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Original Article

Elevating Therapeutic Potential: Levofloxacin-Loaded Ocular Films for Conjunctivitis Management

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ABSTRACT

Background: The study aimed to develop ocular films containing levofloxacin to treat conjunctivitis. These films were meticulously prepared using a combination of Gelatin, Aloe barbadensis leaves mucilage, and HPMC K4M, by the solvent casting technique, with the primary objective of enhancing the therapeutic efficacy of levofloxacin for this specific eye condition. **Materials and Methods:** A comprehensive evaluation was carried out to ensure the quality and reliability of the films, encompassing parameters such as film thickness, weight variation, content uniformity, percentage moisture loss, and absorption capacity. In addition, *in vitro* drug discharge studies were conducted to simulate the eye's conditions and understand the controlled discharge of the drug. The study also considered the influence of polymer concentrations, on drug discharge using Design Expert software's Box Behnken Design. **Results:** Notably, the research revealed that the ocular films followed zero-order kinetics, meaning they discharged the drug at a constant rate over time. **Conclusion:** Furthermore, the films demonstrated stability under ambient conditions, making them a promising alternative for prolonged drug delivery and improved therapeutic outcomes in conjunctivitis treatment.

Keywords: Biodegradable, Eye, Film, Levofloxacin, Ocular.

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INTRODUCTION

Eye infections, caused by bacteria, viruses, fungi, or parasites, range from mild to severe and affect the conjunctiva, cornea, and eyelids. Types include conjunctivitis, keratitis, blepharitis, and endophthalmitis. Symptoms include redness, swelling, pain, discharge, itching, blurred vision, light sensitivity, tearing, and crusting. Diagnosis involves an eye exam and possibly lab tests. Treatments are antibiotics for bacteria, antivirals for viral, antifungals for fungal infections, and antihistamines or anti-inflammatory drops for allergies.[1] Prevention includes good hygiene, proper contact lens care, avoiding shared items, eye protection, and prompt medical attention. Early diagnosis and treatment are crucial for effective management and prevention of complications.[2]

Ocular inserts are innovative drug delivery systems that enhance medication administration and efficacy for eye conditions. These small, flexible devices are placed in the conjunctival sac or on the cornea, providing controlled, sustained release of drugs. They offer advantages over eye drops, such as increased bioavailability, reduced dosing frequency, and improved patient compliance. By maintaining steady drug levels, ocular inserts enhance therapeutic effects and minimize side effects. They can deliver a variety of medications, making them versatile tools for managing ocular diseases like glaucoma, dry eye syndrome, and infections.

Levofloxacin (LFX) is a broad-spectrum fluoroquinolone antibiotic used to treat bacterial eye infections like conjunctivitis, keratitis, corneal ulcers, and endophthalmitis. It inhibits essential bacterial enzymes, leading to cell death, and offers high tissue penetration, rapid onset, and prolonged effect. Available in eye drops and sustained-release ocular films, LFX's efficacy is enhanced by advanced drug delivery systems like ocular inserts. These inserts address the limitations of eye drops, such as poor bioavailability and frequent dosing, by providing controlled, sustained drug release, improving therapeutic outcomes, and increasing patient compliance. They also minimize systemic absorption and potential side effects by localizing the drug's action to the infection site.[3]

Hydroxypropyl Methylcellulose (HPMC K4M), gelatin, and Aloe Barbadensis Leaf Mucilage (ABLM) are key components of advanced Ocular Drug Delivery Systems (ODDS) for

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treating bacterial conjunctivitis. HPMC K4M and gelatin are biodegradable and biocompatible, ensuring safety with ocular tissues, while ABLM adds antibacterial, anti-inflammatory, and bioadhesive properties. These polymers' hydrophilic nature enhances bioadhesion and prolongs drug residence time in the eye, allowing sustained drug release and reducing dosing frequency. These advanced formulations aim to increase residence time, extend drug discharge, and improve patient compliance, enhancing the treatment effectiveness for bacterial conjunctivitis.[3,4]

The study aimed to develop ocular films containing levofloxacin for treating conjunctivitis. These films were meticulously prepared using a combination of gelatin, Aloe barbadensis leaf mucilage, and HPMC K4M through the solvent casting technique, with the primary objective of enhancing the therapeutic efficacy of levofloxacin for this eye condition.

MATERIALS AND METHODS

Materials

Levofloxacin was generously provided as a gift sample by Microlabs in Bengaluru, India. HPMC K4M, gelatin, PEG-400, dihydrogen potassium orthophosphate, and sodium hydroxide were procured from Fischer Scientific. Aloe barbadensis leaves were collected from plants growing around Anantapur. All other chemicals used in the study were of analytical grade.

