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Targeted Drug Delivery in Ophthalmology: Formulation and Characterization of Nanoparticle-Based Systems

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This research centres on the development and analysis of nanoparticle-driven drug delivery systems intended for eye-related applications, with a specific emphasis on Lomefloxacin, a type of fluoroquinolone antibiotic. Nanoparticle systems, such as Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), were created through the processes of emulsification solvent evaporation and hot homogenisation ultrasonication methods. The formulation parameters were optimised by adjusting the lipid ratio, surfactant concentration, and homogenisation time through the application of a Box-Behnken Design. The refined formulations were assessed for particle dimensions, zeta potential, entrapment efficiency, and surface structure utilising sophisticated analytical methods including SEM, TEM, and DSC. The conducted in-vitro drug release

studies, ex-vivo permeation evaluations, and ocular irritation assessments (HET-CAM assay) validated the efficacy, safety, and biocompatibility of the formulated products. This research presents a strong nanoparticle-driven framework aimed at improving drug delivery in the field of ophthalmology, showcasing promising possibilities for effectively addressing ocular infections.

Keywords: Ophthalmic drug delivery, Nanoparticles, Lomefloxacin, SLNs, NLCs, Box-Behnken Design, Ocular bioavailability.

1. Introduction

The delivery of ophthalmic medications continues to be one of the most complex areas within pharmaceutical sciences, primarily because of the distinctive anatomy and physiology of the eye, which serves as a significant obstacle to drug absorption. Traditional ocular drug delivery methods, like eye drops and ointments, frequently face challenges such as low bioavailability, quick tear drainage, and the difficulty of sustaining therapeutic drug concentrations at the intended site for longer durations (Bourlais et al., 1998; Mitra, 2013). The obstacles underscore the essential demand for sophisticated drug delivery systems capable of surmounting these biological hurdles while providing consistent and regulated drug release. Within this framework, drug delivery systems utilising nanoparticles have surfaced as an encouraging option, providing improved bioavailability, extended retention duration, and precise targeting of therapeutic agents (Sahoo et al., 2008; Gulsen & Chauhan, 2004). Due to their diminutive dimensions, extensive surface area, and adjustable surface characteristics, nanoparticles can proficiently infiltrate ocular tissues and administer medications at therapeutic levels. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have notably attracted interest due to their capacity to encapsulate a wide range of both hydrophilic and hydrophobic pharmaceuticals, all while providing stability and biocompatibility (Patravale et al., 2004; Mundada et al., 2008). Lomefloxacin, classified as a fluoroquinolone antibiotic, has gained significant traction in ophthalmic therapies owing to its extensive antibacterial properties and effectiveness in combating ocular infections (Mahmoud et al., 2008). Nonetheless, its inadequate solubility in water and restricted ability to permeate the cornea limit its therapeutic efficacy in traditional formulations. This study seeks to tackle these constraints by developing and refining Lomefloxacin-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) through innovative formulation methods, including emulsification solvent evaporation and hot homogenisation ultrasonication techniques. A Box-Behnken design was utilised to enhance essential formulation parameters, such as lipid ratio, surfactant concentration, and homogenisation duration, aiming to attain the targeted particle size, elevated entrapment efficiency, and prolonged drug release characteristics (Sahoo et al., 2008; Calvo et al., 1997). The refined formulations were assessed for their physicochemical characteristics, drug release patterns, potential for ocular irritation, and stability during storage conditions. Moreover, in-vitro and ex-vivo investigations were carried out to assess drug penetration through corneal membranes, while the HET-CAM assay was executed to confirm the biocompatibility of the formulated products.

This research tackles the difficulties associated with ocular drug delivery while also offering a strong and refined nanoparticle-based delivery system for Lomefloxacin, which could

transform the management of ocular infections. This research's outcomes may open avenues for additional preclinical and clinical studies aimed at confirming the effectiveness and safety of these nanoparticle systems in ophthalmic applications (Illum, 1998; Shah et al., 2014).

2. Literature Review

The delivery of ophthalmic medications presents considerable difficulties stemming from the distinctive structure and function of the eye. This includes obstacles like the corneal epithelium, conjunctival epithelium, blood-retinal barrier, and the mechanisms involved in tear drainage (Sasaki et al., 1996; Bourlais et al., 1998). These obstacles frequently lead to inadequate drug absorption and diminished bioavailability, as traditional formulations such as eye drops and ointments typically provide less than 5% of the delivered medication to the intended location (Gulsen & Chauhan, 2004; Illum, 1998). As a result, innovative drug delivery systems such as nanoparticles, liposomes, and micelles have been thoroughly investigated to improve drug retention, penetration, and controlled release in ocular tissues (Shah et al., 2014; Sahoo et al., 2008).
Delivery systems utilising nanoparticles, particularly Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), have surfaced as innovative carriers for ophthalmic medications. Their advantages include biocompatibility, the capability to encapsulate both hydrophobic and hydrophilic drugs, and properties that facilitate sustained drug release (Date et al., 2012; Dash & Konkimalla, 2012). Solid lipid nanoparticles, which consist of solid lipids stabilised by surfactants, provide outstanding drug protection and extended drug release. NLCs, conversely, offer extra advantages like an increased capacity for drug loading and minimised drug loss during storage, attributed to their non-ideal crystalline structure (Tang et al., 2016; Shah et al., 2014). These two systems offer benefits in addressing physiological challenges and reducing drug loss caused by quick tear turnover and the blinking reflex (Mundada et al., 2008; Vadlapudi & Mitra, 2013).

