Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14,issue.24s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Development and Evaluation of Timolol Maleate loaded Bilosomes.

Gangotri Yadav[1], Hrishikesh More[2], Ashish Jain[3], Shubham Waghmare[4], Gaurav Pandhare[5]

[1]Department of Pharmaceutics ,Research Scholar ,Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel.
[2]Department of Pharmaceutics ,Faculty ,Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel
[3]PHD Pharmacognosy , Principal, Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel.
[4]Department of Pharmaceutics ,Research Scholar ,Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel.
[5]Department of Pharmaceutics ,Research Scholar ,Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel.
[6]Department of Pharmaceutics ,Research Scholar ,Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel

Corresponding Author
Hrishikesh More*
Shri D.D.Vispute College of Pharmacy and Research Centre , Panvel
Email ID : hrishikeshmore20@gmail.com

Cite this paper as Gangotri Yadav, Hrishikesh More, Ashish Jain, Shubham Waghmare,Gaurav Pandhare.(2025) Development and Evaluation of Timolol Maleate loaded Bilosomes. . .Journal of Neonatal Surgery, 14, (24s), 1059-1068

ABSTRACT

Objective : This study aimed to enhance anti-glaucoma activity of Timolol maleate by incorporating Timolol maleate into bilosomal pH-responsive in-situ gel for the treatment of Intra-ocular pressure caused by glaucoma.

Material and Method : The thin-film hydration technique was used to create bilosomes with different proportions of cholesterol, bile salts, and edge activators. Since it demonstrated the best drug entrapment efficiency, the thin-film hydration approach was chosen for additional development. At varying concentrations, the optimized bilosomal batch was incorporated to pH-responsive in-situ gels.

Results and Discussion : 0.1% Carbopol(934) gel demonstrated the highest drug content and was used for further analysis. The bilosomes were characterized for particle size (150–400 nm), polydispersity index, and entrapment efficiency (72%–95%). Transmission electron microscopy confirmed spherical morphology. The optimized bilosomal pH-responsive in-situ gel formulation exhibited favorable pH, viscosity, spreadability, and sustained in vitro drug release. Antimicrobial efficacy evaluated via the cup plate diffusion method revealed a larger zone of inhibition for the bilosomal in-situ gel, indicating enhanced therapeutic potential and antimicrobial effectiveness. These findings suggest its potential as an effective formulation for the treatment of Intra-ocular pressure caused by glaucoma .

Keywords: *ocular delivery; Timolol maleate; bilosomes; in-situ gel.*

1. INTRODUCTION

Ophthalmic drug delivery is the most challenging part of drug delivery due to complex nature of eye, which obstructs the delivery of medications to specific ophthalmic locations. Thats why, the structure of eye is a topic of interest among the formulation scientists in the world of pharmaceutics.1 Conventional eye drops in the pharmaceutical market acquires 90% stake of accessible optic formulations is an ideal treatment for ophthalmic diseases.2

Glaucoma is an optic neuropathy which is characterized as neurodegenerative disorder of various origins, resulting in the loss of retinal ganglion cells (RGCs). Intraocular pressure (IOP) stands out as the key identifiable risk factor for glaucoma, and it is currently the only one that can be modified. IOP-induced stress causes injury to the axons of retinal ganglion cells (RGC) at the optic nerve head. Glaucoma is differentiated from other optic neuropathies through its induction of biomechanical changes in the lamina cribrosa, creating a characteristic appearance of the optic nerve head.3-4 The eye features trabecular meshwork, which consists of spongy tissues that operate as a drainage outlet for the eye. The issue occurs when the trabecular meshwork becomes obstructed, hindering the outflow of aqueous humor from the eye. As a consequence, the increased volume of aqueous humor in the eye elevates the intraocular pressure (IOP), affecting the nerve cells by compressing them and eventually resulting in their destruction. Nerve cells play a crucial role in the visual mechanism by transmitting visual information to the brain; therefore, the death of these cells leads to vision loss.5-6

Timolol maleate functions as a non-selective beta-adrenergic blocker, targeting both beta-1 and beta-2 (β-1 and β-2) adrenergic receptors. It does not exhibit significant intrinsic sympathomimetic, direct

myocardial depressant, or local anesthetic (membrane-stabilizing) effects. Timolol maleate effectively decreases intraocular pressure (IOP) and is utilized in the treatment of patients with open-angle glaucoma and aphakic glaucoma. Timolol maleate is utilized for the treatment of hypertension and for the reduction of cardiovascular mortality and the risk of reinfarction in patients who have successfully navigated the acute phase of myocardial infarction and are clinically stable. Available in oral tablet form, as well as for injection and ophthalmic use as separate sterile aqueous solutions, Timolol maleate is usually well tolerated, with the majority of side effects being mild and transient.[7] It Blocks both β-1 and β-2 adrenergic receptors, decreases intraocular pressure by limiting aqueous humor production or possibly improving outflow; decreases blood pressure by obstructing adrenergic receptors and reducing sympathetic outflow, leads to negative chronotropic and inotropic activity.[8]

