IJPSR (2023), Volume 14, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 April 2023; received in revised form, 10 June 2023; accepted 04 July 2023; published 01 December 2023

DEVELOPMENT OF NANOGEL OF OFLOXACIN FOR OCULAR DRUG DELIVERY

Gopa Roy Biswas *, Pritam Dutta, Grihadeep Paul, Soumik Patra and Srijita Paul

Department of Pharmaceutics, Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F Nilgunj Road, Panihati, Sodepur - 700114, Kolkata, India.

Keywords:

Ocular obstacles, Nanogels, Nanoparticles, Gelling agent

Correspondence to Author:
Gopa Roy Biswas

Associate Professor, Department of Pharmaceutics, Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F Nilgunj Road, Panihati, Sodepur - 700114, Kolkata, India.

E-mail: goparoy2020@gmail.com

ABSTRACT: Multiple anatomical and physiological obstacles have always made ocular drug administration difficult. Different novel drug delivery systems, including nanogels, nanoparticles, liposomes, dendrimers, implants, contact lenses, nanosuspensions, microneedles, and in situ thermosensitive gels, have been developed to get around the barriers to improve ocular Bioavailability. For the encapsulation of visitor molecules, nanogel has become a flexible drug delivery technology in this aspect. Numerous techniques for the creation of nanogels have been developed over time, such as Concomitant polymerization and crosslinking, Separate polymerization and crosslinking, Micro templating/crosslinking, *etc*. In this work, Ofloxacin-loaded Nanostructured Lipid Carrier has been prepared with the help of Design expert software (Version 7.1.5) taking amount of liquid lipid, and homogenization speed as independent variables and particle size, spreadability coefficient, % entrapment efficiency as responses. Dynamic Light Scattering (DLS) was used for studying the particle size. The results confirmed the size of the NLC within the nano range (78.79- 648.4 nm). From the experiment, optimized product was found to be 78.79 nm with 66.2% drug entrapment efficiency and 0.359 gm. cm/sec spreadability coefficient. From the optimized formulation drug diffused gradually in increasing order and maintained a constant rate after a few hours with a diffusion rate of 89.79% in 7 hours. In *ex-vivo* permeation study through goat skin, it has been found that the permeation of ofloxacin through goat ocular skin from the optimized product is around 71.33% in 7 hours.

INTRODUCTION: Nanogels are crosslinked hydrogel materials that are water soluble and contain both hydrogel and nanoparticle qualities, as well as the potential to release drugs under regulated conditions. Thus, these carriers offer a polymeric nanotechnological approach with exceptional qualities like high drug loading capacity, high stability, responsiveness to a wide range of environmental stimuli, whereby they may contract or expand in response to a change in pH or temperature, resulting in the release of the drug under particular circumstances [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(12).5619-32

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(12).5619-32

The rapid advancement of nanotechnology has necessitated the development of nanogel systems that have demonstrated their ability to deliver drugs in a controlled, sustained, and targetable manner. With the emergence of polymer sciences, it is now essential to build intelligent nanosystems that can be useful for both therapy and the advancement of clinical trials.

Lipid-nanoparticles, specifically solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), have received a lot of attention recently as efficient, biodegradable, biocompatible, and non-toxic carriers with a variety of suitable properties for dermal application of cosmetics and pharmaceuticals [2]. The "solid lipid" core of SLN emulsion spheres, which have an average diameter of 10–1000 nm, is stabilized by a number of surfactants [3].

In order to prevent the solid lipid matrix from forming into a perfect matrix of solid lipid crystals during storage and to improve the absorption of active ingredients, the liquid lipid component in NLC works to overcome the shortcomings of SLN [4]. As a result of the mixture of solid and liquid lipids that make up NLC, it has an imperfect crystalline structure that leaves greater room between the lipid chains and the matrix [5].

The eye can be the site of many diseases (some of which are sight-threatening), and treating these various diseases has always been a challenge for physicians and the pharmaceutical industry, especially when considering the route of drug administration. The delivery of medications to the eye, a difficult-to-treat region, has made significant strides recently. With numerous defences and processes preventing the ingestion of external substances, the eye is a relatively solitary organ within the body [6]. In treating ocular disorders, polysaccharides such cellulose, hyaluronic acid, alginic acid, chitosan, and polysaccharide derivatives have been utilized successfully to enhance medication delivery [7].

Topical eye drops are widely used and are usually the preferred type of medication administered regarding the anterior segment of the eye [8]. However, these drugs typically do not reach the retina, vitreous, or choroid, for which other preferred routes of drug administration are being developed as novel technologies are developed. It has some challenges like Higher tear dilution, and turnover rate, Bioavailability <5% [9]. These delivery systems can improve the solubility and stability, hide the flavour and odour, and raise the Bioavailability of nutrients and medications that are not well absorbed [10].

Nanostructured lipid carriers (NLC) is a promising DDS able to prolong the delivery, improve the stability, and decrease the systemic toxicity of liposoluble drugs [11]. NLC Surface can be modified with a suitable polymer to make it bio-adhesive and enhance its retention at the disease site. For Ocular Delivery, NLC was prepared using a blend of solid and liquid lipids, biocompatible and stable with the size of 50 to 1000 nm. NLC incorporated into Hydrogel is equal to Nanogel. Ofloxacin is a broad-spectrum antibiotic, useful for the treatment of bacterial infections. Its molecular weight is 361.3675 g/mol. Ofloxacin is bactericidal and prevents bacterial DNA replication by attaching to an enzyme called DNA gyrase [12]. This enzyme is necessary for unwinding one DNA double helix into two, which is how ofloxacin blocks bacterial DNA replication.

Interestingly, the drug's affinity for bacterial DNA gyrase is 100 times greater than mammalian. A broad-spectrum antibiotic with activity against Gram-positive and Gram-negative bacteria is called ofloxacin. Ofloxacin affects the enzymes DNA gyrase and topoisomerase IV, which work similarly to human topoisomerase in preventing excessive DNA supercoiling during transcription or replication. The medication prevents normal cell division by preventing them from performing their role. Ofloxacin has around 98% bioavailability in tablet form. Ofloxacin is primarily removed by the kidneys, where between 65% and 80% of an oral dose is excreted unaltered through urine after 48 hours of administration. Ofloxacin is excreted in the faeces in amounts ranging from 4–8% of dosage, while biliary excretion of the medication is modest [13].

MATERIALS AND METHODS:

Materials: Ofloxacin was received as a gift sample from Unichem Laboratories Ltd. (C-31 &32, Industrial Area, Meerut Road, Ghaziabad: 201003, U.P., India). Stearic acid & Carbopol 934 P were purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra 400005. Castor oil of Dabur was purchased locally. Tween 80 was procured from Merck Specialities Private Limited, Mumbai – 400018). Double Distilled Water was prepared in the Guru Nanak Institute of Pharmaceutical Science & Technology Laboratory, Kolkata 700114; Triethanolamine and Methanol were procured from Merck Life Science Private Limited, Mumbai 400079.

