

EVALUATION OF OCULAR FILMS OF OFLOXACIN FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: The current study emphasizes on the treatment of ocular infection with objectives of reducing the frequency of administration, obtaining controlled release and greater therapeutic efficacy of the drug (ofloxacin) using ocular films.

Methods: Ocular films were designed by solvent evaporation method containing a different combination of polymers. The folding endurance (mechanical strength) was determined by the number of folds at a specific single place required to break the film into two parts. Thickness was measured using screw gauge. The surface pH was done by pH paper. The percentage moisture absorption was carried out by placing the ocular films in a desiccator containing ammonium chloride. Percentage moisture loss was carried out by placing the ocular films in the desiccator containing anhydrous calcium chloride. *in vitro* drug release study were carried by using a modified version of Franz diffusion cell. Stability study were carried using stability chambers as per ICH guidelines. The antibacterial activity was performed by using male albino rabbits.

Results: The thickness and folding endurance of the films were in the range of 44 ± 1.1 to 92 ± 1.8 and 4.5 ± 0.6 to 6.8 ± 0.3 , respectively for different formulations. Surface pH was evaluated in the range of 6.6 to 7.2. Percentage moisture absorption and percentage moisture loss were evaluated in the range of 1.17 ± 1.1 to 6.72 ± 1.5 and 0.58 ± 0.9 to 1.23 ± 0.9 respectively. Microbial growth was not observed in any formulation during sterility testing. The drug release for different batch codes PAH, PBE, PCP, PDC, PEEH, and PFEC was found to be 96.2, 56.9, 93.4, 94.5, 98.4 and 95.9 % respectively up to 12 h. Ocular films of batch code PEEH was optimized for maximum drug release (98.4%). The antibacterial effect was noted periodically (01 to 05 d) after administration of sterile formulation in the treated eyes vs. control eyes of each rabbit. The optimized batch PEEH of ocular films reduced the infection and redness completely within 3 d in a single dose.

Conclusion: The optimized formulation would be able to offer benefits such as increased residence time, prolonged drug release, reduced frequency of administration and improved patient compliance with complete removal of inflammation and redness from the cul-de-sac.

Keywords: Ofloxacin, Ocular films, Solvent evaporation method

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INTRODUCTION

Ophthalmic liquid preparations are insufficient due to poor bioavailability of the drug, caused by fast lachrymal secretion and poor patient compliance [1]. The unique anatomy and physiology of the eye renders it a highly protected and sensitive organ, and the unique structure restricts drug entry at the target site of action. Conventional drug delivery systems including eye drops, suspensions, and ointments for the treatment of various infections in the cul-de-sac are frequently used, but they cannot be considered optimal because most topically instilled drugs do not offer adequate bioavailability due to the wash off of the drugs from the eye through lacrimation and tear dilution [2].

In addition, the human cornea composed of epithelium, substantia propria and endothelium hinders drug entry; consequently, less than 5% of the administered drug enters into the eye. Alternative approaches (ocular inserts/films, corneal shield, sol to gel system etc.) are continuously sought to facilitate significant drug absorption into the eye [3, 4]. In this ocular research films of an antibacterial drug (ofloxacin) was developed and evaluated for antibacterial response with a programmed rate for a longer period by increasing precorneal residence time [5].

The permeability of drugs through the polymeric films is dependent on the characteristics of the polymer, the casting solvent, and the plasticizers used. Plasticizers (phthalate esters, phosphate esters, fatty acid esters, and glycol derivatives) are very useful in the preparation of polymeric ocular films to reduce the brittleness, to impart flexibility, to increase strength, and also to improve adhesiveness of the films with surfaces or membranes [6].

