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FORMULATION AND EVALUATION OF CIPROFLOXACIN NIOSOME GEL FOR OCULAR DELIVERY

Indu Raghunath ^{*1} and T. N. K. Suriyaprakash ²

M. V. M College of Pharmacy ¹, Department of Pharmaceutics, Yelahanka, Bangalore - 560064, Karnataka, India.

School of Pharmacy ², Hindustan Institute of Technology and Science, Padur, Chennai - 603103, Tamil Nadu, India.

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Correspondence to Author:

Indu Raghunath

Associate Professor,
M. V. M College of Pharmacy,
Department of Pharmaceutics,
Yelahanka, Bangalore - 560064,
Karnataka, India.

E-mail: indu.raghunath166@gmail.com

ABSTRACT: This study aimed to formulate and evaluate ciprofloxacin-loaded niosomes for sustained ocular drug delivery and enhanced bioavailability by increasing residence time in the eye. Niosomes were prepared via thin film hydration using Tween 80, 60, and 20 at three different concentrations, keeping cholesterol and drug ratios constant. These vesicles were assessed for drug encapsulation efficiency and *in-vitro* release. Among the formulations, Tween 60-based niosomes in a 1:1 ratio with cholesterol (F4) showed superior drug entrapment and release in phosphate buffer saline (PBS). F4 was incorporated into Carbopol 940 gel base to enhance ocular retention. The resulting gel demonstrated suitable viscosity, pH, and a mean vesicle size of 203.7 ± 1.3 nm with a PDI of 0.337 ± 0.2 and positive zeta potential, indicating uniformity and stability. SEM (Scanning Electron Microscopy) analysis confirmed the spherical shape of the vesicles. *In-vitro* release studies indicated zero-order kinetics, with the Higuchi model suggesting diffusion-controlled release. The Korsmeyer-Peppas model yielded an 'n' value above 0.5, indicating non-Fickian diffusion involving both diffusion and polymer matrix relaxation. Stability testing as per ICH (International Conference on Harmonization) guidelines showed minimal drug degradation under refrigerated conditions, confirming the formulation's stability. The Draize eye irritation test performed in rabbits showed no signs of redness, swelling, or irritation in the cornea, iris, or conjunctiva, establishing the formulation's safety. Overall, the findings support niosomal gel as a promising carrier for sustained ocular delivery of ciprofloxacin, with potential to enhance therapeutic efficacy, reduce dosing frequency, and improve patient compliance.

INTRODUCTION: The human eye, an organ of exceptional sensitivity and importance, is equipped with unique anatomical and physiological safeguards. It enables interaction with the surrounding world by conveying visual stimuli to the brain, serving as a key conduit between neural processing and external reality ¹.

The eye is safeguarded by a combination of dynamic and static protective mechanisms. Dynamic defences such as tear turnover, reflex blinking, and nasolacrimal drainage help eliminate foreign substances from the ocular surface.

Meanwhile, static structures like the eyelid, conjunctiva, and corneal epithelium provide physical protection. Internally, the blood-aqueous barrier (BAB) and blood-retina barrier (BRB) restrict the passage of compounds from systemic circulation into ocular tissues. These barriers are further supported by metabolic enzymes and structural components like the sclera and retina ^{2,3}.

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Despite these defenses, the eye remains susceptible to infections, injuries, and external trauma due to its direct exposure to the environment. To address these challenges, ocular drug delivery systems have been developed with the goals of bypassing ocular barriers, enhancing drug stability and therapeutic effectiveness, extending drug retention time, reducing dosing frequency, allowing for combination therapies, and minimizing adverse drug reactions to improve patient compliance^{3,4}.

Liquid ocular formulations such as eye drops, suspensions, and emulsions are the most widely used, with eye drops alone comprising more than 95% of marketed ophthalmic products. These are primarily intended for treating anterior segment disorders, although their effectiveness is often limited by rapid elimination from the ocular surface. While suspensions and emulsions are advantageous for delivering hydrophobic drugs, they may lead to temporary blurring of vision. Semi-solid forms like gels and ointments can enhance drug retention time on the eye surface, thereby improving drug efficacy^{5,6}.

A major limitation of ocular delivery is the short contact time with ocular tissues, which hinders consistent and effective drug absorption. This challenge highlights the need for rational formulation strategies aimed at increasing drug residence time on the ocular surface. In addition to eye drops, other topical dosage forms such as gels, ointments, and ocular inserts have been developed to extend drug retention. However, their use is often restricted due to discomfort and visual disturbances⁷. Systemic drug administration for ocular conditions is also limited, as the blood supply to corneal tissues is minimal, reducing drug access to the eye. To target posterior eye diseases, intraocular injections are sometimes employed; however, these are invasive, potentially painful, and may affect patient adherence. Furthermore, the rapid drainage of topically applied drugs lowers therapeutic efficacy and necessitates more frequent dosing. This not only burdens the patient but also increases the risk of systemic side effects due to drug absorption through non-ocular pathways^{4,8}.

Nanotechnology in Ocular Drug Delivery: Nanotechnology merges scientific and technological advancements with the capacity to

manipulate materials at the nanoscale typically between 1 and 100 nanometers. This precise control at the molecular level has opened up vast possibilities across various scientific disciplines, including medicine. When applied to healthcare, this approach is referred to as nanomedicine, which encompasses diagnostics, disease management, and therapeutic applications. Since the 1980s, nanotechnology has played an increasingly important role in ocular drug delivery.

The emergence of nanoformulations has provided innovative solutions to longstanding challenges in ophthalmic treatment. These nanoscale systems can effectively bypass ocular barriers, enhance drug retention on the corneal surface, improve tissue permeability and drug bioavailability, and offer protection against degradation of unstable compounds. Moreover, they tend to be better tolerated by patients compared to traditional formulations. Both organic and inorganic nanoparticles (NPs) have demonstrated promising potential in addressing clinical challenges in ophthalmology. These advanced delivery systems offer more efficient and targeted drug transport, thereby significantly enhancing therapeutic outcomes in the treatment of eye diseases⁹⁻¹².

