sciendo Acta Marisiensis - Seria Medica 2021;67(4):227-237 DOI: 10.2478/amma-2021-0032

RESEARCH ARTICLE

Formulation and evaluation of controlled release ocusert of gatifloxacin and prednisolone

Navneet Nagpal[1], Sukhmanpreet Singh[1], Guraasdeep Kaur[1], Parminderjit Kaur[1], Ravi Kumar Dhawan[2], Mandeep Singh Chhina[3], Manisha Arora[3*]

1. Department of Pharmaceutics, Khalsa College of Pharmacy, Amritsar, India
2. Department of Pharmacology, Khalsa College of Pharmacy, Amritsar, India
3. Department of Pharmaceutical Chemistry, Khalsa College of Pharmacy, Amritsar, India

Objective: Ocuserts are sterile, solid, or semisolid dosage forms prepared to attain increased contact time between the drug and the conjunctival tissue to keep up a constant release of drug when placed in the lower cul-de-sac or conjunctival sac of the eye. The aim of formulating this delivery system was to treat both inflammatory and infectious conditions of the eye with increased ocular residence time by releasing drugs at a slow and constant rate. **Method**: Gatifloxacin, and prednisolone ocuserts were prepared by solvent casting method, and evaluated for physical appearance, uniformity of weight, thickness, folding endurance, drug content, surface pH, in-vitro, and ex-vivo release profile. **Results**: All formulated inserts exhibited positive results in terms of their evaluation parameters. Ocuserts were sterile, with no turbidity in selected media during the study, and they were stable throughout six months. **Conclusion**: Results suggested that prepared optimized ocusert formulation would be a suitable alternative to eye drops for treating conjunctivitis and other bacterial infections.

Keywords: eye, ocusert, gatifloxacin, prednisolone

Received 11 August 2021 / Accepted 15 October 2021

Introduction

The eye presents distinctive scope and challenge in the field of pharmaceuticals delivery. Pharmaceutical scientists consider ophthalmic drug delivery as the most compelling and demanding accomplishment [1]. The main challenge to pharmaceutical scientists is to bypass the shielding eye barriers with no major injury to the tissue [2]. The eye is seen as a doorway for the administration of drugs and is mostly used for local treatment than systemic treatment to evade the possibility of harm to the eye due to the high concentration of drugs in the blood of the eye [3].

To eliminate the demerits of the conventional ocular drug delivery systems, new advancements are needed to be made for ocular drug delivery systems to develop an extended period and controlled release approach [4]. Importance to the formulation of new, and advanced ocular drug delivery systems should be given due to the establishment of innovative, and more responsive diagnostic methods, and therapeutic substances [5]. Efforts are made in the past few years to develop a formulation with better ocular drug bioavailability by changing some parameters like viscosity, and the addition of polymers that aims to achieve effective drug concentrations at the desired site of action for a specific period. So far, extended corneal contact time is the main satisfactory development in ocular drug delivery systems. Accordingly, it is sound to consider non-conventional approaches such as nanotechnology, microspheres, liposomes, prodrug, etc. for efficient release, and to enhance ocular drug delivery systems, and decrease after effect [6] .

* Correspondence to: Manisha Arora
E-mail: m.nagpal721@gmail.com

Gatifloxacin, a fourth-generation fluoroquinolone, is the most commonly used drug against most Gram-positive bacteria like *Staphylococcus aureus,* and Gram-negative bacteria like *Haemophilus influenzae*. Gatifloxacin is used against bacterial conjunctivitis, ocular infections, irritations, and for prophylaxis against endophthalmitis in cataract surgery [7]. It has a melting point of 182-185°C and Log P of 2.6. pH of gatifloxacin (0.3 %) eye drop is between 5 to 5.5 and the usual dose is one drop solution every two to three hours in the affected eye. The octanol/water partition coefficient of gatifloxacin at pH 5.1 was found to be 0.044, and at pH 7.0, the value increased to 0.145. Thus, the drug is in its unionized form when the shifting of pH of the formulation toward neutrality (pH of tear fluid) and higher lipid solubility at neutral pH encourages high permeation of gatifloxacin through the cornea [8]. The bactericidal action of gatifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination [9]. It is well absorbed from the gastrointestinal tract after oral administration with bioavailability of 96% and has t1/2 from 7 to 14 hours. Gatifloxacin undergoes limited biotransformation with less than 1% of the dose excreted in the urine as ethylenediamine and methylethylenediamine metabolites. It is primarily excreted in the urine unchanged via glomerular filtration as ethylenediamine and methylethylenediamine metabolites [10]. Figure 1. shows the chemical structure of gatifloxacin [11].

Topical corticosteroids are commonly used as a routine treatment over several weeks to reduce the inflammatory reaction after cataract surgery [12]. Corticosteroids are successful at reducing ocular inflammation because of

their ability to inhibit nearly all chemical mediators in the inflammatory cascade [13]. They downregulate inflammation by inhibiting deoxyribonucleic acid (DNA) transcription in the cell nucleus and interrupt the inflammatory cascade by increasing histaminase production; histaminase is an enzyme that breaks down unbound histamine into an inactive metabolite by inhibiting the production of phospholipase A2, which produces arachidonic acid from phospholipids in cell walls. Arachidonic acid is the main precursor to inflammatory mediators, such as prostaglandins and leukotrienes [14]. Prednisolone acetate 1% has been used for inflammation control [15]. Prednisolone has a melting point of 235° C, Log P of 1.62, and partition coefficient of 0.11. This corticosteroid achieves its highest aqueous level (669.9 ng/ml) within 120 min and maintains a significant level over 24 h; thus, maximum four-daily application of prednisolone acetate 1% may be suitable for various inflammatory conditions [16]. Prednisolone can be reversibly metabolized to prednisone. It is then further metabolized in the liver. Prednisolone and the metabolites are excreted predominantly in the urine [17]. Figure 2. shows the chemical structure of prednisolone [11].

The antibiotic and anti-inflammatory formulation is necessary to treat both inflammatory and infectious conditions of the eye i.e. conjunctivitis, post-operative eye conditions, cataract surgery postoperative management; with increased ocular residence time by releasing drugs at a slow and constant rate with accurate dosing leading to reduction of systemic absorption with better patient compliance [18]. Combined doses with both drugs were developed to reduce the number of applications required and the potentially toxic effects due to preservatives [19]. Even at low concentrations, the preservatives and buffering agents cause some degree of cell damage to ocular tissue as evaluated using corneal and conjunctival cells in tissue culture. The toxicity increases with increasing drug concentrations. Corneal toxicity caused by preservatives may cause ocular discomfort and vision changes and may interfere with patient compliance with the recommended dosage [20].

Eye drops are having the drawback of dose frequency, low bioavailability, and systemic side effects. So, this study is aimed to counter these drawbacks, and hence, increasing patient compliance, and bioavailability, reducing systemic side effects, and frequency of dosing.

