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RESEARCH PAPER

Preparation and characterization of captopril loaded polycaprolactone electrospun nanofibers

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ABSTRACT

Objective(s): Electrospun nanofibrous mats of Polycaprolactone (PCL) and amino modified SBA-15 containing Captopril were prepared and release of drug from prepared nanofibers were investigated in this study.

Materials and Methods: Amino modified SBA-15 synthesized through grafting with aminopropyl triethoxy silane. Then, Captopril which is used as an Angiotensin-Converting Enzyme (ACE) inhibitor was loaded as a model drug into the synthesized particles. Furthermore, silica particles containing different amounts of captopril (7.5, 10, 15 mg) were loaded into PCL nanofibers' structure using electrospinning. Therefore, captopril's release rate in phosphate buffer was analyzed. The analysis of captopril-loaded silica and captopril-loaded nanofibers (without silica) was done in vitro matrix. To identify the acquired nanofibers, FTIR, SEM, SEM mapping, EDX and average diameter calculation were performed.

Results: Comparison between the drug release rate of silica-containing nanofibers and bare silica particles indicated that silica particles positively affected the drug release rate. Moreover, kinetic studies have revealed that the drug release rate follows Higuchi and Korsmeyer-Peppas model. Bare nanofibers' average diameter was 209 nm while silica and captopril containing nanofibers were 286 nm in average diameter.

Conclusion: Therefore, it was concluded that drug-loaded Polycaprolactone nanofibers are potential candidates for biomedical applications.

Keywords: *Captopril release, Electrospinning, Nanofibers, Nanostructure silica, Polycaprolactone*

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INTRODUCTION

Pharmaceuticals are being studied for their therapeutic effects on various diseases and medical conditions, however, their instability and degradation before reaching the target site is a limitation on their capabilities [1]. However, nanofibers are widely known for their applications in biomedical science and especially in drug delivery [2-5]. Different controlled drug release patterns can be achieved using electrospun nanofibers (immediate, delayed, sustained, etc.). However, sustained drug release is more useful because it increases the time and dose of drug delivery to target sites [6]. Electrospinning is a widely applied process to produce nonwoven ultrafine nanofibers and has properties such as simplicity, affordable price, and good pore size distribution [2]. In general, electrospinning can be influenced by three main factors: 1- solution characteristics, 2- processing factors and 3- environmental factors. Even though all these parameters influence the fiber morphology, the properties of the solution still play a more important role than other parameters [7]. However, electrospun nanofibers tend to release drugs rapidly when left unmodified, mainly due to the drug distribution on the nanofiber surface, large surface area, and amorphous nature of the drug inside of nanofibers, which are not conducive to sustained release of medicine. Santa Barbara Amorphous-15 (SBA-15) is a two-dimensional hexagonal mesoporous silica (P6mm) synthesized by Pluronic P123 (EO_{20}-PO_{70}-EO_{20}) copolymer under acidic conditions. The main advantage

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of using this Pluronic copolymer is that the final silica product can retain the hexagonal structure during synthesis at different temperatures and concentrations. Pore size and surface area can also be changed by modifying the synthesis process [8]. The main advantages of SBA-15 are its simple synthesis, easy recycling, non-toxic structure-directing agent (SDA), and high thermal and hydrothermal stability [9]. XRD spectroscopy analysis shows that the hexagonal structure of this compound is preserved even at high temperatures (850 °C). SBA-15 is used as a drug storage unit due to its biocompatibility, thermal stability, high specific surface area (usually 1000 m^2/g), high porosity ((pore volume 0.5-1.5 cm^3/g), (porosity is adjustable (typically 2 to 15 nm)) and non-cytotoxic properties, making it suitable for controlled drug delivery and release. Although SBA-15 has good drug delivery properties, these properties can be further enhanced by grafting SBA-15 onto polycaprolactone nanofibers, which further increases the drug release time compared to SBA-15 and simple polycaprolactone. In this study, SBA-15 was synthesized and functionalized using (3-aminopropyl) triethoxysilane and then incorporated into polycaprolactone (PCL) by electrospinning. Captopril was also used as a model drug to investigate PCL containing SBA-15- NH2. The synthesis and functionalization of SBA-15 were characterized by TEM, DLS, SEM and FT-IR. The PCL nanofibers were also characterized by FT-IR, SEM, SEM mapping and EDX. To confirm the grafting of SBA-15 in PCL, the nanofibers were dissolved in hydrofluoric acid, the disappearance of the Si-O-Si absorption peak in FT-IR and the weight loss confirmed the presence of SBA -15 in PCL nanofibers.

