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Development and characterization of nano-fiber patch for the treatment of glaucoma

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ABSTRACT

In the present work polymeric nano-fiber patches was developed for the effective treatment of glaucoma using timolol maleate and dorzolamide hydrochloride as model drugs. The nano-fibers were prepared by electrospinning technique and were characterized on the basis of fiber diameter, morphology, entrapment efficiency, mucoadhesive strength, and drug release behavior, etc. Final formulations were inserted in the cul-de-sac of glaucoma induced rabbits and the efficacy of the formulation was evaluated. The results clearly indicated the potential of the developed formulation for occur drug delivery. There was a significant fall in the intraocular pressure compared to commercial eye drops.

1. Introduction

Effective ocular drug delivery has remains an unmet challenge till date. Rapid elimination of conventional liquid eye drops is still an unsolved problem in ophthalmic drug delivery. Rapid and high tear turnover due to irritation caused by the drug itself, by the excipients may further elevate the precorneal losses. Administration of larger volume conventional eye drops also remains helpless to improve the drug bioavailability. Due to this rapid precorneal losses the half-life of drugs applied in the form of conventional formulations is considered to be between about 1–3 min and merely 1–3% of the total dose is actually able penetrates through the cornea to reach intraocular tissues (Lee and Robinson, 1979; Patton and Robinson, 1976). Moreover, this poor absorption may leads to drainage of a significant amount of administered drug to the nose or gut area. Both of these are effective portals to deliver the drug to the systemic circulation to evoke unwanted side effects and toxicity of the drug (Gaudana et al., 2010). The delivery of dug to the systemic circulation has completely overshadowed the significance of topical drug delivery. In past few years a lot of efforts have been made to improve the situation but we are unable to achieve the desired goal.

Some attempts have been made to improve bioavailability using viscous or sticky ointments but these formulation leads to a total blurring of vision. Ocular inserts are difficult to administer and sometime they may be more difficult to remove if non-biodegradable in nature. In past few years utilization of colloidal carriers has become a hot area of research. Vesicular structure including liposomes may have the disadvantage of lower stability or eye irritation in case of surfactant based vesicle (niosomes) may be a hindrance. Polymeric nano/microparticles have shown some promise and improved the drug bioavailability to some extent (Valls et al., 2008). These particles can be effectively administered in the liquid form just like eye drop solutions and can improve drug retention by selecting mucoadhesive polymers. However, they are also unable to completely rectify the problem and the particles itself can be spill out with due to blinking and tear turnover.

In the present research work, we developed a nano patch for effective ocular drug delivery. We selected glaucoma as a model disease and a combination of timolol maleate and dorzolamide hydrochloride as model drugs. The nano-fibers patches were developed by using electrospinning. Electrospinning is one of the best methods for the fabrication of nano-fibers with average diameters in sub-micrometer down to nanometer range (Chuangchote and Supaphol, 2006; Huang et al., 2006; Wu et al., 2010). Due to very small nano-fibers diameter and hence exceptionally high surface area significantly higher drug content can be loaded in a very small piece of the Nano patch. The developed nano-fibers patch can be effectively inserted to the cul-de-sac area with a limited contact with the corneal region. The cul-de-sac can transiently retain the maximum volume (~30 μl) of the of the administered eye drop. This is devoid of any problems such as blurred vision, irritation and patient discomfort. The selected polymers i.e., Polyvinyl alcohol (PVA) (Koski et al., 2004; Pham et al., 2006) and polycaprolactone (PCL) (Sharma et al., 2011) are the biodegradable and biocompatible in nature. Therefore, the patch will degrade with time and come out. PVA is the one of the most widely used

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biodegradable polymer in the drug delivery system. Polyvinyl alcohol (PVA) is a semi-crystalline, hydrophilic polymer with good chemical and thermal stability (Koski et al., 2004). The physical characteristics and its specific functional uses depend on the degree of polymerization and the degree of hydrolysis. The excellent chemical resistance, physical properties and biodegradability of PVA have led to the development of many commercial products based on this polymer. Slow degradation rate of PCL makes it suitable for fabrication of nano-fibers patch for prolonged drug delivery. The Food and Drug Administration (FDA) have approved PCL for use in the drug delivery. Moreover, electrospinning was done under UV-light which provides an additional advantage of *in situ* sterilization.