To tackle the ophthalmic constraints of conventional eye drops or other standard ophthalmic products, it is recommended to develop a new formulation that can persist in the eye for an extended time and release the drug to reach the desired concentration. Researchers have reported different types of formulations aimed at improving corneal residence time, such as contact lenses, ointments, collagen shields, and ocular inserts.[9]

Bilosomes is a closed vesicle with a bilayer stabilized by bile salts, composed of a nonionic surfactant akin to niosomes, but incorporating bile salts.[10] Bilosomes represent a type of ultra-deformable vesicle that is flexible and improves the oral administration of bovine serum albumin.[11] Bilosomes improve the stability and encapsulation efficiency of the therapeutic agent. The further incorporation of nanoformulation into an in-situ gel system that employs gelling polymers has significantly increased the duration of corneal contact and the transit time in the cul-de-sac, in addition to minimizing drug elimination. Under normal conditions, the in-situ gel formulation exists in a sol form but changes to a gel after being applied topically to the eye, stimulated by ions, pH, and temperature.[12]

In this study, a blend of carbopol (934P) and hydroxyl propyl methylcellulose (HPMC) was employed for the creation of the in-situ gel. Carbopol is a polymer derived from polyacrylic acid (PAA) and shows a transformation from sol to gel state in an aqueous solution when the pH is increased beyond 5.5. It is a weakly acidic polymer that ionizes under alkaline pH conditions. Therefore, at higher pH levels, the PAA swells due to the electrostatic repulsion among its anionic groups, which leads to the release of the drug contained within the medium. It also exhibited mucoadhesive properties by interacting with mucin through electrostatic interactions, hydrogen bonding, hydrophobic interactions, and interdiffusion.[13] The acidic characteristics of Carbopol might cause eye irritation; thus, the inclusion of HPMC as a viscosity-enhancing agent is recommended to lower the concentration of Carbopol.[14] HPMC is classified as a semisynthetic, inert, nonionic, nontoxic polymer with viscoelastic qualities.[15]

The purpose of this study was to create and evaluate an innovative bilosomal in-situ gel formulation of Timolol Maleate, with the goal of augmenting its anti-glaucoma effectiveness and achieving sustained drug release. Bilosomes, which are characterized by their ultra-deformable vesicular structure, were utilized to encapsulate Timolol Maleate to enhance its bioavailability.

2. MATERIALS AND METHODS

Materials

Timolol Maleate provided by Dhamtec Pharma and Consultants, Navi Mumbai, Maharashtra, India. cholesterol (CHO), Span 60, and sodium deoxycholate (SDC), chloroform, Sodium Hydroxide (NaOH), Calcium chloride ($CaCl_2$), Sodium Bicarbonate ($NaHCO_3$), and Sodium Chloride (NaCl), Carbopol 934P, Hydroxy propyl methyl cellulose K100 M (HPMC-K100M) were purchased from Research-Lab Fine Chem Industries, Mumbai, Maharashtra, India. All other chemicals used were of analytical grade.

Methods

Pre-formulation Study:

The λ_{max} of the Timolol Maleate drug was determined using UV spectrophotometry (Shimadzu—UV 1800), with calibration curves in phosphate buffer (pH 7.4) and methanol. The compatibility between the drug and the excipients was confirmed using the FTIR (Shimadzu IR Affinity-1S CE).

Formulation of Bilosomes:

A thin-film hydration approach was adopted for the formulation of Timolol Maleate loaded bilosomes.[16] Different concentrations of surfactant (Span 60, between 40 and 60 mg) and CHO (10 to 30 mg) were mixed with a fixed quantity of Timolol Maleate (10 mg) dissolved in 10 mL of organic solvent (chloroform, 10 mL) in a round bottom flask. The flask was then placed in a rotary evaporator (BUCHI, Rota vapor R-300, Mumbai, India) to evaporate the organic solvent at 50 °C under reduced pressure. A thin film of the formulation was created on the flask's surface and kept in a desiccator overnight to eliminate moisture. The thin layer was subsequently hydrated with an aqueous solution of SDC (15 to 25 mg in 15 mL)

on a rotary evaporator (1 hour at 80 rpm and 40 °C) to yield the Timolol Maleate bilosomes dispersion. This dispersion was subjected to ultrasonication (UP100H, Hielscher Ultrasonics GmbH, Germany) for 5 minutes in 30-second intervals at 4 °C. The prepared Timolol Maleate bilosomes was stored at 4 °C in borosilicate vials.

Table 1. Formulation table for Timolol Maleate loaded bilosomes by thin-film hydration approach.

Formulation Code	Drug (mg)	CHO (mg)	Span 60 (mg)	SDC (mg)
F1	10	10	40	20
F2	10	20	60	15
F3	10	30	40	20
F4	10	10	50	25
F5	10	20	50	20
F6	10	30	50	15
F7	10	10	60	20
F8	10	20	40	25

The prepared bilosomes were optimized using metrics such as entrapment efficiency, TEM analysis, polydispersibility index, and zeta potential. Optimized bilosomes were further incorporated into in-situ gel.