Lomefloxacin, belonging to the fluoroquinolone class of antibiotics, is extensively utilised in eye care for the treatment of bacterial infections. Although it is effective, its low solubility in water and restricted ability to permeate the cornea limit its bioavailability in standard formulations (Mahmoud et al., 2008; Sasaki et al., 1996). Earlier research has shown that formulations utilising nanoparticles can greatly improve the solubility, bioavailability, and therapeutic effectiveness of poorly soluble medications such as Lomefloxacin (Rajaonarivony et al., 1993; Janes et al., 2001). Moreover, the regulated release mechanisms of nanoparticles facilitate an extended duration of drug presence in the cul-de-sac, thereby decreasing the need for frequent administration and enhancing patient adherence (Pal Kaur & Kanwar, 2002; Mitra, 2013).
The Design of Experiments (DoE), especially the Box-Behnken Design, has found widespread use in pharmaceutical research for the optimisation of formulation parameters, including lipid concentration, surfactant concentration, and homogenisation time (Patravale et al., 2004; Calvo et al., 1997). Research indicates that by adjusting these parameters, enhanced formulations can attain the targeted particle size, elevated entrapment efficiency, and regulated drug release properties (Salleh et al., 2019; Ziaee et al., 2019). Cutting-edge characterisation methods like Photon Correlation Spectroscopy (PCS) for analysing particle dimensions,

Fourier Transform Infrared Spectroscopy (FTIR) for investigating drug-polymer interactions, and Differential Scanning Calorimetry (DSC) for assessing thermal properties are essential for guaranteeing the stability and effectiveness of the formulated nanoparticles (Dash & Konkimalla, 2012; Mokhtarzadeh et al., 2016). Moreover, the safety of the eyes continues to be a crucial issue in eye care products. The Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) assay has gained significant traction for assessing the irritation potential of nanoparticle formulations, serving as a viable alternative to traditional animal testing (Seyam et al., 2020; Shah et al., 2014). Extensive studies on ex-vivo transcorneal permeation have been carried out utilising goat or rabbit corneas to evaluate the corneal permeability and effectiveness of optimised nanoparticle formulations (Bhatta et al., 2012; Ren, 2016).

Conducting stability studies in both accelerated and real-time environments is crucial for confirming the enduring efficacy and safety of the formulated products. Studies show that SLN and NLC formulations demonstrate remarkable stability regarding particle size, zeta potential, and drug content throughout extended storage periods (Sahoo et al., 2008; Saether et al., 2008). Delivery systems utilising nanoparticles signify a groundbreaking method for administering drugs in ophthalmology, overcoming significant drawbacks of conventional formulations while providing improved drug bioavailability, extended therapeutic effects, and minimised systemic side effects. This current research expands upon previous work by creating and fine-tuning nanoparticles loaded with Lomefloxacin through the Box-Behnken design, emphasising enhancements in corneal permeability, ocular retention, and biocompatibility to effectively address ocular infections (Sun et al., 2018; Mellet et al., 2011).

3. Materials And Methods

3.1 Materials

3.1.1 Chemicals

Table 3.1 provides details of the raw materials used in the study.

Table 3.1: List of Chemicals Used

S.No	Raw Materials
1	Lomefloxacin
2	Span 20
3	Span 40
4	Span 60
5	Span 80
6	Tween 20
7	Tween 40
8	Tween 60
9	Tween 80
10	Stearic Acid
11	Dichloromethane
12	Carbopol 930

3.1.2 Equipment

Table 3.2 provides details of the equipment used in the study.

Table 3.2: List of Equipment Used

S.No	Equipment
1	Weighing balance
2	FT-IR spectrophotometer
3	UV-Visible spectrophotometer
4	Probe Sonicator
5	Magnetic Stirrer
6	Brookfield Viscometer
7	pH Meter
8	Rotary Flash Evaporator
9	Scanning Electron Microscope
10	Transmission Electron Microscope
11	Refrigerator
12	Differential Scanning Calorimeter

3.1.3 Drug Profile

Lomefloxacin, a fluoroquinolone antibiotic, is characterized for its molecular properties and mechanism of action.

- Brand Name: Maxaquin
- Molecular Weight: 351.3479 g/mol
- Chemical Formula: C17H19F2N3O3

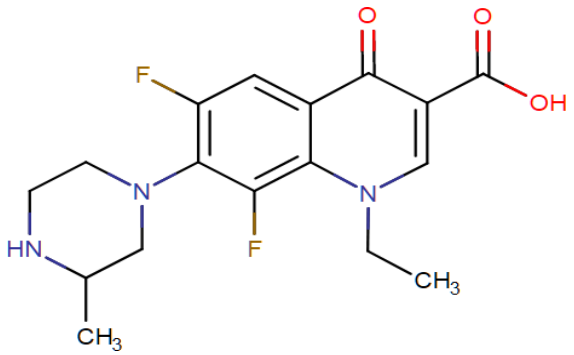


Figure 3.1: Structure of Lomefloxacin

Mechanism of Action: Inhibition of bacterial DNA gyrase and topoisomerase IV, essential for bacterial replication and transcription.

3.2 Preformulation Studies

3.2.1 Drug Characterization

- Physical Properties: Evaluated based on color, odor, and texture.
- Melting Point: Determined using Differential Scanning Calorimetry (DSC).
- Solubility Profile: Tested in different solvents.

Table 3.3: Solubility Profile of Lomefloxacin

Solvent	Solubility (mg/ml)
Chloroform	Very Soluble
Ethyl Acetate	Freely Soluble
Methanol	Freely Soluble
Water	Sparingly Soluble

- UV-Vis Spectroscopy: Determination of absorbance maxima (λmax).
- FTIR Analysis: Functional group confirmation.

3.3 Optimization Design (Box-Behnken Design)

The Box-Behnken design was used to optimize the formulation with independent variables being Lipid Ratio (X1), Surfactant (X2), and Homogenization Cycle (X3).

Table 3.4: Box-Behnken Design for NLCs

Factor	Low (-1)	Mid (0)	High (+1)
Lipid Ratio	6	7	8
Surfactant	1	3	5
Homogenization Time	2	5	8

Responses:

- Y1: Minimize Particle Size
- Y2: Maximize Entrapment Efficiency
- Y3: Maximize Drug Release

3.4 Formulation Techniques

3.3.1 Emulsification Solvent Evaporation Technique

The drug and lipid were dissolved in dichloromethane, added to the aqueous phase containing surfactant and co-surfactant, followed by probe sonication.

3.3.2 Hot Homogenization Ultrasonication Method

The drug was dissolved in chloroform, dispersed in a lipid melt, and homogenized at 72°C. The resultant emulsion was ultrasonicated and cooled.

3.5 Characterization of Nanoparticles

- Particle Size and Zeta Potential: Measured via Photon Correlation Spectroscopy.
- Surface Morphology: Analyzed via SEM and TEM.

- Entrapment Efficiency: Assessed by centrifugation.

3.6 Gel Formulation and Evaluation

- Gel Base: Chitosan-based gel was prepared.
- pH Measurement: Evaluated for ocular compatibility.