Preformulation studies

In the preformulation phase of the study, extensive compatibility assessments were conducted to evaluate interactions between LFX and various excipients. Equal proportions of LFX and excipients were thoroughly blended to create samples, which were then analyzed using Fourier Transform Infrared (FTIR) spectral analysis. This analytical method was employed to detect any potential chemical or physical interactions between LFX and the excipients, providing valuable insights into the suitability of the chosen formulation components for developing the ODDS.

Preparation of ocular films

In the formulation process, the polymer was dissolved in simulated tear fluid with a pH of 7.4 to create the LFX reservoir within a beaker, using a magnetic stirrer for thorough mixing and achieving the required polymer concentrations. LFX (0.5% w/v) was added to the polymer-solvent blend, along with PEG-400 and other additives, under continuous stirring to enhance the formulation's properties.[5] Once the mixture was thoroughly blended, it was poured and cast into films using mercury as a substrate. To account for various formulation variables, 13 batches of cast films were created following a Box Behnken Design approach, facilitated by Design Expert Software (version 11) (Table 1).

EVALUATION

Thickness

To determine the film's thickness, a digital caliper was used to measure three distinct points on the film's surface. The mean film thickness was then calculated by summing these measurements and dividing by three to obtain an average value. Additionally, the standard deviation of the thickness was calculated based on the average thickness value. This standard deviation offers insights into the uniformity or consistency of the film's thickness across its surface, indicating the degree of variation or dispersion in the measurements. These measurements and calculations are crucial for ensuring the quality and consistency of the film in the ocular drug delivery system.[6]

LFX content

To evaluate the uniformity of LFX distribution within cast films, a systematic analysis was conducted. Three distinct inserts were meticulously extracted from various locations within the cast film, each placed in a 100 mL volumetric flask with phosphate buffer at a pH of 7.4 to extract the LFX from the film. Following this extraction, 1 mL of the resulting solution was withdrawn and diluted with pH 7.4 phosphate buffer to bring the LFX concentration within the detectable range. A UV-visible spectrophotometer was employed to measure the absorbance of the diluted solution specifically for LFX at 287 nm, using a blank as a reference. This comprehensive procedure was repeated for all batches of cast films and executed in triplicate to ensure result reliability. Standard deviations were calculated to gauge the consistency of LFX content among the different film batches. To ascertain the precise LFX quantity, a specific formula was applied, considering the dilution factor. This meticulous process is a standard method in pharmaceutical and materials science research for evaluating the uniformity and consistency of LFX distribution in cast films. This rigorous analytical approach helps to ascertain the uniformity and reliability of the LFX content in the ocular inserts across various batches (E.q.1).

$$\text{The amount of LFX in one film is given by } = \frac{AsXGL}{Gr} = -mg \ldots\ldots (1)$$

Where,As=absorbance of sample solution; GL=conc. of LFX in standard solution; and Gr=absorbance of standard LFX solution.

Weight variation

The weight variation test serves as a crucial quality control step in pharmaceutical formulation. In this study, three films were meticulously selected from different areas within the same formulation to assess weight uniformity across the batch. Each film was individually weighed with precision, and their weights were recorded in milligrams (mg). The mean weight of the films was then calculated by summing the weights of these three films and dividing by the number of films. To gauge the spread or variability in film weights, the standard deviation was computed

Table 1: Various film formulae made in the study.

Formulation	Levofloxacin (%)	Gelatin (mg)	ABLM (mg)	HPMC K4M (mg)	PEG-400 (mL)	Benzalkonium chloride (%)	PBS (pH 7.4) q.s
LF-1	0.5	10	5	37.5	0.3	0.002	25
LF-2	0.5	30	5	37.5	0.3	0.002	25
LF-3	0.5	10	10	37.5	0.3	0.002	25
LF-4	0.5	30	10	37.5	0.3	0.002	25
LF-5	0.5	10	7.5	25	0.3	0.002	25
LF-6	0.5	30	7.5	25	0.3	0.002	25
LF-7	0.5	10	7.5	50	0.3	0.002	25
LF-8	0.5	30	7.5	50	0.3	0.002	25
LF-9	0.5	20	5	25	0.3	0.002	25
LF-10	0.5	20	10	25	0.3	0.002	25
LF-11	0.5	20	5	50	0.3	0.002	25
LF-12	0.5	20	10	50	0.3	0.002	25
LF-13	0.5	20	7.5	37.5	0.3	0.002	25

from the mean value. A smaller standard deviation indicates greater uniformity in film weights, while a larger standard deviation suggests increased variability. This meticulous weight variation test ensures that the films within the formulation exhibit consistent weights, a critical factor in ensuring accurate dosing and maintaining high product quality standards.

