

Design, Optimization and Performance Profiling of Flexible Nano-vesicular Ciprofloxacin Gel: A Targeted Therapy for Ocular Bacterial Keratitis

Satyam KHAJURIA*, Pritpal KAUR MALHI**, Ramandeep S NARANG***, Karanpreet KAUR****, Dr Jasjeet KAUR NARANG*****

Design, Optimization and Performance Profiling of Flexible Nano-vesicular Ciprofloxacin Gel: A Targeted Therapy for Ocular Bacterial Keratitis

Esnek Nano-veziküler Siprofloksasin Jelinin Tasarımı, Optimizasyonu ve Performans Profili Oluşturulması: Göz Bakteriyel Keratitine Yönelik Hedefli Bir Tedavi

SUMMARY

Bacterial keratitis (BK) constitutes a severe eye infection capable of causing substantial vision loss, leading to ulceration and Inflammation. BK is a major cause of corneal blindness in developing countries and faces challenges from the eye's protective structures, limiting drug bioavailability from conventional forms. To address the limitations of conventional dosage forms and to enhance drug efficacy, ciprofloxacin loaded nano-vesicular carriers were developed and then integrated into an optimized agent to induce gelling (Carbopol 940) for prolonging the time of contact of the drug with the eye. The prepared ciprofloxacin loaded flexible nano-vesicular carriers and gels (F40.4% w/v, F40.6% w/v, F40.8% w/v and F41% w/v) were evaluated for % entrapment efficiency, vesicle shape, in vitro drug release and, release kinetics, vesicle size, zeta potential, polydispersity index, isotonicity, sterility, degree of deformability, pH, homogeneity and grittiness, rheology, drug content, ex vivo permeation, flux, permeation coefficient, % drug retention, toxicity study, bioadhesion study, cytotoxicity studies and antimicrobial activity. Optimized formulation F4 and gel (F41%w/v) showed maximum entrapment efficiency (99.94±0.009%), particle size (275.0±0.01 nm), PDI (0.272±0.001), zeta potential (-44.3±0.1 mV), spherical shape, isotonicity with blood, sterility, and high deformability, pH 7.0±0.01, drug content 98.5±3.9%, dynamic viscosity 3809 mPa-s, high spreadability, in vitro release 80.5±0.32 %, ex vivo permeation 72.27±0.65%, significantly higher ($p \leq 0.05$) flux, permeability coefficient, drug retention, bioadhesion time, and antimicrobial activity than drug solution gel, with low toxicity and stability. The formulated flexible nano-vesicular ciprofloxacin gel was postulated to be a promising choice for the treatment of Bacterial Keratitis (BK).

Keywords: Bacterial keratitis, ciprofloxacin, nano-vesicular carriers, flexible, intraocular, stability.

ÖZ

Bakteriyel keratit (BK); ülserasyon ve inflamasyona yol açarak ciddi görme kaybına neden olabilen ağır bir göz enfeksiyonudur. BK, gelişmekte olan ülkelerde kornea kaynaklı körlüğün başlıca nedenlerinden biridir ve tedavisi, gözün koruyucu yapıları nedeniyle zorluklar içerir; bu yapılar, konvansiyonel formlardan sağlanan ilaç biyoyararlanımını sınırlar. Konvansiyonel dozaj formlarının sınırlamalarını gidermek ve ilacın etkinliğini artırmak amacıyla, siprofloksasin yüklü nano-veziküler taşıyıcılar geliştirilmiş ve ardından ilacın gözle temas süresini uzatmak için jelleşmeyi sağlayan optimize edilmiş bir ajana (Carbopol 940) entegre edilmiştir. Hazırlanan siprofloksasin yüklü esnek nano-veziküler taşıyıcılar ve jeller (F40,4% w/v, F40,6% w/v, F40,8% w/v ve F41% w/v): % hapsetme verimliliği, vezikül şekli, in vitro ilaç salımı ve salım kinetiği, vezikül boyutu, zeta potansiyeli, polidispersite indeksi, izotonisite, sterilite, deforme olabilirlik derecesi, pH, homojenlik ve pürüzlülük, reoloji, ilaç içeriği, ex vivo permeasyon, akı, permeasyon katsayısı, % ilaç tutulumu, toksisite çalışması, biyoadezyon çalışması, sitotoksitesite çalışmaları ve antimikrobiyal aktivite açısından değerlendirilmiştir. Optimize edilmiş F4 formülasyonu ve jeli (F41% w/v); maksimum hapsetme verimliliği (99,94±0,009), partikül boyutu (275,0±0,01 nm), PDI (0,272±0,001), zeta potansiyeli (-44,3±0,1 mV), küresel şekil, kan ile izotonisite, sterilite, yüksek deforme olabilirlik, pH 7,0±0,01, %98,5±3,9 ilaç içeriği, 3809 mPa-s dinamik viskozite, yüksek yayılabilirlik, %80,5±0,32 in vitro salım ve %72,27±0,65 ex vivo permeasyon değerleri sergilemiştir; ayrıca ilaç çözeltisi içeren jele kıyasla anlamlı derecede yüksek ($p \leq 0,05$) akı, geçirgenlik katsayısı, ilaç tutulumu, biyoadezyon süresi ve antimikrobiyal aktivite göstermiş; düşük toksisite ve iyi stabilite özellikleri sergilemiştir. Formüle edilen esnek nano-veziküler siprofloksasin jelinin, Bakteriyel Keratit (BK) tedavisi için umut verici bir seçenek olduğu öngörülmüştür.

Anahtar Kelimeler: Bakteriyel keratit, siprofloksasin, nanoveziküler taşıyıcılar, esnek, göz içi, stabilite.

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* ORCID: 0009-0005-3427-3423, School of Pharmacy, Khalsa University, Amritsar, Punjab, India

** ORCID: 0009-0002-7481-8721, Department of Pharmaceutics, Khalsa College of Pharmacy, Amritsar, Punjab, India

*** ORCID: 0000-0002-2036-3883, Department of Oral and Maxillofacial Pathology, Sri Guru Ram Das Institute of Dental Sciences and Research, Amritsar, Punjab, India

**** ORCID: 0009-0004-6383-8764, School of Pharmacy, Khalsa University, Amritsar, Punjab, India

***** ORCID: 0000-0002-2265-7711, School of Pharmacy, Khalsa University, Amritsar, Punjab, India

° Corresponding Author; Jasjeet Kaur Narang

E-mail: jasjeet2975@yahoo.com

INTRODUCTION

Bacterial keratitis (BK) represents a serious infection with potential to threaten ocular vision that occurs when corneal epithelial integrity is compromised, typically following trauma (Mayandi, Liu, & Liu, 2019). This disruption allows pathogenic bacteria to invade the corneal stroma, resulting in rapid ulceration, intense inflammation, and potentially significant visual impairment if left untreated (Zhou et al., 2024). The main pathogens associated with Bacterial Keratitis (BK) consist of Gram-positive bacteria like *Staphylococcus aureus*, multiple *Streptococcus* species, *Staphylococcus epidermidis*, and *Bacillus* species (Youssef, Cai, Dudhipala, & Majumdar, 2021). Timely and efficient treatment of Bacterial Keratitis (BK) plays a vital role in preventing grave outcomes like corneal perforation, endophthalmitis, or permanent visual impairment (Ung & Chodosh, 2021). Among available therapies, the use of fluoroquinolones [ciprofloxacin (CIP), ofloxacin (OFX), levofloxacin (LFX), moxifloxacin (MOX)] as monotherapy is widely regarded as the preferred therapy due to their broad-spectrum antimicrobial activity and excellent ocular penetration (Yu et al., 2021; Zhou et al., 2024).

Traditional topical eye drops often face challenges with suboptimal bioavailability. This stems mainly from ocular anatomical barriers and natural defenses, including tear production and fast drainage, which result in rapid drug loss and systemic absorption (Sahu et al., 2025). Consequently, less than 5% of the administered therapeutic agent is typically targeted and localized in the eye (Tang et al., 2025). To overcome these challenges, researchers have investigated several advanced drug delivery approaches, such as drug-eluting contact lenses (Guo, Sun, Qiao, Fan, & Zhang, 2025), *in situ* gels (Paul et al., 2025), polymeric micelles (Nunse, Shevalkar, & Borse, 2025), nanoparticles (Tenpattinam et al., 2025), microemulsions (Gautam, Sharma, Yadav, Rajeswari, & Kesavan, 2025), and liposomes (Karmakar, Manna, Jana, Jana, & Nandi, 2025).

Transfersomes (TFs) represent a promising category of ultra-flexible vesicular systems for ocular drug delivery, providing superior corneal penetration and greater therapeutic effectiveness (Mohapatra & Corson, 2025). Originally developed by Cevc, Gebauer, Stieber, Schätzlein, A. and Blume (1998) in the 1990s as the first generation of highly deformable vesicles (Shrivastava, Dewangan, & Shrivastava, 2025), these structures mainly consist of phospholipids and differ from traditional liposomes through their exceptional flexibility, adaptability under pressure, and stress responsiveness. This is primarily due to an edge activator- a single-chain surfactant featuring a large radius of curvature (Souto et al., 2021). Transfersomes can incorporate phospholipid or non-phospholipid amphiphiles, or mixtures thereof, enabling encapsulation of both water-soluble and fat-soluble compounds. They enable transport across barriers driven by osmotic gradients (Opatha, Titapiwatanakun, & Boonpisuttinant, 2020). Their high elasticity permits squeezing through narrow pores much smaller than their diameter without losing integrity (Rai, Pandey, & Rai 2017). Remarkably, they preserve their size and stability post-passage, thanks to the edge activator, which also boosts hydrophobic drug solubility and entrapment efficiency (Lei, Yu, Lin, & Zhou, 2013; Pandey, Prajapati, Sharma, Shivhare, & Agrawal, 2017).

In spite of many targeted dosage forms developed for the treatment of Bacterial Keratitis, no flexible nano-vesicular ciprofloxacin gel has been reported to date, which makes the research novel. The developed formulation would not only overcome the disadvantages of already reported dosage forms for Bacterial Keratitis, such as tear production, nasolacrimal drainage, GI side effects, resistance development etc. (Alsaidan et al., 2022), but would also improve therapy by providing enhanced drug penetration and controlled release with reduction in systemic side effects (Firdos, Haranath, Yaraswini, Sai, & Satish, 2024).

The developed formulation is intended to treat

bacterial keratitis caused by gram-positive *Staphylococcus aureus*. *S. aureus* is reported to be one of the leading pathogens isolated in Bacterial Keratitis, besides being the most common cause of bacterial keratitis, reported to be prevalent in 53.4 lakh cases. It is also recognized for its strong capability to resist antibiotics (Marquart & O'Callaghan, 2013). Ciprofloxacin achieves 91% clinical improvement and 60% bacteriologic cure in Methicillin-resistant *Staphylococcus aureus* (MRSA), showing 2-3 log better efficacy against *S. aureus* in corneal infections as compared to other fluoroquinolones (Andre, Van Camp, Ung, Gilmore, & Bispo, 2024). In view of the above facts, the study is intended to formulate flexible nano-vesicular ciprofloxacin gel for effective treatment of Bacterial Keratitis as compared to other reported or marketed dosage forms.

In the present investigation, intraocular targeted nano-vesicular carriers loaded with ciprofloxacin to treat bacterial keratitis using the thin-film hydration technique on a rotary evaporator were developed, and to enhance rheological characteristics, these carriers were incorporated into a gelling matrix. Both the resulting suspension and gel formulations underwent comprehensive evaluation for key parameters.

MATERIALS AND METHODS

Materials

Ciprofloxacin was procured from Kwality Pharmaceutical Ltd, Amritsar. Sodium deoxycholate, Soya lecithin, Carbopol 934, potassium chloride, triethanolamine, mannitol salt agar, and nutrient broth were purchased from Hi-Media, Mumbai, India. Tween 80, sodium bicarbonate, Carbopol 940, calcium chloride

dihydrate, and hydrochloric acid were obtained from Qualikems Fine Chem Pvt. Ltd., Vadodara, India. Span 80 and sodium sulphide were procured from Central Drug House, New Delhi, India. Chloroform was procured from Molychem, Mumbai, India. Soyabean casein digest medium (SCDM) as well as fluid thioglycolate medium (FTM) were bought from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Chemicals of analytical/reagent grade were procured and directly used as supplied.