To accomplish the aim of this research the predicted batches of ocular films of different concentration of polymer combinations were optimized through the factorial design study and the final

batches were selected and developed containing hydroxyl propyl methyl cellulose (HPMC K100M), polyvinylpyrrolidone (PVP) and ethyl cellulose in the concentration of 0.5%, 0.5% and 1.0% respectively with polyethylene glycol (PEG 400) 10% w/w as plasticizer. Optimized ocular films were evaluated with various parameters such as folding endurance, the thickness of films, surface pH, percent moisture absorption, percent moisture loss, percent drug release, stability study, sterility testing and *in vivo* study [7, 8].

MATERIALS AND METHODS

Materials

Baxil Pharma Pvt. Ltd., Haridwar, India provided a gift sample of Ofloxacin while all the polymers of analytical grade were purchased from different suppliers of India like Hydroxyl Propyl Methyl Cellulose (HPMC K100M) from Loba Chemie Pvt. Ltd.-Mumbai, Polyvinylpyrrolidone (PVP) and Ethyl Cellulose from Qualikems fine chem. Pvt Ltd.-Delhi. Polyethylene Glycol (PEG 400) was purchased from Antares Chem. Pvt Ltd.-Mumbai and Arachidonic acid was purchased from Hi-Media Labs Pvt. Ltd., Mumbai. Microbial culture (*Staphylococcus aureus*) was purchased from Institute of Microbial Technology (IMTECH), Chandigarh, 06 male Albino rabbits, were provided by research place (animal house of R V Northland Institute) after IAEC protocol approval (Approval No.-1149/PO/ac/07/CPCSEA) through CPCSEA.

Method

Various combinations were designed for different batches of ocular films as per factorial design study with 0.5 % w/v concentration by solvent evaporation method containing a different combination of polymers such as hydroxyl propyl methyl cellulose (HPMC K100M), ethyl cellulose, carbopol 934 and polyvinylpyrrolidone (PVP). The optimized concentration of selected polymers were homogenized to

make a clear solution. When the clear solution was prepared, 25 ml solution for five films was pipetted out, and the drug was added to the solution and homogenized with the aid of stirring. The solution so obtained was poured onto the glass ring placed over the prelubricated petri plates. One glass ring consumed 5 ml of the drug solution. An inverted funnel was placed over the petri plate to allow slow evaporation. The open end of the funnel was plugged with cotton wool. The whole assembly was left undisturbed till the films dried. After the complete drying, the film rings were lifted from the petri plates, and the suitable size was cut from the help of die. Different parameters have been evaluated for optimized ocular films; folding endurance, thickness, surface pH, percentage moisture absorption, percentage moisture loss, *in vitro* drug release study, stability study and antibacterial activity were performed with male albino rabbits.

Physicochemical characterization of ocular films

The ocular films of ofloxacin were evaluated for physicochemical characteristics such as folding endurance, thickness, surface pH, % moisture absorption, % moisture loss, stability study and % drug release. The folding endurance of ocular films was determined by the number of folds at a specific single place required to break the film into two parts. The thickness of the recovered films was measured using screw gauze [9]. After performing the initial settings the film was placed on the anvil such that area where the thickness is to be measured lies. The screw was gently tightened on to the specimen and reading of the gauze was noted to get the thickness of the film. The surface pH determination of the film was done by allowing them to swell by placing 2 drops of distilled water over it. After this the swollen film was taken and pH was determined using pH paper on the surface of the film [10].

The percentage moisture absorption test was carried out to check physical stability or integrity of the ocular films. Ocular films were weighed and placed in a desiccator containing 100 ml of saturated solution of ammonium chloride. After 3 d the ocular films were taken out and reweighed. The percentage moisture absorption was calculated using the formula [11]:

$$\% \text{ moisture absorption} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

Percentage moisture loss was carried out to check the integrity of the film at dry condition. Ocular films were weighed and kept in the desiccators containing anhydrous calcium chloride. After 3 d the ocular films were taken out and reweighed, the percentage moisture loss was calculated using the formula [12]:

$$\% \text{ moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

In vitro drug release study

The inserts were placed on a modified version of Franz diffusion cell using cellophane membrane in contact with isotonic phosphate buffer pH 7.4 kept at 37±1 °C with constant stirring of 50 rpm. 1 ml Sample was withdrawn at different time intervals analyzed for drug content spectrophotometrically (Shimadzu UV 1700) at 294 nm [13, 14].

