

Preparation of the Potential Ocular Inserts by Electrospinning Method to Achieve the Prolong Release Profile of Triamcinolone Acetonide

Shahla Mirzaeei^{1,2*}, Kaveh Berenjian³, Rasol Khazaei³

¹ Pharmaceutical Sciences Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

² Nano Drug Delivery Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

³ Student Research Committee, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Article info

Article History:

Received: 24 September 2017

Revised: 12 February 2018

Accepted: 13 February 2018

ePublished: 18 March 2018

Keywords:

- Ocular
- Insert
- Electrospinning
- Nanofiber
- Chitosan
- Triamcinolone acetonide

Abstract

Purpose: The poor bioavailability of drugs in the ocular delivery systems is an important issue and development of delivery systems with prolonged release profile could be in a major importance. This study aims to develop an ocular delivery system using electrospun nanofibers to be a candidate insert for delivery of triamcinolone acetonide.

Methods: For this purpose, three different chitosan-based formulations were prepared by electrospinning method, and electrospun nanofibers were compared to a formulation comprising hydrophobic polymers (Eudragit S100 and Zein). The electrospun nanofibers were characterized by SEM and FTIR analyses. The release profile and release kinetic models of all the formulations were also examined.

Results: The SEM photographs of electrospun nanofibers revealed that among the four designed formulations, formulation obtained by electrospinning of chitosan and PVP possessed the best quality and the minimum size (120 ± 30 nm), which resulted the most uniform and bead-free nanofibers. This formulation also possessed the prolonged release profile of triamcinolone acetonide and was the only electrospun nanofiber following the zero-order kinetic profile. Due to the small diameter and uniformity of this formulation, the prolonged and well controlled release profile, it could be taken into account as a candidate to overcome the drawbacks of the commonly used ocular delivery systems and be used as ocular insert.

Conclusion: This study confirmed the ability of electrospun nanofibers to be used as ocular inserts for delivery of ophthalmic drugs.

Introduction

Topical formulations of corticosteroids, including drops and ointments, are the most prescribed formulations for treatment of different ocular disorders. Eye drops are the most frequently used ophthalmic drugs, due to the ease of instill and topical administration.¹ This type of administration of ophthalmic drugs affects the anterior segment of the eye and is associated by significant drawbacks; the poor bioavailability of drug (due to nasolacrimal drainage and dilution of drug by tear turnover), requirement of frequent administration, unpredictable and uncontrolled doses, blurred vision, messy and difficult dosing, and short residence time are some of these drawbacks.²⁻⁴

Several efforts have been conducted to attain an effective formulation, with a desired concentration of drug at the intended site of action, for a programmed period of treatment.⁵ The therapeutic efficacy of an ocular drug can be greatly improved by prolonging its contact with the corneal surface.⁶

Ocular inserts are the solid and semi-solid drug-impregnated formulations which could be placed into cul-

de-sac or conjunctiva sac.⁶ These inserts have been used frequently for reducing the frequency of administration, attaining the extended release duration, predictable and controlled release profile, and desired efficacy in treatment of eye infections such as conjunctivitis, keratitis, corneal ulcers.^{3,7-10}

The main objective of this study is to produce the electrospun formulations which could be used as ophthalmic inserts and to incorporate triamcinolone acetonide into these formulations. These formulations hoped to be able to increase the contact time between the drug and conjunctival tissue, decrease the number of administration, achieve a controlled and prolonged release suited for topical treatment of infection, and improve the therapeutic efficacy of drug at the site of action.

Electrospinning is prevailing as a remarkably simple, well-established method which provides a versatile technique for generating various nanofibers with controlled surface morphology from different sources (polymers, ceramics, and composite).¹¹ Electrospun fibers with different characteristics are promising scaffolds for

*Corresponding author: Shahla Mirzaeei, Tel: +98 833 4266780, Fax: +98 833 42764936, Email: shahlamirzaeei@gmail.com