2. Materials and methods

2.1. Materials

PCL with Mn ~ 80 KDa was purchased from Sigma–Aldrich (Bangalore, India). The solvents *N, N*-dimethyl formamide (DMF) and acetone were obtained from CDH Laboratory Reagents (New Delhi, India). PVA with Mw ~ 200 KDa was purchased from CDH Laboratory Reagents (New Delhi, India). Timolol maleate and dorzolamide hydrochloride were obtained as gift samples from MMG Healthcare (Sirmour, India).

2.2. Fabrication of the electrospun nano-fibers patches

PVA and PCL nano-fibers were produced by an electrospinning technique. PVA and PCL (10% w/v) solutions were prepared in deionized water and a mixture of DMF and acetone (1:1 ratio) respectively. Timolol maleate (0.5% w/v) and dorzolamide hydrochloride (2% w/v) solutions were added to the homogeneous polymeric solution with constant stirring. The final solution was subjected to electrospinning using rotating cylindrical drum as a collector placed at a distance of 15 cm from the needle under an applied voltage of 15 KV and flow rate of 0.2 ml/h. The nano-fiber sheets were cut into of 1 cm^2 patches and used for further characterization and evaluation parameters.

2.3. Characterization of nano-fiber patches

Nano-fiber patches were characterized on the basis of various parameters such as scanning electron microscopy, infrared spectroscopy, differential scanning calorimetry, drug loading, entrapment efficiency, degree of swelling, *in vitro* drug release, folding endurance and mucoadhesive strength.

2.3.1. Surface morphology

Surface characteristics such as uniformity in diameter, smoothness of surface etc. are important parameters to evaluate the quality of the developed nano-fibers sheet. Surface morphology of the nano-fibers was analyzed by using scanning electron microscope (EVO M-10, Zeiss, Germany). The vacuum dried nano-fiber patches were coated with gold palladium and observed microscopically at accelerating voltage of 15 kV (Garg et al., 2013).

2.3.2. Evaluation of chemical properties and drug interaction

The infrared spectrum of the drug loaded nano-fibers patches were obtained by using IR spectrometer (Nicolet-380, Thermo, USA). The IR spectra were used to evaluate the interactions between the functional groups of the polymers as well as assure the absence of any significant changes in the chemical composition of the drug after being electrospun into nano-fibers. DSC used to study degree of crystallinity, polymorphic transitions or thermal transitions due to high energy exposure during electrospinning. The phase transitions of different nano-fiber were analyzed by differential scanning calorimetry (DSC 1 STAR system; Mettler Toledo, USA) in a perforated aluminium sealed 50 μl pans at a heating rate of 5 °C/min from 30 to 300 °C using nitrogen as blanket gas (50 ml/s) (Garg et al., 2012).

2.3.3. Evaluation of drug entrapment

Electrospun nano-fibers are expected to possess high drug entrapment efficiency due to presence of exceptionally high surface area. Entrapment efficiency describes the efficiency of the preparation technique to incorporate drug into carrier system (Wang et al., 2004). The entrapment efficiency of nano-fibers was calculated by drying the drug loaded nano-fibers at 40 °C for 5 min inside a hot air oven and then bath sonicated (Steryl Medi Equip. System, Chennai) for 30 min in the simulated lachrymal fluid of 7.4 pH. The amount of drug was then analyzed by UV derivative spectra (Parnami et al., 2013). The entrapment efficiency was calculated using standard formula.

$$\text{Drug entrapment efficiency } (\%) = A_{\text{observed}}/A_{\text{initial}} \times 100.$$

2.3.4. Degree of swelling

Degree of swelling represent the relative measure of the cross linking between the nano-fibers. The degree of swelling of unloaded and drug loaded nano-fiber patches in artificial tear fluid of pH 7.4 at 37 °C were compared at different time intervals (4th, 8th, and 12th h) (Duan et al., 2007). Degree of swelling was calculated according to the following equation as;

$$\text{Degree of swelling } (\%) = (M - M_d)/M_d \times 100$$

where M is the weight of swollen nano-fibers sample and M_d is the weight of dried nano-fiber (at 40 °C).

