Preparation and in vivo evaluation of sodium alginate - poly (vinyl alcohol) electrospun nanofibers of forskolin for glaucoma treatment

Shiva Kumar Yellanki1,2*, Balaji Anna2 and Marupaka Radha Kishan3
1Department of Pharmacy, Jawaharlal Nehru Technological University Kakinada, Kakinada, Andhra Pradesh, India
2Department of Pharmaceutics, Trinity College of Pharmaceutical Sciences, Peddapalli, Karimnagar, Telangana, India
3Department of Pharmacognosy, Govt. Polytechnic for Women, Hanamkonda, Warangal, Telangana, India

Abstract: The present investigation is aiming to prepare Sodium Alginate (SA) - Poly (vinyl alcohol) (PVA) nanofibrous mats of Forskolin (FSK) for ocular delivery to treat the glaucoma. Nanofibers of SA: PVA (1:0.25) load with β-cyclodextrin. FSK solid dispersion were successfully prepared by an electrospinning technique. Eight formulations were Prepared and evaluated for drug content, scanning electron microscopy, degree of swelling, drug release and In Vivo Intra ocular pressure (IOP) reduction studies. The morphological studies revealed that average diameter of prepare nano fibers were decreased for formulations with low polymer concentration. Less diameter and uniform surface was observed for formulations F4 and F8 which are prepared under applied voltage 20kV, Capillary tip-to-Collector distance 15cm conditions. From the degree of swelling studies, it was observed that thinner the nanofiber mats, the greater the degree of swelling. The burst release within one hour was seen for F1 to F4 formulations whereas up to 90 min for F5 to F8 formulations. Release kinetic studies revealed that release of drug from the Nanofibrous mats have followed zero order kinetics. The results of in vivo IOP reduction studies suggested that FSK loaded Nanofibrous mats formulation (F4) produced a significant and controlled reduction in IOP throughout 45h.

Keywords: Electrospinning technique, Forskolin, Solid dispersions, In Vivo Intra ocular pressure (IOP) reduction studies, Nanofibrous mats.

INTRODUCTION

Nanofibers are having vital applications in biomedical field for drug delivery. Due to large surface area of nano fibers, they are more efficient for drug delivery and wound healing (Chew et al., 2006).

By electrospinning method small scale diameter fibers were produced by application of high potential to the fluid polymer solution during ejection on the grounded collector, during this process the liquid solvent evaporate. This formed fibers having major roll for delivery of drugs and in biomedical research (Ashammakhi et al., 2008),(LeDuc et al., 2007). Glaucoma may caused and related to age, other disease conditions and family history, this disease causes nerve ending or optical nerve damage due to more intra ocular pressure (IOP) (Quigley et al., 1997; Quigley, 1996; Sommer, 1996).

Electrospinning is a relatively simple process to produce nanofibrous structures from polymer solutions and is consequently most commonly applied. This technique relies on electrostatic forces realized by an electric field that is applied between the tip of a nozzle, through which the polymer solution is flowing, and a collector plate. This electric field induces a distortion of the polymer solution from a spherical pendent drop to a Taylor cone. Once the electrostatic forces exceed the surface tension of the polymer solution, a jet is drawn from the tip of the Taylor cone. Solvent evaporation and interaction of the charges with the external electric field cause instability of the jet, which in turn causes bending and splaying. As a result, the jet elongates and nanofibres are randomly deposited on the collector plate.

Alginate is naturally occurring polymer and it convert in to gel form due to ion exchange with lachrymal fluid and it is biocompatible (Augst et al., 2006).

Forskolin, a labdane diterpene extracted from the Coleus forskohlii roots (Bhat et al., 1977), is used for hypertension, cardiovascular diseases (Kansal et al., 1978), (Dubey et al., 1997), asthma, (Suryanayanan et al., 1998), (Lazarus et al., 1961). Forskolin activates adenylatecyclase and amount of cyclic AMP (adenosine mono phosphate) in cells this process initiates the activity.

