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Design and optimization of lomefloxacin loaded NLC gel for ophthalmic drug delivery

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Abstract---Lomefloxacin is a fluoroquinolone drug that has broad-spectrum action against Gram-negative as well as Gram Positive pathogens. The drug retention issues of ophthalmic drug delivery are a key parameter to select NLC gel as a deliver system. The purpose of the present investigation was to design, optimize and evaluate Lomefloxacin loaded nano structured lipid gel for Ophthalmic Drug Delivery. Lomefloxacin loaded NLCs were prepared by high-pressure homogenization approach (hot), using Olive oil as liquid lipid, Stearic acid as solid lipid and tween 80 as surfactant. Box-Behnken design was used to optimize Lomefloxacin loaded NLCs using Design-Expert 12 programme. Lipid ratio X1, surfactant concentrationX2, and homogenization cycle X3 are kept as three independent variables particle size (Y1 nm), entrapment efficiency (Y2 percent), and drug release percentage (Y3 percent) as dependent variables. NLCs were characterized for particle size, zeta potential and entrapment efficiency. The effects of composition of lipid materials and surfactant mixture on the particle size, zeta potential, drug entrapment efficiency, and in vitro drug release behavior were investigated. The formulation composed of 8 mg of lipid, 3.19 ml of surfactant, and a homogenization cycle of 2 cycles resulted in the optimal formulation as it has shown a particle size, entrapment efficiency, and drug release percentage within projected limits, at 209.243nm, 81.74 percent, and 89.76 percent, respectively.

Keywords---NLC, Lomefloxacin, Box-Behnken design, particle sizes, Entrapment efficiency, DSC, TEM.

Introduction

In the last decade, nanotechnology is the fastest-growing strategy for increasing the solubility and permeability of drugs. In addition, nanomaterials produced by nanotechnology could avoid the immune response and penetrate multiple biological barriers (Mohammadi et al., 2017). Nanostructured lipid carrier (NLC), lipid-based nanoparticles, has been introduced as a new pharmaceutical delivery system (Beloqui et al., 2016). NLC has certain advantages such as simple manufacturing process without organic solvents and easy scale up (e.g., high pressure homogenization). Moreover, NLC contains both solid and liquid lipids to form unstructured matrices, thus NLC improves drug loading capacity and reduces drug expulsion during storage (Mohammadi et al., 2017). Optimization of the formulation or process is important to improve the regularity of the formulation during the development of the formulation. Systemic design is therefore, used extensively to develop the formulation (Herneisey et al., 2019; Beg et al., 2019; Dahmash et al., 2018). The application of statistical experimental designs, such as design of experiments, would be useful in understanding the relationship between factors and responses in a formulation. Herein, a Box–Behnken design was used to optimize Lomefloxacin-NLC, which is useful for establishing quadratic or cubic response surfaces and constructing second order polynomial models (Gupta et al., 2017).

Lomefloxacin was the model drug selected for present study. Lomefloxacin is a fluoroquinolone drug that has broad-spectrum action against Gram-negative as well as Gram Positive pathogens. The drug retention issue of ophthalmic drug delivery is a key parameter to select NLC gel as a deliver system. The aim of the study was to develop Lomefloxacin-loaded NLC through design of experiments. In addition, characterized for particle size, zeta potential and entrapment efficiency. The effects of composition of lipid materials and surfactant mixture on the particle size, zeta potential, drug entrapment efficiency, and in vitro drug release behavior, Exvivo and in vivo Pharmacokinetic studies confirmed non irritability and enhanced bioavailability of Lomefloxacin-NLC.

Material and Methods

Model drug for the current research selected was Lomefloxacin procured from Sisco RL Pvt Ltd. Mumbai. Other chemicals needed for formulation such as Tween 20, Tween 40, Tween 60, Tween 80, Span 20, Span 40, Span 60, Span 80, Stearic Acid, Dichloromethane and Carbopol 930 of research grade are procured from SD Fine chemicals Mumbai.

Methodology

Selection of Lipids

Lomefloxacin (LMX) solubility was taken into consideration while selecting solid and liquid lipids. An Eppendorf tube with two millilitres of liquid lipids (olive and castor) was filled with 2 millilitres of LMX and vertex it for 15 minutes. Then it was placed in an orbital shaker for 72 hours to allow the lipids to emulsify. For this experiment, the extra amount of LMX was used in the same manner as when

it was introduced to 1 g of solid lipids melted at 5°C above the melting point of Glyceryl Monostearate, Myristic acid, and Palmitic acid. Saturation solubility of LMX was determined when the mixture became less transparent. 5000 rpm, 15 minutes, Remi-centrifuge was used to centrifuge the mixture. Supernatant and LMX concentration were determined by UV-Vis-spectrophotometer (Shimadzu 1800, Kyoto, Japan) at 281 nm after dilution, using the Shimadzu 1800 The proper lipids must be chosen. The selection of lipids, both solid and liquid, was based on their ability to bind the most LMX. (Jeevanandam et al., 2018).

Selection of Surfactant

Choosing the right surfactant Transfuse the surplus LMX into an Eppendorf and centrifuge it for 15 minutes with 1 mL of surfactant (Tween 20, Tween 80, PEG200, Limonene, and Poloxamer). In an orbital shaker, the mixture was let to stand for 72 hours. In order to separate off the supernatant, the mixture had to be centrifuged for around 15 minutes at 5000 rpm. At 281nm (Shimadzu, 1800, Tokyo, Japan), the concentration of LMX was determined using a UV-Vis spectrophotometer.

Development of Lomefloxacin Loaded NLCs (LMX-NLCs)

To formulate the LMX NLCs, the most typical high-pressure homogenization approach (hot). This mixture was first dissolved in water and added to a hot surfactant solution in order to generate an initial emulsion prior to sending the mixture into the high-pressure homogenizer. Different cycles of high-pressure homogenization at the same pressure were used to generate the NLC from the developed primary emulsion (Olbrich et al., 2002).

Statistical Experimentation for Optimization

Box-Behnken statistical design with three variables and three levels was used to optimise LMX-NLCs since it provides an adequate number of experiments. In the Presen work lipid ratio, surfactant concentration, and homogenization cycle are seleced as the three independent variables and (PS (nm), entrapment efficiency (Y2 percent), and drug release percentage (percent) as dependent variables. Using the programme, we were able to generate fifteen trial runs with three different formulas. All of the experiments were 7completed, and the data for the dependent variable was entered into the program for opimizaion. In order to find the best fit model for all three replies (Y1, Y2, and Y3), the data were all fitted into four distinct models: linear, second-order linear, cubic, and quadratic all models were subjected to ANOVA and statistical regression analysis. 3D and contour plots were used to determine the influence of variables on the individual response in 3D and contour plots. Table1 and 2representing Box-Behnken design with all factors in different level for Lomefloxacin loaded NLC and Formulation table of Lomefloxacin NLC using Box-Behnken Design.