MATERIALS AND METHODS

Materials

All the following chemical materials and laboratory equipment which are listed below were purchased from reliable companies. Chloroform ($CHCl_3$), methanol (CH_3OH), tetra ethyl ortho silicate ($Si\ (OC_2H5)_4$), hydrochloric acid (HCl), acetone (C_3H_6O), toluene ($C_6H_5CH_3$), and dichloromethane (CH_2Cl_2) were purchased from Merck company. 3-Triethoxysilylpropylamine ($H_2N(CH_2)3Si\ (OC_2H5)_3$), Pluronic ($EO_{20}PO_{70}EO_{20}$), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate ($KH2PO_4$), deionized water (H_2O), polycaprolactone (PCL), nitrogen gas (N_2).

Dry toluene preparation

The toluene solution was reflexed on the sodium solid. In addition, some benzophenones were added to the previous solution and the reflex continued till the solution's color changed to blue. Then, the obtained solution was kept on the calcium hydride for 24 hr. moreover, the previous solution was distilled and collected in an Erlenmeyer flask containing sodium solid and molecular sieve.

SBA-15 preparation

The SBA-15 was synthesized by Pluronic copolymer P123 (EO20PO70EO20). 4 g of Pluronic P123 were dissolved in 30 mL of deionized water and 120 mL of HCl (2M) at 35 °C. After completely dissolving, 8.5 g of tetra ethyl ortho silicate (TEOS) was added to the obtained solution and stirred for 20 hr at 35 °C. Afterward, the obtained composite was washed with ethanol and DW was kept in the oven for an hour at 100 °C to dry completely. Mesoporous silica was calcined in a lab furnace for 6 hr at 550 °C to separate the total amount of Pluronic from mesoporous silica. 3-aminopropyl (3-triethoxysilane) was used to modify the surface of the synthesized silica. At first, 11 mmol of aminopropyl (3-triethoxysilane) was added to the 3 g of SBA-15 slush in 100 mL of toluene under nitrogen gas. Thereupon, the solution was reflexed for 48 hr at 110 °C. A white precipitate was washed with 100 mL of toluene, dichloromethane, and normal hexane respectively. The obtained white precipitate (SBA-15-NH2) was dried in a lab vacuum pump.

Preparation of drug-loaded silica

At first, 100 mg of the drug and 5 mL of distilled water were added to the balloon then 50 mg of SBA-15-NH2 was added to the previous solution. The obtained solution was stirred for 24 hr. the sediment was separated by centrifuge. The obtained sediment was washed several times and then dried at 60 °C in the oven. To determine drug loading efficiency on SBA-15-NH2 the amount of drug remaining in the solution after washing was calculated using a UV-Vis spectrometer. Loading efficiency was calculated according to equation (1)

$$DL\ (\%) = (M_D/M_S) \times 100 \quad (1)$$

In which M_D is the mass of the loaded drug and M_S being SBA-15-NH_2's mass.

Nanofiber preparation

Incipiently, a mixture of 4 mL chloroform and 1 mL of methanol was prepared. In addition, 0.6 g of polycaprolactone was added to the previous solution and stirred for 3 hr. Then, the silica nanoparticles (SBA-15-NH_2) containing different amounts of captopril (7.5, 10, 15 mg) was added to the obtained solution and stirred. To improve mixing process, the solution was put in an ultrasonic bath for 20 min. The obtained solution was kept at room temperature. The details of the electrospinning machine consist of a 1.5 mL/hr flow rate, 20 kW power, and 20 cm distance. The nanofibers were collected on aluminum foil.

