

ORIGINAL RESEARCH PAPER

Preparation and evaluation of electrospun nanofibers containing pectin and time-dependent polymers aimed for colonic drug delivery of celecoxib

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Received; 9 August 2015

Accepted; 5 October 2015

ABSTRACT

Objective(s): The aim of this study was to prepare electrospun nanofibers of celecoxib using combination of time-dependent polymers with pectin to achieve a colon-specific drug delivery system for celecoxib.

Materials and Methods: Formulations were produced based on two multilevel 2² full factorial designs. The independent variables were the ratio of drug:time-dependent polymer (X_1) and the amount of pectin in formulations (X_2). Electrospinning process was used for preparation of nanofibers. The spinning solutions were loaded in 5 mL syringes. The feeding rate was fixed by a syringe pump at 2.0 mL/h and a high voltage supply at range 10-18 kV was applied for electrospinning. Electrospun nanofibers were collected and evaluated by scanning electron microscopy and drug release in the acid and buffer with pH 6.8 with and without pectinase.

Results: Electrospun nanofibers of celecoxib with appropriate morphological properties were produced via electrospinning process. Drug release from electrospun nanofibers was very low in the acidic media; while, drug release in the simulated colonic media was the highest from formulations containing pectin.

Conclusion: Formulation F2 (containing drug:ERS with the ratio of 1:2 and 10% pectin) exhibited acceptable morphological characteristics and protection of drug in the upper GI tract and could be a good candidate as a colonic drug delivery system for celecoxib.

Keywords: Celecoxib, Colonic delivery, Electrospinning, Nanofiber, Pectin

INTRODUCTION

Electrospinning is a reliable process for the production of fibers in nano scale. Using an electric charge inducing in the surface of a polymeric solution a charged liquid is produced. When the electric field overcomes the surface tension of the liquid a thin jet is ejected from the surface of solution droplet which is elongated through the electric field followed by solvent evaporation and production of fibers that can be collected in the collector plate [1,2]. Electrospun nanofibers have been widely used in biomedical applications such as tissue engineering [3,4], wound dressing [5,6], enzyme immobilization [7,8] and fast [9] or controlled drug delivery [10-12]. Colonic drug delivery has gained interest not just for the treatments of local diseases of colon such as inflammatory bowel

disease (IBD) or Crohn's disease but also as a confident approach for peptide and protein drug delivery [13]. Of the main strategies for orally targeting drug to the colon is using pH- or time-dependent polymers. Regarding the variation of drug release from each of these systems in different physiological situations [14] combination of pH- and time-dependent materials could manage more confident drug release from a colonic delivery system. Despite the potential of using electrospun nanofibers in drug delivery due to their unique characteristics such as large surface-to-volume ratio and utilization of a wide variety of polymers, application of these nanofibers in colonic drug delivery has been rare. There is an investigation in the literature in which electrospun nanofibers of diclofenac sodium were prepared using eudragit L 100-55 as a pH-dependent polymer and the authors concluded that the mentioned formulation has the potential as an oral colon drug delivery system [15]. Meanwhile, any combination of two colonic delivery systems has not been tried yet.

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Note. This manuscript was submitted on August 9, 2015; approved on October 5, 2015

Therefore, the aim of our study was to prepare electrospun nanofibers of celecoxib using separate mixtures of two time-dependent polymers, Eudragit RS and polycaprolactone with pectin as a colon-specific bacterially degradable polysaccharide. Celecoxib was used as a model drug due to effectiveness in colon cancer treatment [16].

On the other hand, poor solubility of indomethacin makes it a promising candidate for substitution in electrospun nanofibers

MATERIALS AND METHODS

Materials

Celecoxib (Darupakhsh, Iran), Eudragit RS 30D (ERS)(Rohm Pharma, Germany), polycaprolactone (PC)(Sigma Aldrich, USA), pectin (Serva, USA), Acetonitrile (Merck, Germany), Ethanol (Merck, Germany), KH_2PO_4 (Merck, Germany), NaOH (Merck, Germany), hydrochloric acid (Merck, Germany) and NaCl (Merck, Germany) were obtained from the indicated sources.

