 60.8x_1x_2 - 2.6x_1x_3 - 1.9x_1x_4 + 2.1x_1x_5 - 32x_2x_3 - 0.87x_4x_5 - 13.3x_2x_5 + 1.76x_3x_4 + 1.2x_3x_5 \quad (5)$$

After analyzing the parameters and their interactions with each other for individual response (y_1), determining the best electrospinning conditions, paying attention to the point that nanofibers diameters of PVA mats must have minimum values, the best amounts of each effective parameter along with the response (y_1) for the electrospinning conditions are as follows: PVA concentration (x_1)=8% (w/v), flow rate (x_2)= 0.8 mL/h, distance between the needle tip to the collector (x_3)=10 cm, applied voltage (x_4)=19.5 kV, and crosslinking time (x_5)=12 h. According to the above conditions, the minimum average diameter of neat PVA nanofibers was about 220 nm. The regression coefficient (r^2) for eq. (5) was estimated to be 0.96.

Similarly, Figure 4(a)-(c) shows the average variations of neat PCL nanofibers diameters with effective interaction parameters, PCL solution concentration (x_6), the distance between the syringe needle tip to the collector (x_3), the voltage (x_4) and the flow rate (x_2). The general equation for the variation of the neat PCL nanofibers diameters (y_2) with the x_2, x_3, x_4, and x_6 factors is as follows:

Table IV. D-Optimal Design to Study the Effects of PCL Concentration (x_6), Flow Rate (x_2), Distance between Needle Tip to Collector (x_3) and Applied Voltage (x_4) on the Nanofiber Diameter (y_2) of Electrospun PCL

Run	x_6: PCL %(w/v)	x_2: Flow Rate (mL/h)	x_3: Distance (cm)	x_4: Voltage (kV)	y_2: Diameter (nm)
1	8	1	10	25	356.0
2	8	0.4	18	25	279.7
3	12	1	18	25	478.8
4	12	1	10	25	741.2
5	12	0.4	10	25	750.0
6	12	0.4	18	25	650.0
7	10	0.7	14	17.5	288.0
8	12	0.4	18	10	800.5
9	8	1	18	10	279.0
10	12	1	10	10	373.8
11	8	1	18	25	305.6
12	8	1	10	10	286.8
13	8	0.4	10	25	443.2
14	12	1	18	10	512.3
15	8	0.4	10	10	460.8
16	12	0.4	10	10	428.0
17	10	0.7	14	17.5	293.0
18	8	0.4	18	10	196.2
19	12	1	10	17.5	484.4
20	12	0.7	18	10	814.8
21	12	0.4	14	25	752.8
22	8	1	10	17.5	532.4
23	8	0.4	14	25	583.3
24	8	0.7	18	10	454.3
25	10	1	18	25	376.2
26	10	1	10	25	564.8

$$y_2 = 5546.7 - 1068.3x_6 + 1790.7x_2 - 28.2x_3 - 41.7x_4 + 50.6x_6^2 - 1366.9x_2^2 + 1.5x_4^2 + 5.3x_3x_6 + 2x_4x_6 - 1.8x_3x_4 \quad (6)$$

In order to get the minimum diameter for PCL nanofibers which is about 430 nm, the optimized conditions for x_2, x_3, x_4, and x_6 parameters from eq. (6) are as follows: PCL concentration (x_6)=9.5% (w/v), flow rate (x_2)=0.45 mL/h, distance between the needle tip to the collector (x_3)=14.5 cm and the applied voltage (x_4)=19.5 kV. The regression coefficient (r^2) for the eq. (6) was calculated about 0.91.

Generally, every parameter eliminated in these equations is because of their unimportance effects on the answers, had a p-value>0.05.

Morphology of Neat and Drug-Loaded Electrospun PVA, PCL, and PVA/PCL Mats. As it was explained, effective parameters in electrospun nanofibrous mats of PVA, PCL and (80/20) PVA/PCL hybrid were optimized by experimental design method in order to get minimum average diameter of nanofibers for all the samples.