% Moisture Absorption

Three ocular inserts were selected from each film within the batch to ensure a representative sample. Each selected insert was individually weighed to determine its initial weight in milligrams (mg). These weighted inserts were then placed in a desiccator maintained at a high humidity level of approximately 75% Relative Humidity (RH). They remained in this controlled environment for three days.[7] After the three-day exposure to high humidity, the ocular inserts were carefully removed from the desiccator and reweighed to determine their final weight. This process assesses the moisture uptake and stability of the inserts under high humidity conditions, providing valuable insights into their performance and suitability for use in ODDS (E.q.2).

$$\%\ moisture\ absorption = \frac{Weight\ (Final) - Weight\ (initial)}{Weight\ (initial)} X100 \ldots\ldots (2)$$

% Moisture Loss

The % moisture loss test was conducted to assess the film's integrity under dry conditions. Initially, the ocular inserts were weighed to establish their starting weight. Subsequently, these inserts were placed inside a desiccator containing anhydrous calcium chloride, creating an extremely dry environment. After a three-day exposure to these dry conditions, the ocular inserts were carefully removed from the desiccator and reweighed.[8] The purpose of this test was to quantify the amount of moisture lost by the inserts when subjected to dry conditions. This evaluation helps determine the inserts' ability to maintain structural integrity and stability in low-moisture environments, which is crucial for ensuring their suitability for ophthalmic applications (E.q.3).

$$\%\ moisture\ loss\ = \frac{Weight\ (initial) - Weight\ (final)}{Weight\ (initial)} X100 \ldots\ldots (3)$$

In vitro LFX Permeation Studies

In the *in vitro* LFX discharge studies, a bi-chambered donor-receiver compartment model was utilized, employing a transparent and regenerated cellulose semi-permeable membrane (Sigma Dialysis Membrane). This model was specifically designed to replicate ocular *in vivo* conditions, particularly the corneal epithelial barrier. Within this model, the ocular insert was placed in the donor compartment, and the semi-permeable membrane served as a mimic for the corneal barrier. To mimic the volume of tear fluid, 0.7 µL of pH 7.4 phosphate buffer was consistently maintained in the donor compartment throughout the study. To simulate the blinking action of eyelids, a reservoir compartment containing pH 7.4 phosphate buffer was continuously stirred at 20 rpm using a magnetic stirrer. Samples were periodically withdrawn from the receiver compartment and replaced with an equal volume of pH 7.4 phosphate buffer. The LFX content in the withdrawn samples was analyzed at 287 nm using a UV-visible spectrophotometer (Shimadzu 1700, Japan), with a reference standard and pH 7.4 phosphate buffer as a blank. The *in vitro* discharge kinetics data were analyzed using various models, including Zero-order, First-order, Higuchi's Diffusion Kinetics, and the Korsmeyer-Peppas model. These analyses provide insights into the discharge behavior and kinetics of LFX from the ocular insert, essential for understanding its performance in a simulated ocular environment.[9] The Korsmeyer-Peppas model helps determine Fickian diffusion (n=0.5), indicating LFX discharge through diffusion, non-Fickian or anomalous

transport (0.5-1), suggesting a combination of diffusion and erosion mechanisms, zero-order discharge (n=1), associated with constant surface area systems, and super case II transport (n>1), indicating relaxation-controlled LFX discharge.[10,11]

RESULTS

Physicochemical analysis

In this study, a comprehensive investigation was undertaken to prepare 13 different LFs using a carefully selected combination of HPMC K4M, gelatin, and ABLM at varying concentrations. These polymers were specifically chosen for their biocompatibility, biodegradability, and their ability to yield uniform and flexible films, crucial attributes for ODDS. Additionally, the incorporation of PEG-400 as a plasticizer further enhanced the films' flexibility, rendering them well-suited for their intended application.

The experimental design and formulation development process were meticulously executed with the aid of Box-Behnken Design (BBD) in Design Expert software. This statistical approach facilitated the systematic exploration of the effects of multiple factors and their interactions on the formulation's properties and performance, ultimately enabling the optimization of the formulation by adjusting the concentrations of the selected polymers and other components.

The successful fabrication of uniform and flexible films through the solvent casting method underscored the efficiency of this technique for creating ocular inserts. These inserts hold significant promise for improving LFX delivery in ophthalmic applications, potentially leading to enhanced therapeutic outcomes and improved patient compliance. The systematic approach adopted in this study, which integrated polymer selection, formulation design, and solvent casting, represents a significant contribution to the advancement of ODDS. Furthermore, the compatibility test conducted between LFX and the selected polymers yielded promising results, as evidenced by the almost identical peaks observed. This high degree of compatibility is essential for formulating films to ensure that LFX and the polymers do not interact in a manner that could compromise the drug's effectiveness.