Ocusert consists of a drug reservoir, sandwiched between two films of the microporous polymeric membrane. Ocuserts are defined as sterile preparations with solid or semisolid consistency, whose size & shape is maintained for easy ocular administration. They consist of a polymeric vehicle containing the drug. Ocuserts are placed in the lower cul-de-sac or conjunctival sac of the eye. The drug release from such a system is controlled by lachrymal fluid, which permeates through the membrane. Sufficient internal pressure is achieved to drive the drug out from the reservoir. The drug delivery rate is controlled by diffusion through the membrane [21]. Ocuserts are available in different types according to their composition and applications. Figure 3. shows the classification of ocuserts [6].

Despite some disadvantages i.e. anxious administration and difficult removal, dislocation in front of the pupil, accidental loss of ocusert while sleeping or rubbing the eyes, and felt like a foreign body in the eye or cutting sensation [22] ocuserts are advantageous in saving time for the healthcare professionals and patients as well [23]. Ocuserts increases the duration of action of a drug improves bioavailability, reduces the frequency of dosing, and therefore have better patient compliance. The drug can also be administered to the inflamed eye due to the controlled release of the medicament. Advantages of the device also include therapeutic effectiveness and continuous release rate, less effect on accommodation, less miosis, convenience for the patient, and reliability in patients who must rely on others for treatment (eg, children and the elderly). Some disadvantages encountered can also be resolved with instruction and encouragement of patients [24]. Overall, when compared to eye drops, the Ocusert system presents many definite advantages and is a highly desirable method of therapy in many eye diseases. A schematic representation of an ocusert is given in figure 4.

Materials and methodology

Materials

Gatifloxacin and prednisolone were procured as a gift sample from Ramson Remedies, Amritsar. Hydroxypropyl methylcellulose (HPMC), Polyvinyl alcohol (PVA), Sodium alginate, and Dibutyl phthalate were purchased from Sigma-Aldrich. All other reagents used were of analytical grade. Shimadzu's Ultraviolet spectroscopy (UV-1800), Thermoscientific's Fourier transform infrared spectroscopy (Nicolet iS5), Mattler Tolido Stare's Differential Scanning Calorimeter (DSC 3) were used for spectroscopic analysis.

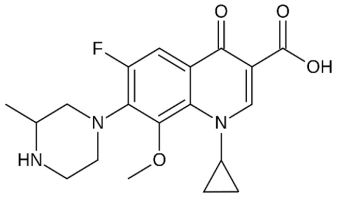

Fig. 1. Chemical structure of gatifloxacin

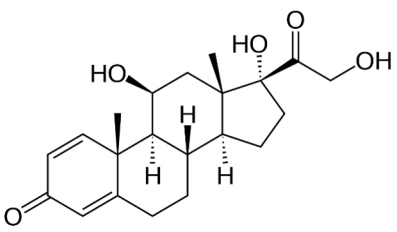

Fig. 2. Chemical structure of prednisolone

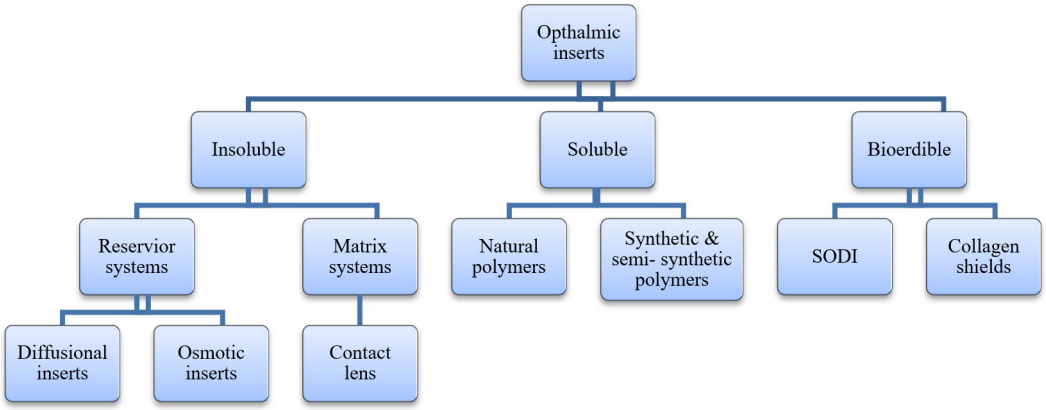

Fig. 3. Classification of ocuserts

Drugs excipient compatibility study

The mixture of drugs and excipients was kept at 50°C for 4 weeks. Characterization of the mixture was done using DSC, FTIR, and UV spectrophotometric methods. The drugs and polymers were weighed separately as per their formulation ratio in a 10 mL glass vial and mixed using a vortex mixer for 2 min. Then in each vial, 10% of the water was added and the drugs-excipient blend was further mixed. Vials were sealed with a Teflon-lined screw cap and stored at 50°C for 4 weeks. The samples were examined for any unusual color change periodically [25].

Characterization by UV spectrophotometer

UV spectrophotometric assay technique has become very significant to validate the quality of drugs and pharmaceutical formulations. The samples were taken from storage after 4 weeks and analyzed using a UV spectrophotometer. The drug content of initial and stored samples was measured. Appropriate dilutions were made and analyzed using a UV spectrophotometer at 285.5 nm for gatifloxacin and 247.5 for prednisolone against blank [26].

FTIR measurement

Assessments of drug-drug and drug-excipient compatibilities were performed by FTIR spectroscopy. Nujol mulling technique was used to prepare the sample film and dried ground fine samples were analyzed in the frequency range between 4000 and 400 cm^{-1}. Dried and fine ground KBr (1%) was mixed with ground drug and excipients and a homogenous, transparent film was prepared by applying pressure of 1000 kN/m^2 with a hydraulic press. Resulted spectrums were compared with the reference spectrum [27].

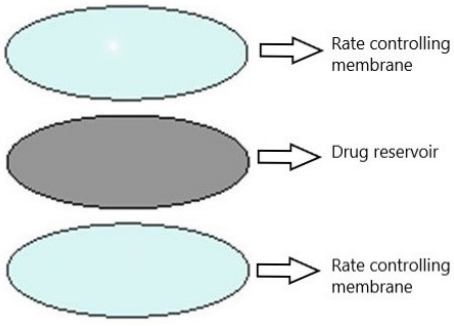

Fig. 4. Schematic representation of ocusert

DSC analysis

DSC is a highly sensitive technique, used widely in the pharmaceutical to determine the thermal transitions of API's and excipients. Drugs-excipient compatibility study was done on the stored mixture (kept at 50°C for 4 weeks) by the DSC curve of heat flux versus temperature or versus time at a rate of 50°C min^{-1} from 50 to 200°C temperature range under nitrogen flow of 25 mL min^{-1} to determine the melting temperature (*T*m) [28, 29].