Formulation of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carriers

Flexible nano-vesicular carriers loaded with ciprofloxacin for intraocular targeting were formulated via the thin-film hydration technique using a rotary evaporator. Edge activators (Tween 80, sodium deoxycholate, Span 80) and Soya lecithin (phospholipid) were dissolved in chloroform at varying concentrations. The solvent was then removed under vacuum at 45 °C, yielding a uniform dry lipid film. Hydration of this film occurred in the final step using distilled water containing the drug (Mohammad, Ali, & Khan, 2024). In order to maintain sterility of the developed formulation, all the formulations were prepared under aseptic conditions. All components (soya lecithin, Tween 80, drug solution) were sterile-filtered through 0.22 µm membranes before mixing in a Class II laminar flow hood according to ISO class 5 guidelines for sterility (ISO, 2015). Specific sterility methods like moist heat, dry heat, etc. were not used as these are reported to be unsuitable for nano formulations (Bento, Gaspar, Coimbra, de Sousa, & Braga, 2023). The different compositions of formulated drug-loaded flexible nano-vesicular carriers are given in Table 1.

Table 1. Different compositions of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carriers

Formulation code	Drug (mg)	Soya lecithin (%)	Sodium deoxycholate (mg)	Tween 80 (mg)	Span80 (mg)	Distilled Water (ml)
F1	30	3	90	-	-	10
F2	30	4	120	-	-	10
F3	30	5	150	-	-	10
F4	30	3	-	90	-	10
F5	30	4	-	120	-	10
F6	30	5	-	150	-	10
F7	30	3	-	-	90	10
F8	30	4	-	-	120	10
F9	30	5	-	-	150	10

Evaluation of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carriers

Entrapment efficiency

Drug-loaded flexible nano-vesicular carriers in Eppendorf tubes were subjected to centrifugation at 4°C and 13,000 rpm for 20 min (Youssef, Dudhipala, & Majumdar, 2020). After discarding the supernatant, vesicle disruption was achieved with methanol:chloroform (2:1), followed by filtration to quantify en-

trapped drug. Entrapped ciprofloxacin was analysed spectrophotometrically at 278nm using a validated method with methanol:chloroform (2:1) as the blank. The analytical method was validated for accuracy, percentage recovery, precision, LOD and LOQ. Entrapment efficiency (EE %) for the nano-vesicular carriers was determined from three replicates and calculated using the formula reported by Zubairu, Negi, Iqbal and Talegaonkar (2015):

$$\% \text{Entrapment efficiency} = \frac{\text{Quantity of added drug} - \text{Quantity of drug in freeform}}{\text{Total Quantity of drug}} \times 100$$

In vitro drug release study: *In vitro* release of drug from ciprofloxacin-encapsulated nano-vesicular carriers and drug solution was assessed using a Franz diffusion cell with a treated dialysis membrane (El-Gizawy, Khattab, & Zein El-Dein, 2020). Simulated tear fluid (pH 7.4), prepared by dissolving sodium chloride (0.670 g), calcium chloride dihydrate (0.008 g), and sodium bicarbonate (0.2 g), in 100 mL distilled water, served as the medium in the receptor compartment. The dialysis membrane was placed between the two compartments of the cell. Precisely 1 ml of each developed dosage form was applied onto the membrane. The assembly was placed on a magnetic stirrer at $37 \pm 2^\circ\text{C}$ with continuous agitation. At predefined time intervals, aliquots were withdrawn, replenished with equal volumes of fresh medium, followed by filtration using a $0.45 \mu\text{m}$ membrane, and analyzed for drug

content via a validated UV spectrophotometric method at 271 nm. Cumulative percent drug release versus time was graphed (Nanda, Mehak, Narang, & Narang, 2022). The procedure was repeated three times.

Determination of release rate orders: The release kinetics was studied by various kinetic models (Zero order, first order, Higuchi, and Korsmeyer-Peppas plot). To study the release kinetics of the formulation, data obtained from *in vitro* drug release studies were plotted in various kinetic models: zero order as % cumulative amount of drug release v/s time, first order as log cumulative % of drug remaining v/s time, Higuchi model as cumulative % of drug release v/s square root of time, and Korsmeyer Peppas as log cumulative drug release v/s log time. The best fit model was confirmed by the value of the correlation coefficient close to 1 (Askarizadeh, Esfandiari, Honarvar, Sajadian, & Azdarpour, 2023; Dhawan, Aggarwal, & Harikumar, 2014).

Vesicle size, zeta potential and polydispersity index of optimized formulation F4: The vesicle size and polydispersity index of the optimized formulation F4 were measured using Litesizer 500 at 25 ° C. Similarly, Zeta potential was determined using Litesizer 500 (Zubairu et al., 2015).

Vesicle shape: The shape of the formulated optimized formulation F4 was observed under the Olympus microscope at 10X and 40X.

Isotonicity: Isotonicity must be preserved to avoid ocular tissue damage or irritation. A blood smear from the optimized F4 formulation, stained with Leishman's stain, was prepared, washed, and air-dried. RBC morphology was examined under optical microscopy at 10× and Olympus microscopy at 40× magnification. Identical procedures were applied to create blood smears with isotonic, hypertonic, and hypotonic solutions. RBC size and shape from the F4-treated sample were compared against those from the control solutions (Isotonic, Hypertonic, Hypotonic) (Mahajan, Patel, & Kareliya, 2020). All observations were conducted in triplicate.

Sterility testing: Sterility testing employed the direct inoculation method. Aseptically, 1 mL of optimized F4 formulation was inoculated into 10 mL each of fluid thioglycolate medium (FTGM) and soybean casein digest medium (SCDM). Incubation of FTGM was done at 32–35 °C, while SCDM was maintained at 20–25 °C for 7 days, with positive controls (uninoculated media) used for comparison (Mahajan et al., 2020).

Degree of Deformability: The degree of deformability was assessed using a DLS (Dynamic Light Scattering) method in conjunction with a microporous filter test, as deformability influences the permeation of intraocular flexible nano-vesicular carrier formulation. In this study, intraocular flexible nano-vesicular carrier formulation (1ml) was passed through a microporous filter (0.22 micrometer). The vesicle size before and after filtration to assess deformability was monitored by DLS measurements, and the deforma-

bility index to calculate the degree of deformability was calculated by the formula given below (Marwah, Badhan, & Lowry, 2022):

$$DI = 100 - LE/L \times 100$$

Where,

DI= Deformability index

LE = Vesicle size after extrusion (nm)

L = Size of the vesicle before extrusion (nm)

Preparation of intraocular flexible nano-vesicular carrier-based gel

Gelling agent selection

Viscosity of the optimized F4 formulation was enhanced by incorporating gelling agents such as Carbopol 940, Carbopol 934, as well as sodium alginate (1% w/v concentration) under continuous stirring. The pH of Carbopol-based formulations was neutralized to 7.0 using triethanolamine, with stirring continued until a homogeneous clear gel formed. Formulations were stored for 24 hours, followed by assessment for grittiness, transparency, as well as phase separation, leading to selection of the most suitable gelling agent based on performance characteristics (Kaur et al., 2018).

Optimization of optimum gelling agent concentration

The gelling agent concentration required to produce a stable as well as clear intraocular flexible nano-vesicular carrier-based gel, with absence of grittiness, was optimized further. Drug-loaded intraocular flexible nano-vesicular carriers containing different concentrations (0.2 %w/v, 0.4% w/v, 0.6% w/v, 0.8% w/v and 1% w/v) of selected gelling agent were formulated and characterized (Dar et al., 2020).

Evaluation of intraocular flexible nano-vesicular carrier-based gel

Physical characterization of intraocular flexible nano-vesicular carrier-based gel: Physical characterization like color, odour, appearance, phase separation and, consistency of prepared gels (F4_{0.2 % w/v}, F40.4%

$F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$) was checked at room temperature (Abou El Ela & El Khatib, 2014). The analysis was performed three times.

pH: The developed formulations ($F4$ at 0.2% w/v, 0.4% w/v, 0.6% w/v, 0.8% w/v, and 1% w/v) were evaluated for pH with the use of a digital pH meter at ambient temperature (Nanda et al., 2022). Measurements were done thrice to ensure reliable as well as reproducible results.

Grittiness and Homogeneity: Grittiness as well as homogeneity of the formulated gels were evaluated manually by pressing a small quantity between thumb and index finger (Nanda et al., 2022). The evaluations were done thrice to ensure reproducibility as well as consistency in texture evaluation.

Viscosity: Viscosity measurements of gel formulations (0.4%, 0.6%, 0.8%, 1% w/v) were performed using a Brookfield viscometer with spindle 64 at $37 \pm 0.5^\circ\text{C}$ and speed of 60 rpm, after a 2-minute wait time. Each determination was conducted in triplicate, with standard deviations calculated (Mehdi-Alamdarloo, Mehrabani, Imani, & Jelvehgari, 2020).

Drug content: Drug content in 1 g samples of ciprofloxacin-loaded intraocular flexible nano-vesicular carrier gels ($F4$ at 0.6% w/v, 0.8% w/v, and 1% w/v; 0.30% w/v drug) was determined by dissolving in 10 mL methanol, shaking for 10-15 minutes, filtering, and diluting 1 mL filtrate using methanol. Absorbance was measured using a UV-visible spectrophotometer at λ max 278 nm and concentrations calculated from a standard curve (Phaladesai, Shabaraya, Shripathy, & Azharuddin, 2014). All analyses were performed thrice.

In vitro drug release study: The release from ciprofloxacin-loaded intraocular flexible nano-vesicular carrier gels ($F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$) and a 1% drug solution gel was studied using a Franz diffusion cell with a treated dialysis membrane. Simulated tear fluid (pH 7.4) served as the receptor medium; 1 g of gel was applied to the donor chamber, maintained at $37 \pm 2^\circ\text{C}$ with constant stirring. At predefined in-

tervals, aliquots were taken, replaced with the same volumes of fresh medium, followed by filtration using a 0.45 μm filter membrane and evaluation using UV spectrophotometry at λ max 271 nm against a ciprofloxacin calibration curve to calculate cumulative percent release, which was plotted versus time (Nanda et al., 2022). All experiments were performed in triplicate.

Rheological studies: For intraocular flexible nano-vesicular carrier-based gels (0.6%, 0.8%, and 1% w/v), rheological characteristics were evaluated by an Anton Paar rheometer at 25°C with a PP25 cone-plate geometry. Viscosity measurements were recorded across 25 data points under linear ramp conditions (Nanda et al., 2022). All analyses were done thrice.

Spreadability: The spreadability of the prepared intraocular flexible nano-vesicular carrier-based gels ($F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$) was assessed using the parallel-plate method. Exactly 1 g of gel, prepared 48 h before testing, was placed between two 20×20 cm plates made of glass. After 1 min, a 500 g weight was applied on top for 1 min. The spreading diameter (d , in mm) of the gel was then measured. Spreadability was quantified as the spreading area (S_p , in mm^2) using the formula:

$$S_p = d^2\pi/4 \quad (\text{Bakhrushina et al., 2022})$$

Ex vivo corneal permeation: This was assessed using a Franz diffusion cell setup with freshly excised goat corneas. One gram each of the optimized $F4$ (1% w/v) nano-vesicular gel and drug solution gel was applied evenly to the donor side of the corneal membrane. The receptor compartment contained simulated tear fluid (pH 7.0), at $37 \pm 0.5^\circ\text{C}$ under magnetic stirring. Samples were taken periodically, replenished with fresh medium and quantified via validated UV spectrophotometry at 271 nm against a standard curve. In order to avoid interference from extracted proteins, the goat cornea was first stabilized using simulated tear fluid pH 7.4 before performing *ex-vivo* studies as per the procedure given by Badran, Alsubaie, Bekhit, Alomrani and Almomen (2025).

For stabilization, the goat corneal membrane was mounted between the receptor compartment, and donor compartment. The simulated tear fluid pH 7.4 present in the receptor compartment was allowed to stir for 15 min on a magnetic stirrer and after 15 min, the solution was removed, and fresh simulated tear fluid pH 7.4 was filled in the Franz diffusion Cell. The temperature of the receptor chamber containing diffusion media (simulated tear fluid pH 7.4) was controlled at 37 ± 1 °C (Dave, Paliwal, Yadav, & Sharma, 2015) while the contents were magnetically stirred for proper mixing. The simulated tear fluid pH 7.4 was replaced every half an hour to stabilize the cornea. The cornea was stabilized for 4-5 hours, with simulated tear fluid being replaced 8 to 10 times. After stabilization, absorbance was recorded using a UV spectrophotometer, and the cornea was considered stabilized when no absorbance was observed (Gaba et al., 2019). Absence of absorbance was considered to be evidence that there was no interference from extracted biological proteins, and cumulative permeation was plotted as percent drug permeated versus time (Pathak, Chhabra, & Pathak, 2013). All runs were done in triplicate.

