EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 9
54-61
Year: 2016

IN SITU DRUG RELEASE OF PVA/PLGA/SA NANOFIBERS FOR INFECTION DRUG DELIVERY SYSTEMS

Behnaz Ardeshirzadeh[1], Noraini Bet Jaafar[1], Hamid Akbari Javar[2], Arash Goodarzi, Mehrdad Mohammadpour[3] and Abbas Bahador

[1]Faculty of Chemical Engineering, Universiti Teknologi of Malaysia(UTM), Johor Bahru, Malaysia.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS), Tehran, Iran
Eye Research Center, Farabi Eye Hospital, Iran.

***Corresponding Author: Behnaz Ardeshirzadeh**
Faculty of Chemical Engineering, Universiti Teknologi of Malaysia(UTM), Johor Bahru, Malaysia.

Article Received on 02/07/2016 **Article Revised on 23/07/2016** **Article Accepted on 14/08/2016**

ABSTRACT
In this study a nanofiber drug delivery system is chosen from the other research using polymer (PVA) with sulfacetamide encapsulated into PLGA (50:50) nanofibers. Sulfacetamid was chosen as the drug due to its bacteriostatic activities in the treatment of eye infections. The aim of this study is to study sustained release from in situ formulation of sulfacetamid loaded nanofibrous implant. The effect of drug loading rate on the drug release profile was determined. Using the PLGA / PVA /SA formulation and 1 wt % SA drug was found to give the best dosage for drug release. The selected formulation is then tested in vitro using dialysis bag and in vivo using rats as animal test. Sulfacetamid loaded PVA/PLGA nanofibrous in situ implants were prepared using coaxial electrospinning and layer nanofibers for the controlled release of sulfacetamid and results indicated the effect of NMP on release profile.

KEYWORDS: Nanofiber, sulfacetamide, electrospinning.

1. INTRODUCTION

Electrospinning offers great flexibility in selecting materials for drug delivery applications. Either biodegradable or nondegradable materials can be used to control whether drug release occurs via diffusion alone or diffusion and scaffold degradation. Electrospun fibers can be oriented or arranged randomly, giving control over both the bulk mechanical properties and the biological response to the scaffold. Nowadays, all kinds of drugs such as antibiotics, anticancer agents and proteins, DNA, and RNA can be incorporated into electrospun scaffolds. Using the various electrospinning techniques the applications of electrospinning in tissue engineering and drug delivery are almost unlimited.

Sill and Recum[1] in their recent review mentioned electrospun nanofibrous scaffolds and their applications in drug delivery and tissue engineering. There are also some review attempts regarding surface engineering and drug releasing scaffolds[2] and suitable polymers in which drug could be loaded[3]. There are various examples of using biodegradable and non-biodegradable polymers for drug delivery in electrospinning process. Kenway et.al[4] prepared nanofibers which were made either from polycaprolactone (PCL) as a biodegradable polymer or polyurethane (PU) as a nonbiodegradable polymer or from blends of the two. Ketoprofen was embedded in polymer solutions, and its release profile was followed by UV–visible spectroscopy in phosphate buffer of pH 7.4 at 37 and 20 ºC. Regardless of the differences in inherent properties of the polymers, the result showed similar release rates of the drug from the polycaprolactone, polyurethane and their blend nanofibers. The significant difference was in visual mechanical properties which was improved in the blend of PCL with PU. They also investigated the release profiles from electrospun mat compared to cast films. In their previous work Kenawy et al[5].

Electrospun nanofibrous mats composed of PLA, poly (ethylene-co-vinyl acetate) (PEVA), and a 50:50 blend of the two polymers have been studied where the release of tetracycline hydrochloride from electrospun mats was examined. They pointed that both polymer composition and drug loading affected the rate of drug release with PEVA demonstrating quicker release than either the 50:50 PEVA/PLA blend or PLA. They also compared the release profile obtained from electrospun mats to those obtained from corresponding cast films. Due to their larger surface areas, electrospun mats tended to give greater release of drug than did the corresponding films. In another work, Zong and coworkers[5] used an electrospinning method to fabricate bioabsorbable amorphous poly (D, L-lacticacid) (PDLA) and semi-crystalline poly (L-lactic acid) (PLLA) nanofiber non-woven membranes for biomedical applications. They apply one of the most popular antibiotics, Mexofin, in polymeric solutions and electrospun into nanofibrous non-woven mats. The resulting membrane exhibits very uniform structures with an average diameter of 160 nm. The release profile of the drug from membranes was determined with the UV spectroscopic technique by measuring the absorbance at 234 nm as a function of time.The study found over 90% typical loading efficiency of Mefoxin in the PDLA sample by electrospinning. Finally, the authors concluded that the drug functionality seems to be completely unaffected by the gentle electrospinning process. PLGA or poly (lactic-co-glycolic acid) is a synthesized copolymer of monomers of poly glycolic acid (PGA) and lactic acid (PLA). The two monomers are linked by ester linkages to form PLGA (Astete, C. E)[6].