Preformulation

Preformulation was performed aiming for achieving the best levels of polymers and drug ratios for experimental design. Ethanol and acetonitrile were used as common solvents of drug-Eudragit RS and drug-polycaprolactone, respectively.

Experimental design

Formulations were produced based on two separate multilevel 2^2 full factorial designs. The independent variables were the ratio of drug:time-dependent polymer (X_1) and the amount of pectin in formulations (X_2). Types and levels of the independent variables are listed in Table 1.

Table 2 depicts the resulted formulations. Formulation F4 was not practically producible and thus was removed from list of runs.

Table 1. Independent variables: types and levels

Variables	Levels	
the ratio of drug:time-dependent polymer (X_1)	1:2	2:1
the amount of pectin in formulations (X_2)(%)	0	10

Preparation of spinning solutions

8% (w/v) solution of drug and 75% (v/v) dispersion of Eudragit RS30D were prepared in ethanol to achieve a 1:2 w/w ratio of drug:polymer. Conversely, 16% (w/v) solution of drug and 25% (v/v) dispersion of Eudragit RS were prepared in ethanol to achieve a 2:1 w/w ratio of drug:polymer. On the other hand, 8% (w/v) solution of drug and 16% (w/v) solution of polycaprolactone were prepared in acetonitrile to achieve a 1:2 w/w ratio of drug:polymer. Conversely, 16% (w/v) solution of drug and 8% (w/v) solution of polycaprolactone were prepared in acetonitrile to achieve a 2:1 w/w ratio of drug:polymer.

Electrospinning process

Electrospinning solutions containing different ratios of drug:polymers were loaded in 5 ml syringes. The feeding rate was controlled by a syringe pump (Cole-Pham®, USA) and was fixed at 2.0 mL/h. A high voltage supply fixed at 10-18 kV was applied, and a piece of aluminum foil was used to collect the ultrafine fibers with a horizontal distance of 15 cm from the needle tip. Nanofibers were collected and stored in a desiccator for more studies.

Scanning Electron Microscopy

The surface morphologies of electrospun nanofibers were assessed using a LEO-rp-1455 scanning electron microscope (SEM). The samples were primarily silver sputter-coated under argon to render them electrically conductive. The pictures were then taken at an excitation voltage of 15 kV.

Dissolution studies

Dissolution studies were performed with a sample of 1×1 cm of each formulation in a dissolution tester (DT800, Erweka, Germany) using USP apparatus I, at a rate of 100 rpm and 37°C , in 900 mL of dissolution medium ($n=3$). For simulating various parts of GI tract, different media were used including HCl solution with $\text{pH} = 1.2$ simulating gastric fluid (SGF), phosphate buffer with $\text{pH} = 6.8$ simulating intestinal fluid (SIF) and phosphate buffer with $\text{pH} = 6.8$ containing pectinolytic enzyme with concentration of 0.6 mg/mL simulating colonic fluid (SCF). Samples were taken and the released drug from pellets was assayed spectrophotometrically by a UV / Visible spectrophotometer (Bio-wave II, WPA, England) at a wavelength of 251 nm.

Table 2. Compositions of formulations (runs)

Run	Celecoxib (%)	EudragitRS (%)	Polycaprolactone (%)	Pectin (%)
F1	33.3	66.6	0	0
F2	30	60	0	10
F3	66.6	33.3	0	0
F4	60	30	0	10
F5	33.3	0	66.6	0
F6	30	0	60	10
F7	66.6	0	33.3	0
F8	60	0	30	10

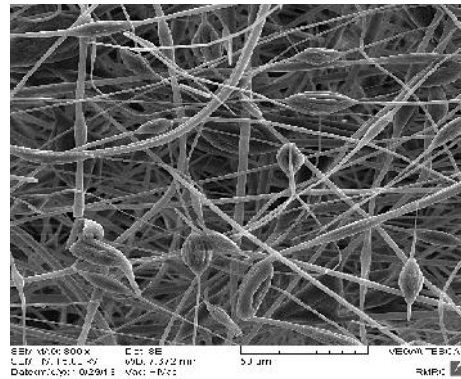
Statistical analysis

The effects of the independent variables on each experimental response were modeled using a second-order polynomial equation:

$$Y = C + b_1X_1 + b_2X_2 + b_3X_{12} + b_4X_{22} + b_5X_1X_2 \quad \text{Eq. 1}$$

