JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 08, ISSUE 04, 2021

Preparation of Polymer Nanofibers via the Electrospinning Technique for Burn Dressing Purposes

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Abstract
Chitosan/polyethylene oxide (CS/PEO) nanofiber containing silver sulfadiazine (AgSD) was successfully prepared and analyzed in the present study. CS nanofiber mats with antibacterial activity against some common bacteria found on burn wounds were provided from a 70% aqueous acetic acid solution containing a different ratio of CS/PEO and AgSD, as a potential antibacterial agent. Several parameters were investigated to optimize the fiber diameter, including polymer concentration, CS to PEO ratio, applied voltage, and needle to collector distance. The obtained nanofibers were analyzed by scanning electron microscopy, transmission electron microscopy, and ultraviolet-visible spectroscopy. The spectroscopy results showed the presence of AgSD in nanofiber mats. The drug release behavior of electrospun nanofiber mats was investigated in phosphate buffer saline (PBS, pH = 7.4) at 37 ˚C. In all tested nanofiber mats, a burst release of the drug was observed after 4hoursof incubation in PBS. The most AgSD released from nanofibrous mats were observed after 40 hours of releasing and it was 76.74%. The results indicated that the new type of CS wound dressing incorporated with AgSD can be a high potential candidate in the treatment ofinfected wounds.

Keywords: Nanofibers, Electrospinning, Chitosan, Silver sulfadiazine, Burn-dressing

1. Introduction

Shortening the time of wound healing has attracted much attention for many years. Accordingly, finding the right path ultimately leading to faster wound healing is one of the most important goals in medicine.Burn is one of the major public health problems worldwide, especially in developing countries. Extensive burn injuries are not only life-threatening for these patients but also have serious effects on their physical, psychological, and economic conditions, their families, and society. Burn injuries and related infections are considered one of the main leading causes of accidental death in all age groups, leading to a significant increase in disease and mortality, especially in children [1-3]. In this regard, proper use of antimicrobial drugs and removal of burned tissues reduce resistance in bacteria, increase drug performance, and finally, decrease the risk of sepsis (blood infection). Current treatments for burns are based on antibiotic therapy methods and plastic surgery. Silver sulfadiazine is an effective and widely used antibiotic for burn injuries in humans [4-7].

The use of nano drug delivery systems will contribute to increasing the effectiveness of drug properties, and these systems have more advantages compared to conventional drug delivery systems. The type of drug, dose, and method of drug delivery systems are highly important in successful treatment. Nano systems can improve therapeutic activity by increasing the half-life of drugs, the solubility of water-insoluble drugs, the reduction of potential immunogenicity, and the better control of drug release. Undesirable side effects and the need for more frequent drug delivery reduce as well [8-15].

Polymeric nanofibers have drawn much attention in recent years [16-17]. The electrospinning technique is a simple and effective method for obtaining ultra-fine fibers with diameters ranging from micrometers to some nanometers. The electrospun fibrous mats present a range of useful characteristics such as a high specific surface area, high aspect ratio, and high porosity while with small pore sizes. The electrospun mats can mimic the extracellular matrix and thus enhance cell migration and proliferation [18-22]. Therefore, these mats are especially suitable for biomedical applications including wound dressing, drug delivery, and tissue engineering [23-29].

Chitos an is a biopolymer that is well known for its application in the healing of wounds in humans [30-34]. Biodegradability, biocompatibility, non-toxicity, anti-bacterial, and antioxidant properties are the most important factors involved in the selection of this polymer for wound dressing. It has also been confirmed that CS confers considerable antibacterial activity against a broad spectrum of bacteria [35-37].

Lu et al. used self-assembly technology for a novel wound dressing based on CS and nano silver an dreported an increase in the rate of wound healing while a decrease in the infection rate [7]. In another study by Shu-Huei Yu et al.,CS/alginate hydrogel membranes containing AgSD were prepared as a wound dressing.The

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controlled antibacterial release of AgSD against Pseudomonas aeruginosa and Staphylococcus aureus suggests that this dressing is effective in suppressing bacterial proliferation and keeping the wounds away from bacterial invasion [39]. In the currentstudy, electrospinning is used to prepare CS/PEO nanofiber containing AgSD for burn dressing purposes.

CS/PEO nanofiber mats includingAgSD are porous and have permeable spaces that act as a permeable membrane allowing the wound to remain moist. Simultaneously, the size of pore spaces blocks the entrance of microorganisms such as bacteria and fungi [38-39]. The use of CS in the structure of the nanofiber promotes the wound healing degree while decreasing the risk of infection due to the bioactivity and antimicrobial properties of CS [40-43].

2. Experimental

2.1. Materials

CS (low molecular weight) and PEO (MW 900 KD) werepurchased from Primex(Iceland)and Acros Organics Company, respectively. Further, glacial acetic acid andAgSDwere provided from Merck Company and Shenyang Funing Pharmaceutical Company, Ltd, respectively.

