Fibers and Polymers 2018, Vol.19, No.7, 1454-1462
DOI 10.1007/s12221-018-8087-1

ISSN 1229-9197 (print version)
ISSN 1875-0052 (electronic version)

Mucoadhesive Chitosan Electrospun Nanofibers Containing Tetracycline and Triamcinolone as a Drug Delivery System

Leila Behbood[1], Soroush Karimi[2], Esmaeil Mirzaei[3], Ghobad Mohammadi[1], Mahsa Azami[4], and Elham Arkan[2]*

[1]*Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah 67149-67346, Iran*
[2]*Nano Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah 67149-67346, Iran*
[3]*Department of Medical Nanotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz 71336-54361, Iran*
[4]*Department of Pharmaceutics, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah 67149-67346, Iran*
(Received February 24, 2018; Revised May 16, 2018; Accepted May 18, 2018)

Abstract: The mucoadhesive Chitosan (CS) nanofibers as a drug delivery system were developed. Chitosan was modified via the immobilization of thiol groups from L-cysteine as a mucoadhesive reagent. The mucoadhesive properties of the chitosan nanofibers were evaluated by tensiometer set and via tensile studies. Drug and mucoadhesive agent loading lead to decrease diameters and increased porous of nanofibers. The release of Tetracycline (Tet) and Triamcinolone (Tri) were increased with increasing immersion time and it became constant at long immersion times. Mucoadhesion studies were done at pH 2-7 and in pH 6 maximum mucoadhesive properties observed. Release studies demonstrated a sustained release of both drug continued up to 48 hours. Microbial studies were performed on the nanofibers. The drug delivery system represented a novel tool for improve the therapeutic efficacy of various drugs that are poorly absorbed from the gastrointestinal tract. Also it is an efficient system for treatment of oral ulceration.

Keywords: Chitosan, Electrospun nanofibers, Mucoadhesive, Drug delivery

Introduction

Bioadhesion can be defined to the phenomenon that natural and synthetic materials stick to biological surfaces for a long time. In the pharmaceutical sciences, bioadhesion could also refer to the use of bioadhesives to bond two surfaces together especially in drug delivery, dental and surgical applications. Therefore, the extensive attention in bioadhesion studies is due to its results for the development of new biomaterials [1,2].

Mucoadhesive drug delivery has a potential for optimize localized drug delivery [3] by retaining a dosage form at the site of action such as within the gastrointestinal tract and in systemic delivery by holding a formulation in intimate contact with the absorption site such as the nasal cavity [4,5].

Recently, advancement has occurred in this area because the need of delivered challenging T molecules such as biopharmaceuticals (proteins and oligonucleotides). There are many applications for mucoadhesive delivery system as therapeutic agents such as coating and protection of damaged tissues (gastric ulcers or lesions of the oral mucosa) or acting as lubricating agents (in the oral cavity, eye and vagina) [6].

Mucoadhesive delivery systems transferred drugs to target site and an long inhabit time at the site of drug absorption and an increase in drug concentration gradient on the mucosa, so they are preferred than non-mucoadhesive delivery systems in many cases [7].

Chitosan is biocompatible and biodegradable polysaccharide which has mucoadhesive properties. There are weak bounds between the positive charges of the polymer and negative charges of mucus glycoproteins. The mucoadhesive properties can be increased Manifold via immersing thiol groups on the polymer structure. Compounds containing thiol such as cysteine are able to modify the structures of Thiomers. Disulfide bond is formed between thiol groups present on the chitosan and thiols being available on the glycoprotein substructures of the mucus the mucoadhesive properties. Beside mucoadhesive properties [8] thiomers demonstrate enhanced enzyme inhibitory [9], permeation enhancing [10] and efflux pump inhibiting properties [11].

Several advantages have been enumerated for electrospun nanofibers in drug delivery such as the high loading capacity, high encapsulation efficiency, easiness of preparation and cost-effectiveness [12].

Electrospinning method is an efficient technique to produce polymeric nanofiber. The nanofibers were formed using an electrostatic field and gather on a collector in the form of a fiber layer.

The system and process parameters are affected on the fiber formation. The process parameters include the intensity of the electric field, the distance between the nozzle and the collector, temperature and humidity of the ambient air, the type of the nozzle and the flow speed of the cooling air during the melt spinning. The system parameters have a

*Corresponding author: elhamarkan@yahoo.com

fundamental role on the structural properties of the nanofiber, such as chemical structure and molar mass of the polymer, flow of polymer, thermodynamics of the polymer interactions, and concentration of the polymer in the solution, viscosity or the dielectric properties of the solution [8,9].

Triamcinolone acetonide is a synthetic corticosteroid with potent anti-inflammatory effects, TA has approved as an injectable suspension for the treatment of different inflammatory conditions.

Tetracycline is an antibiotic that exhibiting activity against a wide range of gram-positive and gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasmas, and rickettsiae, and protozoan parasites. Tetracycline widely was used in the treatment of human and animal infections because it has desirable antimicrobial properties and the absence of major adverse side effects [13].

In the present study, Mucoadhesive chitosan nanofibers contain tetracycline and Triamcinolone acetonide were prepared by the electrospinning method and its application for treatment of oral aphthous ulceration. Then, the effects of contact time, initial polymer concentration, mucoadhesive agent concentration, drug concentration and temperature have been investigated to obtain the optimum conditions. The optimal conditions producing uniform and smooth fibers were chosen to prepare nanofibers that contained tetracycline hydrochloride (TH) and triamcinolone in various amounts. The release characteristics of the drugs from the nanofiber mats were investigated using high performance liquid chromatography (HPLC).