Coleus forskohlii is a botanical that has been used since ancient times and Ayurvedic traditional medicine. The root portion of the plant has been traditionally used for medicinal purposes and contains the active constituent, forskolin. Forskolin was named after the Finnish botanist, Forskal. Historically, it has been used to treat hypertension, congestive heart failure, eczema, colic, respiratory disorders, obesity, asthma, angina, painful urination, insomnia and convulsions (Sangeetha et al., 2011).
In present work, alginate was blended with Poly (vinyl alcohol) (PVA) and electrospun for formation of uniform nano fibrous mats and alginate was cross linked by the calcium. The aim of this work was to formulate ocular nano fibrous mats using ion activated polymer containing Forskolin to be applied topically and to evaluate the in vitro and in vivo performance of the prepared nano fibrous mats.

MATERIALS AND METHODS

Materials
Forskolin (FSK) with purity of >98% was obtained from Madvik Labs, Hyderabad, India. Sodium Alginate (SA) (molecular weight - 195 000g. mol⁻¹) and Poly (vinyl alcohol) (PVA) were procure by SD fine chem., India. High Performance Liquid Chromatography grade solvents (Ethanol, calcium chloride and Methanol) were used for experiment. Triple distilled and deionized water was used throughout the studies.

Method
Preparation of drug polymer solution
Solid dispersions (SD) of Forskolin (FSK) were prepared by kneading method for improving the aqueous solubility using β-cyclodextrin as complexing agent. Drug with β-cyclodextrin ratio 1:1 was kneaded separately in a mortar and pestle using deionized distilled water as solvent for 30 min. The slurry is allowed to dry for 24h in vacuum finally obtained granules were pulverized and passed through sieve no. 60. SA and PVA polymers were dissolved in deionized water with 1:0.25 concentrations and was vortexed at low speed and rotated for 24hr at 37°C using a rotating hybridization incubation. Solid dispersion equivalent to 0.5% w/v of FSK was dissolved in SA-PVA polymer solution and the drug polymer solution was allowed to mix for 5 h on magnetic stirrer at room temperature, the ratio of polymer and drug was maintained constant for all formulations (table 1).

Preparation of Electrospun nanofibrous scaffolds
Electrospinning was employed for preparation of SA-PVA polymeric nano fibrous mats; the polymer solution with drug was filled in syringe (5ml) with 22 gauge needle. The syringe pump (KDS100, USA) is used for fixing the polymer filled syringe to maintain the ejection rate. The high voltage supplier was placed to produce 0-25kV potential, the positive charge was connected to syringe needle and negative pole was connected to grounded alominium foil, which is employed as collector. The distance between syringe tip and collector was adjusted between 5-15cm and constant flow was adjusted (0.8ml h⁻¹). The selection of potential based on the formed fiber uniformity and formed fibers were maintained at 37°C for 48 hr. The neutralization was performed for electrospun Sodium alginate nano fiber mats. Resulting samples were neutralized by immersion in 100 ml of 2% w/v calcium chloride solution for 20 s and washed with distilled water and lyophilized (Christopher et al., 2011). All the above experimentation was carried out under laminar airflow to maintain the sterility conditions of opthalmic products; gamma irradiation was performed using a commercial ⁶⁰Co source to a dose of 20.6 kGy for four days.

Fig. 1: Scanning electron micrographs (a) electrospun droplets of SA- PVA. (b) Uniform fibers (F4).

Characterization of Alginate/PEO Nanofibers
Drug content
A known weight of the mats (10mg) was suspended in 10ml artificial tear fluid (ATF) pH 7.4 and 2ml of methanol. The mats was maintained for 24 h at 60°C in artificial tear fluid (ATF) pH 7.4 solution and the amount
of FSK was determined by UV spectroscopy (Shimadzu, UV-1601, Japan) at 210 nm with reference of standard curve.

\[ \text{Degree of swelling } = \frac{w - w_d}{w_d} \times 100 \]  

(1)

where \( w \) is swollen nanofibers weight, \( w_d \) is the dried weight, calculated by drying the swollen nano fiber mats at 40°C (Marziyeh, Jaleh et al., 2011).