Table 1
Box-Behnken design with all factors in different level for Lomefloxacin loaded NLC

Independent Factor	Low(-1)	Mid (0)	High(+1)
Lipid ratio X1	6	7	8
Surfactant (ml) X2	1	3	5
Homogenization cycles X3	2	5	8
Responses (Y)	Constraints		
Particle size(nm) Y1	Minimum		
Entrapment Efficiency (%) Y2	Maximum		
Drug release %Y3	Maximum		

Table 2
Formulation table of Lomefloxacin NLC using Box-Behnken Design

Formulation Code	Lipid X1	Surfactant X2	Homogenization cycle X3
LMX-NLC1	6	1	5
LMX-NLC2	8	5	5
LMX-NLC3	8	3	8
LMX-NLC4	7	1	2
LMX-NLC5	7	3	5
LMX-NLC6	7	5	2
LMX-NLC7	6	5	5
LMX-NLC8	7	5	8
LMX-NLC9	6	3	8
LMX-NLC10	8	3	2
LMX-NLC11	7	5	3
LMX-NLC12	7	1	5
LMX-NLC13	8	1	5
LMX-NLC14	7	1	8
LMX-NLC15	6	3	2

Characterization of Lomefloxacin loaded NLCs (LMX-NLC) (Wolfgang et al., 2001)

Measurement of particle size and zeta potential of Lomefloxin loaded NLC

The particle size and zeta potential of NLC were measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 50-200 for measurements, and the pH of diluted samples ranged from 6.9 to 7.2. Zeta potential measurements were carried out at 25°C, and the electric field strength was around 23.2 V/cm. Surface morphology was studied using transmission electron microscopy (TEM). After appropriate dilutions

Determination of entrapment efficiency

Entrapment efficiency (EE%) was determined by measurement of the concentration of free drug (unentrapped) in aqueous medium. The entrapment efficiency was calculated by the following equation.

%DEE= (experimental drug loading/Theoretical drug loading (TDL)) x 100

In vitro Drug Release Study

The dialysis bag diffusion approach, as described by Reddy and Murthy (2005) [41], was used to examine in vitro drug release from NLC. Phosphate buffer was used to conduct the drug release tests from NLC (pH 7.4). Using a pre-activated cellulose dialysis bag (Mol. cutoff 12,000; HIMEDIA, Mumbai, India), the nanoparticulate dispersion corresponding to 5 mg of Lomefloxacin was put and sealed at both ends. 500 mL of dissolving medium was kept at 37.2 °C in the dissolution vessels, which held the dialysis bag and were agitated at 100 rpm. An airtight seal on the receptor compartment ensured that dissolving liquid would not evaporate. Receptor samples were taken at pre-determined intervals of 15 minutes, 30 minutes, 1, 2, 4, 8, 12, 24, and 48 hours and the same amount of new dissolving medium was used to replace these samples. A Millipore membrane (0.22 m) was used to filter the methanol-dilution samples, and the samples were examined spectrophotometrically.

Optimization of formulation

The optimization of prepared formulations was done by considering percentage drug entrapment and studying interaction between factors as discussed underneath. Interaction between the factors. The statistical evaluation of all the obtained results data was carried out by analysis of variance (ANOVA) using Microsoft excel version 2007. The ANOVA results (P value) showed the effect of various independent variables on dependent parameter such as percentage drug entrapment. After regression analysis of all formulations, full polynomial model was obtained followed by omission of non-significant terms ($P > 0.05$) to obtain reduced model for the analysis. This equation represents effect of independent formulation variables on entrapment efficiency.

Construction of contour and response surface plots

Both plots were constructed from reduced polynomial equation using sigma plot version 11.0 by keeping one parameter stationary and varying others.

Evaluation of model/check point analysis

Checkpoint analysis was carried out to evaluate the dependability of the model through comparison between experimental and predicted values of the responses.

NLC Gel Evaluation of Lomefloxacin(L-NLCG)

NLC Gel of Lomefloxacin(L-NLCG) was Evaluated for pH A digital pH meter was used to test the pH of the new formulations (Elico Pvt. Ltd. India). In ophthalmic formulations, pH is a critical consideration as formulation should always match the pH of eye environment to prevent irritation and discomfort to the patient. Further the formulated NLC Gel of Lomefloxacin was evaluated for Gelation Temperature, Clarity (by physical method using white and black background) and Gelling Capacity. The gelling capacity of all created formulations was examined to discover gelling systems that may be used in situ. Visually inspecting and recording the gelation time (seconds) was used to estimate the gelling capability of the system, which was placed in a vial of 2 ml of newly generated pH 7.4 simulated tear fluid. The gelation and viscosity properties of the LSNG formulation were taken into consideration. There is no gelation at all. + Slow and dissipates quickly. ++ Gelation occurs immediately and lasts just a short time. +++ Gelation occurs immediately and lasts for a long time.

Ex-vivo Trans corneal Permeation Study

Goat eyes were transported in STF pH 7.4 from the local slaughterhouse. Cornea and 2–4 mm of the surrounding sclera was then gently removed and cleaned with saline solution. NLC and drug-loaded NLC gel permeation over extracted goat cornea was studied using the Franz diffusion cell (NLC-Gel). The epithelial side of the excised goat cornea faced the donor compartment when it was positioned appropriately between the donor and receptor chambers. 8 mL of STF pH 7.4 was added to the receptor chamber, which was agitated at 50 RPM and kept at 37 0.5 °C. The epithelial side of the cornea was coated with 2 cc of NLC gel. STF pH 7.4 was used to replenish aliquots of samples (1 mL) that had been removed at a predefined interval. The UV technique was used to evaluate the materials. It was then determined if the improved formulation was superior to a standard medication solution. (Mangesh et al., 2017).

The HETCAM Corneal Toxicity Assay

An alternative to the Draize in-vivo rabbit eye test is HET CAM (Hen's egg chorioallantois membrane) investigation. The HET-CAM test was used to assess the LSNG8 formulation's potential for causing ocular discomfort. Fertilized eggs were used to form the chorioallantois membrane (CAM). For three days, the eggs were kept at 37 °C± 0.5 °C and 55 % RH. Those eggs that were determined to be nonviable were thrown away on the third day of the experiment. A manual rotation of the egg every 12 hours ensured that the viable eggs were properly incubated for 10 days. The shell was cut off from the air cell side on the 10th day, and it was then removed from the device. After that, 300 l of the improved formulation was injected into the exposed CAM using a micropipette with a plastic tip. CAM was treated with 300 l each of 1 N NaOH (positive control) and 0.9 percent w/v NaCl (negative control) in three separate trials (negative control). Anxiety-inducing factors such bleeding, lysis, and coagulation were looked for in intervals of up to 300 seconds in the CAM.

In-vivo Pharmacokinetic Study for Drug Loaded Lipidic Nano Gel Formulation(Ravi et al., 2014

The experiment was carried out on in rabbits. They were kept in polypropylene cages, under standard situation (12 h light/dark cycle, 24 °C, 35–60% humidity) and drinking water ad libitum. Chromatographic separation of collected samples was carried out by previously validated chromatographic method using HPLC.

Preparation of standard solution

A series of standard solutions of Lomefloxacin ranging from 20 to 1000 ng/mL were prepared in methanol. Samples were prepared by addition of 50 µL of standard solution and 200 µL of acetonitrile to the eppendorf tube containing 100 µL of blank plasma. The mixture was then processed according to the sample treatment procedure described below. Final Lomefloxacin concentrations in plasma were 10–500 ng/mL. Sample treatment procedure the eppendorf tube consisting of an aforementioned mixture was meticulously vortex-mixed (Macro Scientific Work Pvt Ltd, Delhi, India) for 30 s followed by centrifugation at 15,000 rpm for 10 min at −6 °C to separate denatured protein. After centrifugation, 20 µL of the filtered supernatant (0.45 µm membrane filter) was injected and were analyzed by standard HPLC method. Experimental design before dosing, the animals were fasted for the period of 12 h prior and 4 h post with free access to water. Animals were divided into two groups consisting of six animals in each. Control group received a suspension of Lomefloxacin (drug suspended in 0.5% w/v sodium CMC and the test group received the optimized formulation (NLC-8) at a dose of 15 mg/kg body weight.