Preparation of ocuserts

The gatifloxacin and prednisolone ocuserts were prepared by solvent casting method. Preparation consisted of the following three steps:

Step-I: Preparation of the drug reservoir
Step-II: Preparation of the rate-controlling membrane
Step-III: Sealing of the rate-controlling membranes with the reservoir

Step-I: Preparation of the drug reservoir

The required quantity (as shown in table I) of polymers and plasticizer was weighed and dissolved in double-distilled water. The mixture was heated for 2 to 3 hours at 50-60°C on a water bath with frequent manual stirring until the entire polymer was dissolved. The weighed amount (as shown in table I) of gatifloxacin and prednisolone was added and stirred for about 4 hours at 40-50°C on a magnetic stirrer to get uniform dispersion. After complete mixing, 2 mL of the prepared casting solution was poured into glass rings of 8 mm internal diameter, which were lying on the mercury as substrate in the Petri dish as shown in figure 5 (A) and then placed in the hot air oven for 24 hours at 40°C. The Petri dish was covered with an inverted funnel to ensure the slow evaporation of the solvent during heating as shown in figure 5 (B). The dried films were then separated from glass rings carefully [30].

Step-II: Preparation of rate-controlling membrane

A weighed quantity (as shown in table II) of polymer and plasticizer was dissolved in the double-distilled water at room temperature. Continuous and constant stirring was done to obtain a uniform polymeric solution. The solution was then poured into a glass ring, of 9 mm internal diameter which was lying on the mercury as substrate in the Petri dish and then placed in the hot air oven for 24 hours at 40°C. The Petri dish was covered with an inverted funnel to ensure the slow evaporation of the solvent. The dried films were then separated from glass rings carefully [30].

Step-III: Sealing of the films

The drug reservoir was sandwiched between the two rate-controlling membranes and sealing was done by applying chloroform on the edges of the rate-controlling membrane to control the release from the periphery. The prepared ocuserts were stored in an air-tight container until use [31].

Table I. Composition of the reservoir films for 20 rings

S.No.	Ingredients	F1A	F2A	F3A	F4A
1.	Gatifloxacin (mg)	15	15	15	15
2.	Prednisolone (mg)	50	50	50	50
3.	HPMC (mg)	30	50	70	90
4.	PVA (mg)	90	70	50	30
5.	Di-butyl phthalate (% of polymer)	30%	30%	30%	30%
6.	Double distilled water (mL)	5	5	5	5

Table II. Composition of rate-controlling membranes for 20 rings

S.No.	Ingredients	F1B	F2B	F3B	F4B
1.	Sodium alginate (mg)	50	100	150	200
2.	Di-butyl phthalate (% of polymer)	30%	30%	30%	30%
3.	Double distilled water (mL)	5	5	5	5

Characterization of ocuserts

Organoleptic characteristics

Ocuserts were evaluated for the organoleptic characterization i.e. texture, appearance, odor, and color [32].

Uniformity of thickness

The thickness of the ocusert was determined to ensure the uniform distribution of the drug substances. Uniformity of thickness was determined using a Micrometre screw gauge [30].

Uniformity of weight

This was determined to ensure that each film contains the consistent amount of a drug without significant deviation. From each batch, three ocuserts were weighed individually using a digital balance. The mean weight of ocuserts was noted [33].

Table III. Preparation of different formulation batches of ocuserts as per Step-III

Formulations	Drug reservoir + rate-controlling membrane
F1	F1A + F1B
F2	F1A + F2B
F3	F1A + F3B
F4	F1A + F4B
F5	F2A + F1B
F6	F2A + F2B
F7	F2A + F3B
F8	F2A + F4B
F9	F3A + F1B
F10	F3A + F2B
F11	F3A + F3B
F12	F3A + F4B
F13	F4A + F1B
F14	F4A + F2B
F15	F4A + F3B
F16	F4A + F4B

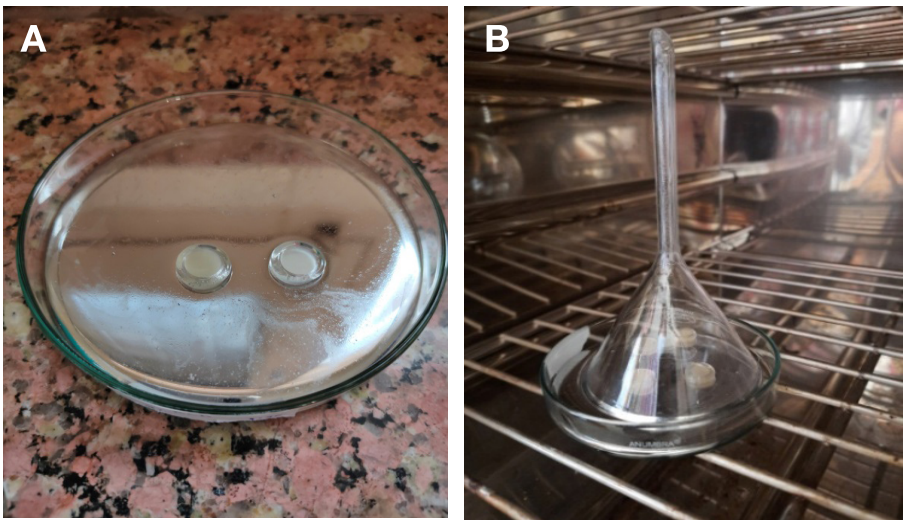

Figure 5. A Glass rings with casting solution,lying on the mercury. B. Evaporation process during preparation of ocuserts

Drug content

Drug content is determined to measure the amount of active ingredients present in each formulation. Ocusert was dissolved in 10 mL of simulated tear fluid (STF) in a beaker [34]. The sample was withdrawn from the above solution and the absorbance was measured by UV-Visible spectrophotometer at 285.5 nm for gatifloxacin and 247.5 nm for prednisolone after suitable dilutions [33].

Percent moisture absorption

This is done to check the physical stability or integrity of the ocuserts in humid conditions. The ocuserts from each batch were weighed and placed in desiccators containing aluminum chloride. The ocuserts were taken out and re-weighed, after a successive period of 3 days [35]. The % moisture absorption was calculated using the following formula,

% Moisture absorption = Final weight – Initial weight / Initial weight x 100

Percent moisture Loss

This is carried out to check the integrity of the ocuserts in dry condition. The ocuserts were weighed and kept in desiccators containing anhydrous calcium chloride. After three days, the ocuserts were taken out and weighed again [35]. The % moisture loss was calculated using the following formula:

% Moisture loss = Initial weight – Final weight / Initial weight x 100

Folding endurance

Folding endurance is determined to check the ability of the ocusert to withstand folding. This also indicates brittleness. Folding endurance was determined by repeatedly folding a small strip of the film at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance. A mean of three readings was recorded [33].

