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

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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

Nano fibers 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 electro spinning 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).

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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.

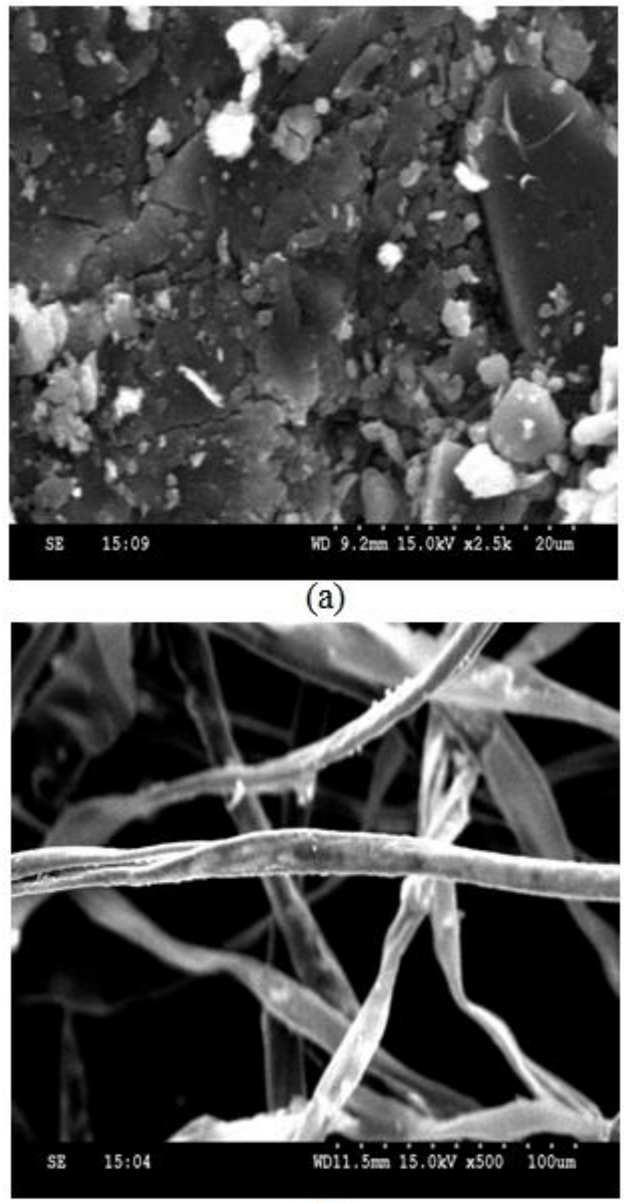
Method

Preparation of drug polymer solution

Solid dispersions (SD) of Forskolin (FSK) were prepared by kneeding 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 $37^{o}C$ 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 $37^{o}c$ for 48 hr. The neutralization was performed for electrospun Sodium alginate nano fiber mats. Resulting samples were neutralized by immersion in 100 ml of 100% ethanol for 10s with slow shaking and immersed in 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 ophthalmic products; gamma irradiation was performed using a commercial ^{60}Co source to a dose of 20.6 kGy for four days.



(b)

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

Characterization of Alginate/PEO Nanofibers

Drug content

Aknown 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 $60^{o}C$ 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.

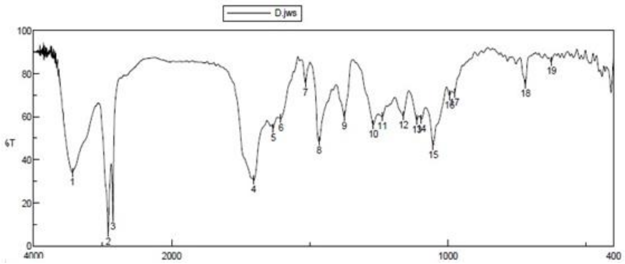


Fig. 2: FTIR spectrum of pure FSK.

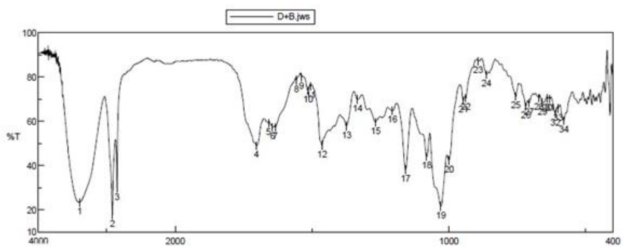


Fig. 3: FTIR spectrum of optimized formulation F4 (Forskolin (% w/v) 0.5, SA: PVA (1:0.25) 2% w/w)

Characterization

All formulated samples were examined by a scanning electron microscope (Hitachi S450, Japan) after drying for a period of 48 hr (Hu, Ji *et al.*, 2005). Fourier-transformed infrared (FTIR) spectroscopy was carried out to evaluate the interaction between formulation ingredients, if any. The FTIR analysis was performed for all ingredients with drug using FTIR spectrophotometer (Shimadzu, Japan) (Pandey *et al.*, 2010).