Pharmacokinetic data analysis

PK solver add-in program for Microsoft excel was used for the estimation of Pharmacokinetic parameters. The maximum plasma concentration (Cmax) and the time to reach maximum plasma concentration (Tmax) were obtained directly from the graph between plasma concentration and time. Area under curve [AUC]0–24 was considered up to last point of measurement. Relative bioavailability (F) was calculated by dividing [AUC]0–24 of formulation with plain drug suspension. Each experiment was carried out in triplicate.

Stability Study

A month's worth of stability testing was done on the improved formulation. An amber glass vial was used to store the drug-loaded NLC gels at 4 °C 2 °C in an oxygen-free stability chamber. After a month, the samples were thrown away. There were no physical changes seen in the NLC Gel compared to the NLC, while there were physical alterations found in the NLC. It was done in threes for all the experiments.

Results and Discussion

Selection of Lipids

The selection of lipids, both solid and liquid, was based on their ability to bind the most LMX. Solubility data in liquid lipid and lipid are represented in table 3& 4 and in fig 1&2.

Table 3
Selection of lipid liquid based on drug solubility

Liquid Lipids	Drug solubility in lipid(mg/ml)
Olive oil	25.16±1.23
Castor oil	20.13±1.11
Peanut oil	15±2.13
Labrasol	6.9±14
Soyabean oil	5.1±1.08

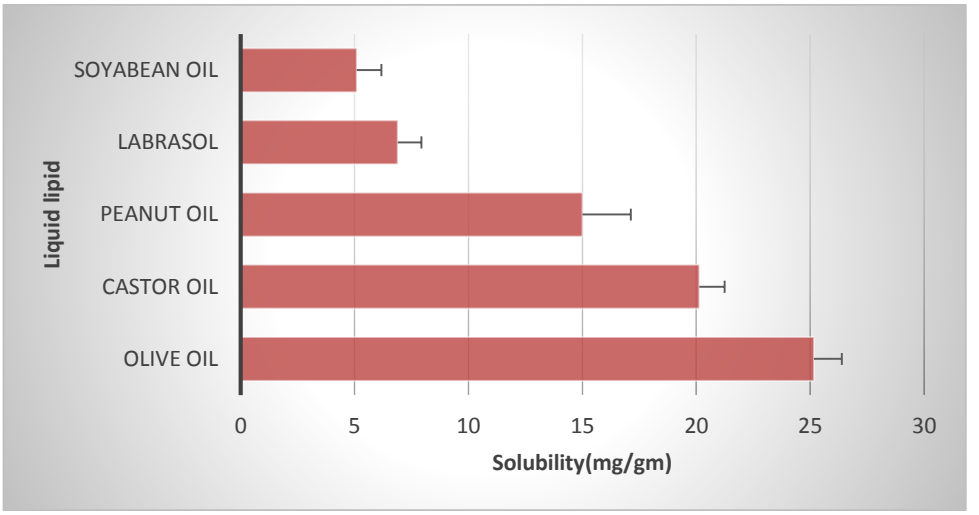


Fig. 1. Selection of lipid liquid based on drug solubility

Table 4
Lomefloxacin's Solubility in Different Lipids

Lipids	Melting point range ^{0}C	Drug solubility in lipid(mg/ml)
Stearic acid	69	115 ±8.01
Glyceryl Monostearate	57	50±4.11
Myristic acid	54.4	48±3.11
Palmitic acid	62.9	45±3.99

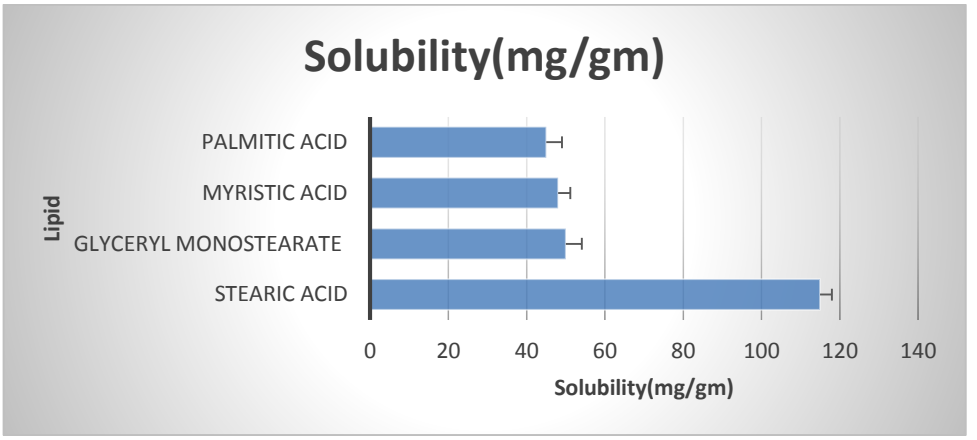


Fig. 2. Screening of lipid based on their ability to solubilize Lomefloxacin

Aspects to Consider in Surfactant Selection

The right surfactant was selectedbased on drug solubility in various Surfactants following recommended method was done. Solubility of surfactants presented in table 5 and in fig 3.

Table 5
Solubility of surfactants

Surfactant	Solubility(mg/g)
Tween 80	30±1.31
Tween 20	26±2.01
PEG 200	18±1.44
Limonene	21±1.03
Polaxamer	19±2.30

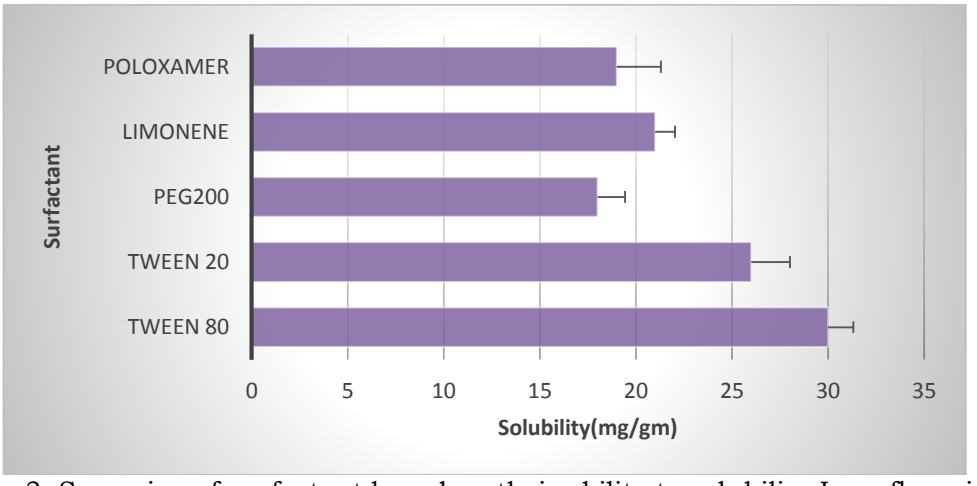


Fig.3. Screening of surfactant based on their ability to solubilize Lomefloxacin

Development of Lomefloxacin Loaded NLCs (LMX-NLCs)

To create the LMX NLCs, we employed the most typical high-pressure homogenization approach (hot). This mixture was first dissolved in water and added to a hot surfactant solution in order to generate an initial emulsion prior to sending the mixture into the high-pressure homogenizer. Different cycles of high-pressure homogenization at the same pressure were used to generate the NLCs from the developed primary emulsion.