©2018 The Authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

drug encapsulation and cell incorporation and also can offer the cells an environment for mimicking the native extracellular matrix (ECM). These nano-scale scaffolds have been studied for adhesion, proliferation, and differentiation of different types of cells.¹² Incorporation of bioactive agents into nanofibers can be occurred in one-step process with almost no loss of the agents. The high surface area to volume ratio of nanofibers, flexibility in surface functionalities,¹³ porous structure, and good mechanical strength make them promising candidates for many biomedical applications,¹⁴⁻¹⁶ such as controlling delivery of antibiotics and other antibacterial compounds.^{17,18} Natural polymers have been implicated as the opportunistic biomedical applications because of their biocompatibility, biodegradability and similarity to the ECM. The most frequently used natural polymers attracting much attention for the biomedical applications are the polysaccharides and proteins.¹⁹ Chitosan is a hydrophobic linear polysaccharide obtained from deacetylated chitin.²⁰ Chitosan was chosen as the main polymer in this study because it is a water insoluble mucoadhesive polymer which can provide the prolong release of drugs and improve the ocular bioavailability of triamcinolone acetonide.^{21,22} Chitosan can interact chemically with the negatively charged mucus layer or the eye tissues and enhance the residence time of drug.³ Based on the literature review, in spite of overwhelming popularity of electrospinning, no study has been devoted to the application of electrospinning in preparation of ocular inserts for delivery of ophthalmic therapeutics. Just besides of this study, one study investigated the application of electrospun PLA/PVA nanofibers as inserts in ocular delivery of dexamethasone.²³ To the best of our knowledge, this is the first attempt towards preparing ocular inserts by chitosan-based electrospun nanofibers, to be used as the ocular inserts capable of controlling and prolonging the delivery of triamcinolone acetonide. In this

study, electrospun chitosan-based nanofibers were fabricated to be used as ocular inserts. Furthermore, a formulation comprising two hydrophobic components (Eudragit/ Zein) was prepared and the results were compared to the chitosan-based nanofibers. Eudragit was chosen because it is suitable for loading the anionic drugs and also can attach to the negatively charged cells.²⁴

Materials and Methods

Chitosan and Zein were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Eudragit S100 was procured from Evonik Industries, Parsippany, NJ, USA. Poly vinyl alcohol (PVA 13-23 kDa, 98% hydrolysis), poly vinyl pyrrolidone (PVP), and ethanol were procured from Merck (Germany). Triamcinolone acetonide was purchased from Crystal Pharma (Spain). All the other compounds were in analytical grade and were performed without further purification.

Preparation of electrospinning solutions

In this study, four formulations were performed for producing triamcinolone acetonide-loaded nanofibers (Table 1). Chitosan-based electrospun nanofibers were obtained from the solution of chitosan in acetic acid. In order to prepare formulation T₂, chitosan was dissolved in 1% acetic acid to obtain the concentration of 6% (w/v) and stirred for 4h at 300 rpm and PVA (12% w/v) was added to the solution. Formulation T₃ comprised of chitosan, PVP, and PVA dissolving in water with concentrations of 6, 3, and 9%, respectively. The formulation T₃ was obtained by co-dissolving chitosan (6% w/v) in acetic acid and addition of PVP (12% w/v) under stirring conditions at 300 rpm. The electrospinning solution of formulation T₁ was prepared by dissolving Eudragit S100 in ethanol and Zein in isopropyl alcohol the solution was stirred for 5h. A drug solution with concentration of 1% w/v in acetic acid was added to each formulation.

Table 1. Different formulations prepared for electrospinning process, the arithmetic mean size and standard deviation of nanofibers.

Formulation	PVA%	PVP%	Zein%	Eudragit%	Chitosan%	Mean* size(nm)	Standard Deviation(nm)	Viscosity (cps)
T ₁	-	-	12.5	5	-	169	36	925
T ₂	-	12	-	-	6	120	30	1850
T ₃	9	3	-	-	6	260	73	1948
T ₄	12	-	-	-	6	172	48	1172

*Arithmetic mean size of nanofibers

Electrospinning

After preparation of electrospinning solutions, the solutions were placed in a 5 mL syringe connected with a metallic needle, with inner and outer diameters of 0.6 and 0.9 mm, at the nozzle. The solutions were pumped at the flow rate of 0.7 mL/h using a syringe pump (SP1000, fanavaran Nano-meghyas, Iran). The positive electrode of the Fanavaran Nano-meghyashigh-voltage power supply (D04X-05TH, Fanavaran Nano-meghyas, Iran) was clamped to the tip of the needle and the electrospun nanofibers were collected on a grounded rotating

cylindrical collector covered by aluminum foil. The needle tip to collector distance was fixed at 8 cm and the solutions were electrospun at a fixed voltage of 22 kV. The electrospinning of solutions were carried out at constant temperature of 40°C by controlling the temperature in a metallic box enclosing the electrospinning apparatus.

Characterization of electrospun nanofibers

The diameter and morphology of electrospun nanofibers were analyzed by Scanning Electron Microscope (SEM,

Hitachi, HT-4160-02, Japan). The nanofiber samples were placed on the aluminum stub and were gold coated prior to SEM imaging. The mean diameter of nanofibers was determined using ImageJ software. The diameter of at least 100 samples was measured and the arithmetic average diameter was obtained. The viscosity of electrospinning solutions was determined using BROOKFIELD ULTRA DV III Rheometer.

