

ORIGINAL ARTICLE

Development of polyvinyl alcohol–gelatin membranes for antibiotic delivery in the eye

Dharmendra Jain¹, Edmund Carvalho¹, Ajit Kumar Banthia² and Rinti Banerjee¹

¹Department of Biosciences and Bioengineering, Indian Institute of Technology, Mumbai, India and ²Materials Science Centre, Indian Institute of Technology, Kharagpur, India

Abstract

Aim: The aim of the present study was to develop biosynthetic hybrid polymer-based ocular insert for topical administration of antibiotics for treatment of ocular infections. **Methods:** Ciprofloxacin hydrochloride-loaded three different inserts were prepared by solution casting method by esterification of a biopolymer (gelatin) with a synthetic polymer (polyvinyl alcohol, PVA). Esterification between PVA and gelatin was confirmed by Fourier transform infrared spectroscopy. **Results:** Inserts were found to be wettable and swellable with simulated tear fluid and had a contact angle $<50^\circ$ with simulated tear fluid. Mechanical properties of PVA–gelatin (10:3 wt%) inserts included a maximal tensile strength of 8.6 ± 2 MPa and the inserts showed adequate mucoadhesion with reconstituted mucin. In vitro drug release of ciprofloxacin hydrochloride for up to 24 hours was observed from the inserts. Inserts were found to be biocompatible using SIRC rabbit corneal epithelial cell line by sulforhodamine B assay and by Draize test in albino rabbits. Further inserts showed higher ocular penetration of sodium fluorescein in goat eye as compared to eyedrop solution. **Conclusions:** In brief, the study suggests that PVA–gelatin polymeric blends are promising as ocular inserts for prolonged release of antibiotic in the eye as compared to eyedrops. Such inserts may also be therapeutically beneficial for treatment of corneal ulcers and external ocular infections.

Key words: Ciprofloxacin hydrochloride, gelatin, ocular inserts, ophthalmic drug delivery, polyvinyl alcohol

Introduction

Medication is generally applied to the eyes in the form of conventional dosage forms like aqueous eyedrops, which comprise 90% of the currently used ophthalmic formulations. The main drawbacks of eyedrops is poor bioavailability because a major part of the administered drug is drained away from the precorneal area because of lachrymal secretion, tear turnover, and nasolacrimal drainage¹. Thus, frequent instillation of concentrated drug solutions is required to achieve desired therapeutic effects leading to pulse kinetics with an initial period of overdosing leading to side effects, followed by a period of underdosing leading to poor availability of the drug². Also, because of nasolachrymal drainage, some drug may get absorbed in the gastrointestinal tract and cause unwanted side effects.

An ideal ocular delivery system should be biocompatible, should increase the contact time of drug in the eye, hence its bioavailability, reduce systemic absorption, and show a sustained and controlled drug release. The drug levels achieved at the target sites should be equal to or above the minimum effective concentrations. Ocular inserts, which are solid devices generally placed in the conjunctival sac, may fulfill these criteria and are feasible as alternatives to conventional ocular delivery systems². Accurate dosing, no requirement of additives, for example, preservatives, reduction of systemic absorption, low risk of side effects, and sustained drug release are other added advantages of the ocular inserts^{3–5}.

Hornof et al.⁴ formulated mucoadhesive inserts based on synthetic polymer thiolated poly(acrylic acid) loaded with a model drug diclofenac sodium. The inserts showed release of diclofenac sodium for 8 hours. Chetoni et al.

Address for correspondence: R. Banerjee, Department of Biosciences and Bioengineering, Indian Institute of Technology, Mumbai 400076, India.
E-mail: rintib@iitb.ac.in

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formulated oxytetracycline containing ocular inserts based on silicone elastomer grafted with polyacrylic acid or polymethacrylic acid. The inserts showed in vitro sustained release of oxytetracycline hydrochloride³. Colo et al. developed ofloxacin-loaded ocular inserts based on poly(ethylene oxide). The study indicates that inserts increased the maximum drug concentration (C_{\max}) with respect to commercial eyedrops⁶. Hsiue et al. investigated pilocarpine trapped in diffusion-controlled hydrophilic inserts of poly(2-hydroxyethyl methacrylate) as sustained drug release systems with an in vitro release of pilocarpine for 24 hours⁷. Clearly, inserts are promising biomaterials for ophthalmic drug delivery. However, there has been some concern regarding the toxicity and biodegradability with the synthetic polymers used in these inserts. The present study is the first study using combinations of synthetic and biopolymers for topical administration of ciprofloxacin in the eye.

In the present study, polyvinyl alcohol (PVA) and gelatin were chosen for development of inserts, because of their biocompatibility and biodegradability. PVA has excellent film-forming and adhesive properties. PVA hydrogel has excellent transparency and is biocompatible⁸. Gelatin is a nonantigenic natural protein obtained by a controlled hydrolysis of fibrous insoluble collagen⁹. It has gained much interest as edible films for its abundance and biodegradability. Gelatin is known to have mucoadhesive properties and is well tolerated on ocular administration^{10,11}. However, a gelatin film by itself has low mechanical properties, which limits its application as a biomaterial. Thus, it is often used after modification through several methods such as cross-linking, grafting, and blending^{9,12}. A rigid polymer like PVA was added to gelatin in this study to create polymeric blends having superior mechanical properties (because of PVA) and improved mucoadhesiveness (because of gelatin).

Ciprofloxacin hydrochloride was chosen as a drug of choice because of its widespread use in combating bacterial infections. Ciprofloxacin has efficacy against gram-positive and gram-negative bacteria and the frequency of spontaneous resistance to ciprofloxacin is very low¹³. It is used as a single agent for treatment of topical ocular infections such as conjunctivitis and keratitis caused by *Staphylococcus aureus*. Ocular infections like corneal ulcers and keratitis are frequently treated by topical application of the ciprofloxacin¹³. It has been approved as a topical preparation for the treatment of corneal ulcer¹⁴.

In the present study, we explored ciprofloxacin hydrochloride-loaded flexible and circular-shaped polymeric blends as matrix-type ocular inserts based on PVA and gelatin for ocular drug delivery.