Experimental

All reagents were of the highest purity available from Merck and Sigma companies and used without further purification. Chitosan (CS) (low molecular weight) was purchased from Eastar Group (Dong Chen) Co. (PubChem CID: 16213812), Iceland. Polyethylene oxide (PEO) (MW826.97KD, PubChem CID: 486583) was purchased from Across Organics Co. Glacial acetic acid (PubChem CID: 176) was purchased from Merck. Double distilled water was used in all experiment. Tetracycline (PubChem CID: 54704426), cysteine (PubChem CID: 5862), glutathione (GSH) (PubChem CID: 124886), thiobutyl amide (TBA) and 2-iminothiolane (Traut's reagent) and Triamcinolone (PubChem CID: 6216) purchased from Sigma. The dialysis-tubing cellulose membrane with 13000 cut off purchased from Sigma-Aldrich Co. (USA). Human Umbilical Vein Endothelial Cells (HUVEC) was obtained from the Pasteur Institute (Tehran, Iran). The HPLC grade water was treated with a Milli-Q Labo purification system in our laboratory.

Apparatus

The electrospinning process was carried out using Electroris (Fanavaran Nano-Meghyas, FNM, Ltd., Iran) as an electrospinner. The Fourier Transform Infrared (FTIR) analysis was used in the range of 400-4000 cm^{-1} to determine the type of functional groups on the prepared nanofibers. The polymer solution was placed into 5 m*l* plastic syringe with diameter of syringe 1.2 mm with a blunt-ended 18G needle. The prepared nanofibers were characterized by FTIR, Scanning Electron Microscope (SEM) and Brunauer-Emmert-Teller (BET) analysis (BET, ASAP 2020). The average diameter and diameter distribution of nanofibers were obtained with an image analyzer (Image-Proplus, Media Cybrernetics).

For evaluation of drug release, Shimadzu LC-10A series chromatographic system (Shimatzu Corporation, Kyoto, Japan) was used. The system consisted of a model SCL-10A controller unit, a model FCV-10AL mixer, a model DGU-14A degasser, a model LC-10AD piston pumps, a model CTO-10A column oven and a model SPD- M10A Ultra-Violet/Visual detector. Analysis was done using a Knauer (Berlin, Germany) CE184 (250 mm×4.6 mm), C18 column.

Preparation of Polymer Solution Containing Tetracycline Hydrochloride and Triamcinolone

CS and PEO solutions were prepared in acetic acid. To elucidate the effect of solution concentration on morphological appearance and size of the obtained fibers, in various ratios (i.e., 70:30, 75:25, 80:20, 85:15, 90:10 w/w) of both solutions were used to different mixtures. They were prepared in acetic acid. Afterward, Thiolated agents such as Cysteine, GSH, Traut's reagentand TBA in constant concentrations were used.

Cysteine solutions of various concentrations (1-4 % w/w) were added to polymer solution.

Also, Tet and Tri with various concentrations (Tet: 5-10 % w/w and Tri: 1-5 % w/w) were added to polymer solutions containing cysteine. In optimum condition, Tet and Tri were blended in CS/PEO/Cys solution with (CS/PEO/Tet/Tri/Cys (70:16:8:4:2 %w/w) percentage and slight stirring was used to speed up the dissolution. Prior to electrospinning, viscosity and conductivity of solutions were measured.

Mucoadhesive Study

The mucoadhesive properties of the nanofibers with above agents were evaluated via the rotating cylinder method and via tensile studies. Among these agents Cysteine was maximum mucoadhesive property than others.

Cysteine solutions of various concentrations (1-4 % w/w) were prepared in distilled water. These solutions were blended in CS/PEO solution (80:20 % w/w) Slight stirring was used to speed up the dissolution. Prior to electrospinning, viscosity and conductivity of solutions were measured using a Brookfield DV-III ULTRA and an Orion 160 conductivity meter, respectively.

Electrospinning Process

The prepared solution was loaded into a 5 m*l* plastic syringe equipped with a syringe needle. This was placed to a KD programmable syringe pump to control the solution flow rate. Then, a high voltage was applied between the needle and the collector. CS/PEO nanofibers were produced on the collector. A voltage of 18 kV, with a tip-collector distance of 12 cm, at a speed of 1 m*l*/h was applied to the solution and nanofibers were collected on the cylindrical collector.

Cross-linking of Nanofibers

Cross-linking of fiber mats of both CS/PEO and CS/PEO containing cysteine was carried out using 25 % GA. The fiber mats (10 cm×20 cm) on the aluminum foil were placed into a sealed chamber saturated with the vapor of 40 m*l* of GA solution. The nanofiber mats were exposed to GA vapor for given periods of time (20, 40, 60 and 120 min) and then heat-treated in an oven at 60 °C under vacuum for 24 h to enhance the cross-linking reaction and to remove the unreacted GA. Maximum stability and minimum swelling was observed at 40 minutes. The fibers were dried initially for 12 h at 60 °C in a vacuum chamber.

Swelling Studies of Electrospun Nanofibers

The swelling studies were carried out on the pure and GTA cross-linked CS /PEO nanofiber. The dry samples were immersed in 30 °C deionized water. The sample was weighted each 2 h. After 2 h this weight stayed less or more constant. These experiments were repeated at five times. The swelling percentage was calculated by equation (1):

$$\text{Swelling } \% = \left(\frac{W_w - W_d}{W_d}\right) \times 100 \quad (1)$$

where W_w and W_d was the masses of the swollen and dried nanofiber mats, respectively.