Vesicular nanocarriers have emerged as a promising strategy for delivering therapeutics in the treatment of various ocular diseases. These systems, which include liposomes, niosomes, micelles, and polymer-based vesicles, offer several advantages such as high drug encapsulation efficiency, enhanced ocular penetration, and improved drug stability. By protecting therapeutic agents from degradation during storage and systemic circulation, they help prolong the drug's half-life and reduce clearance by the reticuloendothelial system. Among these carriers, surfactant-based systems like niosomes have gained particular interest for ophthalmic applications. Niosomes are notable for their structural flexibility, biocompatibility, biodegradability, and the capacity to encapsulate both hydrophilic and hydrophobic drugs. These properties make them especially suitable for improving drug availability at ocular sites and addressing challenges associated with conventional eye therapies^{13,14}.

Niosomes are vesicular drug carriers characterized by an aqueous core surrounded by a lipophilic bilayer, typically formed using nonionic surfactants in combination with stabilizing agents like cholesterol. Their unique structure allows for the encapsulation of both hydrophilic and lipophilic drugs, making them highly versatile for ocular drug delivery. Compared to other vesicular systems such as liposomes, niosomes present multiple advantages. They exhibit superior chemical stability, low toxicity, and favorable biocompatibility, along with being biodegradable and non-immunogenic. Their flexible structure enhances corneal penetration and enables controlled drug release, while also contributing to prolonged drug retention and improved shelf life. From a manufacturing perspective, niosomes offer practical benefits. They can be produced through relatively simple and cost-effective methods and are more stable during large-scale production. Unlike liposomes, niosomes do not require stringent storage conditions there is no need for freezing or inert gas environments making them more convenient and economical for long-term use. Due to these properties, niosomes have shown significant promise as nanocarriers for enhancing ocular drug bioavailability. They have been successfully used to deliver therapeutic agents like acetazolamide, timolol maleate, naltrexone hydrochloride, and doxycycline hyclate, demonstrating their effectiveness in managing various eye diseases and improving treatment outcomes^{15, 16}.

Niosomes can be classified according to their size and the number of lipid bilayers they contain.

Small Unilamellar Vesicles (SUVs) generally range in size from 10 to 100 nanometers, whereas Large Unilamellar Vesicles (LUVs) are typically between 100 and 3000 nanometers. In contrast, Multi-Lamellar Vesicles (MLVs), which consist of multiple bilayers, are larger in size, often exceeding 5 micrometers in diameter. Non-ionic surfactants play a crucial role in the formation of niosomes. These molecules contain both a hydrophilic (water-attracting) head and a hydrophobic (water-repelling) tail. Therefore, hydrophilic drugs are encapsulated in the aqueous core of the vesicle, whereas hydrophobic drugs are incorporated into the lipid bilayer. They contribute to enhancing membrane permeability and improving drug solubility by reducing surface tension. Additionally, using non-ionic surfactants with longer alkyl chains can lead to higher drug entrapment efficiency within the niosomal structure¹³.

In theory, the formation of niosomes necessitates a specific amphiphilic compound and an aqueous medium. Upon hydration, amphiphile monomers self-assemble into vesicles due to the significant interfacial tension between the water and the hydrophobic (typically hydrocarbon) segments of the amphiphile, promoting aggregation. At the same time, the hydrophilic head groups experience steric and electrostatic repulsion, encouraging their exposure to the surrounding water. The balance between these opposing forces results in the creation of a supramolecular structure. Key components involved in niosome preparation include non-ionic surfactants and membrane stabilizers like cholesterol¹⁷.

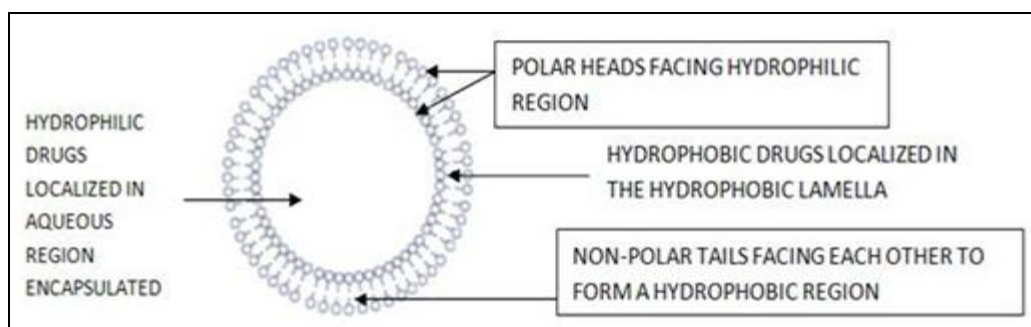


FIG. 1: STRUCTURE OF NIOSOMES

Niosomes are investigated for ocular drug delivery due to their ability to enhance penetration and their low toxicity. This study aims to develop and assess niosomal formulations of the anti-infective drug

Ciprofloxacin to improve its ocular bioavailability, minimize dosing frequency, and ultimately enhance patient compliance. Ciprofloxacin niosomes prepared with various non-ionic surfactants were

subjected to visual inspection, drug encapsulation as well as *in-vitro* drug release studies and the best niosomal formulation was converted to gel using Carbopol 940. Niosomal gel was evaluated for its viscosity, pH, *in-vitro* drug release studies, stability and ocular irritation studies.

MATERIALS AND METHODS: Ciprofloxacin was obtained as a gift sample from Chethana Pharmaceuticals, Perinthalmanna, Malappuram. Tween 80, tween 20, chloroform and Carbopol 940 were obtained from Hi Media. Tween 60 and cholesterol (water soluble) was procured from Sigma Aldrich. All remaining reagents used were of analytical grade.