Figure 5(a)-(f) shows the morphology of nanofibrous mats containing the drug and without it. The samples contain no beads which were confirmed by SEM micrographs. This indicates that tetracycline hydrochloride loaded inside the nanofibrous mats has a suitable compatibility with the polymer-solvent system.[25]

The results indicate that the addition of the drug reduces the average diameter of nanofibers. Figure 6 shows the diameter reduction for neat and tetracycline loaded nanofiber samples, PVA, PCL, and (80/20) PVA/PCL hybrid. Figure 6 also shows that the optimized average diameter of PVA nanofi-

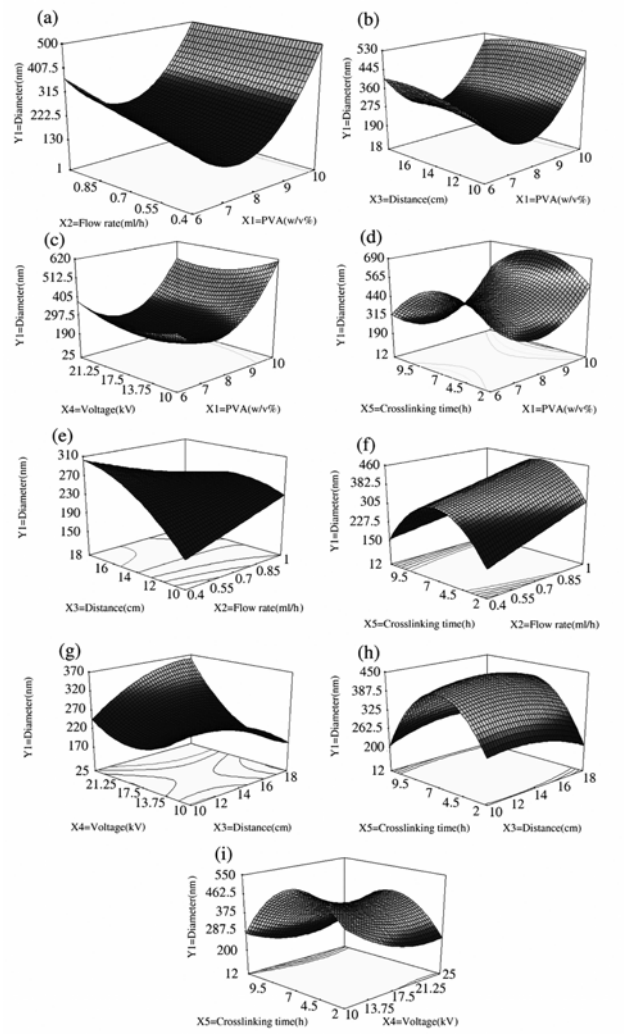

Figure 3. Effect of different electrospinning process variables on average nanofiber diameters for PVA mats.

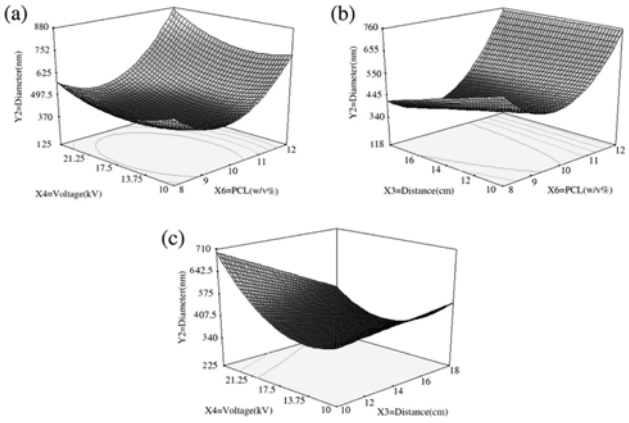

Figure 4. Effect of different electrospinning process variables on average nanofiber diameters for PCL mats.

bers for neat and drug loaded samples were 220 and 160 nm, respectively. This can be explained in terms of viscosity