Characterization of Nanofibers

Morphological studies of the nanofibers evaluated by a JEOL JSM-5200 SEM. For SEM analyzing the immobilized fibers was employed with gold in a vacuum condition. Diameters of the electrospun nanofibers were measured directly from the SEM images.

The functional groups of PEO, CS and CS/PEO nanofibers were characterized by Fourier Transform Infrared.

The BJH theory and Brunauer-Emmet-Teller (BET) method was employed to determine the surface area and pore volume of nanofibers.

Mechanical testing of nanofibers was measured by mechanical testing machine STM50.

The DSC tests (Mettler Toledo, Switzerland) were carried out for pure powder for Tet, Tri, CS, PEO and electrospun nanofibers, thiolated nanofibers and drugs loaded thiolated nanofibers. The amount of sample was placed inside a Seal aluminum pan and then was loaded. Samples in the range of (0-300) °C were heated (10 °Cmin^{-1}) and the thermograms were recorded. Flow rate of nitrogen vapor was 50 m*l* per minute.

Drug Loading and Release

CS/PEO nanofibers containing 10 % Tet and 5 % Tri had an aggressive release during in early hours and this is due to surface-separated drugs that quickly dissolves. For this reason we decided to limit our experiments to 8 % Tet and 4 % Tri drug Loading.

The drug release study was evaluated by dialysis method. The CS/PEO nanofibers containing cysteine were selected for the *in vitro* degradation and drug release measurements.

The membranes were cut to square pieces of 1.0×1.0 cm^2, and their thickness around 80 μm and weight around 20 mg were measured. The CS/PEO nanofiber were placed in dialysis bag containing of PBS (pH 7.4). The dialysis bag containing nanofibers was immersed in the same buffer in 37 °C and 50 rpm stirring. At exact and specified period between 0 and 500 min, 1 m*l* of immersed media was taken out and replaced with phosphate buffer. The HPLC method was used for assay of drugs. Chromatographic conditions was mobile phase consisted of acetonitrile-ammonium phosphate 20 mm (20:80, v/v) which degassed using Elma ultrasonic (Germany). The flow rate of mobile phase was 1 m*l*/min. Sample injection volume was 10 μ*l*. Analysis was carried out at room temperature using UV detection performed at 254 nm. Initially the 20 μ*l* of actonitril and buffer injected on column respectively to regulation the baseline.

Cell Culture

Human Umbilical Vein Endothelial Cells (HUVEC) was obtained from the Pasteur Institute (Tehran, Iran) and maintained at 37 °C in a humidified atmosphere (90 %) containing 5 % CO2. Cells were cultured in Dulbecco's modified Eagle's medium - F12 with 10 % (v/v) heat-inactivated fetal bovine serum, 100 U m*l*$^{-1}$ penicillin and 100 mg/m*l* streptomycin.

To determine if nanofibers exhibit cytotoxic effects on HUVEC, cells (10^4 cells) were seeded in complete medium onto 12-well plates and incubated in a humidified incubator for 24 h. Then, nanofibers, which contain different concentrations of antibiotics (1-5 mg/m*l*), were completely punched and put onto 12-well plate. Cell viability was assayed at 24, 48, 72 and 96 h by MTT assay.

Results and Discussion

Viscosity of solutions has a valid effect on the morphology and size of nanofibers. The results of the viscosity of dissolved agents are reported in Table 1. The viscosity plays an important role in determining the fiber size and morphology during spinning of polymeric fibers. It has been

found that with very low viscosity, there is no continuous fiber formation and with very high viscosity there is difficulty in the ejection of jets from polymer solution, thus there is a requirement of optimal viscosity for electrospinning. Sukigara *et al.* have shown the significant effects of viscosity on silk nanofibers [14].

Table 1. Comparison of the apparent viscocity (g) of chitosan, chitosan-SH and chitosan-SH+Drug without PEO and in the presence of PEO and PEO+cysteine (1 % w/w)

Polymer	Apparent viscosity (mPa·s)		
	Without PEO	With PEO	With PEO±Cys
Chitosan	41.4±3.5	54.1±3.8	33.4±4.2
Chitosan+Drug	45.8±7.7	51.4±5.2	30.1±3.9

*The values presented are means±standard deviation of at least three experiments.

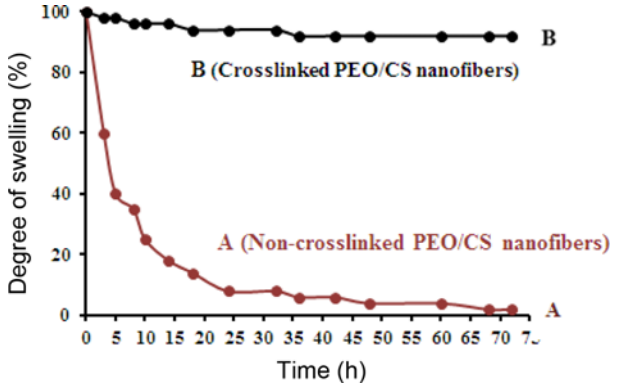


Figure 1. Swelling behavior of nanofibers, (A) unmodified Non-crosslinked CS/PEO nanofibers and (B) modified crosslinked CS/PEO nanofibers.

The solution viscosity has been strongly related to the concentration of the solution and the relationship between the polymer viscosity and/or concentration and fibers obtained from electrospinning has been studied in a number of systems [15,16]. An increase in solution viscosity or concentration gives rise to a larger and uniform fiber diameter. Taken together, these studies indicate that there exist polymer-specific, optimal viscosity values for electrospinning and this property has a remarkable influence on the morphology of fibers.