Stability study of optimized batch of ocular film

Stability studies were carried out on batch code PEEH, according to ICH guidelines by storing replicates of ocular films (packaged in aluminium foil) for a period of 0, 30, 90 and 180 d with a relative humidity 75%±5% at a temperature 40 ±2 °C and 180 d at room temperature using the stability chamber. The sample was collected after 30, 90, 180 d under accelerated conditions and 180 d at RT, respectively and evaluated [15, 16].

Sterility testing of optimized batch of ocular film

Sterility tests were based on the principle that if bacteria or fungi are placed in a medium having moisture and the desired pH and kept at a favorable temperature, the organism will grow and their presence can be indicated by the growth in originally medium [17]. Optimized

batch of ocular films was taken in two different sterile nutrient agar petri dishes. One uninoculated nutrient agar petri dish was taken as a negative control (to test sterility of the medium). A lab isolated culture of *Staphylococcus aureus* was inoculated in one nutrient agar petri dish served as positive control. Both the petri dishes were incubated at 37 °C for 24 h. Results were interpreted with positive and negative control petri dishes. The growth of microorganism was observed in positive control and no growth of microorganisms was observed in the negative control test, which confirmed that all the apparatus used for the test were sterile and aseptic conditions were maintained. Now the sample formulation was placed in the negative control test and incubated at same conditions. There was no growth of a microorganism in the samples under test, confirming the sterility of ocular films. These sterile ocular films were considered suitable for *in vivo* studies [18-20].

Sterilization of optimized batch of ocular film

These formulations were sterilized separately by exposing both sides to UV radiation for 90 min in a cabinet under aseptic conditions and were finally packaged in pre-sterilized aluminium foil [21].

In vivo study

The protocol for *in vivo* studies in rabbit was designed and approved by institutional animal ethics committee of research place. The rabbits were fed balanced diet pellets and maintained in a temperature-controlled room, at 20 °C to 24 °C before the experiment. The antibacterial activity (redness in conjunctiva and zone of inhibition for microbial growth) was carried with all 06 rabbits eyes divided into two groups (left eyes as a positive control group and right eyes as treated group). A microbial culture of *Staphylococcus aureus* 10 µl was instilled into both groups in each rabbit and incubated at room temperature in an aseptic room of microbiology lab for 24 h. The redness and infection in both groups in each rabbit were observed, and treatment was started after 24 h up to 5 d with the administration of sterile ocular inserts containing ofloxacin only in treated groups per day.

Redness was scored as follows: 0= Normal; 1= mild redness; 2= moderate redness; and 3= fully red conjunctiva. With this test, zone of inhibition was also observed for the treated group. Fresh sterile nutrient agar petri dishes were prepared and incubated for 24 h with test organism (*S. aureus* culture) at 37 °C. The fluid tear samples from the treated group were collected at an interval of 2 h with the help of a sterile swab up to 12 h. These swabs (containing released drug from ocular films) were placed on petri dishes of test organism over 24 h and the zone of inhibition were calculated separately with the help of Image-J software [22, 24].

RESULTS AND DISCUSSION

The ocular films of ofloxacin were prepared by a solvent evaporation method using glycerin as a lubricant and characterized on the bases of physicochemical parameters, sterility testing, *in vitro* and *in vivo* release studies. All the formulations with each polymer were evaluated for various parameters such as folding endurance, thickness, surface pH, % moisture absorption, % moisture loss, drug release of ofloxacin (table 1). The nature of rate-controlling membrane had influences on the physicochemical characteristics of the ocular films. The plasticizer is the most important component which may affects the mechanical properties of the films by lowering the glass-transition temperature of the polymer.