The electrospinning processes were conducted using Electroris (FNM Ltd., Iran, www.fnm.ir) as an electrospinner.

2.2. Preparation of CS/PEO nanofibrous mats

To this end,the 6% (w/v) CS solution was prepared using 70% (v/v) aqueous acetic acid solution and stirred at room temperature. Moreover, PEO was dissolved in 70% (v/v) aqueous acetic acid solution under gentle stirring for an hour to obtain homogeneous solutions of 0.67, 1.5, and 2.5% (w/v). The CS/PEO blend solutions were prepared by mixing these two solutions at 90/10, 80/20, and 70/30. AgSD powder was also dissolved in 70% acetic acid. Then, the solution was mixed with the CS/PEO solutions at the ratio of 1:2. The final solutions had total polymer concentrations of 3%.All solutions were kept under constant stirring for one hour at 25˚C to obtain homogeneous solutions. All solutions were then immediately used for electrospinning.

2.3. Electrospinning

In this work, different types of CS/PEO solutions were prepared by dissolving CS and PEO in 70% acetic acid at the CStoPEO ratios of 90/10, 80/20, and 70/30. The polymer solution was placed into a 5 ml plastic syringe with a blunt-ended 18 G needle,which was located in the range of 9-12 cm apart from the grounded collector. A syringe pump was fed the solution to the needle tip at an injection rate of 1 ml/hour. A positive high voltage was connected to the metallic needle, and the collector was connected to the ground. The applied voltage was in the range of 10-25 kV. These experiments were conducted at 25˚C (room temperature) and 28˚C and the collector speed of 0 and 500 rpm (maximum speed). The drum was wrapped in aluminum foil to collect the nanofibers.The details of the experiments are provided in Table 1.

Table 1-Used recipe in electrospinning methods

Experiment #	CS/PEO Ratio	AgSD presence	Tip tocollector distance (mm)	Voltage (kV)	Drum(rpm)	Processing temperature (°C)
1	70/30	☑	100	10	500	25
2	70/30	☑	100	15	500	25
3	70/30	☑	100	20	500	25
4	70/30	☑	100	25	500	25
5	70/30	☑	90	15	500	25
6	70/30	☑	100	15	500	25
7	70/30	☑	110	15	500	25
8	70/30	☑	120	15	500	25
9	70/30	☑	100	15	Zero	25

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10	70/30	☑	100	15	500	28
11	70/30	☑	110	20	500	28
12	70/30	☒	110	20	500	28
13	80/20	☑	110	20	500	28
14	80/20	☒	110	20	500	28
15	90/10	☑	110	20	500	28
16	90/10	☒	110	20	500	28

2.4. Characterization

The morphology of the electrospun nanofibres was analyzed by scanning electron microscopy (SEM) (Zeiss-960A) and transmission electron microscopy(TEM) (Philips, EM 208) operated at 100 kV.In SEM, nanofibers were electrospun onto the aluminum foil and then mounted on the grounded collector plate for 30 minutes. The samples were sputter-coated with gold in a sputter coater for 60 seconds to reduce electron charging effects.In TEM, nanofibers were directly electrospun onto carbon grids (Cu-100 mesh) which were attached on aluminum foil for 30 minutes.

The nanofiber size was measured with Image Analyzer software (SemAfore). For each experiment, the average fiber diameter and distribution were determined from about 25 measurements of the random fibers.

The existence of the AgSD in the CS/PEO solutions was confirmed by monitoring the absorption band using anultraviolet-visible (UV-Vis) spectrophotometer. To create similar conditions for nanofibers and polymer solutions, 0.005 g nanofibers and 1g CS/PEO solutions containing AgSD were diluted with 70% acetic acid. Finally, homogenous solutions were obtained by gentle stirring for an hour.

2.5. Antibiotic-release studies

The electrospun nanofiber webs were cut into approximately 5×5 cm^2 pieces and then accurately weighed to determine the approximate weight of a typical electrospinning nanofiber mat. The average weight of these webs is about 8.4 mg.

Next, the electrospun nanofiber mats with/without drug were cut intonearly 5×5 cm^2 pieces and placed in 500 ml phosphate buffer saline (PBS). Homogenous solutions were obtained by gentle stirring for 2 hours. Finally, the absorbances of the nanofiber solutions with/without the drug were measured at the wavelength of 240 nm using a UV-Vis spectrophotometer. The drug concentrations in the nanofibers were calculated by the calibration curve.