Figure 1 shows the swelling properties of nanofibers. As can be seen, degrees of swelling of CS/PEO nanofibers was decreased after cross-linking with cross linker. The decrease in the degree of swelling of nanofiber with cross linker could be due to the formation of aldimine linkages between the free amino groups of CS and the aldehyde groups of GTA. These linkages caused consecutive fiber segments with increased water diffusivity resistance. This condition decreased the swelling degree of GTA cross-linked nanofibers.

The results of morphological studies are shown in Figure 2. The SEM image and distribution diameter of PEO/CS electrospun nanofiber membrane at applied voltage of 24 kV, tip-collector distance of 10 cm and flow rate of 1 $ml \cdot h^{-1}$ were shown in Figure 2(A). The Figure 2(B) shows the homogeneous fibers with average diameter of 184± 21 nm. Figure 2(C) shows image of final nanofiber that

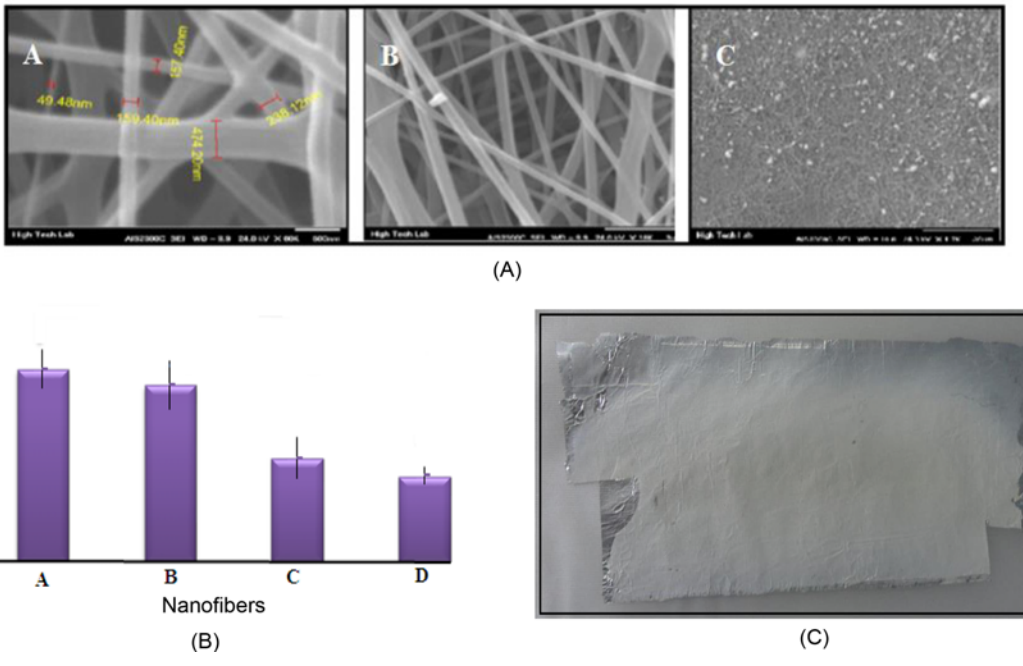


Figure 2. SEM images (scale bar (A=500 nm, B=1 μm and C=5 μm) of (A) electrospun nanofibers, (B) average diameter of electrospun CS/PEO nanofiber and (C) image of final nanofiber (C).

Table 2. Diameters of electrospun fibers, 80 % w/w chitosan by varying concentrations of cysteine ranging from 1 to 5 % w/w with an equal weight ratio

Investigated fibers	Diameters (nm)
CS/PEO 80:20 % w/w	372±41
CS/PEO/Cysteine 1 %	293±36
CS/PEO/Cysteine 2 %	267±32
CS/PEO/Cysteine 3 %	205±29
CS/PEO/Cysteine 4 %	184±21
Cysteine 5 %/PEO/CS	56 ±116
CS/PEO/Cysteine/Drugs	188±27

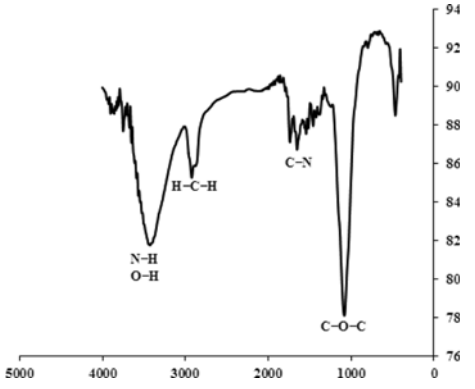


Figure 3. FT-IR spectra of CS/PEO.

optimized.

The Table 2 showed the fibers diameter with different cysteine concentration.

Figure 3 indicates the FTIR results for nanofibers. In CS/PEO nanofiber membrane, a broad bond at 3100-3600 cm^{-1} was assigned to N-H and O-H stretching of the polysacharide molecules. Two bonds at 1560 and 1650 cm^{-1} were attributed to the amide groups in the structure of CS/PEO nanofiber. The broad bond appeared at 2900 cm^{-1} was due to the CH_2 stretching groups. The C-H group peaks were observed at 1340 and 1370 cm^{-1}. The absorption bonds around 1100 cm^{-1} assigned to C-O-C stretching vibration.

Obtained average pore diameter, surface area and pore volume of nanofibers were 3.82 nm, 345.1 m^2g^{-1} and 0.423 cm^3g^{-1}, respectively. The value of pore diameter of fibers indicated that the surface of CS nanofiber was mesoporous and nanofibers have large surface areas. Measured mechanical properties are shown in Figure 4.

