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Original Article

A Comprehensive QbD Study on Bioadhesive Ocular Films for Improved Conjunctivitis Management: Insights from Design Expert Software

Repollu Maddileti[1,*], Haranath Chinthaginjala[2]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University-Anantapur, Ananthapuram, Andhra Pradesh, INDIA.
[2]Department of Pharmaceutics, Raghavendra Institute of Pharmaceutical Education and Research (Autonomous) (Affiliated to JNTU-Anantapur), K.R. Palli. Cross, Ananthapuram, Andhra Pradesh, INDIA.

ABSTRACT

Objectives: 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 release studies were conducted to simulate the eye's conditions and understand the controlled release of the drug. The study also considered the influence of polymer concentrations, on drug release using Design Expert software's Box Behnken Design. **Results:** Notably, the research revealed that the ocular films followed zero-order kinetics, meaning they released 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.

Correspondence:
Mr. Repollu Maddileti
Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University-Anantapur (JNTUA), Ananthapuram, Andhra Pradesh, INDIA.
Email: madhurepalle9160@gmail.com

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INTRODUCTION

The development of Ocular Drug Delivery Systems (ODDS) represents a compelling challenge for pharmaceutical scientists.[1] The eye is a distinctive organ with its own set of complexities, making drug administration within this context a particularly demanding endeavor. The eye is susceptible to a range of diseases, including blepharitis, conjunctivitis, ophthalmia neonatorum, trachoma, iritis, and corneal ulceration.[2] These conditions often arise as a result of the immune system's efforts to eliminate harmful foreign agents and bacterial infections. However, in some instances, these immune responses can be so intense and misguided that they inadvertently cause harm to the host's eye tissues.[3] Therefore, designing effective ODDS that can target and treat these conditions while minimizing side effects is a crucial goal for pharmaceutical researchers.[4]

While the commercial availability of ophthalmics featuring bioadhesive, biodegradable, and herbal polymers remains limited, research in this realm has injected fresh energy and momentum into the quest for innovative and enhanced ophthalmics.[5] This surge in research activity has ushered in a new era of possibilities and creativity within the field of formulation technology. The pursuit of modified or entirely novel ophthalmics has gained significant traction, holding the potential to chart exciting new directions in the world of ophthalmic pharmaceuticals.[6] These endeavors aim not only to improve the efficacy and safety of ocular treatments but also to explore the diverse therapeutic benefits presented by natural and herbal polymers.[7] As researchers continue to push boundaries and unlock the potential of these materials, the future holds great promise for the development of groundbreaking ophthalmic solutions that can address a wide range of eye-related conditions and concerns.

Bacterial infections are a common cause of numerous eye disorders, and Levofloxacin (LFX) stands out as a preferred antibacterial drug for their treatment.[8] In the Indian market, LFX formulations are available in the form of eye drops and eye ointments.[9] However, when administered as eye drops, there have been frequent reports of poor bioavailability due to issues like

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solution drainage, rapid precorneal elimination, and high tear turnover.[10] On the other hand, applying ointment topically to the cornea often leads to blurred vision, a major concern that can reduce patient compliance. This blurriness necessitates frequent instillation of concentrated medication to achieve the desired therapeutic effect.

To address these challenges and improve patient outcomes, it has become crucial for research scientists to develop an advanced ODDS. Such a system could effectively overcome the limitations and reduce the side effects associated with conventional ocular preparations while enhancing bioavailability.[11] The controlled-release focus emerges as a versatile and promising drug carrier system, offering a convenient and efficient means of delivering medication paralleled to traditional ocular dosage forms. By providing a controlled discharge of LFX and mitigating issues like blurred vision and frequent dosing, the ocusert system holds great potential for enhancing the treatment of bacterial eye infections and improving patient comfort and compliance.

LFX, a potent third-generation fluoroquinolone antibacterial, is the optically active L-isomer of ofloxacin and is effectively employed in the treatment of bacterial conjunctivitis.[12] In the formulation of ODDS for this purpose, several key components come into play.

Hydroxypropyl Methylcellulose (HPMC K4M), gelatin, and the herbal-derived Aloe Barbadensis Leaf Mucilage (ABLM) are essential constituents of these formulations. HPMC K4M and gelatin are biodegradable and biocompatible polysaccharides, contributing to the formulation's safety and compatibility with ocular tissues.[12] ABLM, in addition to these properties, offers supplementary benefits like antibacterial, anti-inflammatory, and bioadhesive attributes. This multifaceted herbal ingredient is particularly noteworthy for its ability to form hydrogen bonds with the drug, resulting in prolonged drug discharge.

The inclusion of these polymers in the formulations imparts them with bioadhesive properties, largely owing to their hydrophilic nature. These bioadhesive properties have significant implications for ocular drug delivery.[13] They can enhance residence time within the eye, ensuring the drug remains in contact with the target tissue for an extended period. This, in turn, leads to prolonged drug discharge, reducing the need for frequent administration.

Ultimately, the prepared formulations hold the promise of achieving their intended goals, such as increased residence time, extended drug discharge, reduced dosing frequency, and improved patient compliance. These attributes are vital for overcoming the limitations associated with conventional ODDS, ultimately enhancing the effectiveness and patient experience in the treatment of bacterial conjunctivitis.