Formulation of Bilosomal in-situ gel:

The optimized Timolol Maleate bilosomes were transformed into the sol-gel form by utilizing Carbopol-934P (pH-sensitive) and HPMC K100 M (viscosity enhancer) polymers in various ratios, as detailed in Table 2. The pH-triggered in-situ gelation technique was employed in the formulation's development.[17] According to this technique, a 0.9% w/v NaCl solution was prepared, and the required amount of HPMC (0.2–1% w/v) was incorporated under continuous stirring to create an HPMC dispersion free of lumps. The necessary amount of Carbopol (1–1.8% w/v) was then sprinkled over the mixture and allowed to sit overnight to ensure complete hydration and adequate swelling. A sufficient amount of Timolol Maleate bilosomes dispersion (equivalent to 0.3 w/v of Timolol Maleate) was added to the polymeric solution. Ultimately, a preservative (0.02% v/v benzalkonium chloride) was included to finalize the formulation.[15]

Table 2. Formulation table of Timolol Maleate loaded bilosomes in-situ gel.

Formulation	Carbopol 934P (% w/v)	HPMC K100M (% w/v)	Benzalkonium Chloride (% v/v)	Sodium chloride (g)	Distilled water (mL)
BF5G1	0.1	0.2	0.02	0.90	100
BF5G2	0.2	0.4	0.02	0.90	100
BF5G3	0.4	0.6	0.02	0.90	100
BF5G4	0.6	0.8	0.02	0.90	100

The Bilosomal in-situ gel was evaluated for pH, drug content, viscosity, Gelling capacity, in-vitro drug release studies, sterility testing, and various other characteristics, and an antimicrobial efficacy study was conducted by using the cup plate diffusion method.

Characterization of Timolol Maleate loaded bilosomes

Entrapment Efficiency

Entrapment Efficiency (EE%) is an essential factor in determining the performance of drug-loaded vesicular systems. It signifies the percentage of the drug that is successfully contained within the vesicles in relation to the overall amount of drug employed during the formulation process.

Determination of Vesicle Size, Zeta Potential and PDI

The vesicles' mean diameter, size distribution curve, and electric potential were determined through dynamic light scattering using Malvern Zetasizer (Malvern Pananalytical Ltd., UK). The prepared transferosomal formulation was diluted before the measurement.

Surface morphology study by transmission electron microscope (TEM) Vesicle size morphology determination

The morphology of optimized bilosomes was visualized using transmission electron microscopy (TEM). A 1 ml sample of bilosomes was diluted in 10 ml of distilled water, and a drop of this solution was placed on a copper-coated grid microscopic slide to dry. Following this, the dried sample was stained with a drop of 1% aqueous phosphotungstic acid solution. Excess solution was absorbed with filter paper, and the specimen was then viewed under the microscope to obtain an image.

Evaluation of in-situ gel

Appearance and pH, clarity and drug content:

The pH level of Timolol Maleate bilosomal in-situ gels was assessed utilizing a calibrated digital pH meter (Equiptronics—EQ 610, Mumbai, India) at ambient temperature. The pH measurement was conducted three times, and the mean value was recorded.

Viscosity:

The viscosity of the formulations was measured using the DV-III ULTRA Programmable Rheometer (Model LV) with a spindle-SC4-18 that was immersed in the formulation for testing. The spindle speed was set to 20 rpm, and the temperature was maintained at 25°C. The viscosity values were calculated based on Bingham's calculation. The samples were tested in triplicate to verify that the developed formulations have an adequate viscosity to prevent the rapid pre-corneal elimination of the drug.

Gelling capacity

To determine the composition appropriate for use as an in situ gelling system, the prepared formulations were assessed for gelling capacity. By adding 1 mL of the produced formulation to a test tube holding 5 mL of STF pH 7.4 at 37°C, the gelling capacity was ascertained. Visual observations were made of the time it took for the solution to turn into gel and for the gel to dissolve.Based on the gelation time and the amount of time that the created gel remains that way, the formulations' gelling capacity was categorized into four groups: no gelation (-), poor (+), good (++), and excellent (+++).

In-vitro drug release studies

In-vitro drug release experiments were carried out using the dialysis membrane method in triplicate. To begin with, the dialysis membrane (molecular weight cut-off 12000-14000 Da), which had been soaked overnight in the dissolution medium, was opened like a bag and secured at one end. About 1 mL of the selected formulation, which included a combination of Carbopol®/HPMC, was placed into the tied end of the dialysis bag, followed by the addition of 0.5 mL of STF pH 7.4 to simulate gel formation in the eye after the instillation of the in situ gel preparation. The second end of the dialysis bag was then properly tied and immersed in a beaker containing 100 mL of STF pH 7.4, simulating tear fluid and its pH. The beaker was placed in a shaker water bath set at 50 rpm and 37°C. Approximately 2 mL of the sample was withdrawn at 0.5, 1, 2, 3, 4, 6, and 8 hours, with the same volume of freshly prepared STF added to maintain sink conditions. The samples were analyzed using a UV-Visible spectrophotometer at 294 nm, with STF as a blank. The resulting release data were fitted into various kinetic models, including zero order, first order, Higuchi, Hixon-crowell, and Korsemeyer-peppas, to determine the drug release pattern and mechanism. The time taken to release 50% of the drug (T50%) was calculated and statistically treated using one-way analysis of variance (ANOVA). When a statistically significant difference was found, a post hoc Tukey's HSD (honestly significant difference) test was performed. A p-value of less than 0.05 was considered statistically significant

Sterility testing

Ophthalmic products need to be sterile, thus it is imperative to perform sterility testing. The formulation chosen was aseptically transferred into sterile fluid thioglycolate medium and incubated for no less than 14 days at 35°C to evaluate bacterial growth. The sterility of the formulation was visually confirmed by the clarity of the medium throughout the 14 days.