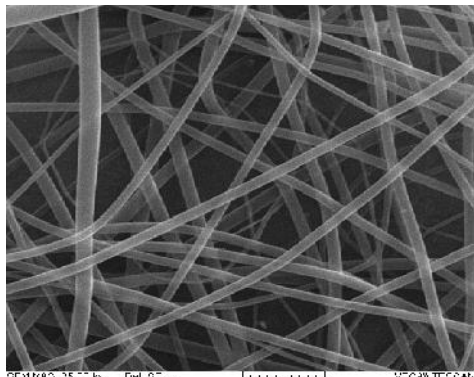
Models were simplified with a backward, stepwise linear regression technique.

Only significant terms ($P < 0.05$) were chosen for the final model. Modeling was performed using SPSS (version 15.0).

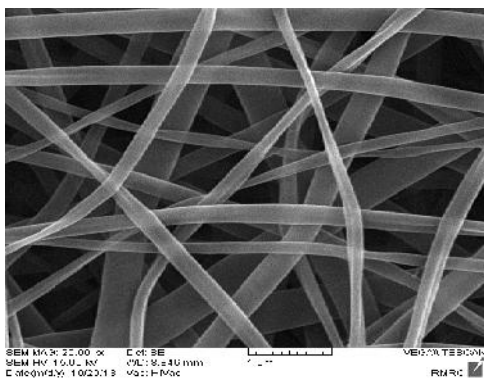


(c)

Fig. 1. SEM images of formulations with drug:PC in the ratios of ; (a) 1:2, (b) 2:1 and (c) 3:1



(a)



(b)

RESULTS AND DISCUSSION

Preformulation

To produce suitable electrospinning solutions and achieve reproducible electrospinning process, selection of solvent is one of the most important factors [17]. The solvent should be able to dissolve the drug easily as well as producing good solution for electrospinning. In this study, preformulations were performed to achieve a suitable common solvent for both drug and polymers, separately.

It was shown that ethanol could be appropriate for dispersing ERS with drug. Also, acetonitrile was an adequate solvent for PC and celecoxib. Preformulation studies also demonstrated that the ratio of drug:time-dependent polymer up to 2:1 could manage appropriate electrospun nanofibers (Fig 1) and the higher amounts of drug resulted beads in the structure of ERS and PC nanofibers (Fig 1c).

According to preformulation, addition of pectin to the formulation structure was possible up to 10% and in the higher ratios; it was not possible to produce electrospuns.

Scanning Electron Microscopy

SEM images of drug loaded nanofibers showed that the incorporation of celecoxib in the composition of formulations up to the ratio of 2:1 related with either ERS or PC is possible and could manage reproducible electrospun with suitable physical characteristics. Addition of drug to the nanofiber structure did not significantly change the size and shape of nanofibers (Fig 2). Also, substitution of ERS with PC did not alter apparent characteristics of nanofibers. Meanwhile, incorporation of pectin in the formulations resulted nanofibers with a little smaller diameter size (Fig 3) and formulation F2 containing pectin did not produce due to process problems.