The structural change during electrospinning and the interaction of different chemical groups were observed by Fourier Transform Infrared (FTIR) spectroscopy using potassium bromide tabs. The range of spectra was in $400\text{--}4000\text{ cm}^{-1}$ with the accuracy of 4 cm^{-1} .

Release study

The *in-vitro* release profile of triamcinolone acetonide from different formulations was determined to obtain the best formulation which is close to the aim of this study. The specified pieces of drug loaded electrospun nanofibers were placed in a dialysis bag (Mw cut-off=12,000–14,000 Daltons; Delchimica Scientific Glassware, Milan, Italy), containing PBS (pH=7.4). The dialysis bag was sunk in PBS (pH=7.4) as release medium. The system was stirred continuously at 100 rpm and maintained at 37°C . At predetermined time intervals, a specified amount of release medium was replenished by fresh medium. The

concentration of triamcinolone acetonide in withdrawn samples at different times was determined using UV spectrophotometer at 238 nm. This study was carried out in triplicate and the release curve of triamcinolone acetonide from each electrospun nanofiber was prepared.

Results and Discussion

Size and morphology of electrospun nanofibers

The SEM photographs of the four electrospun nanofibers with different formulations are demonstrated in Figure 1 (a-d). The size and standard deviation of electrospun nanofibers were determined using ImageJ software, and the results were tabulated in Table 1. The SEM photographs indicated that formulations T₁, T₂, and T₄ resulted in bead-free electrospun nanofibers with a smooth surface and uniform diameters, but in the formulation T₃, some scattered droplets were formed on the nanofiber and its beaded structure made formulation T₃ unsuitable for medical applications. A glance at figure indicated that adding PVP to the structure of chitosan/PVA nanofibers caused bead formation and a decrease in the uniformity of nanofiber. The size of nanofibers was also compared and among the prepared formulations the best one with smallest diameter and standard deviation was formulation T₂ (chitosan/ PVP) with the mean diameter of $120\pm 30\text{ nm}$. The viscosity of electrospinning solutions is also indexed in Table 1.

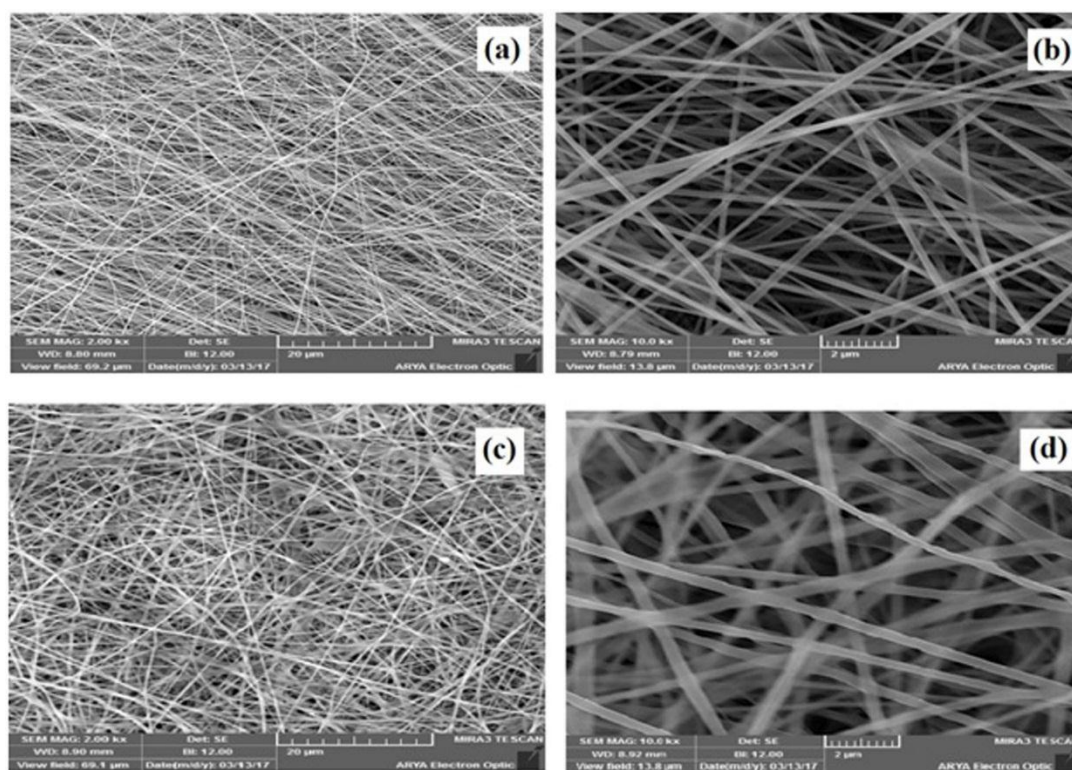


Figure 1. The SEM photographs of formulations (a) T₁, (b) T₂, (c) T₃, and (d) T₄.