The electrospun nanofiber webs were cut into approximately 5×5 cm^2 pieces. In addition, the samples were accurately weighed and then placed in the dissolution test baskets (Dissolution TestErweka-DT800) containing 500 ml of phosphate buffer solution (pH=7.4) at 37 $^{\circ}$C. At predetermined time intervals, the nanofibers sample was taken out from the incubation buffer and put into another fresh buffer solution. The amount of the released drug was determined spectrophotometrically. The UV absorbance of AgSD in the buffer solution was determined at λ_{max} = 240 nm and converted to the AgSD concentration according to the calibration curve of AgSD in the same buffer.

3. Results and Discussion

3.1. Fiber morphology

The optimal process conditions for producing adequate amounts of nanofibers for the next tests such as drug release require the following conditions stated in Table 2.

Table 2- Optimal process conditions to produce nanofibers

Electrospinning parameter	Optimal condition
Voltage	20 kV
Tip to collector distance	110 mm
Feeding rate	1ml/h
Speed of drum	500 rpm
Temperature	28 °C
Time for electrospinning	4 hours

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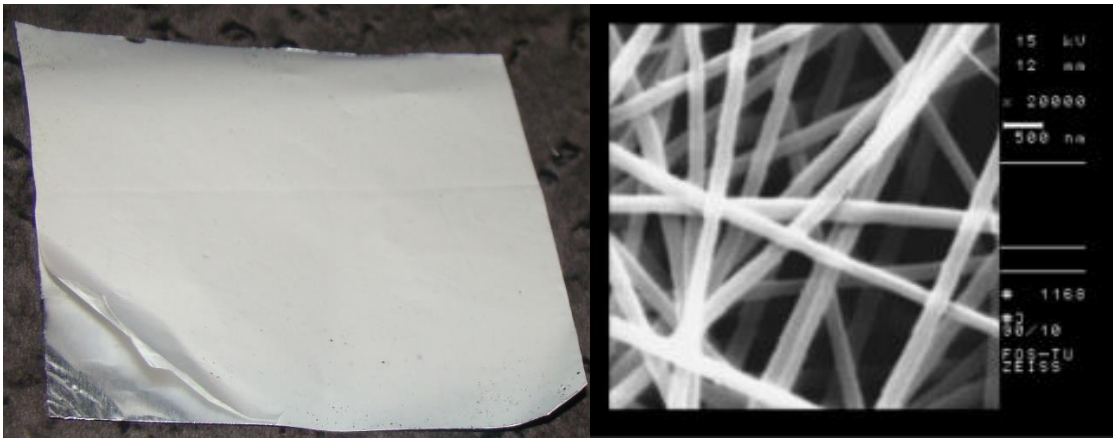
The SEM image of nanofibers mats containing AgSD was analyzed based on the optimal conditions(Table 2). Figure 1 displays the successful electrospinning due to uniform and beadless nanofibers by digital and SEM images of nanofiber mats containing AgSD.



a b

Figure1-(a) Nanofiber mat image and (b) SEM image of nanofiber mat containing AgSD

CS-based nanofibers includinga different ratio of CS/PEO and AgSD, as an antibacterial agent, were successfully electrospun from 70% aqueous acetic acid solutions.

There were two forms of nanofibers at the CS/PEO ratios of 80/20 and 70/30. One group was thinner and discontinuous and had a jagged surface while the other one was thicker and more continuous. The presence of the two forms of CS/PEO nanofibers at the above-mentioned ratiosis depicted in Figure 2. TEM images also confirmed the presence of the two forms of nanofibers at the same ratio (Figure2).

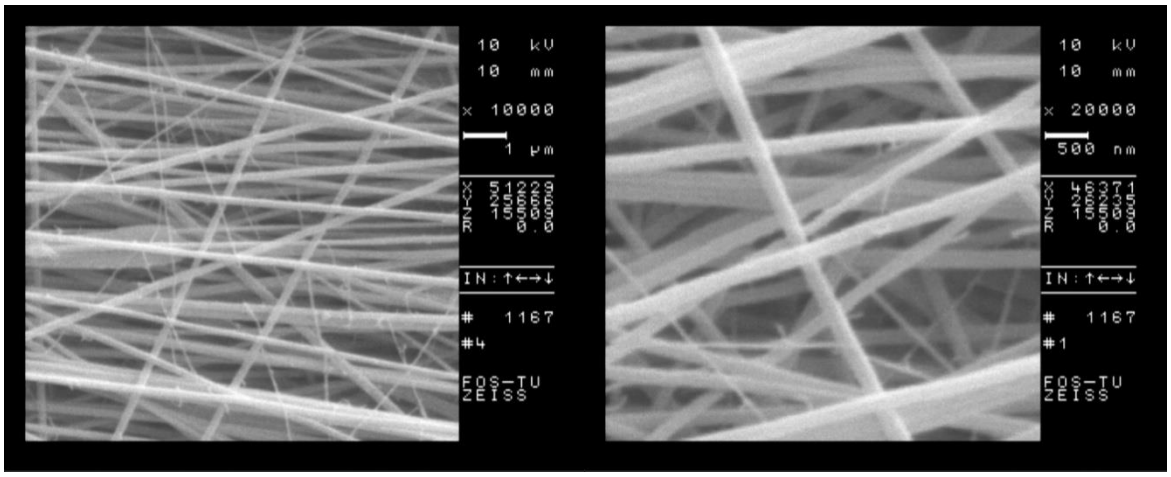


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Figure 2- SEM and TEM images of two forms of CS/PEO nanofiberscontaining AgSD obtained from electrospinning at the ratios of (a) 80/20 and (b) 70/30

