Formulation Optimization and Evaluation of Ocular Inserts Prepared with Sulfoxyamine Modified Chitosan

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Abstract

Aim: The aim of the present research work is to use our previously modified chitosan in formulation development and optimization of ocular insert. **Materials and Methods:** Ocular insert was made from an aqueous dispersion of ofloxacin, polyvinyl alcohol (PVA), modified chitosan, sodium alginate, and dibutyl phthalate by the solvent casting method. It was coated with Eudragit S100 and evaluated for drug-excipient interaction, physicochemical characteristics, and microbiological and *in vitro* release studies. In the present study, 3^2 full factorial designs were employed with the help of Design-Expert software to study the effect of independent variables, i.e., effects of PVA amount (X1) and modified chitosan amount (X2) on the dependent variables, i.e., % moisture absorption and mucoadhesion time. **Results and Discussions:** In Fourier transform infrared study, a major characteristic peak of ofloxacin was found in the entire coated ocular inserts, confirming the presence of the drug in the polymer without interaction. The result of the differential scanning calorimetric (DSC) study shows the thermogram of pure ofloxacin exhibited a sharp endothermic peak at 276.25°C and heat of fusion of 41.97J/G, which corresponds to its melting point. DSC thermogram of the physical mixture, and formulation do not shows peak of pure drug.This revealed that ofloxacin was molecularly dispersed in polymer. The weight and thickness of the inserts were in the range of 3.23–5.75 mg and 0.11–0.21 microns, respectively, for different formulations. The *in vitro* drug permeation studies were carried out using an all-glass modified Franz diffusion cell. The drug concentration and mucoadhesion time of the ocular insert were found satisfactory. The ocular insert coated with Eudragit S100 showed maximum drug permeation through Millipore membrane and excised goat cornea compared to other formulation. In goat corneas permeation studies, the formulation OFXF8 showed a 17.06% release at the end of 4 h which is more as compared to other formulation. Ocular inserts using PVA and modified chitosan as a polymer were successfully prepared and can be effectively used for sustained ocular delivery over a period of 24 h. An optimization study was conducted using 3^2 factorial design. **Conclusion:** Based on these findings, it was concluded that the designed ocular inserts are a realistic alternative to conventional eye drop by virtue of its ability to improve bioavailability and improving patient compliance.

Key words: Design expert, modified chitosan, mucoadhesion time, ocular inserts, ofloxacin, solvent casting method

INTRODUCTION

Chitosan, a unique cationic natural polysaccharide obtained commercially by deacetylation of chitin, has received considerable attention during the last decades due to its favorable properties, including biocompatibility, biodegradation, and non-toxicity.[1,2] Previously many researchers are worked on to improve the Chitosan functionality and better control these

physicochemical properties.[3] Chemical modification of gums not only minimizes these drawbacks but also enables their use for specific drug delivery purposes but also alters their physicochemical properties[4,5] and it is used to improve their functional properties a number of physical and chemical modification approach has been employed.[5]

The field of ocular drug delivery is one of the interesting and challenging endeavors facing the pharmaceutical scientist.[6] The cornea is the avascular and transparent tissue that plays an important role in optical clarity and visual acuity.[7] About 90% of the dose applied topically from such solutions is lost due to pre-corneal losses (lacrimation and drainage), which leads to poor availability, and frequent dosing is required for the installation to achieve an adequate level and therapeutic effect. The standard treatment of severe bacterial keratitis requires frequent administration of fluoroquinolone eye drops.[8] However, this regimen is not only disruptive to the patient and usually necessitates hospitalization but also it has also been associated with toxicity to the corneal epithelium. To overcome these limitations, ocular inserts seem promising by prolonging the contact time with improved efficiency of the therapy and patient compliance. Ocular inserts offer many advantages over conventional dosage forms such as increased ocular residence, possibility of releasing drugs at a slow and constant rate, accurate dosing, and avoidance of toxicity due to preservative and increased shelf life.[9] Ocular inserts of ofloxacin have been designed to improve ocular availability.

Ofloxacin chemically is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.[10] The half-life of ofloxacin is 4–5 h and bioavailability is 85–95%. The purpose of selection ofloxacin for this research work is that it is a synthetic fluoroquinolone agent widely used in ocular gingivitis, ocular conjunctivitis, and other ocular disorders for symptomatic relief of pain and inflammation.[11] Ofloxacin is a broad-spectrum antibacterial agent with activities against Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Serratia* species, *Proteus* species, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*) and Gram-positive bacteria (*Staphylococcus* species and *Streptococcus enterococci*).[12] It inhibits the enzyme bacterial DNA gyrase, which nicks double standard DNA, introduces negative supercoil, and then reseals the nicked ends. This is necessary to prevent excessive positive supercoiling of the strands when they separate to permit replication or transcription. The bactericidal action probably results from the digestion of DNA by exonucleases whose production is signaled by the damaged DNA. Taking the above information in account, the purpose of the current study was to formulate ocular inserts of ofloxacin that would be capable of prolonging the contact time, thereby potentially enhancing intracorneal delivery of ophthalmic medicament.[13]

Therefore, in the present study, the water-soluble Chitosan derivative was prepared by reacting it with thionyl chloride followed by ammonia as per our previously described method.[14] The synthesized polymer was used in the preparation of ophthalmic insert. Ocular insert was made from an aqueous dispersion of ofloxacin, polyvinyl alcohol (PVA), modified chitosan, sodium alginate, and dibutyl phthalate by the solvent casting method. It was coated with Eudragit S100 and evaluated for drug-excipient interaction, physicochemical characteristics, and microbiological and *in vitro* release studies.