Methodology:

Compatibility Study of the Materials: In this work, FTIR has been chosen to study the compatibility between the drug and other materials [14]. KBR pellets were prepared and scanned to get FTIR spectra of the drug (Ofloxacin), materials (Stearic acid, Castor oil, Carbopol 934 P, etc.) individually, and of the mixture (Nanostructured

Lipid Carrier). FTIR spectrophotometer (Perkin Elmer Spectrum Two) was used to obtain the spectra. Sample preparation involved mixing the sample with Potassium bromide, triturating it in a glass mortar, and placing it in the sample holder. The spectrum was scanned over a 4000 – 400 cm-1 15 frequency range.

Experimental Design for the Preparation of Nanostructured Lipid Carrier (NLC): Face-Centered Central Composite Design (α = 1) was selected for the preparation of a Nanostructured Lipid Carrier (NLC) with the help of Design – Expert Software (Version 7.1.5) [16]. Liquid Lipid (mL) and Homogenization Speed (RPM) were taken as independent variables and set at high and low levels [17]. As a result, 13 runs have been found. The coded values of the independent variables have been mentioned in **Table 1.**

TABLE 1: CODED VALUES (HIGH AND LOW VALUES) OF INDEPENDENT VARIABLES

Independent Variables	Unit	Low Actual (- 1 Level)	High Actual (+1 Level)	-alpha	+alpha
Liquid Lipid	mL	2.00	5.00	2.00	5.00
Homogenization Speed	RPM	3000.00	7000.00	3000.00	7000.00

According to the Face–Centered Central Composite Design, 13 runs have been found and mentioned in **Table 2.**

TABLE 2: FACE–CENTERED CENTRAL COMPOSITE DESIGN

Run	Factor 1 (Liquid Lipid in mL)	Factor 2 (Homogenization Speed in RPM)
1	3.50	5000.00
2	2.00	5000.00
3	3.50	5000.00
4	3.50	5000.00
5	5.00	7000.00
6	5.00	5000.00
7	3.50	5000.00
8	5.00	3000.00
9	3.50	7000.00
10	2.00	7000.00
11	2.00	3000.00
12	3.50	5000.00
13	3.50	3000.00

Among 13 runs, it has been found that 5 runs are repeated, hence the total number of runs has been considered to be 9.

Method of Preparation of Nanostructured Lipid Carrier (NLC): The lipid mixture was prepared by mixing Stearic acid (Solid Lipid) and Castor oil (Liquid Lipid) by continuous stirring in a hot condition (60º-70ºC), with the help of a magnetic stirrer (REMI 1MLH) of Remi Elektrotechnik Ltd. (Vasai, India). An aqueous phase of ofloxacin was mixed with the lipid phase in a hot condition (60º-70ºC) in the presence of a surfactant (Tween 80). The mixture (kept in a beaker), was placed under a homogenizer (REMI Motor of Remi Elektrotechnik Ltd., Vasai, India) for 15-20 minutes at 3000-7000 rpm [18]. Throughout the process, the temperature was kept high. The mixture was transferred to the homogenizer tube for further homogenization [19]. The mixture was sonicated for 30 minutes using Ultrasonic Sonicator (Labman Scientific Instruments Private Limited, Chennai – 600118, India) and kept for 48 hours at room temperature. Then the formulations were lyophilized using a lyophilizer (Optics Technology, Delhi – 34, India) [20]. The composition of different formulations has given in **Table 3.**

TABLE 3: COMPOSITION OF DIFFERENT NLC (NANOSTRUCTURED LIPID CARRIER) FORMULATIONS

Formulation Number	Amount of Stearic Acid (gm)	Amount of Castor Oil (mL)	Amount of Ofloxacin (gm)	Amount of Double Distilled Water (mL)	Amount of Tween 80 (mL)	Homogenization Speed (RPM)
NLC_1	6.50	3.50	0.01	10.00	0.3	5000.00
NLC_2	8.00	2.00	0.01	10.00	0.3	5000.00

NLC_5	5.00	5.00	0.01	10.00	0.3	7000.00
NLC_6	5.00	5.00	0.01	10.00	0.3	5000.00
NLC_8	5.00	5.00	0.01	10.00	0.3	3000.00
NLC_9	6.50	3.50	0.01	10.00	0.3	7000.00
NLC_10	8.00	2.00	0.01	10.00	0.3	7000.00
NLC_11	8.00	2.00	0.01	10.00	0.3	3000.00
NLC_13	6.50	3.50	0.01	10.00	0.3	3000.00

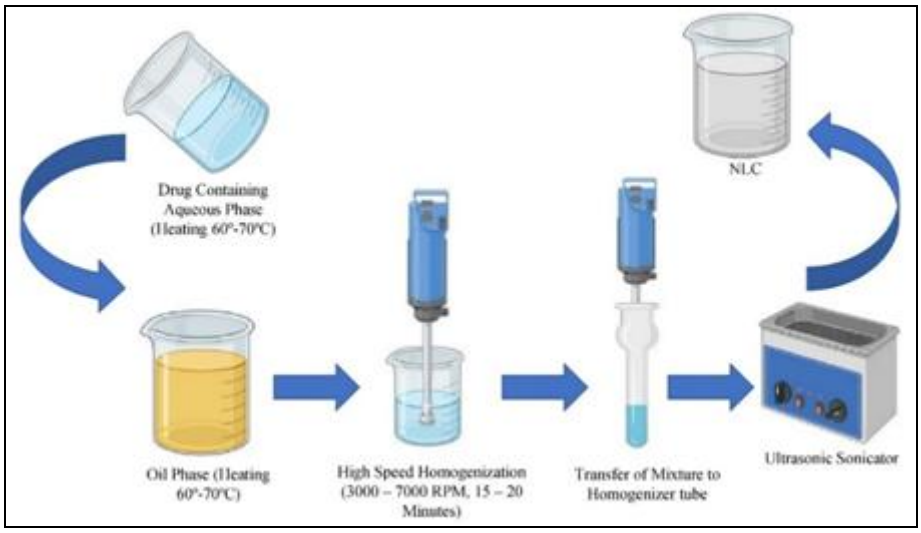

FIG. 1: PREPARATION OF NANOSTRUCTURED LIPID CARRIER (NLC)

Method of Preparation of Nanogel: The prepared NLC (Approx 10% W/V) was incorporated in Hydrogel formed by Carbopol 934 P dissolved in DDW in the concentration of 1.25% (W/V) [21]. It was mixed by stirring keeping on a magnetic stirrer. Tween 80 (0.15 mL) was incorporated for the stabilization of the formulation. At the end, 1 – 2 drops of Triethanolamine were added to the mass to get the proper consistency of the product.

Evaluation of Ofloxacin-loaded Nanogel:

Physical Appearance: The physical appearance and the homogeneity of the nanogel formulations were tested by visual observations [19].

pH Determination: The nanogel formulations' pH was determined using a pH meter.

Determination of Particle Size: Dynamic light scattering (DLS) was used to measure the particle size of the NLC formulations at 25°C using a Zetasizer (Version 7.11) device (Malvern Instruments Ltd, UK) [15]. The particle size and polydispersity index of the NLC formulations were measured after they had been dissolved in methanol and 100-fold diluted with it.

SEM Analysis: Scanning electron microscopy was performed to confirm the particle size and shape. The NLC-loaded hydrogel was lyophilized, and that material was analyzed in SEM (Zeiss Evo 18, Carl Zeiss Microscopy, Penta Fet X 3). The diameter of the particles present in the nanogel was found between 100 to 200 nm.