Materials and methods

Materials

PVA (molecular weight 125,000 g) was purchased from S.D. fine-chem Ltd., Mumbai, India. Gelatin,

sulforhodamine B (SRB), globulin, lysozyme, and porcine mucin were purchased from Sigma-Aldrich, St. Louis, MO, USA. Trichloroacetic acid was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Ciprofloxacin hydrochloride I. P. was provided as a free gift by Cadila Pharmaceuticals Ltd., Ahmedabad, India. Glycerol, sodium chloride (extrapure AR), bovine serum albumin (96–98% purity) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. D-Glucose, calcium chloride, disodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, glacial acetic acid, and hydrochloric acid were purchased from Qualigens Fine Chemicals, Mumbai, India. Simulated tear fluid (STF) was freshly prepared according to the method described by Cohen et al., by dissolving bovine serum albumin (0.268 g), globulin (0.134 g), calcium chloride (0.008 g), sodium chloride (0.658 g), D-glucose (0.650 g), and lysozyme (0.268 g) in 100 mL Milli-Q water¹⁵. SIRC (rabbit corneal epithelial cells) cell lines were obtained from National Centre for Cell Science, Pune, India. Minimum essential medium eagle (MEM), Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), antibiotic-antimycotic solution, luria agar, and luria broth were purchased from Himedia Laboratories, Nashik, India. Ninety-six-well tissue culture plates were purchased from Nunc, Roskilde, Denmark. All other reagents were of analytical grade and were used as received. Milli-Q water was used as a solvent if not otherwise stated.

Method

PVA–gelatin ocular inserts were prepared by esterification of hydroxyl group of PVA with carboxyl group of gelatin similar to the procedure of Pal et al., with slight modifications¹⁶. Three different membranes were prepared by varying concentration of gelatin to be added in the membrane. A clear aqueous solution of PVA (12.5%, w/v) was prepared by dissolving 1.0 g PVA in 8.0 mL deionized water at 70°C. Three different gelatin solutions were prepared by dissolving 100, 200, and 300 mg of gelatin in 2.0 mL deionized water and adding to previously prepared PVA solution. The final concentration of PVA and gelatin in PVA–gelatin blend was 10% (w/v) for PVA and 1%, 2%, and 3% (w/v) for gelatin. Five hundred microliters of glycerol was added to above blend as plasticizer. Esterification between hydroxyl groups of PVA and carboxylic groups of gelatin was initiated in acidic medium by adding hydrochloric acid (50–100 μ L). Simultaneously, ciprofloxacin hydrochloride was added to the blend under magnetic stirring for homogenous dispersion. The resulting solution was stirred at 70°C for 30 minutes. The dispersion so obtained was converted to membranes by solution casting method on Petri plates. The membranes were dried at room temperature in a laminar airflow chamber under sterile conditions. Finally, the dried membranes were carefully removed and rinsed with sodium hydroxide solution. Inserts of

6 mm diameter and thickness of 0.3–0.4 mm were punched out from the membranes and stored in sterile containers until further use. Unesterified inserts [PVA alone (10%, w/v, 10 mL) and gelatin alone (13%, w/v, 10 mL)] were also prepared by solution casting method and served as controls.

Physicochemical properties

Inserts' characteristics and morphology

The diameter and thickness of the inserts were measured with electronic vernier calipers with sensitivity of 0.01 mm. About 5–10 thickness measurements were made on each insert and average was taken. Samples were stored at 27°C and $50 \pm 5\%$ relative humidity prior to further characterization. Surface morphology of inserts was studied using scanning electron microscope (SEM) and atomic force microscopy (AFM). Inserts were sputtered with gold and fixed evenly on stubs using adhesive tapes to analyze surface morphology by SEM.

Fourier-transform infrared spectroscopy

The interactions which resulted in the formation of inserts were investigated by Fourier-transform infrared (FT-IR) spectroscopy. Fourier-transform infrared spectra of ocular inserts were recorded at room temperature using Spectrum One FT-IR spectrometer (PerkinElmer, Shelton, CT, USA) in the range of 400–4000 cm^{-1} . Insert was mounted directly in the sample holder. Spectra of PVA and gelatin were recorded with KBr pellet.

Wettability (contact angle)

Wetting is the contact angle between a liquid and a solid surface, resulting from intermolecular interactions when the two are brought together. The primary measurement to determine wettability is a contact angle measurement. This measures the angle between the surface of the solid and the surface of a liquid droplet placed on the solid.

Wettability of inserts was determined by measuring contact angle using Milli-Q water and STF¹⁷. Contact angles of inserts were measured by sessile drop technique using a CAM-100 optical contact angle meter (KSV Instruments, Helsinki, Finland). A drop of wetting liquid (Milli-Q water/STF) was carefully deposited with a syringe onto the surface of an insert mounted on glass slide. The image of droplet was analyzed by an automated curve-fitting program using inbuilt software. The contact angle was measured as an average angle between the surface and the tangent drawn to the surface of droplet at interface at right and left extremes of the drop. All measurements were made immediately following deposition of the drop. All reported data are mean values of nine independent measurements.

Swelling study

Swelling of the inserts was determined by placing drops (10 μL) of STF on inserts at regular time intervals till 360 minutes at $35 \pm 2^\circ\text{C}$, as the temperature of ocular

surface is 35°C ¹⁸. After regular periods of time, inserts were wiped with lint-free tissue paper to remove excess surface STF, weighed, and returned to the same container. The procedure was repeated until there was no further increase in the weight, that is, equilibrium swelling of the inserts had been reached. All measurements were made in triplicate for each insert. The increase in weight relative to the dry weight was evaluated as a measure of swelling.

Mechanical properties

Mechanical properties of the inserts such as tensile stress, elastic modulus, and elongation to break were measured with a computer-controlled universal testing machine (UTM; Tinius Olsen, Surrey, England) according to ASTM standard method 5K ASTM D3500 (Tensile strength) [XHead]. Mechanical properties of the inserts were determined at room temperature and $50 \pm 5\%$ relative humidity. The dried membranes were cut as thin strips and fixed between the rubber grips of UTM. Initial grip separation was set at 50 mm¹⁹. The crosshead speed was set at 30 mm/min. Mechanical properties were determined from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of original length). Tensile strength measures the force required to elongate a material to the point of break. Elastic modulus, a measure of intrinsic stiffness of materials, is the slope of the linear range of stress-strain plot. Mechanical properties for each type of insert were replicated for at least three samples, and average values are reported as the results.