Surface pH

The ocusert was allowed to swell in 1 mL of double-distilled water in a closed petri dish at room temperature for 30 min. Then the surface pH was determined by a digital pH meter [35].

Swelling index

Ocusert was weighed and placed 4 mL of simulated tear fluid in a beaker. After 5 minutes, the ocusert was removed and the excess simulated tear fluid on the ocuserts was wiped and the ocusert was weighed again.[36] The % swelling index was calculated by the following formula,

%Swelling index = (Weight of swollen ocusert after time t – original weight of ocusert) / original weight of ocusert x 100

In-vitro drug release study

In-vitro diffusion study is carried out using a bi-chambered donor receiver compartment model (Diffusion cell). The diffusion cell membrane (pre-hydrated cellophane) is tied to one end of the open cylinder, which acts as a donor compartment. The ocusert is placed on the membrane, which is in contact with the receptor medium comprising of simulated tear fluid. The receptor fluid is maintained at 37 ± 0.5ºC with constant stirring, using a magnetic stirrer.1 mL sample is withdrawn from receptor compartment at specific time intervals and is analyzed spectrophotometrically. Each sample withdrawn is replaced with an equal volume of simulated tear fluid. An aliquot is then analyzed using a UV spectrophotometer at 285.5nm and 247.5nm [37].

Ex-vivo permeation studies

The *ex-vivo* permeation study is carried out using a diffusion cell. The goat cornea is removed from the goat eye and mounted onto a diffusion cell in a way that the corneum side of the layer should remain in contact with the ocusert in the donor compartment. The receptor fluid is maintained at 37 ± 0.5ºC with constant stirring, using a magnetic stirrer.1 mL sample is withdrawn from receptor compartment at specific time intervals and is analyzed spectrophotometrically at 285.5nm and 247.5nm. Each sample withdrawn is replaced with an equal volume of tear fluid [38].

Kinetics of drug release

To understand the mechanism and kinetics of drug release the results of the *in-vitro* drug release and *ex-vivo* permeation study were applied with kinetic equations of zero order, first order, Higuchi, and Korsmeyer-Peppas [39].

Sterility test

Sterility test is performed according to Indian Pharmacopoeia. 2 mL of prepared ocusert solution is removed and is aseptically transferred to fluid thioglycollate medium and soybean-casein digest medium separately. The media are then incubated for not less than 14 days at 30°C to 35°C in case of fluid thioglycolate and 20°C to 25°C in the case of soybean-casein digest medium [40].

Stability study

Stability study is done as per ICH guidelines, to predict the shelf-life of a product by accelerating decomposition, mostly increasing the temperature. Characteristics such as changes in the drug concentration, color, folding endurance, etc. can be monitored during stability studies. Ocuserts were wrapped in aluminum foil and stored in a glass bottle at 40 ºC and 75% relative humidity (RH) in the stability chamber. Ocuserts were analyzed for physical appearance, % weight variation, folding endurance, and drug release, after a period of 0, 1, 2, 3, and 6 months [41].

Results and discussion

Drug-excipient compatibility studies

No color change was observed in the drug-excipient mixture during and after 4 weeks of storage. The UV assay of drugs from the drugs-excipients mixtures was found to be decent after 4 weeks (table IV), indicating the stability of the gatifloxacin and prednisolone with used excipients.

The transmittance peaks were identical to API spectra and showed no interaction or interference of impurities in FTIR spectroscopy. The data obtained by this study indicated no interaction between the drugs, and excipients, hence drug and excipient combination was safe to formulate in a novel dosage form as shown in figure 6.

DSC of gatifloxacin and prednisolone with used excipients is shown in figure 7. Thermograms of gatifloxacin and prednisolone with excipients showed a sharp peak at viz 183.8°C and 235.7°C indicated no degradation of drugs as well as no interaction between drugs and polymer.