In the current study, the development of ODDS involved the utilization of key components, including gelatin, ABLM, and HPMC K4M. These polymers play a crucial role in the formulation of the ODDS. Additionally, PEG-400, a plasticizer, was incorporated into the formulations to enhance their flexibility and drug discharge assets.

To ensure the quality and effectiveness of these ODDS, various evaluation parameters were employed. These parameters likely encompass a range of tests and assessments to assess the characteristics and performance of the formulations. Common evaluation parameters for such ODDS may include tests for film thickness, weight variation, content uniformity, % moisture loss, absorption capacity, and *in vitro* drug discharge studies.

These evaluations serve to confirm the consistency, stability, and effectiveness of the formulations. They help researchers and scientists gauge the suitability of the developed ODDS for their intended purpose, which is often aimed at improving drug delivery to the eye, enhancing therapeutic efficacy, and ensuring patient comfort and compliance.

The goal of the study was to create levofloxacin-containing ocular films for the treatment of conjunctivitis. Using a combination of gelatin, mucilage from Aloe barbadensis leaves, and HPMC K4M, these films were painstakingly created using the solvent casting technique. The main goal was to increase the therapeutic efficacy of levofloxacin for this particular eye ailment.

MATERIALS AND METHODS

Materials

Levofloxacin (LFX) was generously provided as a gift sample by Microlabs in Bengaluru, India. HPMC K4M and gelatin, PEG-400, dihydrogen potassium orthophosphate, and sodium hydroxide were acquired, from Fischer Scientific. Aloe barbadensis leaves were collected from the 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 the interactions between the LFX and various excipients. This involved the thorough blending of equal proportions of the LFX and excipients to create samples. These samples were then analyzed using Fourier Transform Infrared (FTIR) spectral analysis. These analytical methods were employed to detect any potential chemical or physical interactions between the LFX and the excipients, providing valuable insights into the suitability of the chosen formulation components for the development of the ODDS.

Preparation of ocular films

In the formulation process, the polymer was dissolved in a simulated tear fluid with a pH of 7.4, creating the LFX reservoir within a beaker. This was achieved by employing a magnetic stirrer to ensure proper mixing of the polymer and obtain different

concentrations of each polymer as needed. LFX (0.5% w/v) was then added to the polymer-solvent blend. Additionally, PEG-400 and other additives were introduced into the solution under stirring conditions to enhance the formulation's properties.[14,15]

Once the mixture was thoroughly blended, it was poured, and films were cast using mercury as a substrate. To account for various formulation variables, a total of 20 batches of cast films were created following a Box Behnken Design approach,[16] facilitated by Design Expert Software (version 11). From these cast films, ocular inserts with a specified diameter of 8 mm were precisely cut using a cork borer. These fabricated ocular inserts named LOF (Levofloxacin ocular Films) underwent a series of *in vitro* evaluation tests, including assessments of LFX entrapment efficiency and collective LFX discharge over a 24 hr period (Table 1). These evaluations are crucial in determining the performance and effectiveness of the developed ODDS.[17]

Evaluation

Thickness

To determine the thickness of the film, a digital caliper was employed to make measurements at three distinct points on the film's surface. Subsequently, the mean film thickness was calculated by summing these three measurements and dividing by three to obtain an average value. Additionally, the standard deviation of the thickness was figured based on the average thickness value. This standard deviation provides information about the degree of variation or dispersion in the thickness measurements, offering insights into the uniformity or consistency of the film's thickness across its surface.[18] These measurements and calculations are essential for ensuring the quality and consistency of the film in the ODDS.

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 then 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.[19] To ascertain the precise LFX quantity, a specific formula was applied, considering the dilution factor. This meticulous process

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
LOF-1	0.5	10	5	37.5	0.2	0.002	20
LOF-2	0.5	30	5	37.5	0.2	0.002	20
LOF-3	0.5	10	10	37.5	0.2	0.002	20
LOF-4	0.5	30	10	37.5	0.2	0.002	20
LOF-5	0.5	10	7.5	25.0	0.2	0.002	20
LOF-6	0.5	30	7.5	25.0	0.2	0.002	20
LOF-7	0.5	10	7.5	50.0	0.2	0.002	20
LOF-8	0.5	30	7.5	50.0	0.2	0.002	20
LOF-9	0.5	20	5	25.0	0.2	0.002	20
LOF-10	0.5	20	10	25.0	0.2	0.002	20
LOF-11	0.5	20	5	50.0	0.2	0.002	20
LOF-12	0.5	20	10	50.0	0.2	0.002	20
LOF-13	0.5	20	7.5	37.5	0.2	0.002	20
LOF-14	0.5	20	7.5	37.5	0.2	0.002	20
LOF-15	0.5	20	7.5	37.5	0.2	0.002	20
LOF-16	0.5	20	7.5	37.5	0.2	0.002	20
LOF-17	0.5	20	7.5	37.5	0.2	0.002	20
LOF-18	0.5	20	7.5	37.5	0.2	0.002	20
LOF-19	0.5	20	7.5	37.5	0.2	0.002	20
LOF-20	0.5	20	7.5	37.5	0.2	0.002	20

is a standard method in pharmaceutical and materials science research for evaluating the uniformity and consistency of LFX distribution in cast films.[20] This rigorous analytical approach helps to ascertain the uniformity and reliability of the LFX content in the ocular inserts across various batches (Eq.1).