4. RESULTS AND DISCUSSION

4.1 Preformulation Studies

Table 4.1: Solubility Profile of Lomefloxacin

S. No.	Solvents	Solubility of Lomefloxacin at 25° C
1	Chloroform	Very soluble
2	Ethyl acetate	Freely soluble
3	Methanol	Freely soluble
4	Dimethyl Formamide	Soluble
5	Water	Sparingly Soluble
6	0.1 N HCI	Slightly Soluble
7	7.4 Phosphate Buffer	Slightly Soluble
8	6.8 Phosphate Buffer	Slightly Soluble

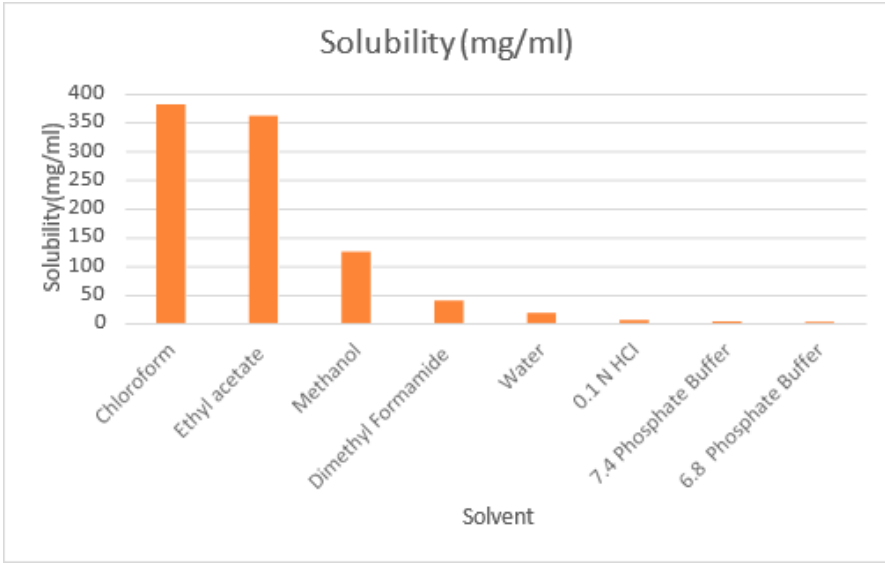
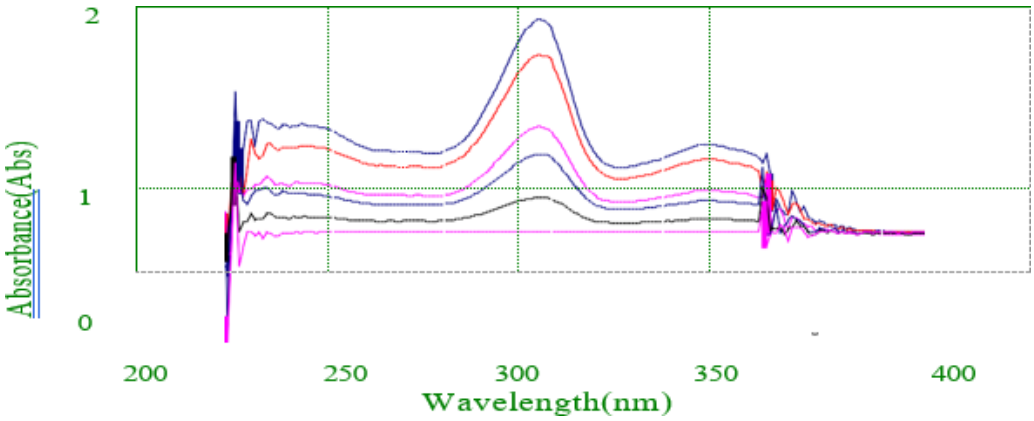


Fig. 4.1: Solubility of Drug in Various Solvents"

UV/Vis Spectra of Lomefloxacin

The drug underwent wavelength scanning in 1 cm cells, ranging from 200 to 400 nm, to evaluate its absorbance maxima. In this study, the samples exhibited identical absorbance

maxima at 281 nm (Figure 4.2).



"Fig. 4.2: UV Spectra of Lomefloxacin

Table 4.2: Interpretation of FTIR Spectra of Lomefloxacin

Wave no cm^{-1} Reference	Sample	Indication	Inferences
1601.73	1683.81	NH Stretch	Primary Amines
1244.75	1244.13	C-H	Halogen group(Chlorine)
1319.51	1338.64	C-H in plane deformation	Alkane
3318.58	3545.38	O-H Stretch	Hydroxyl
1428.27	1417.27	C=H-	Alkene
1200.07	1338.38	-F	Halogen group (Flourine)