Determination of flux and permeability coefficient: The quantity of ciprofloxacin permeating through excised goat cornea from the intraocular flexible nano-vesicular carrier gel (F4 1% w/v) and drug solution gel—expressed as Q (g/cm^2)—was plotted versus time (h). Flux at steady-state (J_{ss} , $\text{g}/\text{cm}^2/\text{h}$) was obtained from the slope of the linear regression under steady-state conditions, while the permeability coefficient (k_p) was calculated by dividing (J_{ss}) by the initial drug concentration (C_o) in the donor chamber (Badran, Alomrani, Alharbi, & Alanazi, 2024). Measurements were performed in triplicate.

Determination of drug retention in the corneal membrane: Residual formulation on the excised goat corneal membrane was removed using cotton soaked in 0.05% sodium lauryl sulfate, followed by rinsing with distilled water. The membrane was then weighed,

minced into small pieces, followed by sonication in methanol for 15 min to extract ciprofloxacin. The solution obtained was centrifuged, filtered, and analyzed for the content of the drug ($\mu\text{g}/\text{cm}^2$) using UV spectrophotometry (Nanda et al., 2022). Experiments were conducted in triplicate.

Toxicological evaluation: They were performed to estimate the ocular safety of the developed formulation. The following assessments were performed to investigate potential adverse effects on the corneal tissue (Zubairu et al., 2015).

Corneal Hydration: Corneal hydration levels were estimated by utilizing the same goat corneas that were used in the study for estimating permeation. After the experiment, the weight of each cornea was taken, immersed in methanol (1 mL), dried overnight, and weighed again. Corneal hydration was estimated from the weight difference (Nanda et al., 2022). Experiments were performed in triplicate.

Histopathological evaluation: Following the *ex vivo* permeation study, each cornea of the goat was removed from the Franz diffusion cell and preserved in 10% formalin solution. Two untreated corneas served as controls, maintained separately in potassium chloride and normal saline solutions before formalin fixation. Sections of cornea were then sliced, stained using hematoxylin and eosin, and examined microscopically (Zubairu et al., 2015).

Effect of formulation on corneocytes LDH assay: To understand the effect of formulation on corneocytes, the assembly was set following the procedure used for setting the assembly for the *ex vivo* permeation study. The drug-loaded formulation and normal saline were placed in the donor compartment in separate diffusion cells. Normal saline was employed as a negative control. The receptor compartment was filled with Tyrode solution. After 60 minutes, the samples were withdrawn at predetermined times from the receptor compartment and analysed by using the Coral Clinical Systems LDH assay kit (Zubairu et al., 2015). The experiment was repeated thrice.

Bio adhesion study: The study followed the procedure outlined by Nanda and coworkers with minor changes. An agar plate was prepared, and the test sample was positioned at its center. After 5 minutes, the plate was mounted on an IP disintegration tester and moved up and down in simulated tear fluid maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$, with the sample fully immersed at the lowest point and exposed at the highest. Visual observation of the formulation's retention on the plate determined the time of residence of the elastic liposome-based gel (Nanda et al., 2022). Tests were conducted in triplicate.

Antimicrobial activity and determination of zones of inhibition: *Staphylococcus aureus* suspension was prepared by dissolving lyophilized powder in 100 mL nutrient broth, and then incubating it at 37°C for 24-48 hours. A 50 μL aliquot was then diluted in 500 μL sterile saline to get a final concentration of 5×10^7 CFU/mL (Pandey et al., 2017). From this suspension, 50 μL was aseptically spread onto nutrient agar plates using a sterile cotton swab by rotating the plate 60° between applications and pressing firmly around the edges. Plates were dried at room temperature with lids closed. Three 3-mm wells were punched using a sterile cork borer, filled respectively with nano-vesicular carrier gel ($\text{F4}_{1\% \text{ w/v}}$; 1 g containing 3 mg ciprofloxacin), plain gel (1 g), and drug-loaded gel (1 g containing 3 mg ciprofloxacin), then refrigerated for 2 hours to promote drug diffusion. Plates were incubated for 24-72 hours at 37°C , with zones of inhibition measured and compared across formulations (Upadhyay, Kumar, & Pathak, 2016). All experiments were repeated thrice.

Evaluation of the safety of the developed formulation using cell lines by determining % cell viability using MTT cytotoxicity assay: The cytotoxicity profile of the different formulations of ciprofloxacin was evaluated on L929 cells (Fibroblast-like cells originating from the subcutaneous connective tissue of C3H/An mice) using the MTT assay. The cytotoxicity study was performed on L929 cells, as L929

cells (mouse fibroblasts) are the globally standardized model reported in the literature for *in vitro* cytotoxicity testing under ISO 10993-5 (Ramakrishnan & Daly, 2026). The cell line has been reported in the literature for studying the cytotoxicity of nanofibrous and film-structured ciprofloxacin hydrochloride inserts for sustained ocular delivery, thus confirming their use for studying ocular formulations (Taghe, Mirzaeei, & Ahmadi, 2023). DMEM media, supplemented with 10% v/v 10% Fetal Bovine Serum (FBS), 100U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin, and 2mmol/l L- glutamine and maintained at 37°C with 5% CO_2 was used to grow cells. For the assay, Cells were seeded into 96-well plates at 1×10^4 cells/well, followed by incubation to achieve 80-90% confluency. Cells were then treated with various groups ($\text{F4}_{1\% \text{ w/v}}$ nano-vesicular gel, F4 nano-vesicular suspension, placebo gel, drug-loaded gel, drug solution) at ciprofloxacin concentrations of 12.5, 25, 50, and 100 $\mu\text{g/mL}$ for 24 hours (Taghe et al., 2023). Equivalent dilution volumes of placebo gel were applied to expose cells to the formulation excipients. Post-treatment, cells underwent PBS washing, followed by incubation with MTT solution (5 mg/mL) for 3 hours; subsequently, 100 μL DMSO was added per well for dissolving the purple formazan crystals. Absorbance readings were obtained at 570 nm via an ELISA multi-plate reader (Ghasemi, Turnbull, Sebastian, & Kempson, 2021), using a scanning multiwall spectrophotometer, and based on this absorbance value, the following equation was utilized for calculating % cell viability (Rai et al., 2025).

Cell Viability % = $\frac{\text{Absorbance of cells after treatment}}{\text{Absorbance of cells without treatment}} \times 100$ (Nigam, Atanassova, & Mishra, 2019)

Stability studies: The optimized drug-loaded intraocular flexible nano-vesicular carrier gel (F4 , 1% w/v) underwent stability testing over 30 days under room temperature and refrigerated ($4 \pm 1^{\circ}\text{C}$) conditions. Post-storage evaluation included assessments for creaming, physical stability, phase separation, par-

ticle size, drug content, and zeta potential (Shakeel, Baboota, Ahuja, Ali, & Sheikh, 2008).

Statistical analysis: Data are presented as mean $\pm$ standard deviation (SD). Statistical analysis of results from different groups employed GraphPad InStat 3 software with two-tailed unpaired t-tests. Statistical significance was assigned at $p \leq 0.05$ (Lu et al., 2020).

RESULTS

Entrapment efficiency: The entrapment efficiency of the developed formulations was determined by using a validated UV spectroscopy method. The method exhibited good accuracy with a mean recovery of 99.82%. The individual recovery values ranged from 98.52% to 102.65%. The precision was acceptable, with a relative standard deviation (RSD) of 1.31%. The sensitivity of the method was supported by a limit of detection (LOD) of 1.03 $\mu\text{g/ml}$ and a limit of quantification (LOQ) of 3.12 $\mu\text{g/mL}$. The entrapment efficiency (EE) of ciprofloxacin-loaded flexible nano-vesicular carriers ranged from 82.53% to 99.94%, with optimized formulation F4 consisting

of Tween 80 as edge activator showing the highest Entrapment of 99.94% among all formulations.

In vitro evaluation: The *in vitro* release of the developed formulations was evaluated using a validated UV spectroscopic method. The method exhibited good accuracy with a mean recovery of 99.75%. The individual recovery values ranged from 94.91% to 104.04%. The precision was acceptable, with a relative standard deviation (RSD) of 1.78%. The sensitivity of the method was supported by a limit of detection (LOD) of 2.27 $\mu\text{g/ml}$ and a limit of quantification (LOQ) of 6.88 $\mu\text{g/ml}$. The optimized ciprofloxacin-loaded formulation (F4) exhibited a sustained release profile over 360 min, with cumulative release increasing progressively from $6.76 \pm 0.019\%$ at 5 min to $98.92 \pm 0.008\%$ at 360 min, achieving near-complete release within 6 hours. Conversely, the plain ciprofloxacin solution displayed rapid release, reaching $93.70 \pm 0.049\%$ ($0.8031 \pm 0.011 \mu\text{g/mL}$) by 120 min, followed by a plateau at nearly 92–93% from 180–360 min. Figure 1 illustrates the *in vitro* release profiles of both F4 formulation and drug solution.

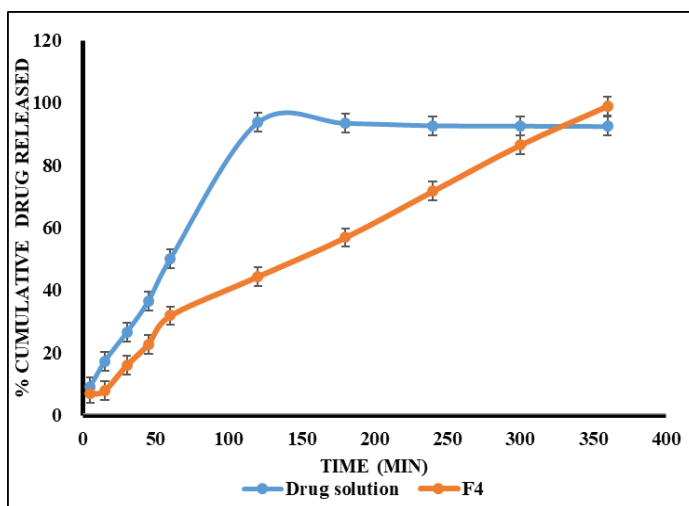


Figure 1. *In vitro* release profile of optimized formulation F4 and drug solution

Determination of release rate orders: The release kinetics of the optimized ciprofloxacin-loaded flexible nano-vesicular carrier (formulation F4) were analyzed to determine the most appropriate release rate mod-

el. The release kinetics data are illustrated in Table 2 and Figure 2. The correlation coefficient (R^2) values were calculated for different kinetic models, namely zero order, first order, Higuchi, and Peppas-Korsmey-

er. The R^2 values were found to be 0.987 for the zero order model, 0.799 for the first-order model, 0.984 for the Higuchi model, and 0.975 for the Peppas-Korsmeyer model. Among these, the zero-order model exhibited the highest correlation coefficient, closest to unity, indicating that the release of ciprofloxacin from the nano-vesicular formulation F4 followed a zero-order release profile.

Table 2. Correlation coefficient (R^2) values for different kinetic models

Kinetic model	Correlation coefficient (R^2) values
Zero-order	0.987
First-order	0.799
Higuchi	0.984
Peppas-Korsmeyer	0.975

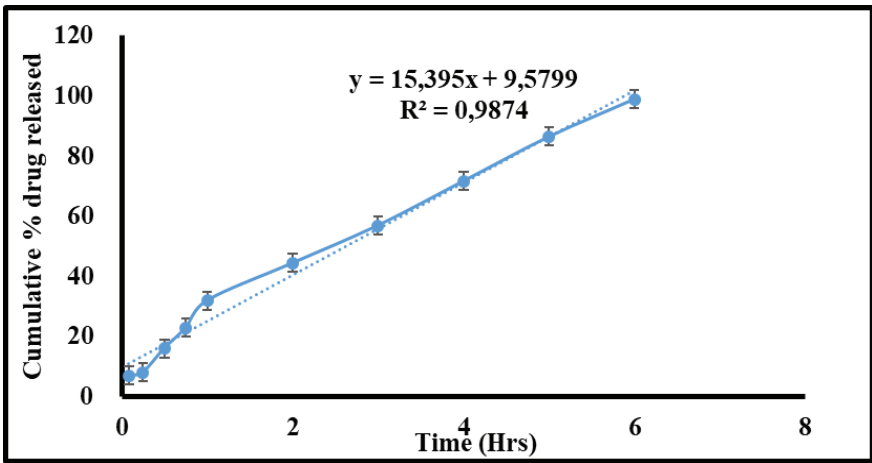


Figure 2. Kinetic profile of optimized formulation F4 by Zero-order release

Vesicle size, polydispersity index and zeta potential of formulations: Vesicle size, polydispersity index and zeta potential of the optimized formulation were estimated as per the procedure. The physico-chemical characterization of formulation F4 revealed a vesicle size of 275.0 ± 0.01 nm (Figure 3.), indicating the formation of nanosized vesicles suitable for drug delivery applications. The graph in Figure 3 has an interval of 100 to 1000 on the X-axis. Since the reported interval 100-1000 is large, the reported value (275nm) seems closer to 100nm. The polydispersity index (PDI) was determined to be 0.272 ± 0.001 , suggesting a moderately uniform size distribution within the formulation (Ali & Hussein, 2017). Moreover, the zeta potential was found to be -44.3 ± 0.1 mV (Figure 4.).