Characterization of electrospun fibers morphology

The morphology of SBA-15, SBA-15-NH2 and electrospun nanofibers was characterized by scanning electron microscopy (SEM; Vega II XMU instrument Tescan, Czech Republic). The specimens were coated with gold for 20 seconds and imaged with a backscatter detector. The fiber diameters of the scaffolds were calculated from their corresponding SEM images using image analysis software (Image J, NIH). The structural and chemical properties of SBA-15, SBA-15-NH2 was investigated by transmission electron microscopy (TEM; Zess. EM10, Philips CM120).

Fourier transform infrared spectroscopy (FTIR)

Chemical analysis of SBA-15, SBA-15-NH2, captopril and also electrospun PCL/ SBA-15-NH2 and PCL/Captopril nanofibers was performed by ATR-FTIR spectroscopy. ATR-FTIR spectra of scaffolds were obtained on an Equinox 55 spectrometer (Bruker optics, Germany).

Drug release from prepared nanofibers

Releasing the drug from nanofibers out of the body has been done in phosphate buffer solution (pH=7.2 and volume of 800 mL) at 37 °C under a stirring situation. 0.1 mg of nanofiber was put in a permeable membrane. Then, some samples were obtained in different periods. The buffer solution sampling in specific periods and the changes in concentration in the maximum wavelength were investigated by UV-vis (λmax=226 nm). The main concentration of each level was calculated by the equation (2).

$$C_n = C_N + \frac{C_{n-1}V_1}{V_t} \qquad (2)$$

Where C_n is the real concentration, C_N is the appearing concentration, V_1 is the measured volume, and V_t is the total volume.

Investigating the kinetic of drug release

For most drug delivery systems, the process of drug release is usually investigated by two equations. The first one is classic fickian which has been known as Higuchi equation which describes the releasing drug from an insoluble matrix system. The second equation which is more comprehensive to describe drug release which has known as Korsmeyer-Peppas equation. To find the mechanism of drug release 60% of the data must be matched with Korsmeyer-Peppas.

$$M_t/M_\infty = kt^n \qquad (3)$$

According to equation 3, if n<0.45 the mechanism of the drug release follows the fickian mechanism.

RESULT AND DISCUSSION

SEM and TEM images of SBA-15 and SBA-15-NH2

According to the Fig. 1 and 2, synthesized silica (SBA-15) has filamentary morphology and these particles have around 1.2 µm length. The

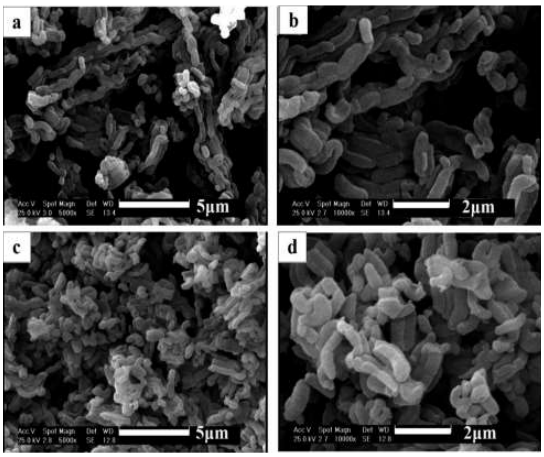

Fig. 1. SEM images of SBA-15 before modification (a,b) and SBA-15-NH2 (c,d) after modification process

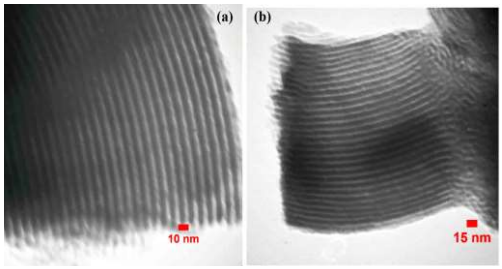

Fig. 2. TEM images of SBA-15 before modification (a) and SBA-15-NH2 (b) after modification process