Statistical Experimentation for Optimization

Box-Behnken statistical design with three variables and three levels was used to optimise LMX-NLCs since it provides an adequate number of experiments. We used lipid ratio, surfactant concentration, and homogenization cycle for the three independent variables (PS (nm), entrapment efficiency (Y2 percent), and drug release percentage (percent)). Using the programme, we were able to generate fifteen trial runs with three different formulas. All of the experiments were completed, and the data for the dependent variable was entered into the program.ly, the software is complete. In order to find the best fit model for all three replies (Y1, Y2, and Y3), the data were all fitted into four distinct models: linear, second-order linear, cubic, and quadratic All models were subjected to ANOVA and statistical regression analysis. 3D and contour plots were used to determine the influence of variables on the individual response in 3D and contour plots.

Characterization of Lomefloxacin loaded NLCs (LMX-NLC)

Lomefloxacin loaded NLCs (LMX-NLC) were Characterized for Particle size(nm) , EE% and drug release % as per recommended procedure and data obtained from studies were presented in table 6 and in fig 4 & 5.

Table 6
Characterization of Lomefloxacin loaded NLCs (LMX-NLC)

Formulation Code	Particle size(nm)	EE%	Drug Release%
LMX-NLC1	250.1	71	88.12
LMX-NLC2	231.4	73	84.16
LMX-NLC3	244.3	74	89.47
LMX-NLC4	182.4	86	92.13
LMX-NLC5	288.1	71	86.47
LMX-NLC6	261.2	72	80.37
LMX-NLC7	273.4	80	76.35
LMX-NLC8	178.5	88	94.14
LMX-NLC9	213.4	72	79.24
LMX-NLC10	241.6	70	81.57
LMX-NLC11	299.4	73	84.65
LMX-NLC12	225.1	70	83.24
LMX-NLC13	198.1	89	92.14
LMX-NLC14	213.7	62	81.45

LMX-NLC15	200.8	65	78.12

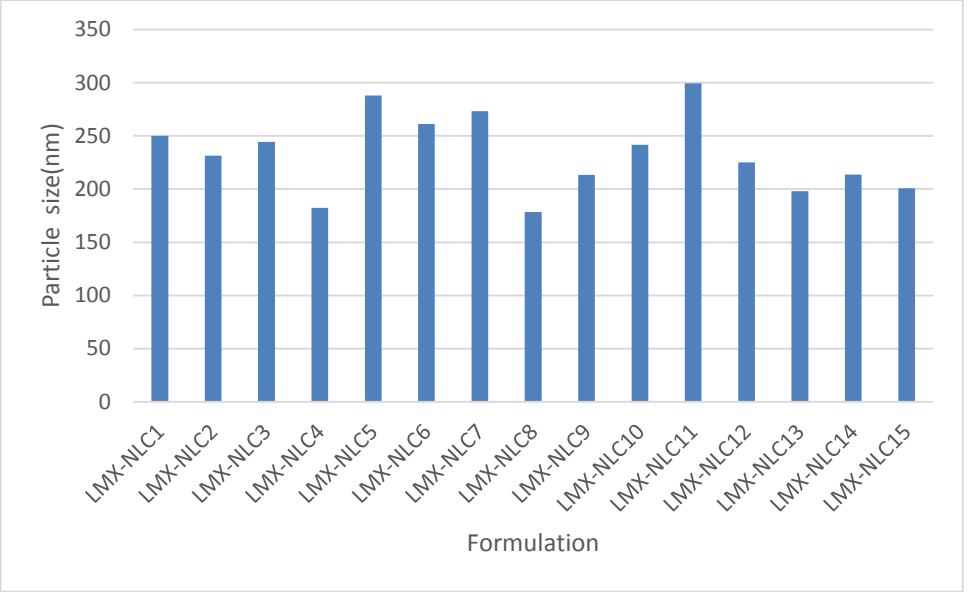


Fig 4. Particle size of LMX-NLC

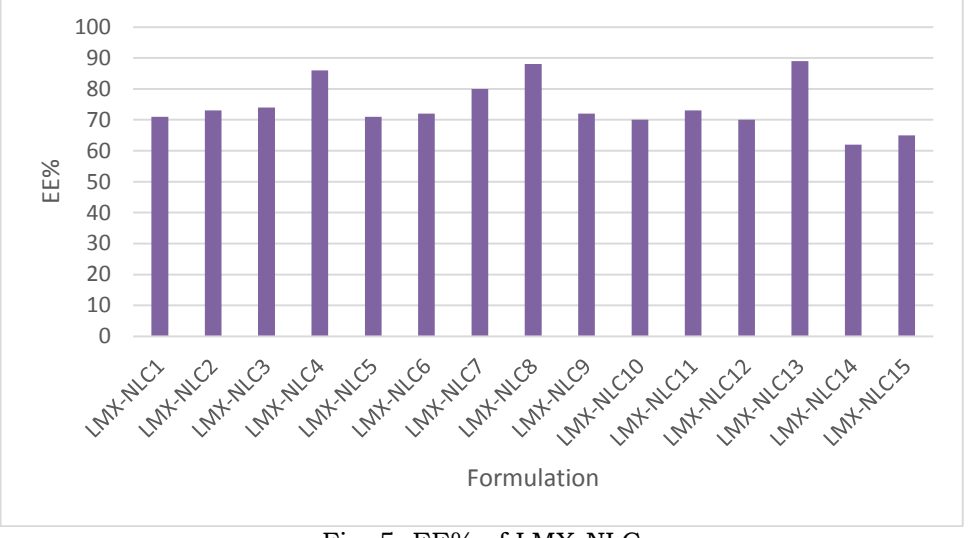


Fig. 5. EE% of LMX-NLC

Table 7
OPTIMIZATION of LMX-NLC Formulation for particle size

ANOVE of Quadratic Model for Response 1 Particle size						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	14459.68	9	1606.63	62.78	0.0221	Significant
X1-Lipid ratio	1097.46	1	1097.46	1.22	0.0203	

X2-Surfactant	871.53	1	871.53	0.9660	0.0108	
X3-Homogenization cycle	3240.13	1	3240.13	3.59	0.0166	
X1X2	267.32	1	267.32	0.2963	0.0096	
X1X3	106.09	1	106.09	0.1176	0.0456	
X2X3	2016.01	1	2016.01	2.23	0.0652	
$X1^2$	6294.12	1	6294.12	6.98	0.0459	
$X2^2$	466.27	1	466.27	0.5168	0.0544	
$X3^2$	115.27	1	115.27	0.1278	0.0353	
Residual	4511.02	5	902.20			
Lack of Fit	4351.84	3	1450.61	18.23	0.1525	not significant
Pure Error	159.18	2	79.59			
Cor Total	18970.69	14				