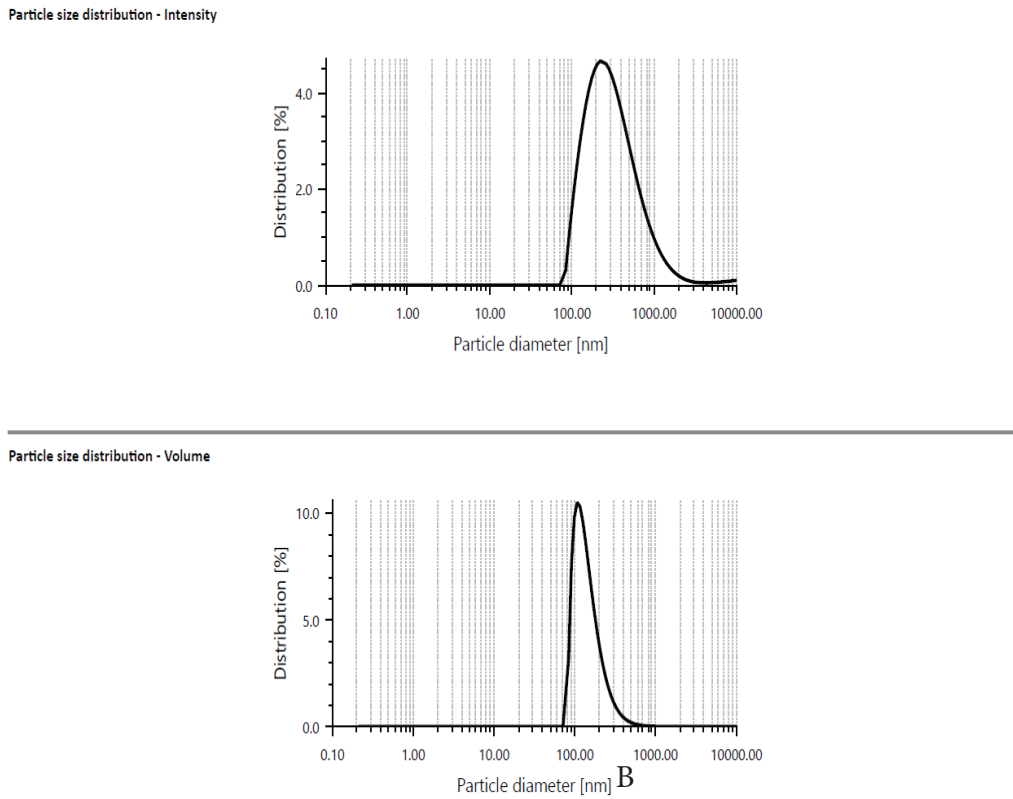


Figure 3. Vesicle size and PDI of formulation F4

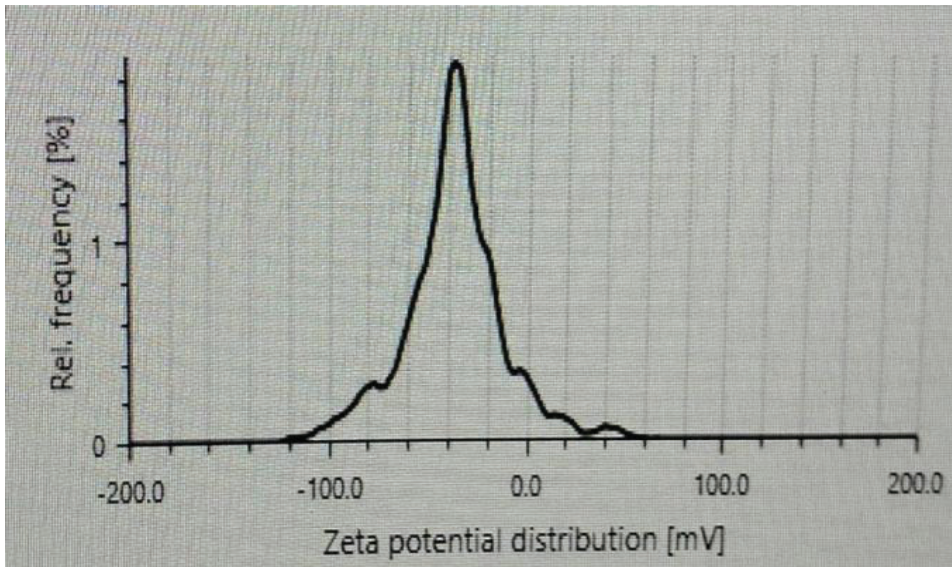


Figure 4. Zeta potential of formulation F4

Vesicle shape: The shape of the optimized formulation was observed as per the procedure. The shape of the vesicles of the optimized formulation was found to be spherical with significant core and coat parts (Figure 5A-B.).

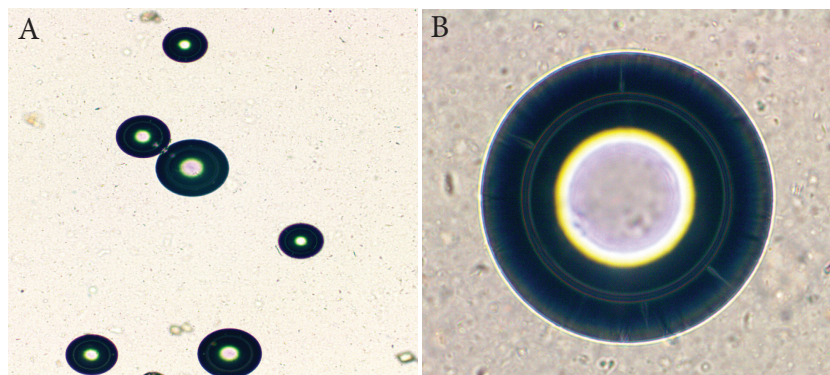


Figure 5. Representative images of optimized nano-vesicular carrier formulation (F4) with vesicle size in micrometers observed under Olympus microscope A) at 10X and B) at 40X

Isotonicity: Isotonicity of ciprofloxacin-loaded flexible nano-vesicular carrier formulation F4 was determined as per the procedure. After observing the blood cells using a light microscope and Olympus microscope, it was concluded that Blood cells exposed to hypertonic solution shrank, while those treated with

hypotonic solution swelled. The cells treated with the ciprofloxacin-loaded flexible nano-vesicular carrier F4 suspension did not cause lysis of the blood cells and maintained their shape similar to that observed after treatment with normal saline. The results are illustrated in Figure 6 (A-D) and Figure 7 (E-H).

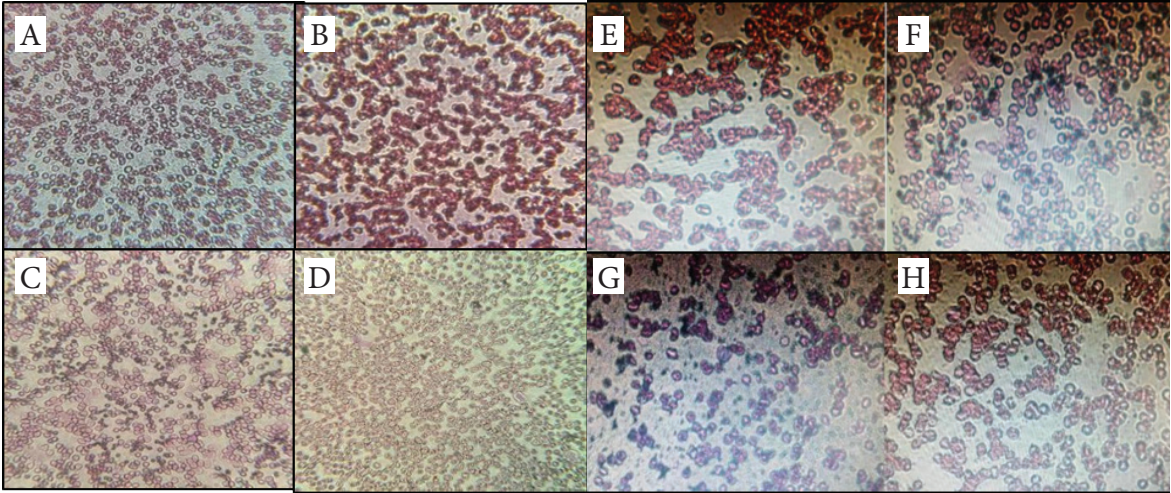


Figure 6. Images under normal microscope at 10 X A) RBCs in isotonic solution B) RBCs in hypotonic solution C) RBCs in formulation D) RBCs in hypertonic solution

Figure 7. Images under olympus microscope at 40 X E) RBCs in hypotonic solution F) RBCs in isotonic solution G) RBCs in hypertonic solution H) RBCs in formulation

Sterility testing: The sterility testing of the optimized ciprofloxacin-loaded flexible nano-vesicular carrier formulation F4 was determined as per procedure. Results indicated no turbidity in the formulation when incubated in fluid thioglycolate medium at 32 °C to 35 °C and soyabean casein digest medium at 20

°C to 25 °C for 7 days, confirming the sterility of the formulation.

Degree of deformability: The degree of deformability of the optimized ciprofloxacin-loaded flexible nano-vesicular carrier F4 formulation was calculated as per the procedure. The degree of deformability of

the optimized ciprofloxacin-loaded flexible nano-vesicular carrier formulation was evaluated by measuring the particle size before and after extrusion. The particle size before to extrusion was 275.0 ± 0.01 nm, and after extrusion, it slightly decreased to 274.5 ± 0.50 nm. This minimal change indicated no significant difference ($p < 0.05$), confirming that the vesicles retained structural integrity during the extrusion process. The degree of deformability was recorded as 1.247 ± 0.010 , while the deformability index was calculated to be 0.181 ± 0.050 .

Table 3. Composition and observations of intraocular flexible nano-vesicular carrier-based gel formulations formulated using different gelling agents

S.No.	Polymers	Concentration (w/v)	Observations
1	Carbopol 934	1%	Translucent and stable
2	Carbopol 940	1%	Clear and stable with good consistency
3	Sodium alginate	1%	Poor consistency, not clear

Based on the observations, it was concluded that gels formulated using Carbopol 940 were stable, clear, and exhibited good consistency. Based on the conclusions, Carbopol 940 was selected as the agent for inducing gelling. Further gels with different concentrations of carbopol 940 (from 0.2% to 1% w/v) containing optimized formulation F4 ($F4_{0.2\% \text{ w/v}}$, $F4_{0.4\% \text{ w/v}}$, $F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$) were formulated.

Evaluation of prepared intraocular flexible nano-vesicular carrier-based gel

Physical characterization: The different gels ($F4_{0.2\% \text{ w/v}}$, $F4_{0.4\% \text{ w/v}}$, $F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$) prepared were assessed for physical appearance under

Preparation of intraocular flexible nano-vesicular carrier-based gel

Gelling agent selection: Various gel formulations containing 1% of different gelling agents were prepared to identify the optimal agent. These gels underwent characterization for key parameters, with results summarized in Table 3. Carbopol 940 emerged as the most suitable based on these findings and was chosen for formulating intraocular flexible nano-vesicular carrier gels.

normal daylight conditions. Even though, all formulations were satisfactory in physical characteristics with no phase separation and slight cloudiness, formulations $F4_{1\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$, and $F4_{0.6\% \text{ w/v}}$ were chosen for further studies as they exhibited satisfactory flow properties and good consistency due to a more gelling effect, which could favour retention of the dosage form in the eye (Cassano, Di Gioia, & Trombino 2021). The formulation $F4_{0.2\% \text{ w/v}}$ and $F4_{0.4\% \text{ w/v}}$ had high flow properties and less viscosity when observed, which was inappropriate for intraocular retention and was therefore not taken up for further studies. The results are presented in Table 4.

Table 4. Physical characterization of intraocular flexible nano-vesicular carrier based gel ($F4_{0.2\% \text{ w/v}}$, $F4_{0.4\% \text{ w/v}}$, $F4_{0.6\% \text{ w/v}}$, $F4_{0.8\% \text{ w/v}}$ and $F4_{1\% \text{ w/v}}$)

S.no	Property	$F4_{0.2\% \text{ w/v}}$	$F4_{0.4\% \text{ w/v}}$	$F4_{0.6\% \text{ w/v}}$	$F4_{0.8\% \text{ w/v}}$	$F4_{1\% \text{ w/v}}$
1	Colour	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
2	Appearance	Clear	Clear	Clear	Clear	Clear
3	Odour	Odourless	Odourless	Odourless	Odourless	Odourless
4	Phase separation	No	No	No	No	No
5	Consistency	+	++	++	+++	++++

pH: The pH of the prepared intraocular flexible nano-vesicular carrier-based gels (F4_{0.6 w/v}, F4_{0.8 w/v} and F4_{1.0 w/v}) was found in the range of 7.0-7.35, which is the physiological range to avoid irritation after intraocular administration. The results are given in Table 5.

