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Controlled delivery of cefixime trihydrate from organic-inorganic nanofiber composites

Arash Ramedani [1], Yeganeh Hatefi [2], Masoud Mozafari [3,*]

[1]Institute for Nanoscience & Nanotechnology (INST), Sharif University of Technology, P.O. Box, 89694-14588, Tehran, Iran
[2]Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, P.O. Box, 91775-1793, Mashhad, Iran
[3]Bioengineering Research Group, Nanotechnology and Advanced Materials Department, Materials and Energy Research Center (MERC), P.O. Box 14155-4777, Tehran, Iran

*corresponding author e-mail address: ***mozafari.masoud@gmail.com***

ABSTRACT

One of the challenges in medicine today is to develop solutions to improve public health worldwide. Drug delivery systems have become a success key of modern medicine. Electrospun nanofibers have big promise for developing many types of drug delivery systems due to their special characteristics and the simple but effective and useful manufacturing methods. Nanofibers are known as drug delivery systems using cefixime trihydrate as an antibiotic drug. In this research, the release of cefixime trihydrate from electrospun nanofibers was studied by UV–VIS spectroscopy. For this purpose, the nanofibers were prepared from poly(ε-caprolactone) (PCL), gelatin, and a composite of the three components (PCL/Gelatin/Hydroxyapatite). The nanofibers were electrospun from trifluoroethanol (TFE) solutions. The gelatin containing cefixime trihydrate was electrospun from 14% w/v solution in TFE, with a flow rate of the polymer solution between 18 and 21 ml/h. PCL containing cefixime trihydrate was electrospun from 14% w/v solution in TFE with a flow rate of 3 ml/h. Composite of 50:45:5 (PCL/Gelatin/Hydroxyapatite) containing 5% cefixime trihydrate were spun at a flow rate of 10–13 ml/h. According to the obtained results, it was concluded that the release of cefixime from (PCL/Gelatin/Hydroxyapatite) composite followed a nearly zero-order kinetic, which was characterized by a slower but linear release over 100 days without initial drug burst. This research proved that organic-inorganic nanofiber composite shows a sustained release rate compared with the the mats derived from PCL or pure gelatin.

KEYWORDS: *Nanofiber; Cefixime Trihydrate; Controlled delivery; Composite*

1. INTRODUCTION

Targeted delivery systems are used to improve the effectiveness of treatment and reducing side effects by delivering them at a specific rate in the tissue of interest for a period of time to the site of action. However drug delivery system exhibit significant advantage but individual systems have due cause to serious problems such as second surgery for remove implant, negative effect on osteoconduction, adverse tissue response of biodegradable polymers, and poor degradability with ceramics [1–4]. Therefore, the option for an organic–inorganic composite material would be more rational than individual categories of carrier materials. Multiple components mixed to provide better performance, composites are expected to provide desired mechanical stability, improve tissue integration, and retard drug release better.

Table 1. Composites Studied for Drug Delivery.

Composite		Drug	References
Inorganic	Organic		
β-Tricalcium phosphate	PCL	Gatifloxacin hydrate	20
Tricalcium phosphate	PCL	rhBMP-2	22
Bioglass	PLLA–PMMA	Gentamicn sulfate	33
Hydroxyapatite	Anionic collagen gel	Norfloxacin, Ciprofloxacin, Gentamicin	34
Tetra calcium phosphate and Dicalcium phosphate	Collagen	Estradiol	40

Table 1 presents a representative list of studies published based on organic–inorganic composites as drug delivery systems. BCC Research reported an overview of the world market for drug delivery. The global market is expected to reach about 80 billion in 2019 [5].

There are several methods of producing nanofibers. Polymeric nanofibers can be processed by a number of techniques such as Drawing, Template Synthesis, Phase Separation, Self-Assembly and Electrospinning. A comparison of the various issues relating to these processing methods can be found in Table 2.

Table 2. Comparison of processing techniques.