The film thickness across all LFswasconsistently uniform, ranging from 0.15±0.01 to 0.19±0.01 mm. These slight variations in thickness were likely attributed to the combined weight of the polymer and plasticizer. However, despite these variations, the average area of the film was measured at 0.502 cm^2, confirming the uniformity of thickness with minimal deviation.

Moreover, the LFX content in all LFs exhibited a high level of uniformity, ranging from 88.00±3.99 to 97.03±0.95%. Notably, LF-6 displayed the highest LFX content. Additionally, the weight of the LFs showed uniformity, with values ranging from 65.54±0.7 to 61.85±0.4 mg. These low standard deviation values across all LFs underscored the reproducibility of the manufacturing process, highlighting consistent thickness, weight, and LFX content.

Furthermore, the % moisture loss test indicated that when subjected to very dry conditions, the LFs experienced a maximum moisture loss ranging from 7.98±0.5 to 9.55±0.4. This moisture loss could be attributed to the reduced burden presented by gelatin and the plasticizer PEG-400 in the film.

Conversely, the % moisture absorption test revealed that LFs containing hydrophilic polymers exhibited higher moisture absorption. For instance, LF-6, containing more amount of gelatin, demonstrated the highest moisture absorption at 18.05±0.7%, while LF-5, with less hydrophilic polymer (gelatin), exhibited the lowest moisture absorption at 12.65±0.8%. These results suggested that gelatin had a greater tendency to absorb moisture. Importantly, despite moisture absorption, the film's integrity remained intact, as observed through its unchanged physical appearance (Table 2).

In vitro diffusion studies

In vitro diffusion studies were conducted in triplicate to evaluate the LFX diffusion profiles from the ocular inserts. Samples were withdrawn at various time intervals, and the % of LFX permeated and retained was calculated based on the mean LFX content in the respective films. Among the LFs, LF-6 exhibited the highest LFX permeation, reaching 96.08% at the end of 24 h. This was followed by LF-4 (94.44%), LF-2 (92.87%), and LF-11 (90.96%). The % of LFX permeation profiles for all LFs were plotted over time, illustrating the discharge kinetics.

To further understand the discharge mechanism, the data were subjected to kinetic analysis. The cumulative % LFX permeation versus time (Figure 1) exhibited regression coefficients ranging from 0.986 to 0.999. Additionally, the regression coefficients for the log cumulative % of LFX remaining versus time for the first-order plot ranged from -0.898 to -0.992. Although the zero-order curves were linear, they had different slopes, indicating variations in zero-order kinetics. These kinetic analyses provided insights into the discharge behavior of LFX from the ocular inserts, essential for understanding their performance in a simulated ocular environment (Table 3).

The data were analyzed using Korsmeyer's equation to confirm the precise discharge mechanism. The regressions indicated fairly linear curves and slope values were computed. LF-8, containing HPMC K4M (50%), gelatin, (30 mg), and ABLM (7.5 mg), displayed the most favourable discharge profile, with 93.05% permeation at the end of 24 hr. The prolonged permeation observed in this formulation was attributed to the formation of hydrogen bonds between the LFX and the polymer, contributing to controlled LFX discharge. Gelatin, known for its adhesive properties, further enhanced the formulation's performance when inserted into the cul-de-sac of the eye.

Table 2: Physicochemical constraints of the formulated films.

Formulation	Weight (mg)	Thickness (mm)	Content uniformity (%)	Moisture loss (%)	Moisture absorption (%)
LF-1	65.54±0.7	0.17±0.01	88.00±3.99	9.11±0.1	14.59±0.5
LF-2	63.93±0.5	0.18±0.01	93.60±2.67	9.08±0.3	16.62±0.5
LF-3	64.92±0.4	0.17±0.01	90.36±1.85	9.09±0.4	14.01±0.6
LF-4	62.75±0.3	0.16±0.01	93.32±3.03	9.55±0.4	14.85±0.2
LF-5	61.85±0.4	0.18±0.02	85.66±2.31	8.82±0.6	16.72±0.6
LF-6	62.04±0.1	0.16±0.01	97.03±0.95	8.74±0.5	18.05±0.7
LF-7	62.07±0.6	0.17±0.02	93.65±1.08	8.94±0.3	13.05±0.8
LF-8	63.08±0.2	0.15±0.01	95.07±2.57	8.97±0.6	16.62±0.5
LF-9	64.01±0.4	0.18±0.01	88.52±2.02	9.13±0.1	14.64±0.2
LF-10	62.54±0.4	0.17±0.02	90.20±5.24	9.05±0.2	15.52±0.4
LF-11	63.34±0.5	0.19±0.01	96.65±3.64	7.98±0.5	14.08±0.6
LF-12	64.08±0.3	0.17±0.01	97.00±5.26	9.06±0.2	12.65±0.8
LF-13	63.42±0.2	0.18±0.01	92.24±2.32	8.77±0.2	14.52±0.5

Values in mean±SD; *n*=3.