Preparation of Ciprofloxacin Niosomes: Ciprofloxacin niosomes were formulated using a modified version of the conventional thin film hydration technique. Non-ionic surfactants Tween 80, Tween 60, and Tween 20 were tested at three

different concentrations (ratios of 1, 1.5, and 2) while keeping the drug and cholesterol ratios constant, in order to determine the optimal conditions for vesicle formation **Table 1**. The mixture of vesicles forming ingredients like surfactants (tween 20, 60, 80) and cholesterol are dissolved in a volatile organic solvent (chloroform) in a round bottom flask. Chloroform was evaporated at 66°C under reduced pressure using a rotary evaporator set at 120 rpm for one hour, resulting in the formation of a thin lipid film on the inner wall of the flask. To eliminate any remaining traces of the organic solvent, the flask was kept in a desiccator overnight. The dried lipid film was then hydrated with 10 mL of PBS (pH 7.4) by manually shaking the flask for 30 minutes **Table 1**. The resulting aqueous niosomal suspension was allowed to stabilize and then stored at 4°C in a refrigerator for further analysis^{18, 19}.

TABLE 1: COMPOSITION OF SURFACTANT AND CHOLESTEROL FOR NIOSOME PREPARATION

Sl. no.	Formulations	Surfactant	Drug: Surfactant: Cholesterol	Weight taken (mg)		
				Drug	Surfactant	Cholesterol
1	F1	Tween 80	1:1:1	10	10	10
2	F2		1:1.5:1	10	15	10
3	F3		1:2:1	10	20	10
4	F4	Tween 60	1:1:1	10	10	10
5	F5		1:1.5:1	10	15	10
6	F6		1:2:1	10	20	10
7	F7	Tween 20	1:1:1	10	10	10
8	F8		1:1.5:1	10	15	10
9	F9		1:2:1	10	20	10

Determination of λ_{max} of Ciprofloxacin: The UV-Visible spectrophotometric analysis of Ciprofloxacin was carried out by scanning its standard solution within the wavelength range of 200–400 nm. A 10 µg/mL solution of Ciprofloxacin was prepared using PBS at pH 7.4, and the spectrum was recorded using a Shimadzu (UV-240) double beam spectrophotometer. The observed absorption maximum matched the position and relative intensity of the reference spectrum, confirming the identity of the compound.

Development of Standard Curve of Ciprofloxacin: An accurately weighed 50 mg of Ciprofloxacin was first dissolved in 50 mL of PBS (pH 7.4). This solution was then diluted to obtain a concentration of 100 µg/mL, and further diluted to 10 µg/mL using the same buffer. From this stock solution, a series of dilutions were prepared to

achieve concentrations of 10, 20, 30, 40, and 50 µg/mL. The absorbance of each solution was recorded at 271.6 nm using a UV spectrophotometer. A standard calibration curve was constructed by plotting absorbance values against their corresponding concentrations²⁰.

Evaluation of Niosomes:

Visual Appearance and Clarity: The niosomal formulations were examined visually to assess their color, clarity, and uniformity by observing them under light against both white and black backgrounds²¹.

Determination of Encapsulation Efficiency: The entrapment efficiency of the niosomal formulations (F1-F9) was evaluated using the exhaustive dialysis technique. A specific volume of the niosomal suspension was placed into a dialysis tube sealed

with an osmosis cellulose membrane. This setup was then immersed in 100 mL of PBS (pH 7.4) and continuously stirred using a magnetic stirrer. The free (unentrapped) drug diffused out through the membrane into the external medium. The buffer was completely replaced with fresh medium every hour for a duration of 3 to 4 hours, until the absorbance readings stabilized, indicating the absence of further unentrapped drug. The amount of drug retained within the vesicles (entrapped drug) was then quantified using UV spectrophotometry at a wavelength of 271.6 nm²².

Entrapment efficiency (%) = Amount of encapsulated drug / Total amount of drug x 100

In-vitro Drug Release Studies: The *in-vitro* release of ciprofloxacin from the niosomal formulations (F1-F9) was assessed using the membrane diffusion method. A quantity of the formulation equivalent to 10 mg of ciprofloxacin was placed into a glass tube (2.5 cm in diameter, 8 cm effective length), which had one end sealed with a pre-soaked osmosis cellulose membrane, serving as the donor compartment. This tube was positioned in a beaker containing 100 mL of PBS (pH 7.4), acting as the receptor compartment. The setup was arranged so that the membrane end of the glass tube was immersed approximately 1–2 mm into the diffusion medium. The receptor phase was maintained at a constant temperature of $37 \pm 1^\circ\text{C}$ and stirred at 100 rpm using a magnetic stirrer. At predetermined intervals, 5 mL samples were withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were analyzed at 271.6 nm using a double beam UV-VIS spectrophotometer, with PBS (pH 7.4) as the blank²³.

Formulation of Ciprofloxacin Niosome Gel: The most effective niosomal formulation, identified from the previous evaluation, was incorporated into a gel dosage form. Ciprofloxacin-loaded niosomes, prepared using Tween 60 as the non-ionic surfactant with 1:1 ratio of tween 60 & cholesterol, F4, were utilized for this purpose. A gel base was formulated using deionized water as the solvent and Carbopol 940 as the gelling agent at a concentration of 1.5% (w/w). To prepare the gel, 150 mg of Carbopol 940 was first fully hydrated in 6.5 ml of deionized water. Following hydration, 3.5

ml of F4 comprising Tween 60 and cholesterol in a 1:1 molar ratio was added. The final formulation yielded 10 grams of gel containing 0.3% (w/w) ciprofloxacin²⁴.

Evaluation of Niosome Gel:

Viscosity & pH of Niosome Gel: The viscosity of ophthalmic formulations plays a crucial role in influencing the drug's retention time within the eye. To evaluate this parameter, a Brookfield viscometer equipped with spindle type S64 was employed, operating at various rotational speeds 10, 20, 50, 60, and 100 rpm. This instrument determines viscosity by rotating a spindle immersed in the sample fluid, where resistance from the fluid (viscous drag) causes a calibrated torsion spring to twist. The degree of spring deflection is directly related to the torque exerted, which is then used to calculate viscosity. The shear rate generated during the process is a function of both the spindle design and its rotation speed. For the measurement, 5 grams of the gel formulation was placed in a beaker, the spindle was immersed for approximately five minutes, and readings were recorded accordingly^{25, 26}. Measurements were done in triplicates.