2.3.5. In vitro drug release

The drug release from electrospun nano-fiber patches was measured by placing a 1 × 1 cm^2 of drug loaded nano-fiber patch into an activated cellophane membrane, that was then kept in a beaker containing 50 ml artificial tear fluid (pH-7.4) at 37 °C and being stirred continuously. Samples (1 ml) were taken at different time intervals (0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h) and the released drug content was analyzed using UV spectroscopy (Taepaiboon et al., 2007).

2.3.6. Folding endurance

Folding endurance describe the flexibility of the formulation. Folding endurance was measured by folding the nano-fiber patch at the one point till it breaks (Kusum Devi et al., 2003). This property can ensure the behavior of the developed formulation during formulation, packaging, transportation and application.

2.3.7. Mucoadhesive strength

Mucoadhesive strength represents the adhesiveness of formulation with the mucous membrane. The extent of mucoadhesive strength will directly reflect the residence time of the patch in the eye. Mucoadhesive strength of each formulation was determined in the form of force required to detach corneal membrane from the formulation using the Brookfield texture analyzer, USA. The film was applied to the upper probe with the help of a double-sided adhesive tape. The corneal membrane was fixed on lower disc. The upper probe was then lowered at a speed of 0.1 mm $s^{-1}$to touch the surface of the nano-fiber. A force of 0.1 N was applied for 5 min to ensure intimate contact between the membrane and the nano-fiber patch. The force required to detach the film from the tissue surface was determined while moving the upper probe of the texture analyzer was moved up at a speed of 0.1 mm s^{-1} (Semalty

et al., 2008). The surface area of exposed mucous membrane was kept constant (1.13 cm^2) for better comparison.

2.3.8. In vivo studies

The studies were carried out accordance to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3.8.1. Glaucoma inhibition studies. Nine New Zealand white Albino rabbits (having weight 3–5 kg) were divided into three groups. Glaucoma was induced by installing 1.0% w/v sterile topical formulation of Prednisolone Acetate, USP thrice a day in the eyes of rabbits for 2 weeks. The rabbits with intraocular pressure (IOP) ranging from 25 to 30 mm Hg were considered as glaucomatous. The regular IOP was measured by using Shiotz Tonometer (Riester, Germany) at different time intervals (0th, 0.5th, 1st, 2nd, 3rd, 4th, 6th, 8th,12th and 24th h). The reduction in intraocular pressure with time was calculated to evaluate the efficacy of the developed formulations (Gupta, 2011).

2.3.8.2. Eye irritation studies. Eye irritation was also calculated based upon different parameters like blinking rate, redness and discharge volume at different time intervals the results were compared with the response of the control group animals (Johnson and Goodwin, 1985).

3. Results and discussion

3.1. Surface morphology

Diameter and shape of the nano-fibers was determined using SEM. The diameter of all nano-fibers was found to be in the range of 200–400 nm. The drug loaded nano-fibers showed an increase in diameter compared to the blank nano-fibers. It could be attributed due to insufficient evaporation of the solvent from drug loaded nano-fiber, resulting higher specific volume per unit mass of the nano-fiber. Further presence of relatively higher quantity solvent shows a more homogeneous distribution of the formulation content resulting in a smooth surface of the nano-fiber, which is suitable for ocular drug delivery. Fig. 1 shows the SEM images of drug loaded PVA nano-fibers (a), (b) and PCL nano-fibers (c), (d).

3.2. Chemical characterization and drug interaction

Infrared spectroscopy was done for analyzing the compatibility between polymer and drugs in the final nano-fiber formulation. The FTIR spectra's of drugs loaded PCL nano-fiber and PVA nano-fibers are shown in Fig. 2(a) and (b) respectively. The peaks found to be concordant with functional groups present in structure of respective polymers and drugs. Therefore, the results confirmed that there was no interaction between polymers and drugs. The results were further supported by DSC thermographs. The DSC thermographs of PVA and PCL nano-fibers also showed characteristic endothermic peaks at 192.03 °C (Fig. 3(a)) and 60 °C (Fig. 3(b)) respectively.