The elastic modulus of various nanofibers containing CS/PEO, drugs loaded nanofibers, drug loaded thiolated nanofibrs, were demonstrated in Figure 5.

According to the results, as the polymer concentration is increased, Followed by the elasticity (elastic module) of nanofibers increased. Meanwhile it can be reduced by adding drug to the polymer solution and after adding cysteine to the polymer elasticity is reduced. There is little

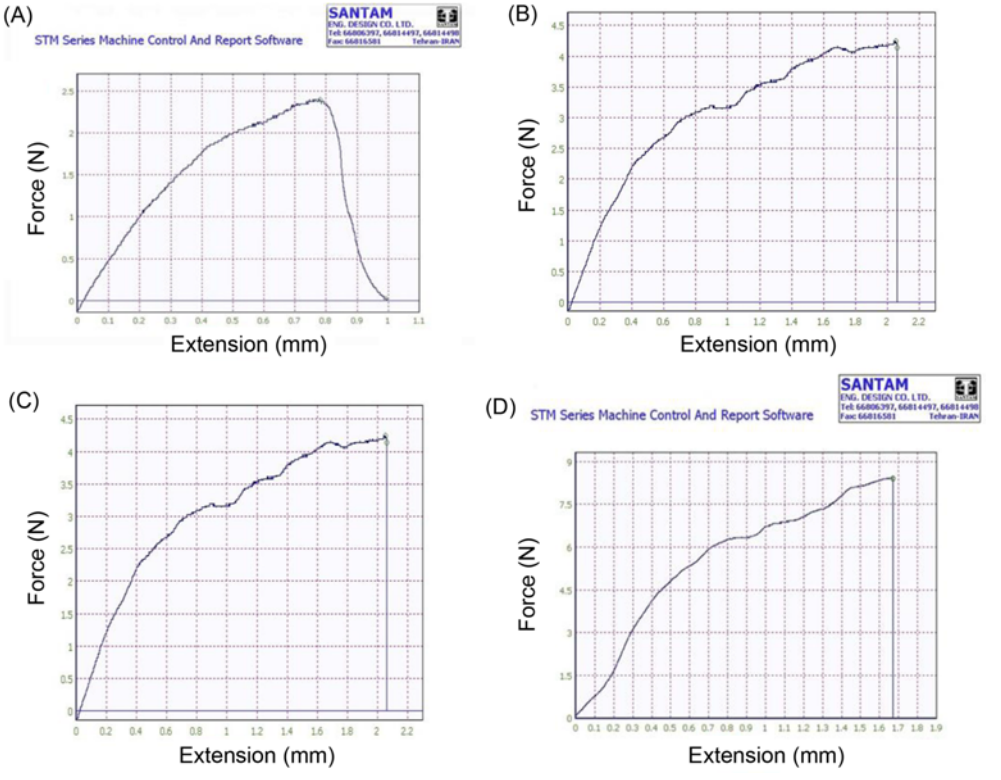


Figure 4. The mechanical measuring nanofiber; (A) CS, poly-ethylene oxide, (B) nanofiber containing drugs, (C) nano fiber with mechanical measurement cysteine and (D) thiol-containing CS nanofibers loaded with drugs.

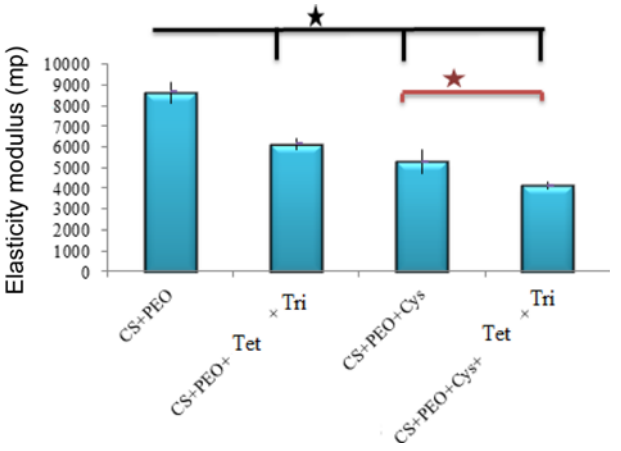


Figure 5. The modulus of elasticity of the different nanofibers.

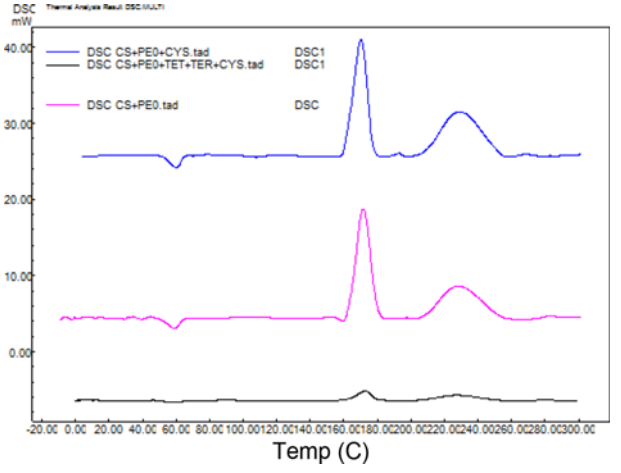


Figure 6. The DSC thermograms of different nanofibers.

difference between the modulus of elasticity of polymers and thiolated polymer. Addition of drug in the system is ineffective on results.