In vitro mucoadhesion study

The mucoadhesive property of inserts was evaluated by means of a tensile test, where the measurement of maximum force to detach an insert from reconstituted mucin after an initial period of intimate contact was done. The force required to evaluate the mucoadhesive potential of PVA–gelatin inserts was measured by a UTM (Tinius Olsen) consisting of a lower and an upper plate and connected with computer-integrated data acquisition software. The lower plate was fixed and the upper plate was connected with a movable load cell, measuring the force of detachment. For mucoadhesion testing, insert was attached to the upper plate and reconstituted mucin was spread carefully on the lower plate. Subsequently, intimate contact between insert and mucin was assured by lowering the upper plate until it touched the insert's surface. The surface of the insert was ensured to remain wet with mucin during the study period. After a predetermined time period (60 minutes), the upper plate was elevated at the speed of 0.1 mm/min until complete detachment of the insert from the mucin occurs, and the force of detachment was measured with the help of the instrument software. In a control experiment, only mucin was spread between the plates and the force of detachment of plates was measured. Mucoadhesion

study of the inserts was performed at room temperature and $50 \pm 5\%$ relative humidity. All detachment tests were carried out at room temperature. At least six measurements were performed for each type of insert and average values are reported.

In vitro drug release study

The in vitro release study was performed using a specially designed continuous flow-through apparatus in triplicate. Insert of known dimensions and weight was placed in donor compartment and flow rate of phosphate buffered saline (PBS) (pH 7.4) was maintained at 0.60 ± 0.15 mL/h over the insert²⁰. The amount of drug released was determined by collecting aliquots from the receiving chamber at prefixed time intervals. Temperature was maintained at $35 \pm 2^\circ\text{C}$ throughout the study. Absorbance was measured by ultraviolet (UV) spectrophotometry at 275 nm using a UV-Visible spectrophotometer (PerkinElmer). Finally, the concentration corresponding to absorbance was determined from the calibration curve. The drug release profile of ciprofloxacin from inserts after predetermined time intervals is expressed as percentage of drug loading.

Antimicrobial efficacy study

Microbiological studies were carried out to ascertain the biological activity of the drug-loaded inserts against microorganisms. *Staphylococcus aureus* and *Escherichia coli* were used as the test microorganisms. A layer of sterilized luria agar (20 mL) was solidified in Petri plates. Overnight cultures of test microorganisms were spread in agar plates. Sterile inserts and control were placed on the plates and incubated at 37°C for 24 hours. Results were compared with placebo formulations. The entire study except incubation was carried out in a laminar air-flow chamber. The zone of inhibitions (ZOIs) measured around each insert were compared with documented ZOIs of a corresponding amount of free drug. The study was done in triplicate and average of diameters of all ZOIs was taken.

Hemolysis study

To check the hemocompatibility of the inserts, an in vitro hemolysis study was performed using red blood cells (RBCs). The RBC membrane remains intact in normotonic solutions, for example, normal saline and with biocompatible materials, but lyses and releases hemoglobin in the presence of non-biocompatible materials. This property of RBCs was utilized to check the hemocompatibility of inserts.

Five milliliters of whole blood was collected from healthy individual and EDTA was used as an anticoagulant. Blood was centrifuged at $1500 \times g$ for 20 minutes. The buffy coat was removed and the packed cells (RBCs) were washed thrice with normal saline. Normal saline was added to the cells to get 40–50% hematocrit.

Hemolysis experiments were done through a method similar to that used by Sharma and Sharma, with slight modifications²¹. About 100 μL of RBCs were diluted to 3 mL with normal saline. This sample served as negative control because RBCs do not lyse in normotonic conditions. Again 100 μL RBC was diluted to 3 mL with double-distilled water. This sample showed 100% hemolysis, that is, positive control, as RBCs tend to lyse in hypotonic medium. Inserts were incubated at 37°C for 1 hour with 100 μL RBCs in test tubes and volume was made up to 3 mL with normal saline. All the inserts were tested for hemolysis in triplicates. The blood samples were then centrifuged at $795 \times g$ for 20 minutes and the absorbance of the supernatant was measured at 540 nm by a PerkinElmer UV-Vis spectrometer. Percentage hemolysis was calculated using the following formula:

$$\% \text{ Hemolysis} = \frac{\text{Absorbance of sample}}{\text{Absorbance of 100\% hemolysis}} \times 100. \quad (1)$$

Cytocompatibility study

The cytocompatibility of inserts was assessed with SIRC cells by SRB assay²². SRB assay is a quick, effective method for testing mitochondrial impairment and correlates with cell viability.

PVA-gelatin (10:3 wt%) inserts were taken for cytocompatibility study. The cells were grown in modified MEM, supplemented with 10% FBS and 1% antibiotic-antimycotic solutions, and incubated at 37°C temperature under 5% CO_2 and saturated humid environment. Nearly confluent cells in 25 cm^2 tissue culture flasks were trypsinized by trypsin-EDTA solution and resuspended in fresh media. Cell counts were done by hemocytometry. Resuspended cells were diluted accordingly and were plated at a concentration of 1×10^4 cells per well in a 96-well tissue culture plate and incubated in a CO_2 incubator (Jouan IG150). PVA-gelatin inserts' extracts (4 mg/mL) were prepared by incubating inserts in MEM for 24 hours at 37°C . After 24 hours, old media were removed from each well and inserts' extracts were added in each well in quadruplicate and kept for incubation in the CO_2 incubator for 24 hours at 37°C . After 24 hours, SRB assay was conducted. In brief, old media with the inserts' extracts were discarded and 200 μL fresh medium was added. Then cells were fixed by adding 50 μL of ice-cold 50% trichloroacetic acid slowly to the medium and incubated at 4°C for 1 hour. Then, the plates were washed 5 to 10 times with deionized water and dried in air. One hundred microliter of 0.4% SRB (Sigma, St Louis, MO, USA) dissolved in 1% acetic acid was added to the fixed cells and kept at room temperature for 20 minutes. Then the plates were washed with 1% acetic acid to remove unbound dye and dried at room temperature. One hundred microliter of 10 mM