% ***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.

$$\%\,\text{Degree of swelling} = \frac{w - wd}{wd} \times 100 \qquad (1)$$

Where *w* is swollen nanofibers weight, *wd* is the dried mass, calculated by drying the swollen nano fiber mats at 40°C (Marziyeh, Jaleh *et al.*, 2011).

In vitro % drug release

The known mass with 2.5 × 2.5cm 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 Vidya Peeth 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-Benutzungdes 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:

$$\Delta\text{IOP} = \text{IOP Dosed eye} - \text{IOP Control eye} \qquad (2)$$

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 7cm distance between Capillary tip -to- Collector. fig. 1(b) representing the uniform fibers with the condition of 2% polymer, applied potential 20 kV and 15cm 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

Ingredients	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Forskolin (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SA: PVA (1:0.25)	2%	2%	2%	2%	4%	4%	4%	4%
Experimental Parameters								
Applied voltage (kV)	15	20	15	20	15	20	15	20
Capillary tip-to- Collector distance (cm)	10	10	15	15	10	10	15	15

voltage and 10-15 cm distance between Capillary tip -to-Collector.

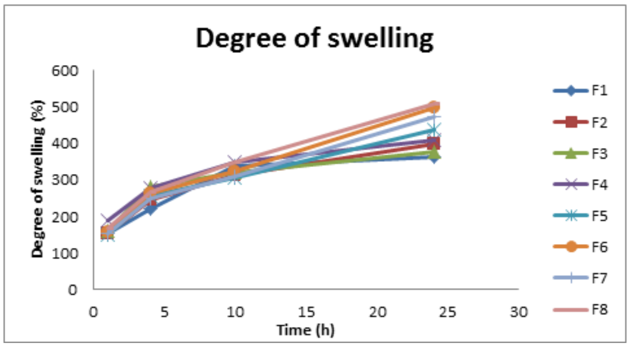


Fig. 4: Degree of swelling for Nanofibrousmats

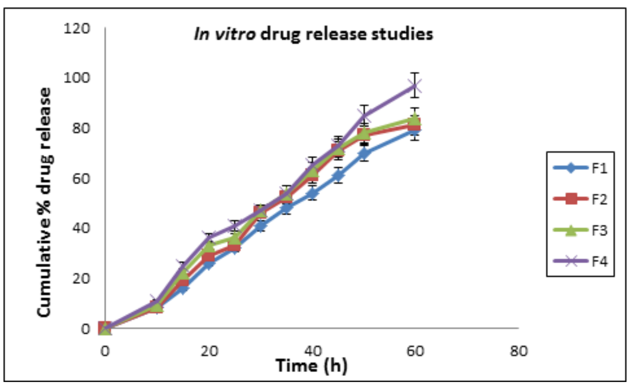


Fig. 5: *In vitro* drug release profile for F1 to F4.

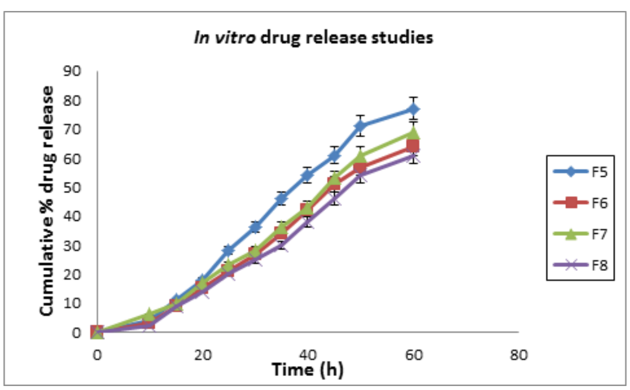


Fig. 6: *In vitro* drug release profile for F5 to F8.

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.

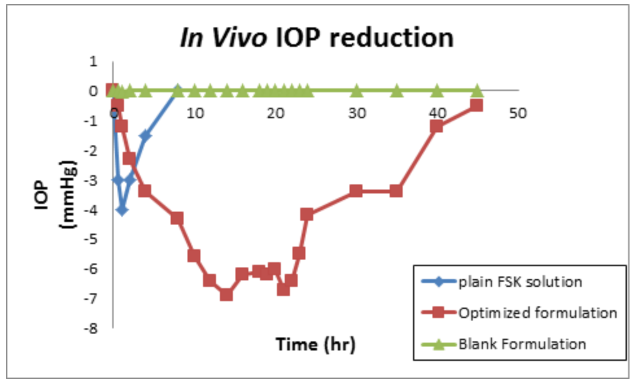


Fig. 7: *In Vivo* Intra ocular pressure (IOP) reduction profile.

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 gelleing agent which prolonged the release and it 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 45h (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.

ACKNOWLEDGMENT

The authors are also thankful to Dr. Ravi Kumar, Principal, Geethanjali College of Pharmacy, Keesara, RR District for their kind cooperation and providing necessary facilities to conduct *In Vivo* studies.

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