Elasticity modulus was determined 5,000 MPa for polymer and 4000 MPa for drugs loaded thiolated polymers which is suitable for the intended purpose. Since the nanofibers are designed for mouth infections, it seems that the elasticity is relatively good and suitable for administration in mouth tissue.

The DSC thermograms of nanofibers are shown in Figure 6.

According to DSC studies the changes in physical and chemical structure of compounds in a certain range of temperature are examined. In obtained thermograms from two polymer nanofibers containing CS and polyethylene oxide, an endo thermic peak is observed in the 52-62 °C.

Endo thermic peak at 60 °C is caused of the evaporation of water from the polymer. Exo thermic peak in 173 °C and 232 °C, resulting from the decomposition of ethylene oxide and CS, respectively [17]. It also increases with increasing temperature and crystallinity of the polymer amorphous phase during heat transfer in a two-step change in the physical structure and chemical CS.

Effect of Mucoadhesive Agents

Several mucoadhesive agents were used to create mucoadhesive property at the nanofibers. These compounds were included glutathione, Cysteine, 4-thio-butyl-amide (CS-TBA). Nanofibers with different types of mucoadhesive compounds were prepared. Mucoadhesive strength of the nanofibers to the oral mucosa in adult sheep was investigated using Tensiometer designed in Shahid Beheshti University I.R. Iran.

Tensiometer breaks the bond between the mucoadhesive nanofiber and oral mucous of sheep and determines the mucoadhesive strength.

Since pH of oral mucosa increases in patients with oral ulceration, Mucoadhesive strength of the hydrated nanofibers evaluated in the presence and absence of a phosphate buffer (pH=5.6, 6, 7) at 37 °C and for 10, 15, 20, 25 minutes in contact with oral mucosa in adult sheep. Among the mentioned nanofibers with various mucoadhesive agents, Nano fibers modified with cysteine showed mucoadhesive strength more than others and it can be used to preparation of mucoadhesive drug delivery systems (Table 3).

Cysteine solutions of various concentrations ranging between 1-4 % w/w were prepared in distilled water. Concentrations of cysteine increased due to increasing in mucoadhesive property. The higher the concentration of 4 % morphological properties was undesirable. The hydration of nanofibers is an important influence on the mucoadhesive strength. In absence of proper hydration on excessive hydration, Mucoadhesive property greatly reduced. In addition, pH has an obvious effect on mucoadhesive property, and formation of strong hydrogen bonds between the electrospun nanofibers and mucosa.

The CS/PEO and thiolated drug loaded nanofibers have shown excellent in-situ Mucous membrane properties, with a clear correlation between the total amounts of polymer-linked thiol groups.

Furthermore, the combination of mucoadhesive and

Table 3. Mucoadhesive effect of different concentrations of adhesion nanofibers

Adhesion strength (phosphate buffer)			Mocuadhesive agent in nanofibers
pH=7	pH=6	pH=5.6	
365.1±14.2	324.5±21.6	293.0 ±10.3	Glutathione
411.8±16.2	586.4±18.1	474.4±14.6	Thio-butyl-amide (TBA)
379.3±15.6	486.1±21.3	452.4±23.6	Iminothiolane
653±13.7	703.1±12.7	686.2±8.4	Cysteine 1 %
687±15.1	734±18.3	722±11.9	Cysteine 2 %
714±24.6	754±19.6	768±24.5	Cysteine 3 %
783±20.6	763±28.1	789±23.0	Cysteine 4 %
721±19.8	734±25.3	738±17.2	Cysteine 5 %

cohesive features is a pre-requisite for effective mucoadhesive oral drug delivery systems.

The In Vitro Release and Kinetic Studies

Figure 7 shows the cumulative release of investigated drugs versus time. Cumulative amount of drug in each sample was determined using its calibration curve obtained by HPLC analysis.

Model-dependent approaches were used for comparison of release profiles. The release data were fitted to kinetic models including the zero-order (equation (2)), first-order (equation (3)) and Higuchi (equation (4)) release equations to obtaining the best model [18].

$$C = K_0 t \quad (2)$$

$$\text{Log } C = \text{Log } C_0 - Kt/2.303 \quad (3)$$

$$Q = Kt^{1/2} \quad (4)$$

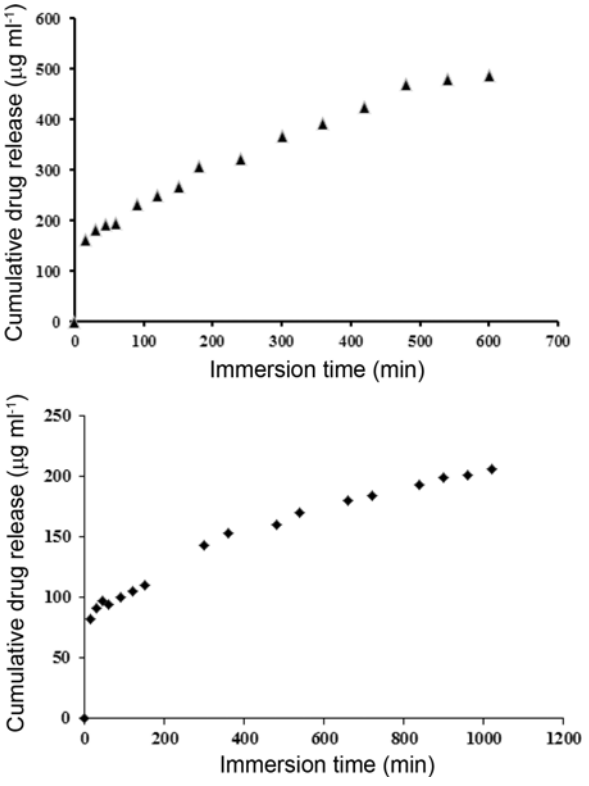


Figure 7. Cumulative release of triamcinolone (▲) and tetracycline (♦) mucoadhesive fibers.