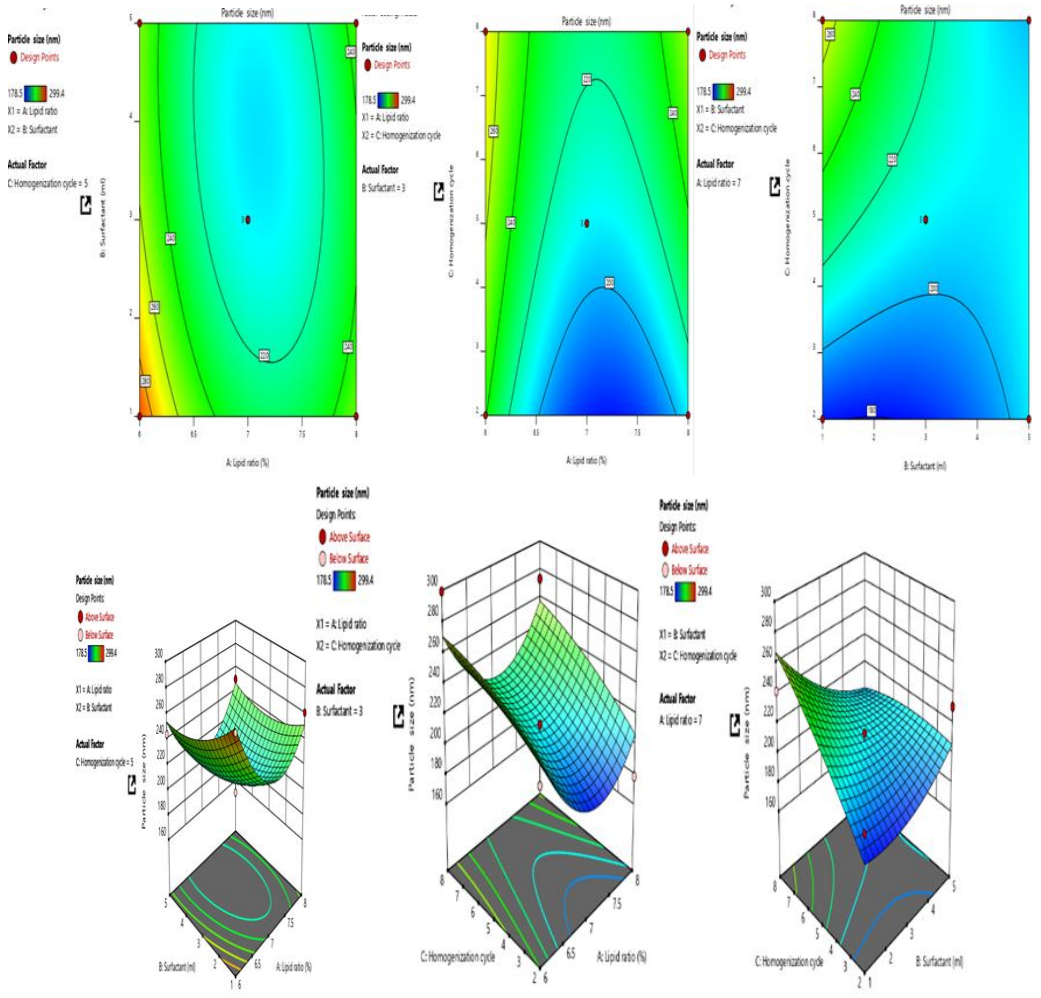


Fig. 6. Counter Plot & 3D Surface Plot for Particle size

Effect on Particle Size(Y1)

The produced nano lipidic carrier's particle size varied noticeably and significantly depending on the independent factors. When a factor in the regression equation has a positive value before it, it means that the response will grow as the factor rises, and vice versa (Rawat and Saraf, 2009). For the given equation, the (r2) correlation coefficient was determined to be 0.9984, the F value for Model was 62.7, p value is 0.0221 (significant), and the Lack of fit was 0.1525 (not significant), which suggested a satisfactory fit. The positive number in the quadratic equation shows that X1 had a large and positive impact on Y1. The particle size of all 15 batches of Lomefloxacin loaded NLC (LMX-NLC) were in the range 178.5 nm to299.4 nm for various factor-level combinations (Table 7). The effect of independentvariablesonparticle sizecanbeexplained bythefollowing quadratic equation:

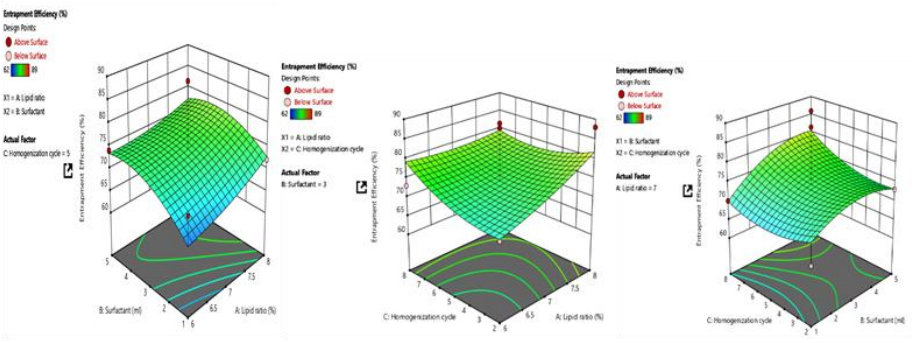
$Y1=287.77+2.79X1-12.52X2-4.51X+2.50X1X2-2.47X1X3-28.50X2X3-16.72X1^2-32.80X2^2-46.02X3^2$

With the increase in concentration of Lipid, the mean particle size significantly increased while negative indication before X2 exhibits the particle size decreased when the surfactant concentration was increased. This might be because larger surfactant concentrations inhibit coalescence by stabilizing the internal structure of the dispersion. Prior to X3, a negative number indicated that homogenization speed had a detrimental influence on particle size. A negative influence on particle size was found in the X1X2 interaction term, whereas positive effects on particle size were found in the terms X2X3 and X1X3.fig 6 shows Counter Plot & 3D Surface Plot For Particle size.

Table 8
Optimization of LMX-NLC Formulation for EE%

ANOVE of Quadratic Model for Response 2 EE%						
Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	293.18	9	32.58	43.124	0.0150	Significant
X1-Lipid ratio	66.13	1	66.13	0.5600	0.0280	
X2-Surfactant	84.50	1	84.50	0.7156	0.0362	
X3-Homogenization cycle	21.13	1	21.13	0.1789	0.0499	
X1X2	4.00	1	4.00	0.0339	0.0212	
X1X3	30.25	1	30.25	0.2562	0.0343	
X2X3	16.00	1	16.00	0.1355	0.0279	
$X1^2$	3.39	1	3.39	0.0287	0.0721	
$X2^2$	40.01	1	40.01	0.3388	0.0458	
$X3^2$	22.31	1	22.31	0.1890	0.0819	
Residual	590.42	5	118.08			
Lack of Fit	217.75	3	72.58	0.3895	0.7760	not significant

Pure Error	372.67	2	186.33			
Cor Total	883.60	14				



Effect on entrapment efficiency (Y2)

Counter Plot & 3D Surface Plot For Entrapment Efficiency%

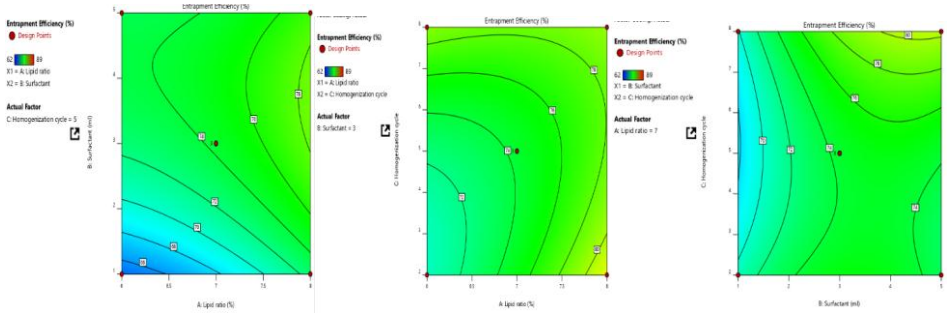


Fig 7. Counter Plot and 3D Surface plot for Entrapment

Efficiency

The effect on the efficiency of entrapment (Y2)