Homogeneity and grittiness: The homogeneity as well as grittiness of the prepared gel dosage forms were evaluated, revealing that all formulations were

uniform and free of gritty particles.

Viscosity: The viscosity of F4_{1.0 w/v} was found to be maximum, whereas formulations F4_{0.6 w/v} and F4_{0.8 w/v} had medium viscosity. Also, formulations F4_{0.2 w/v} and F4_{0.4 w/v} were having comparatively poor viscosity, which was not ideal for intraocular administration and therefore were not taken up for further studies (Dasankoppa et al., 2017). The results are presented in Table 5.

Table 5. Results of various nano-vesicular carrier-based gel formulations

S.no.	Formulation code	pH ± S.D (n=3)	Homogeneity and grittiness	Viscosity (cP) ± S.D (n=3)	Drug content
1	F4 _{0.6 w/v}	7.02± 0.018	Homogeneous and no grittiness	3263 ± 0.1	98.53 ± 0.091
2	F4 _{0.8 w/v}	7.32 ± 0.013	Homogeneous and no grittiness	3519 ± 0.3	98.07 ± 0.005
3	F4 _{1.0 w/v}	7.26 ± 0.011	Homogeneous and no grittiness	6659 ± 0.1	99.20 ± 0.017

Drug content: The drug content of prepared intraocular flexible nano-vesicular carrier-based gel formulations (F4_{0.6 w/v}, F4_{0.8 w/v} and F4_{1.0 w/v}) was evaluated and was found to range from 98.53% to 99.20%. Table 5 represents the results of the drug content determined. Among the intraocular flexible nano-vesicular carrier-based gels evaluated, the formulation containing 1% w/v Carbopol 940 (F4_{1 w/v}) exhibited

maximum drug content of 99.20%.

Rheological study: Rheological studies revealed that as the gelling agent concentration was increased, there was a proportional enhancement in the formulation's viscosity. The different gel formulations (F4_{0.6 w/v}, F4_{0.8 w/v} and F4_{1.0 w/v}) exhibited viscosities of 1634 ± 0.5, 2689 ± 0.2, 3809 ± 0.3 mPa.s, respectively. The results are given in Figure 8 to 10.

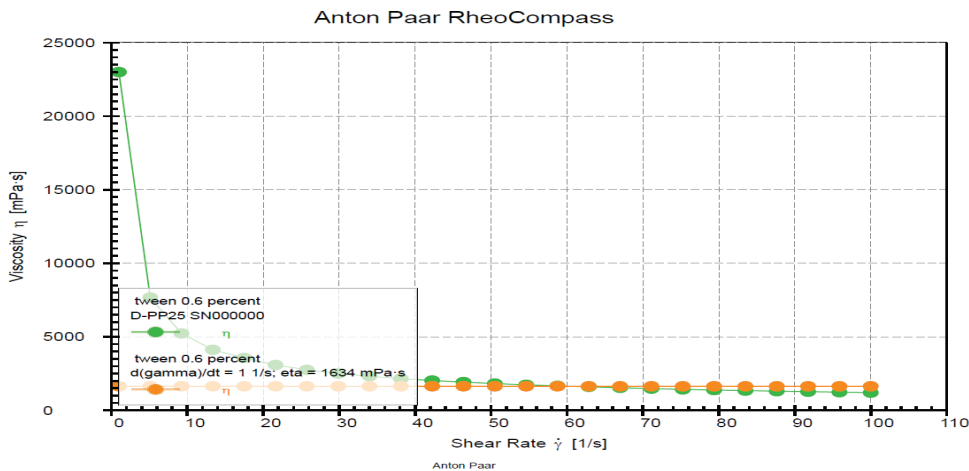


Figure 8. Rheogram of intraocular nano-vesicular gel F4_{0.6 w/v}

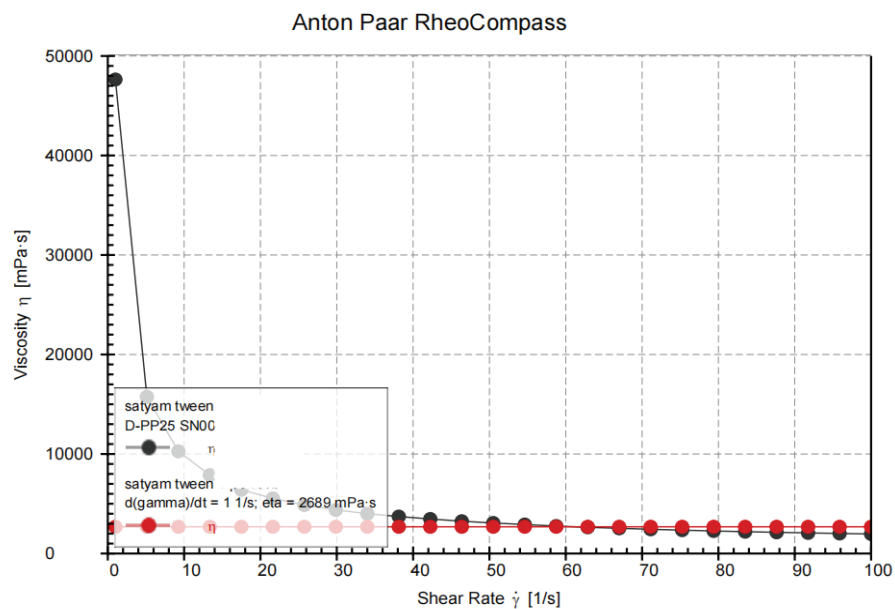


Figure 9. Rheogram of intraocular nano-vesicular gel F4_{0.8w/v}

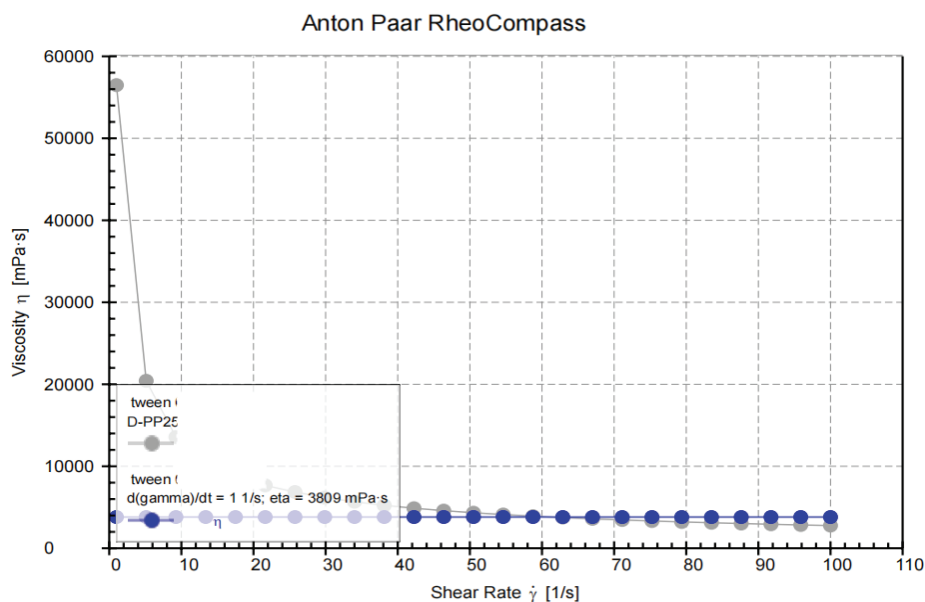


Figure 10. Rheogram of intraocular nano-vesicular gel F4_{1w/v}

In vitro drug release: The cumulative percentage release of drug from the drug solution gel was observed to be significantly ($p \leq 0.05$) more (92.28 ± 0.081) when compared with release from the intraocular flexible nano-vesicular carrier-based gel over a six-hour period. A six hours cumulative percentage release of drug from various intraocular flexible na-

no-vesicular carrier-based gel formulations (F4_{0.6w/v}, F4_{0.8w/v} and F4_{1.0w/v}) was observed to be $91.12 \pm 0.022\%$, $88.32 \pm 0.011\%$ and $83.07 \pm 0.004\%$, respectively. *In vitro* release profile of different intraocular flexible nano-vesicular carrier-based gels as well as drug solution gel is represented in Figure 11.

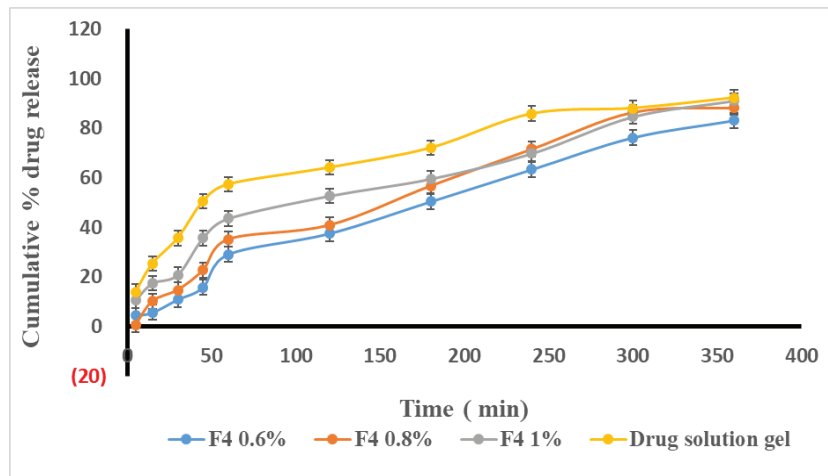


Figure 11. Comparison of *in vitro* drug release profile of F4_{0.4% w/v}, F4_{0.6% w/v}, F4_{1% w/v} and drug solution gel

Based on the results of physical characterisation, pH, homogeneity and grittiness, viscosity, *in vitro* release, drug content, as well as rheological study, among all the intraocular flexible nano- vesicular carrier based gels formulated, formulation F4_{1% w/v} was chosen as the optimized gel dosage form and taken up for further evaluations.

Ex vivo corneal permeation: The analytical UV method used for *ex vivo* permeation studies showed good accuracy with a mean recovery of 99.75%. The individual recovery values ranged from 94.91% to 104.04%. The precision was acceptable, with a relative standard deviation (RSD) of 1.78%. The sensitivity of the method was supported by a limit of detection

(LOD) of 2.27 µg/ml) and a limit of quantification (LOQ) of 6.88 µg/ml. The optimized flexible nano-vesicular carrier based gel formulation F4_{1% w/v} and the drug solution gel were subjected to a corneal permeation study with the help of a Franz diffusion cell and goat corneal membrane (Figures 14 and 15) for a six-hour period. The flexible nano-vesicular carrier-based gel F4_{1% w/v} exhibited a significant increase in permeation ($p \leq 0.05$), that, is $64.98 \pm 0.009\%$, in comparison to the drug solution gel $48.90 \pm 0.087\%$ after a 6-hour study. Percentage cumulative drug permeated versus time plot for flexible nano-vesicular carrier-based gel formulation F4_{1% w/v} and drug solution gel formulation is given in Figure 12.

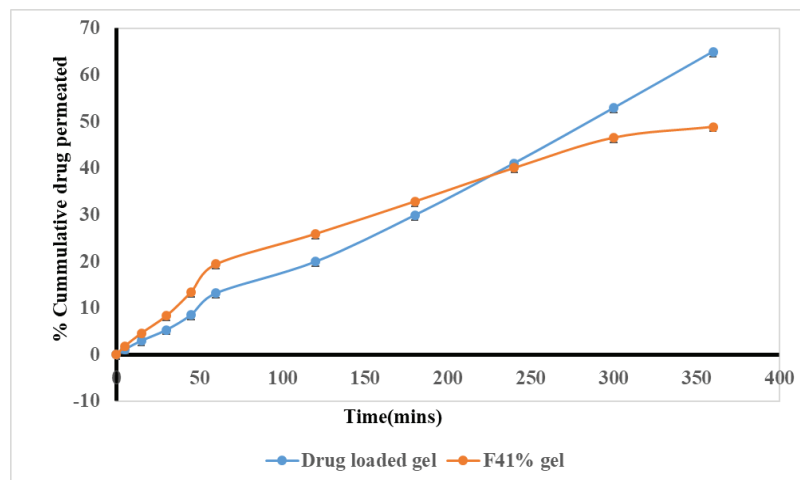


Figure 12. % Cumulative drug permeated vs time plot of optimized gel Formulation F4_{1% w/v} and drug-loaded gel formulation

Data analysis (flux determination and permeability coefficient determination): Flux as well as permeability coefficient of the optimized flexible nano-vesicular carrier-based gel formulation ($F4_{1\% \text{ w/v}}$) and the drug solution

gel were determined. The flux and permeability coefficient of the optimized flexible nano-vesicular carrier-based gel $F4_{1\% \text{ w/v}}$ were significantly higher ($p \leq 0.05$) as compared to the drug solution gel. The results are shown in Table 6.