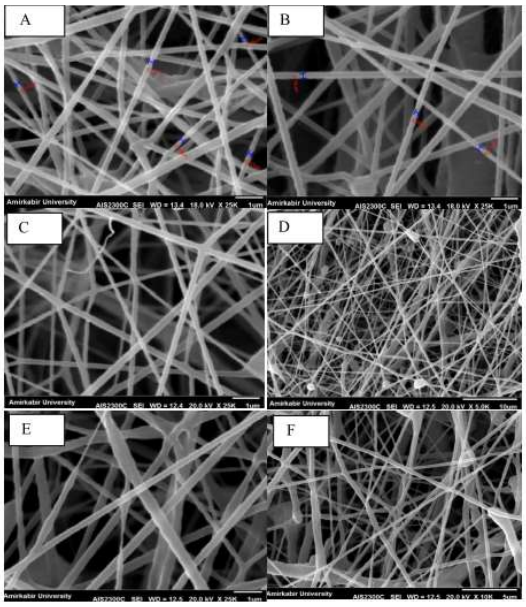

Fig. 3. SEM images of PCL nanofibers (A,B) PCL- Captopril nanofibers (C,D) and SBA-15-NH2 - Captopril nanofibers (E,F) (scale bar = 1 µm)

aminated silica (SBA-15-NH2) has the same morphology as SBA-15. These figures reveal that the surface modification process hasn't any effect on particle shape. A glance at the figures provided reveals that SBA-15 and SBA-15-NH2 are porous and the porous structure of silica remained the same during the aminate process.

SEM image of polycaprolactone nanofiber

Fig. 3 shows the SEM images of prepared nanofibers. Fig. 3A-B demonstrates the PCL nanofibers. By SPSS and Image j applications the average diameter of polycaprolactone nanofibers was around 209 nm. In addition, Fig. 3C-D shows the morphology of the drug-containing polycaprolactone nanofibers. According to Fig. 3 E-F by increasing the silica as a nanocarrier, the diameter of the nanofiber was increased from 209 to 286 nm. It could be due to the increase in solution concentration. As shown in image 4, the distribution of the silica particles is demonstrated by the SEM-mapping for silicon elements. Fig. 4a reveals the distribution of the silica particles in polycaprolactone nanofiber. Fig. 4b demonstrates the silica on the nanofiber. Also, it shows the distribution of oxygen, silicon, and carbon elements which are shown with red points.

FTIR of SBA-15 , SBA-15-NH2 and SBA-15-NH2-Capt

Fig. 5 reveals the FTIR analysis of SBA-15, SBA-15-NH2, and SBA-15-NH2-Capt. The

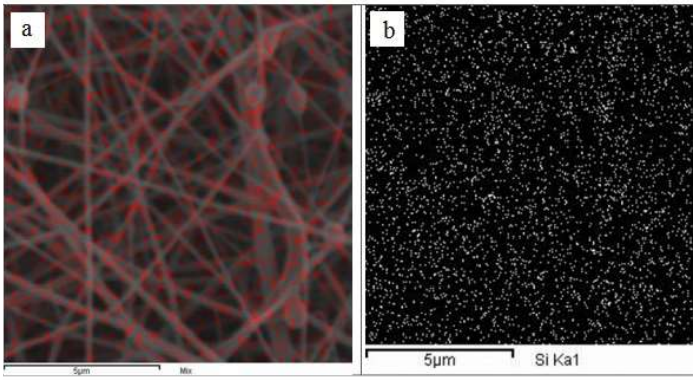

Fig. 4. SEM mapping of PCL-SBA-15-NH2-Capt nanofibers (scale bar = 5 µm)