Table 3: Kinetics of LXM diffusion from the films.

Formulation	Zero-order		First-order			Higuchi		Koresmeyerpeppas		
	Slope (n)	r	Slope (n)	Ko= -slope X 2.303	r	Slope (n)	r	Slope (n)	Constant (k)	r
LF-1	3.753	0.995	0.032	0.076	-0.961	21.082	0.965	1.022	0.366	0.997
LF-2	3.762	0.998	0.031	0.073	-0.983	23.064	0.945	1.125	0.453	0.988
LF-3	3.662	0.994	0.030	0.078	-0.948	24.013	0.934	1.022	0.328	0.916
LF-4	3.694	0.996	0.036	0.088	-0.946	25.653	0.985	1.036	0.397	0.994
LF-5	3.659	0.994	0.032	0.078	-0.939	25.684	0.929	1.057	0.317	0.977
LF-6	3.163	0.999	0.029	0.069	-0.987	29.350	0.916	1.036	0.401	0.968
LF-7	3.395	0.993	0.036	0.079	-0.958	20.274	0.936	1.054	0.389	0.989
LF-8	3.085	0.996	0.030	0.069	-0.948	21.279	0.941	1.036	0.325	0.997
LF-9	3.075	0.992	0.034	0.079	-0.928	26.335	0.907	1.149	0.338	0.997
LF-10	3.378	0.993	0.364	0.831	-0.919	23.664	0.931	1.098	0.374	0.954
LF-11	3.457	0.994	0.351	0.801	-0.947	29.657	0.948	1.052	0.358	0.927
LF-12	3.678	0.986	0.371	0.868	-0.997	26.456	0.968	1.097	0.394	0.931
LF-13	3.494	0.995	0.352	0.812	-0.926	27.248	0.919	1.192	0.346	0.967

The linearity of the discharge profiles suggested that the permeation of LFX from these ocular inserts was primarily governed by a diffusion-controlled mechanism. These findings underscore the potential of these LFs for achieving controlled and sustained LFX permeation for the treatment of ocular conditions.

The fit summary is given in the Table 4.

The Model F-value of 1298.91 strongly indicates the model's significance, with only a 0.01% chance that such a high F-value could result from noise. *p*-values below 0.05 show that the model terms are significant; specifically, terms A, B, C, AB, AC, BC, A^2, B^2, and C^2 are significant. On the other hand, values above 0.1 suggest non-significant terms, implying that reducing the model by removing these terms could improve its performance if they are not necessary for maintaining hierarchy.

Adeq Precision measures the signal-to-noise ratio, with a ratio above 4 being desirable. The obtained ratio of 114.086 demonstrates an excellent signal, confirming the model's adequacy for exploring the design space. The coefficient estimate indicates the expected change in response per unit change in

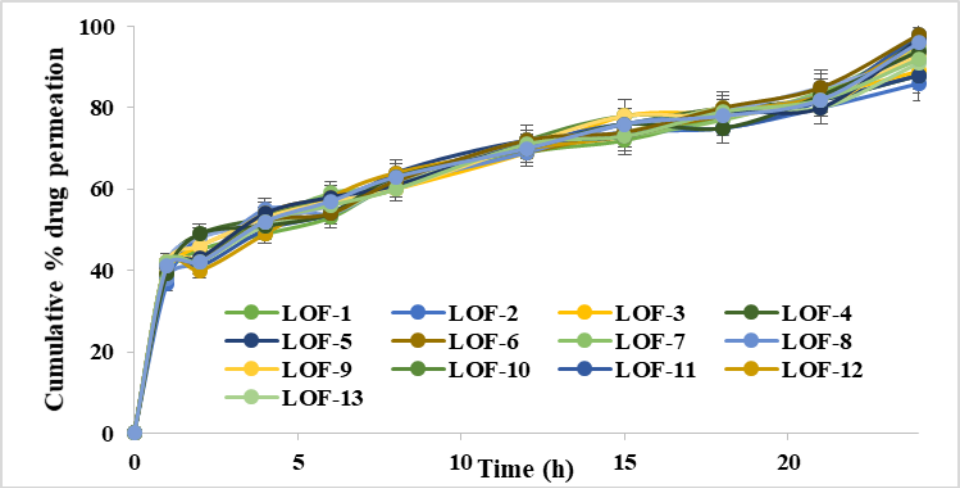

Figure 1: *In vitro* discharge plots from the films.

Table 4: ANOVA details.