3.3. Drug entrapment efficiency

The developed formulations possess an exceptionally high surface area and it was also reflected in the results of drug entrapment. We observed that the entrapment efficiency was found to be around 100% w/w (96.4 w/w for PVA nano-fiber and 95.2% w/w PCL nano-fiber). Higher entrapment efficacy is attributed to passive drug loading technique involves the incorporation of therapeutic agent into the polymeric solution to be spun. This slight decrease in the entrapment efficiency could be due to the decreasing potential drainage capacity of collector as the fiber get deposited on the surface of the collector leading to distraction of fibers from the collector site. Higher entrapment efficiency further allows significant flexibility to miniature the size of nano-fiber film for ocular administration. Higher entrapment efficiency led to smaller film size making patient convenient for self-administration and resulted in lesser ocular irritation.

3.4. Degree of swelling

Degree of swelling plays an important role in the drug release behavior from the nano-fiber patch. The degree of swelling was found be higher in case of PVA nano-fibers compared to the PCL nano-fibers (Fig. 4). The reason behind this pattern could be small diameter of PVA nano-fibers and due to smaller diameter they have more pockets for absorbing the liquid media and space for swelling. Furthermore PCL have relatively hydrophobic nature due to which it absorbs least and degree of swelling was least in the case of PCL nano-fibers. The degree of swelling decreased after the 12 h. This could be due to degradation of polymeric nano-fibers in ATF 7.4.

3.5. In vitro drug release

In vitro release studies showed that the developed nano-fibers are capable of controlled drug delivery up to 24 h (Fig. 5). There was an initial burst release of the drug followed by controlled release. The initial burst release may be attributed to the enormous surface area of the nano-fibers followed by controlled release of the drug from the core of the nano-fibers. The release behavior is also supported by the swelling studies where the degree of swelling was found to be increased after 4 h, indicated that the release mechanism is predominantly governed by diffusion process. Extended drug release from the experimental nano-fiber membrane is further associated to its micro-porous structure which restrict the free access of the dissolution medium into the fiber matrix. The release from the PCL nano-fibers was slower than the PVA nano-fibers. It could be explained on the basis of hydrophobic nature of PCL, lower degree of swelling. The solvent influx in the PCL nano-fibers was slower resulting in a slow release of the drug.

3.6. Folding endurance and mucoadhesive strength

Folding endurance indicates the mechanical strength of the developed nano-fibers patch. An optimal folding endurance level is essential from day 1 (the production) of the patch up to its application. Both the nano-fiber patches showed the good folding endurance, which revealed that nano-fibers have enough flexibility and can easily get adjusted in ocular tissue. The folding endurance of PVA and PCL nano-fiber formulations are 327 ± 3 and 415 ± 5 respectively. The selected polymers exhibit high mucoadhesive property. Mucoadhesive strength of the developed nano-fibers patch was found to be 212 gm/cm^2 (Fig. 6a) and 159 gm/cm^2 (Fig. 6b) for PVA and PCL respectively. The results revealed that both nano-fibers patches have the good mucoadhesive nature and so can be retained in the ocular region for a longer duration. The PVA nano-fiber patches showed higher mucoadhesion as comparative to PCL nano-fibers.

3.7. Reduction in IOP

In vivo studies results showed that the nano-fiber formulation showed the significant reduction in IOP for the prolonged time period (Fig. 7). Marketed formulation resulted in a rapid onset of ac-