Antimicrobial efficacy studies

The antimicrobial efficacy assessments were performed through the disk diffusion technique to determine the biological activity of the selected formulation. Conventional eye drops were used as a reference, and the chosen formulation was placed into cups drilled into sterile Muller Hinton Agar medium that had been previously seeded with Escherichia coli. The plates were incubated for 24 hours at 37°C, and the zone of inhibition was recorded in millimeters. Each formulation was evaluated in triplicate. The results were statistically analyzed using the Independent samples t-test, with a p-value of less than 0.05 considered statistically significant.

3. RESULTS AND DISCUSSION

Preformulation Studies

Timolol Maleate showed a specific λ_{max} at 294 nm in methanol and at 298 nm in phosphate buffer (pH 7.4), confirming its effectiveness for spectrophotometric analysis. The compatibility of Timolol Maleate with the excipients incorporated in the formulation was investigated using Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu IR Affinity-1S CE). The FTIR spectra of pure Timolol Maleate were compared to those of the physical mixture that contained the drug and selected excipients. The absence of significant shifts or the disappearance of characteristic peaks in the physical mixture indicated that no chemical interactions took place, thereby confirming the compatibility of the drug with the formulation excipients in the bilosomal in-situ gel system.

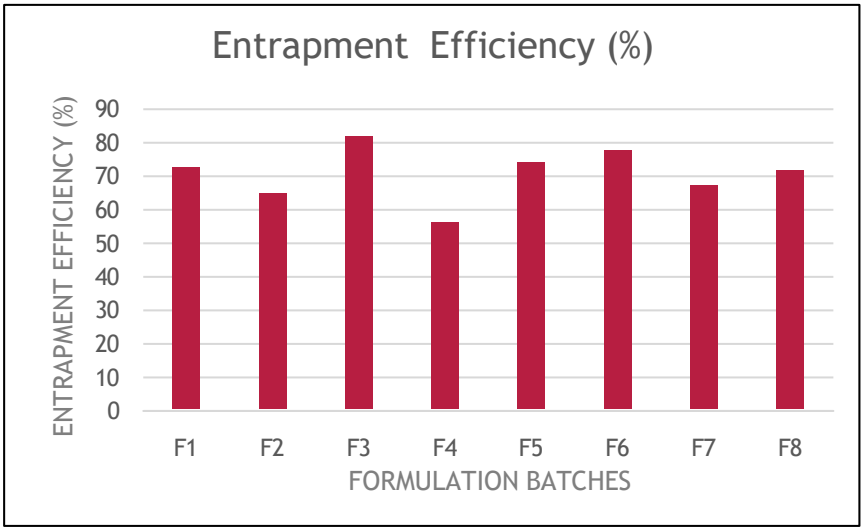
Characterization of Timolol Maleate loaded bilosomes

Entrapment Efficiency

Entrapment efficiency (EE) serves as a critical parameter that signifies the ability of a bilosomal system to encapsulate and retain the drug. Achieving a high EE is crucial to ensure that the therapeutic concentration of the drug is sufficient at the target site, especially in ocular delivery systems like in-situ gels. In this investigation, EE was evaluated for each formulation (F1 to F8), and the findings are presented in Table 6. The EE values ranged from 56.35% (F4) to 82.00% (F3). Among all formulations, F3 showed the highest entrapment efficiency, suggesting an optimal lipid-to-drug ratio and favorable conditions for vesicle formation. Other noteworthy formulations with high EE include F6 (77.80%), F5 (74.05%), and F1 (72.54%). Conversely, formulation F4 had the lowest EE, potentially due to suboptimal surfactant or lipid concentration, which may have led to inadequate drug encapsulation. These variations in EE could be attributed to differences in polymer and surfactant concentrations, vesicle stability, and bilayer integrity. Formulations with appropriate surfactant-lipid combinations (e.g., F3 and F6) likely facilitated improved entrapment of Timolol Maleate, thereby enhancing the therapeutic potential of the delivery system. The optimized batch BF3G1, based on a bilosomal base similar to high-EE batche (F3), exhibited satisfactory entrapment and was selected for subsequent in-situ gelation and evaluation studies.