FTIR results

In drug delivery systems, it could be an important issue to find out the possible interactions of drug and polymer. The FTIR analysis could help the identification of these interactions based on the alteration of the spectra of the

samples. In the present study, the FTIR spectra of pure polymers (Eudragit S100, Zein, chitosan, PVA, and PVA) and free drug were compared with the spectra of the four formulations. The FTIR spectrum of triamcinolone acetonide is demonstrated in Figure 2. FTIR spectrum of

triamcinolone acetonide included the hydroxyl group stretching vibration at 3392 cm^{-1} , the carbonyl group ($\text{C}=\text{O}$) band at 1708 cm^{-1} , $\text{C}=\text{C}$ stretching vibration band at 1662 cm^{-1} , and $\text{C}-\text{O}$ stretching vibration at 1207 cm^{-1} . In the spectrum of Eudragit S100, the characteristic peak at 1724 cm^{-1} could be attributed to the esterified carbonyl group. Two peaks were observed at $1150, 1245\text{ cm}^{-1}$ corresponding to the ester vibration. The broad peak from 3224 to 3452 cm^{-1} could be assigned to the absorption band of the hydroxyl group. The spectrum of Zein revealed characteristic peaks of amide I and amide II at 1651 and 1523 cm^{-1} , which were respectively due to $\text{C}=\text{O}$ stretching vibrations, $\text{N}-\text{H}$ bending, and $\text{C}-\text{N}$ stretching vibrations. The FTIR spectra of formulation T_1 clarified that no significant interaction could be observed between the components of this formulation and drug because of the presence of major bands of components and drug, as well as the absence of new characteristic peaks. The typical infrared bands corresponding to the OH group of chitosan at 3410 cm^{-1} , the NH_2 group of chitosan at 1641 cm^{-1} , and stretching band of the $\text{C}-\text{O}$ functional group at

1365 cm^{-1} could be observed in the spectra of formulations $\text{T}_2, \text{T}_3,$ and T_4 , while there were no significant shifting and alteration in the position of these peaks. The characteristic peaks of triamcinolone acetonide were identified in the spectra of these three formulations, at the same wavelengths ($3292, 1708, 1600-1690,$ and 1234 cm^{-1}), compare to those of pure drug.

In the spectra of the formulation T_2 and T_3 , the characteristic peaks of PVP (1716 cm^{-1} for $\text{C}=\text{O}$ vibration, 1238 cm^{-1} corresponding to $\text{C}-\text{N}$ vibration, and $3066-3522\text{ cm}^{-1}$ corresponding to OH functional group) and those corresponding to PVA (at 3296 cm^{-1} attributing to the OH vibration, the range of $2850-3000$ representing the alkyl vibration) were observed without any alteration in the wavelength of the peaks.

The characteristic peaks of these three formulations seem to be the superposition of their components (polymers and drug) and the FTIR results of these formulations exhibited that drug has been physically dispersed into the polymeric chain, without the presence of detectable chemical interactions.