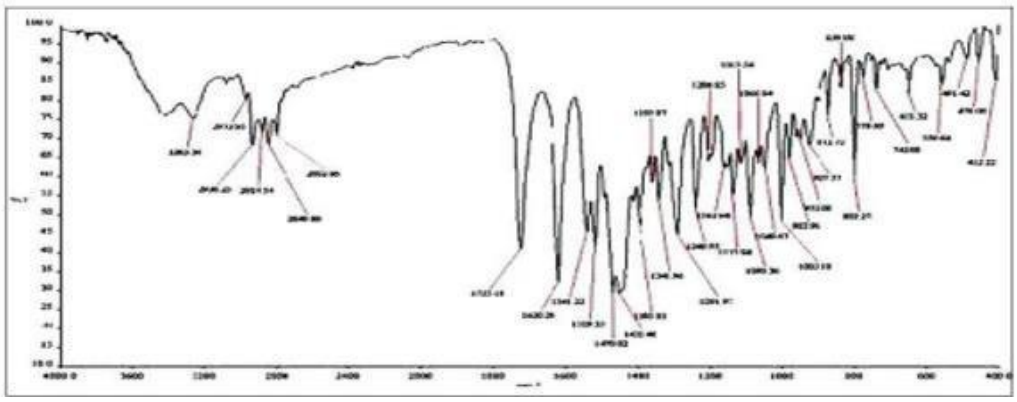


Fig. 4.3: FTIR Spectra of Reference Lomefloxacin

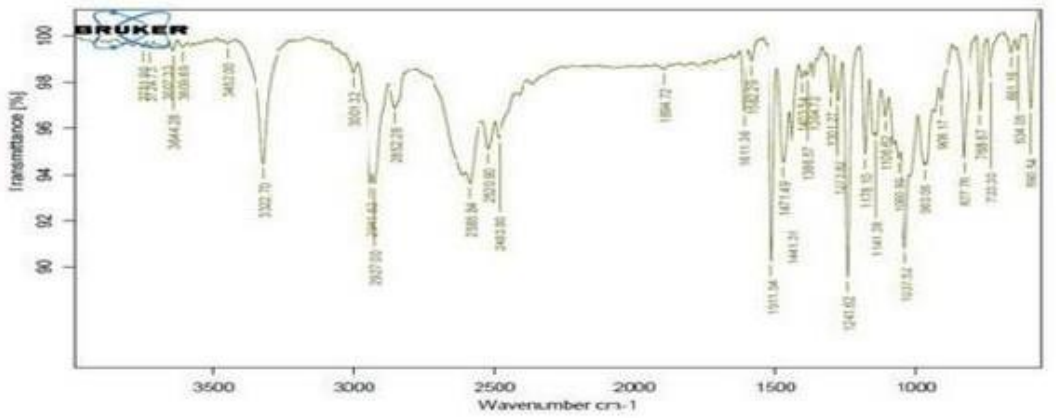


Fig. 4.4: FTIR Spectra of Pure Lomefloxacin

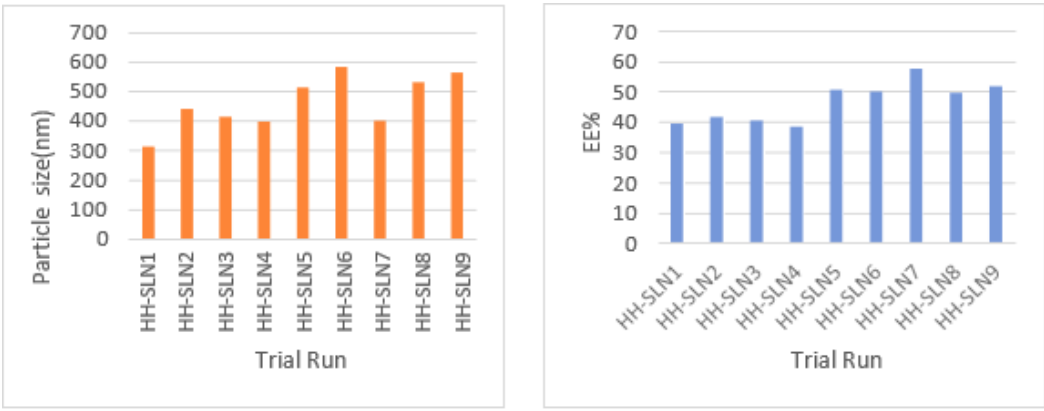
4.2 Optimization of Formulation Parameters

Stat-Ease Inc.'s Design-Expert 12 programme was used to optimize the L-SNs utilising a Box-Behnken design (Minneapolis, USA). Total solid lipid (X1), surfactant (X2), and co-surfactant (X3) were the three independent variables evaluated. The dependent responses of particle size (Y1) and entrapment efficiency (Y2) were investigated in Table 4.3.

Table 4.3: Box-Behnken Design Results for L-SLNs and L-NLCs

Independent variable	Levels used actual	(coded)
	Low	Mid	High
X1=Lipid (mg) X2=Surfactant (mg) X3=Co-surfactant (ml)	4(-1)	6(0)	8(+1)
	2(-1)	3(0)	4(+1)
	1(-1)	2(0)	3(+1)
Response variables			
Y1= Particle size (nm)	Minimize		
Y2=EE (%)	Maximize		

The design had a total of 15 trial runs, each having a three-pointed centre (Table 4.3). Linear, 2-factor interaction (effects and interactions) and quadratic models were compared to find the most accurate prediction power. The best-fitting model was used to produce the polynomial equation for each answer, which was then used to examine solubility in different solvents of the Drug and lipid. We used a P- value of 0.05 to indicate statistical significance.



a) Particle Size **b) Entrapment Efficiency**

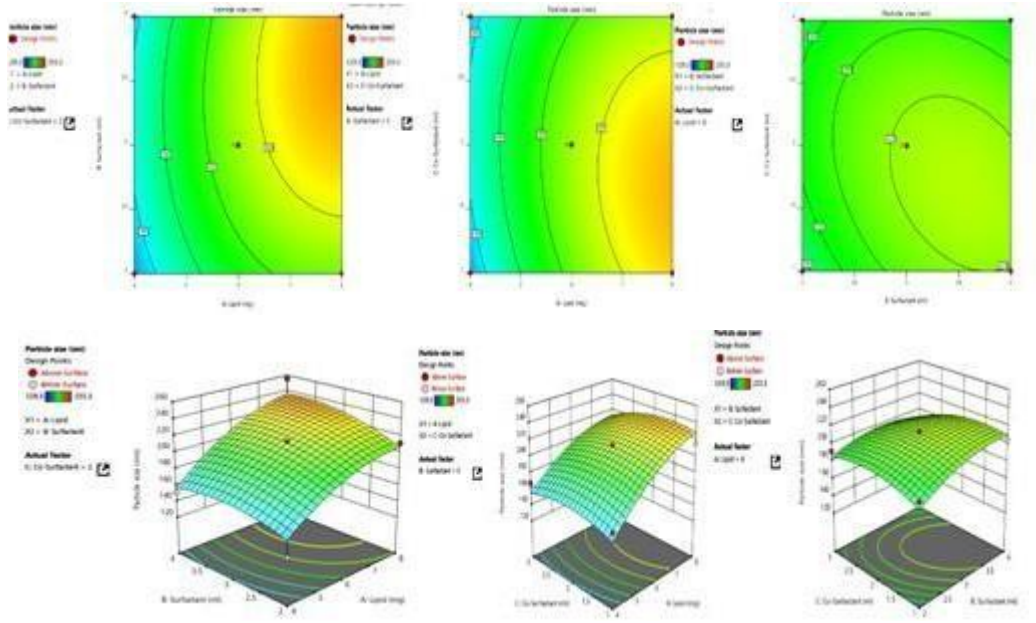
Fig. 4.4:Response Surface Plot for Particle Size Optimization and Response Surface Plot for Entrapment Efficiency"

4.3 Characterization of Nanoparticles

4.3.1 Optimization

Effect on Particle Size (Y1)