Table 3. Entrapment Efficiency of Bilosomes

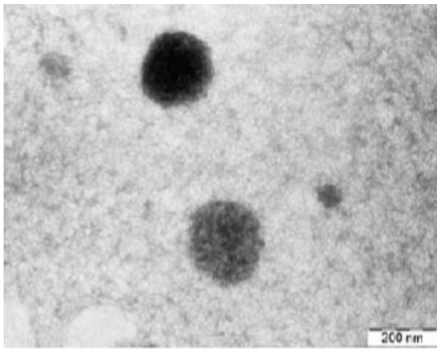
Sr No.	Formulation	Entrapment Efficiency (%)
1.	F1	72.54
2.	F2	65
3.	F3	82
4.	F4	56.35
5.	F5	74.05
6.	F6	77.80
7.	F7	67.25
8.	F8	71.77

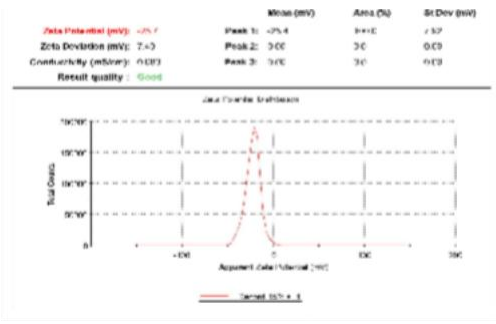


% Entrapment efficiency of prepared formulation

Determination of PDI, Vesicle Size, Zeta Potential and TEM

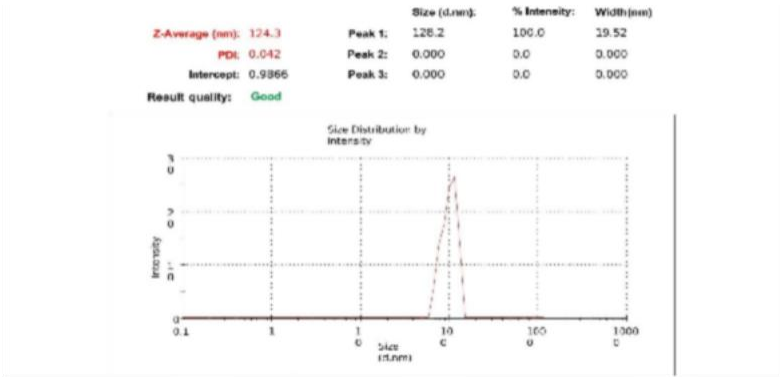
The optimized Timolol Maleate-loaded bilosomal in-situ gel (BF3G1) displayed ideal physicochemical and structural attributes for ocular delivery. The vesicles exhibited a uniform particle size of 124.3 nm with a low PDI of 0.042, indicating both uniform size distribution and homogeneity. TEM imaging confirmed spherical, well-dispersed bilosomes within the nanometric range. The zeta potential of -25.7 mV confirmed good stability, and the formulation showed a high entrapment efficiency of 82%, along with appropriate pH, viscosity, and gelling behavior. In-vitro release studies confirmed sustained drug release for as long as 8 hours. Sterility testing indicated no microbial growth, and antimicrobial efficacy was maintained. In summary, BF3G1 presents a promising, stable, and patient-friendly alternative to conventional eye drops by prolonging ocular residence time and decreasing dosing frequency in glaucoma therapy.





TEM image of BLO formulation

Zeta potential



Size distribution and PDI

Appearance and clarity, pH and drug content

The prepared formulations (BF3G1 to BF3G4) were observed to have a turbid white appearance and were visually cloudy.

This turbidity can be linked to the rising concentrations of both Carbopol® 934P and HPMC K100M, which impacted the clarity of the formulations. Specifically, at higher polymer concentrations (such as BF3G4 with 0.6% Carbopol and 0.8% HPMC), the formulation remained stable but did not exhibit transparency, indicating an increase in viscosity and a gel-like consistency. Therefore, no further increases in polymer concentrations were attempted. The pH of the optimized formulation was determined to be 6.43, which is within the acceptable ocular pH range and is expected to adjust towards the pH of lacrimal fluid (7.4) upon instillation, thereby minimizing irritation and ensuring patient comfort.[19,20] The combination of Carbopol® and HPMC effectively moderated the acidity of the solution. The viscosity of optimized batch was noted to be 68.4 cP, indicating good retention potential on the ocular surface. Furthermore, the formulations showed uniform dispersion, suggesting consistent mixing and homogeneity. The drug content of all formulations was found to be within the acceptable range of 98.03% to 99.53%, demonstrating effective incorporation and uniform distribution of the drug throughout the formulations, thereby supporting their suitability for ophthalmic administration.

Formulation	Clarity	pH	Drug Content (%)	Viscosity (cP)	Gelling Capacity
BF3G1	Cloudy	6.4	99.43 ± 0.15	68.43	+
BF3G2	Cloudy	6.32	99.07 ± 0.51	70.25	+
BF3G3	Cloudy	6.87	98.17 ± 0.31	80.32	++
BF3G4	Non-transparent	7.04	99.33 ± 0.45	128.03	++

Table 4: Results of physicochemical characterization of in situ gel formulations.