CS concentration had a significant effect on the average fiber diameter. More precisely, by increasing the CS concentration, the average fiber diameter decreased from 327 to 184 nm in the presence of AgSD and from 233 to 130nm in the absence of AgSD .

SEM images were analyzed by the SemAfore program to obtain the morphologies and diameters of nanofibers. Table 3 presents the morphology and diameter of nanofibers.Based on the results, the presence of AgSD had a significant effect on nanofiber diameter.

Table 3- Nanofiber's morphology and their diameter with different CS to PEO ratio with/without AgSD

The ratio of CS to PEO	AgSD presence	Average nanofiber diameter (nm)	Range of nanofiber diameter (nm)	Nanofiber's morphology
90:10	☒	130.33	55-194	Uniform fibers without bead
90:10	☑	184.44	105-250	Uniform fibers without bead
80:20	☒	186.5	111-264	Two form fibers without bead
80:20	☑	220.22	166-325	Two form fibers without bead
70:30	☒	233.64	140-337	Two form fibers without bead
70:30	☑	327.25	203-444	Two form fibers without bead

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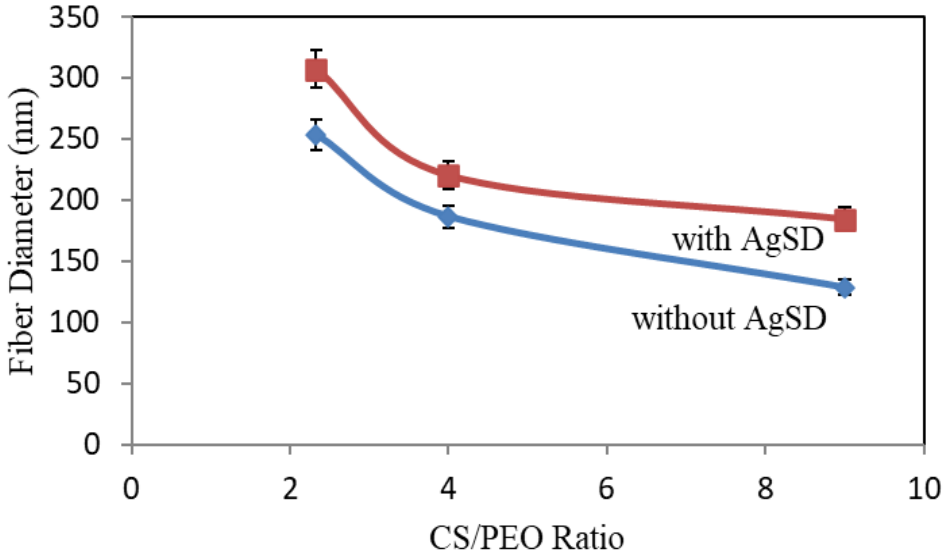

Figure 3-Effect of the CS/PEO ratio on the average nanofiber diameter in the presence and absence of AgSD

The presence of nano particles is illustrated in the TEM micrographs of nanofibers obtained from the electrospinning of the CS/PEO solution in 70% acetic acid at the ratio of 70/30 in Figure 4.

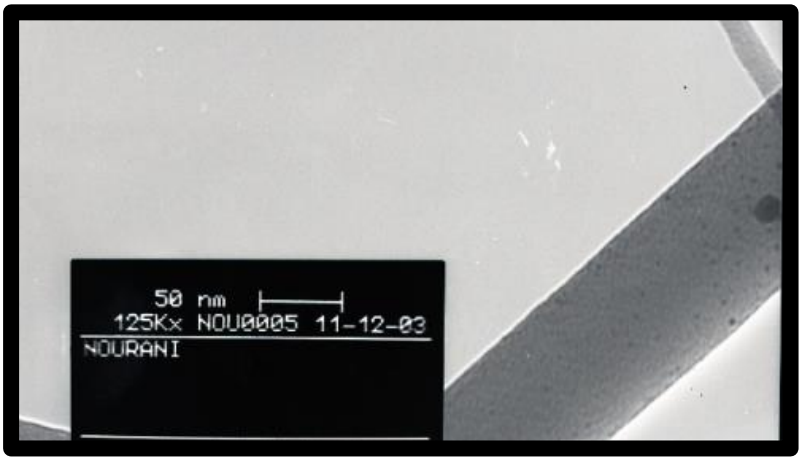

Figure 4-TEM micrographs of nanofibers obtained from the electrospinning of the CS/PEO solution in 70% acetic acid at the ratio of 70/30 and in the presence of nanoparticles