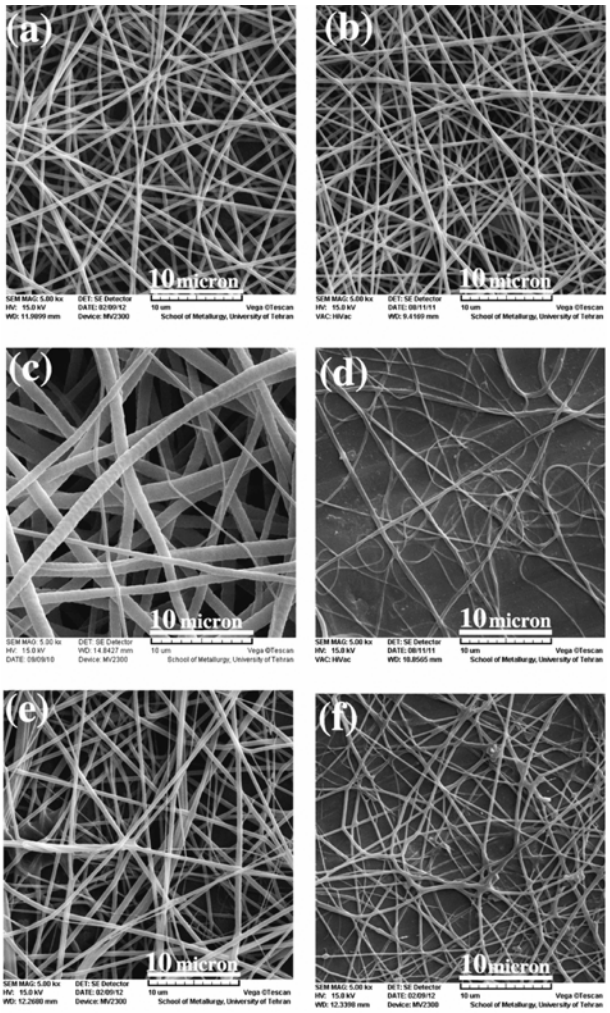

Figure 5. SEM micrographs of nanofibrous mats prepared from PVA (8% (w/v)) solution (a) neat and (b) with loaded tetracycline hydrochloride, PCL (9.5% (w/v)) solution (c) neat and (d) with loaded tetracycline hydrochloride and (80/20) PVA/PCL hybrid (e) neat and (f) with loaded tetracycline hydrochloride (500 μg/mL) concentration.

reduction of polymeric solutions such as the PVA. The drug behaves as a plasticizer between the polymer chain layers.[21]

Generally, a polymer solution with a higher viscosity during electrospinning process resists elongation. In other words, viscosity reduction of polymer solution results in better stretching of injected jet and easier elongation and the results to finer fibers. Figure 5(c,d) also shows a remarkable reduction in nanofibers diameter for neat PCL (9.5% (w/v)) and drug loaded samples with 500 μg/mL concentration. The observed variations for nanofibers diameters were from 430 nm for neat PCL to 240 nm for drug loaded samples. Tetracycline hydrochloride is poorly water soluble and PCL has a hydrophobic nature, therefore drug loaded samples have a better compatibility relative to neat PVA and this results in a remarkable reduction in nanofiber diameter. In the case of

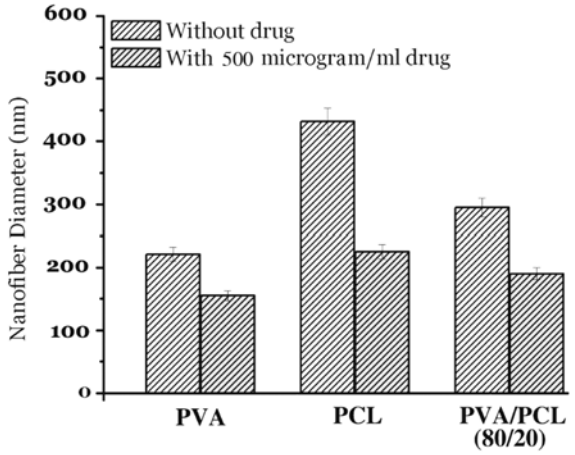

Figure 6. Variations of average nanofiber diameters of neat and tetracycline hydrochloride loaded PVA, PCL, and (80/20) PVA/PCL hybrid.