The pH of the niosomal gel was determined using a digital pH meter by immersing the electrode directly into the formulation. The readings were recorded in triplicate to ensure accuracy.

Physicochemical Characterization of Niosome Gel: Particle size distribution, PDI and zeta potential of the submicron niosomal particles were assessed using a Malvern Zetasizer, which operates based on laser light scattering technology.

To minimize the risk of particle aggregation, the samples were appropriately diluted with deionized water prior to analysis. Measurements were taken at a controlled temperature range of 20–25°C and at a detection angle of 90°, using clean, intact sample cells. The analysis was performed on 0.1% aqueous dispersions of the niosomal gel formulation suitably diluted with deionized water²⁷. Zeta potential was determined using undiluted niosomal dispersion placed directly into appropriate cuvettes for analysis. Measurements were repeated three times to confirm reproducibility.

SEM Analysis: The morphology, size, and surface features of F4 were examined using SEM. An environmental SEM (model JSM 6390, USA) was employed to capture images of the ciprofloxacin-loaded niosomal gel in order to assess its structural attributes following complexation. A thin film of the gel was mounted onto an aluminum stub and then coated with a thin layer of gold to enhance conductivity. Imaging was conducted at an accelerating voltage of 15 kV with a magnification of 20,000 X²⁸.

In-vitro Release Studies of Niosome Gel: The release profile of ciprofloxacin from F4 was evaluated using the membrane diffusion method. The formulation was placed into a glass tube measuring 2.5 cm in diameter and 8 cm in length, the end of which was covered with a pre-soaked osmosis cellulose membrane, serving as the donor compartment. This tube was then positioned vertically in a beaker containing 100 ml of PBS (pH 7.4), which functioned as the receptor compartment. The setup was arranged so that the lower end of the tube was submerged approximately 1–2 mm into the diffusion medium. The receptor medium was maintained at a constant temperature of $37 \pm 1^\circ\text{C}$ and stirred at 100 rpm using a magnetic stirrer. At regular intervals, 5 ml samples were withdrawn and immediately replaced with fresh buffer to preserve sink conditions. The collected samples were analyzed for ciprofloxacin content at 271.2 nm using a double beam UV-Visible spectrophotometer, with phosphate buffer (pH 7.4) serving as the blank²³.

Accelerated Stability Studies: Stability testing was conducted in accordance with ICH guidelines. A short-term accelerated stability study was performed over a duration of three months to evaluate the stability of the niosomal gel formulation.

Freshly prepared samples were placed in borosilicate glass vials, securely sealed, and stored under different conditions: $3-5^\circ\text{C}$, $25 \pm 2^\circ\text{C}$ with 60% relative humidity (RH), and $40 \pm 2^\circ\text{C}$ with 75% RH, using a stability chamber. At predetermined intervals 0, 1, 2, and 3 months the samples were examined for any alterations in physical characteristics such as pH, drug content, color, odor, and texture²⁹.

Ocular Irritancy Studies: F4 was subjected to *in-vivo* evaluation using the Draize test on Albino rabbits. The study protocol received approval from the Institutional Animal Ethics Committee Reg.no-PRC/expt/11/2013-2014 dated 08/01/2014 and F no 25/03/09-AWD, GOI. As per the Draize test procedure, approximately 100 μl of the test formulation was administered into the lower conjunctival sac of one eye, while the other eye served as an untreated control. A single drop, around 0.04 ml, was instilled twice daily, allowing the rabbit to blink naturally. Observations were recorded at intervals of 1, 24, 48, and 72 hours, as well as one-week post-application. Ocular responses were assessed using a standardized scoring system that evaluates changes in the eyelids, conjunctiva, cornea, and iris³⁰.

RESULTS:

Determination of λ_{max} Of Ciprofloxacin and the Development of Standard Curve: λ_{max} of ciprofloxacin was found to be 271.2 nm in PBS pH 7.4

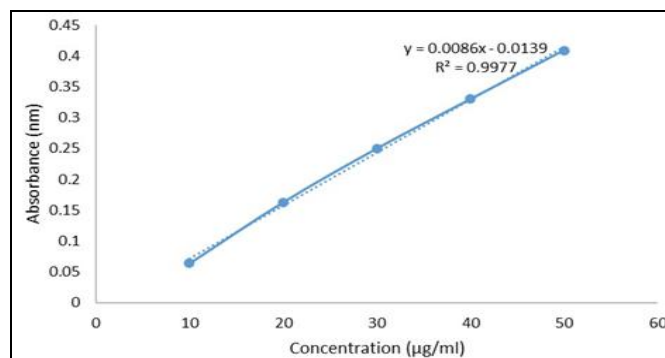


FIG. 2: STANDARD PLOT OF CIPROFLOXACIN IN PBS 7.4

Evaluation of Visual Appearance and Clarity:

TABLE 2: PHYSICAL APPEARANCE OF CIPROFLOXACIN LOADED NIOSOMES

Formulation code	Visual appearance	Clarity
F1	White	Clear
F2	White	Clear
F3	White	Clear
F4	White	Clear
F5	White	Clear
F6	White	Clear
F7	White	Clear
F8	White	Clear
F9	White	Clear

All the ciprofloxacin loaded niosomal formulations were found to be white in color and clear in

appearance. These are especially important in sterile ophthalmic preparations indicating their stability **Table 2**.

Encapsulation Efficiency of Niosomes: Niosomal formulations formulated with Tween 60 demonstrated superior drug entrapment efficiency compared to those prepared with Tween 20 and Tween 80. Among the various formulations, Tween 60 based niosomes prepared with cholesterol in different molar ratios 1:1, 1:1.5, and 1:2 exhibited entrapment efficiencies of 58.6%, 50.2%, and 53.3%, respectively. F4 prepared with a 1:1

cholesterol to Tween 60 ratio achieved the highest entrapment of ciprofloxacin, with an efficiency of 58.6% **Fig. 3**.