There are a number of different drug loading methods like coatings, embedded drug, and encapsulated drug (coaxial and emulsion electrospinning)[7]. These techniques can be used to give precious control over drug release kinetics[8].

Controlled drug delivery systems have gained much attention in the last few decades. This is due to the many advantages compared with the conventional methods such as improving therapeutic efficacy and reducing toxicity by delivering drugs at a controlled rate[9]. Electrospun nanofibers often have higher drug encapsulation efficiency than other nanotechnologies. The main advantages of the fibrous carriers are that they offer site-specific delivery of drugs to the body. Also, more than one drug can be encapsulated directly into the fibers[10]. In situ formation may also occur when material absorbs water from surrounding environment and expand to occur desired space[11]. This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Nmethyl pyrrolidone (NMP) has been shown to be a useful solvent for such system. Chemical reactions that results in situ gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes [12]. In situ forming will improve patient acceptance. It will prolong the residence time and the ocular bioavailability of the drug improves[13]. The hardening of the polymer in the body after injection could be induced by solvent exchange, temperature, or pH change.

The conventional delivery system involves three parts: suspensions, solutions, and ointments. All these system have some advantages and disadvantages[14]. The advantages can be mentioned as: easy way to produce, low cost of fabrication, and easy way for administration by patients. On the other hand there may be some disadvantages like short contact time with ocular surface and fast removal from eye. The ophthalmic drug delivery should prolong the activity of the active factor in target site[15]. The novel in situ gels for ocular healing of ocular disease is an optimum drug delivery system. The reason is that, these gels are easily administered, more stable, do not make any blurring of the eye; it is patient compatible and improves the bioavailability of drug, reducing the dosing frequency - up to once daily as required than in case of the conventional dosage forms[16]. The systems which are built in the position, mostly a large amount of the drug is released before reaching a firm release volume, which is known as drug burst or primary release[17]. One of the primary types of system is using drug coating on the core and then coat it by natural polymer. Ion exchange resins have also been proposed to control drug release[18].

It is very important to choose the therapeutic dosage in which the drug density sets exactly in therapeutic range and in long term. Usually, for all common drugs, when they are used according to their usage (for example, 4 pills daily), at the beginning, the drug density in blood will increase fast in which it becomes more than its therapeutic range. In therapeutic window, the amount of drug density is up to give effective results. If the drug density is less than this amount, the medicine will not be effective; on the other hand if it is more than the determined amount, it will cause some peripheral bad results such as: headache, vomiting, dim sight, itching, low pressure, and mental depression.

Figure 1 illustrates the drug density in blood in two cases: one in usual repeating usage of drug, the other one in ideal case of controlled release. According to these figures, in the case of normal usage of drug as shown in Figure 1a, the amount of drug in blood may reach higher than the therapeutic window. Then, for a short period, it may reach the amount of therapeutic window and finally the drug density may go lower than this limit and pharmacologically the drug will become ineffective. More repetition of drug usage and long biological half-life, can result in building up of this drug in blood.

When the next dosage of drug is used, its density will be repeated in accordance to this cycle. As a result the drug will show its preferred results in a short period of time. Clearly if there is formalization in which medicine becomes released in a control way as in Figure 1b, the effective factor in therapeutic limit will be released for longer period of time and, as far as possible, with less fluctuation. Moreover for some disease such as cancers, physical surgery, it is required to prevent the cell growth in that specific point but prevent accessibility of medicine to other parts of body. Here the controlled release systems which work in specified points (such as pumps) will be used.