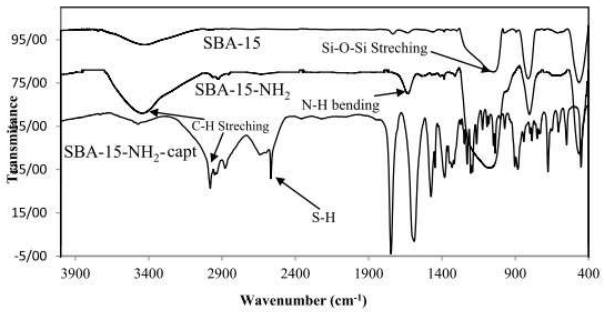

Fig. 5. FTIR of SBA-15, SBA-15-NH2 and SBA-15-NH2-Capt

spectrum at 1070-1220 cm^{-1} is about asymmetric tensile absorption of the Si-O-Si bond which demonstrates the dense lattice of silica. There is a spectrum at 3400 cm^{-1} which is about hydroxyls on the surface. The bends at 470 and 800 cm^{-1} are about symmetrical stretching pulsation and the deformation modes of Si-O-Si [9]. By comparing the FTIR analysis of the SBA-15 and SBA-15-NH2, the spectrum of SBA-15-NH2 at 1200-1350 cm^{-1} is about the C-N bond which is covered by Si-O-Si in the SBA-15 structure. The peak at 1600 cm^{-1} is about the asymmetrical bending pulsation of the NH2 and it reveals that the aminated process of the SBA-15 was successful. In the aliphatic area, there is adsorption at around 2920 cm^{-1} which is about stretching pulsation of the C-H groups. An adsorption spectrum at 3400 cm^{-1} is about N-H groups [9]. So, investigating the FTIR reveals that the amine groups fixed on the surface of the SBA-15 and turned to SBA-15-NH2.

FTIR of PCL nanofibers and drug-containing PCL nanofibers

As shown in Fig. 6, there is a peak at 1200 cm^{-1} which is about stretching C-O and there is a peak at 1725 cm^{-1} which demonstrate carbonyl ester. Aliphatic C-H bond has been revealed at 2945 cm^{-1}. According to the figure, there is a wide peak at 3435 cm^{-1} which demonstrates the terminal hydroxyls in polycaprolactone structure. In the spectrum which is about drug-containing polycaprolactone, some peaks can be seen which are about sulfhydryl and carbonyl. These two peaks are a sign of the presence of the drug in polycaprolactone. Adsorption bonds of carbonyl can be seen at 1685 cm^{-1} while the peak of hydroxyl shifted to a lower wavelength because of the hydrogen bond between molecules of drug and silica.

Presence of silica in prepared nanofibers

Dissolving the nanofiber in hydrofluoric acid (HF) is one of the ways to demonstrate the presence of silica in nanofiber. Fluorine ion reacts with the silicon atom and hydrogen reacts with the oxygen atom. The weight of the nanofiber declined and proved the presence of silica in the nanofiber structure. So, 200 mg of drug-containing nanofiber and SBA-15-NH2-cap-containing nanofiber were dissolved in a beaker by HF for 6 hr, separately and dried at room temperature for 12 hr. The weight loss of the sample containing the drug was around 15% while the weight loss of the sample containing silica was around 50% which proved the presence of silica in nanofiber. In some cases, toluene and tetrahydrofuran were used along HF in the order to enhance the situation [11,12]. To