Spreadability Coefficient: The spreadability Coefficient was determined by Spreadability determinant apparatus. It consists of a wooden block that gets attached to a pulley at one end. The Spreadability Coefficient was measured based on the slip and drag characteristics of nanogel. A glass side was fixed on the wooden block. Some pinch of nanogel was placed on the glass slide and sandwiched between two slides. A measured quantity of weight (20 gm) was placed in the pan attached to the pully. The time (in Seconds) required by the slide to cover a distance of 5 cm was noted. A shorter interval signifies a better spreadability coefficient [22].

Determination of % Entrapment Efficiency: 1 gm of drug-loaded Nanostructured Lipid Carrier (NLC) was mixed with 5 mL of Methanol and it was cold centrifuged (5° - 8°C) at 12,000 RPM for 30 minutes, using a cooling centrifuge of REMI ELEKTROTECHNIK LTD., Vasai, India. After centrifugation, the supernatant fluid was filtered

with filter paper (Mesh No.: 11 µm). The filtered material was poured into a petri dish and allowed to dry. After drying, 10 mL Phosphate Buffer of pH 7.4 was poured into the petri dish and mixed with a glass rod.

Then the material was again filtered and assayed spectrophotometrically at 294 nm using UV Visible Spectrophotometer (Jasco V – 630 Spectrophotometer), to get the concentration of the entrapped drug. The same process was followed for blank NLC whose value was subtracted from the previous result. The formula below was used to compute the percentage of EE [23].

% EE = (Experimental Drug Content) / (Theoritical Drug Content) × 100

Optimization of the Formulation: The formulations were optimized concerning the response such as particle size, spreadability, and % EE with the help of Design - Expert Software (Version 7.1.5). At first, the model was analyzed by doing ANOVA and R squared test. Then the 3D response curve and the perturbation plot were obtained from the model graph. Then, the numerical and graphical optimization was done by the software [16].

***In-vitro* Drug Diffusion Study using Dialysis Membrane:** It was performed through dialysis membrane 50 (Hi-Media) by using Franz Diffusion Cell apparatus with a receptor compartment capacity of 45 ml and cross-sectional area of 0.785 cm^2. The prepared Nanogel was spread over the membrane. It was then fixed in the donor compartment. So that the membrane faces toward the receiver compartment.

The Phosphate Buffer pH 7.4 was filled in the receiver compartment. Magnetic beads were continually agitating the receptor solution while keeping the temperature at 32 ± 0.5 °C. The samples were withdrawn at different intervals, and the drug was analyzed [24].

5 ml of receptor solution was withdrawn, and an equal volume of fresh buffer pH 7.4 was replaced [25]. The samples were analyzed for drug content in UV Visible Spectrophotometer at 294 nm. The slope is obtained from the equation y = 0.032x and Regression Coefficient is 0.9987.

Permeation Study using Goat Skin:

Preparation of Goat Skin: Fresh ocular skin of a goat was collected from a slaughterhouse and used in the permeation experiment. It was hydrated in Phosphate Buffer pH 7.4 for an hour.

***Ex-vivo* Permeation Study:** The Franz Diffusion cell of 45 ml capacity was carried out for the ex vivo skin permeation study [26].

1 gm of nanogel was spread on the goat ocular skin towards the donor compartment. Then the skin was placed on the receiver compartment of the diffusion cell containing Phosphate Buffer pH 7.4. Magnetic beads were continually agitating the receptor solution while keeping the temperature at 32 ± 0.5 °C. The samples were withdrawn at different time intervals, and the drug was analyzed. 5 ml of receptor solution was withdrawn and an equal volume of fresh buffer pH 7.4 was replaced. The samples were analyzed for drug content in UV Visible Spectrophotometer at 294 nm [27].

RESULTS:

Compatibility Study of the Materials: Drug Excipient interaction, one of the essential parameters, are studied before the development of the formulations. Ofloxacin, Excipients, and Excipients mixed with the drug were mixed separately with IR grade KBr in the ratio 1:100, and corresponding pellets were prepared by applying 5.5 metric ton pressure in a hydraulic press [15].

Excipients were Stearic Acid, Castor oil, Tween 80, and Carbopol 934 P. The pellets were scanned over a wave number range of 4000 – 400 cm^{-1} in FTIR Spectroscope. The peaks in single compound spectra are also not of many deviations from the spectra of the mixture.

The exclusive peaks of the drug, ofloxacin (1079 cm^{-1} for R-F where C – F Stretch is present, 1758 cm^{-1} for RCOOH where monomer C = O is present, 2819 cm^{-1} for RCOOH where dimer OH is present, etc.) were observed in the spectra of the mixture (1065 cm^{-1} for R-F where C – F Stretch is present, 2879 cm^{-1} for RCOOH where dimer OH is present, *etc.*). By properly analyzing the results, it can be interpreted that this combination can be used to proceed with nanogel formulations.

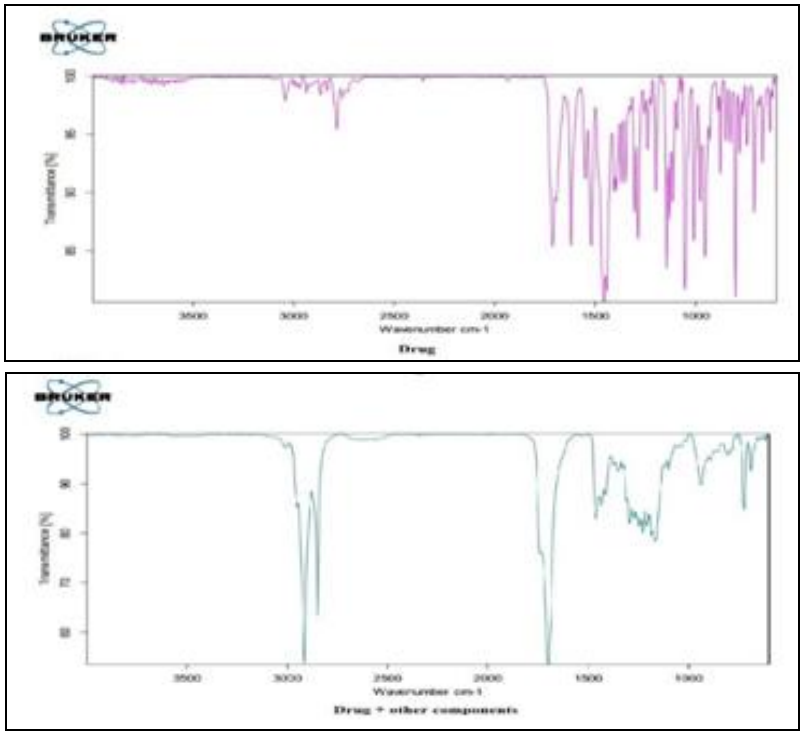

FIG. 2: COMPATIBILITY STUDY OF DRUG (OFLOXACIN) & DRUG WITH OTHER COMPONENTS

Evaluation of Ofloxacin–loaded Nanogel:

Physical Appearance: After developing the products (Nanostructured Lipid Carriers), it has been shown that NLC_1, NLC_9, and NLC_13 are more suitable than the rest for preparing nanogel considering their homogeneity, texture, and state.

pH Determination: pH of the nanogel formulations was found to be in the range of 6.98 to 7.57. pH of Formulation 1(NLC_1) loaded gel was found to be 7.43, pH of Formulation 2(NLC_2) loaded gel was found to be 7.39, pH of Formulation 5(NLC_5) loaded gel was found to be 7.57, pH of Formulation 6(NLC_6) loaded gel was found to be 6.98, pH of Formulation 8(NLC_8) loaded gel was found to be 7.32, pH of Formulation 9(NLC_9) loaded gel was found to be 7.04, pH of Formulation 10(NLC_10) loaded gel was found to be 7.05, pH of Formulation 11(NLC_11) loaded gel was found to be 7.25, and pH of Formulation 13(NLC_13) loaded gel was found to be 7.15.