% Degree of swelling

The swelling of nanofiber mats were calculated. The test was conducted using medium with pH 7.4 (artificial tear fluid) at 37°C at various time points by placing the 10 mg of nano fibrous scaffolds.

\[ \% \text{ Degree of swelling} = \frac{w - w_d}{w_d} \times 100 \]  

(1)

In vitro % drug release

The known mass with 2.5 × 2.5 cm dimension of fibers was used to evaluate the drug release. The weighed fibers sample was placed in 20ml of ATF at pH 7.4, 37°C under 20 rpm. At various intervals (1 h, 2 h up to 48 hours) 1 ml of sample was withdrawn from ATF, replace with 1 ml of freshly ATF and analyzed by UV spectroscopy (Shimadzu, UV-1601, Japan) at 210 nm (Marziyeh et al., 2011). The release kinetic was calculated by Bharti Vidyapeeth University, Poona College Of Pharmacy (PCOP) dissolution software to estimate the release mechanism.

In vivo intra ocular pressure (IOP) reduction studies

The Forskolin (FSK) nanofiber mats were tested for their IOP lowering effects on adult normotensive male New Zealand albino rabbits and the obtained results were compared with that of plain nanofiber formulations as well as plain FSK solution. The induced IOP was attained by 5% glucose solution infusion (15 ml/kg of body weight) through marginal ear vein and accomplished within 20 s. The IOP was measured by standardized Schiotz tonometer (Zur-Benutzung des Schioetz, Germany) (Kaur et al., 2000; Monem et al., 2000). Before the measurement of the tension, the cornea was anaesthetized with 2 or 3 drops of xylocaine (1% w/v). After 2 min, the eyelids were retracted gently with one hand, without exerting pressure on the eye ball. The lower cul-de-sac of right eye of each rabbit of the group (n = 4) received 10 mg of the optimized formulation (F4) while the contra lateral eye (left) received no drug and served as a control. The IOP of both eyes of each rabbit was measured immediately before the administration of formulation (zero reading), 60 min after instillation and the every hour for a period of 48 h. The similar procedure was adapted for the measurement of IOP after instillation of 25µl of plain FSK solution and plain nanofiber formulation, respectively. The change in IOP (Δ IOP) was determined by following equation:

\[ \Delta \text{IOP} = \text{IOP Dosed eye} - \text{IOP Control eye} \]  

(2)

All the observations were taken in triplicate and the mean values were reported. All the measurement periods began during the same hour on each day and all the data were recorded with the same tonometer.

RESULTS

% Degree of swelling

From degree of swelling it was observed that thinner the nano fiber mats, the greater the degree of swelling. However, the degree of swelling after 24 hours was greater for the thicker nanofibers. fig. 4 the degree of swelling for Nano fibrous mats in the release medium (ATF, pH 7.4) at 37°C for 1, 4, 10 and 24 hours. From the results at 1 hour F4 formulation showed more degree of swelling (190±1%) and F5 formulation showed less degree of swelling (152±2%).

Characterization

Fig. 1(a) representing SEM potographs of the electrospun droplets with 2% polymer concentration, applied potential 10 kV and 7 cm distance between Capillary tip -to- Collector. fig. 1(b) representing the uniform fibers with the condition of 2% polymer, applied potential 20 kV and 15 cm distance between Capillary tip -to- Collector (F4). It was observed that uniform fibers with 200 nm to 700 nm prepared without any irregular surface under the condition of 2-4% polymer concentration with 15-20 kV applied
Table 1: Composition of FSK Nanofibrous mats

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<tr>
<th>Ingredients</th>
<th>Formulations</th>
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<td>F1</td>
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<tr>
<td>Forskolin (% w/v)</td>
<td>0.5</td>
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<tr>
<td>SA: PVA (1:0.25)</td>
<td>2%</td>
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<tr>
<th>Experimental Parameters</th>
<th>Applied voltage (kV)</th>
<th>Capillary tip-to- Collector distance (cm)</th>
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The drug content of Nanofibrous mats was ranging from 94.70±0.26 to 96.90±0.58%. FTIR spectra analysis (figs. 2 and 3) revealed no significant interaction between various rational combinations containing physical mixture of drug with polymers.