Tris base (Sigma) was added to each well and kept for 20 minutes to solubilize the dye. Thereafter, the plates were placed on a shaker to allow mixing and the absorbance of each well was read in a plate reader (Thermo Electron Corporation, Vantaa, Finland) at 560 nm. Cell viability was measured as following:

$$\% \text{ Viability} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100. \quad (2)$$

Acute ocular toxicity study (in vivo tolerance assay)

Ocular toxicity studies were performed according to the Draize technique²³ on New Zealand white albino rabbits of 1.62–1.66 kg weight and 5–6 months age at National Toxicology Centre, Pune. Three male and three female rabbits were selected for the study and evaluated to ensure normal eyes. The animals were housed singly in standard cages, in a light-controlled room (12 hours light and 12 hours dark cycles) at 20–24°C and 30–75% relative humidity, with no restriction of food or water. All experiments were carried out under veterinary supervision and were approved by the ethical committee of the institute. Sterile inserts were administered into the lower conjunctival sac of the left eye of the rabbits and the right eye served as the control. Evaluations were made every 24 hours up to 9 days after insert application. Ocular changes were graded by a scoring system (Table 1) for sign of lachrymation, irritation, erythema, edema, and any other reaction to the eyes of rabbits till the end of the study. All measurements were made by the same operator. The study was conducted in compliance with the US Food and Drug Administration guidelines following Good Laboratory Practices regulations. The animals were observed daily for degradation and disappearance of inserts to check the biodegradability of the inserts.

Table 1. Scoring system for assessment of ocular changes on application of inserts.

Assessment	Score
Cornea	
(a) Opacity—degree of density (from scattered or diffuse area and visible iris, translucent area, opalescent area, to opaque area and invisible iris)	0–4
(b) Area of cornea involved (from less than one quarter, greater than one quarter, less than three quarters, to greater than three quarters or whole area)	0–4
Iris (from normal reaction to no reaction to light, hemorrhage)	0–2
Conjunctivas	
Redness of palpebral conjunctivas	0–3
Chemosis (normal swelling, obvious swelling with partial aversion of lids, swelling with lids about half closed to intense swelling with half to completely closed lids)	0–4
Discharge (small discharge, discharges with moistening of the lids, to intense discharge with moistening of the lids and considerable area around the eye)	0–3

Ex vivo eye penetration study

Freshly excised eyeballs of the goat were obtained from the local slaughterhouse immediately after slaughter and washed thoroughly with PBS, graded amounts of alcohol (i.e., 5%, 15%, and 35%, w/v), and antibiotic-antimycotic solution and were used for an ex vivo eye penetration study. DMEM (with added FBS and antibiotic-antimycotic solution) was used as eyeball-nourishing and -preserving media. Eyeball was placed in a specially designed container filled with DMEM in such a manner that only the cornea and part of the conjunctiva remained exposed to the external environment and the rest of the eyeball remained immersed in DMEM. Sodium fluorescein was selected as the model dye for the penetration study because of its easy availability and ease of estimation by fluorimetry. PVA–gelatin (10:3 wt%) insert loaded with sodium fluorescein was applied on the corneal surface of the eyeball. Ten microliters of eyedrop containing (2%, w/v) sodium fluorescein was administered as control. Eyeballs were washed with STF (30 µL/min for 3 minutes) to simulate the instantaneous blinking effect of the eyes on application of dosage forms. Further, the flow of STF at the rate of 2.2 µL/min was maintained on the ocular surface for 3 hours to simulate the lachrymal secretion and tear turnover effect. The 3 hours period was chosen because the eyedrop was completely washed off from the corneal surface in 3 hours, whereas inserts showed release of the dye even after 3 hours. The experiment was carried out in a laminar airflow chamber and the whole setup was maintained at 35°C. After 12 hours, the eyeball was dissected to separate out cornea, iris, aqueous humor, vitreous humor, and retina. Each tissue was homogenized in 5 mL Milli-Q water and the fluorescence was observed using a spectrofluorometer at 520 nm emission wavelength (excitation wavelength 494 nm) to ascertain the penetration of dye in the internal ocular tissues.

Statistical analysis

The statistical analysis was performed by one-way analysis of variance followed by Bonferroni comparison test using OriginPro 7.5 software. *P*-values less than 0.05 were considered statistically significant.

Results

Inserts' characteristics and morphology

The inserts prepared were transparent, and had a diameter of 6 ± 0.5 , 0.3–0.4 mm thickness, and a weight of 10 ± 2 mg. The morphology of PVA–gelatin inserts was studied through the analysis of images obtained by SEM and AFM. SEM and AFM images showed that inserts' surface was smooth, continuous, and homogeneous (Figure 1). No phase separation was observed, indicating good compatibility between insert matrix and drug.

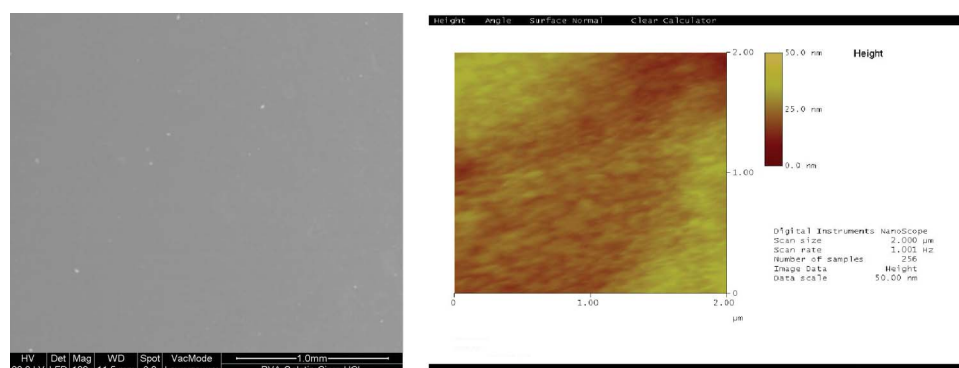


Figure 1. SEM and AFM image of ciprofloxacin-loaded PVA-gelatin inserts.