Determination of Particle Size: Face Centered Central Composite Design ($\alpha = 1$) was used for the preparation of NLC Formulations with the help of Design–Expert Software (Version 7.1.5) [15]. In this design, Liquid Lipid (mL) and Homogenization Speed (RPM) were selected as independent variables and set at high and low levels. The low level for Liquid Lipid (mL) was 2.00 and the high level for Liquid Lipid (mL) was 5.00 [17]. On the other hand, the low level for Homogenization Speed (RPM) was 3000.00 and the high level for Homogenization Speed (RPM) was 7000.00.[16] In this way, 13 formulations have been found. The particle size of the formulations was found to be 78.79 nm to 648.4 nm. In case of Formulation 1(NLC_1), the particle size was found to be 78.79 nm. In case of Formulation 6(NLC_6), the particle size was found to be 648.4 nm. Out of 9 formulations obtained from Face Centered Central Composite Design, it has been observed that NLC_1, NLC_9, and NLC_13 showed a particle size of less than 200 nm whereas the rest showed a particle size in the range of 259.5 to 648.4 nm. Figure 3 shows the DLS curve of the formulated NLC preparation.

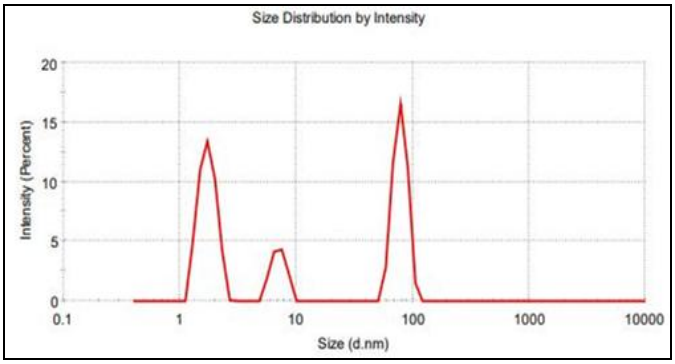

FIG. 3: DLS CURVE OF THE FORMULATED NLC PREPARATION

SEM Analysis: Fig. 4 shows the SEM pictures of the nanogel formulations. The picture shows large number of particles indicating the distribution of NLC in the hydrogel to get proper nanogel. It clearly shows that the particulates are within the nano range (67.69 to 170.9 nm).

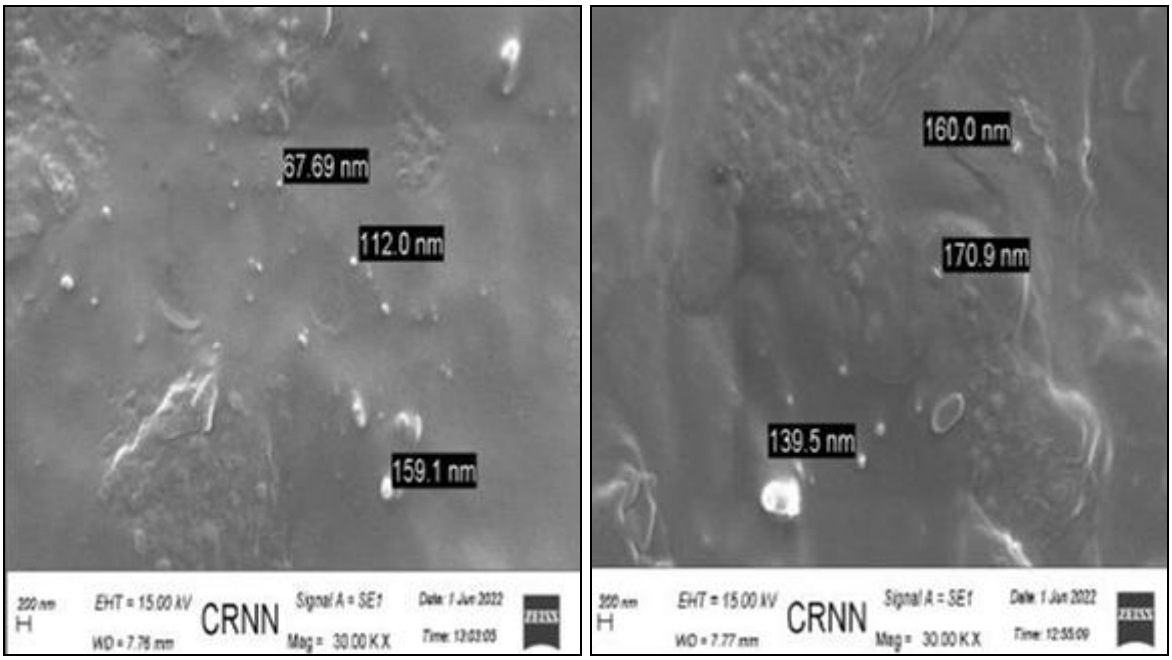

FIG. 4: SEM IMAGES OF NANOGEL FORMULATIONS

Spreadability Coefficient: The Spreadability Coefficient (gm. cm/sec) of different NLC-loaded Gel formulations has been measured. The spreadability coefficient of the nanogel formulations was found to be in the range of 0.259 to 0.359 gm. cm/sec. In Formulation 1(NLC_1) loaded gel formulation, the spreadability coefficient was found to be 0.359 gm. cm/sec. In Formulation 6(NLC_6) loadedgel formulation, the spreadability coefficient was found to be 0.259 gm. cm/sec. It has been found that particle size is inversely proportional to the spreadability coefficient of nanogel formulation. The more the particle size, less the spreadability coefficient.

Determination of % Entrapment Efficiency: % Entrapment Efficiency of the NLC formulations was found to be in the range of 48.7% to 93.05%. In Formulation 8(NLC_8), the entrapment efficiency was found to be 48.7%. In Formulation 10(NLC_10), the entrapment efficiency was found to be 93.05%. It has been found that particle size is directly proportional to the % Entrapment Efficiency (% EE) of nanogel formulation. The more the particle size, the more the % Entrapment Efficiency. **Table 4** shows the particle size, spreadability coefficient, and % Entrapment Efficiency of the NLC Formulations.