MATERIALS AND METHODS

Materials

Ofloxacin was obtained from Matrix Laboratories (India) as a gift. Sodium alginate was obtained from S D Lab chemicals, Mumbai. Eudragit S-100 (anionic methacrylic acid-methyl methacrylate copolymer) was obtained from Jubilant Organosys (India) as gift samples. PVA and dibutyl phthalate were obtained from the Central Drug House (India) and S.D. Millipore filter paper (0.8 µm) was procured from HiMedia Lab. Pvt. Ltd., Mumbai. Fresh goat eyeball was obtained from a butcher's shop. All other chemicals were of analytical grade.

Methods

Modified Chitosan was synthesized as per as per our previously described method [14] and used in this study.

Formulation of ocular inserts[15]

The ocular inserts of ofloxacin were formulated using the solvent casting method with a combination of polymers (sodium alginate, PVA, and modified chitosan) and dibutyl phthalate (w/v of polymer) used as a plasticizer for the uniform strength of insert, as per composition given in Table 1. The required amount of polymers was dissolved in two different beakers in the required quantity of distilled water (50 ml) and stirred on a magnetic stirrer until completely dissolved than both solutions of polymers are mixed together and then dibutyl phthalate (w/v of polymer) was added as a plasticizer to the solution under stirring condition. The weighed amount of drug (100 mg) was added to the above solution and stirred for 1 h to get uniform dispersion. The solution of polymers was sonicated for 30 min to remove the air bubbles. After proper mixing, the casting solution is poured in a clean glass Petri dish. The dried films, thus, obtained were cut into circular pieces of definite size (5.5 mm diameter). The ocular inserts were then wrapped in aluminum foil and were stored in an airtight container (desiccators), the ocular insert (OFXF7 and OFXF9) was coated with Eudragit polymer (0.2% w/v) in isopropyl alcohol and acetone by the dip and dry method. The polymers used were Eudragit S-100 (OFXF7-OFXF9).

Table 1: Compositions of ofloxacin insert formulations for ocular delivery							
Formulation code	Drug (mg)	PVA (mg)	Modified chitosan (mg)	SA (g)	Dibutyl phthalate (ml)	Distilled water (ml)	Coating material 0.2%w/v
OFXF1	100	1.5	7	1	0.3	q. s	-
OFXF2	100	2	7	1	0.3	q. s	-
OFXF3	100	2.5	7	1	0.3	q. s	-
OFXF4	100	1.5	11	1	0.3	q. s	-
OFXF5	100	2	11	1	0.3	q. s	-
OFXF6	100	2.5	11	1	0.3	q. s	-
OFXF7	100	1.5	15	1	0.3	q. s	Eudragit S100
OFXF8	100	2	15	1	0.3	q.s	Eudragit S100
OFXF9	100	2.5	15	1	0.3	q.s	Eudragit S100

OFX: Ofloxacin, PVA: Polyvinyl alcohol, SA: Sodium alginate

The weight gain of ocular insert after coating with polymer was found to be in range 23.4–29.6%.

Optimization of ocular inserts using full factorial designs

In the present study, a 3^2 full factorial design was employed to study the effect of independent variables, i.e., effects of PVA amount (X1) and modified chitosan amount (X2) on the dependent variables, i.e., % moisture absorption and mucoadhesion time.

Factorial design of formulated batches is shown in Table 2.

As per provision of Design-Expert software following batches of Ocular Insert was prepared shown in Table 1.

Fourier transform infrared (FTIR) spectroscopy[16,17]

The FTIR spectra of the pure drug (ofloxacin) and physical mixture (modified chitosan, sodium alginate, PVA, and Eudragit S100) were taken as KBr pellets in the range of 4000–650 cm^{-1} (Perkin Elmer Model 1600 FTIR spectrophotometer, USA).

Differential scanning calorimetric (DSC)[17]

DSC studies of pure drug, physical mixture, and formulation were characterized using a Shimadzu DSC 60 at a heating rate of 10°C/min. The measurements were performed at a heating range of 50–400°C under nitrogen atmospheres.

Thickness uniformity[17]

Insert thickness (5.5 mm diameter) was measured at five different points using a micrometer screw gauge (Mitutoyo Co., Japan) and mean insert thickness was noted ($n = 3$).

Table 2: 3^2 Factorial design		
Formulation code	Variable (X$_1$)	Variable (X$_2$)
OFXF1	−1	−1
OFXF2	0	−1
OFXF3	+1	−1
OFXF4	−1	0
OFXF5	0	0
OFXF6	+1	0
OFXF7	−1	+1
OFXF8	0	+1
OFXF9	+1	+1

OFX: Ofloxacin

Uniformity of weight[18]

Ocular inserts were randomly selected and weighed individually using a calibrated digital balance. The average weight of the prepared ocular insert was then calculated.

Surface pH determination[18]

For the determination of surface pH, the ocular insert was allowed to swell in the Petri dish at room temperature for 30 min in distilled water. The pH paper was kept on the surface, and after 1 min, the color that developed was compared with the standard color scale.

Drug content uniformity[19]

Three inserts were taken out from the film and drug content determined. The ocular insert was dissolved in 10 ml of 0.1 mol/L HCl while the coated insert was dissolved in 10 mL of acetone. The volume was made up to 0.1N HCl and the solution was filtered. The amount of drug in the filtrate was analyzed by measuring absorbance at 295 nm in a spectrophotometer (1601 Shimadzu, Kyoto, Japan). The experiment was done in triplicate.