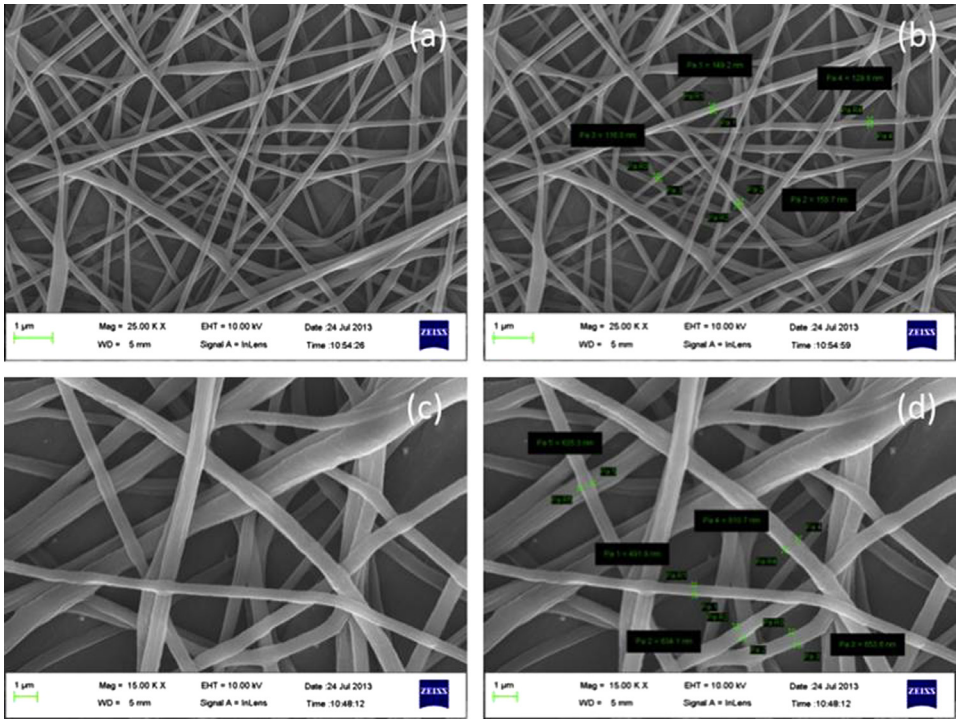

Fig. 1. SEM images of drug loaded PVA nano-fibers (a & b) and PCL nano-fibers(c & d).