Table 4. The R^2 and release rate constant values from in vitro release kinetics

Released drug	Zero order		First order		Higuchi	
	R^2	K (h^{-1})	R^2	K (h^{-1})	R^2	K (h^{-1})
Tetracycline	0.832	0.138	0.92	0.002	0.964	7.18
Triamcinolone	0.88	0.646	0.925	0.001	0.988	16.96

The R^2 and dissolution rate constant (K) of release data on various kinetic models is reported in Table 4. The best model for release, selected based on the value of the regression coefficient (R^2) for the results. Regarding the release model of all formulations, it was found that the prolonged release characteristic of investigated drugs were well fitted to Higuchi's square root model, as has been reported for nano structured systems. Linear fits were obtained, indicating that the release profile of Tri from this matrix systems is diffusion-controlled [19].

The CS-PEO thiolated with cysteine conjugate was able to guarantee a significant delay in the drug release in comparison to nonthiolated-CS, leading to a sustained release over a much longer time period. Such a cross-linking of the polymeric chains results in a highly stable, three-dimensional matrix that allows a controlled drug release.

Cell Viability Assay

The percent of cell viability under different treatment conditions on HUVEC cells revealed that 24, 48, 72 and 96 hours treatment with nonofibers had no cytotoxic effect at concentration up to 5 mg/m*l*.

CS represents, primarily due to its mucoadhesive properties, a valuable tool for non-invasive drug delivery. The longer residence time of formulations based on mucoadhesive polymers at the absorption site is believed to contribute to an increased absorption rate of the incorporated drug. However, such an enhanced bioavailability can be achieved only if a controlled release of the active agent out of the formulation is provided.

Thiolated CS also displays, beside their strong mucoadhesive and permeation enhancing properties, excellent cohesive properties [20-22]. The reduced thiol functions on the CS backbone enable thiolated CS not only to form disulfide bonds with mucus glycoproteins, but also to form inter- as well as intra-molecular disulfide bonds. Such a crosslinking of the polymeric chains results in a high stability of drug carrier systems based on thiolated CS. The free thiol groups of thiolated CS can, besides interacting with the mucus, form inter- and intermolecular disulfide bonds, leading to a high cohesiveness of the polymeric network. This theory was confirmed by the decrease in free thiol groups within thiomers during an oxidation experiment, resulting in an increased viscosity (Bernkop-Schnürch *et al.* 2003).

Antibacterial Activity of Mucoadhesive Nanofiber

In this study, blood agar medium was used for the growth and recognition of Gram-positive bacteria and EMB medium for Gram-negative bacteria. Bacteria on blood agar medium had positive growth and without hemolysis. The results of antibacterial tests of nanofibers in Figure 8(A), demonstrate that zone diameters of bacterial growth inhibition was increased with increasing concentrations of the drug in nanofibers. The nanofibers without Tet and Tri did not show

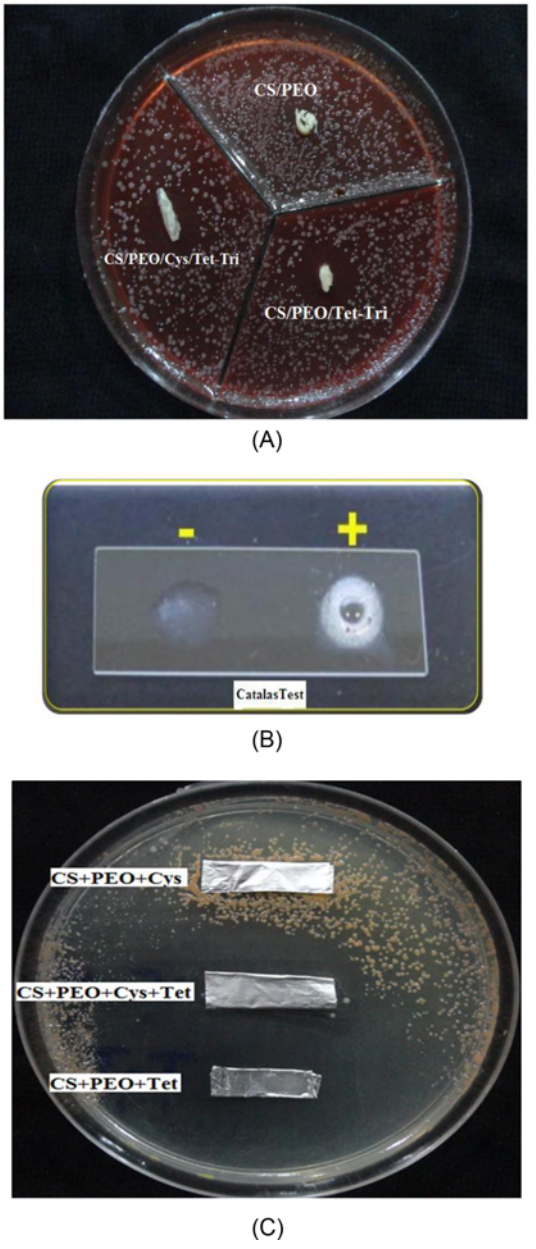


Figure 8. (A) The blood agar medium and antibacterial activity of nanofibers containing drugs, (B) EMB medium for gram-negative bacteria (B) catalase-negative test of bacteria and the samples are Streptococcus (C) the antimicrobial activity of mucoadhesive nanofibers.

any antibacterial effect. Also, it shows the test results Streptococcus on Mueller Hinton agar. Mucoadhesive CS nanofibers drug-free were used as a negative control in both tests.