Fourier-transform infrared spectroscopy

Spectral analysis of inserts confirmed the esterification of the inserts. FT-IR spectra of PVA, gelatin, and PVA-gelatin inserts are shown in Figure 2. FT-IR spectra of pure PVA showed peak at 3445 cm^{-1} , indicating stretching of O-H group. The peak around 2800 cm^{-1} is because of C-H stretching. The absorption peak at about 1382 cm^{-1} is for C-O group. The characteristic FT-IR spectra of gelatin showed peaks at 3434 cm^{-1} because of N-H stretching of amide. The C=O stretching vibration of carboxylate anion appeared at 1635 cm^{-1} . The PVA-gelatin insert showed characteristic spectrum different from those of component polymers. The FT-IR spectrum of the PVA-gelatin esterified insert showed the peak of gelatin at 1635 cm^{-1} shifted to 1725 cm^{-1} , indicating the esterification of PVA and gelatin. This is in agreement with Pal et al.¹⁶ The peak around 3400 cm^{-1} indicates the presence of O-H group with polymeric association and

a secondary amide. Again, broadening of the peak of gelatin at 3434 cm^{-1} and lowering of the wave number indicates polymeric association via hydrogen bonding. From the FT-IR spectra of PVA-gelatin inserts, we can conclude that the inserts had an ester linkage and a secondary alcoholic group. Further, the FT-IR results suggest a complete esterification of the carboxylic acid of gelatin.

Wettability (contact angle)

Measurement of contact angles gives an insight of wettability and adhesion properties of the specimens. The glass-water and glass-STF contact angle was observed at $26.9 \pm 1.7^\circ$ and $28.7 \pm 1.3^\circ$, respectively. The contact angle values of the inserts were observed to be $47.5 \pm 2.7^\circ$, $48.2 \pm 3.4^\circ$, and $49.1 \pm 2.9^\circ$ with water and $48.3 \pm 3.2^\circ$, $47.4 \pm 2.5^\circ$, and $49.2 \pm 3^\circ$ with STF for PVA-gelatin (10:1 wt%), PVA-gelatin (10:2 wt%), and PVA-gelatin

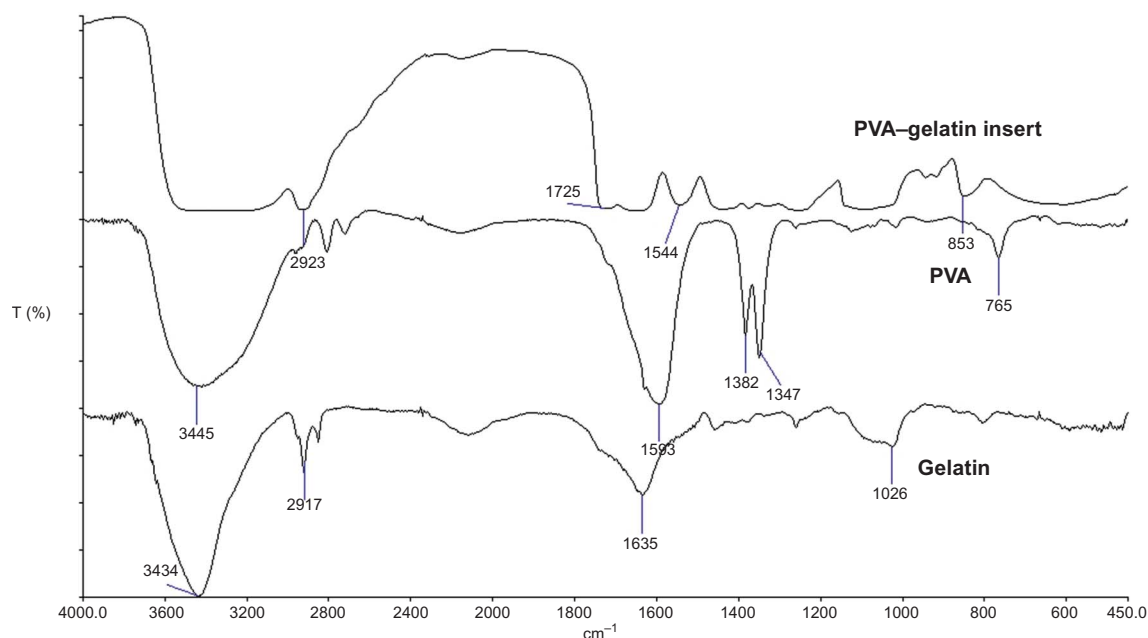


Figure 2. FT-IR spectra of PVA, gelatin, and PVA-gelatin inserts.

(10:3 wt%) inserts, respectively. The contact angles of all the inserts were $<50^\circ$, suggesting the hydrophilic character of inserts, which may be because of the hydrophilic nature of both PVA and gelatin. The corneal surface also shows hydrophilic character; thus, the hydrophilic character of inserts is preferable for good contact with the ocular mucosa.

Swelling study

Inserts were observed to swell rapidly during initial 60 minutes. Upto $101.5 \pm 9.1\%$, $93.3 \pm 3.8\%$, and $101.4 \pm 2.2\%$ swelling was observed with the PVA-gelatin (10:1 wt%), PVA-gelatin (10:2 wt%), and PVA-gelatin (10:3 wt%) esterified inserts, respectively, within 60 minutes. Unesterified inserts started swelling rapidly on hydration with STF, and up to $105 \pm 3.3\%$, $111.8 \pm 3.5\%$, and $114.4 \pm 7.4\%$ swelling was observed with PVA-gelatin (10:1 wt%), PVA-gelatin (10:2 wt%), and PVA-gelatin (10:3 wt%) unesterified inserts, respectively. Statistical analysis showed that the swelling of unesterified inserts after 60 minutes was significantly greater than those of the esterified inserts ($P < 0.05$) for PVA-gelatin (10:2 wt%) and PVA-gelatin (10:3 wt%) ratios, whereas no significant difference was observed in the inserts prepared with different amounts of gelatin. Further, unesterified inserts started deforming and disintegrating within 60 minutes, whereas esterified inserts were found to be stable without deformation during the study period. This suggests esterification provided stability and slowed down the dissolution of the inserts.