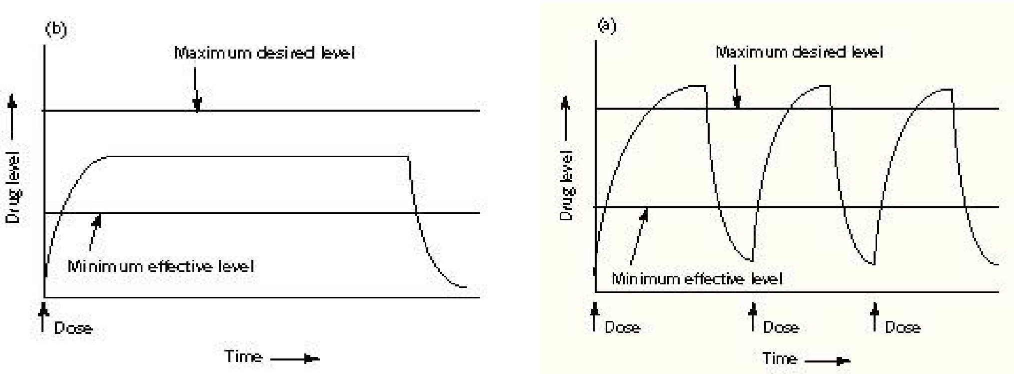


Figure 1 the drug density in blood [91]

91. Maa Y., Heller J., "Controlled release of Naltrexone pamoate from linear poly (orthoesters)", J. Controlled Rel.,14, 21-28, 1990.
(a) Frequency usage of drug usage normal way
(b) One time drug usage in ideal way of controlled release.

2. Experimental

2.1 Materials

Poly(D,L-lactide-co-glycolide) (PLGA, LA/GA 85/15, Mw = 200,000) and poly(vinyl alcohol) (PVA, Mw = 72,000) were purchased from Sigma–Aldrich (Germany). N, N-dimethyl formamide (DMF) solvent was provided from Merck Company (Germany).

2.2 Preparation of solutions

Two different phase were prepared, The outer phase (sheath) was a solution of actual (50:50) ratio of 9.5% PLGA dissolved in N, N-dimethylformamide (DMF) as solvent. The formulations were dissolved by gentle stirring for 4 h at room temperature. The inner phase consisted of 0.05 mg of SA dissolved in a 10% (w/v) PVA solution prepared in DW(SA 1:10 PVA) and gently stirred for 9 hours at 45 ° C with stirrer speed of 1000 rpm. In order to completely remove bubbles formed in the polymer solution prepared samples were placed for 4 hours at ambient temperature.

2.3 Coaxial and layer electrospinning

Two variations of PLGA/PVA were fabricated i.e. type I (core-shell) and type II (layer nanofibers). In type I core-shell nanofibers, the core solution is a blend of PVA/SA/DW (10% PVA) (PVA 10:1 SA), the shell solution in this case was PLGA/DMF blend of 10 % PLGA. Electrospinning unit uses a 19-gauge inner needle concentrically positioned within an outer needle of 25-gauge diameter. In type II layer nanofiber, three types of nanofibers were fabricated; the first type consist of the PLGA/DMF blend of 10% PLGA solution which were electrospun using 19-gauge needle; then the second type which is the PVA/SA/DW (10% PVA) (PVA 10:1 SA) solution using 19-gauge needle in section three the other half of the PLGA/DMF blend of (9.5%PLGA) solution were electrospun on top of the rest of nanofibres. Variable high voltage (DC) power was connected to the spinneret tip to spread out charge into the polymer solution. The instrument was activated at 20 kV for synthesis. The flow rate of the drug-polymer electrospining process was kept at 0.2mL/hr. The nanofibers created in the process were collected over an aluminum foil which covered the collector and positioned at 15 cm from the spinneret. The nanofibers were carried out on conditional time under a controlled encasement temperature of 37ºC and constant humidity. After deposition the fibers were removed from the metallic collector and kept in a desiccator overnight in order to remove the solvent. Figure 2 shows the SEM images of PLGA/PVA/SA coaxial nanofiber.