Process	Usability	Large-scale production	Repeatability	Control on fiber dimensions
Drawing	Laboratory	×	×	×
Melt Blown	Laboratory	✓	×	×
Phase Separation	Laboratory	×	×	×
Polymer Blend Technique	Laboratory	✓	×	×
Template Synthesis	Laboratory	×	✓	✓
Fibril in matrix method	Laboratory	×	×	×
Self-Assembly	Laboratory	×	✓	×
Electrospinning	Laboratory and Industrial	✓	✓	✓

Electrospinning is the attractive technique for preparing nanofibers from a very broad range of polymer biomaterials with the opportunity for control the thickness of mat by adjusting the collection time during the electrospinning, the dimensions and surface morphologies by altering the solution properties and processing parameters. Electrospinning process, in its simplest form consisted of a pipette to hold the polymer solution, two electrodes and a high DC voltage supply. The polymer drop from the tip of the pipette was drawn into a fiber due to the high voltage. The jet was electrically charged and the charge caused the fibers to bend in such a way that every time the polymer fiber looped, its diameter was reduced. The fiber was collected as a web of fibers on the surface of a grounded target. A schematic description of electrospinning is shown in Figure 1.

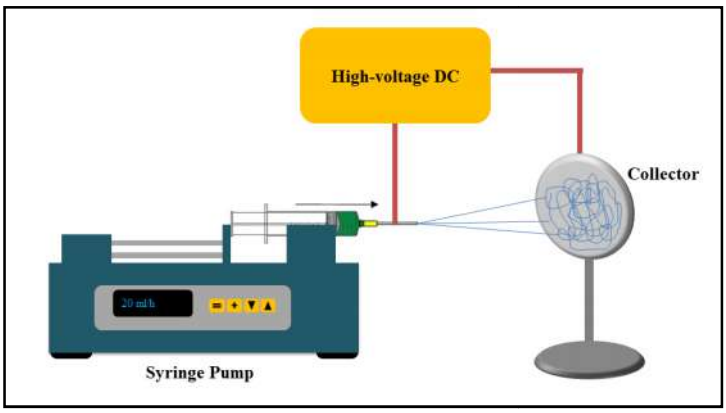

Figure 1. Schematic of electrospinning system.

The fibers are derived by charging a liquid typically to 5–30 kV vs. a ground a short distance away, which leads to charge injection into the liquid from the electrode. The sign of the injected charge depends upon the polarity of the electrode; a negative electrode produces a negatively charged liquid. The charged liquid is attracted to the ground electrode of opposite polarity, forming a so-called Taylor cone at the needle tip and, eventually, a fiber jet. The basic elements of a laboratory electrospinning system are simply a high voltage supply, collector (ground) electrode, source electrode, and a solution or melt to be spun. Electrospinning is a straightforward method of producing fibrous polymer mats with fiber diameters in the range of ca. 3 nanometer to several tens of micrometer [6–9]. Such materials may be useful for many applications in medicine such as wound dressings and scaffolds for tissue engineering [10, 11]. Recent studies [9] have indicated that a single fiber is generated in the electrospinning process, and that the mat is created from the single fiber rather than finely splayed fibers. The simplicity of the electrospinning process itself, the ability to vary the fiber diameter by changing the solution concentration and/or surface tension of the liquid [12], and the ability to incorporate therapeutic compounds into the mats during spinning afforded the prospect of preparing useful polymer systems for controlled drug delivery. Flat mats that can be either fabricated or cut to almost any size represent an attractive form for topical delivery applications, other shapes can be can be constructed using different target geometries. Moreover, a significant implication of the mechanism of electrospinning is that materials derived from polymer blends will likely not be an admixture of two different fibers but rather fibers containing both components, in principle offering another and quite unique means of controlling release rates. Poly(ε-caprolactone), gelatin and Hydroxyapatite are both biomaterials approved by the U.S. Food and Drug Administration (FDA) and Conformit Europe (CE), which have gained widespread acceptance in clinical applications [13]. Electrospun PCL fiber materials have been widely used as the materials of choice in tissue engineering and drug delivery applications due to its favorable mechanical and biodegradable properties. However, PCL fails to provide a desired micro-environment for cell adhesion due to a lack of biological recognition sites and its intrinsic hydrophobicity [14]. PCL-gelatin (PG) composite fibers (CFs) provide new biomaterials for overcoming the shortcomings of natural and synthetic polymers; blending gelatin in the PCL fibers can enhance the biocompatible properties with cell adhesion and proliferation [15]. The PG fibrous scaffold with desirable mechanical, physical, chemical, and biological performances would be an ideal candidate for tissue engineering and drug delivery applications [16].