% Moisture absorption study

Some of the ocular inserts were taken and their initial weight was noted and then these were placed in the desiccators containing aluminum chloride within high (80%) humidity condition for 3 days. After 3 days, ocular inserts were reweighed and then % moisture was calculated using the following formula:

$$\% \text{ Moisture Absorption Study} = \{(wt-wo)/wo\} \times 100$$

Where,
Wt. = Final weight of ocular insert
Wo = Initial weight of ocular insert

Moisture loss study

Some of the ocular inserts were taken and their initial weight was noted and kept in desiccators containing anhydrous calcium chloride for 3 days, and after 3 days, inserts were taken out and reweighed. The percentage of moisture loss was calculated using formula.

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$$\% \text{ Moisture loss study} = \{(wo-wt)/wo\} \times 100$$

Where,
Wt. = Final weight of ocular insert
Wo = Initial weight of ocular insert

Mucoadhesion time[20]

The mucoadhesion time was determined (in triplicate) after application of coated or uncoated ocular inserts (5.5 mm diameter, 0.40–0.45 mm thickness) on a freshly cut goat eyelid. The eyelid was fixed on the bottom of a beaker with cyanoacrylate glue. Ocular insert (coated or uncoated) was attached to the mucosal surface of the eyelid by applying a light force with a fingertip for 20 s. The beaker was filled with 800 mL of bicarbonate Ringer's solution pH 7.4 and stirred at a rate of 150 rpm at room temperature. The time needed for complete detachment of the insert from the mucosal surface was considered as mucoadhesion time.[17]

In vitro permeation study[21]

The *in vitro* drug permeation studies were carried out by putting the ocular insert (5.5 mm in diameter) on Millipore membrane filter (0.15 mm); between the donor and receptor compartments of an all-glass modified Franz diffusion cell. The Millipore membrane filter was used to simulate the corneal epithelial barrier as isolated cornea will not remain viable beyond 4 h. To simulate the tear flow, the donor compartment was infused with bicarbonate Ringer, pH 7.4, at a flow rate of 20 µL/min throughout the study. 1.0-ml sample was withdrawn at hourly intervals from the receptor compartment (containing 10 ml bicarbonate Ringer, pH 7.4, under stirring at 37°C) and the drug permeated was measured. Each withdrawn sample was replaced with an equal volume of fresh bicarbonate Ringer's solution. Drug permeation experiments were also carried out using freshly excised goat cornea for 4 h. At the end of the experiment, % drug permeation was calculated using the following.

Release kinetics[19,20]

The mechanism of drug release was investigated by fitting the drug release data into zero-order, first-order, Higuchi kinetics, and Korsmeyer–Peppas equations. The goodness of fit of drug release was evaluated by the regression coefficient (R^2) value.

Antibacterial activity[21-24]

The microbiological studies were carried out to ascertain the biological activity of the optimized formulation and marketed eye drops against microorganisms. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as the test microorganisms. A layer of nutrient agar (20 ml) seeded with the test microorganism (0.2 ml) was allowed to solidify in the Petri plate. Cups were made on the solidified agar layer with the help of a sterile borer of 4-mm diameter. Then, the volume of the formulations (optimized formulation and marketed eye drops) containing equivalent amounts of the drug was poured into the cups. After keeping Petri plates at room temperature for 4 h, the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured using an antibiotic zone finder.