It was determined that the aforementioned equation's (r2) correlation coefficient was a good match at 0.9979, and the F value for model was 43.124, p value is 0.0150 (significant), and the Lack of Fit was 0.7760 (not significant). The positive number in the quadratic equation shows that X1 had a large and positive impact on Y1. The entrapment efficiency% of all 15 batches of Lomefloxacin loaded NLC (LMX-NLC) were in the range 62% to89% for various factor-level combinations (Table 8). To understand how independent factors affect the entrapment efficiency percentage, we may use the quadratic equation below.

$$Y1=71+2.25X1+.6250X2+0.3750X3-6.25X1X2+0.7500X1X3+1.15X2X3+0.2500X1^2+0.7X2^2-0.13X3^2$$

The entrapment efficiency increased considerably when the lipid content, surfactant concentration, and homogenization speed increased. The interaction term X1 X2 has a negative effect on EE percent, whereas the interaction terms X2

X3 and X1 X3 have a favorable effect. 3D Surface Counter Plot for Entrapment Efficiency are presented in the fig 7.

Effect on % Drug Release at 12hr (Y3)

The value of (r^2) correlation coefficient for the above equation was found to be 0.9979, The F value for Model was 22.36, p value is 0.023(significant) and the Lack of fit was 0.5989(not significant) which indicated good fit. The positive number in the quadratic equation shows that X1 had a large and positive impact on Y1. The entrapment efficiency% of all 15 batches of Lomefloxacin loaded NLC (LMX-NLC) were in the range 76.35% to94.14 % for various factor-level combinations (Table 9). The following quadratic equation explains how independent factors affect entrapment efficiency percent.

$Y1=86.06+3.19X1-2.35X2+1.51X3+0.9475X1X2+1.70X1X3+6.11X2X3-2.90X1^2+2.03X2^2-1.07X3$

With the increase in concentration of lipid, the drug release% significantly increased, the surfactant concentration has negative effect on Drug release% and homogenization speed have a positive effect on Drug release% The interaction term X1X2shows positive effect on Drug release% while X2X3 and X1X3 revealed positive effect on EE%. Counter plots for % drug release at 12hr given fig 9

Table 9
ANOVE of Quadratic Model for Response 3 DR% at 12hr

ANOVE of Quadratic Model for Response 3 DR% at 12hr						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	212.84	9	23.65	22.369	0.0238	Significant
X1-Lipid ratio	2.94	1	2.94	0.0704	0.0113	
X2-Surfactant	93.50	1	93.50	2.24	0.0347	
X3-Homogenization cycle	3.08	1	3.08	0.0737	0.0169	
X1X2	5.64	1	5.64	0.1351	0.0182	
X1X3	92.16	1	92.16	2.21	0.0374	
X2X3	5.11	1	5.11	0.1224	0.0407	
$X1^2$	5.45	1	5.45	0.1307	0.0325	
$X2^2$	1.40	1	1.40	0.0335	0.0620	
$X3^2$	2.97	1	2.97	0.0712	0.0603	
Residual	208.70	5	41.74			
Lack of Fit	113.51	3	37.84	0.7950	0.5989	not significant
Pure Error	95.19	2	47.60			
Cor Total	421.54	14				