Our research team has recently showed a method of preparing a drug delivery system from electrospun polymer fibers based on poly (ε-caprolactone), gelatin, and their composite with hydroxyapatite. Cefixime trihydrate was selected as a model drug due to interest in cefixime trihydrate–PCL monolithic fibers for the treatment of periodontal disease [17]. We do note that poly (ε-caprolactone) particles containing proteins have been in fact prepared by electrostatic extrusion (effectively electrospraying) [18].

2. EXPERIMENTAL SECTION

2.1. Materials.

Poly (ε-caprolactone) (PCL, Mw = 70 000−90 000) was purchased from Aldrich cefixime trihydrate was also obtained from Sigma. Gelatin (pharmaceutical grade), 2,2,2-trifluorothanol (TFE, 99.8%, molecular biology grade) were purchased from Aladdin Chemistry Co. Ltd.

2.2. Analytical.

Solution viscosities were measured using a Brookfield Model RVDV-III instrument equipped with a low viscosity probe for measurements down to ca. 3 centipoise (cP). UV–VIS measurements were obtained using a Perkin-Elmer UV/VIS Lambda 40 Spectrophotometer. The molar extinction coefficient for cefixime trihydrate in tris buffer was found to be 20800 ($L.mol^{-1}.cm^{-1}$) from a linear Beer–Lambert plot of absorbance at 283 nm vs. concentration.

Release of cefixime trihydrate was determined by placing a known mass of polymer and drug in tris buffer and monitoring the absorbance at 283 nm as a function of time. The buffer solution was changed if the released drug gave absorbance higher than 2.0. Data are reported as the percent cefixime trihydrate released based

upon the calculated amount in the samples from the feed composition. As a check of the validity of the absorbance data, a parallel study of release in pure water was conducted and mats were checked for nitrogen content (2 N per cefixime trihydrate) by elemental analysis (Schwarzkopf Laboratories, Woodside, NY). Pure water was used to avoid errors in N content that might arise to absorption of some tris buffer by the mats.

2.3. Electrospinning.

Electrospinning was carried out using 14% w/v solutions of PCL, Gelatin, and a 50:45:5 composite of the three components (PCL/Gelatin/Hydroxyapatite) in trifluoroethanol (TFE). Cefixime trihydrate was solubilized in TFE and added to the polymer solutions. The resulting solutions were indicated homogeneous solubilization of both the polymer and drug.

The electrospinning set-up consisted of a syringe and needle, a ground electrode (stainless steel sheet on a drum whose rotation speed can be varied) ca. 25 cm from the pipette, and a high voltage supply (model CZE1000R, Spellman). A positive voltage (15 kV) was applied to the polymer solution. The solutions were delivered via syringe pumps to control the mass flow rate, which ranged from 3 to 21 ml/h. As the electrical potential was applied, a jet was created. The jet, formed by electrical forces, followed a complicated stretching and looping trajectory as it solidified. The resulting fibers were collected on a rotating metal drum to produce a sheet of non-woven fabric. Sheet thicknesses ranged from 100 to 250 mm. The gelatin containing cefixime trihydrate was electrospun from 14% w/v solution in TFE, with a flow rate of the polymer solution between 18 and 21 ml/h. PCL containing cefixime trihydrate was electrospun from 14% w/v solution in TFE with a flow rate of 3 ml/h. Composite of 50:45:5 (PCL/Gelatin/Hydroxyapatite) containing 5% cefixime trihydrate were spun at a flow rate of 10–13 ml/h.

For comparative purposes, cast films were made from PCL, gelatin, and a 50:45:5 (PCL/Gelatin/Hydroxyapatite) inorganic-organic composite, in all cases containing 5% cefixime trihydrate. The solutions in TFE were cast onto glass petri dishes, left at room temperature until the TFE was evaporated, and then dried at 25 ºC under vacuum for 3 h.

3. RESULTS SECTION

Extensive chain entanglements are necessary to produce electrospun fibers, the consequence being that lower solutions concentrations lead to electrospraying rather than spinning [7, 12]. To that end, the Brookfield viscosities of PCL and gelatin solutions in TFE were determined (Figure 2) to assist in choosing minimum concentrations for spinning. We had found earlier that ca. 14–15% w/v of PCL is necessary to achieve electrospun fibers, and this appears to correspond to the onset of significant chain entanglements in the Brookfield viscosity data in Figure 2. Gelatin begins to spin well at lower concentrations, near 7–8% w/v, in agreement with the earlier onset of the rise in viscosity with concentration for gelatin (Figure 2) and the result of the higher molecular weight of the gelatin. Therefore, solution concentrations of 14% w/v for both polymers in TFE were selected to ensure electrospinning into fibrous mats.