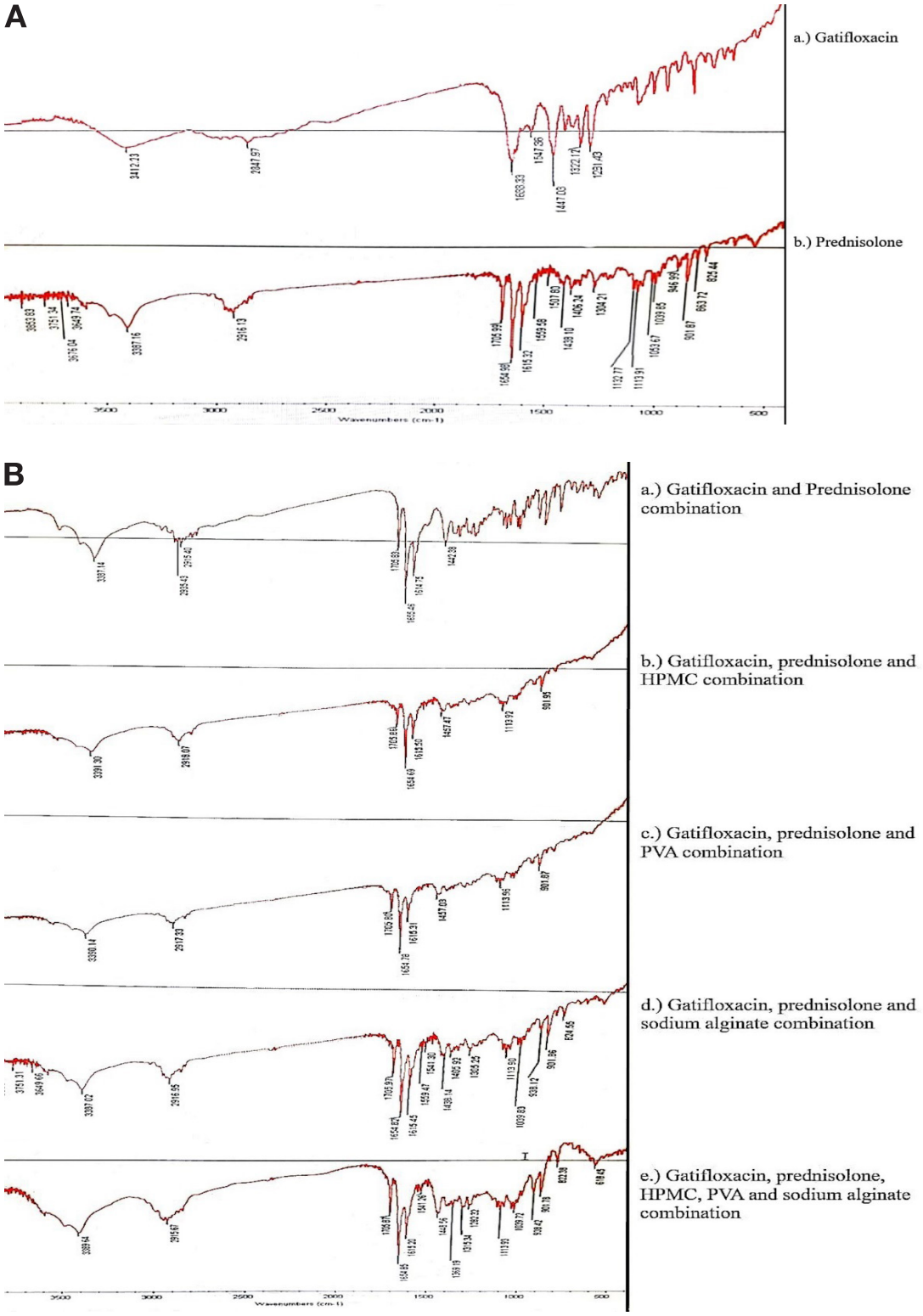

Fig. 6. A. FTIR spectrum of gatifloxacina and prednisolone. B. FTIR spectrum of gatifloxacin, prednisolone and polymers combination

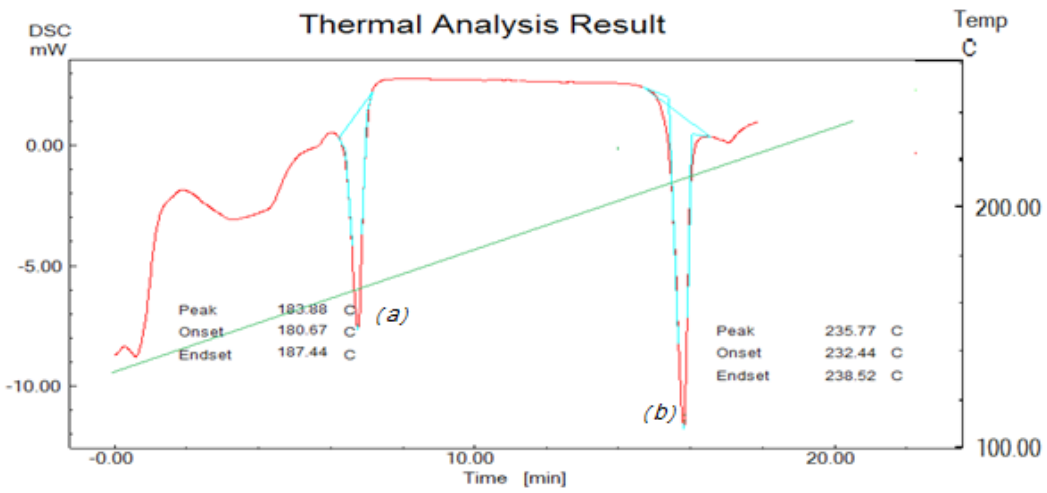

Fig. 7. DSC thermogram showing sharp endothermic melting peaks of (a) gatifloxacin at 183.88°C and (b) prednisolone at 235.77°C

Evaluation of ocuserts

Organoleptic characteristics of prepared ocuserts

Prepared ocuserts (all batches) were analyzed for color, odor, appearance, and texture. The results are given in table V.

Uniformity of Weight

This was determined to ensure that each film contains the consistent amount of a drug without significant deviation.

Table IV. UV assay of gatifloxacin and prednisolone

Storage condition	Gatifloxacin	Prednisolone
Initial	99.41±0.27	99.79±0.27
After 4 weeks storage at 50°C with excipients	99.35±0.27	99.72±0.27

Data is represented as Mean ± SD, n=3

Table V. Organoleptic properties of prepared ocuserts

S.no.	Parameters	Observation
1.	Color	Pale Yellow
2.	Appearance	Uniform
3.	Texture	Smooth
4.	Odor	Odorless

Table VI. Results of various evaluation parameters of prepared ocusert batches

Formulation batch	Weight (mg)	Thickness (mm)	Surface pH	Folding endurance
F1	5.27±0.11	0.112±0.007	7.23±0.04	103±2.64
F2	6.94±0.14	0.127±0.012	6.91±0.09	119±4.00
F3	8.32±0.15	0.135±0.008	7.54±0.17	172±6.55
F4	9.21±0.09	0.143±0.006	7.12±0.39	210±3.60
F5	5.37±0.13	0.115±0.010	7.63±0.24	110±4.58
F6	7.13±0.17	0.129±0.015	7.68±0.10	146±2.00
F7	8.45±0.11	0.137±0.013	7.49±0.15	198±5.00
F8	8.97±0.23	0.141±0.009	7.35±0.12	229±16.52
F9	5.95±0.19	0.113±0.011	6.89±0.27	123±10.53
F10	7.86±0.10	0.125±0.007	7.67±0.19	189±8.00
F11	8.16±0.16	0.136±0.014	7.48±0.31	221±3.00
F12	9.58±0.13	0.142±0.017	7.72±0.29	284±8.54
F13	4.94±0.25	0.117±0.011	7.04±0.21	173±5.56
F14	7.39±0.21	0.128±0.016	6.95±0.07	215±6.00
F15	8.72±0.10	0.132±0.018	7.81±0.13	293±9.16
F16	9.51±0.12	0.139±0.008	7.22±0.18	306±7.54

Data is represented as Mean ± SD, n=3

The average weights of ocuserts were found to be in the range of 4.94±0.25 mg to 9.58±0.13 mg as shown in table VI. The uniformity of weight suggested good distribution of the drug, polymer, and plasticizer.

Uniformity of thickness

The thickness of the ocusert was determined to ensure the uniform distribution of the drug substances. The average thickness of Ocuserts was between 0.112±0.007 mm to 0.143±0.006 mm as shown in table VI. There were no major variations in the thickness of ocuserts indicating the uniform distribution of constituents as well as uniformity of product.

Drug content

Drug content is determined to measure the amount of active ingredients present in each formulation. The drug content was found in the range of 87.23±0.32% to 99.74±0.10% for prednisolone and 85.12±0.10% to 99.48±0.27% for gatifloxacin as shown in table VII.

Percent moisture absorption

This is done to check the physical stability or integrity of the ocuserts in humid conditions. The observed % moisture absorption is in the range of 2.44±0.08 % to 10.26±0.15 % as shown in table VII. It was observed that moisture absorption was increased with an increase in the concentration of hydrophilic polymer.

Percent moisture loss

This is carried out to check the integrity of the ocuserts in dry condition. The observed % moisture loss is in the range of 4.09±0.10 % to 9.04±0.10 % as shown in table VII. It was observed that moisture loss decreased with the decrease in the concentration of hydrophilic polymer.