Table 6. Observation of flux (JSS) and permeability coefficient (K_p) of $F4_{1\% \text{ w/v}}$ and drug solution gel

S.No.	Formulations	Flux (J_{ss}) ($\mu\text{g}/\text{cm}/\text{h}$) $\pm$ S.D (n=3)	Permeability coefficient (K_p) $\pm$ S.D (n=3)
1	$F4_{1\% \text{ w/v}}$	0.1752 ± 0.003	$5.84 \times 10^{-3} \pm 0.017$
2	Drug solution gel	0.1326 ± 0.001	$4.42 \times 10^{-3} \pm 0.001$

% drug retention determination in excised goat corneal membrane: The results revealed that the flexible nano-vesicular carrier-based gel ($F4_{1\% \text{ w/v}}$) achieved significantly higher ($p \leq 0.05$) ciprofloxacin localization in the cornea when compared to the drug solution gel formulation. Specifically, the optimized

nano-vesicular gel ($F4_{1\% \text{ w/v}}$) showed drug retention of 0.0497 ± 0.012 mg and $4.971 \pm 0.001\%$. In contrast, the drug solution gel demonstrated lower retention values of 0.0271 ± 0.021 mg and $2.711 \pm 0.017\%$, respectively. Detailed results are presented in the accompanying Table 7.

Table 7. Determination of drug retention and percentage drug retention in excised goat corneal membrane

S.No.	Formulation	Drug retention in corneal membrane (mg) $\pm$ S.D (n=3)	% Drug retention in the corneal membrane $\pm$ S.D (n=3)
1	$F4_{1\% \text{ w/v}}$	0.0497 ± 0.012	4.971 ± 0.001
2	Drug solution gel	0.0271 ± 0.021	2.711 ± 0.017

Toxicological evaluation

Test for Corneal hydration: The corneal hydration test results revealed a hydration percentage of $78.45\% \pm 0.23\%$, which lies comfortably in the normal range of 75–80% (Zubairu et al., 2015).

Histopathological evaluation: A histopatholog-

ical evaluation was conducted to evaluate the safety of the formulation on corneal tissues. Examination of goat corneas exposed to potassium chloride solution showed significant tissue damage (Figure 13.). In contrast, no noticeable structural changes were observed in corneas treated with the flexible nano-vesicular carrier-based gel ($F4_{1\% \text{ w/v}}$) (Figure 14.).

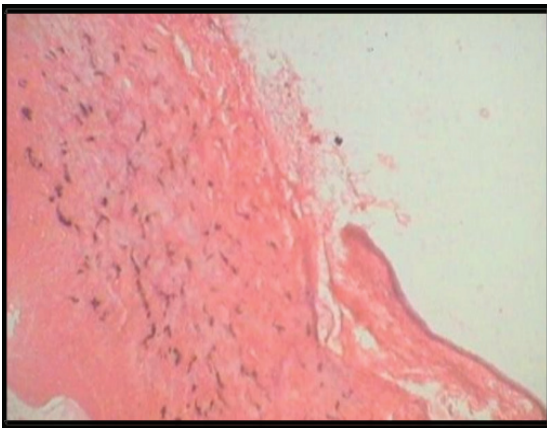


Figure 13. KCl treated excised goat corneal membrane

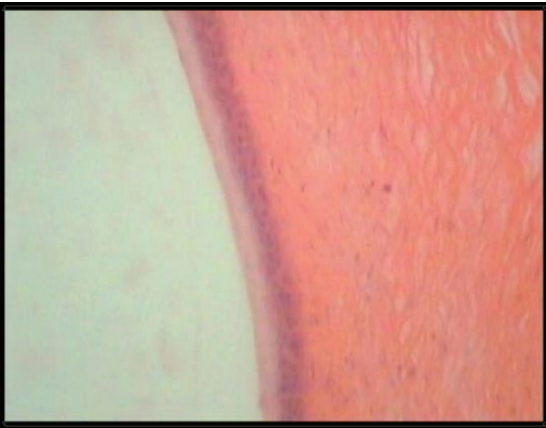


Figure 14. Formulation treated excised goat corneal membrane

Formulation effect on corneocytes: LDH assay

Biochemical estimation of LDH was determined by utilizing a Coral Clinical Systems kit for LDH. The results indicated no significant changes in LDH activity between the goat cornea treated with normal saline (10.5 ± 1.0 units/cm²) and the goat cornea treated with the nano-vesicular gel formulation F4_{1% w/v} (11.5 ± 1.0 units/cm²).

Bioadhesion study: The bioadhesive property of the intraocular flexible nano-vesicular gel formulation (F4_{1% w/v}) was evaluated in comparison to a con-

ventional drug-loaded gel. The nano-vesicular gel (F4_{1% w/v}) exhibited a significantly prolonged adhesion time ($p \leq 0.05$) on the agar surface (21.09 ± 0.017 minutes) compared to the drug solution gel (5.10 ± 0.021 minutes).

Spreadability: The spreadability of the intraocular flexible nano-vesicular carrier-based gel F4_{1% w/v} was determined, and Table 8 represents the results, which show the diameter of the circle formed to be 44 ± 0.5 mm and a high spreadability value of 1521.14 ± 0.17 mm².

Table 8. Spreadability of intraocular flexible nano-vesicular carrier-based gel F4_{1% w/v}

S.No.	Formulation code	Diameter of circle (cm) ± SD (n=3)	Diameter of circle (mm) ± SD (n=3)	Spreadability (mm ²)
1	F4 _{1% w/v}	4.4±0.5	44±0.5	1521.14±0.17

Antimicrobial assay and determination of zones of inhibition: The inhibition zones surrounding the formulations (NVD, PG and DSG) were measured. A significantly larger zone of inhibition ($p \leq 0.05$) was observed for flexible nano-vesicular carrier-based

gel F4_{1% w/v} (40 ± 0.6 mm) as compared to drug solution gel (19 ± 1.2 mm) and plain gel formulation (no zone observed) after a period of 48 hours. The results are given in Table 9 and Figures 15 to 18.

Table 9. Inhibition Zones for various formulations containing ciprofloxacin against *Staphylococcus aureus*

Formulations Time	Inhibition Zone (mm)± S.D (n=3)	
	24 Hrs	48 Hrs
Nano-vesicular gel (NVG)	38±0.17 mm	40±0.6 mm
Plain gel (PG)	0	0
Drug solution gel (DSG)	18±1.3 mm	19±1.2 mm

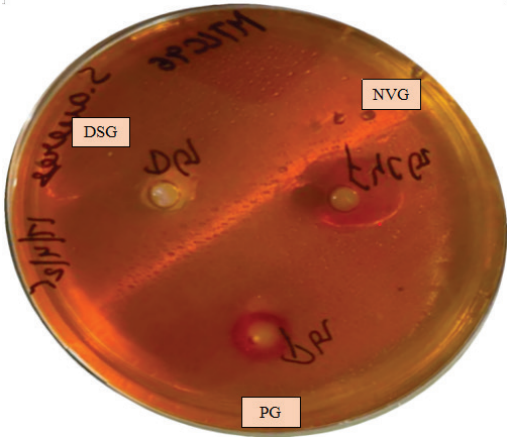


Figure 15. Zones of Inhibition for different formulations after 0 Hours

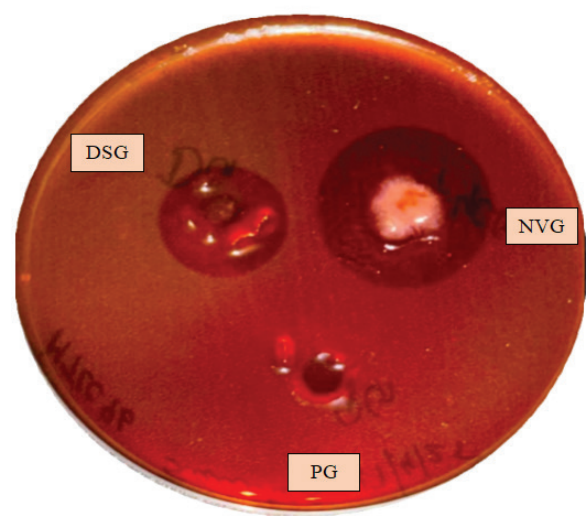


Figure 16. Zones of Inhibition for different formulations after 24 Hours

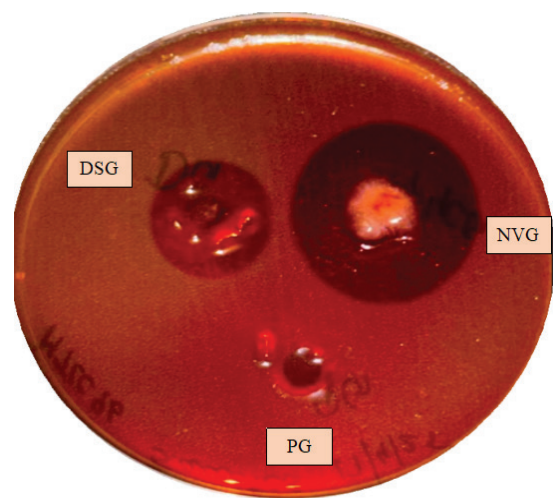


Figure 17. Zones of Inhibition for different formulations after 48 Hours

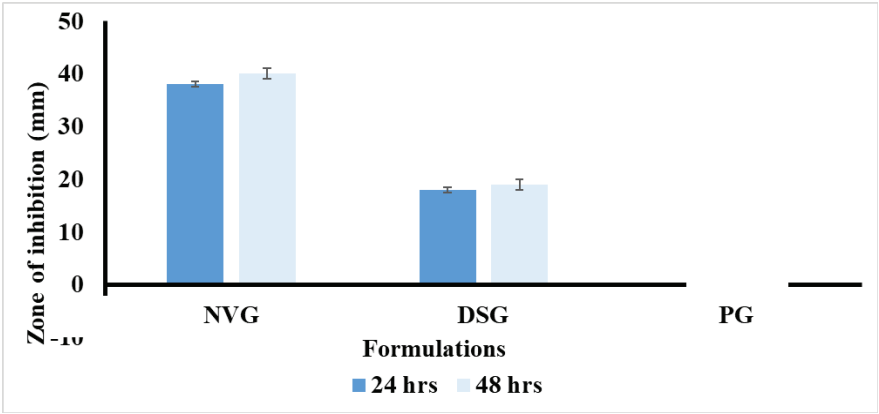


Figure 18. Zones of inhibition comparison of various formulations against *Staphylococcus aureus* after incubation time of 24 hours and 48 hours

Evaluation of the safety of the developed formulation using cell lines by determining % cell viability using MTT cytotoxicity assay: The cytotoxicity was examined for various drug concentration levels of ciprofloxacin (12.5, 25, 50, 100 µg/mL) (Figure 19 and Table 10.). L929 cells exhibited dose- dependent cytotoxicity. It was observed that prepared ciprofloxacin loaded F4_{1%w/v} nano-vesicular gel showed signifi-

cantly lesser cytotoxicity ($p \leq 0.05$) (the cell viability $84.58\% \pm 0.037$ to $50.85\% \pm 0.044$ on L929 cells) as compared to ciprofloxacin drug solution which exhibited cell viability of $56.46\% \pm 0.070$ to $33.51\% \pm 0.037$ in the respective concentration range 12.5- 100 µg/mL. The representative images of L929 cells treated with F4_{1%w/v} nano-vesicular gel before treatment are given in Figure 20.