RESULTS AND DISCUSSION

FTIR spectroscopy

FTIR spectra of the drug, polymer, and their physical mixtures are depicted in Figure 1. The drug sample showed aromatic C=C stretching at 1616.35, 1521.84, and 1452.40 cm^{-1} besides, spectra also showed carboxylic acid C=O stretching at 1708.93 cm^{-1}, C-N stretching at 1355.96 cm^{-1}, 2789.07 cm^{-1} due to CH$_3$ of a methyl group, peak at 1242.16 cm^{-1} due to stretching vibration of oxo group, and peak of 1051.20 cm^{-1} due to C-F stretching. FTIR spectrum of modified chitosan showed a peak at 3226.91 cm^{-1} due to amine, 1060.85 cm^{-1} due to sulfoxy group, 1143.79 cm^{-1} due to C-O stretching in ether, and
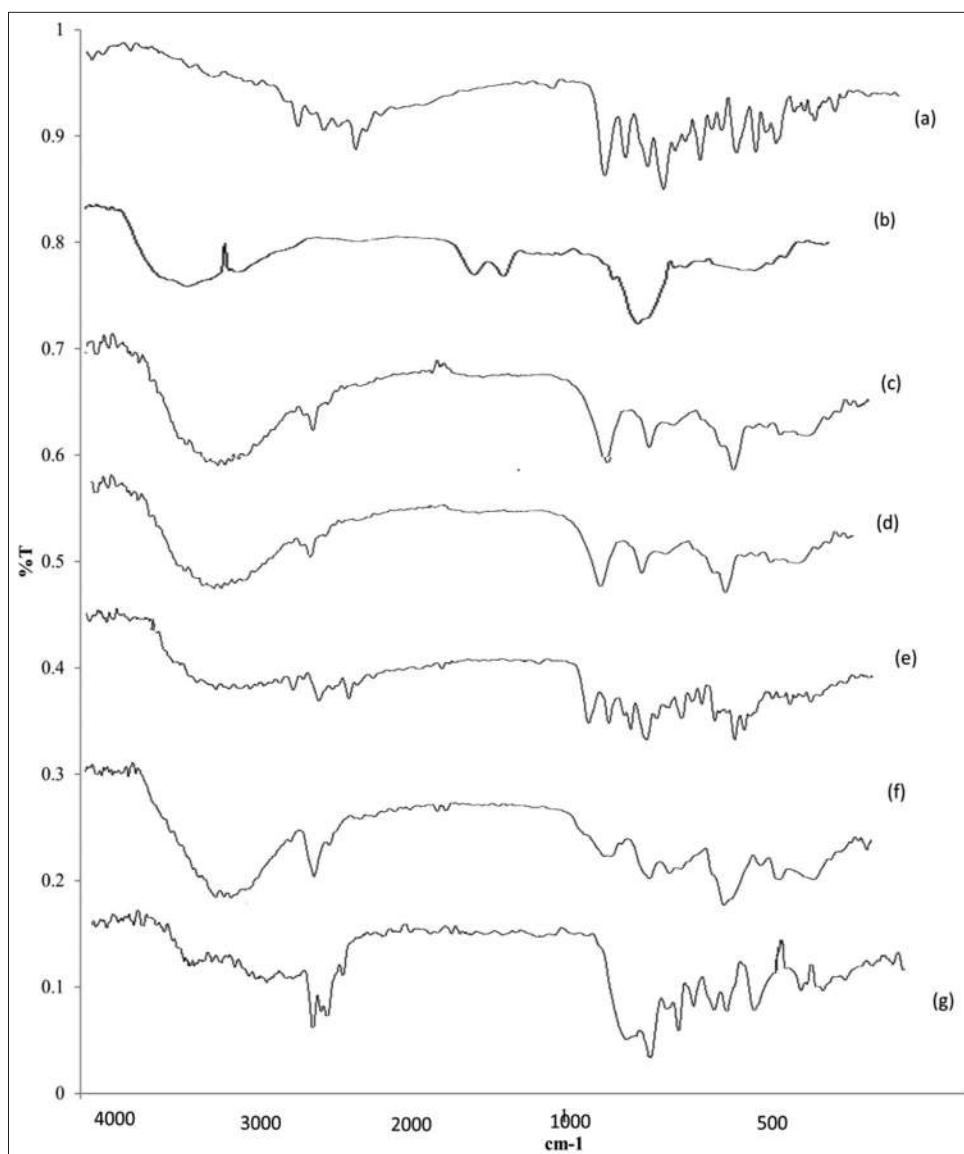
Figure 1: Fourier transform infrared spectra of (a) ofloxacin, (b) modified chitosan, (c) sodium alginate, (d) polyvinyl alcohol, (e) physical mixture, (f) control inserts (uncoated), (g) optimal formulation (Eudragit S100)

1527.62 cm^{-1} due to aliphatic CH. FTIR spectrum of sodium alginate showed a peak at 2924.09 cm^{-1} due to C-H stretching and 1026.13 cm^{-1} due to C-O-C stretching. FTIR spectrum of PVA showed a peak at 3475.73 cm^{-1} due to stretching of a hydroxyl group and 1082.12 cm^{-1} due to C-O stretching. FIIR spectrum of a physical mixture of drug, PVA, modified chitosan, SA, showed peaks at 3475.73 cm^{-1} due to stretching of the hydroxyl group, 2926.01 cm^{-1} due to C-H stretching, and 1051.20 cm^{-1} due to C-O-C stretching (which appears to be contributed by PVA M.C SA), along with pure drug peaks. FTIR spectra of optimized formulations showed a peak at 1618.28 cm^{-1}. FTIR spectrum of optimized ocular inserts (coated) showed aromatic C=C stretching at a usual position, indicating incorporation of ofloxacin and peak for ester at 1620.21 cm^{-1}. As the acrylate polymer is esters. Major characteristic peaks of ofloxacin were found in the entire coated ocular inserts, confirming the presence of the drug in the polymer without interaction. The melting point of the modified chitosan is 230°C

DSC

The result of the DSC study is shown in Figure 2. The thermogram of pure ofloxacin exhibited a sharp endothermic peak at 276.20°C and heat of fusion of 41.97J/G, which corresponds to its melting point. In the DSC thermogram of the physical mixture, and formulation showed peak for pure drug did not appear. This revealed that ofloxacin was molecularly dispersed in polymer.