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

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

Uniformity in weight

The weight variation test is an essential quality control step in pharmaceutical formulation. In this study, three films were meticulously chosen from different areas within the same formulation to assess weight uniformity across the batch. Each film was individually weighed with precision, recording their weights in milligrams (mg). By summing the weights of these three films and dividing by three (the number of films), the mean weight of the films was calculated. To gauge the spread or variability in film weights, the standard deviation was computed from the mean value. A smaller standard deviation indicates greater uniformity in film weights, while a larger standard deviation suggests increased variability.[21] 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 taken from each film within the batch. This selection ensured a representative sample of the inserts. Each of the selected inserts was individually weighed to determine their initial weight in milligrams (mg). The weighted inserts were then positioned in a desiccator maintained at a high humidity level of approximately 75% Relative Humidity (RH). They were left in this controlled environment for three days.[22] After the three-day exposure to high humidity, the ocular inserts were carefully removed from the desiccator and reweighed to determine their final weight (Eq.2).

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

% moisture loss

The % moisture loss test was conducted to assess the film's integrity under dry conditions. In this test, the ocular inserts were initially weighed to establish their starting weight. Subsequently, these inserts were positioned inside a desiccator containing anhydrous calcium chloride, which created an extremely dry environment. After a three-day exposure to these dry conditions, the ocular inserts were carefully removed from the desiccator and reweighed. The purpose of this test was to quantify the amount of moisture lost by the inserts when subjected to dry conditions.[19] 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 (Eq.3).

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

In vitro LFX release 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 *p*H 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 *p*H 7.4 phosphate buffer as a blank.[23-25] The *in vitro* release 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 release behavior and kinetics of LFX from the ocular insert, which is essential for understanding its performance in a simulated ocular environment.

The last model helps to find Fickian diffusion (n=0.5), which indicates LFX discharge by diffusion through a porous matrix/ membrane), non-Fickian or anomalous transport (0.5-1), which suggests a combination of both diffusion and erosion mechanisms, zero-order release (n=1), which is typically related to LFX discharge from systems with a constant surface area and is independent of time and super case II transport (n>1), which implies relaxation-controlled LFX discharge.

RESULTS

Physicochemical analysis

In this study, a total of 20 different LOFs were meticulously prepared using a combination of HPMC K4M, gelatin, and ABLM at various concentrations. These polymers were chosen for their biocompatibility, biodegradability, and their ability to form uniform and flexible films, which are essential properties for ODDS. The addition of PEG-400 as a plasticizer further enhanced the flexibility of the films, making them suitable for the intended application.

The experimental design and formulation development process were facilitated by the use of Box-Behnken Design (BBD) in Design Expert software. This statistical approach helps systematically explore the effects of multiple factors and their interactions on the formulation's properties and performance. It allows for the optimization of the formulation by varying the concentrations of the selected polymers and other components.

The successful production of uniform and flexible films through the solvent casting method demonstrates the efficiency of this technique for creating ocular inserts. These inserts hold promise for improved LFX delivery in ophthalmic applications, with the potential to enhance therapeutic outcomes and patient compliance. The systematic approach used in this study, combining polymer selection, formulation design, and solvent casting, contributes to advancements in ODDS.

The compatibility test conducted between the LFX and the selected polymers revealed that they exhibited almost identical peaks, indicating a high degree of compatibility. This compatibility is a crucial aspect of formulating films to ensure that the LFX and polymers do not interact in a way that could compromise the LFX's effectiveness.

The film thickness across all LOFs was consistently uniform, falling within the range of 0.19±0.02 to 0.16±0.01 mm. The slight variations in thickness were likely attributed to the combined weight of the polymer and plasticizer. Despite these variations, the average area of the film was measured at 0.502 cm^2, confirming that the thickness remained uniform with minimal variation.

The LFX content in all LOFs was found to be in the range of 92.72±2.1 to 98.00±1.4 of LFX, demonstrating a high level of uniformity and LOF-6 has the highest LFX content. The weight of the LOFs also exhibited uniformity, with values ranging from 61.26±0.9 to 66.07±0.1 mg. The low standard deviation values across all LOFs indicated the reproducibility of the manufacturing process, highlighting consistent thickness, weight, and LFX content.

The % moisture loss test revealed that when the LOFs were subjected to very dry conditions, the maximum moisture loss diverse between 8.28±0.8 to 9.82±0.4 This moisture loss was likely due to the reduced burdenpresented by gelatin and the plasticizer PEG-400 in the film.