The UV-Vis results demonstrated that when the concentration of CS was increased, there was a better absorbance of AgSD.The amount of AgSD was 0.684 mg in an8.4 mg nanofiber (the average weight of nanofibers) with a ratio of 90/10 while it was0.445 mg with a CS/PEO ratio of 70/30 and decreasing the amount of CS. Themost likely reason forthisdifferenceis that the solubility of the drug enhancesby theincreasingamount of CS.

The results also showed that nanofibers have more absorbance compared to polymer solutions that are very likely due to errors in weighing nanofibers.

3.2. Drug release studies

In this regard, 5 mg of the drug was dissolved in 500 ml PBS. The absorption of the samples was 240 nm against PBS as a blank using a UV-Vis double beam.

The relationship between absorbance and AgSD concentration for standard drug concentrations in PBS is as follows:

Y= 0.2267X where Y and X represent the absorbance at the wavelength of 240 nm and AgSD concentration in PBS, respectively.

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The average weight of the electrospun nanofiber webs is 8.4 mg. The drug concentrations in the nanofibers were calculated by the calibration curve by multiplying the calculated concentration from the calibration curve by the volume (500 ml). Table 4 provides the results of the calibration curve.

Table4- Results of the calibration curve

Ratio of CS/PEO	Absorbance of the solution at 240 nmOD	AgSD concentration from calibration curve (ppm)	Amount of AgSD in 8.4 mg nanofiber(mg)
90/10	0.31	1.36	0.68
80/20	0.26	1.15	0.57
70/30	0.20	0.89	0.44

The electrospun nanofiber webs were cut into approximately 5×5 cm^2 pieces. Thesamples were accurately weighed and then placed in 500 ml of phosphate buffer solution (pH=7.4) at 37°C. The nanofiber sample was taken out from the incubation buffer and put into another fresh buffer solution at predetermined time intervals. The amount of the released drug was determined spectrophotometrically. Moreover, the UV absorbance of AgSD in the buffer solution was determined at λ_{max} = 240 nm and converted to the AgSD concentration according to the calibration curve of AgSD in the same buffer. Cumulative drug release was calculated as a function of time and the percentage of drug release was determined based on the initial weight of the loaded drug in the electrospinning mat. The drug release test results are presentedin Table 5and the cumulative release profile of AgSD from electrospun nanofibers is displayed in Figure5

Table5- Drug release test results

Time interval	Absorbance of solution at 240 nm (OD)	AgSD concentration from calibration curve (ppm)	Amount of AgSD in 8.4 mg nanofiber (mg)
30 min	0.003	0.01	0.006
1 hour	0.02	0.12	0.06
2 hours	0.04	0.20	0.10
4 hours	0.06	0.30	0.15
8 hours	0.09	0.42	0.21
12 hours	0.10	0.48	0.24
16 hours	0.12	0.54	0.27
20 hours	0.11	0.52	0.26
24 hours	0.12	0.56	0.28
32 hours	0.13	0.60	0.30
40 hours	0.15	0.68	0.34

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48 hours	0.15	0.66	0.33
56 hours	0.15	0.67	0.33
64 hours	0.15	0.66	0.33
72 hours	0.14	0.64	0.32