Physicochemical evaluation and mucoadhesion time of ocular insert

Mechanism of mucoadhesion is adhesion between two substances takes place out which one is biological in nature. Depend on the charge on polymer, they bind with Mucin present in body. The thickness of ocular inserts was found
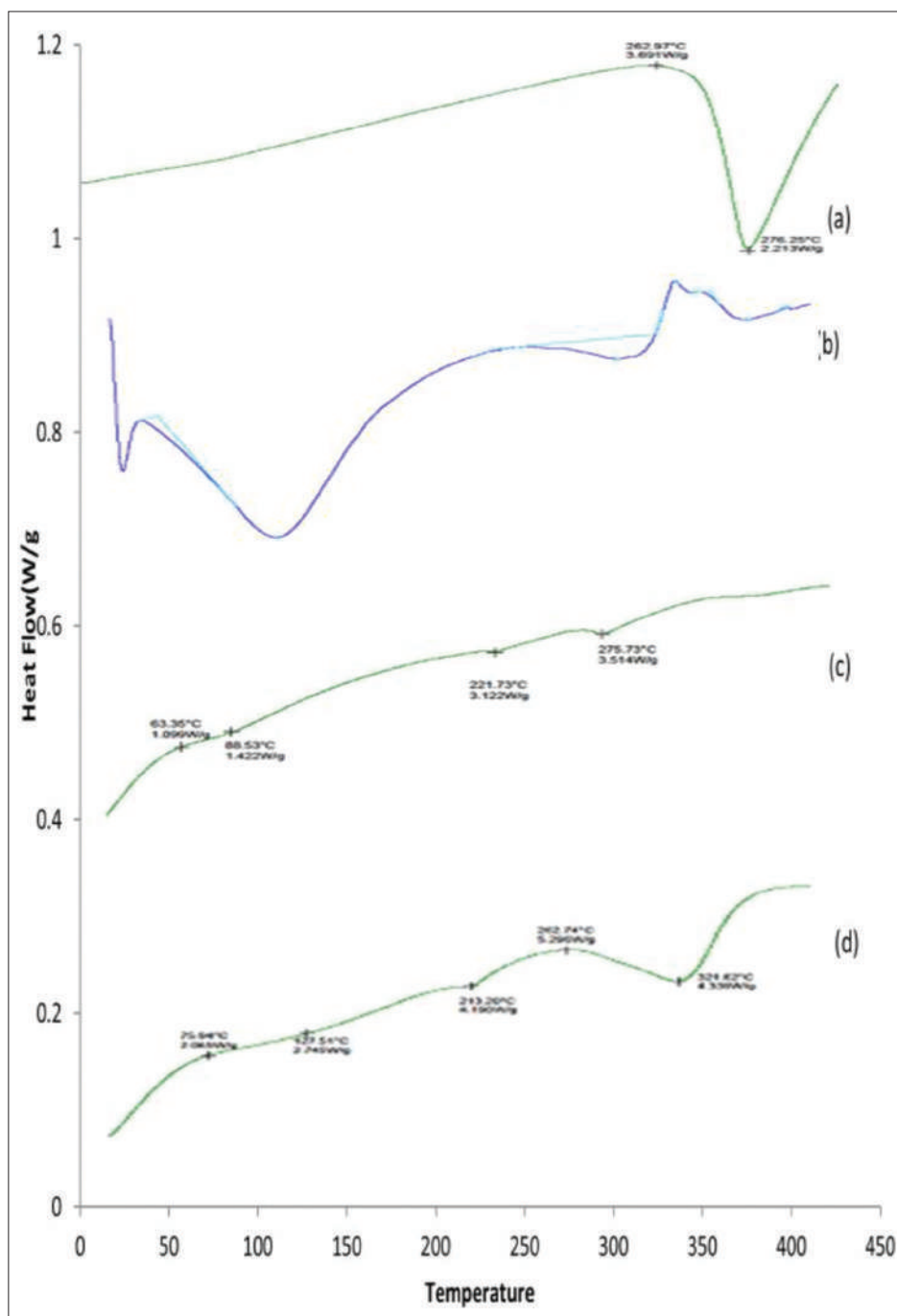
Figure 2: Differential scanning calorimetric of (a) pure drug (b) modified chitosan (c) physical mixture (d) formulation

to be 0.11–0.23 mm. The highest thickness was found in formulations OFXF8 with the highest polymeric mass. Uniformity of weight for all formulations was found to be 3.82–5.75. The weight of formulations OFXF9 was showed maximum due to increase the concentration of polymers and plasticizer effect. The surface pH of the prepared ocular inserts was found to be in between 5.72 and 7.01. This indicates that the prepared inserts would not alter the pH of tear fluid (7.4) in the eye. The drug content of all the formulations was found to be in the range of 0.081 mg–0.110 mg. Content uniformity data showed that product uniformity is good and there is not much variation in content in each

insert. The content uniformity was slightly affected by the content of polymeric mass. Increase polymeric mass, increases the drug content.

The % moisture absorption repose surface and contour plot presenting the effects of PVA amount (X1) and modified chitosan amount (X2) on the % moisture absorption shown in Figures 3 and 4.

The % moisture absorption was calculated for all nine formulations, as shown in Table. According to the result obtained, the moisture absorption is more in the formulations

Formulation code	Thickness (mm)	Weight variation (mg)	Surface pH	Drug content (mg)	% moisture absorption	% moisture loss	Mucoadhesive Time (h)
OFXF1	0.11	3.42	6.54	0.081	4.23	3.72	0.86
OFXF2	0.12	3.63	6.74	0.082	6.76	4.60	0.88
OFXF3	0.12	3.23	5.72	0.083	6.34	5.03	0.87
OFXF4	0.15	4.21	5.90	0.088	8.12	5.84	0.91
OFXF5	0.11	4.82	6.83	0.094	8.54	7.37	0.89
OFXF6	0.14	3.90	6.72	0.104	7.68	7.02	0.90
OFXF7	0.13	5.20	6.92	0.106	7.37	7.25	1.15
OFXF8	0.21	5.75	7.01	0.107	9.42	8.42	1.25
OFXF9	0.13	5.01	6.85	0.110	7.87	7.45	1.10

Table 3: Physicochemical parameter of ofloxacin ocular insert
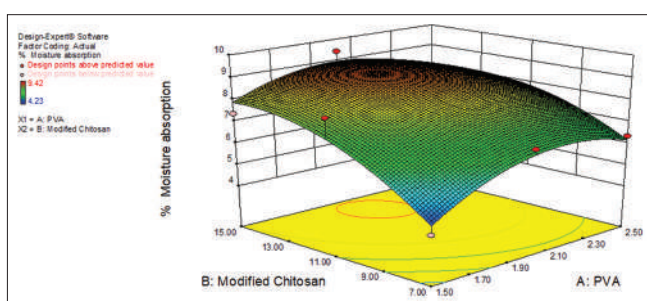
OFX: Ofloxacin