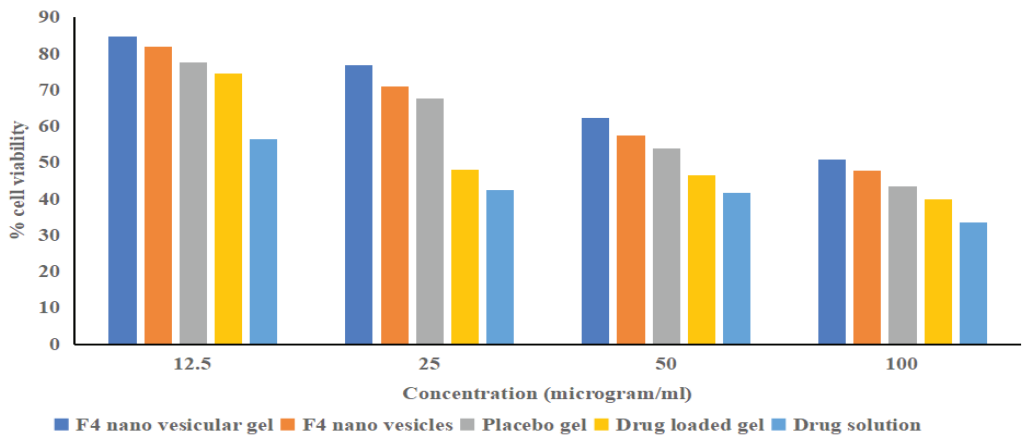


Figure 19. Comparison of % cell viability of F4_{1%w/v} Nano-vesicular gel, Placebo nano-vesicular gel, F4 Nano-vesicular carriers, drug-loaded gel, and drug solution

Table 10. Observation of *in vitro* cytotoxicity study of F4_{1%w/v} nano-vesicular gel, F4 nano-vesicular carriers, Placebo gel, drug-loaded gel, and drug solution

% Cell viability ± S.D (n=3)					
Concentration µg/ml	F4 _{1%w/v} Nano vesicular gel	F4 Nano-vesicular carriers	Placebo gel	Drug-loaded gel	Drug solution
12.5	84.58±0.037	81.83±0.060	77.63±0.006	74.41±0.056	56.46±0.070
25	76.82±0.027	70.83±0.055	67.60±0.004	48.08±0.025	42.27±0.008
50	62.29±0.062	57.39±0.032	53.93±0.061	46.37±0.087	41.53±0.054
100	50.85±0.044	47.73±0.016	43.38±0.021	39.82±0.016	33.51±0.037

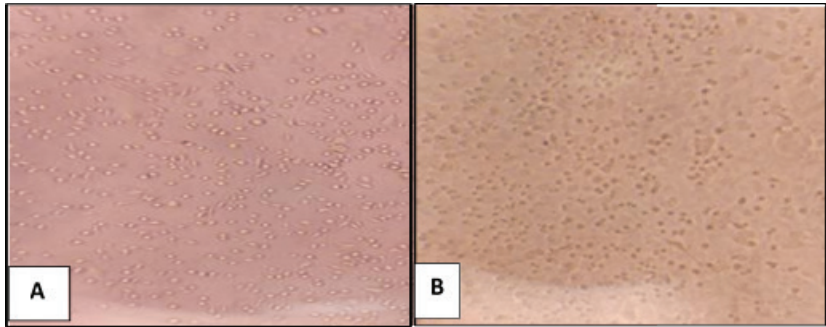


Figure 20. A) L929 cells treated with F4_{1%w/v} nano vesicular gel before treatment B) L929 cells treated with F4_{1%w/v} nano-vesicular gel after treatment

Stability studies: The stability of the flexible nano-vesicular carrier-based gel (F41% w/v) was assessed under two storage conditions: 4±1 °C and room temperature. Samples containing 1 mg of the formulation were taken on various days (0,15 and 30 days) and evaluated for various parameters including pH, viscosity, drug content, zeta potential, percentage of drug remaining, log percentage of drug remaining, and signs of phase separation. At 4±1°C, no significant changes ($p \leq 0.05$) were noticed in pH, viscosity, zeta potential, or drug content over the 30 days pe-

riod, and no phase separation occurred. Analysis of the log % drug remaining over time showed that only 0.08% of the drug underwent degradation after 30 days, indicating excellent stability under refrigerated conditions. In contrast, phase separation was noted in the gel stored at room temperature, suggesting reduced stability under those conditions. Results concluded that F4_{1% w/v} nano-vesicular gel had enhanced stability when kept at 4±1°C. The detailed results of the stability study conducted at 4±1 °C are presented in Table 11 and Figure 21.

Table 11. Results of the stability study of optimized flexible nano-vesicular carrier-based gel F4_{1% w/v} kept at 4 °C

Time (Days)	pH ± S.D (n=3)	Viscosity (mPa-s) ± S.D (n=3)	Zeta potential (mV) ± S.D (n=3)	Drug content (mg) ± S.D (n=3)	% Drug remaining	Log % drug remaining	Phase separation
0	7.26±0.055	3809±0.4	-44.3±0.3mV	2.976±0.03	100	2	No
15	7.28±0.017	3810±0.2	-44.5±0.1mV	2.972±0.05	99.96	1.9998	No
30	7.30±0.001	3812±0.1	-44.1±0.2mV	2.969±0.06	99.92	1.9996	No

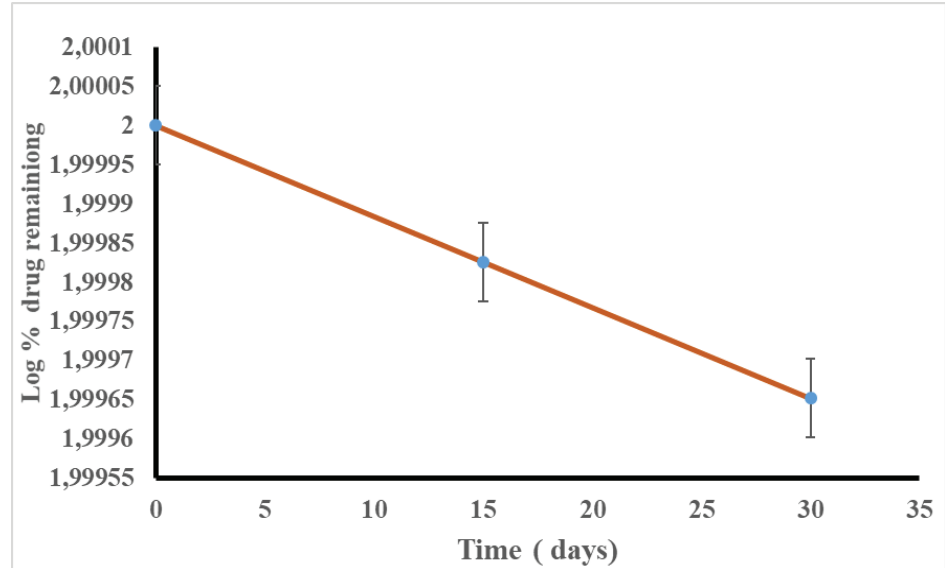


Figure 21. Log % remaining drug v/s Time (days) at 4 °C

For the flexible nano-vesicular carrier-based gel (F4_{1% w/v}) stored at room temperature, phase separation was observed after 15 days; therefore, further study was stopped. The flexible nano-vesicular carrier-based gel F4_{1% w/v} showed phase separation after storage at room temperature after 15 days.

DISCUSSION

Preparation of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carriers

Different concentrations of edge activators (Sodium deoxycholate, Tween 80 and Span 80) and phospholipid (Soya lecithin) were utilized in the prepa-

ration of intraocular targeted ciprofloxacin-loaded nano-vesicular carriers for treatment of Bacterial Keratitis, by using a Rotary evaporator (thin film hydration method) to overcome the drawbacks related to traditional dosage forms. The optimized ciprofloxacin-loaded flexible nano-vesicular carrier formulation F4 demonstrated promising results for intraocular drug delivery.

Evaluation of intraocular targeted ciprofloxacin loaded flexible nano-vesicular carriers

Entrapment efficiency

The ciprofloxacin-loaded flexible nano-vesicular carrier (F4), prepared using Tween 80, demonstrated excellent performance, having the highest entrapment efficiency (Zubairu et al., 2015). The high entrapment efficiency achieved by using the thin film hydration technique is in accordance with the results of Akhter et al. (2022), who reported entrapment efficiency of more than 90% by the thin film hydration method (Akhter et al., 2022). The entrapment efficiency was calculated in methanol:chloroform (2:1) by a validated analytical method at lambda max 278nm.

For calculating entrapment efficiency, separation was achieved by ultracentrifugation at 13,000 rpm for 20 min at 4 °C. The centrifugation study parameters used have been reported by Youssef et al. (2020) to produce denser pellets of vesicles, which results in effective separation of the untrapped drug from the vesicles (Youssef et al., 2020). Besides this, the drug content was also determined in supernatant after centrifugation to confirm that untrapped drug was separated from vesicles (Kamboj, Saini, & Bala, 2014).

In vitro evaluation

The *in vitro* release pattern of ciprofloxacin-loaded flexible nano-vesicular carrier (F4) showed a controlled and sustained drug profile, reaching (98.92 ± 0.008%) within 6 hours, in sharp contrast to the immediate release of drug solution (93.70 ± 0.049%) within 2 hours. The prepared flexible nano-vesicular

carrier suspension showed a sustained and gradual release over a 6-hour interval. The slow release of drug from flexible nano-vesicular carrier can be attributed to the chemical attributes of the drug, which allow a preferred binding of the drug with spherical bilayer structure chemical components (phospholipids and surfactants) of flexible nano-vesicular carriers (Cristiano et al., 2020).

Determination of release rate orders

F4 formulation showed the highest linearity ($R^2=0.987$) in the zero-order kinetic model indicating, zero-order release. Zero-order kinetics suggests that the drug is released at a constant rate over time, independent of its concentration within the vesicular system. This behavior is desirable for controlled drug delivery because it maintains a relatively constant drug concentration and minimizes fluctuations in therapeutic levels. The release profile indicates that the ultradeformable flexible nanovesicular carriers acted as a reservoir system, providing sustained drug release throughout the study period (Askarizadeh et al., 2023).

Vesicle size, polydispersity index (PDI) and, zeta potential of formulations

Vesicle size demonstrated the formation of nano-sized vesicles suitable for drug delivery applications. Polydispersity index suggested a uniform size distribution within the formulation. A low polydispersity index ($PDI < 0.3$) indicates a narrow and uniform particle size distribution, which is essential for the physical stability and reproducibility of vesicular systems (Ali & Hussein, 2017). Such uniformity minimizes aggregation and sedimentation, ensures consistent drug release behavior, and enhances ocular tolerability by preventing the presence of large, irritating aggregates. Consequently, low PDI is considered a critical quality attribute for achieving stable and effective ocular drug delivery systems (Danaei et al., 2018). Zeta potential indicated good electrostatic stability of the vesicles due to strong negative surface charge, which helps in the prevention of aggregation while contributing

to the overall formulation stability, physical integrity, uniform dispersion, and predictable performance over time (Wen, Farid, & Kassem, 2014). Vesicles with adequate electrostatic repulsion resist destabilizing processes such as sedimentation and coalescence. This ensures that the vesicle size distribution remains relatively constant during storage, which is crucial for long-term drug delivery performance (Kamble et al., 2022).

Vesicle shape

The shape of the vesicles of the optimized formulation was found to be spherical with significant core and coat parts (Ahmed, Kassem, & Sayed, 2020). Spherical vesicle morphology is considered highly advantageous for ocular drug delivery due to the unique physiological and anatomical constraints of the eye. The smooth and uniform curvature of spherical vesicles minimizes mechanical irritation to the sensitive ocular surface, thereby improving tolerability during topical administration (Gaudana, Ananthula, Parenky, & Mitra, 2010). In addition, this geometry facilitates even spreading across the precorneal tear film, ensuring uniform distribution of the formulation over the ocular surface despite rapid tear turnover and blinking (Achouri, Alhanout, Piccerelle, & Andrieu, 2013). Furthermore, their organized bilayer structure supports predictable and controlled drug release, which is essential for maintaining therapeutic concentrations in the eye where rapid elimination is a major limitation (Akhtar et al., 2022). Overall, spherical vesicles contribute to improved formulation stability, reduced irritation potential, enhanced precorneal residence, and more reliable ocular drug delivery performance.

Isotonicity

Isotonicity study is a critical quality evaluation for ocular formulations because the eye is highly sensitive to osmotic variations. The findings indicated that the ciprofloxacin-loaded flexible nano-vesicular carrier (F4) was isotonic. This is particularly important for

ophthalmic drug delivery, as deviations from isotonicity can lead to ocular irritation, tearing, pain, corneal damage, and reflex blinking, all of which significantly reduce drug residence time and bioavailability (Mahajan et al., 2020).

Sterility testing

Sterility testing was done to confirm the sterility of all the formulations. Sterility is a critical and non-negotiable quality requirement for ocular formulations because the eye is a highly sensitive and infection-prone site with limited natural defense against microbial contamination. Unlike other routes of administration, ophthalmic preparations are applied directly onto the corneal surface, conjunctiva, and tear film, where even a small microbial load can lead to severe infections such as keratitis, conjunctivitis, or endophthalmitis, potentially resulting in vision impairment or loss (Vashikar, RahulChavan, Patil, Toraskar, & Patil, 2026). The sterility test indicated that the optimized formulation F4 was sterile, as there was no growth of microorganisms in it.