The catalase test was used for identification of Streptococcus and isolation of Staphylococcus. In the Catalase test the H_2O_2 indicator was used. Some of the bacteria grown from the patient sample are poured into the KOH solution. The release of the bubbles in this test represents a catalase-negative test of bacteria and the samples are Streptococcus (Figure 8(B)).

The antimicrobial properties were evaluated by the Disk Diffusion method. In this method Streptococcus was cultured on Mueller Hilton Agar and used for antimicrobial susceptibility testing. Nanofibers-containing drug and drug-free were placed on the plate with a distance and were incubated for 24 hours at 37 °C. The zone diameters of bacterial growth inhibition were measured (Figure 8(C)).

Conclusion

The mucoadhesive nanofibers fabricated successfully. The obtained fibers characterized by means of FTIR, SEM, DSC, BET and mechanical analysis technique. Release of Tet and Tri from electrospun mats of thiolated CS/PEO was studied and it was found that electrospun fibers have release of drug over about 5 days. The usefulness of thiolated CS as carrier matrices for controlled drug release was demonstrated in the study. The Tet is well-established as an antibiotic drug in the treatment of oral ulceration infections. Both thiolated CS/PEO nanofibers remained stable during the whole experiment (72 h) and no disintegration could be observed. Regarding the release model it was found that the prolonged release characteristic was well fitted to Higuchi's square root model indicating that the release profile of drug from these matrix systems is diffusion-controlled. The antibacterial study demonstrate that zone diameters of bacterial growth inhibition was increased with increasing concentrations of the drug in nanofibers and nanofibers without drug did not show any antibacterial effect. 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 exploring electrospun systems for the controlled release of therapeutic macromolecules.

Acknowledgments

The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 97213) for the financial support.

References

1. J. Gu, J. Robinson, and S. Leung, *Critical Reviews in Therapeutic Drug Carrier Systems*, **5**, 21 (1987).
2. A. B. Brennan, R. H. Baney, M. C. Turnage, T. G. Estes, A. W. Feinberg, L. H. Wilson, and J. F. Schumacher, "Surface Topographies for Non-toxic Bioadhesion Control", Google Patents, 2015.
3. T. Potrč, S. Baumgartner, R. Roškar, O. Planinšek, Z. Lavrič, J. Kristl, and P. Kocbek, *Eur. J. Pharm. Sci.*, **75**,

101 (2015).
4. C. K. Mylangam, S. Beeravelli, J. Medikonda, J. S. Pidaparthi, and V. R. M. Kolapalli, *Drug Delivery*, **23**, 195 (2016).
5. C. Rieger, D. Kunhardt, A. Kaufmann, D. Schendel, D. Huebner, K. Erdmann, S. Propping, M. P. Wirth, B. Schwenzer, and S. Fuessel, *Int. J. Pharm.*, **479**, 357 (2015).
6. V. M. Lenaerts and R. Gurny, "Bioadhesive Drug Delivery Systems", CRC Press, 1989.
7. J. Mašek, D. Lubasova, R. Lukáč, P. Turanek-Knotigova, P. Kulich, J. Plockova, E. Mašková, L. Prochazka, Š. Koudelka, and N. Sasithorn, *J. Controlled Release*, **249**, 183 (2017).
8. A. Bernkop-Schnürch, V. Schwarz, and S. Steininger, *Pharm. Res.*, **16**, 876 (1999).
9. A. Bernkop-Schnürch, H. Zarti, and G. F. Walker, *J. Pharm. Sci.*, **90**, 1907 (2001).
10. A. E. Clausen, C. E. Kast, and A. Bernkop-Schnürch, *Pharm. Res.*, **19**, 602 (2002).
11. F. Föger, T. Schmitz, and A. Bernkop-Schnürch, *Biomaterials*, **27**, 4250 (2006).
12. X. Hu, S. Liu, G. Zhou, Y. Huang, Z. Xie, and X. Jing, *J. Controlled Release*, **185**, 12 (2014).
13. I. Chopra and M. Roberts, *Microbiol. Mol. Biol. Rev.*, **65**, 232 (2001).
14. S. Sukigara, M. Gandhi, J. Ayutsede, M. Micklus, and F. Ko, *Polymer*, **44**, 5721 (2003).
15. H. Jiang, D. Fang, B. S. Hsiao, B. Chu, and W. Chen, *Biomacromolecules*, **5**, 326 (2004).
16. P. Gupta and G. L. Wilkes, *Polymer*, **44**, 6353 (2003).
17. L. Li and Y.-L. Hsieh, *Carbohydr. Res.*, **341**, 374 (2006).
18. E. Rostami, S. Kashanian, and A. Azandaryani, *Molecular Biology Reports*, **41**, 3521 (2014).
19. A. Hemati Azandaryani and K. Derakhshandeh, *Int. J. Nanomed.*, **6**, 2393 (2011).
20. A. Bernkop-Schnürch, M. Hornof, and T. Zoidl, *Int. J. Pharm.*, **260**, 229 (2003).
21. F. Laffleur, A. Fischer, M. Schmutzler, F. Hintzen, and A. Bernkop-Schnürch, *Acta Biomater.*, **21**, 123 (2015).
22. Y. Zhou, J. Zhao, X. Sun, S. Li, X. Hou, X.-B. Yuan, and X. Yuan, *Biomacromolecules*, **17**, 622 (2016).