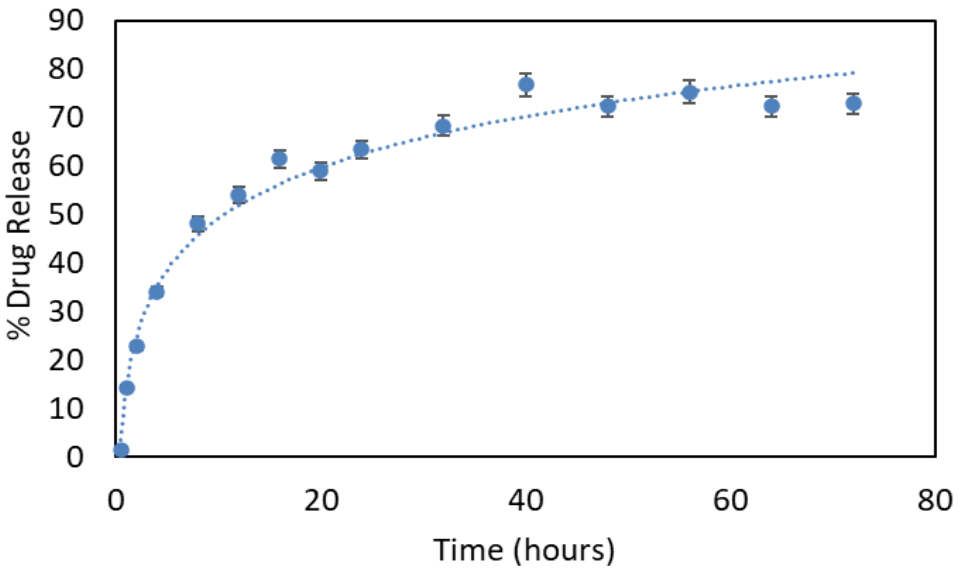

Figure 5-The cumulative release profile of AgSD from electrospun nanofibers at the ratio of 70% CS to 30% PEO in PBS (pH=7.4)

CS/PEO nanofibers with the ratio of 70/30 had the release of 34.15% in the first four hours. This amount reached 48.08% in the second four hours and kept its release at the same rate on the first day. It was 54.04 and 61.64% in the third and fourth four hours, respectively, and reached 63.37% at the end of the first day, indicating that more than half of the drug has released on the first day. After 32 hours, the drug release was 68.31% and reached its most AgSD release at 76.74% after 40 hours and then had a fixed rate. Accordingly,the drug release rate decreases with time. In all tested nanofiber mats, a burst release of the drug was observed after four hours of incubation in PBS.

It is noteworthy that adding drugs to polymer solutions leads to changes in the diameter and morphology of electrospun nanofibers due to variations in viscosity and solution conductivity. A reduction in viscosity whilean increase in solution conductivityled to the preparation of nanofibers with less diameter [11, 14]. In this study, adding the drug to the CS/PEO solution increased nanofiber diameter. Considering that the drug is water-insoluble, the addition of the drug to the polymer solution can reduce solution conductivity, leading to an increase in nanofiber diameter.

Barat et al. studied the release of metronidazole from CS films at a pH of 6.6 and atemperature of 37°C, followed by investigating the influence of crosslinking and drug loading on in vitro metronidazole release. All films showed a burst release at the end of 24 hours regardless of the drug-to-polymer ratio and crosslinking. They found that the first 24-hour burst release of the drug is due to the trapped drug at the surface level.The next release of the drug was slow and took over six days.Based on their report, crosslinking the films inhibited the burst release of metronidazole by 30%,and the minimum burst drug release was related to the crosslinking sample which was 68% [44].

In another study,Kumari et al.evaluated the in-vitro release of metformin hydrochloride from glutaraldehyde crosslinking CS/methylcellulose films in the phosphate buffer with a pH of 7.4 and the temperature of 37°C. Based on their results, the rate of drug release increased by a reduction in the crosslinking degree. Accordingly, they considered this issue as a result of a reduction in the pore size of the polymer network due to crosslinking.Finally, the percentage of the drug release for CS/MC was 14.5 after 24 hours [12].

In this study, the CS/PEO nanofibers containing AgSD represented the burst AgSD release at its first drug release. Drug release from nanofiber mats is strongly under the influence of the polymer structure. The first burst

drug release of AgSD from CS/PEO nanofiber mats is due to poor physical interaction between hydrophilic CS, PEO, and water-insoluble drugs and the position of the drug on the surface of nanofibers.

4. Conclusions

In the present study, CS-based nanofibers were first fabricated by the electrospinning of a 70% aqueous acetic acid solution with different ratios (90/10, 80/20, and 70/30) of CS/PEO containing AgSD. AgSD release was studied by placing the electrospun nanofibers in dissolution test baskets containing PBS (pH=7.4). The structure of these webs, type of the applied polymer, and the controlled release of AgSD indicated that the obtained nanofibers containingAgSD can be a proper potential candidate for burn dressing with the antibacterial capability to prevent injured skin from infections due to biodegradability, biocompatibility, antibacterial, and excellent biological properties of CS.

Acknowledgments

This work was supported by Islamic Azad University - Science and Research Branch, Tehran, Iran, and Islamic Azad University- Pharmaceutical Science branch, Tehran, Iran.