Degree of deformability

A low deformability index value of 0.181, i.e., near zero indicated very high deformability of the flexible nano-vesicular carrier F4 formulation, which minimized the risk of rupture when penetrating the cornea while enhancing drug bioavailability in deeper tissues. The elasticity and deformability index of vesicles (275nm) was evaluated as per the already reported method by Marwah et al. (2022) by using the formula $D = J \times (r_v / r_p)$ which considers extruded volume (J), vesicle size after extrusion (r_v) and filter pore size (r_p) as critical variables and is the standard formula reported in literature by Cevc et al. (1998) for calculating the degree of deformability of elastic vesicles including ethosomes, transethosomes and spanlastics etc. The extrusion was performed at a constant controlled pressure of 1.5-2 bar, which addresses the pressure-dependence concern for vesicles (Griddalur & Uppala, 2020).

Preparation of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carrier-based gel

The optimized ciprofloxacin-loaded flexible nano-vesicular carrier F4 formulation was integrated into a gel matrix to enhance the retention time of the developed formulation in the eye. Enhancing the retention would boost its therapeutic outcomes by limiting tear washout of the ciprofloxacin-loaded flexible nano-vesicular carrier F4 formulation (Sayeh, Abdelkader, & El-Gizawy, 2014).

Evaluation of intraocular targeted ciprofloxacin-loaded flexible nano-vesicular carrier-based gel

Physical characterization

The optimized formulation F4_{1% w/v} exhibited desirable physicochemical attributes with slight cloudiness, which can be attributed to the presence of edge activators (Tween 80/Span 60) that self-assemble into bilayers, creating a colloidal dispersion, that appears translucent due to light scattering (Bhujbal, Rupenthal, Patravale, & Agarwal, 2025; Dina, Mowafaq, & Mohammed, 2023).

pH

The pH study indicated that the pH of all formulated intraocular flexible nano-vesicular carrier-based gels was within the range (7.0–7.35), indicating that prepared formulations were safe for ocular administration and would be well tolerated upon administration (Mazyed & Abdelaziz, 2020).

Homogeneity and grittiness

The intraocular flexible nano-vesicular carrier-based gels was uniform in texture, smooth, and devoid of gritty particles indicating that the gel can be applied comfortably, without any uneasiness (Mazyed & Abdelaziz, 2020).

Viscosity

The viscosity of intraocular flexible nano-vesicular carrier-based gel (F4_{1.0% w/v}) was found to be good. The viscosity of an intraocular flexible nanovesicular carrier-based gel is a critical rheological parameter

that directly influences its performance in ocular drug delivery. The formulation F4 (1.0% w/v) exhibited the highest viscosity, indicating a well-developed gel network with good structural consistency. This increased viscosity is beneficial in ophthalmic applications because it enhances the residence time of the formulation on the precorneal surface by reducing the rate of drainage through the nasolacrimal duct (Pawar, Rathod, & Jagadale, 2024).

Drug content

Among the intraocular flexible nano-vesicular carrier-based gels evaluated, the formulation containing 1% w/v Carbopol 940 (F4_{1% w/v}) was found to have a maximum drug content of 99.20%, showing good content uniformity. High drug content with minimal variation reflects a well-optimized formulation process in which the drug was uniformly distributed throughout the nano-vesicular system and subsequently within the gel base. This uniformity is essential for ensuring dose precision, particularly in ocular drug delivery, where even minor inconsistencies can significantly affect therapeutic outcomes due to the small administered volumes (Mazyed & Abdelaziz, 2020).

Rheological study

Rheological study revealed that gel viscosity increased proportionally with increasing gelling agent concentration, which had the potential to prolong residence time in the eye by increasing contact time of the intraocular flexible nano-vesicular carrier-based gel (F4_{1.0% w/v}) on the corneal surface (Mazyed & Abdelaziz, 2020). Rheograms showed pseudoplastic (shear-thinning) flow behavior. The observed pseudoplastic (shear-thinning) flow behavior is therapeutically critical for ocular delivery (Kapadia et al., 2022). At rest, the 1% gel exhibited optimal viscosity (3809 cP), which is postulated to create a viscous barrier that would resist nasolacrimal drainage and extend precorneal residence time (Ranch et al., 2019). The sheer thinning effect would not only reduce the viscosity dramatically during blinking, thereby enabling uniform corneal spreading without visual disturbance, but is also

reported to maintain patient comfort during blinking (Sadeq, Sabri, & Al-Kinani, 2022, Shoaeba, Sharav, Jain, Sahu, & Meshram, 2021) by dropping the viscosity during the blink which in turn minimizes frictional shear stress between the eyelid and the eye, avoiding the gritty “blinking friction” commonly associated with highly viscous Newtonian formulations. This ensures that the formulation does not cause blurred vision, thereby enhancing overall patient compliance and ocular tolerance (Kapadia et al., 2022).

***In vitro* drug release**

The results revealed that increasing the gelling agent concentration reduced that release of drug from the gel dosage forms. Drug release from the solution gel was higher than that from the intraocular flexible nano-vesicular carrier-based gel, which exhibited sustained release of ciprofloxacin due to the reservoir effect of the nano-vesicular carriers. The sustained release resulted from the gradual drug leakage from the vesicles into the gel matrix followed by diffusion into the release medium. This sustained delivery may reduce dosing frequency and improve patient compliance (Adepu & Ramakrishna, 2021).

***Ex vivo* corneal permeation**

The study highlighted the advantage of the flexible nano-vesicular carrier-based gel (F4_{1% w/v}), which exhibited significantly higher drug permeation (64.98%) and retention in the corneal tissue (4.97%) compared to the drug solution gel. This provides insight into the superior ability of flexible vesicular gels to localize as well as retain the drug at the action site, potentially reducing dosing frequency (Zubairu et al., 2015). The improved permeation could be due to incorporation of an edge activator, which helped in enhancing permeation and improved the *ex vivo* corneal penetration (Tiwari, Tiwari, & Singh, 2020).

Toxicological evaluation

Test for corneal hydration

The corneal hydration was found to be 78.45% $\pm$ 0.23%, which was within the range of 75-80%,

showing minimal damage to the cornea. Corneal hydration is a widely accepted parameter for assessing potential corneal damage and irritation following exposure to ophthalmic formulations. Under normal physiological conditions, the corneal hydration level is typically maintained within the range of 75–80%, which is essential for preserving corneal transparency, structural integrity, and normal visual function. Values exceeding this range generally indicate corneal edema and tissue damage, whereas lower values may suggest dehydration and disruption of normal corneal physiology (Zubairu et al., 2015).

Histopathological evaluation

Histopathological examinations revealed that the flexible nano-vesicular carrier-based gel (F4_{1% w/v}) did not induce any structural alterations in the cornea, thereby preserving the normal histoarchitecture of all layers of the cornea, including the epithelium, endothelium, and stroma, indicating safe application of flexible nano-vesicular carrier-based gel (F4_{1% w/v}) into the eyes (Nanda et al., 2022).

Formulation effect on corneocytes: LDH assay

The lactate dehydrogenase (LDH) assay is a widely accepted method for evaluating the cytotoxicity and biocompatibility of pharmaceutical formulations by assessing the integrity of the cell membrane. LDH is a stable intracellular enzyme that is normally retained within viable cells. However, when cell membrane damage occurs, LDH is released into the extracellular environment. Therefore, the extent of LDH release serves as a sensitive indicator of cellular injury and membrane disruption. In the present study, the LDH assay demonstrated that the intraocular flexible nano-vesicular carrier-based gel formulation F4 (1.0% w/v) exhibited excellent biocompatibility, as no significant increase in LDH release was observed compared with the control. The low level of LDH leakage indicates that the formulation did not induce appreciable cellular damage or compromise membrane integrity (Cho, Shi, & Ben Borgens, 2010).

Bio-adhesion study

The bio-adhesion study indicated that the intra-ocular flexible nano-vesicular carrier-based gel (F4_{1.0% w/v}) had a greater bio-adhesion time when compared with the drug solution gel. Due to better bio-adhesion time, the intraocular flexible nano-vesicular carrier-based gel F4_{1% w/v} dosage form would have improved retention time intraocularly, resulting in improved efficacy with minimal frequency of dosing (Nanda et al., 2022).

Spreadability

The intraocular flexible nano-vesicular carrier-based gel (F4_{1.0% w/v}) displayed good spreadability, ensuring uniform application into the eye with little stress. Good spreadability ensures that the formulation rapidly forms a thin and continuous film over the ocular surface, thereby maximizing the contact area between the drug-loaded vesicles and the corneal epithelium. Uniform spreading facilitates consistent drug availability at the absorption site and helps achieve reproducible therapeutic outcomes. In addition, a formulation that spreads readily requires less mechanical force during blinking to distribute the gel across the eye, thereby minimizing stress and irritation to the delicate ocular tissues (Mazyed & Abdelaziz, 2020).

Antimicrobial assay and determination of zone of inhibition

The ciprofloxacin loaded F4_{1%w/v} nano-vesicular gel showed enhanced antimicrobial performance, indicating larger zones of inhibition against *S. aureus*, which confirmed the formulation's therapeutic potential and enhanced penetration of ciprofloxacin from a flexible nano-vesicular carrier-based gel (Basha, El-Alim, Shamma, & Awad, 2013). As per the reports in the literature, the MIC of ciprofloxacin is 0.125–2.0 µg/mL against *Staphylococcus aureus* (Smith & Eng, 1985). The dose of formulation was 3 mg/ml with over 90% of the drug released over a period of 6 hours. Since the concentration of ciprofloxacin released from the formulation exceeds 2.0 µg/mL (highest MIC), it can be concluded that the formulation would

demonstrate therapeutic efficacy for treating bacterial keratitis. The results are in accordance with reports of Gonzalez et al. (1984). The results can be correlated with results of *in vitro* release studies where the concentrations were above MIC.

Evaluation of the safety of the developed formulation using cell lines by determining % cell

viability using MTT cytotoxicity assay

The cytotoxicity study was performed on L929 cells as L929 cells (mouse fibroblasts) are the globally standardized model reported in the literature for *in vitro* cytotoxicity testing under ISO 10993-5 (Ramakrishnan & Daly, 2026). The cell line has been reported to be used by Taghe et al. (2023) for studying the cytotoxicity of nanofibrous and film-structured ciprofloxacin hydrochloride inserts for sustained ocular delivery, confirming their use for studying ocular formulations (Taghe et al., 2023). Ciprofloxacin loaded F4_{1%w/v} nano-vesicular gel showed significantly lesser cytotoxicity ($p \leq 0.05$) (the cell viability from $84.58\% \pm 0.037$ to $50.85\% \pm 0.044$) on L929 cells, suggesting it as a safe carrier system, as a value above 80% is considered non-toxic (Taghe et al., 2023). The dose used for cell line studies conducted was in accordance with a study by Taghe et al. (2023), who reported that ciprofloxacin in a concentration of 100 µg/mL was suitable for application in spite of low cell viability (Taghe et al., 2023).

Stability studies

Stability testing demonstrated that the gel remained highly stable at $4 \pm 1^\circ\text{C}$ for at least 30 days without significant changes in viscosity, pH, zeta potential, and drug content, while room-temperature storage caused phase separation, highlighting the importance of refrigerated storage for long-term use (Gupta, Aggarwal, Singla, & Arora, 2012).

CONCLUSION

The optimized ciprofloxacin-loaded flexible nano-vesicular gel (F4_{1% w/v}), presents a robust nanotechnology-driven platform for treating localized ocular

infections. By integrating deformable nano-vesicles within a gel matrix, the formulation effectively addresses the critical limitations of conventional aqueous eye drops, such as tear dilution, nasolacrimal drainage, and rapid precorneal clearance. It demonstrated remarkable sustained-release characteristics and significantly enhanced transcorneal permeation, which ensures that therapeutic drug concentrations would be maintained at the infection site over an extended period of time. Notably, the formulation exhibited superior antimicrobial efficacy against *Staphylococcus aureus*, a leading pathogen in ocular keratitis. This enhanced potency, combined with verified ocular safety in corneocytes and the results of histopathological investigation and biocompatibility, underscores its potential to accelerate healing and reduce the risk of bacterial resistance. Based on the results, it has been concluded that the formulated flexible nano-vesicular gel offers a highly viable, patient-centric alternative to traditional ocular therapies by significantly reducing dosing frequency and improving overall treatment adherence. However, future studies to evaluate its long-term safety, retention time, and overall drug bioavailability in *in vivo* animal models and clinical setups need to be done to confirm its therapeutic superiority over conventional dosage forms.

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AUTHOR CONTRIBUTION

Experimental work and present study (SK), Statistics, Hypothesis development, Data interpretation and analysis of data (JKN), Literature review (KK, PKM), Histopathological study and data interpretation (RSN).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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