The slope values were calculated from the results it can be noticed that swelling rate is 9.6% and 10.5% per 60 min for 200 to 300μm and 400 to 600μm fibers. The thicker fibers were composed with the interior layers this layer controls the penetration of liquid content form environment and takes much time to complete swelling the fibers.

**In vitro % drug release**

Fig. 5 and 6 displays the cumulative drug release of FSK from Nanofibrous mats up to 48 hours. The results showed that rapid initial release. The burst release within one hour was seen for F1 to F4 formulations whereas up to 90 min for F5 to F8 formulations. The variation in burst effect is due to the smaller diameter and low concentration of polymer in Nanofibrous mats for F1 to F4. After a quick burst release, the profiles of release followed a linear fashion with a very slow rate. The release constants were calculated from the slope of the respective plots. It indicates that release of drug from the Nanofibrous mats (F1 to F8) have followed zero order kinetics (0.984, 0.844, 0.915, 0.934, 0.987, 0.912, 0.844, 0.833). Higher correlation was observed in the Higuchi equation. The observations led us to conclude that, all the selected ocular Nanofibrous mats followed diffusion controlled zero order drug release. F4 formulation selected as optimized formulation based on degree of swelling and drug release characters. Selected optimized formulation (F4) was studied for in vivo IOP reduction studies.
**In vivo intra ocular pressure (IOP) reduction studies**

The In vivo Intra ocular pressure (IOP) reduction studies were conducted successfully the results suggested that the hypotensive activity of FSK loaded Nanofibrous mats formulation (F4) was comparable to that of the plain drug solution. Initially, IOP decreased sharply for the first 1 h in case of plain FSK solution whereas IOP was observed to decrease slowly in case of FSK loaded Nanofibrous mats formulation (F4).

**DISCUSSION**

Alginate is used as gelling agent which prolonged the release and is biocompatible with adhesive nature (Rowley et al., 1999). Nanofibrous mats were prepared successfully by Electrospinning method. Effect of polymer concentration, applied voltage (kV) and distance between Capillary tip -to- Collector distance (cm) on Nanofibres diameter.

Different Nanofibrous mats were prepared (table I), from the SEM studies formulations prepared with 20 kV applied voltage and 15 cm distance between Capillary tip -to- Collector were showed uniform size and less diameter. By experimentation it was identified that applied potential, concentration of polymer and distance between collector and syringe effecting the uniformity of formed fibers. The FTIR spectra of drug-polymer mixture confirmed neither any shift in the wave numbers of the peaks nor in the intensity, construed lack of interaction.

Degree of swelling results concludes that Nano fibrous mats with small diameter showed rapid degree of swelling (Marziyeh et al., 2011). The thicker fibers entrapped more amount of liquid and swells more than other fibers. The strength of layers in fibers was affecting the swelling nature of immersed fibers (Marziyeh et al., 2011).

All formulations showed controlled release up to 48 hours and showed maximum drug release for less polymer concentration Nanofibrous mats (F1 to F4) whereas F5 to F8 formulations showed less drug release.

The IOP was immediately and noticeably reduced up to 1 h after instillation of plain FSK solution, but increased slightly over the rest of the period of observation. This type of fluctuation was not observed in case of optimized formulation (F4), where the IOP continued to drop. The results suggested that FSK loaded Nanofibrous mats formulation produced a significant and controlled reduction in IOP throughout 45 h (fig. 7).

**CONCLUSION**

New system for the delivery of FSK as anti-glaucoma drug in the electrospun fibers was developed by electrospinning technique. FTIR studies confirmed absence of any physiochemical interaction between drug and other ingredients. Physicochemical characters of all formulations were in the limit. Based on the morphological studies, degree of swelling and release kinetics F4 was selected as optimized formulation and performed for in vivo studies. The Intra ocular pressure (IOP) reduction studies suggested that formulation (F4) produced a significant and controlled reduction in IOP throughout 45 h.

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**REFERENCES**


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