Mechanical properties

The strength of ocular inserts is an important consideration with respect to resistance to damage during handling and long-term durability, and may govern some aspects of the insert performance. The strength and flexibility of inserts is expressed by its tensile stress (ultimate tensile strength) and elongation to break¹². Mechanical properties of various inserts are presented in Figure 3. The mechanical properties of esterified inserts were observed to be superior as compared to the inserts made of PVA and gelatin alone. This may be

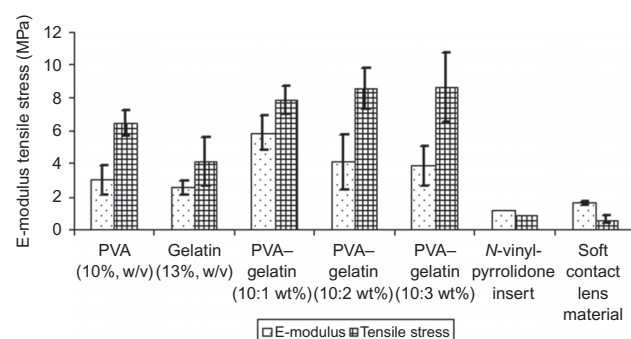


Figure 3. Elastic modulus and tensile stress of various inserts.

because of the intermolecular interactions and esterification between PVA and gelatin. Maximum tensile strength of 8.6 ± 2 MPa was observed with ciprofloxacin-loaded inserts containing 3% (w/v) gelatin. Tensile strength was found to increase slightly with increasing concentration of gelatin, which may be because of greater molecular interactions between PVA and gelatin molecules. The elastic modulus of various inserts was in the range of 3.9–5.8 MPa. The higher values of elastic modulus suggest that the inserts are flexible and thus are easier to administer. Maximum elastic modulus and tensile stress of the *N*-vinylpyrrolidone inserts is observed to be 1.09 and 0.8134 MPa, respectively²⁴. Similarly maximum elastic modulus and tensile stress of the various soft contact lens materials are reported to be 1.6 and 0.57 MPa, respectively²⁵. Clearly the values of elastic modulus and tensile stress of PVA-gelatin inserts is much higher than the above-mentioned products. Percent elongation of the inserts was found to be $284 \pm 38\%$, $342 \pm 70\%$, and $348 \pm 65\%$ with PVA-gelatin (10:1 wt%), PVA-gelatin (10:2 wt%), and PVA-gelatin (10:3 wt%) inserts, respectively, whereas the elongation of plain gelatin membrane and PVA membrane was in the range $114 \pm 27\%$ and $305 \pm 31\%$, respectively.

In vitro mucoadhesion study

The force of detachment of the inserts from the mucin-coated surface was observed to be 1.2 ± 0.90 , 1.6 ± 1.1 , and 1.8 ± 0.97 N with PVA-gelatin (10:1 wt%), PVA-gelatin (10:2 wt%), and PVA-gelatin (10:3 wt%) inserts, respectively. In a control experiment when only mucin was applied between plates, the force of detachment was observed to be 0.05 N. Clearly this force is negligible in comparison of inserts, indicating adhesive interactions of insert polymers with mucin layer. Results of mucoadhesion study reveal that the force of detachment with PVA-gelatin inserts is significantly higher as compared to that with mucin alone ($P < 0.05$).

In vitro drug release study

The average cumulative percent drug release profile of various inserts at prefixed time intervals is presented in Figure 4. The in vitro drug release profile of ciprofloxacin

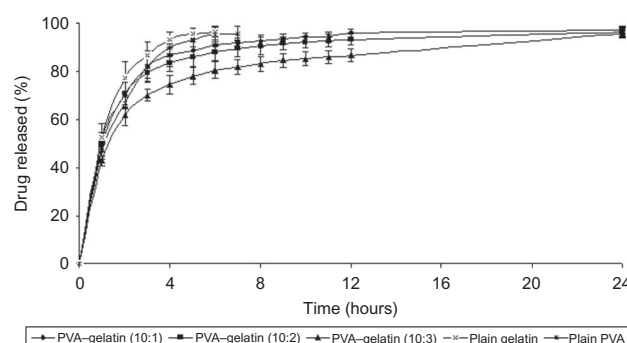


Figure 4. In vitro drug release of ciprofloxacin from various inserts.

from inserts after predetermined time intervals is expressed as percentage of the drug loading. A biphasic release pattern was observed from the inserts. Initially, up to 42–52% burst release was observed with various inserts, which may be because of the immediate release of drug adsorbed on or near the surface of inserts on coming in contact with the release media. After that release was prolonged till 24 hours, PVA-gelatin (10:3 wt%) inserts showed slower release as compared to other inserts. Complete drug release from plain PVA and plain gelatin inserts was observed for 6–7 hours only; after that, these inserts were disintegrated and dissolved. The complete drug released from eyedrop solution was observed within 30 minutes. Hundred percent release from the inserts was significantly longer as compared to the release from the plain polymer-alone inserts and eyedrop solution ($P < 0.05$). The drug release profile is affected by the diffusion kinetics and the degradation profile of the insert, which can be modulated by varying the cross-linking efficiencies.

Antimicrobial efficacy study

Diameter of ZOI depends on several factors such as inoculum size, temperature, growth media, and the type of organism. The inserts were found to be active against selected organisms, as indicated by clear ZOIs (Table 2) which are well matching to the range of standard ZOI of corresponding concentration of ciprofloxacin against *S. aureus* and *E. coli*²⁶.

Hemolysis study

RBCs incubated with PVA-gelatin inserts showed negligible hemolysis of 0–3%, similar to that of normal saline-treated erythrocytes.

Cytocompatibility study

SRB assay was conducted to check biocompatibility and cytocompatibility of the inserts. All the inserts showed excellent cell viability of SIRC cells after incubation for 24 hours in an in vitro cytocompatibility study by SRB assay. The viability of control cells was taken as 100%, and >95% cell viability was observed with the inserts.