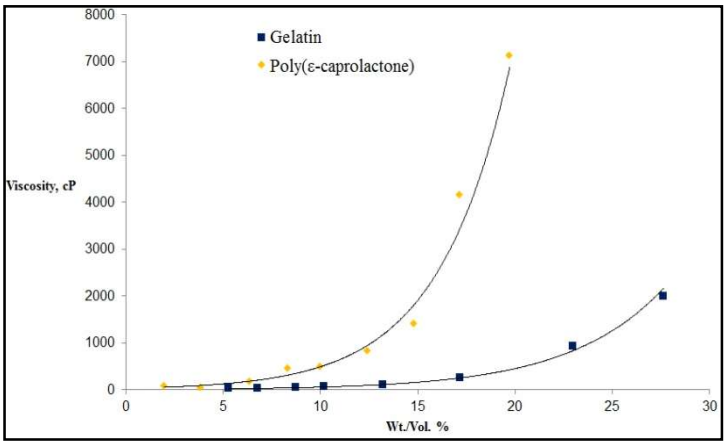

Figure 2. Brookfield viscosity data for gelatin and poly (ε-caprolactone) in TFE.

Our system is capable of preparing polymer mats with areas of ca. 200 cm^2. The mats are opaque due to light scattering by the fine fibers, yet retain the general mechanical characteristics of the base polymer, such as elasticity in the case of PCL. As expected, mats spun from a (PCL/Gelatin/Hydroxyapatite) compositeare stiffer than mats of PCL alone, although we have not as yet measured mechanical properties as a function of composition (Figure 3).

Figure 3. Example of PCL electrospun sheet.

The release profiles of cefixime trihydrate from electrospun fibers and the cast films are shown in Figures 4 and 5. Electrospun PCL shows a higher release rate than the mats derived from (PCL/Gelatin/Hydroxyapatite) composite or pure gelatin. Electrospun PCL released 65% of its drug content within 120 hours, whereas the composite material released about 50% over the same time period (Figure 4). Mats of gelatin fibers exhibit some instantaneous release, most probably from cefixime trihydrateon the fiber surfaces with negligible release over 50 h. This is likely due to the partial crystallinity of gelatin, which limits the diffusion of the aqueous environment into the polymer inner layers and consequently limits the diffusion of the drug from the fibers. The time scale of our release experiments is too short to expect significant release of drug resulting from hydrolysis of gelatin. We believe this is linked to the partial crystallinity of

gelatin which inhibits release over short times. Long term release from gelatin mats via hydrolytic degradation is under investigation.

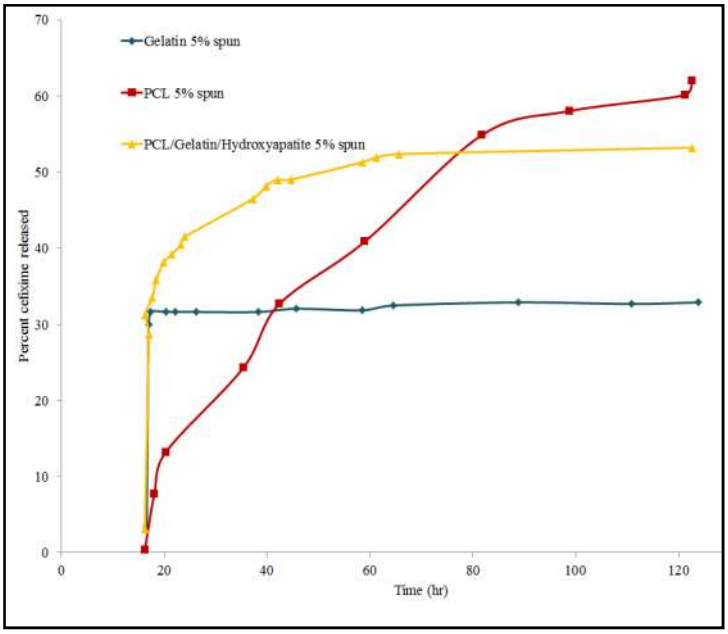

Figure 4. Percentage release of cefixime from electrospun mats vs. time.