Folding Endurance

The folding endurance was measured in the range of 103±2.64 to 306 ±7.54 as shown in table VI. This test

Table VII. Results of various evaluation parameters of prepared ocusert batches

Formulation code	%Moisture absorption	%Moisture loss	Swelling index (%)	Drug content (Prednisolone) (%)	Drug content (Gatifloxacin) (%)
F1	2.89±0.16	4.93±0.13	2.13±0.15	95.20±0.08	96.41±0.19
F2	3.47±0.07	6.34±0.19	3.04±0.11	91.48±0.23	90.25±0.07
F3	5.12±0.13	7.69±0.17	3.45±0.25	92.76±0.14	93.33±0.11
F4	8.52±0.29	8.57±0.08	3.60±0.10	94.89±0.12	91.28±0.16
F5	2.44±0.08	4.09±0.10	2.37±0.31	97.44±0.19	92.30±0.08
F6	3.59±0.21	5.75±0.29	2.55±0.50	96.59±0.07	94.35±0.24
F7	4.05±0.19	6.03±0.36	3.86±0.35	99.74±0.10	98.96±0.36
F8	9.46±0.11	8.47±0.23	4.15±0.21	97.02±0.16	99.48±0.27
F9	5.94±0.13	7.39±0.28	3.94±0.20	98.72±0.09	95.30±0.18
F10	7.16±0.09	8.01±0.15	4.36±0.06	95.74±0.21	97.43±0.11
F11	8.86±0.24	8.21±0.09	4.84±0.15	94.46±0.13	89.23±0.21
F12	8.53±0.14	8.24±0.11	5.28±0.31	92.34±0.29	87.17±0.14
F13	6.76±0.10	5.46±0.07	4.97±0.25	98.29±0.15	95.38±0.09
F14	8.34±0.17	7.98±0.25	5.48±0.12	89.36±0.07	86.15±0.26
F15	9.58±0.06	8.60±0.17	5.90±0.05	87.23±0.32	88.20±0.15
F16	10.26±0.15	9.04±0.10	6.04±0.11	90.63±0.26	85.12±0.10

Data is represented as Mean ± SD, n=3

shows the flexibility of ocuserts. This test ensures that the prepared ocuserts are flexible films that can withhold breaking and tearing.

Surface pH

The surface pH of the prepared ocuserts was found in the range of 6.89±0.27 to 7.81±0.13 as shown in table VI. The surface pH of all formulations was found to be near to tear fluid pH.

Swelling Index

The swelling index was found in the range of 2.13±0.15 to 6.04±0.11 as shown in table VII. The result showed good swelling of the prepared ocuserts and no significant variation in absorption properties of the formulations.

In-vitro drug release study

% Cumulative drug release was found to be 79.85- 98.77% for gatifloxacin and 81.36- 99.52% for prednisolone as shown in figure 8.

Ex-vivo permeation studies

Ex-vivo permeation study was done for the selected batch (F7). The study showed the permeation of viz. 98.85±0.28% and 99.38±0.13% for gatifloxacin and prednisolone, respectively through the goat cornea.

Kinetics of drug release

Various release kinetic models such as zero order, first order, Higuchi, and Korsmeyer-Peppas were applied to the *in-vitro* release data. All the formulations were found to be following the zero-order release profile as shown in table VIII. In *ex-vivo* studies, various release kinetic models such as zero order, first order, Higuchi, and Korsmeyer-Peppas were applied to formulation F7 permeation data. The formulation (F7) was found to be following zero-order release as shown in table IX.

Sterility test

A sterility study was done for the best-optimized formulation of prepared ocuserts (F7). Results showed no turbidity

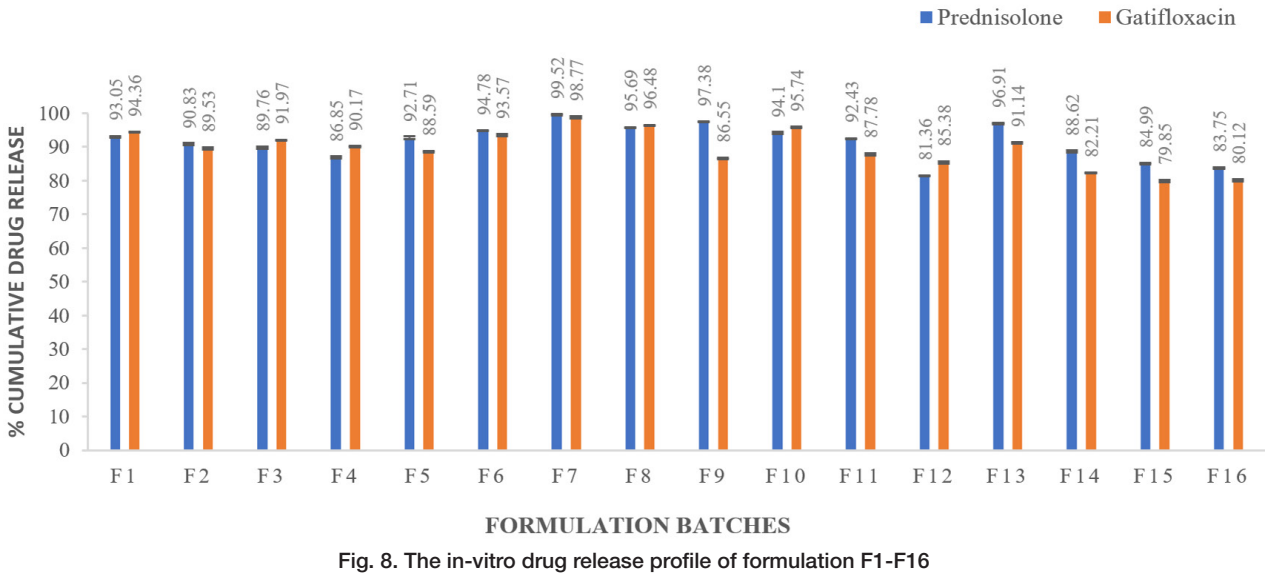

Fig. 8. The in-vitro drug release profile of formulation F1-F16

Table VIII. Correlation coefficient of kinetic modelling

Sr. no.	Formulation code	Coefficient of regression (R^2 value) for prednisolone				Coefficient of regression (R^2 value) for gatifloxacin			
		Zero order	First order	Higuchi	Korsmeyer-Peppas	Zero order	First order	Higuchi	Korsmeyer-Peppas
1.	F1	0.996	0.732	0.925	0.849	0.997	0.718	0.922	0.817
2.	F2	0.994	0.698	0.929	0.806	0.994	0.723	0.916	0.828
3.	F3	0.995	0.781	0.892	0.867	0.996	0.775	0.897	0.888
4.	F4	0.992	0.671	0.952	0.786	0.997	0.730	0.933	0.847
5.	F5	0.997	0.771	0.899	0.870	0.992	0.736	0.936	0.889
6.	F6	0.996	0.687	0.944	0.794	0.996	0.728	0.933	0.859
7.	F7	0.995	0.653	0.948	0.759	0.998	0.742	0.918	0.843
8.	F8	0.994	0.680	0.948	0.794	0.998	0.738	0.915	0.839
9.	F9	0.998	0.755	0.917	0.877	0.995	0.772	0.909	0.877
10.	F10	0.994	0.696	0.941	0.810	0.990	0.673	0.924	0.770
11.	F11	0.987	0.643	0.965	0.761	0.991	0.671	0.954	0.783
12.	F12	0.991	0.767	0.923	0.896	0.995	0.750	0.929	0.878
13.	F13	0.997	0.706	0.939	0.822	0.995	0.787	0.901	0.884
14.	F14	0.993	0.707	0.931	0.835	0.993	0.741	0.936	0.881
15.	F15	0.993	0.706	0.924	0.813	0.989	0.688	0.921	0.782
16.	F16	0.995	0.757	0.908	0.875	0.992	0.701	0.934	0.820