Acute ocular toxicity study (in vivo tolerance assay)

Following insertion in rabbit's eyes, inserts started hydrating and adhered to the application site. No clinical signs of toxicity to eyelids, cornea, iris, and conjunctiva were observed in any of the animals during the study. Excellent ocular tolerance was observed with the

inserts with an irritancy index of zero. This suggests that inserts are nonirritant to ocular mucosa. The inserts were found to be mucoadhesive and biodegradable.

Ex vivo eye penetration study

To evaluate the penetration capacity of the inserts, ex vivo eye penetration study was performed. Percent penetration of sodium fluorescein penetrated in ocular tissues from the inserts to control eyedrop solution is presented in Figure 5. Average percent penetration of sodium fluorescein from the inserts in the cornea, iris, vitreous humor, and retina was almost 26-, 23-, 90-, and 7.6-fold higher as compared to the eyedrops. Average 67% sodium fluorescein of the initial amount was observed to be penetrated in the eyeball, whereas rest of the dye remained in the insert. In case of eyedrop solution, only 2.1% dye was observed to have penetrated. The percent penetration of sodium fluorescein in the various ocular tissues from the insert was significantly greater than in the eyedrop solution ($P < 0.05$). The results suggest that inserts can prolong the release of dye. Penetration of dye from inserts in the posterior segment tissues shows that the inserts remained adhered to the surface of eyeball and released the dye.

Discussion

Ocular inserts increase the residence time in the precorneal area, and thereby improve the bioavailability and therapeutic efficacy of drugs with respect to traditional eyedrops. Other attractive features of ocular inserts are their sustained release characteristics; thus, less frequent administration of drug is required to produce a prolonged pharmacological activity.

There are no studies in the published literature of ocular inserts using combinations of synthetic and biopolymers for the delivery of antibiotics. Various commercially available ocular inserts have certain limitations; for example, solid ophthalmic drug insert shows a drug release profile only for 1 hour. Ocusert and Ocufit SR are made of synthetic polymers, that is, ethylene-vinyl acetate and silicone elastomer, respectively²⁷. In this

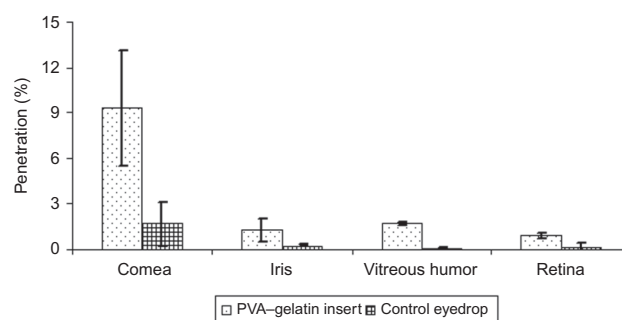


Figure 5. Percent penetration of sodium fluorescein in various ocular tissues.

Table 2. Zone of inhibition of various microorganisms observed with PVA-gelatin inserts.

Organism	Standard ZOI (mm)	Observed ZOI of inserts (mm)
<i>Staphylococcus aureus</i>	22–30	31.6 ± 1.0
<i>Escherichia coli</i>	30–40	32.6 ± 2.2

study, we formulated ocular inserts using a synthetic polymer (PVA) as well as a biopolymer (gelatin), to combine the mechanical properties of PVA with the biocompatibility and mucoadhesion of gelatin. Gelatin can also be used instead of collagen as a biomaterial for aiding wound healing. Although collagen is known to have antigenicity in physiological conditions, gelatin is nonantigenic in nature. In addition to this, gelatin is more cost-effective than collagen²⁸. Plasticizers are generally used to ensure flexibility in polymeric inserts. Glycerol is nontoxic and well known for its plasticizing properties. Addition of glycerol provides elasticity to inserts. Although pre-swelling with an aqueous medium softens the inserts, the handling and brittle nature of the inserts requires an additional plasticizer such as glycerol. Glycerol is used in many ocular formulations and is known to be safe for ocular use. Ciprofloxacin is used as a drug for treatment of bacterial keratitis and external ocular infections. The advantage of using PVA-gelatin inserts as a drug carrier is that upon hydration they soften, making them comfortable in the eye, and the mucoadhesive nature secures its place in the cul-de-sac even during blinking, avoiding the risk of expulsion. Consequently, the contact time of drugs with ocular tissues will be prolonged. Formation of complete esterification between PVA and gelatin was confirmed by FT-IR spectroscopy. Additionally, some hydrogen bonding between the polymers was also observed.

Inserts prepared without esterification disintegrated and dissolved within minutes after coming into contact with STF. To decrease the rate of dissolution and to increase the corneal contact time of inserts, esterification of hydroxyl groups of PVA with carboxyl groups of gelatin was carried out. By esterification, we regulated the dissolution of inserts and consequently the release of drug from the inserts in tear fluid.

Inspection of surface of inserts macroscopically and by SEM and AFM showed smooth and homogeneous surfaces, indicating homogeneous incorporation of drug and matrix without phase separation or precipitation. Also, because of the smooth surface, grittiness or unwanted irritation on application was minimized. On the other hand, it has been reported that randomly distributed discontinuous zones characterized by cracks are observed in gelatin-alone membranes¹².

The hydrophilicity of inserts is evident from contact angle measurements where the inserts are wettable with STF, which suggests better contact of inserts with the hydrophilic corneal surface. The STF-absorbing capacity of inserts was established by the swelling study. The swelling character of inserts has a great impact on their adhesive properties, drug release kinetics, and onsite stability. The rate of swelling is dependent on the chemical structure, cross-linking density, pH of the swelling media, the temperature of environment, and availability of the hydrophilic groups for interaction with water molecules²⁹. Rapid hydration and swelling of polymer

favors interdiffusion between polymers and mucus layer resulting in stronger adhesion⁴. It was observed that unesterified inserts swelled higher than esterified inserts, and disintegrated and deformed on hydration with STF, whereas esterified inserts were found to be stable for relatively longer periods, which results in stability and slower degradation of the esterified inserts.