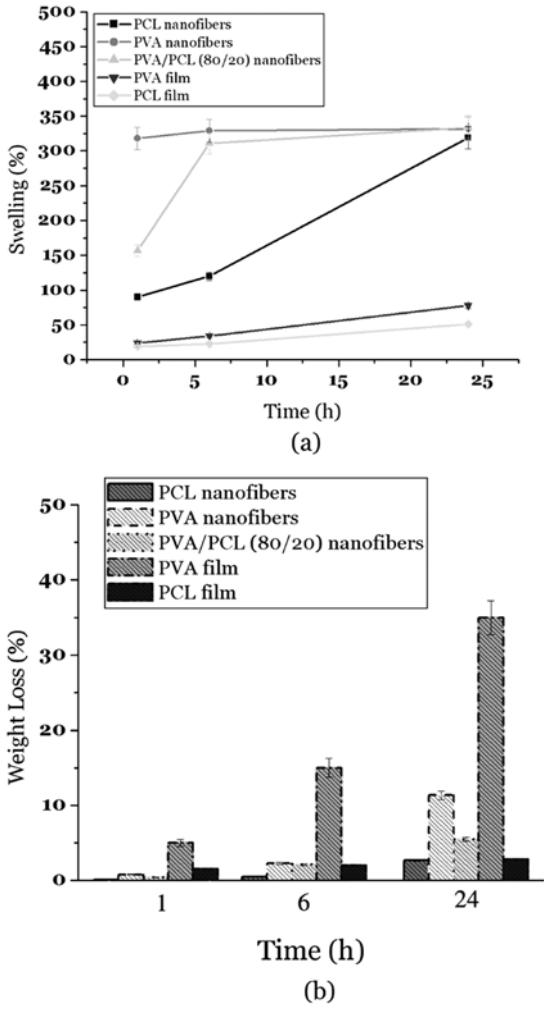

Figure 7. (a) Degree of swelling and (b) weight loss for neat PVA, PCL and (80/20) PVA/PCL hybrid nanofibrous mats, and also for PVA and PCL films in PBS after 1, 6, and 24 h.

neat and drug loaded (80/20) PVA/PCL samples the diameters of nanofibers were reduced from 315 to 200 nm respectively (Figure 5(e, f)).

Finally, it was decided for predetermined optimized conditions of electrospinning process and tetracycline hydrochloride concentration 500 μg/mL, as a model drug, nanofiber solution samples PVA (8% (w/v)), PCL (9.5% (w/v)) and (80/20) PVA/PCL hybrid were selected for further investigations.

Degree of Swelling, Weight Loss and Biodegradability of the Optimized Samples. Figures 7(a, b) show the degree of swelling and weight loss in nanofibrous mats PVA, PCL, (80/20) PVA/PCL hybrid and also PVA, PCL cast films for neat and drug loaded sample in a drug release environment (PBS, pH=7.2) at 37 °C for 1, 6, and 24 h. As it can be seen in Figure 7(a), the degree of swelling which is one of the important factors in controlling the drug release rate for different prepared samples are as follows: PVA nanofibers>(80/20) PVA/PCL nanofibers>PCL nanofibers>PVA cast films>PCL cast films. These properties depend on hydrophilic/hydrophobic degree and also structure of nanofibers and cast films. In all the investigated samples, the percentage swelling of nanofibers were much greater than cast films. This is due to the high porous nature of electrospun nanofibrous mats. The high swelling percentage results the loaded drug molecules in the samples to release the drug much easier and more complete to the intended environment. PVA nanofibers due to their hydrophilic and also porous nature had the most swelling percentages about 300% to 350% while these variations for PVA cast films were about 25% to 75% during 1 to 24 h period. Figure 7(b) shows weight reduction variations of the samples in PBS environment as a result of biodegradation. However, weight reduction order of the samples were as follows: PVA cast films>PVA nanofibers>(80/20) PVA/PCL nanofibers>PCL cast films>PCL nanofibers.

PVA is hydrolyzed by water molecules. The water molecules attack -OH groups in PVA and break it at 3300 cm^{-1}. Figure 8(a)-(f) shows ATR-FTIR of nanofibers PVA, PCL and (80/20) PVA/PCL hybrid samples without drug loading before and after placing them in PBS environment at 37 °C for 24 h. In the hydrophilic degradation mechanism of the polyesters, the water molecules attack the polyester bond -CO-O at a 1168 cm^{-1} wave number before biodegradation test (Figure 8(c)) and shift it to 1175.08 cm^{-1} (Figure 8(d)) after degradation respectively. The variation in FTIR peaks for (80/20) PVA/PCL hybrid sample before and after biodegradation test is a mixture of PVA and PCL nanofibers degradation but with a reduced variation. This is due to the added percentage of PCL to PVA in the hybrid sample which results to hydrolytic degradation prevention of -OH bonds in PVA. The biodegradation test results show that the degree of degradation for PVA is much greater than PCL because PVA is a hydrophilic and PCL is a hydrophobic polymer.