Conversely, the % moisture absorption test showed that LOFs containing hydrophilic polymers exhibited higher moisture absorption. For instance, LOF-6, which contained gelatin, demonstrated the highest moisture absorption at 18.05±0.9%, whereas LOF-5 with a less hydrophilic polymer, exhibited the lowest moisture absorption at 11.02±0.6%. These results indicated that gelatin had a greater tendency to absorb moisture related to gelatin. Importantly, despite moisture absorption, the film's integrity remained intact, as observed through its unchanged physical appearance.

Overall, these findings suggest that the selected polymers and formulation methods were suitable for creating ocular inserts with consistent LFX content, thickness, and weight. The compatibility of the LFX and polymers, along with their performance under various moisture conditions, underscores the potential of these inserts for use in ODDS (Table 2).

In vitro diffusion studies

In vitro diffusion studies were conducted in triplicate to assess the LFX diffusion profiles from the ocular inserts. At various time intervals, samples were withdrawn, and the collective % of LFX permeated and LFX retained was calculated based on the mean amount of LFX present in the respective films.

Among the LOFs, LOF-8 exhibited the highest collective LFX permeation, reaching 83.00% at the end of 24 hr. This was followed by LOF-5 (81.00%), LOF-2 (80.00%), and LOF-11 (78.3%). The collective% LFX permeation profiles for all LOFs were plotted over time, showing the release kinetics.

To further understand the release mechanism, the data were subjected to kinetic analysis. The collective % LFX permeation versus time exhibited (Figure 1) regression coefficients ranged from exhibited regression coefficients from 0.977 to 0.999. Additionally, the regression coefficients for the log cumulative % LFX remaining versus time for the first-order plot ranged from -0.898 to -0.992. Additionally, the zero-order curves were linear, they had different slopes, indicating variations in zero-order kinetics (Table 3).

To confirm the precise release mechanism, the data were analyzed using Korsmeyer's equation. The regressions indicated fairly linear curves and slope values were computed. LOF-8, containing HPMC K4M (50%), gelatin, (30 mg), and ABLM (7.5 mg), displayed the most favourable discharge profile, with 83% permeation at the end of 24 hr. The prolonged permeation observed in this formulation was attributed to the formation of hydrogen bonds between the drug and the polymer, contributing to controlled LFX release. Gelatin, known for its adhesive properties, further enhanced the formulation's performance when inserted into the cul-de-sac of the eye.

The linearity of the release 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 LOFs for achieving controlled and sustained LFX permeation for the treatment of ocular conditions.

The statistical analysis indicates several important findings like the Adjusted r^2, a measure of the goodness of fit for the regression model, is 0.1350 for EE and 0.4249 for the % CDR factors.

Table 2: Physicochemical constraints of the formulated films.

Formulation	Weight (mg)	Thickness (mm)	Content uniformity (%)	Moisture loss (%)	Moisture absorption (%)
LOF-1	61.26±0.9	0.18±0.01	92.72±2.1	9.28±0.2	12.51±0.8
LOF-2	62.03±0.5	0.19±0.02	97.11±5.7	9.02±0.3	15.02±0.5
LOF-3	61.92±0.2	0.17±0.01	97.23±3.8	9.31±0.5	12.01±0.1
LOF-4	63.05±0.3	0.16±0.01	97.02±2.5	9.82±0.4	14.05±0.2
LOF-5	61.85±0.4	0.15±0.02	96.98±4.5	8.28±0.8	11.02±0.6
LOF-6	61.94±0.1	0.16±0.01	98.00±1.4	8.48±0.5	18.05±0.9
LOF-7	62.03±0.5	0.17±0.02	97.02±2.6	8.64±0.5	13.05±0.8
LOF-8	62.08±0.2	0.16±0.01	97.03±2.4	8.97±0.6	16.02±0.5
LOF-9	62.09±0.4	0.18±0.01	95.08±2.2	9.03±0.1	13.64±0.6
LOF-10	64.54±0.9	0.17±0.02	96.99±3.3	9.45±0.8	15.62±0.4
LOF-11	63.38±0.5	0.18±0.01	97.04±3.1	7.18±0.5	14.08±0.2
LOF-12	66.07±0.1	0.17±0.02	94.02±3.2	9.36±0.2	13.65±0.8
LOF-13	61.82±0.2	0.17±0.01	96.89±2.4	8.26±0.3	14.82±0.7
LOF-14	62.07±0.2	0.17±0.02	97.01±1.6	7.06±0.5	15.02±0.3
LOF-15	62.65±0.5	0.17±0.01	96.97±3.7	8.30±0.7	14.75±0.7
LOF-16	63.05±0.8	0.17±0.02	94.96±2.4	9.31±0.8	13.55±0.6
LOF-17	65.56±0.7	0.18±0.01	97.06±1.9	8.46±0.6	14.61±0.7
LOF-18	63.48±0.1	0.16±0.01	95.01±0.5	7.09±0.7	15.31±0.2
LOF-19	62.67±0.6	0.16±0.01	96.92±0.3	9.30±0.8	13.82±0.1
LOF-20	63.07±0.3	0.17±0.02	97.03±0.2	9.71±0.6	14.72±0.4

Values in mean±SD; n= 3.