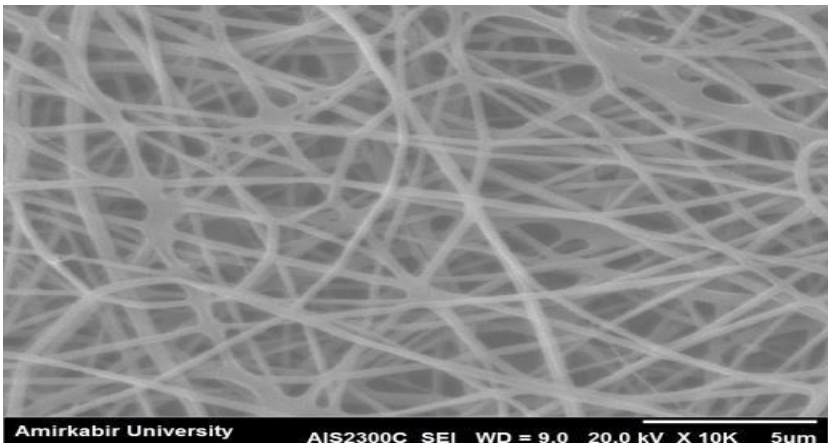
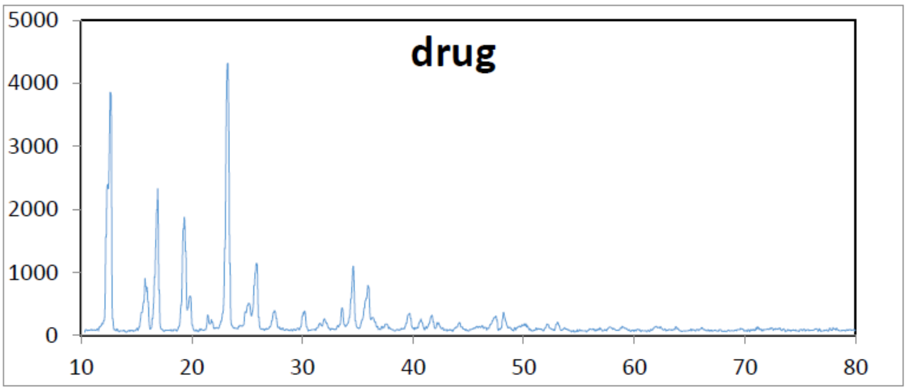


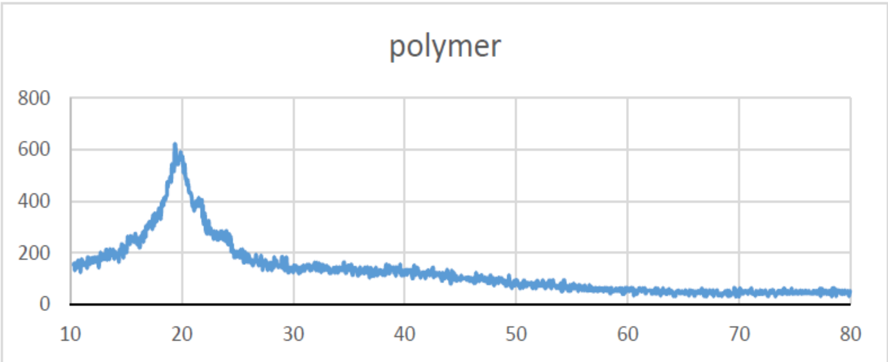
Figure 2 SEM image PLGA/PVA/SA nanofibers with (DMF)

XRD is a highly reliable technique used to search for the nature of any crystalline compounds. Figure 3 (a) presented the XRD pattern of pure SA and nanofiber mats. Figure 3 (b) represented more about the spectra of the PLGA/PVA nanofiber. As semi-crystallite in nature, it can be observed that it does not have any logical peaks except the one which represent its semi-crystalline nature. While in comparison to Figure 3 (c) with its other modified counterparts prepared from electrospinning of SA-containing nanofiber, the spectra shows some additional peaks at the same diffraction angles of the SA peaks with identical location which emphasized that the nanofibers involve SA and simultaneously supports the SEM, TEM, AFM results. The intensity of peak obtained from XRD analysis and the interesting results which concur with the presence of crystalline SA shows that the intensity of peaks were increasing with the presence of SA in the solutions.