Viscosity

Viscosity is a crucial aspect in the design of ophthalmic formulations, as it directly impacts the residence time of the drug on the ocular surface. Low-viscosity solutions may drain from the eye too quickly, leading to a decrease in therapeutic efficacy, while excessively high viscosity can obstruct instillation and hinder spreadability. In this study, the viscosity of bilosome-loaded in-situ gel formulations (BF3G1 to BF3G4) was measured to range from 68.43 cP to 128.03 cP, indicating a direct correlation with increasing polymer concentration. Among all formulations, BF3G1 was selected as the optimized formulation, with a viscosity of 68.43 cP, which ensures a balanced profile providing adequate ocular retention while maintaining ease of instillation and good spreadability. As the concentration of Carbopol® 934P and HPMC K100M increased in BF3G2, BF3G3, and BF3G4, a corresponding rise in viscosity was noted: 70.25 cP, 80.32 cP, and 128.03 cP, respectively. These increases also led to enhanced gelling capacities, shifting from mild gelation (+) in BF3G1 and BF3G2 to stronger gelation (++) in BF3G3 and BF3G4. The results confirm that viscosity and gelling capacity are closely dependent on the polymeric concentration.[21] Importantly, all formulations maintained viscosities within an acceptable range, ensuring that none posed challenges during instillation. The optimized formulation, BF3G1, provided an ideal combination of moderate viscosity, effective in-situ gelation, acceptable pH (6.4), and high drug content (99.43 ± 0.15%), making it suitable for safe and effective ophthalmic delivery.

Gelling capacity

An effective in-situ gelling ophthalmic formulation must possess a balanced gelling capacity and suitable viscosity, ensuring that it can be easily administered as a liquid and undergoes a rapid sol-to-gel transition upon contact with the ocular surface. This transition is critical for enhancing the drug's residence time in the pre-corneal area, which is primarily influenced by the formulation's viscosity and polymer concentration. In this research, all bilosome-loaded in-situ gel formulations (BF3G1–BF3G4) remained in a sol state at room temperature and at the formulation's pH, but they underwent in-situ gelation when exposed to simulated tear fluid (STF, pH 7.4). The gelling capacity progressively increased with higher polymer concentrations. BF3G1 and BF3G2, which contained lower concentrations of Carbopol® 934P and HPMC K100M, exhibited mild gelation (+), while BF3G3 and BF3G4 demonstrated stronger gelation (++) with more structured and stable gel formation. The enhanced gelling behavior can be attributed to the hydrophobic backbone of Carbopol® and its increased ionization at elevated pH, resulting in greater electrostatic repulsion among polymer chains. This leads to the expansion of the polymer network and the formation of a three-dimensional viscoelastic gel matrix. The synergistic interaction between Carbopol® and HPMC further strengthens this network, improving the formulation's mechanical integrity. Such in-situ gels are capable of forming a stable gel layer upon ocular administration, which facilitates sustained drug release at the target site by retaining their structure without rapid erosion or dissolution, thus improving therapeutic efficacy and patient compliance.[22,23]

Gelling capacity

In-vitro drug release studies

The in-vitro release behavior of Pure Timolol Maleate (TM), TM-loaded bilosomes (TM-BLO), and TM-loaded bilosomes incorporated into an in-situ gelling system (TM-BLO-IG) was analyzed over an 8-hour timeframe. The drug release profiles are represented in Figure 1 and summarized in Table 5. Pure TM displayed a rapid drug release, with approximately 97.8% of the drug released within 8 hours, and 51.1% released in the first hour. This burst release is due to the free, unencapsulated state of the drug and the lack of any barrier system to hinder its diffusion. In contrast, TM-BLO exhibited a more controlled release, with 80% of the drug released by the end of 8 hours. The bilosomal vesicular system effectively delayed the drug release due to the encapsulation of TM within lipid bilayers, which act as a diffusion barrier. This confirms the potential of bilosomes to sustain drug release and minimize burst effects typically observed in conventional dosage forms. The most significant delay in drug release was noted in TM-BLO-IG, where only 64.4% of the drug was released over 8 hours. The cumulative release at 1 and 2 hours was 28.9% and 40%, respectively. This slow and sustained release profile is attributed to the formation of a viscous gel matrix upon exposure to simulated tear fluid (pH 7.4), which creates a physical barrier that restricts drug diffusion and enhances ocular residence time. Among the three formulations, TM-BLO-IG (BF3G1) was identified as the optimized batch based on its prolonged release profile, effective in-situ gelation, and moderate viscosity. The $T_{50\%}$ value (the time required to release 50% of the drug) for Pure TM, TM-BLO, and TM-BLO-IG was approximately 1.2 h, 2.1 h, and 4.5 h, respectively, clearly demonstrating the superior sustained release performance of the in-situ gel system. To elucidate the drug release mechanism, the data were fitted into various kinetic models, including zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas models. Among these, TM-BLO-IG demonstrated the best adherence to zero-order kinetics, indicating a constant release rate that is independent of the drug concentration. Moreover, the Korsmeyer–Peppas model showed an n value between 0.43 and 0.85, which points to a non-Fickian (anomalous) diffusion mechanism, where the drug release is controlled by both diffusion and the erosion of the polymer matrix. These findings affirm that the integration of bilosomal drug delivery with a pH-responsive in-situ gel system effectively prolongs drug release, potentially enhancing ocular bioavailability while decreasing dosing frequency. Thus, BF3G1 (TM-BLO-IG) is a promising method for the sustained ocular delivery of Timolol Maleate in the treatment of glaucoma.