Figure 3: Repose surface plot presenting the effects of polyvinyl alcohol amount (X1) and modified chitosan amount (X2) on the % moisture absorption
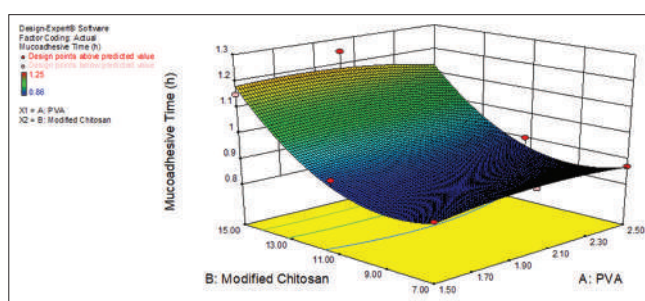

Figure 5: Repose surface plot presenting the effects of polyvinyl alcohol amount (X1) and modified chitosan amount (X2) on the mucoadhesion time
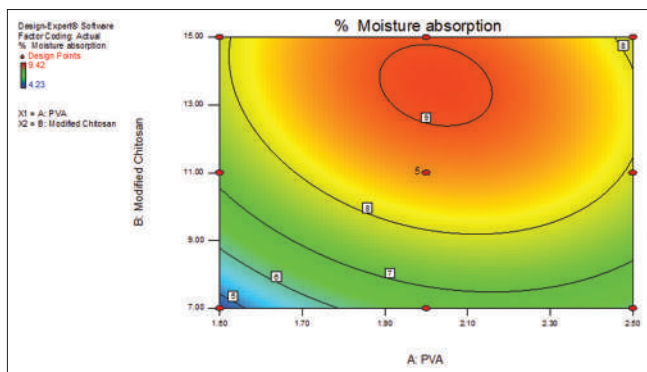

Figure 4: Contour plot presenting the effects of polyvinyl alcohol amount (X1) and modified chitosan amount (X2) on the % moisture absorption
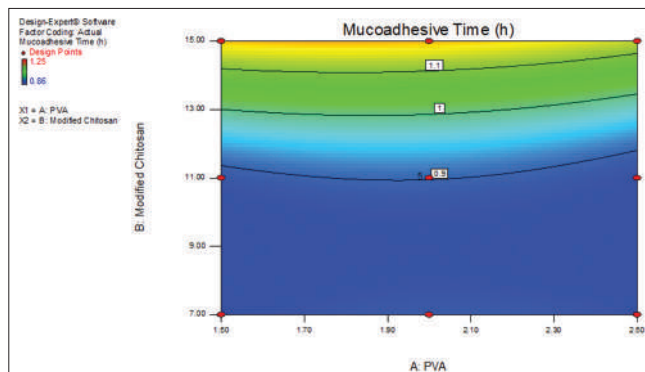

Figure 6: Contour plot presenting the effects of polyvinyl alcohol amount (X1) and modified chitosan amount (X2) on the mucoadhesion time

in which hydrophilic polymers are present. The highest moisture absorption was marked for OFXF8 formulations. This may be due to the presence of PVA, which is relatively more hydrophilic in nature. As a higher amount of moisture, the film becomes soft and maybe effect on formulation integrity. The formulations OFXF1 have shown the minimum % moisture absorption.

The % moisture loss was determined in triplicate shown in Figures 4 and 5. The moisture loss was occurred, when all the formulations were kept in very dry conditions. The

moisture loss for all formulations varied between 3.72 and 8.42. Formulations OFXF8 showed maximum moisture loss, i.e., 8.42 ± 0.42 under dry conditions.

Result of Design-Expert software-assisted batches of ofloxacin ocular insert shown in Table 3.

Mucoadhesion time

Mechanisms of mucoadhesion is binding of the dosage form to the gastric epithelial cell surface so its stick to the gastric

Table 4: Result of ANOVA								
Response model	**Sum of square**	**Degree of freedom**	**Mean square**	**F value**	**P value**	**R square**	**Ade. precision**	**CV %**
% Moisture absorption	21.5	12	4.22	15.99	0.0010 Significant	0.9195	12.79	6.44
Mucoadhesion time	0.19	12	0.037	28.61	0.0002 Significant	0.9533	13.48	3.78

mucosa. Repose surface and contour plot presenting the effects of PVA amount (X1) and modified chitosan amount (X2) on the mucoadhesion time shown in Figures 5 and 6.

The mucoadhesion time of various ocular insert was found to be 0.86–1.25 h. The formulations OFxF8 was showed maximum mucoadhesion time, due to the presence of hydroxyl group in the inserts (contributed by PVA and modified chitosan), which could form additional bonding with the mucosa.

Result of ANOVA study

Responses observed for nine formulations were fitted to the Design-Expert software for ANOVA study and result of it shown in Table 4. Statistical analysis data suggested the quadratic model for % moisture absorption and mucoadhesion time variables.

Mathematical relationship in the form of the polynomial equation for the measured response % moisture absorption

% Moisture absorption = $+8.68+0.36*+1.22B*-040*A*B-1.12*A^2-0.93*B^2$.

Mathematical relationship in the form of polynomial equation for the measured response % mucoadhesive time is given in equation below.