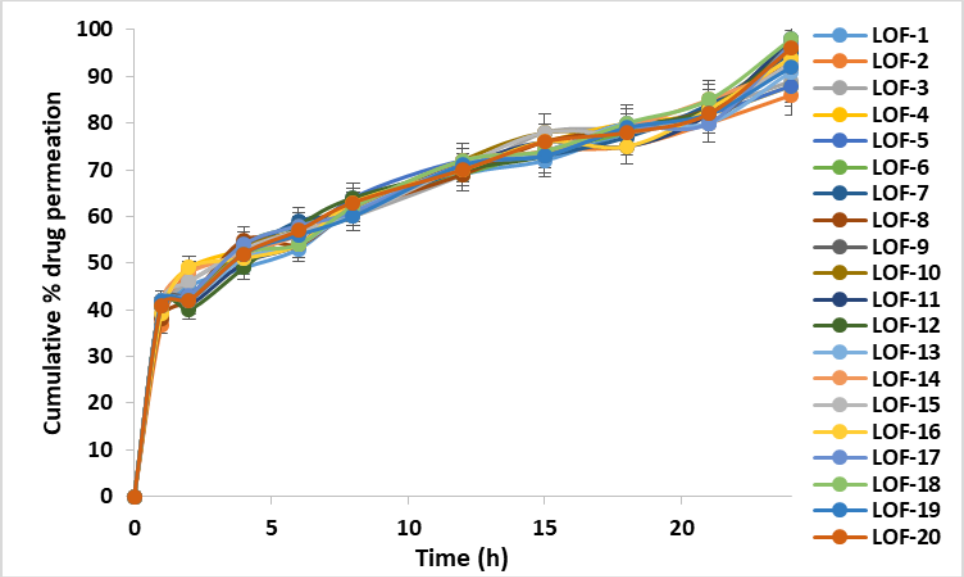

Figure 1: *In vitro* release plots from the films.

The Predicted r^2, which assesses the model's ability to generalize to unseen data, is -0.2292 for EE and 0.0798 for % CDR. The Adequate Precision values are greater than 4, with 61.012 for EE and 20.933 for % CDR, which is desirable. The Lack of Fit tests for EE and % CDR reveal non-significant F-values (0.82 and 0.35, respectively) relative to the pure error. This implies that the model fits the data well, with a low probability (52.16% for EE and 78.75% for % CDR) that the Lack of Fit F-value could occur due to random noise.

Table 3: Kinetics of LXM diffusion from the films.

Formulation	Zero-order			First-order			Higuchi		Koresmeyer peppas		
	Slope (n)	Ko=-slope	r	Slope (n)	Ko=-slope X 2.303	r	Slope (n)	r	Slope (n)	Constant (k)	r
LOF-1	3.751	3.751	0.995	0.033	0.075	-0.963	21.001	0.961	1.023	0.365	0.995
LOF-2	3.763	3.763	0.993	0.032	0.073	-0.983	23.082	0.945	1.125	0.452	0.981
LOF-3	3.652	3.652	0.994	0.031	0.071	-0.948	24.013	0.934	1.025	0.328	0.912
LOF-4	3.693	3.693	0.992	0.036	0.082	-0.947	25.698	0.987	1.036	0.398	0.994
LOF-5	3.658	3.658	0.997	0.031	0.071	-0.939	25.684	0.928	1.057	0.318	0.978
LOF-6	3.123	3.123	0.999	0.029	0.066	-0.987	29.386	0.916	1.035	0.401	0.966
LOF-7	3.325	3.325	0.992	0.031	0.071	-0.952	20.274	0.936	1.054	0.388	0.985
LOF-8	3.025	3.025	0.994	0.029	0.068	-0.948	21.252	0.941	1.035	0.325	0.991
LOF-9	3.015	3.015	0.993	0.032	0.074	-0.928	26.341	0.902	1.147	0.337	0.993
LOF-10	3.375	3.375	0.994	0.364	0.838	-0.918	23.688	0.931	1.098	0.374	0.954
LOF-11	3.458	3.458	0.990	0.351	0.808	-0.947	29.657	0.948	1.054	0.356	0.928
LOF-12	3.658	3.658	0.995	0.374	0.861	-0.992	26.456	0.964	1.097	0.394	0.931
LOF-13	3.415	3.415	0.994	0.352	0.810	-0.925	27.152	0.918	1.194	0.344	0.960
LOF-14	3.654	3.654	0.997	0.384	0.884	-0.898	25.515	0.924	1.065	0.392	0.925
LOF-15	3.349	3.349	0.996	0.314	0.723	-0.905	24.657	0.930	1.032	0.332	0.913
LOF-16	3.164	3.164	0.995	0.328	0.755	-0.925	23.331	0.922	1.078	0.364	0.908
LOF-17	3.627	3.627	0.998	0.371	0.854	-0.936	27.082	0.945	1.048	0.421	0.962
LOF-18	3.204	3.204	0.994	0.332	0.764	-0.961	26.631	0.960	1.061	0.382	0.975
LOF-19	3.809	3.809	0.995	0.329	0.757	-0.938	24.625	0.933	1.070	0.411	0.939
LOF-20	3.629	3.629	0.992	0.308	0.709	-0.946	24.186	0.918	1.033	0.368	0.977

Furthermore, the *p*-values for both factors (EE and % CDR) are less than 0.05 (< 0.0001), indicating that these model terms are statistically significant. In this case, terms B, AB, AC, BC, A^2, B^2, and C^2 are considered significant contributors to the model, suggesting their importance in explaining the response variable.