Both the degree of swelling and the weight loss of these samples are discussed along with the results on the release

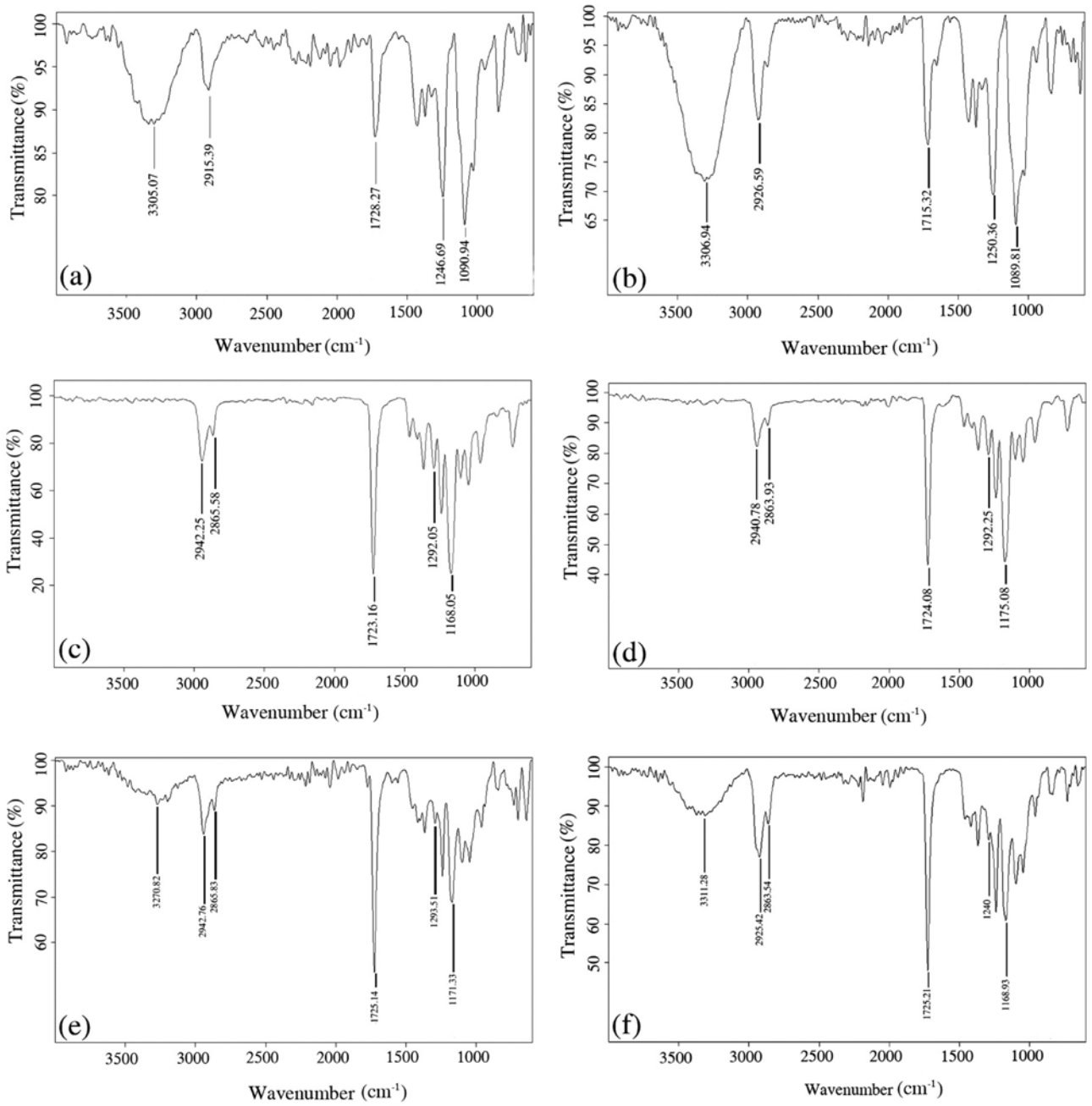

Figure 8. FTIR spectra of PVA nanofibers (a) before and (b) after biodegradation, PCL nanofibers (c) before and (d) after biodegradation, and 80/20 PVA/PCL hybrid (e) before and (f) after biodegradation.

characteristics of the model drug from these samples in the following section.