TABLE 4: PARTICLE SIZE, SPREADABILITY COEFFICIENT, AND % ENTRAPMENT EFFICIENCY OF NLC FORMULATIONS

Formulation Number	Particle Size (nm)	Spreadability (gm. cm/sec)	% Entrapment Efficiency
NLC_1	78.79	0.359	66.2%
NLC_2	389.4	0.302	86.2%
NLC_5	323.8	0.306	64%
NLC_6	648.4	0.259	57.65%
NLC_8	319.4	0.309	48.7%
NLC_9	148.1	0.344	69.7%
NLC_10	288.5	0.311	93.05%
NLC_11	259.5	0.316	73.57%
NLC_13	196.9	0.325	53.52%

Optimization of the Formulation: Face–Centered Central Composite design approach was used to optimize ofloxacin loaded nanogel formulations. The design displayed 13 formulation compositions

with five common compositions to check for errors in the results. The software was modified to incorporate the data of particle size, spreadability, and % Entrapment Efficiency to interpret the findings. The software generated predicted values, polynomial equations, contour graphs, and 3D response surface graphs to assess the effects of independent factors on dependent components. When the anticipated value produced by the program was compared to the actual particle size and entrapment efficiency, the result was found to be closer to the predicted value. **Table 5** shows a summary of the regression analysis for particle size and entrapment effectiveness. All responses fit best with a quadratic model with the highest correlation coefficient (R^2). The chosen independent variable had an individual as well as a combined impact on the dependent variables, making the quadratic response optimal for optimization. For both replies, it was discovered that the expected R^2 and the adjusted R^2 were in good agreement. The F value displayed a value larger than 4, and the ANOVA result was determined to be significant. The ANOVA of the quadratic model for responses of developed ofloxacin-loaded nanogel has been mentioned in **Table 6**. To assess the software's accuracy, a percentage prediction calculation was made. The polynomial equation's positive sign encourages the interaction of independent variables with the dependent variables, whereas its negative value denotes the opposite relationship. The tested independent variable was deemed suitable for the optimization since the total combined desirability was closer to unity [25].

TABLE 5: STATISTICAL MODEL SUMMARY OF REGRESSION ANALYSIS RESULTS FOR RESPONSE PARTICLE SIZE, SPREADABILITY, AND % ENTRAPMENT EFFICIENCY

Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV	Remark
Particle Size						
Linear	0.0610	- 0.1268	- 0.5362	179.64	78.68	
2FI	0.0614	- 0.2515	- 0.9520	189.31	82.92	
Quadratic	0.7536	0.5776	- 0.8394	109.98	48.17	Suggested
Spreadability						
Linear	0.0445	- 0.1466	- 0.5800	0.034	10.22	
2FI	0.0446	- 0.2739	- 1.3010	0.035	10.78	
Quadratic	0.7725	0.6100	- 0.7314	0.020	5.96	Suggested
% Entrapment Efficiency						
Linear	0.9156	0.8987	0.8377	3.80	5.63	
2FI	0.9181	0.8909	0.7695	3.94	5.84	
Quadratic	0.9967	0.9944	0.9750	0.90	1.33	Suggested

TABLE 6: ANOVA OF QUADRATIC MODEL FOR RESPONSES OF DEVELOPED OFLOXACIN-LOADED NANOGEL

ANOVA Results	Particle Size (nm)	Spreadability (gm. cm/sec)	% Entrapment Efficiency
Regression			
Some of square	2.590E+005	9.103E-003	1705.16
Degree of Freedom	5	5	5
Mean Square	51792.62	1.821E-003	341.03
F – Value	4.28	4.75	424.48
P	0.0419	0.0326	< 0.001
Influence	Significant	Significant	Significant
Lack of fit–test			
Some of square	84675.24	2.681E-003	5.62
Degree of Freedom	3	3	3
Mean Square	28225.08	8.937E-004	1.87
Residual			
Some of square	84675.24	2.681E-003	5.62
Degree of Freedom	7	7	7
Mean Square	12096.46	3.830E-004	0.80

Effect of Independent Variables on Particle Size: The range of particle sizes for several batches was discovered to be between 78.79 (NLC_1) to 648.4 nm (NLC_6). Due to the variation in the composition of the Liquid Lipid, Homogenization

Speed, a considerable difference in particle size was seen.

The following independent variables impact particle size as shown by the polynomial equation (1), 3D response plot **Fig. 5.**

Particle Size = +1159.56183 – 846.80747*A + 0.15156*B – 2.05000E–003*AB+128.05900*A^2–1.45668E–005*B^2... (1)

Where, A = Liquid Lipid, and B = Homogenization Speed.

Particle Size was negatively impacted by liquid lipid. Hence, the ideal lipid content is required to achieve the minimal size. Due to the availability of higher concentration liquid lipid, the particle size decreases with an increase in liquid lipid concentration. Homogenization Speed displayed a positive impact on particle size. The particle size increases as the homogenization cycle increases.

Equation (1) also showed the combined effect of Liquid Lipid and Homogenization speed on particle size. This combination displayed a negative effect on particle size. So, from equation (1), it can be concluded that liquid lipids had a more prominent effect on particle size than homogenization speed.

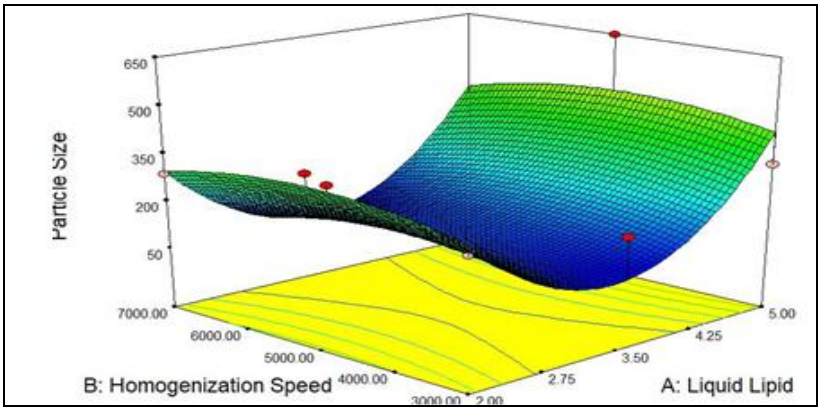

FIG. 5: 3D RESPONSE SURFACE PLOT SHOWING THE EFFECT OF INDIVIDUAL VARIABLES ON PARTICLE SIZE

Effect of Independent Variables on Spreadability Coefficient: The range of spreadability coefficient for several batches was discovered to be between 0.259 (NLC_6 based gel) to 0.359 gm. cm/sec (NLC_1 based gel).

Due to the variation in the composition of the Liquid Lipid, Homogenization Speed, a considerable difference in spreadability was seen.

The following independent variables impact the spreadability coefficient as shown by the polynomial equation (2), 3D response plot **Fig. 6.**

Spreadability = + 0.098376 + 0.15542*A – 4.19253E-006*B +1.66667E–007*AB–0.023195*A^2+4.52586E–010*B^2 …(2)

Where, A = Liquid Lipid, B = Homogenization Speed.