Source	ANOVA for the LFX content		
	Sum of Squares	F-value	*p*-value
Model	155.97	1298.91	< 0.0001
A-Gelatin	34.49	2584.87	< 0.0001
B-ABLM	2.11	158.26	0.0011
C-HPMC K4M	109.67	8219.97	< 0.0001
AB	1.74	130.60	0.0014
AC	0.4160	31.18	0.0113
BC	0.4422	33.15	0.0104
A^2	3.14	235.53	0.0006
B^2	0.1457	10.92	0.0456
C^2	0.8229	61.68	0.0043
Residual	0.0400		

a factor's value when all other factors are held constant. In an orthogonal design, the intercept represents the overall average response from all runs, with coefficients indicating adjustments around that average based on factor settings.

Variance Inflation Factors (VIFs) greater than 1 indicate multicollinearity, with higher values showing a more severe factor correlation. VIFs below 10 are generally acceptable, ensuring that multicollinearity does not significantly affect the model. This thorough statistical analysis highlights the model's robustness and the significant contributions of specific terms, providing reliable guidance for optimizing the formulation. The coded equation can be representeas follows.

$$DC=+92.24+2.08A+0.5137B+3.70C-0.66AB-0.3225AC-0.3325BC-1.17A^2+0.2525B^2+0.6C^2$$

In this 92.24, indicates the baseline value of the LFX content when all independent variables are at their central levels. The main effects show that increases in A, B, and C result in increases in LFX content, with coefficients of 2.08, 0.5137, and 3.70, respectively. Interaction effects between these variables reveal that combinations of A and B, A and C, and B and C negatively impact LFX content, as indicated by the negative coefficients -0.66, -0.3225, and -0.3325. Quadratic effects are also considered, with $-1.17A^2$ indicating a significant decrease in LFX content at higher levels of A, while $0.2525B^2$ and $0.6C^2$ show slight increases in LFX content at higher levels of B and C. This coded equation provides a comprehensive mathematical model to understand the individual and combined effects of these factors, enabling optimization of their levels to achieve the desired LFX content.

The normal plots (Figure 2A), Run Order Plots (Figure 2B), and Cook's Distance plots (Figure 2C) in the analysis assess the relationships between the independent variables (Gelatin, ABLM, and HPMC K4M) and the response variable (drug content). These diagnostic plots help understand the distribution of residuals,

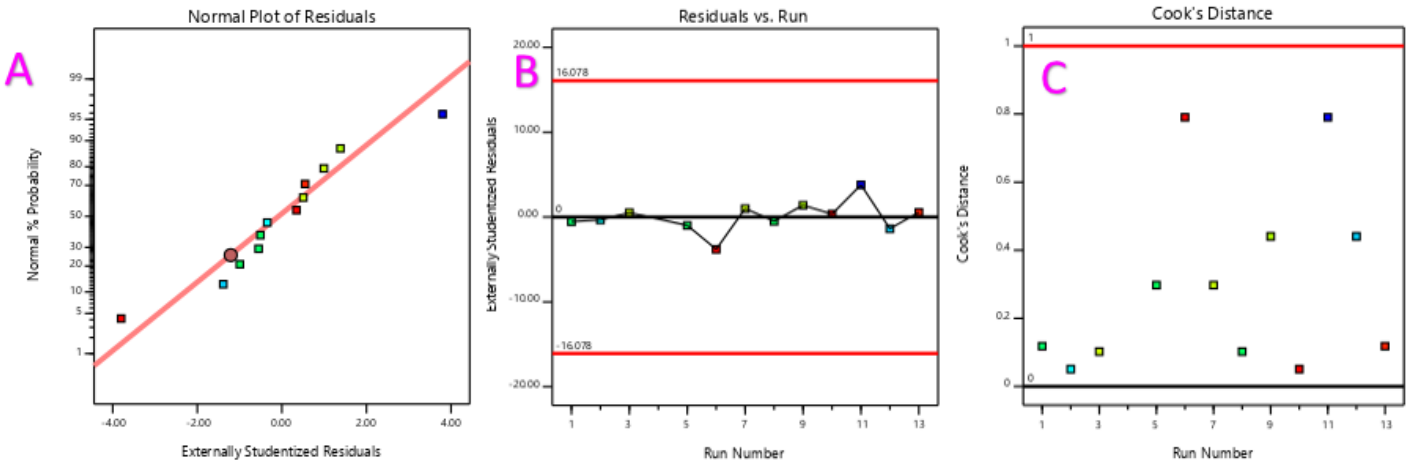

Figure 2: A) Normal plot of residuals; B) residual vs. run; C) cook's distance representing the impact of independent variables on the LFX content.