TABLE 3: ENCAPSULATION EFFICIENCY DATA	
Formulation code	Encapsulation efficiency (%)
F1	41.14±1.02
F2	35.6±0.61
F3	39.07±0.91
F4	58.6±1.06
F5	50.2±1.23
F6	53.3±1.14
F7	39.1±0.81
F8	33.71±0.46
F9	36.9±0.92

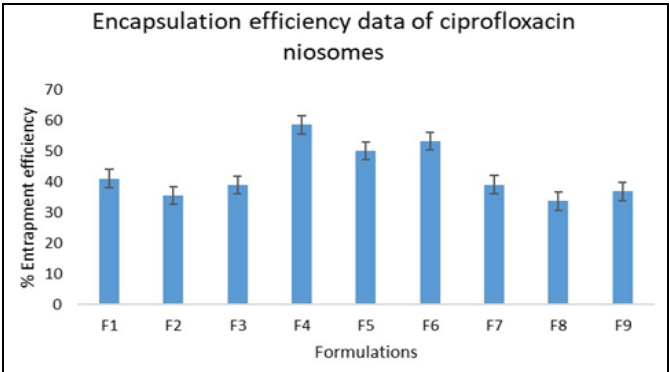


FIG. 3: ENCAPSULATION EFFICIENCY DATA OF CIPROFLOXACIN LOADED NIOSOMES

In-vitro Drug Release Studies:

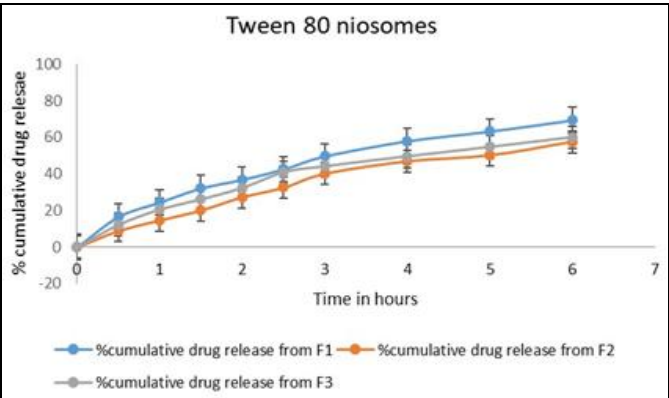


FIG. 4: % DRUG RELEASE FROM TWEEN 80 NIOSOMES

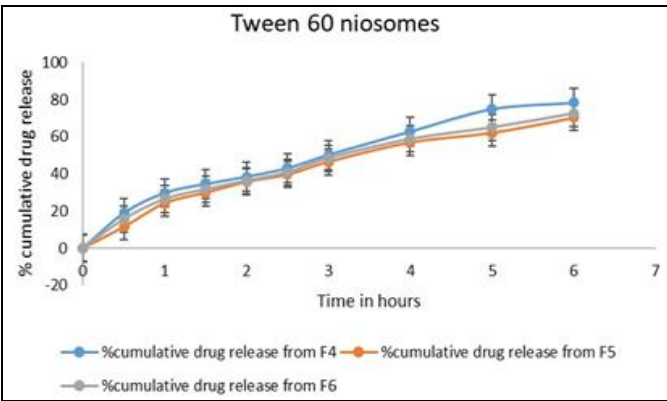


FIG. 5: % DRUG RELEASE FROM TWEEN 60 NIOSOMES

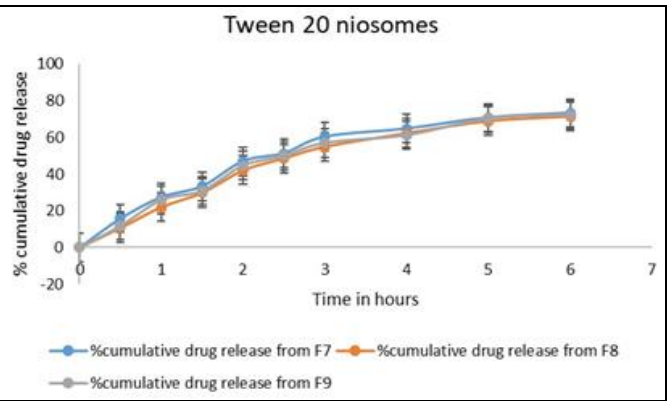


FIG. 6: % DRUG RELEASE FROM TWEEN 20 NIOSOMES

Tween 80 and tween 20 niosomes showed a lesser release of ciprofloxacin compared with tween 60 niosomes. Among the tested formulations, niosomes prepared with Tween 60 demonstrated superior ciprofloxacin entrapment and a more rapid drug release profile compared to those made with Tween 20 and Tween 80. Specifically, the F4 formulation, consisting of a 1:1 ratio of Tween 60 to cholesterol, achieved the highest encapsulation efficiency (58.6%) along with a sustained and accelerated release of approximately 78% within 6 hours **Fig. 4, 5, 6**. Given its promising characteristics, the F4 niosomal formulation was further developed into a gel using Carbopol 940 as the gelling agent.

Incorporating the drug into a gel base enhances ocular residence time, potentially reducing the frequency of administration and thereby improving patient adherence to treatment.

Viscosity & pH of Niosome Gel: Viscosity of F4 niosomes after being converted to gel was measured by Brooke field viscometer and was found to be 168 ± 198 cps at 10 rpm. pH of F4 was found to be 7.2 ± 0.6 .

Physicochemical Characterization of Niosome Gel: Average particle diameter of F4 was found to be 203.7 ± 1.3 nm with a PDI of 0.337 ± 0.2 . F4 exhibited a positive zeta potential of 14.07 ± 0.03 .