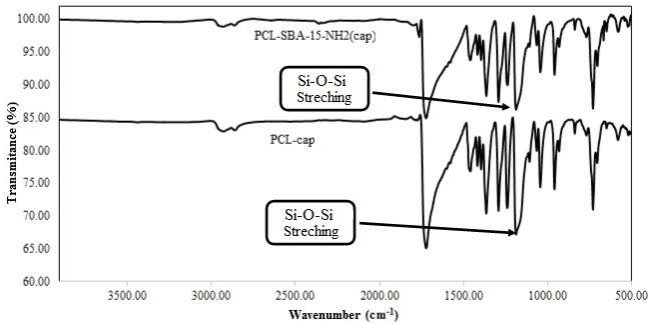

Fig. 6. FTIR of PCL nanofibers and drug-containing PCL nanofibers

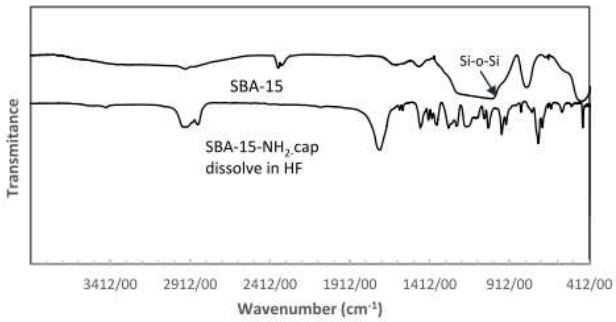

Fig. 7. FTIR of SBA-15 and SBA-15-NH2-Capt dissolved in HF

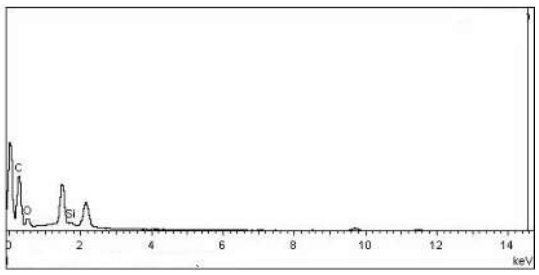

Fig. 8. EDX analysis of PCL-SBA-15-NH2-Capt nanofibers

prove the process, an FTIR analysis (Fig. 7) was taken and the adsorption spectrum at 1070-1220 cm^{-1} demonstrates the silica was removed completely. According to Fig. 8, the EDX analysis proved the presence of the silicon particles on the surface of the samples. The percent composition of silica, oxygen, and carbon was investigated by EDX analysis which has been written in Table 1.

In vitro drug release

According to the Fig. 9 and 10, drug release was studied from different silica samples containing the drug. The amount of drug released for each sample was measured in triplicate. The results showed that the drug released after 72 hr became unchanged. While the drug was attached to the silica-free nanofibers, the release was like an explosion and within 12 hr it reached a maximum amount of about 1.3 mg. Thus, the SBA-15- NH2 particle has an aminopropyl effect to release the drug due to its polarity. As a result, the release process becomes slower. The drug release from the silica channel consists of two steps: first step is to diffuse the solvent into the silica channel to dissolve

Table 1. EDX analysis results

Element	Frequency of atoms	Wt (%)	Wt% sigma	At (%)
Carbon	88.82	71.69	1.18	77.57
Oxygen	9.11	26.69	1.20	21.68
Silicon	1.35	1.62	0.14	0.75

Wt (%): Weight percent, At (%): Atomic percent

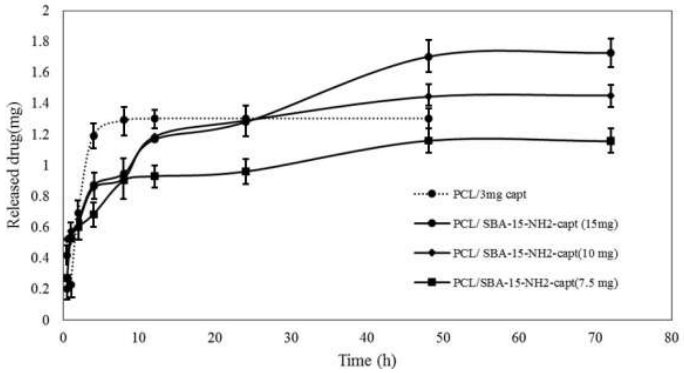

Fig. 9. Drug release from PCL nanofibers (n=3)

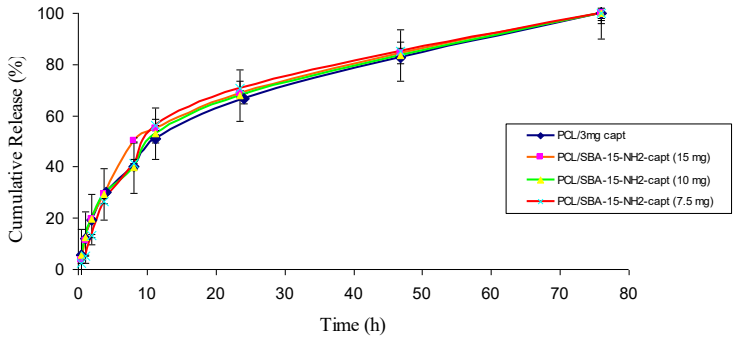

Fig. 10. Cumulative release of captopril from PCL nanofibers (n=3)