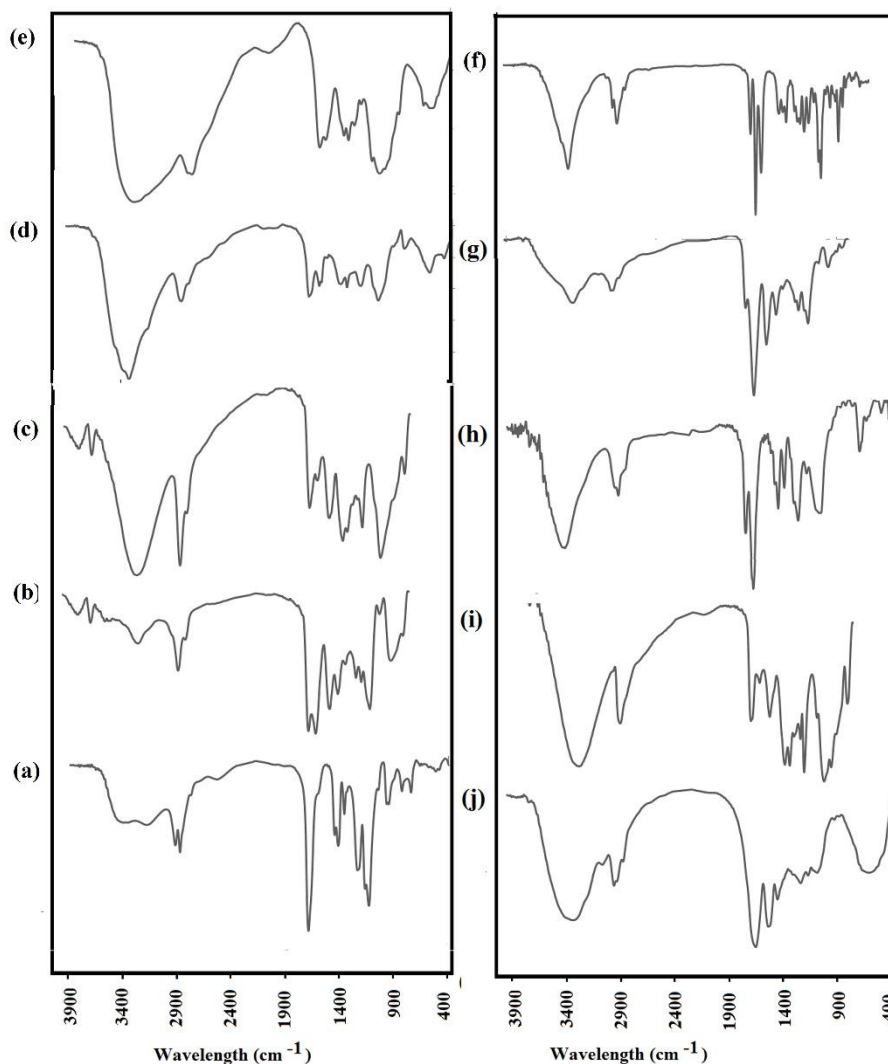


Figure 2. The FTIR spectra of pure Eudragit S100 (a), Zein (b), PVP (c), PVA (d), chitosan (e), triamcinolone acetonide (f), and electrospun nanofibers T_1 (g), T_2 (h), T_3 (i) and T_4 (k).

Release study and release kinetic

In order to study the release pattern of triamcinolone acetonide from different electrospun nanofibers, the concentration of drug in release medium at specified time intervals were determined using a UV-Vis spectrophotometer. The absorbance band of solutions was recorded and the concentration of drug was calculated using the standard calibration curve, which correlated the concentration of triamcinolone acetonide to the absorbance intensity. The calibration curve followed the below equation:

$$y=0.035x+0.0038 \quad R^2 = 0.9998$$

Where y is the absorbance intensity at 238 nm and x is the concentration of triamcinolone acetonide in release medium.

The release pattern of different electrospun nanofibers is represented in Figure 3.

A glance at figure indicated that triamcinolone acetonide released from these electrospun nanofibers T₁, T₂, and T₃ without inducing burst release, but the formulation T₄ released up to 30% of the loaded drug within the first hour. The absence of the burst release in the release profile of these nanofibers showed the potential of these formulations to control the release and delivery of triamcinolone acetonide. These formulations could be advantageous over the other forms of chitosan-based inserts because the release profile of drug contained burst release and those inserts were not able in controlling the release rate and delivery of drugs. According to the figure, the release profile of these formulated electrospun nanofibers was prolonged and these insert candidates released the drug for up to 4 days, while the release profile of free triamcinolone acetonide has been reported more than 70% within 8h.²⁴ The slowest release rate was achieved for formulation T₁, in which the EudragitS100 and Zein were performed in the electrospinning solution, following the formulation T₂ (chitosan/PVP nanofiber). In order to compare the release rate of these formulations the time corresponding to 80% release of triamcinolone acetonide ($t_{80\%}$) was recorded. The $t_{80\%}$ was found up to 23, 25, and 44.8 h for formulations T₄, T₃, and T₂ respectively, while formulation T₁ did not release 80% of the loaded drug within the study period. The completed release of drug (about 99% release) was achieved for electrospun nanofiber T₂ within 4 days, while the nanofiber T₁ released about 77% of the loaded drug within this period. The slow release pattern of formulation T₁ could be due to the hydrophobic nature of the polymers (Eudragit and Zein) and lower the dissolution rate of these polymers in release medium. The release profile of triamcinolone acetonide from Eudragit RS100 nanofibers with 20% polymer concentration showed a burst release within the first hour following the plateau release and 65% of drug released the nanofiber within 8h,²⁴ while in the present study the release of 65% of loaded triamcinolone acetonide from Eudragit/Zein nanofibers took more than 70 h.