In this study, polyethylene glycol (PEG-400) at a concentration of 10% w/w of total polymer was selected since it gave sufficiently pliable films to allow for uniform subdivision into films without breaking the film. Ocular films of hydroxyl propyl methyl cellulose (HPMC K100M) with rate-controlling membranes of ethyl cellulose (EC) and polyvinylpyrrolidone (PVP) were flexible and elastic. Thickness and folding endurance were optimum in all batches and the % cumulative drug release (table 2) was evaluated with all six batches, the batch code PEEH was optimized with a maximum drug release (98.4±1.1) having surface pH (7.2), % moisture absorption (2.97±1.1w/w) and % moisture loss (0.58±0.9 w/w).

Table 1: *in vitro* evaluation of optimized batches

Batch code	Thickness (μm)	Folding endurance (no. of folds)	Surface pH	% Moisture absorption (w/w)	% moisture loss (w/w)	% Drug release
PAH	5.6 \pm 0.2	73 \pm 1.2	6.9	6.72 \pm 1.5	1.02 \pm 0.8	96.2 \pm 0.9
PBE	4.6 \pm 0.1	92 \pm 1.8	7.1	1.17 \pm 1.1	0.58 \pm 1.7	56.9 \pm 1.2
PCP	6.8 \pm 0.3	47 \pm 0.9	6.6	4.88 \pm 1.2	0.98 \pm 1.0	93.4 \pm 1.2
PDC	6.3 \pm 0.2	44 \pm 1.1	7.1	6.15 \pm 1.6	1.23 \pm 0.9	94.5 \pm 1.1
PEEH	4.9 \pm 0.2	82 \pm 1.1	7.2	2.97 \pm 1.1	0.58 \pm 0.9	98.4 \pm 1.1
PFEC	4.5 \pm 0.6	48 \pm 0.7	7.1	3.04 \pm 1.2	0.69 \pm 1.8	95.9 \pm 0.9

Values are expressed as mean \pm SD (n=5)

Table 2: % cumulative release of optimized batches of ocular films of ofloxacin

Time (h)	PAH	PBE	PCP	PDC	PEEH	PFEC
0.5	9.14	8.06	6.03	9.10	9.70	6.00
1	18.07	13.36	15.02	16.10	11.09	15.76
1.5	27.02	19.61	25.66	23.10	28.83	22.20
2	34.10	24.05	39.91	33.11	34.78	32.65
3	45.32	25.69	45.08	40.26	47.86	45.66
4	50.95	32.76	48.79	55.88	55.22	48.96
5	65.15	37.00	61.86	64.60	59.86	58.72
6	73.18	42.91	74.57	72.63	67.83	73.62
7	80.51	45.41	75.46	77.13	81.27	76.85
8	83.97	48.29	83.02	85.59	88.08	85.25
10	90.62	53.51	88.94	92.64	94.46	91.87
12	96.20	56.90	93.40	94.50	98.40	95.90

Stability study

On the bases of physicochemical characteristics and *in vitro* comparative release study of all six batches, the stability study was performed for batch code PEEH with accelerated study from 0 d to 6 mo and up to 6 mo with room temperature and the folding endurance, thickness, surface pH, % moisture absorption (table 3) and % drug release (table 4) were further evaluated. From the

accelerated stability studies, performed at elevated temperature and humidity revealed that no significant changes were observed in film thickness, folding endurance and surface pH [25]. Ocular films could be stored safely at study storage conditions. However, storage temperature not in excess of 40 °C and moisture-proof packing are recommended to ensure the stability of the formulation. Overall the degradation was less than 5%, a tentative shelf-life of more than years may be assigned to the formulations as per the ICH guidelines.