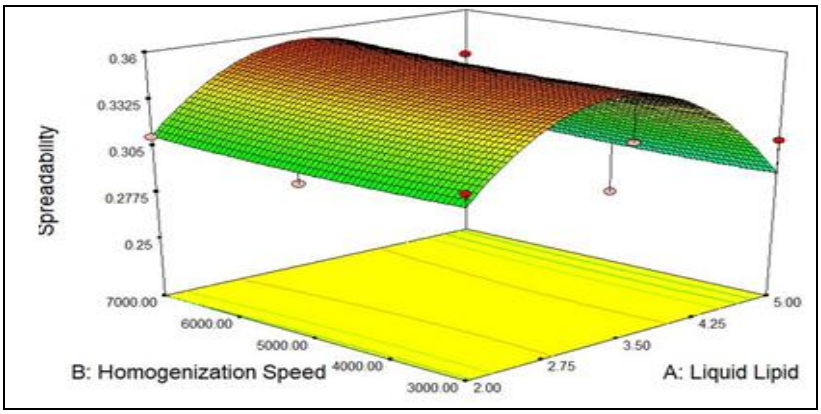

FIG. 6: 3D RESPONSE SURFACE PLOT SHOWING THE EFFECT OF INDIVIDUAL VARIABLES ON THE SPREADABILITY COEFFICIENT

Spreadability was positively impacted by liquid lipid. Hence, the ideal lipid content is required to achieve better spreadability. Due to the availability of higher concentration liquid lipid, the spreadability coefficient increases with an increase in liquid lipid concentration. Homogenization Speed displayed a negative impact on the spreadability coefficient. The spreadability coefficient increases as the homogenization cycle decreases. Equation (2) also showed the combined effect of Liquid Lipid and Homogenization speed on spreadability coefficient. This combination displayed a positive effect on particle size.

Effect of Independent Variables on % Entrapment Efficiency (% EE): The range of % Entrapment Efficiency for several batches were discovered to be between 48.7 (NLC_8) to 93.05 nm (NLC_10). Due to the variation in the composition of the Liquid Lipid, and Homogenization Speed, a considerable difference in % Entrapment Efficiency was seen.

The following independent variables have an impact on % Entrapment Efficiency as shown by the polynomial equation (3), 3D response plot **Fig. 7.**

$$\% EE = + 87.16606 – 28.98806*A + 0.013930*B – 3.48333E–004*AB + 3.07977*A^2 – 8.46379E–007*B^2 ..(3)$$

Where, A = Liquid Lipid, and B = Homogenization Speed.

% EE was negatively impacted by liquid lipid. Due to higher concentration liquid lipid availability, the % EE decreases with an increase in liquid lipid concentration. Homogenization Speed displayed a positive impact on particle size. The % EE increases as the homogenization speed increases. Equation (3) also showed the combined effect of Liquid Lipid and Homogenization speed on % EE. This combination displayed a negative effect on % EE. So, from equation (3), it can be concluded that liquid lipids had a more prominent effect on % EE than homogenization speed.

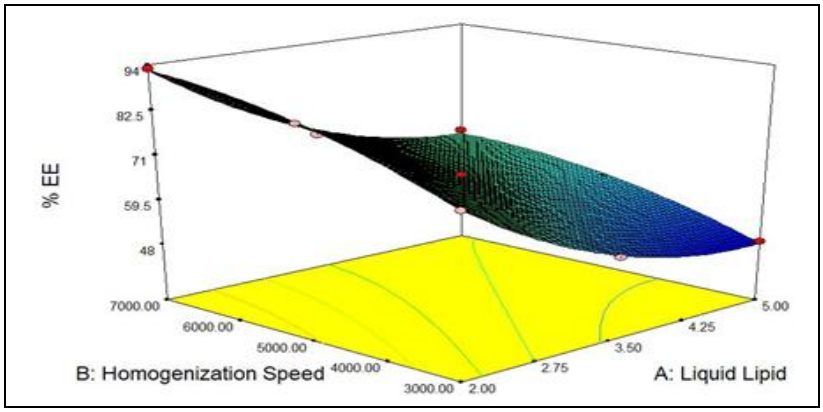

FIG. 7: 3D RESPONSE SURFACE PLOT SHOWING THE EFFECT OF INDIVIDUAL VARIABLES ON THE % ENTRAPMENT EFFICIENCY

By doing the "Numerical Optimization", 39 solutions have been found. In every solution, desirability is 1.000.

Table 7 represents the "Solutions obtained from Numerical Optimization".

TABLE 7: SOLUTIONS OBTAINED FROM NUMERICAL OPTIMIZATION

Solution Number	Liquid Lipid (mL)	Homogenization Speed (RPM)	Desirability
1	3.5	7000	1.000
2	3.5	5000	1.000
3	3.5	3000	1.000
4	2.3	3400	1.000
5	5	3000	1.000
6	2.3	5400	1.000
7	2.3	6600	1.000
8	5	7000	1.000
9	5	5000	1.000
10	4.9958	3366	1.000
11	4.1621	6548.4	1.000

12	3.1709	6530.8	1.000
13	4.6154	5360.8	1.000
14	3.8807	5766.8	1.000
15	2.6552	3950.8	1.000
16	3.7718	3675.2	1.000
17	3.2552	5136.4	1.000
18	3.92	4298.8	1.000
19	4.0421	5518.4	1.000
20	3.47	3060.8	1.000
21	4.8083	5944.8	1.000
22	4.9766	5902.4	1.000
23	2.3321	4724	1.000
24	4.5398	5233.6	1.000
25	4.3541	6228.4	1.000
26	4.3313	6342.4	1.000
27	2.0468	3498.4	1.000
28	2.5997	3856	1.000
29	3.8339	3488	1.000
30	4.7573	5917.6	1.000
31	3.8864	5560	1.000
32	4.6277	5663.2	1.000
33	3.1544	5865.6	1.000
34	3.4685	5661.6	1.000
35	4.271	6238.4	1.000
36	3.8429	6912.8	1.000
37	2.1683	4212.8	1.000
38	3.9455	4045.2	1.000
39	2.7617	6806.8	1.000

The Design – Expert Software (Version 7.1.5) provided 39 optimized formulations. Among them, it has been found that the formulations where liquid lipid quantity was equal to 2.00 mL, were not in the optimization list. Hence those formulations have been discarded. The Design – Expert Software provided 13 runs by Face–Centered Central Composite Design model where 5 formulations were repeated.

Hence 9 formulations were taken into consideration. Among these 39 formulations, 6 formulations have been included by the software in the optimization list. Among them, depending on the particle size, Formulation 1(NLC_1) (repeated in the Numerical Optimization list) has been considered as an optimized product, and further studies have been performed on this product.

***In-vitro* Drug Diffusion Study using Dialysis Membrane:** The *in-vitro* drug diffusion profile of Ofloxacin from Formulation 1(NLC_1) was reported in **Fig. 8**. The in vitro drug diffusion study was performed for 7 hours. It has been observed that the drug diffusion from the optimized formulation gradually increased and maintained a constant rate after 5 hours. The diffusion rate of ofloxacin was 89.79 % in 7 hours [28].