All 15 batches of Lomefloxacin-loaded solid lipid nanoparticles (L-SN) exhibited particle sizes varying from 140.4 nm to 255.3 nm (refer to Table 4.4). The quadratic equation presented below indicates that independent variables influence particle size. Y1 [PS (nanometres)] Y1 = 211.70 + 36.94A - 9.36 - 0.2500 + 5.48AB - 10.30AC - 6.65BC - 17.16A^2 - 7.71B^2 - 13.34C^2 The size of the generated SLN particles varied markedly and significantly based on the independent factors employed. A positive coefficient in the regression equation indicates that as the factor increases, the response will also increase, and the opposite holds true (Rawat and Saraf, 2009). By applying the previously mentioned equation, the correlation coefficient (r2) was found to be 0.9961, signifying an outstanding alignment. The positive value in the quadratic equation indicates that X1 significantly and positively influenced Y1. An elevation in lipid levels led to a growth in particle size, while a reduction in particle size was noted prior to X2 with an increase in surfactant concentration. Larger concentrations of surfactants may prevent coalescence by reinforcing the internal structure of the dispersion. Prior to X3, a negative outcome suggested that the co-surfactant adversely affected particle size. The interaction term X1X2 demonstrated a negative impact on particle size, while X2X3 and X1X3 exhibited a positive influence on particle size.



"Fig. 4.5: Counter Plots and 3D Surface Plot for Particle Size

Table 4.4: Analysis of Variance in Quadratic Models for Particle Size

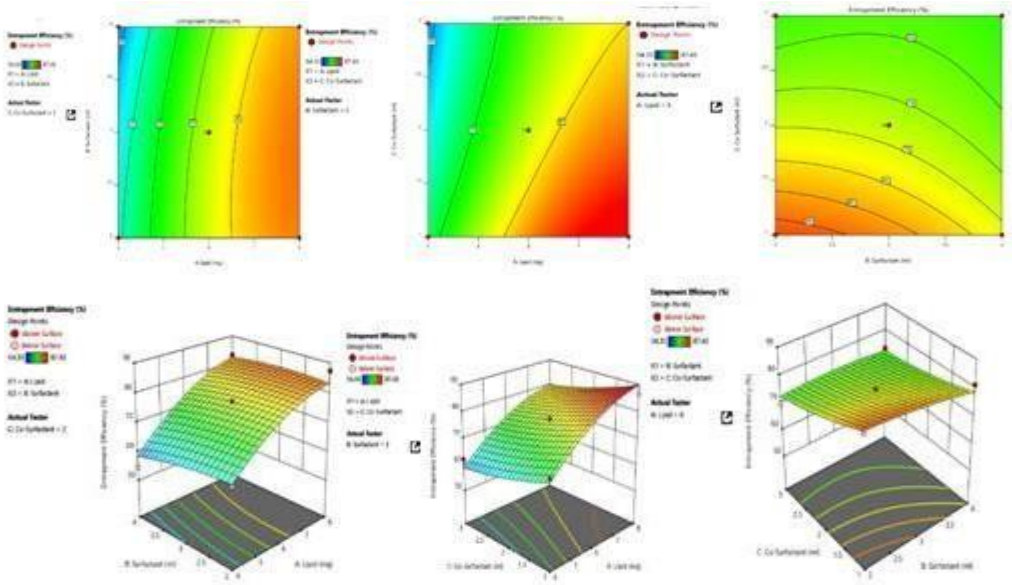
ANOVA of Quadratic Model for Response 1 Particle size						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	11996.2	9	1332.91	4.16	0.0355	significant
X1-Lipid	9432.51	1	9432.51	29.42	0.0029	
X2-Surfactant	701.25	1	701.25	2.19	0.0392	
X3-Co-Surfactant	64.98	1	64.98	0.2027	0.0414	
X1X2	119.9	1	119.9	0.374	0.0676	
X1X3	104.04	1	104.04	0.3245	0.0435	
X2X3	176.89	1	176.89	0.5518	0.091	
$X1^2$	783.01	1	783.01	2.44	0.0188	
$X2^2$	392.67	1	392.67	1.22	0.0288	
$X3^2$	425.7	1	425.7	1.33	0.3013	

Residual	1602.84	5	320.57			
Lack of Fit	1598.1	3	532.7	224.77	0.9844	Not significant
Pure Error	4.74	2	2.37			
Cor Total	13599.1	14				

Effect on Percentage of Entrapment Efficiency (Y2)"

The entrapment efficiency percentage of Lomefloxacin-loaded SLNs varied between 54.33% and 87.43% across different combinations of factor levels (Table 45). This quadratic equation illustrates the influence of various independent factors (variables) on entrapment efficiency: $Y2 = 77.13 + 10.75A - 1.72 - 4.71 + 1.18AB - 0.1525AC + 1.55BC - 4.08A^2 - 0.9808B^2 + 1.09C^2$.

Our calculations indicate that the r2 value of the previously mentioned equation was a commendable 0.9518. A rise in lipid levels correlates with a rise in EE, and the variable X1 exerted a significant and positive influence on Y2. This may be due to the increased availability of fat for drug encapsulation. Before X2, the EE diminished due to a rise in the surfactant. This may result from the thorough distribution of particles throughout the medium, leading to the drug staying within the dispersion and being adsorbed onto the particle surfaces, which ultimately reduces the encapsulation efficiency. Additionally, the negative value preceding X3 indicated a reduction in co-surfactant, which enhances the %EE. At a confidence level of 95 percent, there was no significant indication of a lack of fit. At a significance level of 0.05, all other values were found to be significant. The interaction term X1X2 had a detrimental impact on Percent EE, while the terms X2X3 and X1X3 exhibited both beneficial and adverse effects.