Final Equation in Terms of Coded Factors

$$EE=+81.06+8.99A+6.29B-3.94C+11.87AB-1.04AC-2.35BC-18.15A^2-2.74B^2-8.37C^2$$

$$DR=+59.61+0.4038A-11.68B+1.72C-5.03AB+8.72AC-3.18BC+8.06A^2-4.71B^2+5.60C^2$$

The normal plots (Figure 2A and 2D), Run Order Plots (Figure 2B and 2E), and Cook's Distance plots (Figure 2C and 2F) in the analysis serve to assess the relationships between the independent variables (Gelatin, ABLM, and HPMC K4M) and the response variables (EE and % CDR). These diagnostic plots help in understanding the distribution of residuals, detecting potential outliers or influential data points, and evaluating the robustness of the statistical model.

Subsequently, the contour plots (Figure 3A and 3C) and 3D plots (Figure 3B and 3D) are utilized to visually represent the relationships between the same independent variables and the response variables (EE and % CDR). These graphical representations offer insights into how changes in the independent variables influence the response variables and assist in identifying regions in the factor space where the response is optimized or minimized. Contour plots display constant response values on two-dimensional planes, while 3D plots provide a three-dimensional view of the response surface, aiding in the exploration of complex interactions and optimal factor settings for EE and % CDR.

DISCUSSION

The study presented here revolves around the development and evaluation of Lamifloxacin Ocular Films (LOFs) 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.

Table 4: ANOVA for Quadratic model.

Source	ANOVA for the Response 1 (% EE)			ANOVA for the Response 2 (% CDR)		
	Sum of Squares	*F*-value	*p*-value	Sum of Squares	*F*-value	*p*-value
Model	3989.28	413.88	< 0.0001	2101.38	36.44	< 0.0001
A-Gelatin	646.02	603.21	< 0.0001	1.30	0.2035	0.6615
B-ABLM	316.14	295.19	< 0.0001	1091.61	170.36	< 0.0001
C-HPMC K4M	124.03	115.81	< 0.0001	23.74	3.70	0.0832
AB	563.35	526.02	< 0.0001	101.30	15.81	0.0026
AC	4.33	4.04	0.0722	304.50	47.52	< 0.0001
BC	22.00	20.54	0.0011	40.45	6.31	0.0308
A^2	1506.55	1406.72	< 0.0001	297.30	46.40	< 0.0001
B^2	34.41	32.13	0.0002	101.22	15.80	0.0026
C^2	319.97	298.77	< 0.0001	143.46	22.39	0.0008
Residual	10.71			64.08		
Lack of Fit	2.79	0.8227	0.5216	8.46	0.3548	0.7875