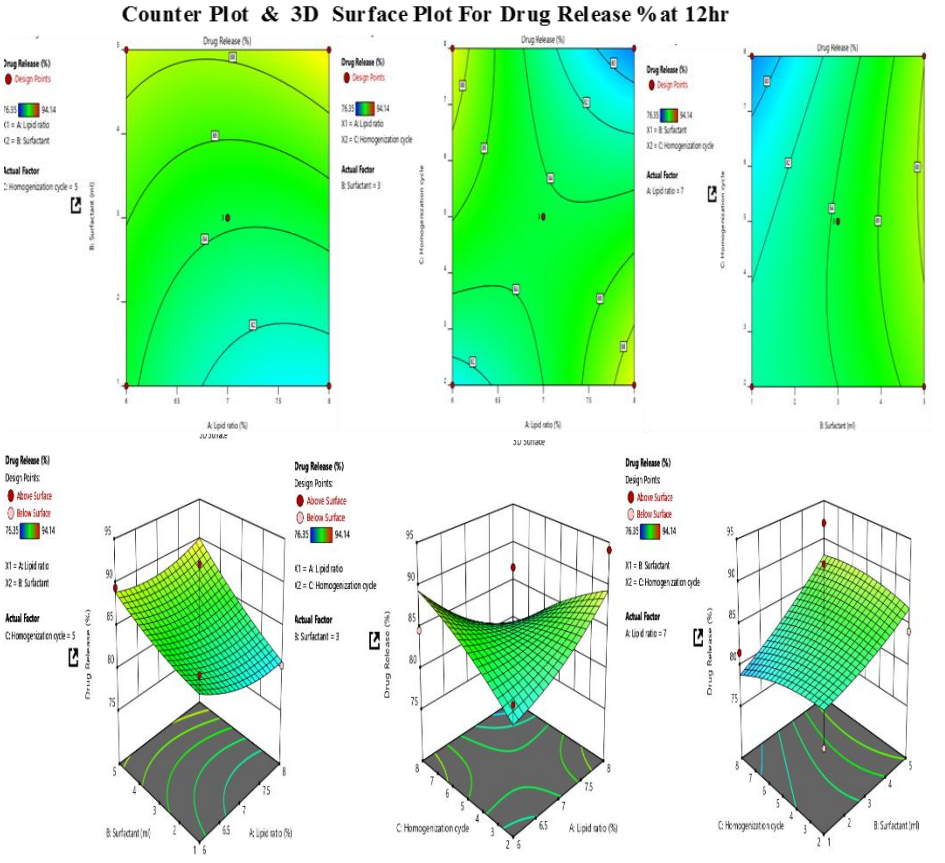


Fig. 9. Counter Plot & 3D Surface Plot for Drug Release % at 12 hr

Desirability

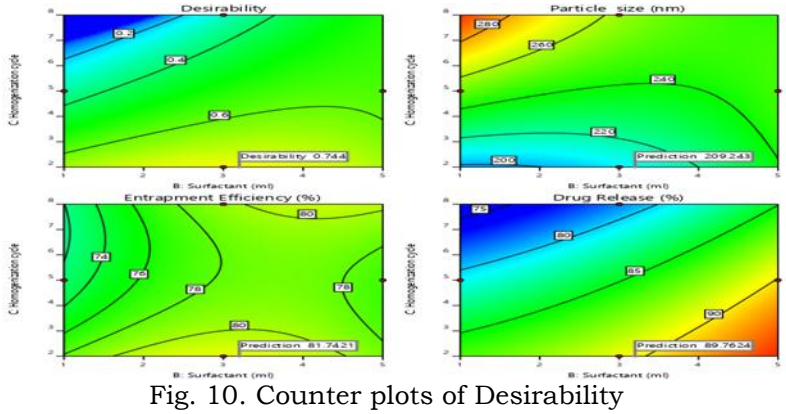


Fig. 10. Counter plots of Desirability

Point of Prediction data is presented in the fig10 and table 10

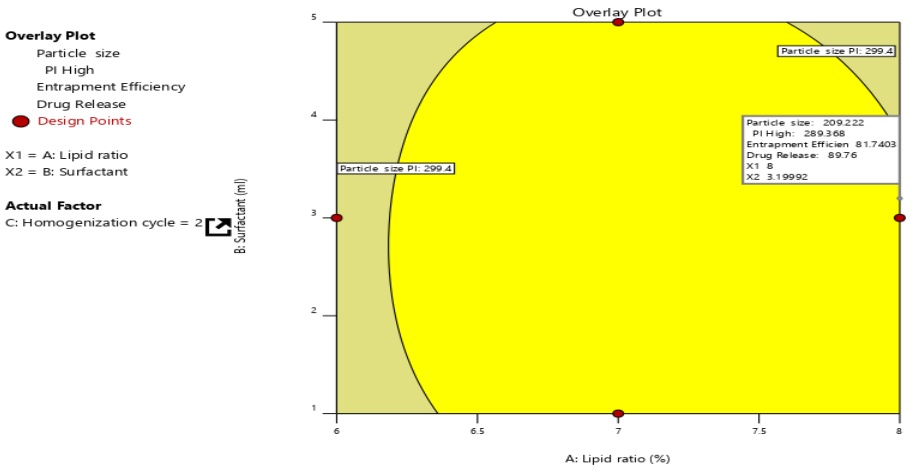


Table 10
Confirmation Location for Opt-LMX-NLC

Factor	Name	Level
A	Lipid ratio	8
B	Surfactant	3.19
C	Homogenization cycle	2

Design-Expert® software V.12 was used to examine the desirability function in order to come up with the best formulation. Particle size minimization and maximal entrapment efficiency were key considerations in developing the ideal formulation. A second batch of formulation factor-containing LMX-NLCs was generated to verify whether the optimization approach was effective. The formulation factor including 8 mg of lipid, 3.19 ml of surfactant, and a homogenization cycles of 2 cp resulted in the optimal formulation. The improved formulation's particle size, entrapment efficiency, and drug release percentage were all within projected limits, at 209.243nm, 81.74 percent, and 89.76 percent, respectively. (Table 11).

Table 11
Responses of formulation factors

Responses	Name	Predicted Mean
R1	Particle size	209.243
R2	Entrapment Efficiency	81.7421
R3	Drug Release	89.7624

Characterization of Opt-LMX-NLC

Morphology, Zeta Potential and Particle Size Distribution

Data is presented in fig 12, 13 &14

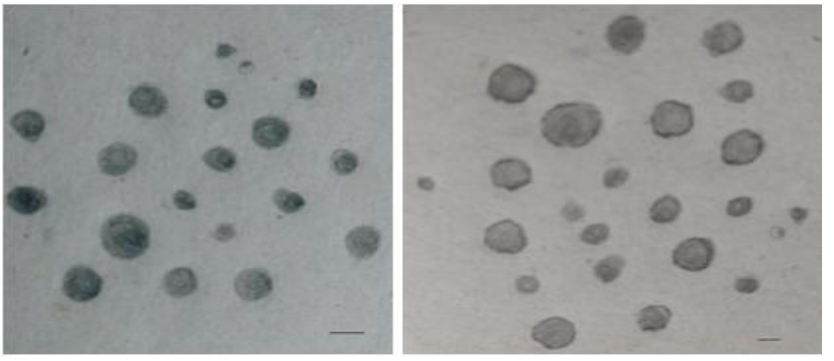


Fig. 12. TEM images of Opt-LMX-NLC

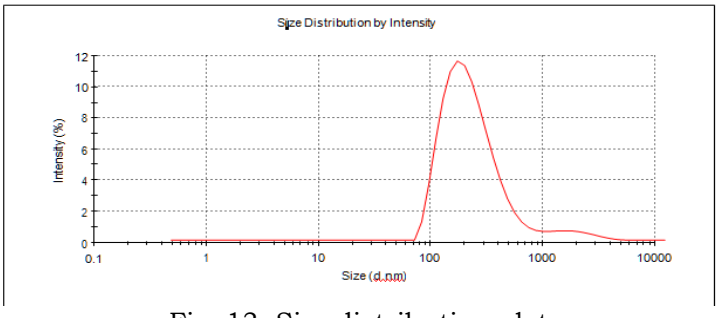


Fig. 13. Size distribution plot

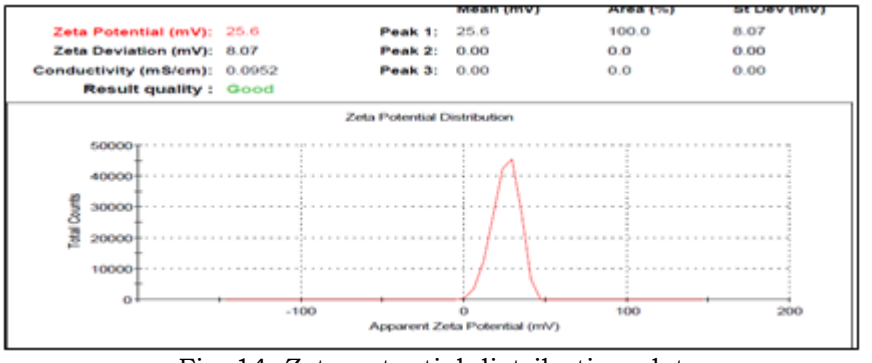


Fig. 14. Zeta potential distribution plot

NLC Gel Evaluation of Lomefloxacin(L-NLCG)

The formulated LMX-NLC gel are evaluated for Gelation Temperature, clarity, pH and Gelling capacity of L-NLC-Gel and outcome result data is presented in table 12.

Table 12
Gelation Temperature, clarity, pHand Gelling capacity ofL-NLC-Gel

S.No	Formulation Code	Gelation Temperature	Clarity	pH	Gelling Capacity
1	L-NLCG1	32.22±0.20	Clear	6.24	++
2	L-NLCG2	31.33±0.21	Clear	6.19	+++
3	L-NLCG3	32.23±0.10	Clear	6.12	++
4	L-NLCG4	37.44±0.21	Clear	6.18	+++
5	L-NLCG5	36.23±0.20	Clear	6.12	+++
6	L-NLCG6	33.44±0.10	Clear	6.03	+++
7	L-NLCG7	30.11±0.30	Clear	6.21	+++
8	L-NLCG8	37.23±0.10	Clear	6.12	+++
9	L-NLCG9	31.26±0.11	Clear	6.31	+++
(+)gels after few minutes dissolves rapidly,(++) gelation immediate remains for fewhour (+++)gelation immediate remains forextendedperiod(n=3,Mean±SD)					

Ex-vivo Permeation Study

Lomefloxacin loaded NLC from chitosan gel and commercial eye drop solution of Lomefloxacin around the isolated porcine cornea is shown in this article. Epithetic Lomefloxacin-loaded chitosan gels had the same interpenetration characteristics as a commercial product. Furthermore, there is not infrequent discrepancy between the values of fluxes (Jss) and apparent permeability (K). Until recently, the chitosan gels and the chemical preservation agent were the most effective means of conveying Lomefloxacin 1% w/v in a dose form that resembled commercial dosage forms. Chitosan's growth in popularity has led to an increase in flux and permeability interdependently, which in turn boosts the transport rate, resulting in an improved DTMAP impact. Optimized formulation drug permeation studies Opt-LMX-NLC Gel and commercial formulations have recognized the benefits enlisted in the table 13.