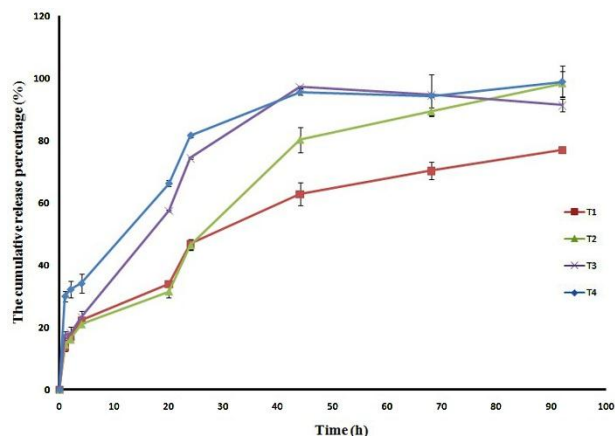


Figure 3. The release profile of triamcinolone acetonide from different electrospun nanofibers.

In order to perform a further comparison the triamcinolone acetonide release from different electrospun nanofibers, the kinetic study was conducted to find the best model correlating the release percentage to the time. The release data of each formulation were fitted to three commonly used empirical models (Higuchi, Zero-order, and First-order) and the amount of R^2 corresponding to each model was compared (Table 2). The kinetic study suggested that the Higuchi was the best fitting model for formulations T₁, T₃, and T₄, while formulation T₂ fitted to the Zero-order kinetic model. Zero-order kinetic with constant release rate and independent drug concentration was known as the ultimate goal of controlled-release delivery systems. The zero-order release profile of formulation T₂, the small size, and size distribution of nanofibers suggested that this formulation is the closest one to the aim of this study.

Table 2. The R-squared of the fitted kinetic models for different formulations.

Formulation	Higuchi	Zero-order	First-order
T ₁	0.9443	0.8546	0.8861
T ₂	0.9074	0.9461	0.9060
T ₃	0.9863	0.9514	0.9337
T ₄	0.9550	0.8725	0.9313

The well-controlled and monotonic release profile of this formulation could be attributed to the monodispersity of the size of nanofibers. The nanofibers with different sizes released the drug with different rates, but the monodisperse nanofibers with homogenous sizes released the drug more predictably. This formulation was selected as the best electrospun nanofiber to be used as an ophthalmic insert. The release study also revealed the promising ability of Eudragit and Zein to prolong the release time of triamcinolone acetonide and achieve the sustained release, but based on the hydrophobic nature of the polymers, the swelling ability of this formulation in the cul-de-sac must be further investigated. It should be noted that although the release rate of the drug from

hydrophobic formulation T₁ was slower than formulation T₂, the formulation T₂ could be advantageous over T₁. Based on the water solubility (and consequently the high swelling ability) of polymers and muco-adhesive nature of chitosan, the formulation T₂ could be swelled in neutral pH of the lacrimal fluid and the solid inserts could be converted to a gel phase and could interact with mucus within muco-adhesion process.³ Therefore, the controlled drug lixiviation for a prolonged period could be achieved. While the hydrophobic nature of formulation T₁ could possibly cause the degradation and erosion of the insert and dispersion into cul-de-sac, which might cause the problems such as blurred vision within the treatment period. Therefore the formulation T₂ could be suggested for further investigations as a desired candidate for alternating the typical ocular delivery systems with ophthalmic inserts. This formulation also could be advantageous over the other ocular inserts suggested in the previous study, according to the monotonic, well-controlled, and well-prolonged release profile of triamcinolone acetonide.

The previous studies showed the lack of control over the release profile of ocular therapeutics from chitosan-based inserts (prepared by different methods), attributing the accumulation of drug on the surface of inserts and existence of burst release in their profile.²⁵⁻²⁷ Jahangiri *et al.*²⁸ used the electrospray method to prepare the triamcinolone acetonide-loaded PLGA nanobeads and nanofibers as the alternative delivery system for ophthalmic drops. In spite of the advantages of the prepared formulations as the ophthalmic inserts, the release profile included the initial burst release following the plateau release. Beside, the release period was much shorter than that of the electrospun formulations in this study. Prajapati *et al.* used the PVA/chitosan films as the ocular insert for prolonging the delivery of ofloxacin. The release of ofloxacin from the films was completed within the first 6h.²⁹

The facility of the electrospun nanofibers administration on the ocular surface could be another important advantage of the suggested formulation. Furthermore, the other advantage of these inserts over the conventional ophthalmic delivery systems such as drops was that the multiple-dose typical ophthalmic delivery systems (such as drops) required and contained preservatives (such as benzalkonium chloride) in the formulation for protecting the therapeutics against the contamination by microorganisms.³ The presence of preservatives could mediate inflammation and corneal and conjunctival cell death,²⁹ while these solid-state inserts did not require the addition of any preservative and could overcome the side effects corresponding to the presence of preservatives.