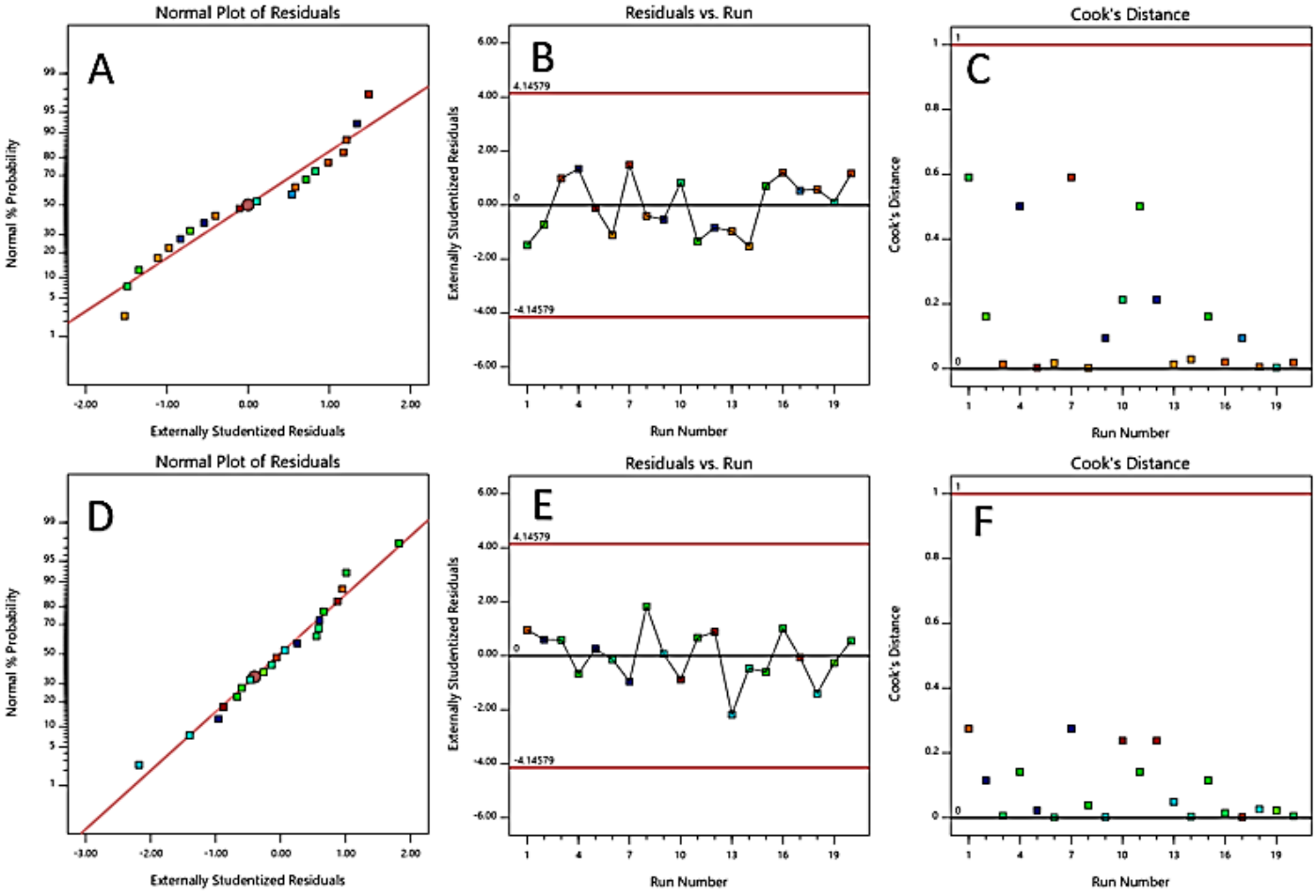

Figure 2: Normal plots of (A & D), residual vs. run plots (B & E), and cook's distance plots (C & F)of the impact of independent variables with the responses (EE and % CDR).

One notable aspect of this study is the selection of polymers for LOF formulation. The use of a combination of HPMC K4M, gelatin, and ABLM in a factorial design is a novel approach, and it is highlighted that these polymers are biocompatible and biodegradable. Such characteristics are essential for ocular applications to ensure safety and compatibility with delicate eye tissues.

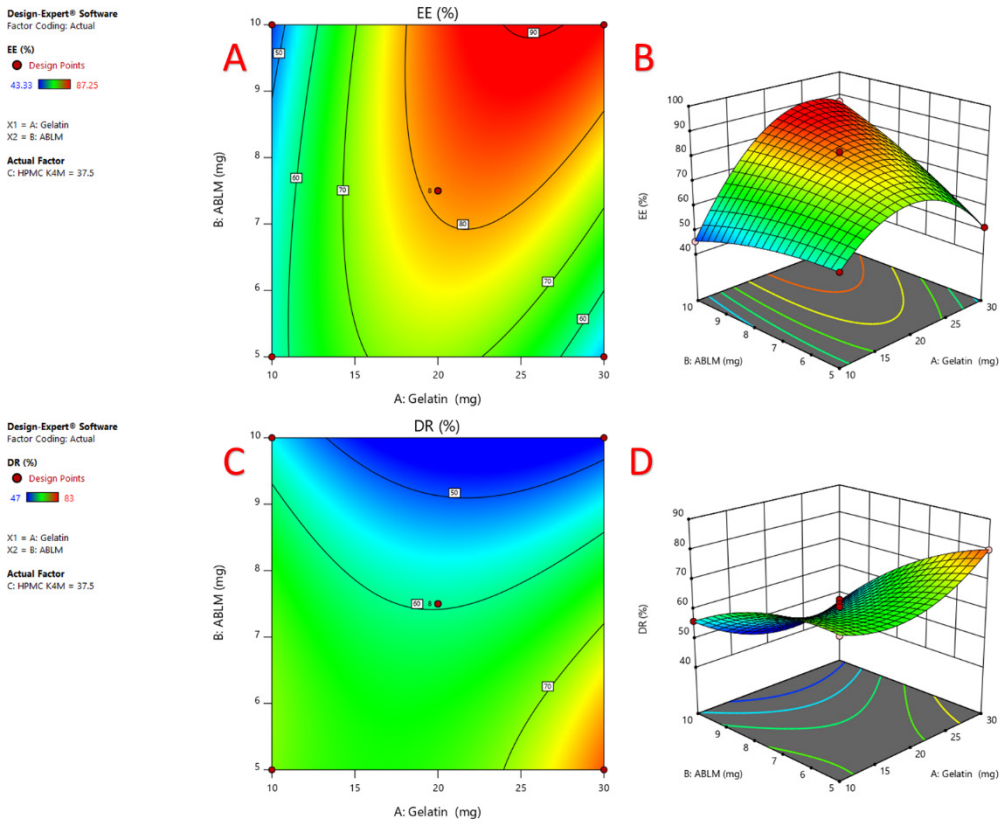

Figure 3: Contour (A & C) and 3D plots (B & D) of independent variables on the responses.

A common practice in ocular film development has been the use of PEG-400 as a plasticizer.[26,27] 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.