Table IX. Kinetics of drug permeation of batch F7

Formulation code	Release of prednisolone (R^2 value)				Release of gatifloxacin (R^2 value)			
	Zero order	First order	Higuchi	Korsmeyer-Peppas	Zero order	First order	Higuchi	Korsmeyer-Peppas
F7	0.997	0.719	0.932	0.836	0.996	0.706	0.937	0.837

and no microbial growth during and after the completion of the sterility test. As there was no appearance of microorganism, the prepared ocusert (F7) can be used for the ophthalmic purpose.

Stability studies

The stability of the prepared ocusert formulation (F7) was assessed by the mentioned parameters (Table X). It was found that the F7 formulation was stable throughout the period.

Discussion

An eye is extensively impervious to foreign particles due to its unique anatomy, physiology, and biochemistry, and is the key challenge to the researcher to circumvent the protective barrier of the eye without causing any permanent tissue damage. Currently, the knowledge in this ocular delivery system is rapidly developing and various polymeric materials are employed to serve as means of diagnostic tools or to deliver therapeutic agents the targeting of a specific site in a controlled manner. To enhance the amount of active substance reaching the target tissue or exerting a local effect in the cul-de-sac the residence time of the ocular insert should be lengthened. Moreover, combination medication provides additive effects for lowering bacterial infection and inflammation. In the most common incarnation of this ocular drug delivery system, a core of the drug-matrix is surrounded by a permeable polymeric membrane whose thickness and permeability control the release rate of the drug into the eye. The release kinetics of this system suggests that if the drug concentration within the reservoir is constant, the driving force of the drug release is constant diffusion through the polymer coating, and zero-order release kinetics can be achieved. Ocusert offers a promising avenue to fulfill the need for an ophthalmic drug delivery system that can localize and maintain drug activity at the site of action for a longer period thus allowing a sustained action, minimizing the frequency of drug administration with patient compliance. The ocusert of gatifloxacin and prednisolone containing polymer matrices of HPMC, and PVA, with a rate-controlling membrane, having sodium alginate was found to be promising ocular delivery systems for the treatment of conjunctivitis.

Conclusion

Prepared ocuserts were smooth, flexible, and were uniform in weight and thickness. The result of the *in-vitro* study suggested that the rate-controlling membrane played an important role in retarding the release of the drug from the reservoir. Various mathematical release kinetic models were

Table X. Stability studies of batch F7

F7	Duration of time				
Formulation parameters	Initial	1 month	2 month	3 month	6 month
Appearance	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Weight variation (mg)	8.39±0.13	8.38±0.11	8.38±0.18	8.37±0.09	8.37±0.18
Folding endurance	198±2.00	197±1.00	197±1.00	197±3.00	195±3.00
% CDR (Gatifloxacin)	98.77±0.29	98.70±0.21	98.68±0.21	98.56±0.27	98.50±0.34
% CDR (Prednisolone)	99.52±0.20	99.48±0.36	99.39±0.19	99.34±0.25	98.74±0.42

Data is represented as Mean ± SD, n=3; %CDR = % Cumulative drug release

applied to the ocuserts drug release data. All the formulations were found to be following the zero-order release. The formulation batch F7 was the best amongst the sixteen formulations in terms of their evaluation parameters. F7 showed better *in-vitro* drug release and also showed good ocular permeation. The results of short-term stability studies indicated that the formulated ocuserts of batch F7 were showed negligible or no changes in evaluation parameters when stored in the stability chamber as per ICH guidelines. It was found stable throughout six months. A sterility test was performed for the best-optimized formulation (batch F7), and it was found sterile, with no turbidity in the selected media during the study. So, from the tests performed HPMC, PVA, and Sodium alginate were found to be good polymeric agents for the formulation of controlled release ocusert delivery system for gatifloxacin and prednisolone. These polymers were hydrophilic in nature and biodegradable. Results suggested that prepared optimized ocusert formulation would be a suitable alternative to eye drops to treat conjunctivitis, and other bacterial infections with better bioavailability, and less frequent dosing.

Authors' contributions

NN (Conceptualization; Data curation; Writing – original draft, Supervision)
SS (Investigation; Validation; Writing – review & editing)
GK (Investigation; Validation; Writing – review & editing)
PK (Data curation; Methodology; Visualization)
RKD (Visualization; Resources; Writing – review & editing)
MSC (Formal analysis; Validation; Writing – review & editing)
MA (Conceptualization; Validation; Writing – original draft)

Conflict of interest

None to declare.