a



b



c

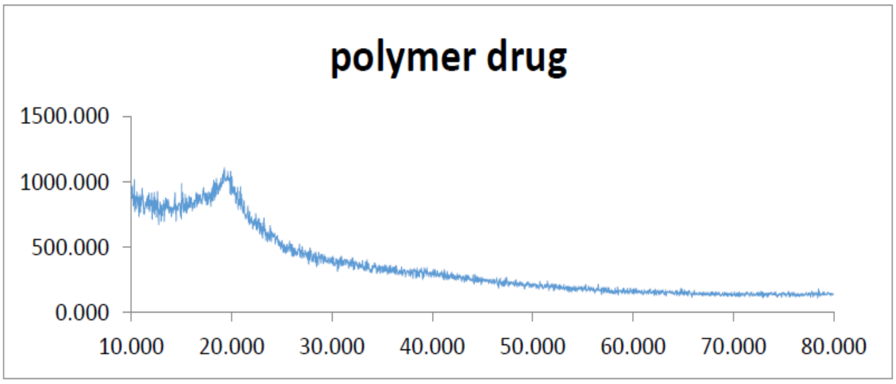


Figure 3 XRD results for the (a) pure SA, (b) PLGA/PVA nanofiber, (c) drug loaded nanofiber

2.4 Stability of Nanofiber Scaffold

To investigate the stability of the nanofiber scaffold in the body, the crosslinked nanofiber scaffold (CN) and not crosslinked nanofiber scaffold (NCN) were immersed for 24 hours at 37 ° C in a solution of acetate buffer. The samples were removed from the solution after 24 hours at room temperature to dry the nanofibers completely.

The structure changes of the nanofiber scaffolds was studied by scanning electron microscopy before and after immersion in acetate buffer solution. Figure 4 (a) and (b) show the SEM image of crosslinked and non croslinked nanofibers containing the drug. As can be observed from the figures for the uncrosslinked nanofibers samples, on exposure to acetate buffer solution for more than 24 hours; some part of the nanofibre structure has been lost to the film layers between the fibers. On the other hand, as expected, crosslinked sample has retained its structure. The stability of the crosslinked nanofiber scaffolds were due to joining of the polymer chains.

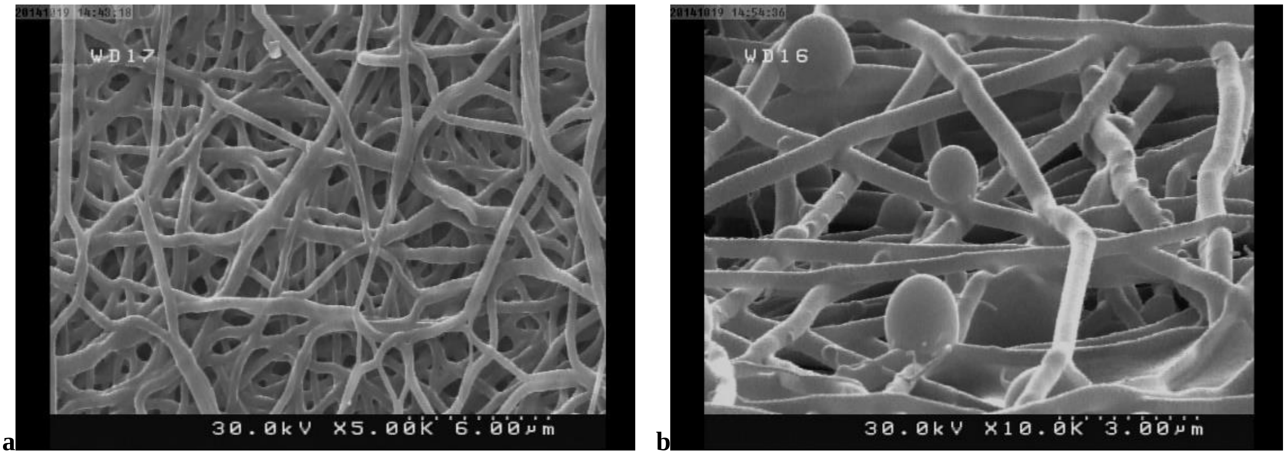


Figure 4 SEM images of nanofibers at initial time, (a) crosslinked (b) and non-crosslinked nanofibers in acetate buffer for 24 hours at 37 ° C

2.5 Sustained Release from the Nanofiber Scaffolds

In order to study the sustained release of SA drug from in vitro nanofiber scaffolds, prepared nanofiber scaffolds were immersed in phosphate buffer solution at pH 7.4 at 37 ° C . In the time periods specified the amount of drug released was measured in the ambient. Because the nanofiber scaffolds maintained their structure in the buffer solution drug released from them could be studied. For obtaining sulfacetamide calibration curves, different concentrations of sulfacetamide were prepared and their UV absorbance was measured at λmax of 256 nm, respectively, by a UV-VIS spectrophotometer (Jasco V-530, Jasco co., Japan).