Table 13
Porcine corneal Lomefloxacin permeability and steady-state flux

Formulation	FluxJss(μg/h/cm^2)	Apparentpermeability coefficientKp(10^{3}cm/h)
Opt-LMX-NLC Gel	71.32	6.86
Marketed Product	72.23	7.15

The HETCAM Corneal Toxicity Assay

It was considered to be one of the most sensitive and powerful assays for predicting ocular irritation qualities, especially for small-scale disrupting compounds. When it comes to startling formulations, the irritancy records or keeping an eye on (0.9 percent NaCl) and the Opt-LMX-NLC Gel are recommended as a nonirritating type of startling formulations according to the spectacular scoring system. But in contrast to this, it was recorded nine times that congelation together with spill blood occurred in just 0.5 minutes while lysis was remembered in the second minute for one of the irritating substances (0.1N NaOH). Were presented in table 14.

Table 14
Irritation testing with a scoring system (HET-CAM assay)

TestFormulation	Effect	Score			Net Score	Inference
		0.5min	2min	5min		
Control(0.9%Nacl)	Lysis	--	--	--	0	Non –Irritant
	Haemorrhage	--	--	--		
	Coagulation	--	--	--		
Irritant(0.1NNaOH)	Lysis		2		0	Severe irritant
	Haemorrhage	6	--	--		
	Coagulation	8	--	--		
Opt-LMX-NLC-Gel	Lysis	--	--	--	0	Non –Irritant
	Haemorrhage	--	--	--		
	Coagulation	--	--	--		

In-vivo Pharmacokinetic Study for Drug Loaded Lipidic Nano Gel Formulation

The equivalent dose of Lomefloxacin 1% w/v was used in pharmacokinetic studies. The in-vivo pharmacokinetic were performed in rabbits by optimized formulations (Opt-LMX-NLC-Gel) and marketed formulation. In Physico chemical evaluation studies for all formulations shows the satisfactory results, Opt-LMX-NLC-Gel, Gel shows the excellent formulation. Aqueous concentrations of Lomefloxacin (1%) were used to calculate the in-vivo parameters at various time periods. The maximum values of Cmax, Tmax, and AUC 0- are calculated.

Plasma Drug Concentration Graph for Various Optimized Nano Lipidic Gel (Opt-LSLN gel, Opt-LMX-NLC gel) and Marketed Formulation

The C_{max} for (Opt-LMX-NLC gel) and marketed formulation was 612.3ng/ml and 721.08ng/ml, T_{max} for (Opt-LMX-NLC gel) and marketed formulation was 2.1 hr and 0.94hr, AUCo-∞ for (Opt-LMX-NLC gel) and marketed formulation are 3211.31 and 2537.1, AUMCt-∞ for (Opt-LMX-NLC gel) and marketed formulation are 3354.4 and 2641.3. MRT for (Opt-LMX-NLC-gel) and marketed formulation are 3.12 and 1.99 hr. (Table. 15 and fig 15)

Table 15
Various parameters for marketed formulation versus optimized formulation (Opt-LSLN and Opt-LMX-NLC gel)

Parameters	Marketedformulation	TestFormulation (Opt-LMX-NLC gel)
Cmax (ng/mL)	721.08	612.3
Tmax (hr)	0.94	2.1
AUCo-∞(ng.h/mL)	2537.1	3211.31
AUMCt-∞(ng.^{2}h/mL)	2641.3	3354.4
$t_{1/2}$(hr)	2.37	3.54
MRT (hr)	1.99	3.12

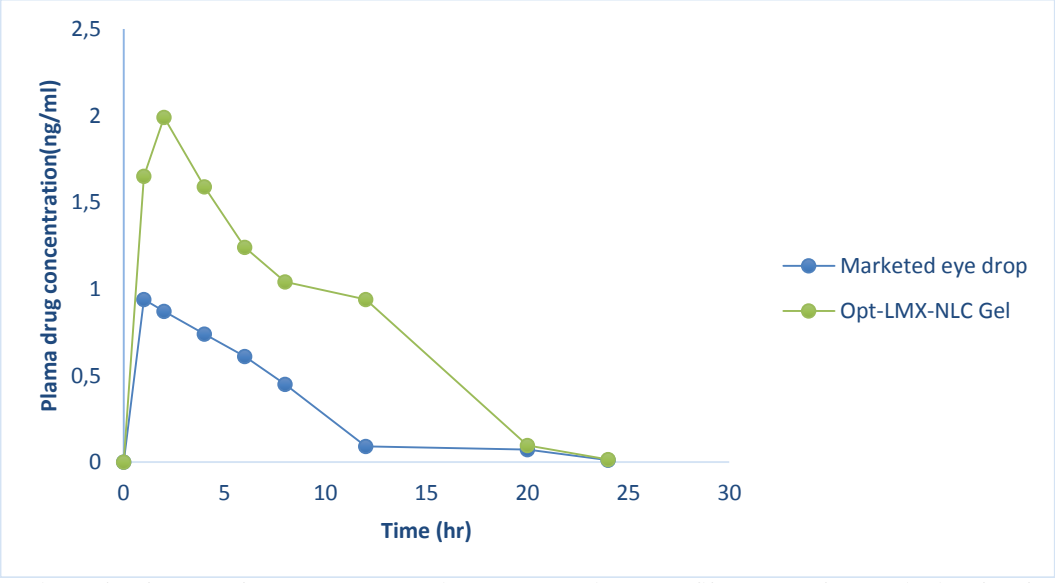


Fig. 15. Plasma drug concentration versus time profile curve for optimized gel formulation (opt-LMX-NLC gel) and marketed formulation

Conclusion

Developed drug-loaded nano lipidic gels for ocular drug delivery system" was the goal of the current piece of art. Topical gel applications are one of the most generally acknowledged methods for the administration of ocular solution over 90% of the time. It is anticipated that the improved bioavailability and prolonged action of topically applied Lipidic Nano gels (Drug-loaded NLC) will lead to accurate case of compliance and longer action times due to the increased retention in the eye. Optimization studies of LMX-NLC gel shown that with the increase in concentration of Lipid, the mean particle size significantly increased while negative indication before X2 exhibits. The particle size decreased when the surfactant concentration was increased. This might be because larger surfactant concentrations inhibit coalescence by stabilizing the internal structure of the dispersion. Prior to X3, a negative number indicated that homogenization speed had a detrimental influence on particle size. A negative influence on particle size

was found in the X1X2 interaction term, whereas positive effects on particle size were found in the terms X2X3 and X1X3.

The entrapment efficiency increased considerably when the lipid content, surfactant concentration, and homogenization speed increased. The interaction term X1 X2 has a negative effect on EE percent, whereas the interaction terms X2 X3 and X1 X3 have a favorable effect. Effect on% Drug Release at 12hr (Y3) shown, with the increase in concentration of lipid, the drug release% significantly increased, the surfactant concentration has negative effect on Drug release% and homogenization speed have a positive effect on Drug release%. The interaction term X1X2shows positive effect on Drug release% while X2X3 and X1X3 revealed positive effect on EE%. Further optimized formulation Opt-LMX-NLC Gel was exposed for six weeks stability studies at 40°C and 75% RH were performed. Outcome of present work is the medication, lipids and surfactant stored in an accelerated stability chamber has shown no transformation.FTIR and DSC measurements were used to verify that drugs, lipids, and polymers had no major physical or chemical incompatibility.

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