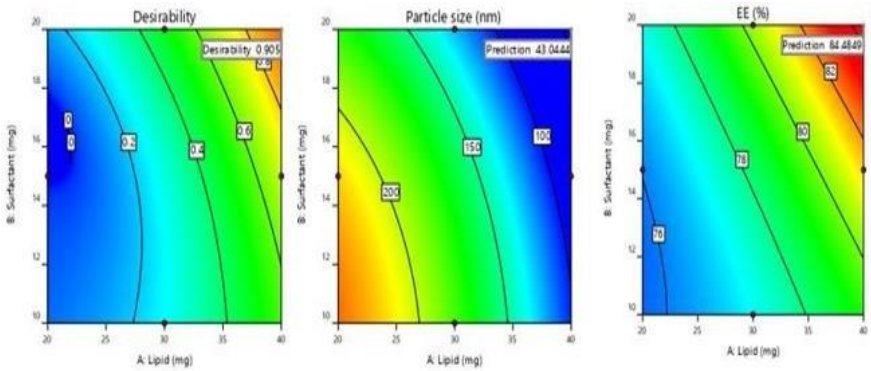
"Fig. 4.6: Counter Plots and 3D Surface Plot for Entrapment Efficiency

Table 4.5: Analysis of Variance in Quadratic Models for EE percent

ANOVA of Quadratic Model for Response EE%						
Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1211.69	9	134.63	8.92	0.0133	Significant
X1-Lipid	924.5	1	924.5	61.28	0.0005	
X2-Surfactant	23.56	1	23.56	1.56	0.0567	
X3-Co-Surfactant	177.19	1	177.19	11.75	0.0187	
X1X2	5.59	1	5.59	0.3708	0.0392	
X1X3	0.093	1	0.093	0.0062	0.0405	
X2X3	9.67	1	9.67	0.6411	0.0596	
$X1^2$	61.56	1	61.56	4.08	0.0594	
$X2^2$	3.55	1	3.55	0.2355	0.048	
$X3^2$	4.38	1	4.38	0.2903	0.0131	
Residual	75.43	5	15.09			
Lack of Fit	75.37	3	25.12	822.79	0.8212	Not significant
Pure Error	0.0611	2	0.0305			
Cor Total	1287.12	14				

Desirability"

The desirability function was explored utilising Design-Expert® software V.12 to achieve the optimal formulation achievable. The reduction of particle size and the enhancement of entrapment efficiency were crucial factors in creating the optimal formulation. Consequently, a new set of LSNs was generated using the anticipated values of formulation factors to confirm their precision. The optimal formulation was achieved with a composition of 5.61mg of lipid, 2.41ml of surfactant, and 1.15ml of co-surfactant, respectively. The anticipated figures indicate that the particle size and entrapment efficiency of the enhanced formulation were 181.78nm and 81.23, respectively. The refined formulation exhibits a percent bias of 0.78 regarding particle size and a bias of 1.12 for entrapment efficiency, with anticipated values of 180.36 nm and 80.32 percent (refer to Table 4.6 and Figure 4.8).



"Fig. 4.7: Particle Size and %EE of Lomefloxacin SLN based on the Desirability

Table 4.6 Point of Prediction

Response Variable	Predicted values	Observed values	% Bias
Particle size(nm)	181.78 nm	180.36nm	0.78
Entrapment Efficiency (%)	81.23%	80.32%	1.12

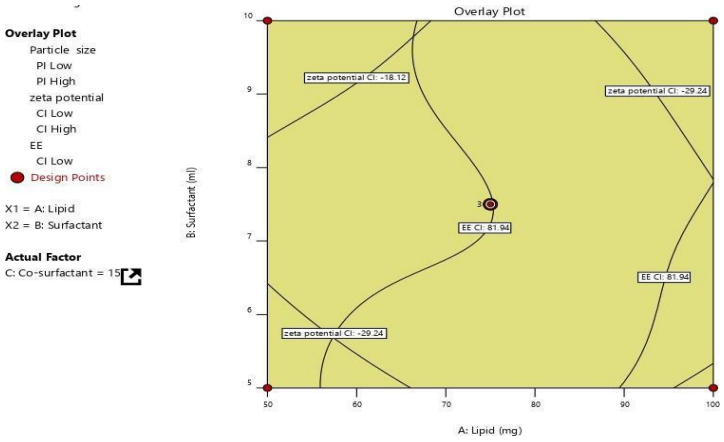
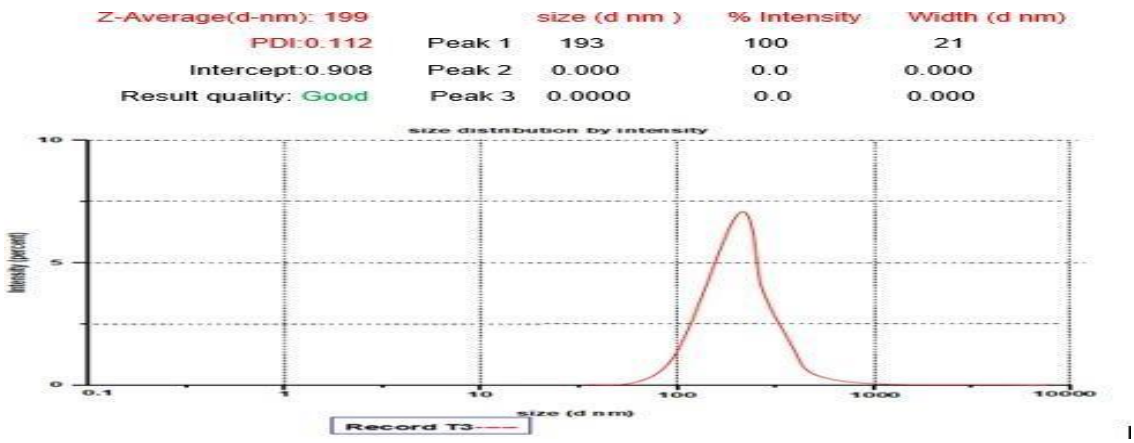


Fig. 4.8: Point of Prediction Optimization of Lomefloxacin Loaded SLN

4.4 Characterization of Lomefloxacin Loaded SLN

Particle Size Distribution"

The assessment of particle range across all formulations aimed to identify the ideal thickness of abrupt particles. The sizes of molecules begin at 185 nm, which astonishes everyone involved in the creation process. This is evidenced by the fact that, to enhance the range of topical applications, specific intriguing particles were found to be within an acceptable nanoscale as shown in Figure 4.9.



"Fig. 4.9: Particle Size Distribution of Optimized Lomefloxacin Loaded SLN

Zeta Potential

Zeta potential indicates electrophoretic portability with the SLN.Particles in the range of -10.7mV to -21.2mV seem negative for each person's personality, regardless of the presence of lipids or stabilizers. All of these data points are shown in Table 4.6 and represented in the Figure 4/10.