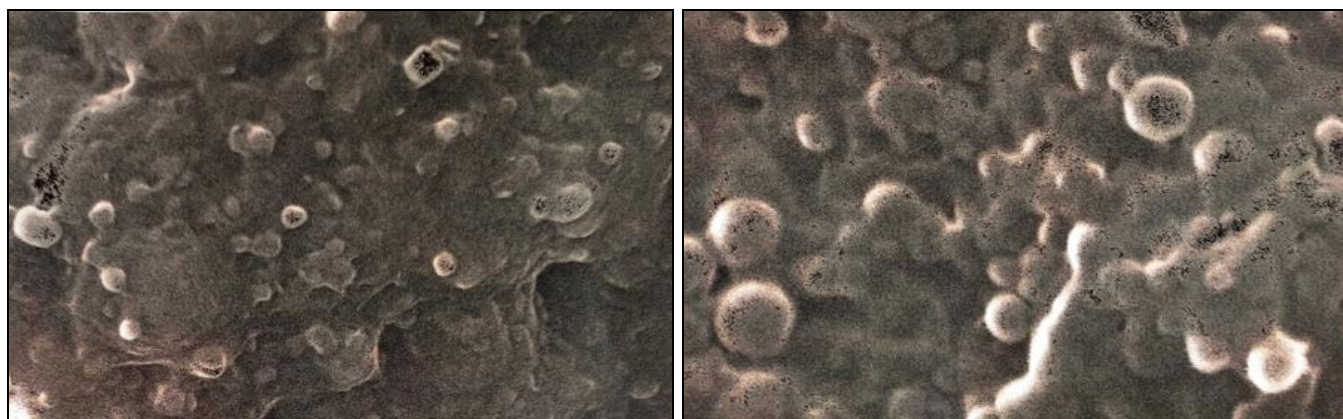


FIG. 7: SEM IMAGES OF (F4) CIPROFLOXACIN LOADED NIOSOMAL GEL

From the SEM images shape and surface characteristics of ciprofloxacin loaded niosomal gel were studied. F4 niosomes were found to be spherical in shape **Fig. 7**.

In-vitro Release Studies of Niosome Gel: *In-vitro* release studies of F4 showed that almost 77% of drug was released from the niosomes at the end of

6 hours in PBS. Formulation showed a burst release of 28% within 1st hour followed by a sustained release pattern. Almost 50% of ciprofloxacin was released into PBS by 3rd hour. Pure ciprofloxacin was almost completely released within 2 hours into PBS **Fig. 8**.

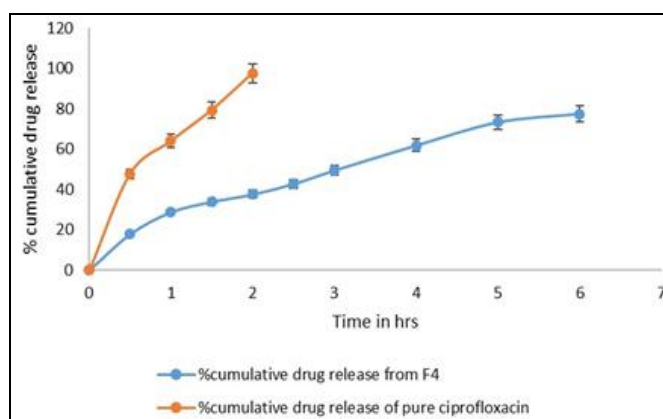


FIG. 8: IN-VITRO RELEASE PROFILE OF CIPROFLOXACIN IN PBS FROM NIOSOMAL GEL AND PURE CIPROFLOXACIN

Release Kinetics: Release of ciprofloxacin from F4 was evaluated using different kinetic models namely zero-order, first-order, Higuchi, and Korsmeyer-Peppas to assess the drug release profile and elucidate the mechanism of release²³.

TABLE 4: R² VALUES OF DIFFERENT KINETIC MODELS

Formulation Code	Zero Order	First Order	Higuchi Matrix	Korsemayer Peppas Plot	
				r ²	n -value
F4	0.9857	0.9825	0.9831	0.9886	0.5876

The release kinetics of F4 revealed a zero-order drug release profile, suggesting a consistent release rate over time. The Higuchi model further supported that the drug release occurred through a diffusion-controlled process. Additionally, the Korsmeyer-Peppas model yielded an 'n' value greater than 0.5, indicating that the gel followed a non-Fickian diffusion mechanism, involving a combination of drug diffusion and polymer relaxation **Table 5**.

Accelerated Stability Studies:

TABLE 5: ACCELERATED STABILITY STUDY DATA OF F4 GEL

Temperature	Evaluation parameters	Observation (months)			
		0	1	2	3
3-5°C	Physical appearance	Creamy white	No change	No change	No change
	Drug content (%)	86.03±0.32	86.01±0.5	85.76±0.51	85.43±0.7
25±2°C 60% RH	Physical appearance	Creamy white	No change	No change	No change
	Drug content (%)	86.03±1.01	85.80±1.92	85.23±1.89	85.01±1.76
40±2°C 75% RH	Physical appearance	Creamy white	No change	No change	No change
	Drug content (%)	86.03±1.2	85.03±1.81	84.1±±1.79	82.76±1.99

Stability studies were conducted over a period of three months following ICH guidelines. Evaluations were performed at the end of the 1st, 2nd, and 3rd months to assess parameters such as drug content, pH, and physical appearance. The niosomal gel was specifically examined for its visual characteristics, pH, and drug content. After three months, no noticeable changes were observed in terms of appearance or clarity. Although minor fluctuations in pH were detected, they remained within acceptable limits (±0.5), indicating good formulation stability **Table 5**.