Table 5: In-vitro drug release data

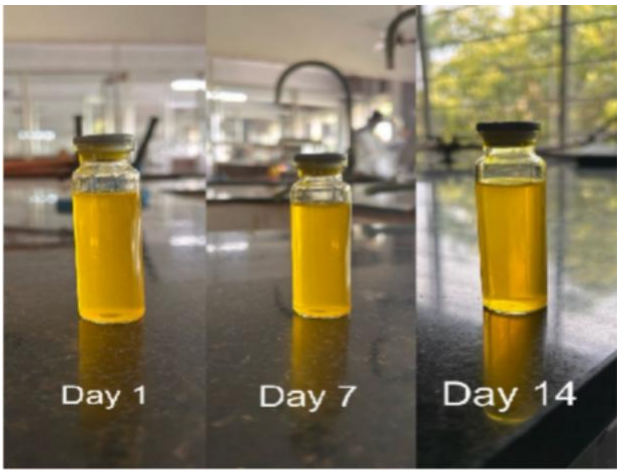
Time (h)	Pure TM(%)	TM-BLO(%)	TM-BLO-IG(%)
0.5	26.7	22.2	13.3
1	51.1	37.8	28.9
2	68.9	51.1	40
4	82.2	62.2	48.9
6	91.1	71.1	57.8
8	97.8	80	64.4

Antimicrobial efficacy studies

The antimicrobial effectiveness of the optimized formulation was assessed through the agar diffusion method, with the results outlined in Table 5 and visually represented in Figure 3. The inhibition zone around the sample indicates the formulation's antimicrobial effectiveness. The findings revealed that the diameter of the inhibition zone was over 18 mm, suggesting that the formulation has very active antimicrobial activity. This level of inhibition is regarded as pharmacologically significant and demonstrates that the antimicrobial agent retained its efficacy even after being integrated into the in-situ gel matrix. The results confirm that the bilosome encapsulation process and in-situ gel formulation did not compromise the antimicrobial activity of the active pharmaceutical ingredient. The sustained release property of the gel may further enhance prolonged antimicrobial action at the application site, potentially lowering the risk of microbial growth and secondary infections in ocular therapy. Consequently, the in-situ gelling system incorporating Timolol Maleate-loaded bilosomes (BF3G1) not only provides extended drug release and improved ocular retention but also maintains effective antimicrobial protection, thereby enhancing the therapeutic potential and safety profile of the formulation.

Sterility testing

Sterility testing was executed to evaluate the microbiological quality of the optimized formulation BF3G1, which contains Carbopol® 934P and HPMC K100M. The assessment was performed using fluid thioglycollate medium (FTM) and incubated for 14 days at 35 ± 2°C, in accordance with pharmacopeial regulations. As depicted in Figure 4, there was no visible turbidity or signs of microbial growth noted during the incubation period on Day 1, Day 7, and Day 14. The test medium remained clear and transparent, indicating the absence of bacterial or fungal contamination. These outcomes confirm that formulation BF3G1 fulfills the sterility criteria necessary for ophthalmic preparations. The lack of microbial growth validates that aseptic processing was preserved during formulation and filling, ensuring the product is microbiologically safe for ocular administration.



Sterility testing

4. CONCLUSION

This study successfully developed and assessed a novel bilosomal in-situ gel system that incorporates Timolol Maleate to enhance ocular drug delivery for glaucoma management. The optimized formulation (BF3G1) displayed nanosized vesicles measuring 124.3 nm, a high entrapment efficiency of 82%, a low polydispersity index of 0.042, and a stable zeta potential of –25.7 mV, which indicates the uniformity and stability of the bilosomal system. When integrated into a pH-sensitive in-situ gel using Carbopol® 934P and HPMC K100M, the formulation exhibited excellent gelling capacity, suitable viscosity, and sustained drug release over a period of 8 hours. In-vitro studies confirmed that the drug release followed zero-order kinetics and a non-Fickian diffusion mechanism, while antimicrobial and sterility testing affirmed its safety and efficacy for ophthalmic applications. In summary, the developed bilosomal in-situ gel presents a promising, stable, and patient-friendly platform for enhancing the therapeutic effectiveness and dosing convenience of Timolol Maleate in the treatment of glaucoma.

Acknowledgements

The authors are thankful to Shri D.D. Vispute College of Pharmacy and Research Center, Panvel, India.