Figure 5 shows the graph of SA drug released from the nanofiber scaffolds. Concentrations of SA throughout release time intend using the calibration equation (drug release (%) = (Ct ×10-3/Ci) x 100), where Ct is the concentration of drug released at time t and Ci is the initial drug concentration. The graph is based on the percentage of drug released relative to time. In theory, drug release behavior from nanofiber scaffolds are made up of four stages. The first phase is the sudden release of drug. At this stage, a significant amount of is drug released at high speed. However this burst release phase is not exhibited by the fabricated nanofiber. Instead the drug release rate from the nanofiber is slow as can be observed in Figure 6 in the early hours; thus it is concluded that the burst release of drug is not shows a significant release in the study and this is one of the important advantages of using scaffolding nanofibers. Although drug released in the first hours plays a significant role in producing phagocytes but the small amounts of drug released on remaining hours may not be very desirable. Since the goal is this system remain in the tissues for a 15 days and release its drug during these days, the obtained drug release time of about 15 days can be desired for such a system specially the burst release of about 30% can provide the initial dose. In fact, the amount of drug on the surface of the nanofiber, washed with buffer solution and distributed to the surrounding ambient from the pores in the polymer nanofibers.

The second stage of drug release from nanofiber scaffold is called stage after burst. At this stage the drug cross over into the buffer from the pores of the nanofiber scaffolds. Then the drugs which were trapped in the scaffolding were delivered. In fact, at this stage, drugs trapped in the middle of the scaffolds are released into the buffer medium due to degradation of polymers, oligomers, and monomers production, and broken bond. The drug release stages outlined cannot be determined distinctly in the study because after the start of the release stage the polymer gradually began to be destroyed. After the second stage the drug released reached its maximum value. Penetration phenomenon at this stage will continue and eventually in the last stage, the concentration of drug in nanofiber is reduced and even with the destruction of polymer nanofibers release speed is very low and shows low slope, while some drug still remains in the nanofibers.

As can be seen in Figure 6, the highest release of drug from the nanofiber scaffold containing 1 wt % SA is in the first half hour at 22.18% and the release after one hour is 32.98 % and after 24 hours the release reached 40.38%. With the passage of time the release rate gradually decreased where the release was measured up to 336 hours (14 days). The maximum release were found to be 50% in 48 hours and 89% in 336 hours for nanofiber scaffolds containing 1 wt. % SA drug in formulation which is obtained 20 kv voltage, 9.5(wt.%) PLGA concentration and 10(wt. %) PVA concentration as describing factors. The formulation is the optimum formulation with lowest PLGA concentration which is made that a reasonable formulation as a matter of cost of the study, however no significant difference is observing.

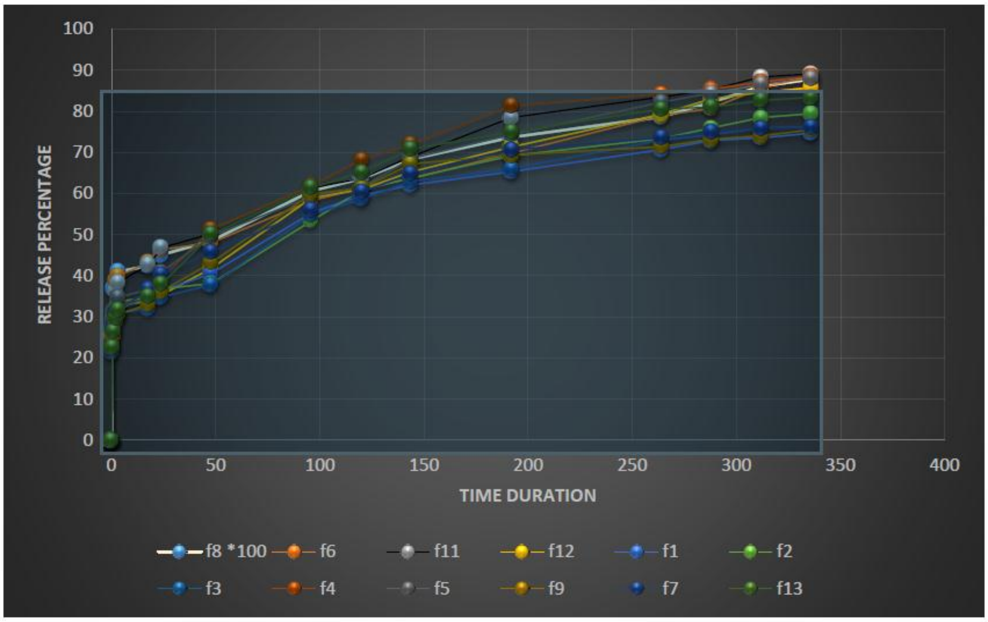


Figure 5 Graph of Sustained SA Drug Release from Nanofiber Scaffolds.;(f1-f13 refers to the formulations used for the nanofibers).