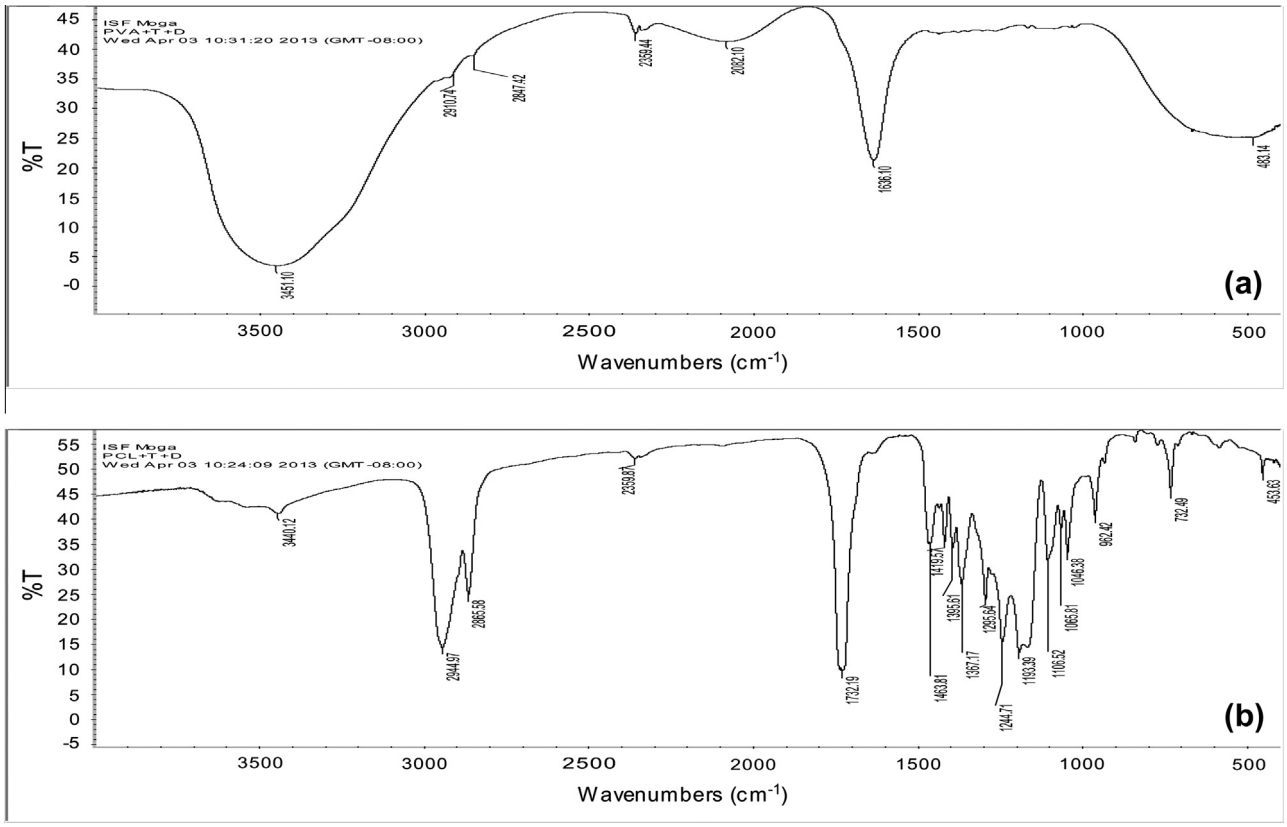

Fig. 2. FTIR Spectra of (a) PVA with drugs (timolol and dorzolamide) (b) PCL with drugs (timolol and dorzolamide).

tion (<1 h) but maintained for a shorter duration. The maximum reduction in case of conventional eye drop was obtained within 4 h. However, a constant reduction in the IOP was for a longer duration and the peak effect was observed after 16th and 20th h in case of PVA and PCL nano-fibers respectively. They maintained the IOP up to 72 h. This could be attributed to the controlled drug