The (PCL/Gelatin/Hydroxyapatite) composite have about 5% release of cefixime trihydrate within 5 h with a smooth and regulated release thereafter. A (PCL/Gelatin/Hydroxyapatite) composite with 25 wt% cefixime trihydrate (Figure 4) releases the drug much more rapidly than the 5% sample, and we suggest that this is due to surface-segregated cefixime that quickly dissolves. For this reason we decided to confine our experiments to 5% drug loading. As a check of our release data monitored spectrophotometrically, electrospun mats of PCL, gelatin and 50:45:5 (PCL/Gelatin/Hydroxyapatite) inorganic-organic composite were prepared with cefixime trihydrateand release of the latter was monitored in pure water by UV–VIS followed by elemental analysis (EA). The results are summarized as follows (percent released via elemental analysis/percent released via UV–VIS): PCL-56/54; gelatin -18/16; PCL/Gelatin/Hydroxyapatite -48/50. The data are in good agreement, giving us confidence that the UV–VIS release data in tris buffer are reliable.

It is interesting that the perecent cefixime released after 5 days from electrospun samples containing 5% cefixime is higher than the percent released from film samples (Figure 5).

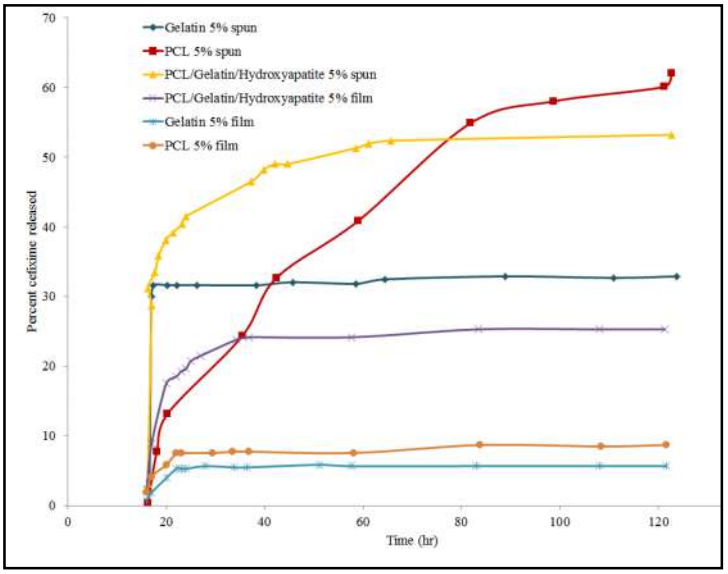

Figure 5. Percentage release of cefixime from films and electrospun mats vs. time.

A reasonable explanation lies in the fact that the film composition is prepared by melt composite of polymer and cefixime trihydrate, and the resulting fibers show evidence of much surface segregation of cefixime and the development of significant porosity upon release. Thus, a fiber scafold has a high internal surface area that in part mimics the high surface area of our fibrous mats, accounting for the smaller than expected differences in total cefixime released. A comparison of the actual amount of cefixime trihydrate released vs. time, shown in Figure 5, reveals that both electrospun samples and film samples. Thus, two potential advantages of the electrospinning approach are the avoidance of melt processing which is especially important for heat-sensitive drugs, and minimization of the initial burst.

In general, the total percent released from the cast films (Figure 5) were lower than that of the electrospun mats, as would be expected due to the much lower surface area of the former. Interestingly, the (PCL/Gelatin/Hydroxyapatite) composite film showed a rather substantial 25% release in about 120 h, and we speculate that this may be the result of accelerated diffusion at PCL/Gelatin/Hydroxyapatite phase boundaries in the films.

4. CONCLUSIONS

Release of cefixime from electrospun mats of PCL, gelatin and a (PCL/Gelatin/Hydroxyapatite) composite was studied and it was found that electrospun PCL and 50:45:5 PCL/Gelatin/Hydroxyapatite mats gave relatively smooth release of drug over about 120 hours. The simplicity of the electrospinning process and the wide selection of polymers that can be processed by this means suggest that electrospun polymers matrices may have broad applicability in controlled release technology. We are currently exploring electrospun systems for the controlled release of therapeutic macromolecules.

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