2.6 Kinetic Studies

Kinetics studies were performed to determine the mechanism of drug release from the fabricated nanofiber. The kinetics of drug release were observed and compared to the known pharmacokinetic models namely Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models. The SA release data were fitted to the established model to evaluate the nanofiber SA release mechanism. The results are presented in Table 1 By comparing the correlation coefficients, it was found that the zero order kinetic model ($R2 > 0.94$) fitted best to the SA release data. The obtained n values ($n<0.45$) of Korsmeyer-Peppas equation indicated that the mechanism of SA release from the prepared nanofibers was by Fickian diffusion. Thus it can be inferred that the release of SA from nanofibers was dependent on drug diffusion rate from nanofibrous formulations.

Table 1 Pharmacokinetic Results for Zero-Order, First-Order, Higuchi and Korsmeyer-Peppas equations

Formula No.	Zero-order		1^{St} order		Higuchi		Korsmeyer-Peppas		
	Release rate ($\%.hr^{-1}$)	r^2	Release rate ($\%.hr^{-1}$)	r^2	Release rate ($\%.hr^{-1}$)	r^2	N	K	r^2
F4	0.1557	0.942	0.0039	0.756	5.332	0.771	0.1779	29.05	0.928
F8	0.1634	0.947	0.0041	0.789	5.667	0.751	0.1824	26.53	0.934
F11	0.1723	0.951	0.0045	0.743	5.732	0.782	0.1923	28.31	0.942
F12	0.1629	0.944	0.0043	0.752	5.823	0.761	0.2094	27.34	0.931

2.7 Drug Release from Crosslinked and Unlinked Nanofiber Scaffold

Drug release characteristics from crosslinked and un-crosslinked core-shell nanofibers were investigated. The drug loaded nanofibers consisting of layer type and coaxial type were trimmed into small pieces weighing 4 mg; then the nanofibers were subjected to crosslinking in Glutaraldehyde vapor for 15 min at 37o C in a controlled environment. All of crosslinked nanofibers were placed in PBS medium after drying. At the end of 24 hours, the amount of drug released is estimated by measuring the absorbance of the release medium (i.e. PBS) at 256 nm, separately. In this study, in order to explicate the effect of crosslinking time on drug release profile, the equivalent amount of samples from f nanofibers (layer type and coaxial type) were utilized. In order to estimate the amount of drug released from the fibers, the linear equation obtained from the standard plot for sulfacetamide in PBS was used. This study was carried out at 37°C in static sink conditions. The release media was centrifuged at low speed (2000 rpm for 2 minutes) and the supernatant was taken for UV-VIS analysis to avoid interference of polymeric fibers in the UV-VIS observation. About 30 mg of nanofibers (crosslinked for 15 mins) were immersed in 70 mL PBS (pH 7.4) medium for conditional (for duration between 0-336 hours) time period under static conditions. Then 5 ml of release medium was retracted for UV-VIS absorbance studies at specific time point. For maintaining constant (volume) sink conditions during the release study, an equivalent amount of release medium was added. The standard curve of sulfacetamide demonstrated the absorbance value for the release medium is at 256 nm for calculating the amount of drug released. The difference between layer type and coaxial type fibers and the effect of solvent used on drug release are also shown in this study. The results showed that more sustained release is shown by crosslinked samples. This is because it is maintaining its structure in buffer solution. All of the samples in the experiment were utilized in triplicates (n=3). Figure 7 shows the cumulative percentage of drug released relative to time.

Cumulative percentage of drug released = (Ct ×10-3/Ci) x 100Mt /M∞ x 100
Where, Ct - concentration of drug released at time,
Ci – The initial Concentration of drug.