Ocular Irritation Studies:

TABLE 6: DRAIZE TEST

Observation	Cornea	Iris	Conjunctiva	Chemosis
Grade	0	0	0	0

Male albino rabbits, each weighing between 1.5 to 2 kg, were used for the ocular irritation study. F4 was administered twice daily over a duration of 7 days. Throughout the study, the animals were regularly monitored for signs of ocular irritation, including redness, swelling, and excessive tearing. Assessment was carried out in accordance with the Draize test protocol. The results indicated that the niosomal gel was well-tolerated, showing no signs of irritation, damage, or abnormal clinical responses in the cornea, iris, or conjunctiva **Table 6**.

aimed at treating a range of bacterial eye infections. A major limitation of ocular drug delivery is the low bioavailability, primarily due to factors such as nasolacrimal drainage, tear turnover, and various anatomical barriers.

To overcome these challenges and enhance corneal retention and ocular bioavailability, several approaches have been explored. In our work, ciprofloxacin loaded niosomes were formulated using different non-ionic surfactants, including Tween 20, Tween 60, and Tween 80. Among the prepared compositions, the one showing optimal drug encapsulation efficiency and sustained drug release was selected for incorporation into a gel

DISCUSSION: This study focused on the development of a niosomal ophthalmic formulation

base using Carbopol 940 as the gelling agent. The resulting niosomal gel offers increased viscosity, which helps extend the corneal residence time and potentially enhances drug permeation through the ocular tissues⁹. Niosomes are bilayered vesicular carriers capable of encapsulating both hydrophilic and lipophilic drugs **Fig. 1**. Their use in ocular drug delivery offers several advantages, including enhanced bioavailability, improved drug permeation, and reduced systemic side effects. Compared to liposomes, niosomes exhibit greater physical and chemical stability, fewer sterility-related issues, and are more suitable for large-scale production^{17, 21}.

In this study, niosomes were prepared using three different concentrations (1, 1.5, and 2) of non-ionic surfactants Tween 20, Tween 60, and Tween 80 while maintaining a constant ratio of drug to cholesterol **Table 1**. The formulations were developed using the conventional thin-film hydration method and evaluated for drug encapsulation efficiency and *in-vitro* release profiles. Niosomal formulations were found to be clear and white in appearance without any phase separation indicating the formulation stability **Table 2**. Among the formulations, those containing Tween 60 demonstrated superior encapsulation efficiency and drug release characteristics compared to the others (**Fig. 3**) **Table 3**.

The length of the surfactant's alkyl chain plays a significant role in determining the drug entrapment efficiency of niosomal formulations. Surfactants with longer alkyl chains, such as Tween 80, tend to exhibit lower drug encapsulation compared to those with shorter chains like Tween 60. This difference can be attributed to the influence of alkyl chain length on the HLB value of the surfactant. A higher alkyl chain length generally correlates with a higher HLB value, which in turn can reduce the surfactant's ability to effectively encapsulate the drug. Consequently, surfactants with higher HLB values typically result in reduced entrapment efficiency, highlighting the importance of surfactant selection in optimizing niosomal formulations³¹. Incorporating cholesterol at a 1:1 molar ratio enhances both the hydrophobic and hydrophilic characteristics of the vesicle membrane, creating favorable conditions for encapsulating hydrophilic compounds. When the

bilayer has low microviscosity, it becomes less capable of retaining water-soluble substances within the vesicles. Therefore, the HLB of the surfactant not only influences its vesicle-forming capability but also impacts the size of its polar head group, which in turn affects vesicle stability and drug entrapment efficiency³².

The *in-vitro* drug release of all prepared niosomal formulations was assessed using the membrane diffusion method. Results indicated that niosomes formulated with Tween 80 and Tween 20 released ciprofloxacin at a slower rate compared to those prepared with Tween 60. The length of the surfactant's alkyl chain significantly influenced the release behavior; niosomes incorporating Tween 80, which possesses a longer and more unsaturated alkyl chain, exhibited a lower drug release than formulations containing Tween 60 or Tween 20. Moreover, formulations with a 1:1 surfactant-to-cholesterol ratio demonstrated an overall faster release rate across all types (**Fig. 4, 5, 6**). The extended and unsaturated alkyl chain of Tween 80 contributes to the formation of a denser and more rigid bilayer structure, restricting drug diffusion and thereby slowing the release. Additionally, Tween 80's higher HLB value results in the creation of a more compact vesicular membrane, further limiting drug permeation. The increased bilayer stability associated with Tween 80 also helps retain the drug within the vesicle for longer durations, ultimately contributing to the slower release observed³³.

Formulation F4, which contained Tween 60 and cholesterol in an optimal ratio and demonstrated superior encapsulation efficiency and ciprofloxacin release, was further developed into a niosomal gel by incorporating Carbopol 940 as the gelling agent. Embedding the vesicular system within a gel matrix can extend the duration of drug release and increase corneal residence time, thereby improving overall ocular bioavailability. Encapsulating the drug within a gel formulation increases its retention time on the ocular surface, which may decrease the required dosing frequency and enhance patient compliance with the treatment regimen².

Viscosity plays an important role in spreadability, drug retention as well as release rate of the formulation. The prepared niosomal gel exhibited a

viscosity of 168 cps, which is considered optimal for an ophthalmic formulation, as it provides sufficient thickness to enhance precorneal retention without causing discomfort or blurred vision during application^{13, 18}. The niosomal gel exhibited a pH of 7.2, which lies within the normal physiological range of the eye, helping to reduce the potential for irritation and promoting greater comfort and tolerance in patients upon application³⁴.

Niosomal gel exhibited an average particle diameter of 203.7 ± 1.3 nm with a PDI of 0.337 ± 0.2 with a positive zeta potential. Vesicle size plays a critical role in the effectiveness and clearance of drugs delivered through niosomes. Several factors influence the size of these vesicles, including the type of surfactant used and the amount of cholesterol present in the formulation. Additionally, the HLB of surfactants significantly affects niosomal size. The HLB, a dimensionless value, serves as a practical reference for selecting suitable surfactants and optimizing both entrapment efficiency and vesicle size. For nonionic surfactants, the HLB scale ranges from 0 to 20, where values below 9 indicate lipophilic (oil-soluble) properties and values above 11 suggest a more hydrophilic (water-soluble) nature. Tween 60, with an HLB value of 14.9, is considered highly hydrophilic, resulting in a lower hydrocarbon chain volume relative to its hydrophilic surface area. Niosomes with smaller particle sizes are generally more effective at crossing ocular barriers than larger ones. Cholesterol, a key structural component of niosomes, contributes to the stability and mechanical integrity of the vesicles. Literature reports also suggest that a higher cholesterol concentration can lead to the formation of smaller niosomes, likely due to the enhanced lipophilic interactions within the vesicular bilayers¹⁸.