References

1. Zachariah, J. R., Rao, A. L., Prabha, R., Gupta, A. K., Paul, M. K., & Lamba, S. (2012). Post burn pruritus—A review of current treatment options. *Burns*, *38*(5), 621-629.
2. Al-Qattan, M. M., & Al-Zahrani, K. (2009). A review of burns related to traditions, social habits, religious activities, festivals and traditional medical practices. *Burns*, *35*(4), 476-481.
3. Brusselaers, N., Pirayesh, A., Hoeksema, H., Verbelen, J., Blot, S., & Monstrey, S. (2010). Burn scar assessment: a systematic review of different scar scales. *Journal of Surgical Research*, *164*(1), e115-e123.
4. Lansdown, A. B. (2002). Silver. I: Its antibacterial properties and mechanism of action. *Journal of wound care*, *11*(4), 125-130.
5. Atiyeh, B. S., Costagliola, M., Hayek, S. N., & Dibo, S. A. (2007). Effect of silver on burn wound infection control and healing: review of the literature.*burns*, *33*(2), 139-148.
6. Fox, C. L., & Modak, S. M. (1974). Mechanism of silver sulfadiazine action on burn wound infections. *Antimicrobial agents and chemotherapy*, *5*(6), 582-588.
7. Lu, S., Gao, W., & Gu, H. Y. (2008). Construction, application and biosafety of silver nanocrystalline chitosan wound dressing. *Burns*, *34*(5), 623-628.
8. Luong-Van, E., Grøndahl, L., Chua, K. N., Leong, K. W., Nurcombe, V., & Cool, S. M. (2006). Controlled release of heparin from poly (ε-caprolactone) electrospun fibers. *Biomaterials*, *27*(9), 2042-2050.
9. Jayakumar, R., Prabaharan, M., Reis, R. L., & Mano, J. (2005). Graft copolymerized chitosan—present status and applications. *Carbohydrate Polymers*, *62*(2), 142-158.
10. Kenawy, E. R., Bowlin, G. L., Mansfield, K., Layman, J., Simpson, D. G., Sanders, E. H., & Wnek, G. E. (2002). Release of tetracycline hydrochloride from electrospun poly (ethylene-co-vinylacetate), poly (lactic acid), and a blend.*Journal of controlled release*, *81*(1), 57-64.
11. Kim, K., Luu, Y. K., Chang, C., Fang, D., Hsiao, B. S., Chu, B., & Hadjiargyrou, M. (2004). Incorporation and controlled release of a hydrophilic antibiotic using poly (lactide-co-glycolide)-based electrospun nanofibrous scaffolds. *Journal of Controlled Release*, *98*(1), 47-56.
12. Kumari, K., Sharma, C., & Kundu, P. P. (2009). In-vitro release of metformin hydrochloride from films of chitosan-methylcellulose blends. *Asian journal of chemistry*.
13. Natu, M. V., Gil, M. H., & de Sousa, H. C. (2008). Supercritical solvent impregnation of poly (ε-caprolactone)/poly (oxyethylene-b-oxypropylene-b-oxyethylene) and poly (ε-caprolactone)/poly (ethylene-vinyl acetate) blends for controlled release applications. *The Journal of Supercritical Fluids*, *47*(1), 93-102.
14. Taepaiboon, P., Rungsardthong, U., & Supaphol, P. (2007). Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E. *European Journal of Pharmaceutics and Biopharmaceutics*, *67*(2), 387-397.
15. Huang, Z. M., He, C. L., Yang, A., Zhang, Y., Han, X. J., Yin, J., & Wu, Q. (2006). Encapsulating drugs in biodegradable ultrafine fibers through co-axial electrospinning. *Journal of Biomedical Materials Research Part A*, *77*(1), 169-179.
16. Gunn, J., & Zhang, M. (2010). Polyblend nanofibers for biomedical applications: perspectives and challenges. *Trends in biotechnology*, *28*(4), 189-197.
17. Subbiah, T., Bhat, G. S., Tock, R. W., Parameswaran, S., & Ramkumar, S. S. (2005). Electrospinning of nanofibers. *Journal of Applied Polymer Science*,*96*(2), 557-569.
18. Bognitzki, M., Czado, W., Frese, T., Schaper, A., Hellwig, M., Steinhart, M., ... & Wendorff, J. H. (2001). Nanostructured fibers via electrospinning. *Advanced Materials*, (13), 70.