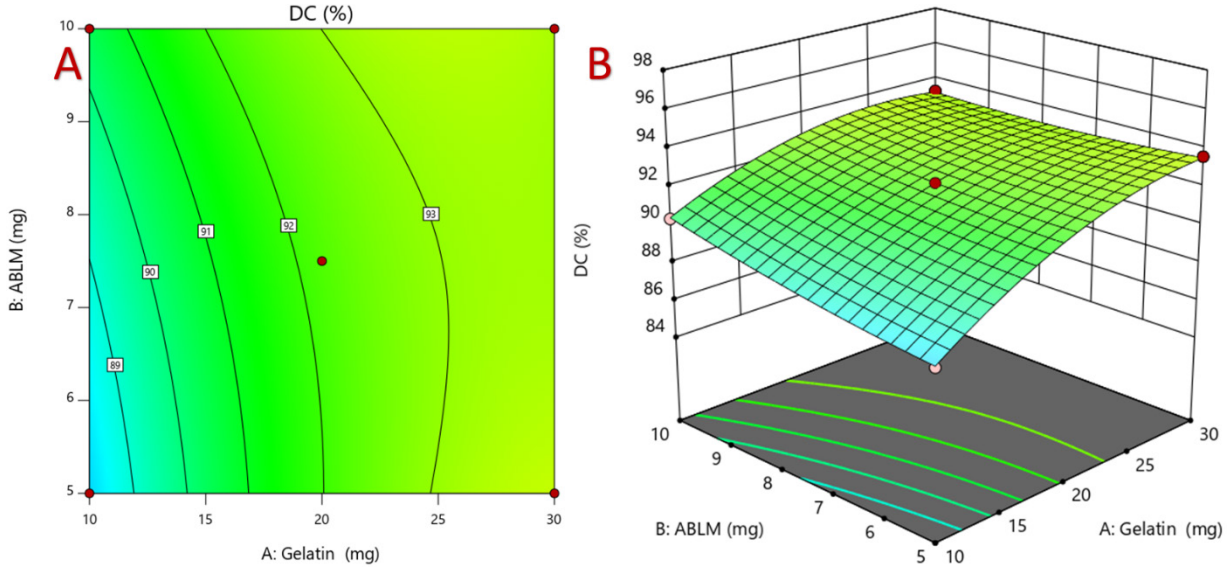

Figure 3: A) Contour and B) 3D plots for the response towards inputs.

detect potential outliers or influential data points, and evaluate the robustness of the statistical model. Subsequently, the contour plots (Figure 3A) and 3D plots (Figure 3B) visually represent the relationships between the same independent variables and the response variable. These graphical representations provide insights into how changes in the independent variables influence the response variable, identifying regions in the factor space where the response is optimized or minimized. Contour plots show constant response values on two-dimensional planes, while 3D plots offer a three-dimensional view of the response surface, aiding in the exploration of complex interactions and optimal factor settings for LFX content.

DISCUSSION

The study presented here revolves around the development and evaluation of Lamifloxacin ocular Films (LFs) to enhance LFX delivery in ophthalmic applications. The research explores a systematic approach that combines polymer selection, formulation design, and solvent casting to optimize ocular inserts, which are expected to improve therapeutic outcomes and patient compliance. One notable aspect of this study is the selection of polymers for LF formulation. The use of a combination of HPMC K4M, gelatin, and ABLM in a factorial design is a novel approach, highlighting that these polymers are biocompatible and biodegradable. Such characteristics are essential for ocular applications to ensure safety and compatibility with delicate eye tissues. A common practice in ocular film development has been the use of PEG-400 as a plasticizer. While this plasticizer has been employed in many previous studies, the current research sets itself apart by employing a unique combination of polymers, offering an alternative approach for the formulation of ocular inserts.[12]

The utilization of the BBD within the Design Expert software for optimization is a noteworthy choice, as it allows for efficient exploration of the parameter space. Notably, only a few attempts

have been made to optimize ophthalmic patches using this design approach, indicating that the study seeks to advance the field by employing a systematic and efficient optimization method. One of the pivotal findings of this study is the high degree of compatibility between the selected polymers and the LFX. Compatibility is crucial in ocular film formulation, as any interaction between the LFX and polymers could compromise the LFX's efficacy. The successful demonstration of compatibility between the LFX and polymers is a significant step in developing reliable ocular inserts. Additionally, the uniformity in film thickness, LFX content, and weight across all LFs is indicative of a highly reproducible manufacturing process. This consistency is vital in ensuring that each ocular insert delivers the intended dose effectively, which is crucial in ophthalmic LFX delivery.[13]

Moisture tests reveal the behavior of LFs under different environmental conditions, showing that the films exhibit both moisture loss and moisture absorption properties. Notably, gelatin-containing films have a higher moisture absorption capacity, providing insights into the effects of different polymers on moisture handling. Importantly, the films maintain their integrity despite moisture absorption, which is a promising characteristic for their stability in ocular use. The *in vitro* diffusion studies provide critical information about the LFX discharge profiles of the LFs. Significantly, LF-8 exhibited the highest collective LFX permeation at the end of 24 hr, suggesting that the combination of HPMC K4M, gelatin, and ABLM in this formulation is particularly effective in achieving the desired LFX discharge profile. The formation of hydrogen bonds between the LFX and the polymer, along with the adhesive properties of gelatin, contribute to controlled LFX discharge, which can be highly advantageous in the treatment of ocular conditions.