***In vitro* Drug Release from the Optimized Nanofibrous Mats and Films.** Figure 9 shows accumulative release of tetracycline hydrochloride 500 μg/mL from nanofibrous mat samples of PVA, PCL, PVA/PCL (80/20) nanofibers hybrid and also cast films of PVA and PCL under optimized conditions in PBS environment at pH=7.2 for a period of 48 h. From swelling and weight reduction results of the samples, the final release rate of the drug tetracycline hydrochloride for nanofibrous mats were about 90% for PVA nanofibers, (80/20) PVA/PCL hybrid, 85%, PVA cast film, 65%, PCL nanofibers, 60%, and PCL cast film, 30%.

We deduced that although phosphate buffer solution environment (200 mL of a phosphate buffer solution prepared with 0.057 M NaH_2PO_4 and 0.043 M KOH at pH=7.2)[26] can produce a homogenous solution of PVA and tetracycline hydrochloride, but incompatibility between hydrophilic PVA and hydrophobic tetracycline hydrochloride allows increased immigration of drug molecules to the surface of nanofibers

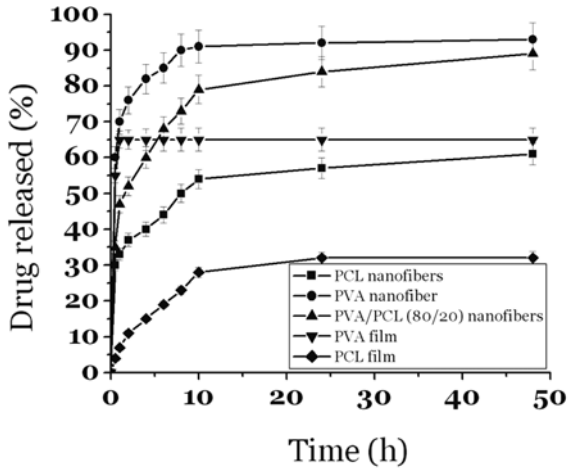

Figure 9. Release profiles of tetracycline hydrochloride with 500 μg/mL concentration from (■) PCL nanofibers, (●) PVA nanofibers, (▲) PVA/PCL (80/20) hybrid nanofibers, (▼) PVA films, and (◆) PCL films.

during rapid elongation and fast evaporation of the solvent during the electrospinning process. Similar behavior was observed for the drug ciprofloxacin by Breda *et al*..[27] These superficial drug molecules can easily diffuse into the aqueous medium and cause an initially fast burst release. Figure 9 clearly shows this behavior for nanofibers samples of PVA and (80/20) PVA/PCL and also PVA cast film. After a quick burst release for PVA nanofibrous mats and films the profile of release followed a linear fashion with a constant rate. Also this behavior has been seen for PCL films with a very slow rate of the drug release in plateau region which is negligible.

Figure 9 also shows that the drug release rate profiles for nanofibrous mats of (80/20) PVA/PCL and PCL samples have a bimodal behavior. The bimodal drug-release pattern could be explained on the basis of a two-release mechanism, as proposed by Jannesari *et al*..[28] These samples also have a relatively fast drug release rate profile before a 10 h period and after that a gentle slope and a gradual drug release rate up to 48 h.

Table V. Regression Coefficient of Tetracycline Hydrochloride Release from PVA, PCL, PVA/PCL (80/20) Nanofibrous Mats and Their Films with 500 μg/mL Drug Calculated by Peppas Equation ($M_t/M_\infty=kt^n$) (n=0.5)

Sample	R^2±SD
PVA Nanofibrous Mats (8%w/v)	0.93±0.08
PCL Nanofibrous Mats (9.5%w/v)	0.98±0.06
PVA/PCL (80/20) Hybrid Nanofibrous Mats	0.94±0.02
PVA Films (8%w/v)	0.90±0.05
PCL Films (9.5%w/v)	0.99±0.04