References

1. Zaki I, Fitzgerald P, Hardy J, Wilson C. A comparison of the effect of viscosity on the precorneal residence of solutions in rabbit and man. Journal of pharmacy and pharmacology. 1986;38(6):463-6.
2. Lee VH, Robinson, R J. Topical ocular drug delivery: recent developments and future challenges. Journal of ocular pharmacology and therapeutics. 1986;2(1):67-108.
3. Karthikeyan D, Bhowmick M, Pandey VP, Nandhakumar J, Sengottuvelu S, Sonkar S, et al. The concept of ocular inserts as drug delivery systems: An overview. Asian Journal of Pharmaceutics. 2014;2(4):192-200.
4. Friedrich SW, Saville BA, Cheng Y-L, Rootman DS. Pharmacokinetic differences between ocular inserts and eyedrops. Journal of ocular pharmacology and therapeutics. 1996;12(1):5-18.
5. Schoenwald R. Ocular pharmacokinetics/pharmacodynamics. Drug and the pharmaceutical sciences. 1993;58:83-110.
6. Kumar KS, Bhowmik D, Harish G, Duraivel SJ. Ocular inserts: A novel controlled drug delivery system. The Pharma Innovation Journal. 2013;1(12, Part A):1-16.
7. Cervantes LJ, Mah FS. Clinical use of gatifloxacin ophthalmic solution for treatment of bacterial conjunctivitis. Clinical ophthalmology (Auckland, NZ). 2011;5:495-502.
8. Mohd Abul K, Aws A, Ibrahim AA, Anil KM, Yasmin S. Delivery of gatifloxacin using microemulsion as vehicle: formulation, evaluation, transcorneal permeation and aqueous humor drug determination. Drug Delivery. 2014;23(3):886-97.
9. Pawar P, Katara R, Mishra S, Majumdar DK. Topical ocular delivery of fluoroquinolones. Expert opinion on drug delivery. 2013;10(5):691-711.
10. Perry CM, Balfour JAB, Lamb HM. Gatifloxacin. Drugs. 1999;58(4):683-96.
11. Indian Pharmacopoeia. Government of India, ministry of health and family welfare. Delhi: Controller of Publications. 2010:1402,951.
12. Laurell CG, Zetterström C. Effects of dexamethasone, diclofenac, or placebo on the inflammatory response after cataract surgery. Br J Ophthalmol. 2002;86(12):1380-4.
13. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br J Pharmacol. 2006;148(3):245-54.
14. Ilyas H, Slonim CB, Braswell GR, Favetta JR, Schulman M. Long-term safety of loteprednol etabonate 0.2% in the treatment of seasonal and perennial allergic conjunctivitis. Eye contact lens. 2004;30(1):10-3.
15. Campos M, Muccioli C, Malta JB, Gerade RA, A LAS, Belfort R. Efficacy and tolerability of a combined gatifloxacin plus prednisolone formulation for topical prophylaxis after LASIK. Clinical ophthalmology (Auckland, NZ). 2011;5:209-14.
16. Awan MA, Agarwal PK, Watson DG, McGhee CN, Dutton GN. Penetration of topical and subconjunctival corticosteroids into human aqueous humour and its therapeutic significance. Br J Ophthalmol. 2009;93(6):708-13.
17. Kiningham KK. Prednisolone. xPharm: The Comprehensive Pharmacology Reference. 2007:1-6.
18. Cunha PA, Shinzato FA, Tecchio GT, Weber SL, Brasil A, Avakian A. Efficacy and tolerability of a gatifloxacin/prednisolone acetate fixed combination for topical prophylaxis and control of inflammation in phacoemulsification: a 20-day-double-blind comparison to its individual components. Clinics (Sao Paulo, Brazil). 2013;68(6):834-9.
19. Ayaki M, Taguchi Y, Soda M, Yaguchi S, Iwasawa A, Koide R. Cytotoxicity of topical medications used for infection and inflammation control after cataract surgery in cultured corneal endothelial cells. Biocontrol science. 2010;15(3):97-102.
20. Ayaki M, Yaguchi S, Iwasawa A, Koide R. Cytotoxicity of ophthalmic solutions with and without preservatives to human corneal endothelial cells, epithelial cells and conjunctival epithelial cells. Clinical & experimental ophthalmology. 2008;36(6):553-9.
21. Banu S, Farheen SA. Ocular Drug Delivery System: A Novel Approach. Asian Journal of Research in Pharmaceutical Science. 2019;9(2):97-106.
22. Kumari A, Sharma P, Garg V, Garg G. Ocular inserts - Advancement in therapy of eye diseases. J Adv Pharm Technol Res. 2010;1(3):291-6.
23. Rajesh A, Poonam D, Sangeeta A. Ocular drug delivery system: ocular insert. International Journal of Universal Pharmacy and Bio Sciences. 2012;1(2):30-8.
24. Kumari A, Sharma PK, Garg VK, Garg G. Ocular inserts - Advancement in therapy of eye diseases. J Adv Pharm Technol Res. 2010;1(3):291-6.
25. Ghosh S, Barik B. Formulation and in vitro evaluation of once daily sustained release formulation of aceclofenac. Tropical journal of pharmaceutical research. 2010;9(3):265-73.
26. Freire F, Aragão C, de Lima e Moura T, Raffin F. Compatibility study between chlorpropamide and excipients in their physical mixtures. Journal of thermal analysis calorimetry. 2009;97(1):355-7.
27. Renu C, Swati B. Drug–excipient compatibility screening—Role of thermoanalytical and spectroscopic techniques. Journal of Pharmaceutical and Biomedical Analysis. 2014;87:82-97.
28. Oliveira G, Ferraz H, Matos J. Thermoanalytical study of glibenclamide and excipients. Journal of thermal analysis calorimetry. 2005;79(2):267-70.
29. Ibrahim HK, El-Leithy IS, Makky AA. Mucoadhesive Nanoparticles as Carrier Systems for Prolonged Ocular Delivery of Gatifloxacin/Prednisolone Bitherapy. Molecular Pharmaceutics. 2010;7(2):576-85.
30. Tanwar Y, Patel D, Sisodia S. In vitro and in vivo evaluation of ocular inserts of ofloxacin. DARU. 2007:139-45.
31. Karthikeyan D, Bhowmick M, Pandey V, Sengottuvelu S, Sonkar S, Gupta N, et al. Design and evaluation of ofloxacin extended release ocular inserts for once a day therapy. Res J Pharm Tech. 2008;1(4):460-8.
32. Kalyanwat R, Shrivastava B, Pathak K. Preparation and Evaluation of bioadhesive ocular inserts of aceclofenac. International Journal of Pharmaceutical Sciences Review and Research. 2016;41:207-13.
33. Saroot R, Gilhotra RM. Design and characterization of bioadhesive ophthalmic films of flurbiprofen. Thai Journal of Pharmaceutical Science. 2011;35:29-39.
34. Pawar S, Pawar R, Gadhve M. Controlled release in situ forming gatifloxacine HCl for ophthalmic drug delivery. Int Res J of Phar.

2012;3:86-9.
35. Patel UL, Chotai NP, Nagda CD. Design and evaluation of ocular drug delivery system for controlled delivery of gatifloxacin sesquehydrate: In vitro and in vivo evaluation. Pharmaceutical development technology. 2012;17(1):15-22.
36. Mathurm M, Gilhotra RM. Glycerogelatin-based ocular inserts of aceclofenac: physicochemical, drug release studies and efficacy against prostaglandin E2-induced ocular inflammation. Drug delivery. 2011;18(1):54-64.
37. Charoo NA, Kohli K, Ali A, Anwer A. Ophthalmic delivery of ciprofloxacin hydrochloride from different polymer formulations: in vitro and in vivo studies. Drug development industrial pharmacy. 2003;29(2):215-21.
38. Chandran S, Roy A, Saha RN. Effect of pH and formulation variables on in vitro transcorneal permeability of flurbiprofen: a technical note. Aaps Pharmscitech. 2008;9(3):1031-7.
39. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.
40. Indian Pharmacopoeia. Government of India, ministry of health and family welfare. Delhi: Controller of Publications. 1996;2(35):448-89, A-147.
41. ICH harmonized tripartite guideline. Stability testing of new drug substances andproducts Q1A (R2).1-18.