Conclusion

For the first time in the present study, hydrophilic chitosan-based ocular inserts were developed for delivery of triamcinolone acetonide by electrospinning method and the results were compared with a hydrophobic (Eudragit/Zein) electrospun insert candidate. The prepared

ophthalmic delivery system could achieve prolonged therapeutic drug concentrations and could be promising candidates as ocular inserts and it could be used in a single dose at longer time intervals. The Zero-order release profile indicated the prolonged, monotonic, and well-controlled release of drug from the suggested formulation, and consequently, limited systemic exposure and side effects and the possibility to improve the patient adherence to therapy could be taken into account as some of the advantages of the suggested formulation. The suggested formulation could be converted from solid to gel form after exposition into the ocular pH, and the muco-adhesive nature of the suggested formulation caused the longer period for control of lixiviation than other similar formulations. Based on the advantages of the suggested formulation, it could be performed for ocular delivery of other similar drugs.

Acknowledgments

The authors would like to acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant number: 95416) for financial support of this work. Also faithfully thanks to Mr Komail Sadrjavadi and Miss Marziyeh Hajialyani for their assistances and valuable ideas.

Ethical Issues

Not applicable.

Conflict of Interests

The authors declared no conflict of interest for this study.

References

1. Kumar BP, Harish G, Bhowmik D. Ocular inserts: A novel controlled drug delivery system. *Pharm Innov* 2013;1(12).
2. Tanwar Y, Patel D, Sisodia S. In vitro and in vivo evaluation of ocular inserts of ofloxacin. *DARU* 2007;15(3):139-45.
3. De Souza JF, Maia KN, Patrício PSDO, Fernandes-Cunha GM, Da Silva MG, Jensen CEDM, *et al.* Ocular inserts based on chitosan and brimonidine tartrate: Development, characterization and biocompatibility. *J Drug Deliv Sci Tec* 2016;32:21-30. doi: 10.1016/j.jddst.2016.01.008
4. Vaishya RD, Khurana V, Patel S, Mitra AK. Controlled ocular drug delivery with nanomicelles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2014;6(5):422-37. doi: 10.1002/wnan.1272
5. Ananthula HK, Vaishya RD, Barot M, Mitra AK. Duane's Ophthalmology. In: Tasman W, Jaeger EA, editors. *Bioavailability*. Philadelphia: Lippincott Williams & Wilkins; 2009.
6. Madhuri B, Gawali Vikas B, Mahesh B. Ocular inserts: A rate controlled drug delivery system—a review. *Int J Pharmaceut* 2012; 2(1): 49-63.
7. Di Colo G, Zambito Y, Burgalassi S, Serafini A, Saettone MF. Effect of chitosan on in vitro release and ocular delivery of ofloxacin from erodible inserts