The utilization of the Box-Behnken Design (BBD) within the Design Expert software for optimization is a noteworthy choice, as it allows for efficient exploration of the parameter space. However, it is highlighted that only a few attempts have been made to optimize ophthalmic patches using this design approach.[28,29] This indicates that the study seeks to advance the field by utilizing 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 a crucial factor 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 the development of reliable ocular inserts.

The uniformity in film thickness, LFX content, and weight across all LOFs 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.[30]

Moisture tests reveal the behavior of LOFs under different environmental conditions. The results show that the films exhibit both moisture loss and moisture absorption properties.[31] The observation that gelatin-containing films have a higher moisture absorption capacity is valuable, as it provides insights into the effects of different polymers on moisture handling. Importantly, the integrity of the films remains intact 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 release profiles of the LOFs. The fact that LOF-8 exhibited the highest collective LFX permeation at the end of 24 hr is significant.[32] This suggests that the combination of HPMC K4M, gelatin, and ABLM in this formulation is particularly effective in achieving the desired LFX release profile. The formation of hydrogen bonds between the LFX and the polymer, as well as the adhesive properties of gelatin, contribute to controlled LFX release, which can be highly advantageous in the treatment of ocular conditions.

The linear release profiles of LFX from the ocular inserts indicate a diffusion-controlled release mechanism. This controlled and sustained LFX permeation is a desirable feature for the treatment of ocular conditions, as it can help maintain therapeutic LFX levels over an extended period, reducing the need for frequent dosing and improving patient compliance.[33]

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 both the Energy Efficiency (EE) and Percentage Cumulative Drug Release (% CDR) factors. This reveals that % CDR plays a more substantial role in explaining the variability in the response variable, while the adjusted r^2 penalizes an excessive use of predictors, ensuring a more conservative estimation of the model's explanatory power.[34]

Additionally, the Predicted r^2 is employed to evaluate the model's ability to generalize to new, unseen data points, demonstrating a negative value for EE, which implies potential limitations in predicting new data, and a positive value for % CDR, indicating a moderate level of generalization.

Furthermore, Adequate Precision values exceeding 4 for both EE and % CDR are considered desirable, signifying that the model possesses adequate signal and can proficiently navigate the design space, especially within the context of optimizing experimental or design parameters.

The Lack of Fit tests for EE and % CDR both result in non-significant F-values compared to pure error, suggesting that the model fits the data well, and there is a low probability that the Lack of Fit F-value is due to random noise.[35]

Lastly, the *p*-values for both EE and % CDR are less than 0.05, indicating the statistical significance of these model terms. This implies that terms such as B, AB, AC, BC, A^2, B^2, and C^2 are considered crucial contributors to the model, underscoring their importance in explaining the response variable.[36]

In Design-Expert software, a normal plot serves as a graphical tool to assess the normality of residuals in a regression or Analysis of Variance (ANOVA) model. It displays the observed residuals against the quantiles of a theoretical normal distribution. A straight line in the plot indicates that the residuals follow a normal distribution, while deviations suggest departures from normality, including outliers and skewness. This plot is essential for checking the assumption of normally distributed residuals, which is crucial for the validity of statistical analyses in experimental design and regression modeling, guiding decisions on potential data transformations or alternative modeling techniques.[37]

The Run Order Plot is a graphical representation used to assess the behavior of residuals (the differences between observed and predicted values) to the order in which data points were collected or run. It allows us to identify patterns, trends, and potential outliers in the residuals based on their run order. This plot is essential for evaluating the model's validity, as systematic trends or outliers may indicate unaccounted factors or data collection issues. It is a valuable diagnostic tool in experimental design and regression modeling, 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; larger values indicate 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 and whether model improvements are needed to account for influential observations. It's a valuable tool for model validation and understanding the impact of individual data points on regression outcomes.[38]

The contour plot is a visualization tool used in experimental design and response surface methodology for understanding the relationships between two independent variables (Gelatin, ABLM, and HPMC K4M) and the response variables (EE and % CDR). 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.[39]

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 (EE and % CDR). It displays a response surface, allows to visualization of complex factor interactions, assesses factor optimization, and gains 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

In 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:** Ultra violet; **QbD:** Quality by Design; **ODDS:** Ocular drug delivery systems; **LFX:** Levofloxacin; **ABLM:** Aloe barbadensis leaf mucilage; **PEG:** Polyethylene Glycol; **LOF:** Levofloxacin ocular Films; **hr:** Hour; **mg:** Milligram; **%:** Percent; **q.s:** Quantity sufficient; **RH:** Relative humidity; **ANOVA:** Analysis of varience; **EE:** Entrapment efficiency; **CDR:** Cumulative Drug Release.

SUMMARY

The study describes the Levofloxacin ocular films with polymers like HPMC K4M, gelatin, and Aloe Barbadensis Leave Mucilage (ABLM) Gelatin, ABLM, and HPMC K4M were chosen as independentfactors, with entrapment efficiency and % cumulative drug release as dependent variables.

Levofloxacin ocular films were developed.

The quantitative impact of independent variables at diverse levels on response variables is forecast by a polynomial equation.

The rapport between independent variables and dependent variables was further explicated via contour and 3D plots.

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