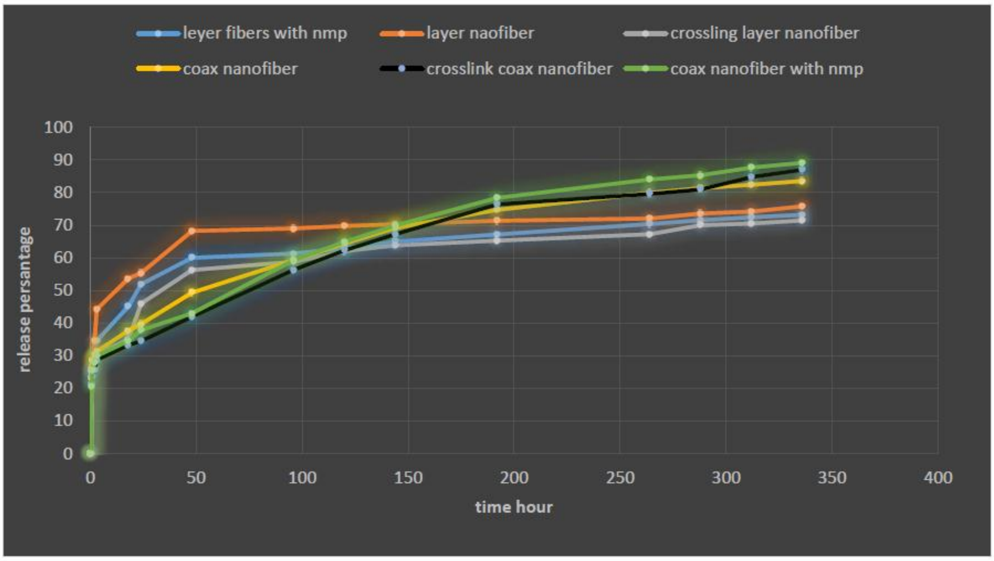


Figure 6 Release of SA from PLGA / PVA /SA in situ drug delivery systems according to types of nanofibers.

In this study the release of SA from PLGA / PVA /SA in situ implant drug delivery systems using NMP solvent and without NMP solvent and the release from two types of nanofiber preparation being coaxial nanofiber and layer nanofibers were investigated and the results are shown in Figure 7 which obtained as expected more burst release in layer type nanofibers compared to coax. The results indicate that by adding NMP the release curve shows higher slope and higher release percentages, which shows that NMP is a decisive factor in the burst release phenomena and the control of drug release, so burst release is reduced and the overall delivery in time duration increases.

3. CONCLUSION

In this study results indicated that the surface plot of PLGA concentration versus PVA ratios revealed that the increase in PLGA concentrations led to increase in SA release percentages. The higher release percentage in higher PLGA concentrations could be attributed to the increase in the free volume of electrospun nanofibers and consequently, enhancement of water sorption into the pores of nanofibers. And also indicated that the higher release percentages of SA from electrospun nanofibers were obtained at middle values of PVA concentration. By increasing PVA concentration, the homogeneous thin nanofibers were produced which resulted in steady release of SA from nanofibers.

The SA release percentage increased by increasing applied voltage. When the applied voltage increases, the electrical field strength increases which causes fiber diameters to decrease. Therefore, the increases in applied voltage resulted in decreasing fiber diameter and subsequently, the higher SA amounts was penetrated from pores of nanofibers.

XRD results which shows drug containing, while in comparison Figure 3.c with its other modified counterparts prepared from electrospinning of SA-containing, the spectra shows some additional peaks at the same diffraction angles of the SA peaks with identical location which is emphasized that nanofibers involve SA and simultaneously supports the SEM, TEM, AFM results. Infrared spectroscopy results do show that presents of SA in the composite nanofiber scaffold PLGA / PVA as well as the interaction between the polymer solutions is very low.

Results shows scanning electron microscopy samples of crosslinked and non croslinked nanofiners containing the drug. As can be observed in the result of scanning electron microscopy samples of crosslinked and non croslinked nanofiners containing the drug the uncrosslinked nanofibers samples on exposure to acetate buffer sulotion, after 24 hours, some parts of the nanofibres structure has been lost for film layers between the fibers. But as expected crosslinked sample has retained its structure. The stability of the crosslinked Nanofiber scaffolds were due to joining the polymer chains. In testing the stability of nanofiber scaffolding it observed that non crosslinked nanofiber loses part of its structure in the acetate buffer but after doing crasslink on nanofibers it shows more stability in the acetate buffer. The results of the drug release from nanofiber scaffolds contain SA also show that the release of the drug substance is taken with a certain rate.

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