Mucoadhesive Time = $+0.90-8.33*A+0.15B*-0.015*A*B-0.026*A^2+0.13*B^2$

In vitro permeation studies

The *in vitro* permeation studies of ofloxacin ocular inserts were carried out through the Millipore membrane filter (0.15 µm) shows in Table 5 and Figure 7 and freshly excised goat cornea clamped between donor and receptor compartment of all glass modified Franz diffusion cell. The results are shown in Table 5, indicating that the *in vitro* drug release from plain ocular inserts (OFXF1-OFXF3) was sustained for 4 h. The drug release from (OFXF4-OFXF6) was sustained for 5 h, while OFXF7-OFXF9 could sustain drug release up to 7 h. After coating with Eudragit, drug release from ocular inserts was found to be sustained due to the presence of the polymeric film. The Eudragit S100 is a polymer of methacrylic acid ester containing 5% and 10% trimethyl ammonium methacrylate

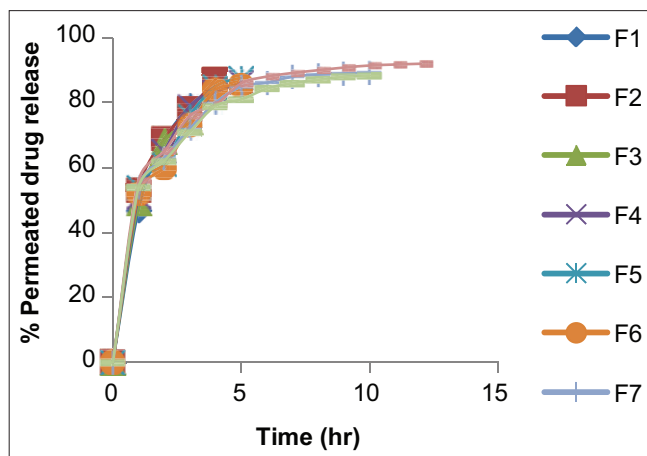| Table 5: *In vitro* permeation of ofloxacin ocular insert through Millipore membrane filter and goat cornea		
Formulation	**Millipore membrane filter**	**Goat cornea**
	Drug permeated (%)	**Drug permeated (%)**
OFXF1	85.12±0.61 (4)	13.05±0.72 (4)
OFXF2	87.21±0.66 (4)	14.03± 0.63 (4)
OFXF3	84.24±0.60 (4)	13.07±0.56 (4)
OFXF4	86.31±0.52 (5)	15.01±0.51 (4)
OFXF5	87.64±0.33 (5)	16.01±0.67 (4)
OFXF6	85.13±0.45 (5)	15.12± 0.52 (4)
OFXF7	89.23±0.46 (10)	16.11± 0.39 (4)
OFXF8	92.10±0.44 (12)	17.24±0.37 (4)
OFXF9	88.34±0.49 (10)	16.14±0.43 (4)

OFX: Ofloxacin

Figure 7: Comparative *in vitro* permeation profile (%) of all formulation of ofloxacin inserts with Eudragit S100 coating, through the Millipore membrane filter. Data are represented as mean ± SD (*n* = 3)

chloride. Among all formulation, OFXF8 showed the best sustaining effect. The OFXF8 formulation gave 92.12% drug permeated through the Millipore membrane filter after 12 h.

The *in vitro* drug permeation studies of ocular inserts formulations of ofloxacin through excised goat corneas are shown in Table 5 and Figure 8. To mimic real-life condition, excised goat corneas were used for permeation studies and experiment was conducted for 4 h considering cornea viability

Table 6: Release kinetics regression coefficient (r^2) values of drug release data calculated from various drug release kinetic models and "n" value (diffusional exponent) in accordance with Korsmeyer–Peppas

| Formulation code	Zero-order r^2		First-order r^2		Higuchi r^2		Hixson Crowell r^2	
	Millipore membrane	Goat cornea	Millipore membrane	Goat cornea	Millipore membrane	Goat cornea	Millipore membrane	Goat cornea
OFXF1	0.8634	0.9511	0.9930	0.4849	0.9738	0.9831	0.9790	0.9755
OFXF2	0.8111	0.9604	0.9915	0.4835	0.9961	0.9899	0.9963	0.9937
OFXF3	0.8420	0.955	0.9782	0.4848	0.9758	0.9993	0.9766	0.9948
OFXF4	0.7931	0.9734	0.9703	0.4813	0.9609	0.9881	0.9544	0.9995
OFXF5	0.8072	0.9638	0.9729	0.4806	0.9655	0.9804	0.9706	0.9965
OFXF6	0.8111	0.9748	0.9570	0.4811	0.9659	0.9796	0.9591	0.9964
OFXF7	0.6406	0.9625	0.9399	0.4805	0.9211	0.9621	0.9079	0.9861
OFXF8	0.6724	0.9484	0.8982	0.4801	0.8768	0.9599	0.8577	0.9849
OFXF9	0.6445	0.9559	0.9636	0.4799	0.9428	0.9507	0.9356	0.9647

OFX: Ofloxacin
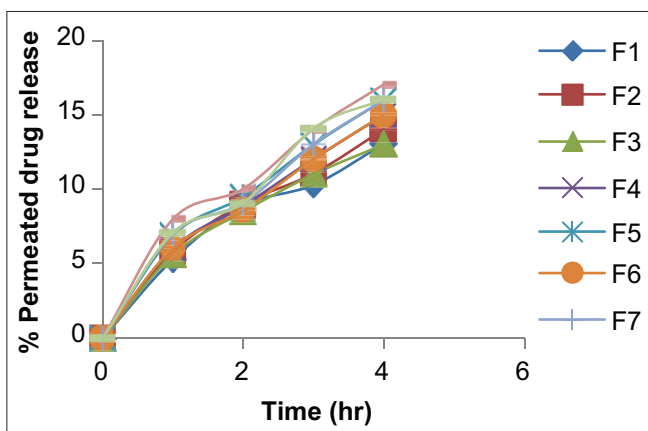
Figure 8: Comparative *in vitro* permeation profile (%) of all formulation of ofloxacin inserts with Eudragit S100 coating, through the excised goat cornea. Data are represented as mean ± SD (*n* = 3)
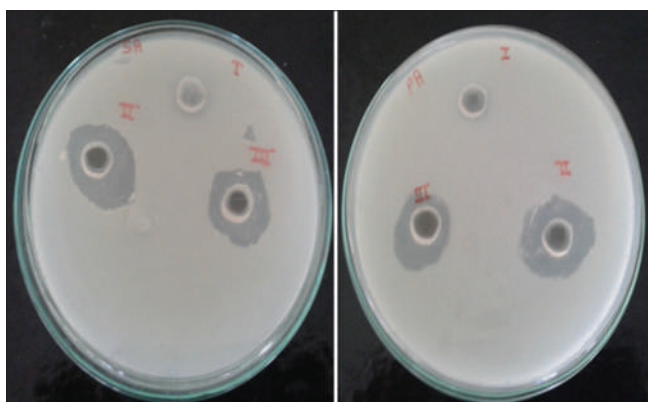