19. Dersch, R., Liu, T., Schaper, A. K., Greiner, A., & Wendorff, J. H. (2003). Electrospun nanofibers: Internal structure and intrinsic orientation. *Journal of Polymer Science Part A: Polymer Chemistry*, *41*(4), 545-553.
20. Ohkawa, K., Cha, D., Kim, H., Nishida, A., & Yamamoto, H. (2004). Electrospinning of chitosan. *Macromolecular Rapid Communications*, *25*(18), 1600-1605.
21. Seeram, R., Kazutoshi, F., LT-C, T. W. E., & Zuwei, M. (2005). An introduction to electrospinning and nanofibers.
22. Huang, Z. M., Zhang, Y. Z., Kotaki, M., & Ramakrishna, S. (2003). A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites science and technology*, *63*(15), 2223-2253.
23. Boudriot, U., Dersch, R., Greiner, A., & Wendorff, J. H. (2006). Tissue Engineering, Drug Delivery, Wound Healing via Polymer Nano Fibers and Nanotubes. *Artificial Organs*, *30*, 785-792.
24. Dersch, R., Steinhart, M., Boudriot, U., Greiner, A., & Wendorff, J. H. (2004). Nanoprocessing of polymers: applications in medicine, sensors, catalysis, photonics. *Polymers for Advanced Technologies*, *16*(2-3), 276-282.
25. Ma, Z., Kotaki, M., Inai, R., & Ramakrishna, S. (2005). Potential of nanofiber matrix as tissue-engineering scaffolds. *Tissue engineering*, *11*(1-2), 101-109.
26. Şenel, S., & McClure, S. J. (2004). Potential applications of chitosan in veterinary medicine. *Advanced drug delivery reviews*, *56*(10), 1467-1480.
27. Schiffman, J. D., & Schauer, C. L. (2008). A review: electrospinning of biopolymer nanofibers and their applications. *Polymer Reviews*, *48*(2), 317-352.
28. Sill, T. J., & von Recum, H. A. (2008). Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials*, *29*(13), 1989-2006.
29. Zhang, Y., Lim, C. T., Ramakrishna, S., & Huang, Z. M. (2005). Recent development of polymer nanofibers for biomedical and biotechnological applications. *Journal of Materials Science: Materials in Medicine*, *16*(10), 933-946.
30. Di Martino, A., Sittinger, M., & Risbud, M. V. (2005). Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials*, *26*(30), 5983-5990.
31. Hudson, S. M., & Jenkins, D. W. (2001). Chitin and chitosan, encyclopedia of polymer science and technology.
32. Jayakumar, R., Prabaharan, M., Nair, S. V., & Tamura, H. (2010). Novel chitin and chitosan nanofibers in biomedical applications. *Biotechnology advances*,*28*(1), 142-150.
33. Khor, E., & Lim, L. Y. (2003). Implantable applications of chitin and chitosan.*Biomaterials*, *24*(13), 2339-2349.
34. Li, Q., Dunn, E. T., Grandmaison, E. W., & Goosen, M. F. A. (1992). Applications and properties of chitosan. *Journal of Bioactive and Compatible Polymers*, *7*(4), 370-397.
35. Son, B., Yeom, B. Y., Song, S. H., Lee, C. S., & Hwang, T. S. (2009). Antibacterial electrospun chitosan/poly (vinyl alcohol) nanofibers containing silver nitrate and titanium dioxide. *Journal of Applied Polymer Science*, *111*(6), 2892-2899.
36. Ueno, H., Mori, T., & Fujinaga, T. (2001). Topical formulations and wound healing applications of chitosan. *Advanced drug delivery reviews*, *52*(2), 105-115.
37. Zhou, Y., Yang, D., Chen, X., Xu, Q., Lu, F., & Nie, J. (2007). Electrospun water-soluble carboxyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration. *Biomacromolecules*, *9*(1), 349-354.
38. Vimala, K., Mohan, Y. M., Sivudu, K. S., Varaprasad, K., Ravindra, S., Reddy, N. N., ... & MohanaRaju, K. (2010). Fabrication of porous chitosan films impregnated with silver nanoparticles: a facile approach for superior antibacterial application. *Colloids and Surfaces B: Biointerfaces*, *76*(1), 248-258.
39. Yu, S. H., Mi, F. L., Wu, Y. B., Peng, C. K., Shyu, S. S., & Huang, R. N. (2005). Antibacterial activity of chitosan–alginate sponges incorporating silver sulfadiazine: Effect of ladder-loop transition of interpolyelectrolyte complex and ionic crosslinking on the antibiotic release. *Journal of applied polymer science*,*98*(2), 538-549.
40. Geng, X., Kwon, O. H., & Jang, J. (2005). Electrospinning of chitosan dissolved in concentrated acetic acid solution. *Biomaterials*, *26*(27), 5427-5432.
41. Homayoni, H., Ravandi, S. A. H., & Valizadeh, M. (2009). Electrospinning of chitosan nanofibers: Processing optimization. *Carbohydrate Polymers*, *77*(3), 656-661.
42. Li, L., & Hsieh, Y. L. (2006). Chitosan bicomponent nanofibers and nanoporous fibers. *Carbohydrate research*, *341*(3), 374-381.

ISSN- 2394-5125 VOL 08, ISSUE 04, 2021

43. Min, B. M., Lee, S. W., Lim, J. N., You, Y., Lee, T. S., Kang, P. H., & Park, W. H. (2004). Chitin and chitosan nanofibers: electrospinning of chitin and deacetylation of chitin nanofibers. *Polymer*, *45*(21), 7137-7142.
44. Barat, R., Srinatha, A., Pandit, J., Anupurba, S., & Mittal, N. (2007). Chitosan inserts for periodontitis: influence of drug loading, plasticizer and crosslinking on in vitro metronidazole release. *Acta Pharmaceutica*, *57*(4), 469-477.