Table 3: stability study of final selected batch PEEH

Parameters evaluated	Accelerated study				Room temp.
	0 d	30 d	90 d	180 d	180 d
Folding endurance	82	81	78	75	81
Thickness (μm)	4.9	4.9	4.9	4.8	4.9
Surface pH	7.2	7.2	7.2	7.3	7.2
% moisture Absorption (w/w)	2.97	3.09	3.18	3.33	2.99
% Drug Release	96.90	95.43	94.63	93.87	95.89

Table 4: Release study of final selected batch PEEH

Time (H)	Drug release				
	Accelerated study				Room temp.
	0 d	30 d	90 d	180 d	180 d
0.5	5.99	5.42	6.76	5.37	7.38
1	15.76	14.25	11.30	8.35	9.46
1.5	22.20	16.98	19.35	17.22	18.92
2	32.65	28.86	26.88	22.04	27.73
3	45.66	37.46	41.38	39.56	35.41
4	48.96	46.52	46.78	46.73	46.76
5	58.72	48.32	52.93	56.25	55.39
6	73.62	59.13	60.10	67.63	68.19
7	76.85	73.53	74.86	76.84	73.45
8	85.25	85.59	82.08	84.58	82.12
10	91.87	92.64	90.14	90.35	89.61
12	96.90	95.43	94.63	93.87	95.89

In vivo study

Redness in conjunctiva was noted periodically (01 to 05 d) in both eyes of each animal after administration of predicted sterile

formulation in the treated eyes vs. control eyes [26]. One-way ANOVA, followed by Tukey-Kramer multiple comparison test were used for comparison of the means of different groups [27]. There is a

significant decrease ($P < 0.01$ and $P < 0.001$) in scores of redness as compared with positive control group. As per the data shows in the

(table 5) the predicted batch PEEH of ocular inserts reduced the redness completely up to 4th day dose.

Table 5: Effect of the anti-bacterial response of predicted batch PEEH (Avg. mean of score with days)

Group (Avg.±SEM)	Days				
	1	2	3	4	5
Control (Left eye)	2.666±0.210	3±0	2.833±0.166	3±0	3±0
Treated (Right eye)	1.833**±0.166	0.833***±0.166	0.333***±0.210	0***±0	0***±0

(** $P < 0.01$, *** $P < 0.001$ vs. control group)

Zone of inhibition study

The bacterial load was noted periodically (02 to 12 h) in treated eyes of each animal after administration of predicted sterile formulation in the treated eyes vs. control eyes. One-way ANOVA, followed by Tukey-Kramer multiple comparison tests was used for comparison of the

means of different groups. There is a significant difference ($P < 0.001$) in the diameter area of zone of inhibition as compared with positive control group. The data shows in the (table 6) the samples were withdrawn from the treated groups of a predicted batch of ocular inserts reduced the maximum bacterial load as per standard test (ciprofloxacin CF30 susceptibility test disc were used for *in vitro* standard test).

Table 6: Effect of the anti-bacterial response of predicted batch PEEH with zone of inhibition (Avg. mean of area in mm²)

Group (Avg.±sem)	Diameter in mm ² of zone of inhibition with time (hrs)					
	2	4	6	8	10	12
Treated (Right eye)	9.5±0.619	15.5±1.056	21.666±0.802	23.166±0.307	23.5±0.223	23.833±0.307

(*** $P < 0.001$ vs. control group)

CONCLUSION

Ocular films of ofloxacin prepared by a solvent evaporation method using a good film-forming hydrophilic polymer hydroxyl propyl methyl cellulose (HPMC K100M) with a satisfactory rate controlling membranes of ethyl cellulose (EC) and polyvinylpyrrolidone (PVP) included polyethylene glycol (PEG 400) as a plasticizer. Various concentrations were designed with different combinations but the concentration of batch code PEEH was smooth, flexible and transparent. Physicochemical parameters viz folding endurance, thickness, surface pH, percentage moisture absorption, percentage moisture loss, stability study, sterility testing were in optimum range for optimized batch of ocular film. *in vitro* and *in vivo* study revealed that the optimized formulation would be able to offer benefits such as increased residence time, prolonged drug release, reduced frequency of administration and improved patient compliance with complete removal of inflammation and redness from the cul-de-sac.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare regarding the publication of this paper.

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