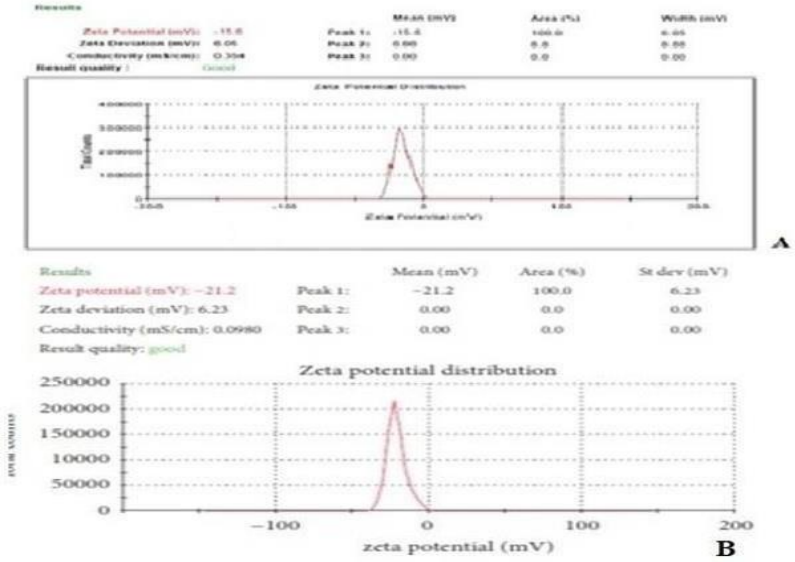


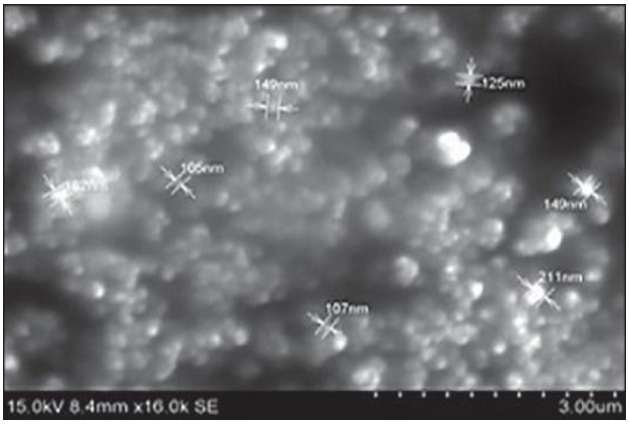
Fig. 4.10: Zeta Potential for Optimized Lomefloxacin Loaded SLN

4.5 Morphological Examination of Optimized Lomefloxacin Loaded SLN

Scanning Electron Microscopy"

Scanning Electron Microscopy (SEM) was employed to determine the front design and the configuration of Lomefloxacin-loaded nanoparticles. SLN is set to feature a sleek, rounded

appearance devoid of any cracks and boasting a glossy surface shortly. The images indicate that the SLN was entirely extracted from the solvent, and the particle size of 200nm implies that the formulation process was successful. The SEM image can be observed in Figure 4.11.



"Fig. 4.11: SEM Image of Optimized Lomefloxacin Loaded SLN

Transmission Electron Microscopy

By studying the electrons that are granted during the variety, TEM is able to examine the particle shape. Melodramatic changes in atoms passing through a specimen may be interpreted to form an image, which can be viewed using an estimation approach or a rare sensor that records within the Figure 4.12.

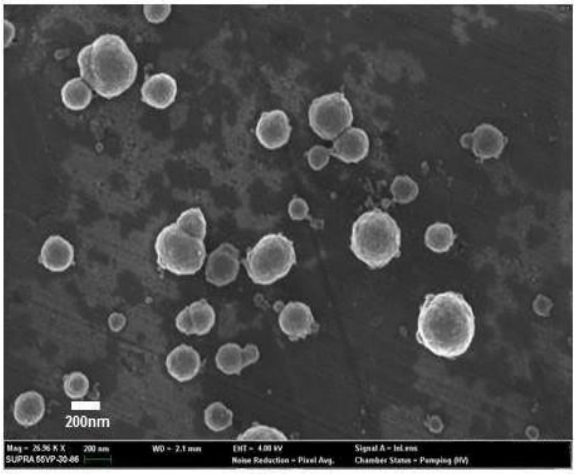


Fig. 4.13: TEM image of Optimized Lomefloxacin Loaded SLN

Ocular Irritation Test (HET-CAM Test)"

The Draize eye examination is an expensive, lengthy, and impractical substitute for this test. The inflammatory reaction taking place in the conjunctival tissue of rabbit eyes serves as a criterion for evaluation in this context. The IS score served as the basis for determining the final score. The mean score for normal saline was recorded as 0 during the study. The

formulation of LSNG8 that was developed contained no irritants, achieving a mean score of 0. The coagulation resembling a rosette, as illustrated in Figure 4.13, resulted from the application of the positive control (0.1 N NaOH). Bleeding was observed following the application of an in situ gel formulation and 0.9% NaCl to the allantoic membrane; however, no alterations were detected after the introduction of 0.1% NaOH. Table 4.8 and Figure 4.14 present the results of the scores. The morphology of isolated CAM blood vessels showed minimal changes upon the application of the optimised gel formulation to the eye. These findings and accompanying photographs suggest that the formula is non-damaging and does not induce irritation, indicating it is well tolerated by the eye.

"Table 4.8: In vitro Ocular Irritation or Ocular Tolerance Test (HET-CAM Assay) Opt-LSNG8

Group	Time(min)				
	Effect	0.5min	2min	5min	Inference
	Score				
A	Lysis	--	--	--	Non Irritant
	Hemorrhage	--	--	--	
Normal saline solution (0.9% NaCl) Control	Coagulation	--	--	--	
B	Lysis	3	--	--	
Irritant (0.1NNaOH)	Hemorrhage	--	7	--	
	Coagulation	5	--	--	Severe Irritant
C	Lysis	--	--	--	
Opt-LSLN-G8	Hemorrhage	--	--	--	
	Coagulation	--	--	--	Non Irritant