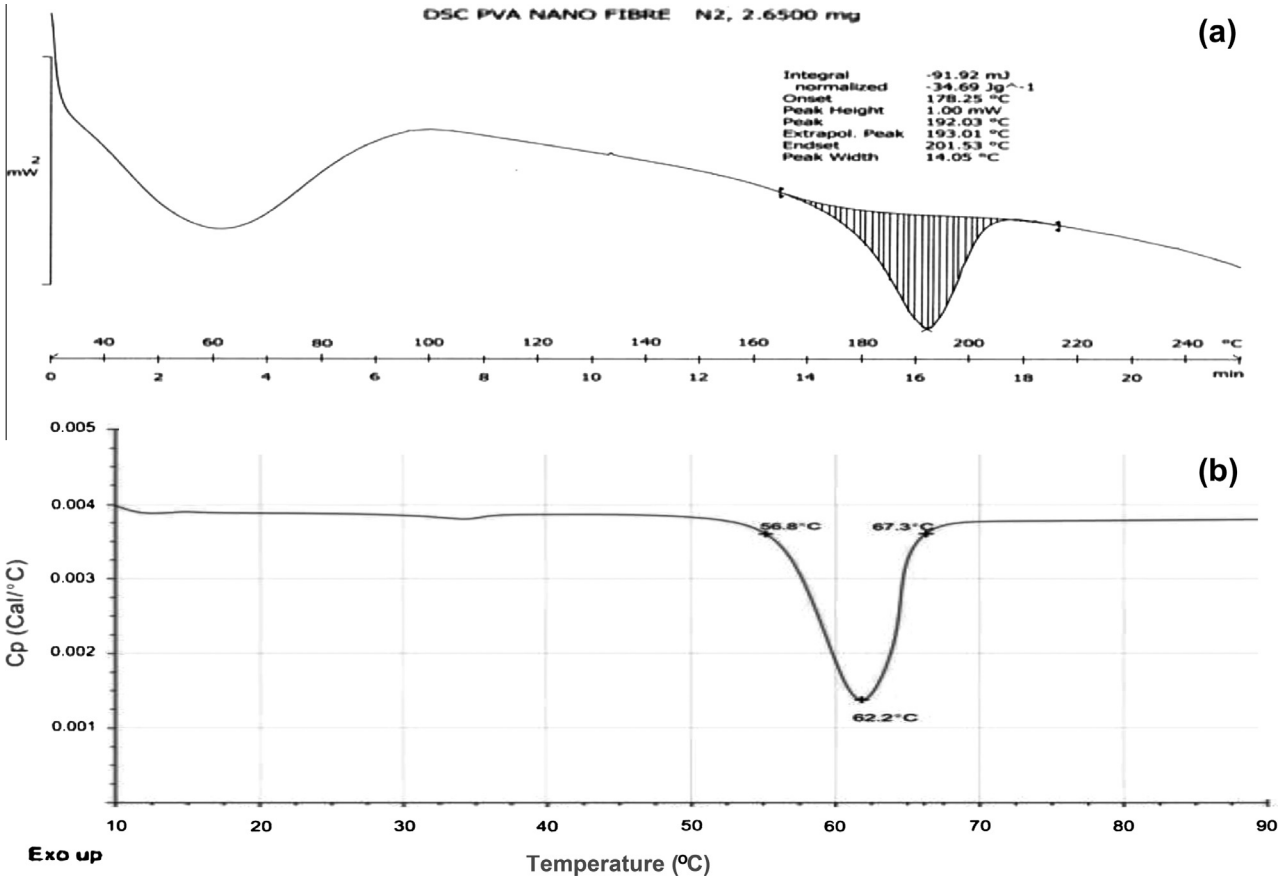

Fig. 3. DSC curve of (a) polyvinyl alcohol Nano-fibers (b) Polycaprolactone Nano-fibers.

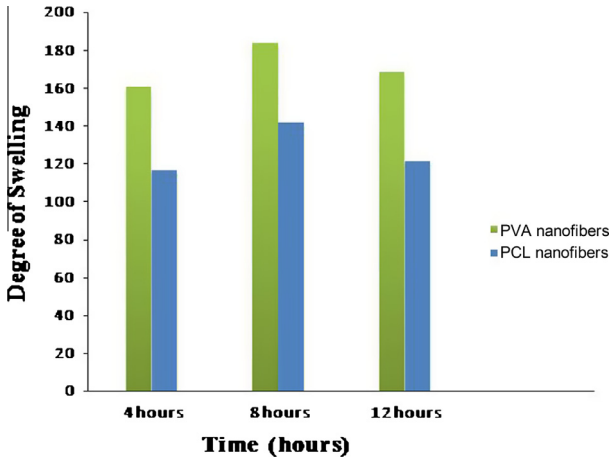

Fig. 4. Degree of swelling of various polymeric nano-fibers.

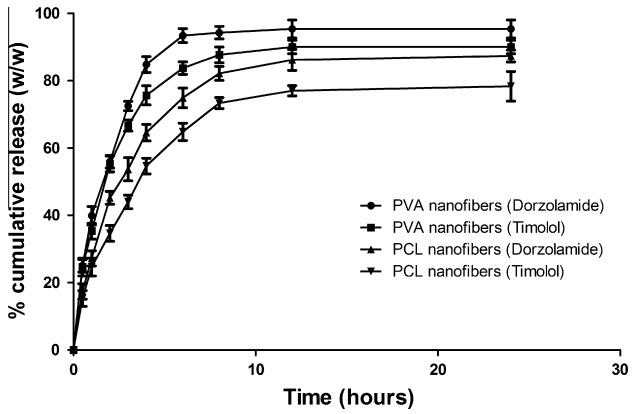

Fig. 5. *In-vitro* release of PVA & PCL polymeric nano-fibers.

release, which allows specific accumulation of drugs within the target region of the ocular cavity, resulting an extended therapeutic activity up to 72 h. Rapid drainage of the drug through the nasolacrimal duct in case of marketed formulation limits its therapeutic activity to 4 h. Further the late peak level in case of PCL over PVA nano-fibers could be associated to polymer degradation and water absorption capacity of the selected polymer under the physiological condition. Since PVA possess higher water absorption capacity than PCL as indicated by swelling study, therefore PCL nano-fibers takes more time to generate therapeutic peak level than PVA nano-fibers.

3.8. Ocular irritation

The ocular irritation was examined by observing the corneal, iris and conjunctivae of the experimental animals (rabbits) according to the Draize test (Table 1). PVA nano-fibers showed no irritation in the rabbit eyes but in case of PCL nano-fibers minor irritation was observed which could be due to its relatively hydrophobic and ionic nature of the polymer.