- based on poly(ethylene oxide). *Int J Pharm* 2002;248(1-2):115-22.
8. Sreenivas S, Hiremath S, Godbole A. Ofloxacin ocular inserts: Design, formulation and evaluation. *Iran J Pharmacol Therapeut (IJPT)* 2006;5(2):159-62.
 9. Reddy ES. Design and characterization of ofloxacin and dexamethasone ocular inserts using combination of hydrophobic and hydrophilic polymers. *Asian J Pharm* 2017;11(01). doi: 10.22377/ajp.v11i01.1089
 10. Reshu S, Laxmi G, Preeti K. Formulation and evaluation of ocular inserts of acyclovir. *Int J Drug Res Tech* 2013;3(4):88-95.
 11. Manea LR, Hristian L, Leon AL, Popa A. Recent advances of basic materials to obtain electrospun polymeric nanofibers for medical applications. *IOP Conf Ser: Mat Sci Eng* 2016;45(3):032006. doi:10.1088/1757-899X/145/3/032006
 12. Da Silva GR, Lima TH, Orefice RL, Fernandes-Cunha GM, Silva-Cunha A, Zhao M, et al. In vitro and in vivo ocular biocompatibility of electrospun poly(varepsilon-caprolactone) nanofibers. *Eur J Pharm Sci* 2015;73:9-19. doi: 10.1016/j.ejps.2015.03.003
 13. Kataria K, Sharma A, Garg T, K Goyal A, Rath G. Novel technology to improve drug loading in polymeric nanofibers. *Drug Deliv Lett* 2014;4(1):79-86.
 14. Villarreal-Gomez LJ, Cornejo-Bravo JM, Vera-Graziano R, Grande D. Electrospinning as a powerful technique for biomedical applications: A critically selected survey. *J Biomater Sci Polym Ed* 2016;27(2):157-76. doi: 10.1080/09205063.2015.1116885
 15. Eatemadi A, Daraee H, Zarghami N, Melat Yar H, Akbarzadeh A. Nanofiber: Synthesis and biomedical applications. *Artif Cells Nanomed Biotechnol* 2016;44(1):111-21. doi: 10.3109/21691401.2014.922568
 16. Duque Sanchez L, Brack N, Postma A, Pigram PJ, Meagher L. Surface modification of electrospun fibres for biomedical applications: A focus on radical polymerization methods. *Biomaterials* 2016;106:24-45. doi: 10.1016/j.biomaterials.2016.08.011
 17. Chen J, Liu Z, Chen M, Zhang H, Li X. Electrospun gelatin fibers with a multiple release of antibiotics accelerate dermal regeneration in infected deep burns. *Macromol Biosci* 2016;16(9):1368-80. doi: 10.1002/mabi.201600108
 18. Nagarajan S, Soussan L, Bechelany M, Teyssier C, Cavaillès V, Pochat-Bohatier C, et al. Novel biocompatible electrospun gelatin fiber mats with antibiotic drug delivery properties. *J Mater Chem B* 2016;4(6):1134-41. doi: 10.1039/C5TB01897H
 19. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliv Rev* 2007;59(4-5):207-33. doi: 10.1016/j.addr.2007.03.012
 20. Alves NM, Mano JF. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *Int J Biol Macromol* 2008;43(5):401-14. doi: 10.1016/j.ijbiomac.2008.09.007
 21. Scherliess R, Buske S, Young K, Weber B, Rades T, Hook S. In vivo evaluation of chitosan as an adjuvant in subcutaneous vaccine formulations. *Vaccine* 2013;31(42):4812-9. doi: 10.1016/j.vaccine.2013.07.081
 22. Laurienzo P. Marine polysaccharides in pharmaceutical applications: An overview. *Mar Drugs* 2010;8(9):2435-65. doi: 10.3390/md8092435
 23. Bhattarai RS, Das A, Alzhrani RM, Kang D, Bhaduri SB, Boddu SHS. Comparison of electrospun and solvent cast polylactic acid (pla)/poly(vinyl alcohol) (pva) inserts as potential ocular drug delivery vehicles. *Mater Sci Eng C Mater Biol Appl* 2017;77:895-903. doi: 10.1016/j.msec.2017.03.305
 24. Payab S, Davaran S, Tanhaei A, Fayyazi B, Jahangiri A, Farzaneh A, et al. Triamcinolone acetone-eudragit(r) rs100 nanofibers and nanobeads: Morphological and physicochemical characterization. *Artif Cells Nanomed Biotechnol* 2016;44(1):362-9. doi: 10.3109/21691401.2014.953250
 25. Fulgencio Gde O, Viana FA, Ribeiro RR, Yoshida MI, Faraco AG, Cunha-Junior Ada S. New mucoadhesive chitosan film for ophthalmic drug delivery of timolol maleate: In vivo evaluation. *J Ocul Pharmacol Ther* 2012;28(4):350-8. doi: 10.1089/jop.2011.0174
 26. Foureaux G, Franca JR, Nogueira JC, Fulgencio Gde O, Ribeiro TG, Castilho RO, et al. Ocular inserts for sustained release of the angiotensin-converting enzyme 2 activator, diminazene aceturate, to treat glaucoma in rats. *PLoS One* 2015;10(7):e0133149. doi: 10.1371/journal.pone.0133149
 27. Verestiuc L, Nastasescu O, Barbu E, Sarvaiya I, Green KL, Tsibouklis J. Functionalized chitosan/nipam (hema) hybrid polymer networks as inserts for ocular drug delivery: Synthesis, in vitro assessment, and in vivo evaluation. *J Biomed Mater Res A* 2006;77(4):726-35. doi: 10.1002/jbm.a.30668
 28. Jahangiri A, Davaran S, Fayyazi B, Tanhaei A, Payab S, Adibkia K. Application of electrospinning as a one-step method for the fabrication of triamcinolone acetone-plga nanofibers and nanobeads. *Colloids Surf B Biointerfaces* 2014;123:219-24. doi: 10.1016/j.colsurfb.2014.09.019
 29. Ammar DA, Kahook MY. Effects of glaucoma medications and preservatives on cultured human trabecular meshwork and non-pigmented ciliary epithelial cell lines. *Br J Ophthalmol* 2011;95(10):1466-9. doi: 10.1136/bjophthalmol-2011-300012