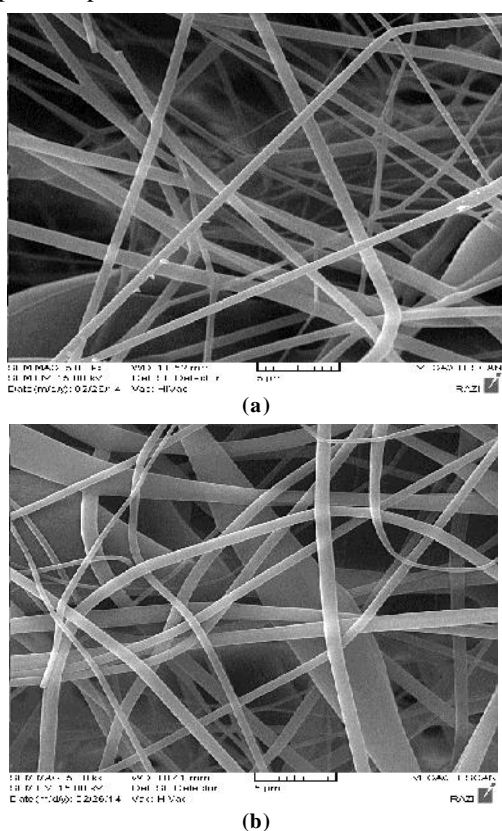


Fig. 2. SEM images of formulations (a) F2 and (b) F3

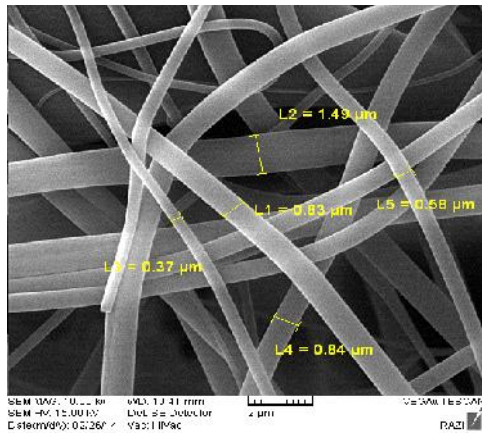
Dissolution studies

Fig 4 depicts dissolution profiles of all the formulations in the acidic media. As shown in Fig 4, drug release from all the nanofibers after 2 h in the simulated gastric fluid was very low. The highest drug release among the formulations was from F3 (containing drug:ERS in the ratio of 2:1); Meanwhile, F2 and F8

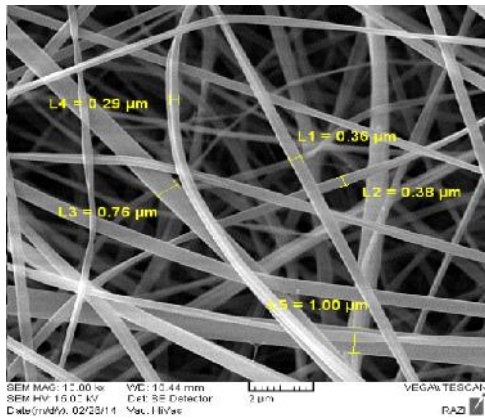
were the most resistant formulations in the acidic media. Low drug release from these two electrospun nanofibers could be due to presence of pectin in their compositions which remains non-ionised in the acidic media and can amplify resistance to drug release [18, 19].

Drug release profiles of formulations in the buffer with pH 6.8 were shown in Fig 5. As can be seen, drug release from all the formulations was below 30% after 3h in the simulated intestinal fluid. Therefore, regarding the relative constant retention time in the small intestine (3h) the whole nanofibers could deliver about more than 70% drug from this region to the colon. There was no significant difference in drug release from electrospun by substitution of ERS with PC and thus both of these time-dependent polymers managed a suitable resistance to drug release by small degrees of swelling during dissolution time and the resulted controlling drug release [20, 21]. F2 and F8 showed the lowest drug release after 10h in the buffer media among the formulations which was compatible with the results of acidic media and verified that the presence of pectin in the structure of nanofibers containing sustained release polymers could delay drug release because of intactness of this polymer in the buffer media.

Fig 6 depicts dissolution profiles of all the formulations in the simulated colonic fluid (SCF). As shown, drug release from electrospun nanofibers without pectin was relatively slow in the colonic media such that the maximum drug release from these formulations was between about 70-85% after 10h. On the other hand, formulations F2, F6 and F8 which contained pectin in their compositions showed fastest drug release in the colonic media. In fact, pectinase enzyme which was presented in the SCF could break down the pectin backbones leading to pore formation in the nanofiber structure. Increase in the porosity of the electrospun due to breaking of pectin polymer chains enhanced drug release from these formulations [22, 23]. Therefore, formulations F2, F6 and F10 were resistant to the upper GI tract media while drug release from these electrospun nanofibers was susceptible to the colonic pectinase enzyme and enhanced in the colonic media. Among these formulations, F6 which composed of PC exhibited the highest drug release in SCF. Meanwhile, formulation F2 which had appropriate size and shape (Fig 1a) and also entirely protected drug in the simulated gastric and intestinal fluid could be more promising as a colonic drug delivery system for celecoxib.