REFERENCES

1. Ahmed S, Amin MM, Sayed S. Ocular Drug Delivery: a Comprehensive Review. AAPS PharmSciTech. 2023 Feb 14;24(2).
2. Agrawal AK, Das M, Jain S. In situ gel systems as ‘smart’ carrier for sustained ocular drug delivery. Expert Opin Drug Deliv. 2012;9(4):383-02

3. Asrani SG, McGlumphy EJ, Al-Aswad LA, Chaya CJ, Lin S, Musch DC, et al. The relationship between intraocular pressure and glaucoma: An evolving concept. Progress in retinal and eye research [Internet]. 2024;103:101303.

4. Iyer JV, Boland MV, Jefferys J, Quigley H. Defining glaucomatous optic neuropathy using objective criteria from structural and functional testing. British Journal of Ophthalmology. 2020 Jul 22;105(6):789–93.

5. El-Feky YA, Fares AR, Zayed G, El-Telbany RFA, Ahmed KA, El-Telbany DFA. Repurposing of nifedipine loaded in situ ophthalmic gel as a novel approach for glaucoma treatment. Biomedicine & Pharmacotherapy. 2021 Oct;142:112008.

6. Fahmy AM, El-Setouhy DA, Ibrahim AB, Habib BA, Tayel SA, Bayoumi NA. Penetration enhancer-containing spanlastics (PECSs) for transdermal delivery of haloperidol: in vitro characterization, ex vivo permeation and in vivo biodistribution studies. Drug Delivery. 2017 Dec 8;25(1):12–22.

7. Kamal, Rathore S, Kumar Nema R, Sidhraj S, Sisodia. TIMOLOL MALEATE A GOLD STANDARD DRUG IN GLAUCOMA USED AS OCULAR FILMS AND INSERTS: AN OVERVIEW. International Journal of Pharmaceutical Sciences Review and Research [Internet]. 2010;3(1).

8. Barnes J, Moshirfar M. Timolol [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2020.

9. Allam A, Elsabahy M, El Badry M, Eleraky NE. Betaxolol-loaded niosomes integrated within pH-sensitive in situ forming gel for management of glaucoma. International Journal of Pharmaceutics. 2021 Apr;598:120380.

10. Al-mahallawi AM, Abdelbary AA, Aburahma MH. Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam. International Journal of Pharmaceutics. 2015 May;485(1-2):329–40.

11. Conacher M, Alexander J, Brewer JM. Oral immunisation with peptide and protein antigens by formulation in lipid vesicles incorporating bile salts (bilosomes). Vaccine [Internet]. 2001 Apr [cited 2019 Aug 3];19(20-22):2965–74.

12. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, et al. Research progress of in-situ gelling ophthalmic drug delivery system. Asian Journal of Pharmaceutical Sciences [Internet]. 2019 Jan 1 [cited 2020 Apr 10];14(1):1–15.

13. Gupta S. Carbopol/Chitosan Based pH Triggered In Situ Gelling System for Ocular Delivery of Timolol Maleate. Scientia Pharmaceutica. 2010;78(4):959–76.

14. Alsaidan OA, Zafar A, Yasir M, Alzarea SI, Alqinyah M, Khalid M. Development of Ciprofloxacin-Loaded Bilosomes In-Situ Gel for Ocular Delivery: Optimization, In-Vitro Characterization, Ex-Vivo Permeation, and Antimicrobial Study. Gels. 2022 Oct 25;8(11):687.

15. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results in Pharma Sciences. 2016;6:1–6.

16. Saifi Z, Rizwanullah Md, Mir SR, Amin S. Bilosomes nanocarriers for improved oral bioavailability of acyclovir: A complete characterization through in vitro, ex-vivo and in vivo assessment. Journal of Drug Delivery Science and Technology. 2020 Jun;57:101634.

17. Kouchak M, Mahmoodzadeh M, Farrahi F. Designing of a pH-Triggered Carbopol®/HPMC In Situ Gel for Ocular Delivery of Dorzolamide HCl: In Vitro, In Vivo, and Ex Vivo Evaluation. AAPS PharmSciTech. 2019 Jun 3;20(5).

18. Kesarla R, Tank T, Vora PA, Shah T, Parmar S, Omri A. Preparation and evaluation of nanoparticles loaded ophthalmic in situ gel. Drug Delivery. 2015 Jan 12;23(7):2363–70

19. Rajoria G, Gupta A. In situ gelling system: A novel approach for ocular drug delivery. Am J Pharmatech Res. 2012;2(4):24-53.

20. Madan M, Bajaj A, Lewis S, Udupa N, Baig JA. In situ forming polymeric drug delivery systems. Indian J Pharm Sci. 2009;71(3):242-51.

21. Pandey A, Mali PY, Sachdeva D, Patel DK, Ramesh R. Development and optimization of levobunolol hydrochloride in-situ gel for glaucoma treatment. Int J Pharm Biol Arch. 2010;1(2):134-9.

22. Asasutjarit R, Thanasanchokpibnll S, Fuongfuchatc A, Veeranondha S. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. Int J Pharm. 2011;411(1-2):128-35.

23. Kanoujia J, Sonker K, Pandey M, Kymonil MK, Saraf SA. Formulation and characterization of a novel pH-triggered in-situ gelling ocular system containing gatifloxacin. Int Current Pharm J. 2012;1(3):43-9.