The SEM images confirmed that the F4 niosomes, loaded with ciprofloxacin, had a spherical shape. These niosomes exhibited a uniform, monodispersed morphology with a smooth surface and well-defined edges (**Fig. 7**). *In-vitro* release of ciprofloxacin from the niosomal gel was studied by membrane diffusion method. At the end of 6 hours, nearly 77% of ciprofloxacin was released from F4, exhibiting a sustained release pattern (**Fig. 8**). This indicates that the formulation was able to maintain a steady and controlled release of the drug over an

extended period of time. The sustained release can be attributed to the encapsulation of ciprofloxacin within the niosomal vesicles, which act as a reservoir for the drug. The niosomal gel likely provides a barrier that slows down the diffusion of the drug, leading to a gradual release into the PBS. The drug release from the niosomal gel exhibited a zero-order release pattern, which indicates that the release rate remained constant over time, regardless of the amount of drug remaining in the formulation.

This type of release is beneficial for maintaining a steady drug concentration, minimizing fluctuations and the need for frequent dosing. The primary mechanism governing the release was diffusion, as confirmed by the Higuchi model, which suggests that the ciprofloxacin is being released from the gel matrix in a diffusion-driven process. Furthermore, the Korsmeyer-Peppas model showed an "n" value greater than 0.5, indicating that the release followed a non-Fickian diffusion mechanism **Table 4**. This means that the release process is not solely dependent on simple diffusion but also involves the interaction between the drug and the gel matrix, particularly the relaxation of the polymer network. The combination of diffusion and polymer relaxation results in a more controlled and sustained release of ciprofloxacin. Therefore, this formulation is well-suited for applications requiring prolonged drug delivery, such as in ocular treatments where continuous drug release is crucial for therapeutic efficacy³⁴.

F4 niosomal gel demonstrated stability in terms of pH, physical appearance, and drug content over a three-month storage period under various temperature and humidity conditions, as outlined by ICH guidelines. Throughout the study, the gel maintained its original color and texture across all tested conditions, indicating good physical stability. Although slight variations in pH were observed, they remained within $\pm 5\%$ of the initial pH values, which is considered acceptable. In terms of drug content, a minimal decrease was noted dropping from 86.03% to 85.43% when stored at refrigerated conditions ($3-5^{\circ}\text{C}$) after 3 months. However, greater reductions were observed at higher storage conditions: 85.01% at $25^{\circ}\text{C}/60\%$ RH and 82.76% at $40^{\circ}\text{C}/75\%$ RH after three months **Table 5**. Based on these findings, storing the ophthalmic niosomal gel at refrigeration

temperatures is recommended to preserve its stability and effectiveness²¹. The Draize rabbit eye test is a widely recognized method for evaluating the potential acute ocular toxicity of chemical substances and formulations. In this study, ocular irritation assessments were conducted following the Draize protocol using male albino rabbits over a 7-day period. A scoring system was employed to grade the severity of any irritation observed: a score of 0 denoted the absence of redness, inflammation, or tearing; a score of 1 reflected mild redness with slight inflammation and minimal tearing; a score of 2 indicated moderate redness accompanied by noticeable inflammation and tearing; and a score of 3 represented severe redness, pronounced inflammation, and heavy tearing. The tested niosomal gel formulation (F4) did not produce any visible signs of irritation, tissue damage, or abnormal clinical effects in the cornea, iris, or conjunctiva. Observations were carried out at multiple intervals 1, 24, 48 and 72 hours post-instillation to monitor symptoms such as discharge, swelling (chemosis), and redness, and no adverse effects were recorded throughout the observation period^{21, 30, 34} **Table 6.**

CONCLUSION: This study focused on the development and evaluation of a niosomal gel formulation of an anti-infective agent, ciprofloxacin aimed at achieving sustained ocular drug delivery for the management of various eye infections. In niosomal drug delivery systems, the active compound is encapsulated within vesicles, which serve as reservoirs, gradually releasing the drug over time. Among the nonionic surfactants tested, Tween 60-based niosomes demonstrated superior performance in terms of both ciprofloxacin entrapment and sustained release.

The optimized formulation, labeled F4 comprising Tween 60 and cholesterol in a 1:1 molar ratio was incorporated into a gel matrix using Carbopol 940 to enhance retention in the ocular region. The resulting gel exhibited appropriate viscosity and a physiologically acceptable pH, along with a vesicle size favorable for ophthalmic use. *In-vitro* drug release studies indicated that F4 followed zero-order kinetics, suggesting a constant drug release rate, and the Higuchi model confirmed that release occurred through a diffusion-based mechanism. The Korsmeyer-Peppas model yielded an 'n' value

greater than 0.5, indicating that the release was governed by a non-Fickian diffusion process, involving both diffusion and polymer matrix relaxation. Stability studies conducted under ICH-recommended storage conditions revealed that refrigeration (3–5°C) better preserved the drug content compared to room temperature and high temperature/humidity environments. Additionally, ocular irritation testing using the Draize method in rabbits confirmed the safety of the F4 formulation, with no adverse effects observed on the cornea, conjunctiva, or iris. Overall, the findings support that the niosomal gel effectively enhances the bioavailability of the drug, enables controlled and prolonged therapeutic action, and may reduce the frequency of administration, thereby improving patient compliance. Further clinical evaluation following scale-up manufacturing is recommended to validate these outcomes for therapeutic use.

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