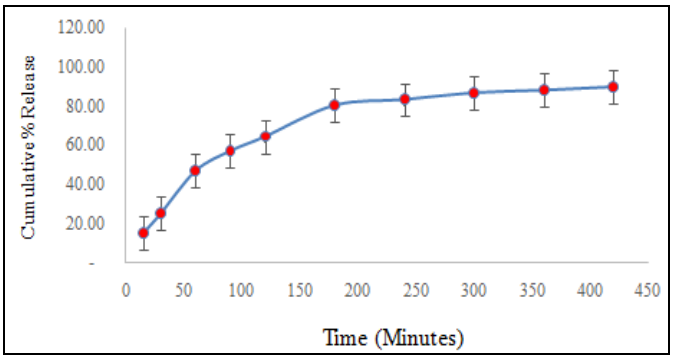

FIG. 8: CUMULATIVE % RELEASE OF OFLOXACIN FROM OPTIMIZED FORMULATION (NLC_1) IN PHOSPHATE BUFFER PH 7.4

***Ex-vivo* Permeation Study using Goat Skin:** After an *in-vitro* drug diffusion study using a dialysis membrane, an *ex-vivo* experiment was conducted to check the correlations between the experiments. The results found were satisfactory to proceed [29]. For this investigation (drug permeation kinetics), healthy adult goat ocular skin has been selected [30]. According to skin permeability, the order reported is mouse > rat > guinea pig > rabbit > monkey > dog > goat>ship>pig>human being [26]. It has been found that the permeation of ofloxacin

through goat ocular skin from the optimized product is around 71.33 % in 7 hours. The drug permeation might be described by zero-order kinetics. The permeability coefficient was calculated using P = K. Vr/S, where S is the effective surface area of the goat ocular skin, Vr is the volume of the receiver chamber, K is the Zero order constant, and P is the Permeability Coefficient [26]. It was found to be 1.141 cm/min.

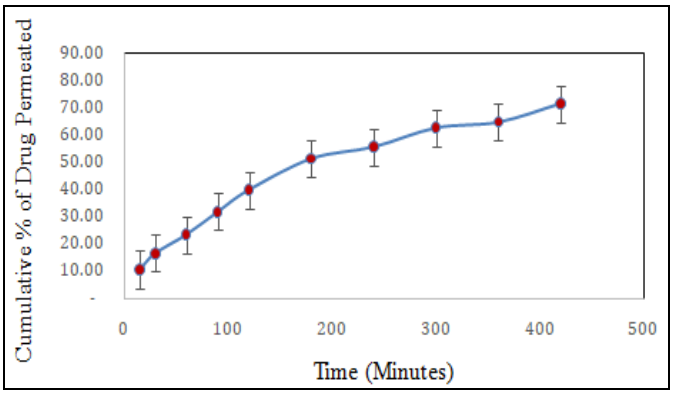

FIG. 9: CUMULATIVE % OF OFLOXACIN PERMEATED, RELEASED FROM OPTIMIZED FORMULATION (NLC_1) BASED GEL

DISCUSSION: As previously noted, this study was conducted using Ofloxacin-loaded NLC. The study aimed to evaluate the efficacy of Ofloxacin-loaded Nanogel for optical administration. First, the compatibility between the drug and excipients was examined using an FTIR analysis. By interpreting the FTIR spectra, it can be concluded that this combination can be used to proceed with nanogel formulations. In order to optimize the formulation based on particle size, spreadability coefficient, and % entrapment efficiency, the formulations were created using Design-Expert software (Version 7.1.5) utilizing a face-centered Central Composite Design. The software provided 13 formulations with 5 repetitions to check and analyze the error. The % entrapment efficiency of the Ofloxacin-loaded NLC formulations was found to be in the range of 48.7% to 93.05%, while the particle size of Ofloxacin-loaded NLC formulations was measured to be in the range of 78.79 nm to 648.4 nm. The spreadability of Ofloxacin-loadednanogel formulations was found to be in the range of 0.259 to 0.359 gm. cm/sec. The ANOVA was used to analyze the results. Polynomial equations and the software's generated 3D response surface graphs were used to describe the impact of Independent Variables (Factor) on the response parameter particle size, spreadability coefficient, and % entrapment efficiency. The software predicted 39 optimum formulations based on the values of the response parameter. A formulation with a low particle size was chosen from the formulations to carry out the additional research. By looking at the formulation's SEM picture, it was possible to verify the surface morphology and particle size distribution. By doing an SEM examination on the optimized formulation, it was discovered that the particle size was between 67.69 and 170.9 nm.

Using a Franz diffusion cell, it was possible to analyze the *in-vitro* drug diffusion using a dialysis membrane and the *ex-vivo* permeation of the optimized formulation through goat optical skin. The in vitro drug diffusion study was performed for 7 hours. It has been observed that the drug diffusion from the optimized formulation gradually increased and maintained a constant rate after 5 hours. The diffusion rate of ofloxacin was 89.79 % in 7 hours.

After *in-vitro* drug diffusion study using a dialysis membrane, an *ex-vivo* experiment was conducted to check the correlations between the experiments. The results found were satisfactory to proceed. It has been found that the permeation of ofloxacin through goat ocular skin from the optimized product is around 71.33 % in 7 hours. The drug permeation might be described by zero-order kinetics. The permeability coefficient was calculated using P = K. Vr/S, where S is the effective surface area of the goat ocular skin, Vr is the volume of the receiver chamber, K is the Zero order constant, and P is the Permeability Coefficient. It was found to be 1.141 cm/min.

Therefore, based on the outcome of the study performed here we can state that the NLC formulations have effective action on the delivery of ofloxacin through the ocular membrane. In the future, by performing these studies the formulation can be more optimized for use in case of ocular drug delivery.

ACKNOWLEDGMENT: The authors are thankful to Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata for providing the necessary facilities to carry out the research work. They are also thankful to Bose Institute, Kolkata

for providing the experimental support of DLS and CRNN, University of Calcutta, for the experimental support of Scanning Electron Microscopy.

CONFLICTS OF INTEREST: The authors declared no conflict of interest.

REFERENCES:

1. Howaili F, Özliseli E, Küçüktürkmen B, Razavi SM, Sadeghizadeh M and Rosenholm JM: Stimuli-Responsive, Plasmonic Nanogel for Dual Delivery of Curcumin and Photothermal Therapy for Cancer Treatment. Front Chem 2021; 8(1): 1-17. doi:10.3389/fchem.2020.602941
2. Vairo C, Collantes M and Quincoces G: Preclinical safety of topically administered nanostructure lipid carriers (NLC) for wound healing application: biodistribution and toxicity studies. Int J Pharm 2019; 569(4): 118484. doi:10.1016/j.ijpharm.2019.118484
3. Kim MH, Kim KT and Sohn SY: Formulation and evaluation of nanostructured lipid carriers (NLCs) of 20(s)-protopanaxadiol (PPD) by box-behnken design. Int J Nanomedicine 2019; 14: 8509-8520. doi:10.2147/IJN.S215835
4. Jafar G, Abdassah M, Rusdiana T and Khairunisa R: Development and characterization of precirol ato 88 base in nanostructured lipid carriers (Nlc) formulation with the probe sonication method. Int J Appl Pharm 2021; 13(3): 43-46. doi:10.22159/IJAP.2021.V13S3.08
5. Shimojo AAM, Fernandes AR V, Ferreira NRE, Sanchez-Lopez E, Santana MHA and Souto EB: Evaluation of the influence of process parameters on the properties of resveratrol-loaded NLC using 22 full factorial design. Antioxidants 2019; 8(8). doi:10.3390/antiox8080272
6. Lynch CR, Kondiah PPD, Choonara YE, du Toit LC, Ally N and Pillay V: Hydrogel Biomaterials for Application in Ocular Drug Delivery. Front Bioeng Biotechnol 2020; 8(3): 1-18. doi:10.3389/fbioe.2020.00228
7. Dubashynskaya N, Poshina D, Raik S, Urtti A and Skorik YA: Polysaccharides in ocular drug delivery. Pharmaceutics 2020; 12(1): 1-30. doi:10.3390/pharmaceutics12010022
8. Gorantla S, Rapalli VK and Waghule T: Nanocarriers for ocular drug delivery: Current status and translational opportunity. RSC Adv 2020; 10(46): 27835-27855. doi:10.1039/d0ra04971a
9. Gilani SJ, Bin Jumah MN and Zafar A:. Formulation and Evaluation of Nano Lipid Carrier-Based Ocular Gel System: Optimization to Antibacterial Activity. Gels 2022; 8(5). doi:10.3390/gels8050255
10. Bashiri S, Ghanbarzadeh B, Ayaseh A, Dehghannya J and Ehsani A: Preparation and characterization of chitosan-coated nanostructured lipid carriers (CH-NLC) containing cinnamon essential oil for enriching milk and anti-oxidant activity. Lwt 2020; 119: 108836. doi:10.1016/j.lwt.2019.108836
11. Guilherme VA, Ribeiro LNM and Alcântara ACS: Improved efficacy of naproxen-loaded NLC for temporomandibular joint administration. Sci Rep 2019; 9(1): 1-11. doi:10.1038/s41598-019-47486-w
12. Mahmood H, Khan IU and Asif M: *In-vitro* and *in-vivo* evaluation of gellan gum hydrogel films: Assessing the co impact of therapeutic oils and ofloxacin on wound healing. Int J Biol Macromol 2021; 166: 483-495. doi:10.1016/j.ijbiomac.2020.10.206
13. Rytwo G and Zelkind AL: Evaluation of kinetic pseudo-order in the photocatalytic degradation of ofloxacin. Catalysts 2022; 12(1): 1-11. doi:10.3390/catal12010024
14. Anwar W, Dawaba HM, Afouna MI, Samy AM, Rashed MH and Abdelaziz AE: Enhancing the oral Bioavailability of candesartan cilexetil loaded nanostructured lipid carriers: *In-vitro* characterization and absorption in rats after oral administration. Pharmaceutics 2020; 12(11): 1-19. doi:10.3390/pharmaceutics12111047
15. Teng Z, Yu M and Ding Y: Preparation and characterization of nimodipine-loaded nanostructured lipid systems for enhanced solubility and Bioavailability. Int J Nanomedicine 2019; 14: 119-133. doi:10.2147/IJN.S186899
16. Jain K, Sood S and Gowthamarajan K: Optimization of artemether-loaded NLC for intranasal delivery using central composite design. Drug Deliv 2015; 22(7): 940-954. doi:10.3109/10717544.2014.885999
17. Hassan H, Adam SK, Alias E, Affandi MMRMM, Shamsuddin AF and Basir R: Central composite design for formulation and optimization of solid lipid nanoparticles to enhance oral Bioavailability of acyclovir. Molecules 2021; 26(18). doi:10.3390/molecules26185432
18. Sethuraman N, Shanmuganathan S, Sandhya K and Anbarasan B: Design, development and characterization of nano structured lipid carrier for topical delivery of aceclofenac. Indian J Pharm Educ Res 2018; 52(4): 581-586. doi:10.5530/ijper.52.4.67
19. Riaz A, Hendricks S and Elbrink K: Preparation and Characterization of Nanostructured Lipid Carriers for Improved Topical Drug Delivery: Evaluation in Cutaneous Leishmaniasis and Vaginal Candidiasis Animal Models. AAPS Pharm Sci Tech 2020; 21(5). doi:10.1208/s12249-020-01717-w
20. Mahmood A, Rapalli VK, Gorantla S, Waghule T, Singhvi G. Dermatokinetic assessment of luliconazole-loaded nanostructured lipid carriers (NLCs) for topical delivery: QbD-driven design, optimization, and *in-vitro* and *ex-vivo* evaluations. Drug Deliv Transl Res 2022; 12(5):1118-1135. doi:10.1007/s13346-021-00986-7
21. Puro D, Athawale R and Pandya A: Design, Optimization and Characterization of Nanostructured Lipid Carriers of Raloxifene Hydrochloride for Transdermal Delivery. Nanosci Nanotechnology-Asia 2018; 10(1): 57-67. doi:10.2174/2210681208666181106124337
22. Ali A, Ali A, Rahman MA, Warsi MH, Yusuf M and Alam P: Development of Nanogel Loaded with Lidocaine for Wound-Healing: Illustration of Improved Drug Deposition and Skin Safety Analysis. Gels 2022; 8(8). doi:10.3390/gels8080466
23. Mahdi WA, Bukhari SI, Imam SS, Alshehri S, Zafar A and Yasir M: Formulation and optimization of butenafine-loaded topical nano lipid carrier-based gel: Characterization, irritation study, and anti-fungal activity. Pharmaceutics. 2021; 13(7). doi:10.3390/pharmaceutics13071087
24. Paliwal S and Kaur G: Formulation and Characterization of Topical Nano Emulgel of Terbinafine. Univers J Pharm Res 2019; 3(6): 28-34. doi:10.22270/ujpr.v3i6.223
25. Thakur B and Kumar I: New Developed and Validated Spectroscopic Method for the Simultaneous Estimation of Terbinafine Hydrochloride and Fluconazole. Int J Pharm Pharm Sci 2020; 12(11): 19-25. doi:10.22159/ijpps.2020v12i11.39344
26. Biswas GR, Majee SB and Roy A: Combination of synthetic and natural polymers in hydrogel: An impact on

drug permeation. J Appl Pharm Sci 2016; 6(11): 158-164. doi:10.7324/JAPS.2016.601125

27. Sandeep DS, Mahitha M and Meghna S: Development, Characterization, and *In-vitro* Evaluation of Aceclofenac Emulgel. Asian J Pharm 2020; 14(3): 330. https://www.asiapharmaceutics.info/index.php/ajp/article/view/3681
28. Farooq U, Rasul A and Sher M: Development, characterization and evaluation of anti-fungal activity of miconazole based nanogel prepared from biodegradable polymer. Pak J Pharm Sci 2020; 33(1): 449-457.
29. Ho HN, Le TG and Dao TTT: Development of Itraconazole-Loaded Polymeric Nanoparticle Dermal Gel for Enhanced Antifungal Efficacy. J Nanomater 2020; 2020. doi:10.1155/2020/8894541
30. Imran M, Iqubal MK and Imtiyaz K: Topical nanostructured lipid carrier gel of quercetin and resveratrol: Formulation, optimization, *in-vitro* and *ex-vivo* study for the treatment of skin cancer. Int J Pharm 2020; 587(7): 119705. doi:10.1016/j.ijpharm.2020.119705

How to cite this article:
Biswas GR, Dutta P, Paul G, Patra S and Paul S: Development of nanogel of ofloxacin for ocular drug delivery. Int J Pharm Sci & Res 2023; 14(12): 5619-32. doi: 10.13040/IJPSR.0975-8232.14(12).5619-32.