Table 2. Captopril release kinetic parameters from PCL nanofibers

Model	Silica type	K	n	Releasing mechanism	R^2
Higuchi	PCL-Cap (3mg)	0.8807	-	-	0.9136
	PCL-SBA-15-NH2-Cap (7.5 mg)	0.4323	-	-	0.8164
	PCL-SBA-15-NH2-Cap (10 mg)	0.4056	-	-	0.8837
	PCL-SBA-15-NH2-Cap (15 mg)	0.7384	-	-	0.5553
Korsmeyer-Peppas	PCL-Cap (3 mg)	0.2070	0.1848	Fickian	0.9666
	PCL-SBA-15-NH2-Cap (7.5 mg)	0.06737	0.4161	Fickian	0.8355
	PCL-SBA-15-NH2-Cap (10 mg)	0.1594	0.2277	fickian	0.9078
	PCL-SBA-15-NH2-Cap (15 mg)	0.00661	0.8813	Non-fickian	0.8223

the drug and the second one is drug release from silica channels. The presence of polycaprolactone nanofibers increased the extent of drug release from the silica structure. While the drug is fixed on the silica, the release becomes slower. But without silica, the drug will be released suddenly. Silica acts as a drug carrier, able to transport large amounts of drug and then release it. According to the literature [13] the pore size of the applied mesoporous support influences the release rate. Thus, the possible explanation of a lower release rate is the smaller pore size of the mesoporous carriers after modification as mentioned by the other researchers [14, 15]. Polycaprolactone was used as the substrate. The amount of drug release with 15 mg of silica is about 70%. As shown in Table 2, the drug released from the nanofiber contains amino silica at concentrations of 7.5 and 10 mg following a Fickian mechanism. Furthermore, 3 mg captopril contains nanofibers that follow a Fickian mechanism while the hydrophobic property of the drug causes it. While the silica concentration increased to 15 mg did not follow the Fickian mechanism. So the correlation coefficient can prove it. If the correlation coefficient reaches 0.1, the activation mechanism will obey the fickian law. Due to the Higuchi equation, as the concentration increases, the correlation coefficient moves further away from 0.1 and shows that as the silica concentration increases, the rate according to the Higuchi equation decreases.

CONCLUSION

In this research mesoporous SBA-15 was first synthesized and then aminated using (3-aminopropyl) trietoxysilane. Then, captopril was loaded into the synthesized silica and UV-Vis was used to determine the amount of drug loaded into silica. Next silica containing polycaprolactone was synthesized using electrospinning and drug release rate when silica was loaded into the nanofibers and when nanofibers were used alone as a drug carrier were analyzed. Results indicate that when SBA-15-NH2 was embedded into the nanofibers, drug release was moderated and the drug was released in a 72 hr period. On the other hand, when the nanofibers were used alone as a drug carrier, releasing procedure was more rapid and the drug was released into the solution in 12 hr. At last, integrity of the nanofibers was analyzed using SEM and drug releasing profile in vitro environment was investigated. Also, drug release kinetics were matched with Higuchi and Korsmeyer-Peppas drug release models. The results showed that drug-loaded Polycaprolactone nanofibers are potential candidates for biomedical applications. Although the potential of nanofibers is enormous, significant challenges lie ahead. Most of the research in this area has been performed on fibers produced on a very small scale, using a needle-based system to electrospin nanofibers from a polymer solution. Fibers produced on large-scale equipment ensure consistent fiber quality and address environmental issues associated with solvent/solution-based electrospinning technology.

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CONFLICTS OF INTEREST

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

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