Analysis of Tetracycline Hydrochloride Release Kinetics from Electrospun PVA, PCL, and PVA/PCL Mats and Their Cast Film. Generally, the drug release rate from a polymer matrix is explained by Fickian diffusion mechanism. In this mechanism, the drug penetration is the determining factor in its release. On the other hand, the drug release from a polymer matrix can be by: drug release out of polymer matrix, or/and drug release due to the degradation of the polymer matrix.[29]

Further studies on drug release rates for tetracycline hydrochloride, cumulative drug release rate profiles by equation Korsmeyer-Peppas and models zero order, first order, Higuchi and Hixon-Crowell were analyzed. Table V shows the results of regression coefficient data analysis by Peppas equation and the value of exponent n. The results show that Fickian diffusion mechanism was appropriate for the drug release for all the samples under investigation. These means that concentration of the loaded drug and the type of polymer used in the nanofibers have no effect on the drug release rate.[24]

Table VI shows the results of regression indices for different drug release rates from different kinetic models. This table shows that in PVA/PCL nanofibers, the high level of initial release (up to 10 h) is probably related to the diffusion of the drug located near the nanofibers surfaces, which can be fitted to a Highchi model. Also drug release from 10 to 48 h for (80/20) PVA/PCL hybrid nanofibers samples has a good fitting with Hixon-Crowell model. This behavior indicates that the main drug release mechanism in (80/20)

Table VI. Regression Coefficient of Different Mathematical Models Fitted to the Release of Tetracycline Hydrochloride from Different Nanofibers and Films with 500 μg/mL Drug (n=0.5)

Sample	Zero Order	First Order	Higuchi	Hixon-Crowell
PVA Nanofibrous Mats	0.75±0.05	0.79±0.05	0.74±0.03	0.95±0.03
PCL Nanofibrous Mats (before 10 h)	0.87±0.05	0.79±0.02	0.87±0.07	0.74±0.03
PCL Nanofibrous Mats (after 10 h)	0.95±0.07	0.91±0.07	0.98±0.05	0.89±0.03
PVA/PCL (80/20) Nanofibrous Mats (before 10 h)	0.81±0.06	0.86±0.04	0.97±0.01	0.88±0.08
PVA/PCL (80/20) Nanofibrous Mats (after 10 h)	0.88±0.02	0.87±0.07	0.8.±0.02	0.94±0.05
PVA Films	0.51±0.09	0.61±0.02	0.52±0.01	0.89±0.04
PCL Films	0.6±0.05	0.41±0.07	0.86±0.03	0.49±0.06

PVA/PCL nanofibers after 10 h is due to the erosion of (80/20) PVA/PCL hybrid nanofibers matrix. For PCL nanofibers sample, the main mechanism for tetracycline hydrochloride before and after 10 h fits Higuchi model. This means that in this case, the mechanism of the drug release is a penetration of drug molecules from outside PCL nanofibers.

Similarly, for PVA nanofibers, PCL and PVA cast film samples containing 500 μg/mL tetracycline hydrochloride the best models are Hixon-Crowell, Higuchi and Hixon-Crowell, respectively.

Conclusions

Homogenously combined neat and tetracycline hydrochloride loaded nanofibers of PVA, PCL, and (80/20) PVA/PCL hybrid samples were prepared. The electrospinning conditions of these samples were optimized by the D-optimal experimental design method. The morphological studies of these nanofibers samples show that the presence of tetracycline hydrochloride, the loaded drug, results in a reduction in the average diameter of the nanofibers. This seems to be due to an increase in surface area to the volume of the nanofibers. Two important and effective parameters in the drug release rates are the degree of swelling and the percentages of weight reduction. The experimental results show that PVA sample has the most swelling percentage in PBS environment and PVA, while PCL cast films due to their nonporous nature and higher interactions with water molecules at the surface of these films have the most weights reduction. The results of hydrolytic degradation of these samples were confirmed by FTIR investigations. The drug release profiles for tetracycline hydrochloride for all the nanofibers and cast film samples show that Fickian diffusion mechanism is dominant. Also, the best solvent in complete agreement with drug release information for these samples was determined. The second part of this work, which is under investigation is the use of PVA, PCL and (80/20) PVA/PCL nanofibers samples for wound dressing. The samples are optimized by experimental design for *in vitro* and *in vivo* investigations.

Acknowledgment. The authors would like to express their sincere gratitude and thanks to Iran National Science Foundation (INSF) for their financial support (grant number: 89001275).

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