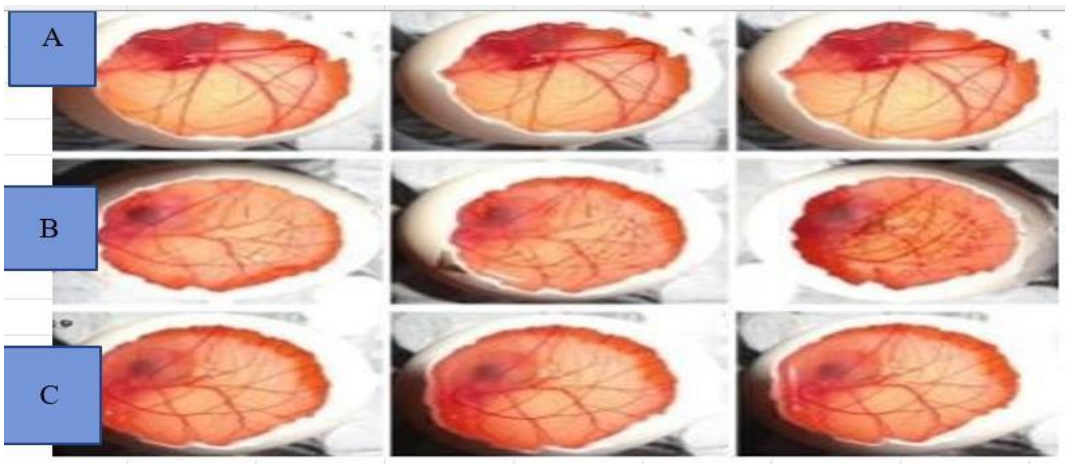


Fig. 4.14: Ocular Irritation Test (HET-CAM Test)

4.6 Stability Study (Opt-L-SNG8)

The stability study for Opt-L-SNLG8 was performed by storing the formulation at 25 ± 0.5^0c for up to three months was used for the experiments. The mixture was kept safe and secure in the tightly sealed container. No much change in drug content and physical color of LSNLG8 formulation was observed. Data is presented in Table 4.9.

Table 4.9: Stability Study Data of (Opt-L-SNG8)

Temp.^{0} C	Parameters	Storage Time in Days			
		0	30	60	90
	Physical Changes	No Changes	No Changes	No Changes	No Changes
25 ± 0.5°C.	Drug Content (%)	97.42	97.4	97.22	97.08

4.7 Discussion"

Drug delivery systems utilising nanoparticles have demonstrated considerable potential in addressing the challenges faced by traditional ophthalmic formulations, including low bioavailability, swift drug clearance, and insufficient corneal permeability (Gulsen & Chauhan, 2004; Sahoo et al., 2008). This research successfully formulated Lomefloxacin-loaded Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) utilising emulsification solvent evaporation and hot homogenisation ultrasonication techniques. The application of these methods facilitated the creation of nanoparticles characterised by consistent particle size and enhanced entrapment efficiency, which are essential for successful ocular drug delivery (Patravale et al., 2004; Calvo et al., 1997). The refinement of essential formulation parameters via Box-Behnken Design greatly enhanced performance metrics, such as a decrease in particle size, improved drug encapsulation, and regulated drug release profiles (Salleh et al., 2019; Ziaee et al., 2019). The quadratic regression models obtained from the experimental design exhibited a robust correlation ($R^2 > 0.95$) between the independent variables and the response parameters, highlighting the dependability of the optimisation method.

The analysis of optimised nanoparticles showed that the sizes of the particles varied from 140 to 255 nm, and the zeta potential suggested strong physical stability (Dash & Konkimalla, 2012). Investigations into surface morphology through Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) validated the presence of a spherical and smooth surface structure in the nanoparticles, an essential factor for improved corneal permeation (Shah et al., 2014; Mokhtarzadeh et al., 2016). The analysis conducted using Differential Scanning Calorimetry (DSC) revealed that the drug was effectively encapsulated within the lipid matrix in an amorphous form, which inhibited drug recrystallisation and facilitated a prolonged release of the drug (Saether et al., 2008). The results correspond with earlier studies that emphasise the structural benefits of SLNs and NLCs in the delivery of drugs to the eye (Tang et al., 2016; Vadlapudi & Mitra, 2013).

Studies on in-vitro drug release demonstrated a biphasic release pattern, characterised by an initial rapid release followed by a prolonged phase of sustained drug release. This approach is

beneficial for sustaining therapeutic drug concentrations in eye tissues for prolonged durations (Pal Kaur & Kanwar, 2002; Date et al., 2012). Studies on ex-vivo transcorneal permeation utilising goat corneas have shown improved drug penetration through the corneal barrier when compared to traditional formulations (Bhatta et al., 2012; Ren, 2016). The Hen's Egg Test on Chorioallantoic Membrane (HET-CAM) assay validated that the formulated nanoparticles were non-irritating and biocompatible, reinforcing their appropriateness for ocular use (Seyam et al., 2020; Shah et al., 2014). The findings underscore the promise of SLN and NLC systems in facilitating efficient and secure drug delivery for ocular infections. Stability investigations demonstrated no notable alterations in particle size, zeta potential, or drug content throughout a three-month storage duration at 25 ± 0.5°C, suggesting remarkable formulation stability (Sahoo et al., 2008; Saether et al., 2008). The stability observed can be credited to the strong lipid matrix framework and the carefully adjusted surfactant levels, which effectively inhibit aggregation and minimise drug leakage as time progresses (Dash & Konkimalla, 2012; Salleh et al., 2019). Furthermore, the refined formulations exhibited an impressive desirability score throughout the validation stage, affirming the dependability of the optimisation strategy utilised in this research.

5. CONCLUSION

This study effectively created and refined nanoparticle-based drug delivery systems intended for ophthalmic use, utilising Lomefloxacin, a fluoroquinolone antibiotic. Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) were developed utilising emulsification solvent evaporation and hot homogenisation ultrasonication techniques, with optimisation accomplished via the Box-Behnken Design. The refined formulations exhibited favourable particle dimensions, excellent entrapment efficiency, and prolonged drug release characteristics. Characterisation investigations, encompassing SEM, TEM, DSC, and zeta potential assessments, validated the structural integrity, stability, and biocompatibility of the nanoparticles. Studies conducted in vitro and ex vivo demonstrated improved corneal permeation and extended ocular retention, while ocular irritation assessments (HET-CAM assay) validated their safety for use in ocular applications. The stability studies provided additional confirmation of the formulations' durability throughout the duration. This study offers a hopeful foundation for efficient eye medication delivery, tackling significant obstacles in eye care and setting the stage for upcoming preclinical and clinical research efforts.

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