Mechanical properties are a significant consideration in the design and quality control of ocular inserts. The mechanical properties of inserts can provide an indication of expected integrity under conditions of stress that may occur during processing, handling, and application of inserts. Stresses in inserts imposed during application or eye movement can cause irreversible deformation or fracture, resulting in loss of performance, user discomfort, or even complete disintegration and thus burst release of drug leading to adverse drug reactions. The inserts developed in this study have the advantages of high tensile strength and flexibility. The mechanical properties of PVA-gelatin inserts are much higher than those of the inserts reported in literature^{24,30}. Tensile strength of PVA-gelatin inserts is observed to be significantly greater (10–15-fold) as compared to inserts composed of copolymers of *N*-vinylpyrrolidone²⁴. Also, the tensile strength and percent elongation of PVA-gelatin inserts are much higher (up to 20–50- and 5–15-fold, respectively) as compared to soft contact lens materials such as 2-hydroxyethyl methacrylate, vinylpyrrolidone, methyl methacrylate, and methacrylic acid²⁵. The mechanical properties of PVA-gelatin inserts were also improved over inserts made of PVA alone and gelatin alone.

Inserts have shown good mucoadhesive performance *in vitro* as observed by force of detachment. Further, in the qualitative assessment of mucoadhesion between inserts and mucin, inserts were found to adhere with mucin layer. The mucoadhesive character of the inserts was further proved in an *in vivo* ocular tolerance assay where inserts were found to adhere to the rabbits' eyes.

Ciprofloxacin is used in the study because of its wide spectrum against most of the pathogenic microbes, for example, *S. aureus* and *E. coli*. The inserts were observed to be effective in inhibiting the growth of microorganisms on incubation for 18 hours as seen by clear ZOIs.

The insert starts hydrating and swelling on contact with PBS, which results in erosion and dissolution of polymers, diffusion of drug in the swelled matrix, and concomitant release of drug. The *in vitro* drug release was observed for 24 hours, which is beneficial over eye-drops. Further, the release of the drug is expected to be more prolonged under *in vivo* conditions, as only a very small volume of the lachrymal fluid will be available to release the drug from the inserts. The release profile is modulated because of diffusion kinetics and the degradation of the insert. The rates can be modulated by the cross-linking efficiency. The amount of the drug released from PVA-gelatin (10:3 wt%) inserts was higher than the minimum inhibitory concentration of ciprofloxacin

against most common ocular pathogens, such as *S. aureus*. Cross-linking of the matrix, solubility of the drug incorporated, degree of swelling, diffusion capacity, and insert erosion are the main factors that control the drug release kinetics of inserts altogether^{4,31}. Also, the complex interplay of osmotic forces and electrostatic interactions between drug and polymer affect the drug release from the inserts³².

Further, the PVA-gelatin inserts in this study show superior mechanical properties compared to inserts based on PVA alone developed by Balasubramaniam et al. and show similar duration of in vitro drug release²⁰. Additionally, the PVA-gelatin inserts were mucoadhesive and biodegradable as observed by in vivo acute ocular toxicity study in albino rabbits. Drug-loaded microparticles and emulsions may also show sustained release of drugs. However, microparticles have a tendency to aggregation and sometimes require use of organic solvents. These concerns are avoided in inserts. Again, insufficient wetting of some microparticles at the mucosal surface may result in low mucoadhesive forces and incomplete drug release³².

The penetration of sodium fluorescein in ocular tissues by applying the inserts to the goat eyeballs shows the ability of inserts to increase the bioavailability of the drugs by application of inserts. Sodium fluorescein was selected as the model dye for the penetration study because of its easy availability and ease of estimation by fluorimetry. Significant dye penetration was observed using inserts as compared to sodium fluorescein-containing eyedrops. The presence of fluorescence in posterior segment tissues such as vitreous humor and retina indicates that the inserts not only prolong the release but are also suitable for deeper penetration. The increased contact time of the inserts to the cornea resulted in increased penetration.

Ocular irritation generally results from cytotoxic reactions of the applied substances with ocular tissues. Hemolysis test is considered as a simple, rapid, and reproducible technique to assess ocular irritation based on damage to RBC membranes. Also, hemolysis assay offers good sensitivity and specificity and shows minimum difference with the Draize test among the different in vitro tests available^{33,34}. PVA-gelatin inserts showed no evidence of RBC membrane damage. Further, an in vitro cytocompatibility study was carried out with SIRC cells, which is a well-established corneal cell line. The viability of the cells was observed >95% in presence of inserts when incubated for 24 hours with SIRC cells. It can be implied from the results of the cytocompatibility study that PVA-gelatin inserts can promote wound healing because cell proliferation plays a major role in wound healing. Further, an in vivo ocular tolerance assay was performed in albino rabbits to check safety of the inserts on actual application. It also provides information regarding the possible hazards and risks likely to arise from exposure to the membrane by the inserts. A

grading system was used to check irritation, edema, erythema, swelling, discharge, and other morphological changes in the external ocular tissues. The results of acute ocular toxicity study clearly indicate that the inserts were well tolerated, and do not show any signs of ocular irritation or toxicity. Further, the inserts remained adhered to ocular mucosa, indicating the mucoadhesive character of inserts, and showed slow degradation.

Conclusions

The ciprofloxacin-loaded PVA-gelatin inserts had smooth and homogeneous surfaces, high light transmittance, hydrophilicity, and superior mechanical and mucoadhesive properties. Greater transparency of the inserts makes them aesthetically acceptable. In vitro drug release study shows that inserts can produce in vitro drug release over 24 hours. The inserts showed greater dye penetration in deeper ocular tissues of goat eyeballs and were nontoxic as determined by in vitro tests and confirmed by acute ocular toxicity studies in albino rabbits. The results suggest that PVA-gelatin inserts may be considered as an effective and promising ocular drug delivery system requiring less frequent administration in comparison to eyedrops and can be used for treatment of external ocular infections and pre- or postoperatively in ocular surgeries. Ciprofloxacin-loaded PVA-gelatin inserts developed in this study can be applied for treatment of corneal ulcers, conjunctivitis, bacterial keratitis, and other corneal infections. Increased cases of these conditions have been reported primarily because of the wearing of (soft) contact lenses.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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