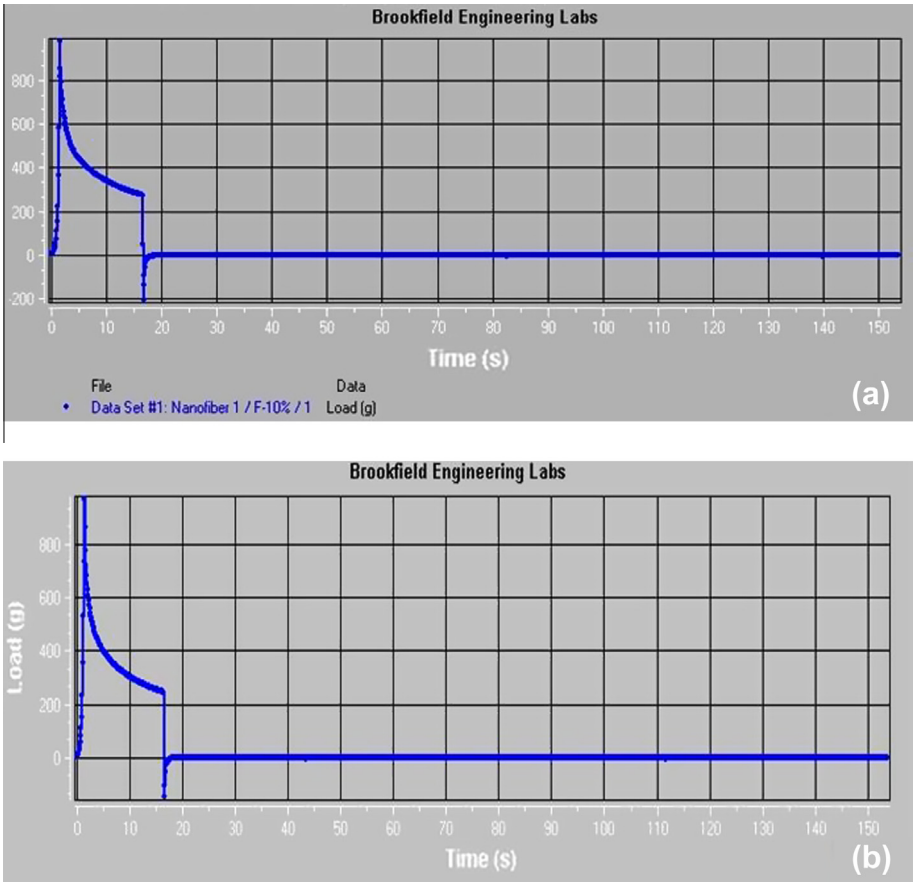

Fig. 6. Mucoadhesive strength of (a) PVA Nano-fibers (b) PCL Nano-fibers.

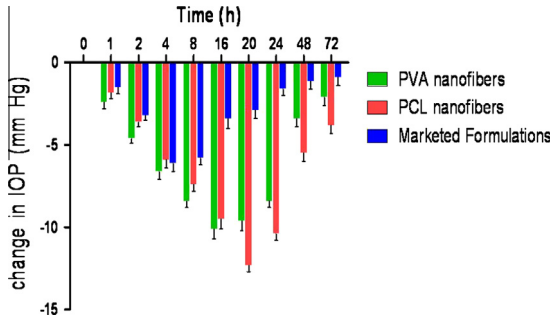

Fig. 7. Ocular hypotensive activity, *in-vivo* activity of nanofiber formulations.

4. Conclusion

In the present work biodegradable polymeric nano-fibers were successfully developed by using electrospinning technique. The microscopic study indicated that the nano-fibers were uniform in diameter with smooth surface. We achieved very high (~100%) entrapment efficiency. The developed formulation possesses very high mucoadhesive strength, thus can be retained for a longer period in the eyes. Moreover, the formulation is capable of maintaining the intraocular pressure for up to 72 h. Therefore, the duration of action can be better controlled by varying these conditions and can be optimized as per our requirement. Hence, the nano-fibers

Table 1
Ocular irritation test according to Draize test.

Formulations	Time (h)	Cornea		Iris	Conjunctivae			Total score value
		Opacity	Area of cornea involved	Interruption for reaction to light	Redness	Chemosis	Discharge	
Control (PBS 7.4)	1	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0
PVA nano-fiber patch	1	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0
PCL nano-fiber Patch	1	0	0	0	1	0	0	2
	24	0	0	0	1	0	0	2
	48	0	0	0	1	0	0	2
	72	0	0	0	1	0	0	2

patch can be effectively utilized for ocular drug delivery in various conditions and not limited to the glaucoma only.

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