(a)



(b)

Fig. 3. SEM images of formulations (a) F7 and (b) F8

CONCLUSION

According to the results of this study, electrospinning was a suitable process for producing nanofibers of celecoxib aimed for colonic drug delivery. It was shown that all of the resulted nanofibers had appropriate morphological properties.

Drug release from electrospun nanofibers was very low in the acidic media.

Meanwhile, drug release in the simulated colonic media was the highest from formulations containing pectin. Among the nanofibers, F2 (containing drug: ERS with the ratio of 1:2 and 10% pectin) with acceptable morphological characteristics and protection of drug in the upper GI tract could be a good candidate as a colonic drug delivery system for celecoxib.

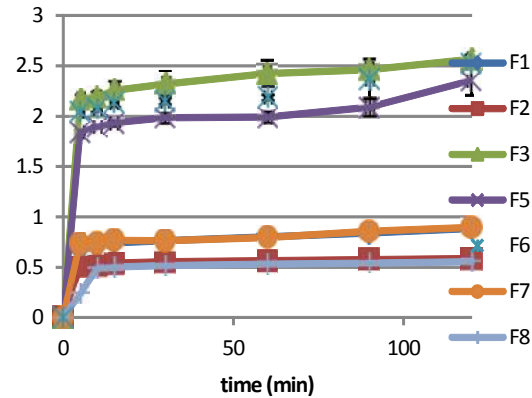


Fig. 4. Dissolution profile of formulations in the acidic media (SGF)

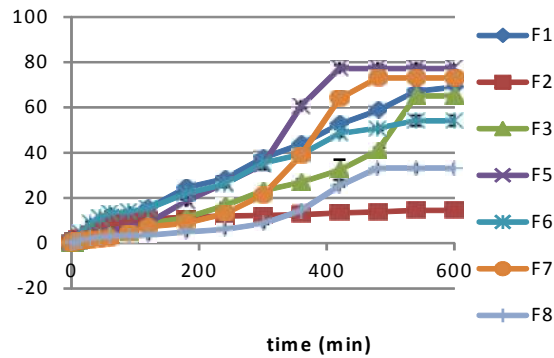


Fig. 5. Dissolution profile of formulations in the buffer media with pH 6.8 (SIF)

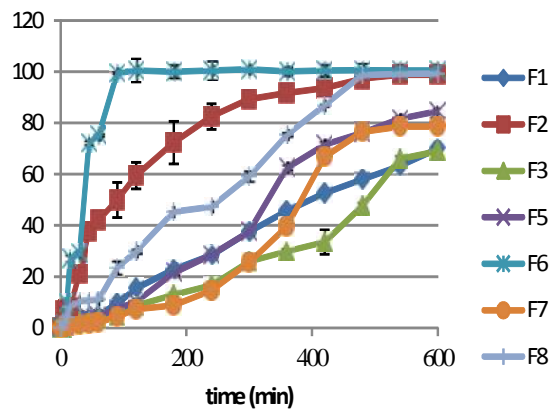


Fig. 6. Dissolution profile of formulations in the buffer media with pH 6.8 with pectinase (SCF)

ACKNOWLEDGMENTS

This work is the Pharm. D thesis of Mr M. Rotubati which is supported by a grant from research chancellor of Ahvaz Jundishapur University of Medical Sciences. The authors would like to thank Darupakhsh pharmaceutical co. for their collaboration and providing samples used in this paper.

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How to cite this article:

Akhgari A, Rotubati MH. Preparation and evaluation of electrospun nanofibers containing pectin and time-dependent polymers aimed for colonic drug delivery of celecoxib. *Nanomed. J.*, 2016; 3(1): 43-48.

DOI:10.7508/nmj.2016.01.005

URL: http://nmj.mums.ac.ir/article_6195_888.html