Figure 9: ZOI of formulation and marketed eye drop seeded with *Staphylococcus aureus* and *P. aeruginosa*. I: Without drug II: Marketed formulation III: Optimized formulation

and the drug permeation from ocular inserts ranged between 13.02 and 17.05, which was less than the permeation observed with the Millipore membrane filter in 4 h. In goat corneas permeation studies, the formulation OFXF8 showed a 17.06 % release at the end of 4 h, which is more as compared to other formulation. Millipore membrane filter acts as a mechanical barrier to drug diffusion while cornea (made of epithelium [lipophilic], stroma [hydrophilic], and endothelium [less lipophilic than epithelium]) acts as a lipophilic-hydrophilic barrier and the drug will have to partition through the barrier for corneal penetration. Accordingly, permeation through the cornea would be lower compared to that across the Millipore membrane filter.

Release kinetics

The release profiles of ocular inserts were treated with Korsmeyer–Peppas equation and slope values of release exponent (n) determined from *in vitro* drug release data of various ofloxacin ocular inserts are shown in Table 6 were <0.85, indicating the anomalous non-Fickian drug release from ocular inserts through the Millipore membrane filter and excised goat cornea (0.480–0.656), i.e., the rate of solvent penetration and drug release is in the same range.

Regression coefficient (r^2) values of drug release data shown in Table 7.

Regression coefficient (r^2) values of drug release data calculated from various drug release kinetic models and "*n*" value (diffusional exponent) in accordance with Korsmeyer–Peppas.

Antibacterial activity

The optimized ocular insert formulation showed antibacterial activity tested microbiologically by the cup-plate technique. The antibacterial efficiency of the selected sustained-release ofloxacin formulation OFXF8 was calculated against *S. aureus, P. aeruginosa* result is shown in Figure 9. The zone

Table 7: Regression coefficient (r^2) values of drug release data

Formulation code	Korsmeyer–Peppas			
	Millipore membrane		Goat cornea	
	r^2	n (slope)	r^2	n (slope)
OFXF1	0.959	0.654	0.985	0.629
OFXF2	0.990	0.538	0.995	0.621
OFXF3	0.965	0.573	0.999	0.611
OFXF4	0.960	0.494	0.993	0.655
OFXF5	0.968	0.480	0.983	0.607
OFXF6	0.972	0.489	0.985	0.656
OFXF7	0.939	0.635	0.960	0.640
OFXF8	0.920	0.656	0.954	0.549
OFXF9	0.955	0.629	0.946	0.616

OFX: Ofloxacin

Table 8: Antimicrobial activity of marketed formulation and optimized formulation

Microbial strain	ZOI (mm) Standard	ZOI (mm) OFXF8
Staphylococcus aureus	20.5	18.13
Pseudomonas aeruginosa	18.2	16.45

OFX: Ofloxacin

of inhibition of standard and ophthalmic formulation was found to be 20.5 mm and 18.2 mm. The zone of inhibition was better with staphylococcus aureus (Gram-positive microorganism) when compared to *P. aeruginosa* for the formulation and marketed eye drop. The zone of inhibition of standard and ophthalmic formulation was found to be almost similar. The inhibition zones were evaluated after 24 h and reduction in the growth of microorganisms was clearly observed. The zone of inhibition increased significantly as the amount of ofloxacin diffused from the ocular insert was increased.

Antimicrobial activity of ocular insert formulation of modified chitosan-based ofloxacin is very effective, as shown in Table 8 can be a promising vehicle for ocular administration of antibacterial against *S. aureus*.

CONCLUSION

In the present work, the ocular insert of ofloxacin was successfully formulated with PVA and modified chitosan. From the preformulation studies, it was found that the ofloxacin was a best-fitted drug for the ocular drug delivery system. The melting point of the drug ranges between 254°C and it is fitted in the criteria. This indicates that the drug is suitable for ocular drug delivery. The physicochemical properties of inserts were found in a satisfactory range. In

ocular inserts were studied for thickness, drug content, mucoadhesion time, and *in vitro* drug release parameters which all are within an acceptable range. The formulation OFXF8 showed a maximum *in vitro* drug profile, better-sustained action, and exhibited Korsmeyer–Peppas drug release kinetics (non-Fickian). Eudragit S100 as a sustained drug release polymer showed promising sustained action *in vitro* drug release. Furthermore, in conclusion, we suggest that the ocular insert formulation of modified chitosan-based ofloxacin can be a promising vehicle for the ocular administration of antibacterial against *S. aureus*. The optimization study was successfully conducted using 3^2 factorial designs. Finally, we concluded that the developed ocular inserts is alternative to conventional ophthalmic products by virtue of its ability to improve bioavailability and improving the patient compliance.

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