The linear discharge profiles of LFX from the ocular inserts indicate a diffusion-controlled discharge mechanism.[14] This controlled and sustained LFX permeation is a desirable feature for treating ocular conditions, as it helps maintain therapeutic LFX levels over an extended period, reducing the need for frequent dosing and improving patient compliance. The statistical analysis yields several noteworthy insights. Firstly, the Adjusted r^2, a measure of the regression model's goodness of fit, is calculated for the LFX content, revealing that % LFX content plays a more substantial role in explaining the variability in the response variable. This implies that terms such as B, AB, AC, BC, A^2, B^2, and C^2 are crucial contributors to the model, underscoring their importance in explaining the response variable.[15]

In Design-Expert software, a normal plot serves as a graphical tool for assessing the normality of residuals in a regression or ANOVA model. It juxtaposes the observed residuals against the quantiles of a theoretical normal distribution. A straight line in the plot indicates that the residuals adhere to a normal distribution, while deviations suggest departures from normality, such as outliers or skewness. This plot is vital for validating statistical analyses in experimental design and regression modeling and guiding decisions on potential data transformations or alternative modeling techniques.

The Run Order Plot is a graphical representation used to assess the behavior of residuals (the differences between observed and predicted values) concerning the order in which data points were collected or run. It helps identify patterns, trends, and potential outliers in the residuals based on their run order. This plot is crucial for evaluating the model's validity, as systematic trends or outliers may indicate unaccounted factors or data collection issues, aiding decisions regarding model refinement and data quality.

The Cook's Distance plot is a diagnostic tool in regression analysis that displays Cook's Distance values for individual data points. Cook's Distance measures the influence of each data point on the regression model, with larger values indicating a more significant impact. This plot helps identify potential outliers or influential data points, assess model robustness, and make informed decisions about whether to include or exclude specific data points or if model improvements are necessary to account for influential observations. It's a valuable tool for model validation and understanding the impact of individual data points on regression outcomes.

The contour plot is a visualization tool used in experimental design and response surface methodology to understand the relationships between two independent variables (Gelatin, ABLM, and HPMC K4M) and the response variable (drug content). It displays a two-dimensional projection of the response surface, where contour lines represent constant values of the response. Contour plots help visualize factor interactions, identify optimal factor settings, and make data-driven decisions to achieve specific response goals, making them invaluable for experimental design and optimization.[16]

The 3D plot is a graphical visualization tool used in experimental design, response surface methodology, and regression analysis to represent the relationship between the independent variables (Gelatin, ABLM, and HPMC K4M) and a response variable. It displays a response surface, allowing visualization of complex factor interactions, assessing factor optimization, and gaining insights into how the response variable changes as factors are varied in a three-dimensional factor space. 3D plots are valuable for exploring data and making informed decisions about experimental design, optimization, and understanding the interplay of factors in achieving desired outcomes.

CONCLUSION

The study successfully developed ocular films containing levofloxacin for the treatment of conjunctivitis. These films were meticulously prepared using a combination of HPMC K4M, gelatin, and *Aloe barbadensis* leaf mucilage, and a comprehensive

evaluation was conducted to ensure their quality and reliability. The research demonstrated that these films followed zero-order kinetics, releasing the drug at a constant rate over time. Moreover, the films showed stability under ambient conditions, suggesting their potential as a promising alternative for prolonged drug delivery and improved therapeutic outcomes in conjunctivitis treatment. This research paves the way for the development of ocular drug delivery systems that can enhance the efficacy and convenience of treatment for this eye condition.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DOE: Design of Experiments; **BBD:** Box Behnken Design; **HPMC:** Hydroxypropyl Methylcellulose; **FTIR:** Fourier-transform infrared; **UV:** Ultraviolet; **QbD:** Quality by Design; **ODDS:** Ocular drug delivery systems; **LFX:** Levofloxacin; **ABLM:** Aloe barbadensis leaf mucilage; **PEG:** Polyethylene Glycol**; LF:** Levofloxacin ocular Films; **hr:** Hour; **mg:** Milli gram; **%:** Percent; **q.s:** Quantity sufficient; **RH:** Relative humidity; **ANOVA:** Analysis of variance.

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